EDWARD TERHEMEN AKANGE

DOCTOR OF PHILOSOPHY

2025

DEVELOPMENT OF AN INDOOR MULTI-TECHNO AQUACULTURE SYSTEM WITH RAPID BIOFLOC FOR THE PRODUCTION OF PACIFIC WHITELEG SHRIMP, *Litopenaeus vannamei*

EDWARD TERHEMEN AKANGE

DOCTOR OF PHILOSOPHY
UNIVERSITI MALAYSIA TERENGGANU

2025

DEVELOPMENT OF AN INDOOR MULTI-TECHNO AQUACULTURE SYSTEM WITH RAPID BIOFLOC FOR THE PRODUCTION OF PACIFIC WHITELEG SHRIMP, *Litopenaeus vannamei*

EDWARD TERHEMEN AKANGE

Thesis Submitted in Fulfilment of the Requirement for the Doctor of Philosophy of Science in the Institute of Tropical Aquaculture and Fisheries Universiti Malaysia Terengganu

February 2025

Pusat Pengurusan Siswazah (PPS)

Borang Pengesahan Penyerahan Tesis / Disertasi/ Laporan Kertas Projek
Declaration Form for Submission of Theses/ Dissertation / Project Paper

Tajuk / *Title* : ……Development of an indoor multi-techno aquaculture system with rapid biofloc for the production of whiteleg shrimp, *Litopenaeus vannamei*…….

Nama Pelajar / *Student's Name* : ……Edward Terhemen Akange……

No. Matrik / *Matric No.* : ……P5482…….

Saya dengan ini mengaku membenarkan Tesis/Dissertasi/Laporan Kertas Projek ini disimpan di Perpustakaan Sultanah Nur Zahirah, Universiti Malaysia Terengganu ("UMT") dan mengakui bahawa UMT mempunyai hak-hak seperti berikut:
I hereby declare that I have allowed this Theses/Dissertation/Project Paper to be kept by Perpustakaan Sultanah Nur Zahirah, Universiti Malaysia Terengganu ("UMT") and acknowledged that UMT reserves the rights as follows:

1. Hak cipta Tesis/Dissertasi/Laporan Kertas Projek ini adalah hak milik UMT.
   The copyright of this Theses/Dissertation/Project Paper belongs to UMT.

2. UMT berhak untuk membuat salinan Tesis/Dissertasi/ Laporan Kertas Projek ini untuk tujuan akademik.
   UMT shall has the right to make copies of this Theses/Dissertation/Project Paper for the academic purpose.

3. UMT berhak membuat salinan Tesis/Dissertasi/Laporan Kertas Projek ini sebagai bahan pertukaran antara institusi pengajian tinggi.
   UMT shall has the right to make copies for this Theses/Dissertation/Project Paper as an exchange between higher learning institutions.

4. Hak-hak UMT yang dinyatakan dalam perkara 2 dan 3 di atas adalah tertakluk kepada kategori Tesis/Dissertasi/Laporan Kertas Projek seperti yang dinyatakan di dalam ini.

The rights of UMT stated in items 2 and 3 above are subject to the category of the Thesis/Dissertation/Project Paper Report as stated herein.

Sila tanda (/) bagi pilihan kategori di bawah:
Please tick (/) from the categories below:

(X)	Tidak Terhad / *Open Access*	
( )	Terhad / *Restricted*	Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. *Contains restricted information as specified by the organization/body where research was done.*
( )	Sulit / *Confidential*	Mengandungi maklumat yang dikategorikan di bawah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972 *Contains information categorized under the security or interests of Malaysia as stipulated in the Official Secrets Act 1972.*

…….

Tandatangan Pelajar /
Student's Signature

……..

Tandatangan Penyelia /
Supervisor's Signature
(Nama dan Cop Rasmi/ *Name & Official Stamp*)

Catatan: Jika Tesis/Dissertasi/Laporan Kertas Projek ini SULIT @ TERHAD, sila lampirkan surat dari pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh Tesis/Dissertasi/Laporan Kertas Projek ini perlu dikelaskan sebagai SULIT atau TERHAD.
Note: If this Theses/Dissertation/Project Paper is CONFIDENTIAL @ RESTRICTED, please attach the letter from the relevant authority/organization stating its reason and duration of this Theses/Dissertation/Project Paper should be classified as CONFIDENTIAL or RESTRICTED.

DEDICATION

Dedicated to

Everyone who believes and stands for real essence of living

and to

My reason for struggle-hood:

Iveren, Shiekuma, MkavTer and Saanmoiyol

I'm indebted to you

To my family and friends

May the Supreme God be with you

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Doctor of Philosophy

DEVELOPMENT OF AN INDOOR MULTI-TECHNO AQUACULTURE SYSTEM WITH RAPID BIOFLOC FOR THE PRODUCTION OF PACIFIC WHITELEG SHRIMP, *Litopenaeus vannamei*

EDWARD TERHEMEN AKANGE

FEBRUARY 2025

Main Supervisor : **Assoc. Prof. ChM. Ts. Nor Azman Kasan, PhD**

Co-Supervisors : **Hajar Rastegari, PhD**

Olumide A. Odeyemi, PhD

Faculty/Institute : **Institute of Tropical Aquaculture and Fisheries**

In 2021, global aquaculture production reached 126 MT, valued at US$296.5 billion. With overfishing limiting wild fish yields, aquaculture has become essential, particularly for intensive shrimp farming. This study aimed to develop and optimise the production conditions for *Litopenaeus vannamei* in an Indoor Multi-techno Aquaculture System (IMTAS). Two 84-day experiments were conducted at the Universiti Malaysia Terengganu. Experiment 1 (14 March–5 June 2023) optimised a conventional biofloc system using the Taguchi L9 method to establish control conditions for the IMTAS. Experiment 2 (2 August–17 October 2023) evaluated IoT-automated IMTAS, which comprised production, flocculation, and phytoremediation units. The IMTAS was tested for water quality and zootechnical and physiological parameters of *L. vannamei*. Nutrient profiles of floc residues and nutrient absorption capacity of *Caulerpa lentilifera* were also assessed. Experiment 1 optimised stocking density, C/N ratio, and probiotic dosage using the Taguchi L9 design and Grey Relational Analysis (GRA). Optimal conditions were stocking density of 200 shrimp/m³, C/N ratio of 15, and probiotic dosage of 14 ml/L. Experiment 2 evaluated IMTAS efficiency, showing that GRG 1 had optimal water quality conditions (total ammonia-nitrogen at 0.90±0.01 mg/L, nitrite at 0.42±0.01 mg/L, phosphate at 0.12±0.00 mg/L, dissolved oxygen at 6.92±0.08 mg/L, electrical conductivity at 44926±784 µS/cm, total dissolved solids at 27411±141 mg/L, salinity at 28.06±0.45

ppm, pH at 6.96±0.05, and temperature at 27.80±0.66°C). Gut microbial analysis also indicated that the *Pseudomonadaceae* family was most prevalent in the Control group, with a relative abundance of 0.80, as compared to 0.15 in GRG 1 and 0.02 in GRG 2, while *Vibrionaceae* was only present in the control group (relative abundance, 0.05). The transcriptome showed an up-regulation in genes controlling growth and immunity in GRG 1 and GRG 2. GRG 1 had significantly higher level of fatty acid essential and non-essential amino acids. *Caulerpa lentilifera* significantly improved water quality by absorbing pollutants, including nitrogen and phosphorus. *Caulerpa lentilifera* demonstrated high efficiency in nutrient storage and water quality improvement, demonstrating its role in wastewater remediation. This study confirms that IMTAS, particularly GRG 1 treatment, significantly enhanced shrimp growth while promoting efficient nutrient recycling and water quality management.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN SISTEM AKUAKULTUR PELBAGAI-TEKNO DALAMAN YANG DISATUKAN DENGAN BIOFLOK PANTAS UNTUK PENGELUARAN UDANG PUTIH PASIFIK, *Litopenaeus vannamei*

EDWARD TERHEMEN AKANGE

FEBRUARI 2025

Penyelia Utama : Profesor Madya ChM. Ts. Nor Azman Kasan, PhD

Penyalia Bersama : Hajar Rastegari, PhD

Olumide A. Odeyemi, PhD

Fakulti/Institut : Institut Akuakultur Tropika dan Perikanan

Pada tahun 2021, pengeluaran akuakultur global mencapai 126 MT yang bernilai AS$296.5 bilion. Dengan penangkapan ikan berlebihan yang menghadkan hasil tangkapan ikan liar, menjadikan akuakultur lebih penting, terutamanya penternakan udang secara intensif. Kajian ini bertujuan untuk membangun dan mengoptimumkan pengeluaran *Litopenaeus vannamei* dalam Sistem Akuakultur Multi-tekno Dalaman (IMTAS). Dua eksperimen telah dijalankan selama 84 hari di Universiti Malaysia Terengganu. Eksperimen 1 (14 Mac – 5 Jun 2023) telah mengoptimumkan sistem bioflok secara konvensional menggunakan kaedah Taguchi L9 bagi mewujudkan keadaan kawalan untuk IMTAS. Eksperimen 2 (2 Ogos – 17 Oktober 2023) telah dijalankan untuk menilai kefungsian automasi-IoT IMTAS, yang terdiri daripada unit pengeluaran, penggumpalan dan fitoremediasi. IMTAS telah diuji untuk mengukur kualiti air, parameter zooteknik dan fisiologi *L. vannamei*. Profil nutrien sisa flok dan kapasiti penyerapan nutrien *Caulerpa lentilifera* juga telah dinilai. Eksperimen 1 telah dijalankan bagi mengoptimumkan kepadatan stok, nisbah C/N dan dos probiotik menggunakan rekabentuk Taguchi L9 dan *Grey Relational Analysis* (GRA). Keadaan optimum didapati pada kepadatan 200 ekor udang/m^3, nisbah C/N 15, dan dos probiotik 14 ml/L. Eksperimen 2 telah dijalankan bagi menilai kecekapan IMTAS, yang menunjukkan *Grey Relational Grade* (GRG) 1 mempunyai keadaan kualiti air yang optimum (jumlah ammonia-nitrogen 0.90±0.01 mg/L, nitrit 0.42±0.01

mg/L, fosfat 0.12±0.00 mg/L, oksigen terlarut 6.92 ±0.08 mg/L, kekonduksian elektrik 44926±784 µS/cm, jumlah pepejal terlarut 27411±141 mg/L, kemasinan 28.06±0.45 ppm, pH 6.96±0.05, dan suhu 27.6°C). Analisis mikrob di usus juga menunjukkan bahawa keluarga *Pseudomonadaceae* adalah paling banyak dalam kumpulan Kawalan, dengan kelimpahan relatif 0.80, berbanding 0.15 dalam GRG 1 dan 0.02 dalam GRG 2, manakala *Vibrionaceae* hanya terdapat dalam kumpulan Kawalan (kelimpahan relatif, 0.05). Transkriptomik menunjukkan peraturan-menaik dalam gen yang mengawal pertumbuhan dan imuniti dalam GRG 1 dan GRG 2. GRG 1 mempunyai tahap asid amino penting dan asid amino tidak penting yang lebih tinggi. *C. lentilifera* telah meningkatkan kualiti air dengan menyerap bahan pencemar, termasuk nitrogen dan fosforus. *C. lentilifera* menunjukkan kecekapan tinggi dalam penyimpanan nutrien dan peningkatan kualiti air, menunjukkan peranannya dalam pemulihan air sisa. Kajian ini mengesahkan bahawa IMTAS, terutamanya dalam rawatan GRG 1, telah meningkatkan pertumbuhan udang yang signifikan, disamping menggalakkan kitar semula nutrien dan pengurusan kualiti air yang efisien.

ACKNOWLEDGEMENTS

I am eternally grateful to God for this wonderful opportunity to learn and research under the supervision of Assoc. Prof Ts. Nor Azman Kasan and the supervisory team comprising Dr Hajar Rastegari and Dr Olumide A. Odeyemi. It was also a great privilege working with the IMTAS research crew made of Mr. Ideris, Mr. Syafiq, Mr. Shuhaimi and Mrs. Amyra. We eventually became a family – I felt the love and companionship in true fraternity. I sincerely appreciate the Higher Institution Centre of Excellence (HiCOE) of the Ministry of Higher Education, Malaysia for funding this research and providing all the necessary support for the success of the IMTAS project.

My wife, Iveren and children, Shiekuma, MkavTer and Saanmoiyol have made invaluable sacrifices during my study on this programme. I have been an absentee husband and father, but I know they understood the situations. Lines on the paper here would not be sufficient to convey my heartfelt appreciation. My parents, siblings, in-laws and friends always kept in touch while I was away for studies. I would not forget the gesture in a haste.

For the wonderful people I met in Malaysia, I say thank you. The graduate room – my second home was always fun-filled. Most of my academic knots were untied during casual discussions while in the company of my friends – Pouya, Donald, Ben, Gary, Pius, Lydia and Shahadat. I did not miss home in your company. And to the administrative staff at AKUATROP, Mdm. Ema, Mdm. Solihah who were always ready to help, I am grateful.

EDWARD TERHEMEN AKANGE

APPROVALS

I certify that an Examination Committee has met on 5th of February, 2025 to conduct the final examination of Edward Terhemen Akange, on his Doctor of Philosophy thesis entitled **"Development of an Indoor Multi-Techno Aquaculture System with rapid biofloc for the production of Pacific whiteleg shrimp, *Litopenaeus vannamei*"** in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

Hamid Amiri Dastnaei, Ph.D
Associate Professor,
Institute of Tropical Aquaculture and Fisheries,
Universiti Malaysia Terengganu.
(Chairperson)

Lilian Wong, Ph.D
Associate Professor,
Institute of Climate Adaptation and Marine Biotechnology,
Universiti Malaysia Terengganu.
(Internal Examiner)

Nita Rukminasari, Ph.D
Professor,
Fakultas Ilmu Kelautan dan Perikanan,
Universitas Hasanuddin Indonesia.
(External Examiner 1)

Ching Fui Fui, Ph.D
Associate Professor,
Borneo Marine Research Institute
Universiti Malaysia Sabah.
(External Examiner 2)

MUHAMMAD IKHWANUDDIN ABDULLAH (Ph.D)
Professor/ Director
Institute of Tropical Aquaculture and Fisheries
Universiti Malaysia Terengganu

Date

This thesis has been accepted by the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Doctor of Philosophy.

MUHAMMAD IKHWANUDDIN ABDULLAH (Ph.D)
Professor/ Director
Institute of Tropical Aquaculture and Fisheries
Universiti Malaysia Terengganu

Date

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UMT or other institutions.

_____

EDWARD TERHEMEN AKANGE

Date:

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Full meaning
BFT	Biofloc technology
CF	Condition factor
CFr	Crude fibre
Cntrl	Control
CP	Crude protein
C/N ratio	Carbon-to-nitrogen ratio
DEG	Differentially expressed genes
DIM	Dimension
DNA	Deoxyribonucleic acid
DOE	Design of Experiments
FCR	Feed conversion ratio
GRA	Grey relational analysis
GRC	Grey relational coefficients
GRG	Grey relational grades
HSI	Hepatosomatic index
IMTAS	Indoor Multi-techno Aquaculture System
KEGG	Kyoto Encyclopaedia of Genes and Genomes
MFW	Mean final weight
MIW	Mean initial weight
MWG	Mean weight gain
PCA	Principal component analysis
ProPO	Prophenoloxidase activity
RNA	Ribonucleic acid
RWG	Relative weight gain
SGR	Specific growth rate
SOD	Superoxide dismutase activity
THC	Total haemocyte count
DM	Dry matter
EE	Ether Extract
ME	Metabolizable energy

NFE	Nitrogen free extract
FD	Freeze-dried
OD	Oven-dried
SFAs	Saturated fatty acids
MUFAs	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids
ADG (%)	Average daily growth
BE (%)	Biofiltration efficiency

LIST OF FORMULA

Equation		Page
2.1	$L_x(n^f)$	37
2.2	$L(y) = k(y-T)^2$	41
2.3	$L(y) = \frac{u}{\Delta^2}(y-T)^2$	41
2.4	$L(y) = k(\frac{1}{y^2})$	41
2.5	$L(y) = ky^2$ y>0	41
2.6	$L(y) = k(y-T)^2 \quad k$	42
2.7	$S{:}N\ Ratio\ = -10\ log[\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}]$	42
2.8	$S{:}N\ Ratio\ = -10\ log[\frac{1}{n}\sum_{i=1}^{n}y_i^2]$	42
2.9	$S{:}N\ Ratio\ = 10log\left[\frac{\bar{y}^2 - {s_i^y}/{n}}{s_i^2}\right] = 10\ [log(\frac{\bar{y}}{s_y})^2 - \frac{1}{n}]$	42
2.10	$C = (F\ \times DM\ \times WF)/CF$	61
2.11	$N = (F \times DM \times WF \times CPF)\ /K$	61
2.12	$C_{req} = N\ \times R$	61
2.13	$C_{molasses} = C_{req} - C_{feed}$	61
2.14	$M_{req} = C_{molasses} \times 0.5$	61
2.15	$TAN_{system} = TAN_{conc} \times Water\ Vol.$	62
2.16	$M_{supplementation} = TAN_{system} \times M_{req}$	62
3.1	$S/N\ ratio = (-10)^{*}log_{10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\chi_{ij}^2$	65
3.2	$S/N\ ratio = (-10)^{*}log_{10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\frac{1}{\chi_{ij}^2}$	65
3.3	$\chi_j^*(p) = \frac{\chi_j(p) - min\ \chi_j(p)}{max\ \chi(p) - min\ \chi_j(p)}$	69
3.4	$\chi_j^*(p) = \frac{max\ \chi(p) - \chi(p)}{max\ \chi_j(p) - \min \chi_i(p)}$	69
3.5	$\Delta_{0j}(p) = \lvert\chi_0^*(p) - \chi(p)\rvert$	70

6.1 $$PO_4^{2-}\ (\text{g}) = \frac{3.066\ X\ P}{1000}$$ 202

6.2 $$ADG\ (\%) = \frac{(\omega_{d7} - \omega_{d0})}{7days} X\ 100$$ 203

6.3 $$BE\ (\%) = \frac{(\alpha - \gamma)}{\alpha} X\ 100$$ 204

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

Aquatic products, including fish, shrimp, and other seafood are essential sources of proteins for human consumption. Global demand for aquatic food is projected to increase by more than 40% between 2018 and 2030, indicating a substantial growth trajectory (Wiloso et al., 2022). The increasing demand for fish and fish products has engendered both impediments and prospects in the aquaculture sector (Seliger et al., 2021). Aquaculture has emerged as a viable alternative source of aquatic animals to meet growing demand due to the overfishing of wild fish, (Bossier & Ekasari, 2017; Nayak et al., 2019; Sampantamit et al., 2020). Many researchers have considered that an increase in aquaculture production would guarantee global food security (Asche et al., 2022; Einarsson & Óladóttir, 2020; Henriksson et al., 2021; Joffre et al., 2021). The aquaculture industry in Malaysia has been associated with numerous economic advantages, such as fulfilling both domestic and international demands to offer a steady income source for farmers, and contributing to the country's food security (Lee et al., 2018). These economic gains have been supported by the development of reliable systems for fish and fishery products exported to the US and EU markets, enhancing the competitiveness of the industry in international trade (Dong et al., 2019). Aquaculture development has paved way for diversification of

species production, promoting sustainability, and addressing current and future challenges in the industry (Bohnes et al., 2022; Hu et al., 2022; Khanjani et al., 2023; Naylor et al., 2021). As the need for aquatic goods continues to increase, the aquaculture sector has emerged as one of the most rapidly expanding sectors. New technologies have been developed to boost sustainable production in the face of climate change and pollution (Johnson et al., 2019; Nácher-Mestre et al., 2018).

Aquaculture systems and technology involve the use of organized arrangements to cultivate aquatic animals, with a focus on implementing routine management practices to enhance production efficiency (Mair et al., 2023; Voicea et al., 2021; Wang et al., 2020). This increasing demand of aquatic products has been met by a shift toward more intensive aquaculture systems (Chen et al., 2018; Coyne et al., 2019). The use of science and advanced technologies in aquaculture has transformed the industry, allowing for increased fish production and improved quality (Irshath et al., 2023). The adoption of modern biotechnological methods, aquaculture systems and technology have the potential to significantly increase fish quality and quantity while also meeting the rising demand for fish sustainably. Aquaculture system optimisation is the process of maximising the efficiency and sustainability of aquaculture operations (Kemal et al., 2023; Ubina & Cheng, 2022). The optimisation process entails finding the best possible configuration and management practices to improve productivity, reduce costs, minimise environmental impacts, and ensure the success of aquaculture operations. Aquaculture system optimisation is achieved by optimising feed management, water quality management, stocking density, disease control measures, and waste management practices (Luna et al., 2020; Prasad et al., 2023). Aquaculture system optimisation aims at enhancing decision-making processes and develop customised solutions for the specific requirements using advanced technologies, data analysis, and modelling techniques, (Kemal et al., 2023). Aquaculture system optimisation entails the use of automated feeding systems, water recirculation systems, genetic selection for disease resistance, and the integration of multi-trophic aquaculture systems (Adegboye et al., 2020).

The emergence of biofloc technology (BFT) has addressed the challenge of harmful waste accumulation in confined aquaculture systems by introducing a

microbial community that co-exists in complex multifunctional interactions, to establish a culture system that promotes optimum growth through self-generated food sources and improved health conditions. BFT system is a zero-water exchange system that relies on the microbial community to detoxify ammonia generated from faecal pellets and unconsumed feed (Minaz et al., 2023). Sustainable aquaculture is affected by an increase in the formation of lethal nitrogenous waste caused by organic residues under intensive culture conditions (Kiani et al., 2020; Nenciu et al., 2022; Nisar et al., 2022). BFT is a sustainable aquaculture technique that depends on the production of microorganisms, including bacteria, algae, protozoa, and nematodes, the majority of which are heterotrophic bacteria (Hamza & Zinjarde, 2023).

Biofloc is a product of the conversion of deleterious metabolic waste generated during the production of aquatic animals raised in the BFT system. Numerous inorganic compounds are released into the environment through the metabolic activities of bacteria, which are useful for other living organisms. Simultaneously, bacteria produce extracellular enzymes that decompose several organic materials, such as keratin, lignin, and other compounds that are not easily transformed (Faizullah et al., 2019; Hernández et al., 2019; Khanjani et al., 2022). Certain species of microorganisms in biofloc systems play a central role in maintaining water quality and promoting the growth and health of cultured animals, thus resolving the growing concern about environmental pollution (Qiu et al., 2023). Heterotrophic bacteria in BFT assimilate inorganic nitrogen and synthesise it into useful energy-rich biomaterials for the trophic utilisation of cultured animals (Da Silva et al., 2023; Hanumantha et al., 2021; Marini et al., 2020), consequently detoxifying the culture system of the deleterious waste generated from faecal pellets and unconsumed feed left in the water medium. Thus, BFT acts as a self-sustaining aquaculture system that enhances the reuse of waste, as well as the creation of resources from harmful waste (Kavitha & Krishna, 2020; Mugwanya et al., 2021).

BFT involves the cultivation of dense microbial aggregates in aquaculture systems or biofloc, creating a dynamic and self-regulating environment that promotes water quality management, waste remediation, and enhanced nutrition for aquatic

organisms (Da Silva et al., 2023; Ghimire et al., 2023). The floc residue formed during this process is considered aquaculture waste, because its accumulation leads to excessive suspended solids, impairs light penetration, and clogs the gills of cultured organisms. The use of biofloc meal as a dietary supplement has been the subject of extensive research, with studies evaluating its impact on growth performance, water quality, and proximate composition of various aquatic species (Binalshikh-Abubkr & Hanafiah, 2022). The addition of biofloc meal to aquafeeds has led to promising results, as demonstrated by studies that have shown improvements in growth rates, enzymatic activity, and nutrient uptake in cultured organisms. Biofloc meal has been recognised as a sustainable alternative to traditional aquafeed ingredients, as it addresses the environmental and resource limitations associated with traditional aquafeed ingredients. The utilisation of biofloc meal as aquaculture feed is an essential link in the aquaculture circular economy (Fraga-Corral et al., 2022; Verreth et al., 2023). Apart from recycling nutrients within the system, biofloc meal reduces the environmental impact but also contributes to the cost-effectiveness of farmers.

The whiteleg shrimp, *Litopenaeus vannamei* is one of the most important species in the global fishery and aquaculture industries. The shrimp aquaculture industry has contributed significantly to the global economy, particularly in middle- and low-income countries. It has been reported that *L. vannamei*, *Penaeus monodon*, and *Macrobrachium rosenbergii* contributed to the annual global shrimp aquaculture production of 6.0 million tonnes in 2018, with an increase to 6.55 million tonnes in 2019 increased to 6.55 million tonnes in 2019, estimated at USD 40.67 billion (FAO, 2019). The economic value of the shrimp aquaculture industry has been vast over the past decade, with an export value of billions of dollars per year. *L. vannamei* is widely distributed and cultivated worldwide, owing to its economic significance in the aquaculture industry. Its several bio-economic advantages make it a commercially important species compared to other shrimp species (Gao et al., 2021; Wang et al., 2019; Yu et al., 2022). Gyan et al., (2022) confirmed a connection between the growth performance and survival rates of the organism and the intestinal microbiota that enhances nutrient digestibility, adaptation to the environment, and resistance to diseases.

1.2 Problem statement

1.2.1 Challenges of poor water quality in shrimp aquaculture systems

Water quality has an enormous impact on shrimp aquaculture in terms of health, productivity and the environment. Many researchers have reported that unfavourable water quality is responsible for disease outbreaks, reduced growth and development, increased mortality, and environmental degradation (Chua et al., 2023). Poor water quality causes stress to shrimp, making them more susceptible to infections and disease outbreaks. Xiong et al., (2017) explained the impact of gut microbiota immaturity and disease-discriminatory taxa in diagnosing the initiation and severity of shrimp disease and illustrated the role of microbial balance in the health of shrimp. The impact of climate change on aquaculture has also resulted in an increase in disease outbreaks, including bacterial and viral pathogen infections, particularly in shrimp ponds (Uawisetwathana et al., 2021). These findings explain the susceptibility of shrimp to disease under poor water quality conditions. These conditions have posed significant risk to shrimp aquaculture production. Moreover, extreme deviations from ideal water parameters can cause increased shrimp mortality, which directly affects farm profitability. A study conducted by Yang et al., (2017) on the changing concentrations of dissolved nutrients in aquaculture shrimp ponds explained the negative role of effluents from these ponds in water pollution. Furthermore, improper waste management and effluent discharge are identified to cause environmental degradation around the culture surrounding. Mahmudi et al., (2022) described the widespread destruction of coastal ecosystems as a result of the rapid growth of shrimp farming associated with poor water quality in aquaculture systems. Additionally, Hardi et al., (2023) explained the decreasing water quality and substrate infertility in shrimp ponds to prove the impact of poor water quality on aquaculture.

1.2.2 Physiological and genetic response of aquatic organism to changes in culture systems

Shrimps undergo physiological adjustments to face environmental challenges that can lead to impaired growth. Low salinity treatments have been observed to retard the growth of black tiger shrimp. It is also reported that low salinity increases the rate of oxygen consumption as an indicator of increased metabolic rate caused by osmotic stress (Rahi et al., 2021). In low-salinity environments, the external concentration of ions differs from that inside the bodies of aquatic animals. To counter this osmotic imbalance, shrimp actively regulate the movement of water and ions across the cell membranes thereby sustaining the energy demands of osmoregulation under challenging low-salinity conditions at the expense of growth (Antony et al., 2019). Temperature changes disrupts hormonal balance that regulate growth, moulting, and reproduction. Genes encoding proteins involved in metabolism, immune responses, and stress tolerance are usually differentially expressed in response to temperature fluctuations (Lou et al., 2019). Proteins are essential for various cellular processes whose proper functioning is temperature-dependent. Enzymes responsible for biochemical reactions are also reported to experience reduced activity at lower temperatures (Ren et al., 2021; Vianna et al., 2020). This slowdown in enzymatic activity affects metabolic processes, nutrient utilisation, and energy production to cause growth challenges in shrimp and other aquatic animals. Shrimps are particularly sensitive to changes in water quality and high ammonia concentrations as significant environmental stressors. Ammonia toxicity (total ammonia nitrogen and un-ionized ammonia) compete with oxygen for binding sites on respiratory pigments. This impairs oxygen transport causing respiratory distress, reduced metabolic efficiency and negative impact on growth. High ammonia levels have been reported to induce secondary stressors that weaken the immune system and make shrimps more susceptible to diseases (Lin et al., 2023; Zhao et al., 2020).

Shrimps show genetic adaptations to temperature changes and activate specific genes that help maintain cellular functions under stress. However, prolonged exposure to temperature fluctuations affect these adaptive responses, leading to disruptions in

gene expression patterns and affecting the health of shrimp. The Pacific white shrimp, *Litopenaeus vannamei*, is one of the most widely farmed species because to its rapid growth, adaptability, and high market value. However, traditional shrimp farming is currently facing persistent challenges such as disease outbreaks, poor water quality, and increased environmental degradation. Biofloc technology (BFT) has emerged as a promising alternative which utilises microbial communities to enhance water quality, nutrient recycling, and disease resistance (Ahmad et al 2017). Recent studies suggest the potential of biofloc technology impacting on the genetic adaptation and physiological responses of cultured shrimp. Metagenomic and transcriptomic analyses entails investigations on the microbial diversity and functional interactions within biofloc systems. Transcriptomic has been reported to provide information about the modulation in gene expression related to immune responses, stress adaptation, and metabolic pathways in *L. vannamei* in biofloc system (Barajas-Sandoval et al 2023). These genetic adaptations often influence the resilience, growth performance, and overall health of shrimp in BFT systems. The adoption of metagenomic studies in biofloc-based shrimp farming is used for characterizing the dynamics of microbial community essential promoting beneficial microbiota and mitigating disease risks (Padeniya et al 2022). Genomic selection techniques often enhance shrimp breeding programmes by identifying genetic markers associated with BFT adaptability, growth efficiency, and disease resistance (Emerenciano et al 2022). As the demand for sustainable aquaculture practices continues to rise, genomic analyses are instrumental in refining BFT applications and ensuring the long-term viability of shrimp farming. Understanding the interaction between biofloc microbial communities, genetic adaptation, and shrimp physiology is vital for promoting sustainable aquaculture strategies and improving production (Gutiérrez-Pérez et al 2022).

1.2.3 Challenges associated with the accumulation of floc in biofloc aquaculture system

BFT is a sustainable aquaculture system that relies on microbial flocs to treat and convert toxic materials, such as nitrogen, into useful proteins that serve as supplementary feeds to fish and crustaceans (Ghimire et al., 2023; Padeniya et al.,

2022). However, the accumulation of floc residues in the system can have negative effects. Contrary to the benefits of the microbial community in a biofloc system, excess floc residues increase biological oxygen demand (BOD) and cause an outbreak of *Vibrio* disease (Kumar et al., 2021). High aeration with strong mixing is also essential to maintain suspended solids in the water column. Sedimented flocs have been reported to cause dissolved oxygen depletion, leading to the release of hydrogen sulphide, methane, and ammonia, which are toxic to shrimp and fish (Chu et al., 2020; Li, et al., 2021).

1.3 Significance of study

Optimum stocking density levels offer essential support for optimum yield and enhanced physiology of shrimp, as well as good water quality in biofloc culture systems. Research has indicated that boosting the stocking density results in a substantial increase in shrimp yield. However, production levels are constrained by diminished growth and survival rates as stocking densities increase (Arambul-Muñoz et al., 2019; Nguyen, 2020). Density-dependent growth and survival are typical responses in intensive shrimp culture, mainly due to a combination of factors including a decrease in the availability of natural food sources and space, an increase in cannibalism, a decrease in water quality, and the accumulation of undesirable sediments. Therefore, it is essential to determine the optimal stocking density based on the specific system conditions to achieve the best results (Chakraborty, 2022).

Various aquaculture systems, such as recirculating aquaculture systems, artificial substrates, BFT, and photoheterotrophic systems, have been used to improve water quality and reduce shrimp stress in intensive systems. Molasses is used to prevent increases in total ammonia nitrogen (TAN) in shrimp cultures under conditions of limited water discharge, thereby contributing to the maintenance of water quality (Das et al., 2023). The addition of molasses has been shown to lead to higher survival rates, mean final weight, and specific growth rate of shrimp, as well as maintenance of water quality and lower concentrations of *Vibrio* sp. Molasses has a fast dissolution

rate, which provides high levels of organic carbon as a substrate for bacteria to metabolise ammonia, thereby controlling the TAN and nitrite levels in culture systems (Verma et al., 2020). Furthermore, molasses has been reported to increase the abundance of beneficial bacteria and decrease the presence of opportunistic pathogenic bacteria, thereby improving the overall quality of biofloc and water in shrimp culture systems (Huang, et al., 2022; Said & Ahmed, 2022). Probiotics act in the conversion of ammonia and other organic wastes into natural food for shrimp to reduce the environmental impacts of aquaculture. This improves intestinal microbial balance in shrimp which promotes better growth, enhanced feed conversion ratios, and reduced water pollution (Butucel et al., 2023; Lavanya & Dayakar, 2022). Reports have indicated that the application of probiotics in biofloc systems provides better protection against infections and diseases, ultimately contributing to the health and productivity of shrimp (de Lima Vieira et al., 2021; Romano, 2021).

The microbial biomass formed during microbial activities in BFT systems is used as an aquafeed. Biofloc residues are rich natural protein-lipid sources formed by the interaction of physical organic substrates and a large range of microorganisms (Chen et al., 2022). Biofloc meal is used for replacing fishmeal as a protein source in fish and shrimp feed because it contains high level of vitamins and minerals. The inclusion of biofloc meal in the diets of fish and shrimp has been reported to promote high feed intake, fish growth, protein efficiency ratio and lower feed conversion ratio (Lin et al., 2023). Seaweed aquaculture has the potential to remove large quantities of nitrogen and phosphorus from culture systems. As reported by Alfeus & Gabriel, (2023), the application of seaweed in biofloc aquaculture systems has proven to be effective in managing water quality and preventing the growth of harmful algae by effectively removing nitrogen and phosphorus from the system.

1.4 Hypotheses of study

Hypothesis I:

H_o = Biofloc aquaculture systems perform best when the operational components are optimised via standard approaches

H_1 = system optimisation does not affect the performance of biofloc aquaculture systems

Hypothesis II:

H_o = Optimum stocking density, C/N ratio, and probiotic dosage influence the growth, physiology and genetic response of *L. vannamei*

H_1 = Optimum stocking density, C/N ratio, and probiotic dosage does not influence the growth, physiology and genetic response of *L. vannamei*

Hypothesis III:

H_o = The floc residue obtained from IMTAS has nutrients in concentrations suitable to be used as unconventional aquafeed materials.

H_1 = The floc residue obtained from IMTAS does not have nutrients in concentrations suitable to be used as unconventional aquafeed materials.

Hypothesis IV:

H_o = Seaweed, *Caulerpa lentilifera* has the absorption capacity to serve as phytoremediation agent in IMTAS

H1 = Seaweed, *Caulerpa lentilifera* does not have the absorption capacity to serve as phytoremediation agent in IMTAS

1.5 Objective of study

The main objective of this research was to determine optimum production conditions for enhanced growth and physiology of *L. vannamei* as well as ensure nutrient and by-product recycling in the Indoor Multi-techno Aquaculture System (IMTAS).

The specific objectives were:

i. to optimize a traditional biofloc system based on water quality conditions and measure its performance using the growth and physiology of *L. vannamei*.

ii. to develop IMTAS from the optimised parameters and compare its performance with the traditional biofloc system using the growth, physiology and genetic response of *L. vannamei*.

iii. to determine the nutrient profile of biofloc meal harvested from IMTAS.

iv. to determine the nutrient absorption capacity and water quality improvement of *Caulerpa lentilifera*. as a phytoremediation agent in IMTAS.

CHAPTER 2

LITERATURE REVIEW

2.1 Developmental trends in fisheries and aquaculture

The fisheries and aquaculture sectors have experienced remarkable advancements due to the integration of cutting-edge technologies and sustainable practices. Innovations such as IMTAS have significantly increased production and minimized environmental impacts. Additionally, the industry is increasingly focusing on the valorization of fishery waste, converting by-products into valuable resources to promote a circular economy.

2.1.1 Global fisheries and aquaculture production

The worldwide production of aquatic animals amounted to 178 million tons, which was marginally lower than the highest reached in 2018 at 179 million tons in 2020 (**Figure 2.1**). This production was mainly derived from two sources, capture fisheries and aquaculture, which accounted for 90 million tons (51 %) and 88 million tons (49 %), respectively. Most of the total production, amounting to 63% or 112 million tons, was derived from marine waters, of which 70% was obtained from fishing

and the remaining 30% from farming. According to reports, approximately 37% of the fishery products originated from inland waters, wherein 83% of the production was attributed to aquaculture, and the remaining 17% came from capture fisheries. According to estimates by FAO, (2022), global production economic value was USD 406 billion at the first sale level. This value was attributed to USD 141 billion for capture fisheries and USD 265 billion for aquaculture. In 2020, the production landscape was extended to include not only aquatic animals but also 36 million tons of algae (by wet weight), mainly derived from aquaculture (Boyd et al., 2022). Marine aquaculture accounts for 97 % of this contribution.

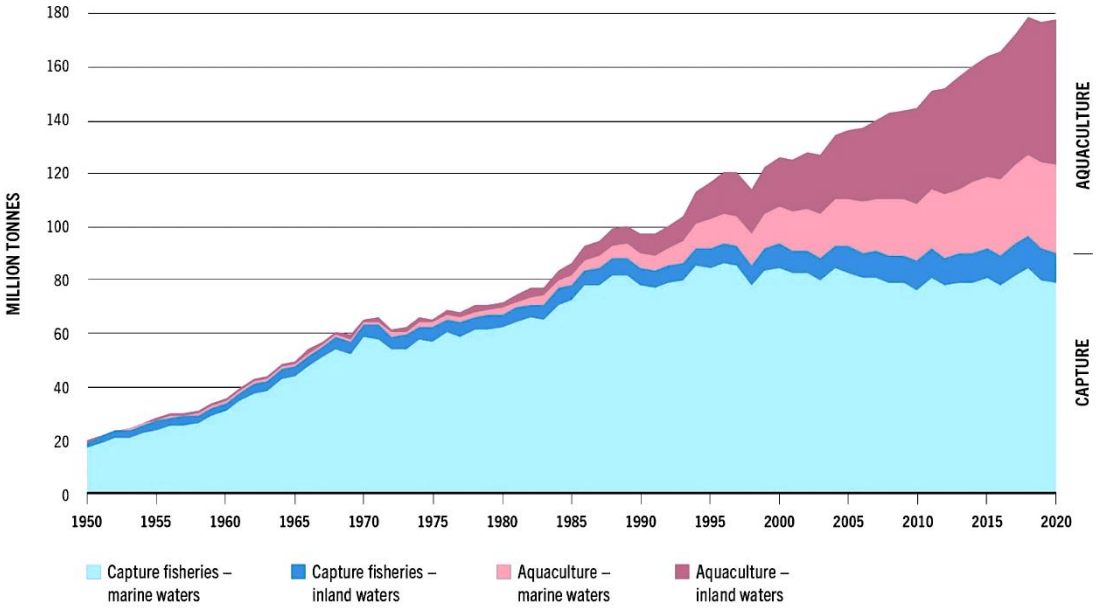


Figure 2.1: Global production trend in fisheries and aquaculture (FAO, 2022)

2.1.2 World aquaculture production

The global aquaculture production in 2020 amounted to 122.6 million tonnes of aquatic animals, primarily used for human consumption, and 35.1 million tonnes of algae, which had both food and non-food applications as shown in **Figure 2.2** (FAO, 2022). Additionally, the production included 700 tons of shells and pearls that were primarily used for ornamental purposes. The total production in live weight reached

87.5 million tonnes of aquatic organisms and 700 tons of shells and pearls. The growth in global meat production from 2018 to 2020 was significant. The estimated farm gate value of this production in 2020 was USD 281.5 billion, reflecting an increase of USD 18.5 billion from 2018 and a rise of USD 6.7 billion from the previous year.

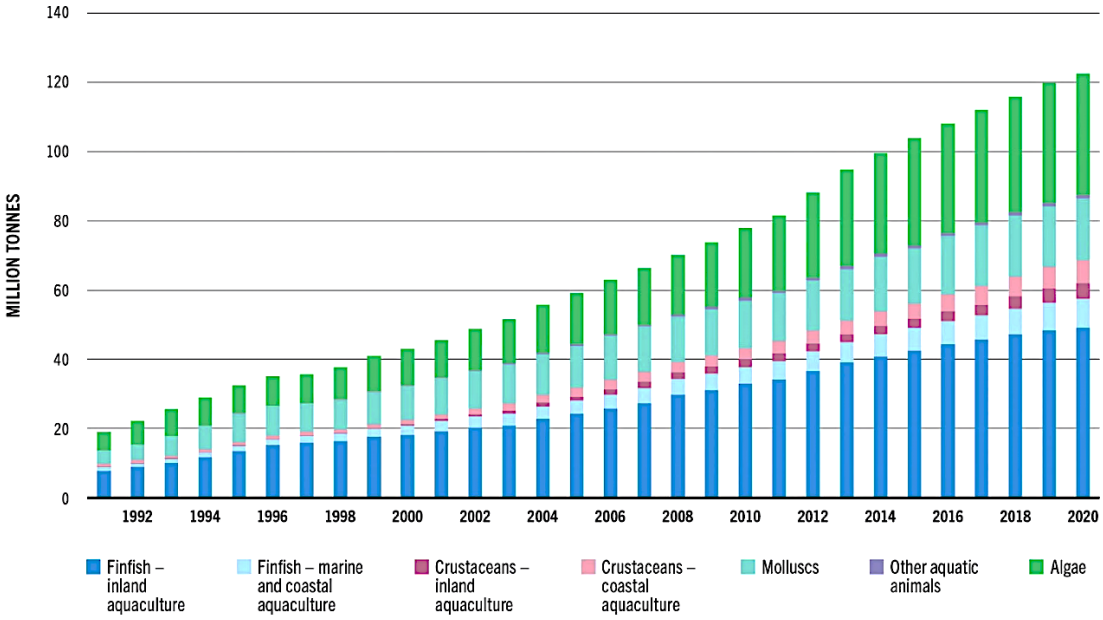


Figure 2.2: Global aquaculture production (FAO, 2022)

According to another module in FAO, (2022), the total world aquaculture output has increased by 609% from 1990 to 2020, with at a mean annual growth rate of 6.7 %. The growth rate experienced a steady decline from 9.5 % during the decade (1990-2000) to 4.6 % during the subsequent decade of 2010-2020. The most recent years (2015–2020) saw a decrease in growth rate to 3.3 % per year. The development of aquaculture has demonstrated varying growth trends across different regions (Naylor et al., 2023). In Asia, the most significant aquaculture-producing region, major aquaculture countries have experienced relatively stable growth over the period 1990-2020, albeit with a notable decrease in growth rates. Some regions experienced considerable variation in their growth during the same period, with certain years showing negative growth (**Figure 2.3**).

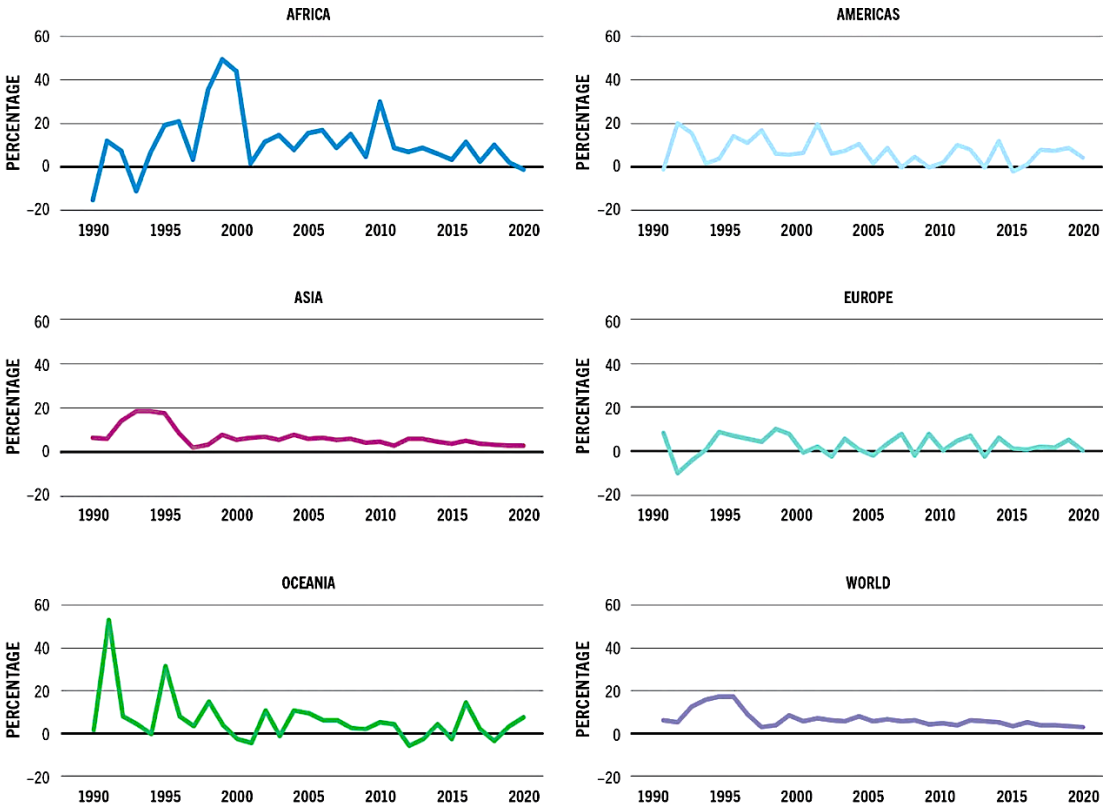


Figure 2.3: Aquaculture growth by continent (FAO, 2022)

In recent years, Asia has emerged as a dominant force in aquaculture worldwide, accounting for an overwhelming majority of the global aquatic animal and algal production. Based on data from 2020, it is astonishing to note that Asia was responsible for producing overwhelming 91.6% of all these products. The differences in aquaculture advancement among Asian countries are significant. Despite the considerable duration that has elapsed, disparities and imbalances in the production and development of aquaculture across various regions and countries have not shown any notable improvement (FAO, 2022).

Approximately 158 million tons of sea food were accessible to Asians in 2019, representing 72 % of the entire global consumption (FAO, 2022). Despite accounting for 60 % of the world's population, only 72 % of total aquatic food is consumed in Asia. The data presented in **Figure 2.4** demonstrate this distinction, where a point of comparison was made in 1961, with Asia accounting for 48% of the aquatic foods

consumed for sustenance. Over several decades, there has been a notable decline in the consumption of aquatic foods in both Europe and the United States. In 1961, the share of sea foods in European diets was 32 %, whereas in the United States it was 9 %. By 2019, the reports of total aquatic product exports meant for Asian markets had decreased to 10 %, while the proportion destined for the European market had dropped to 5 %. The rising rank of Asian countries as major consumers of marine products can be attributed to several interconnected factors. In recent years, the continent has witnessed substantial fiscal development, resulting to a rise in income levels, the growth of a more extensive middle class, and the relocation of rural communities to urban areas, where sea foods are easily accessible (FAO, 2022).

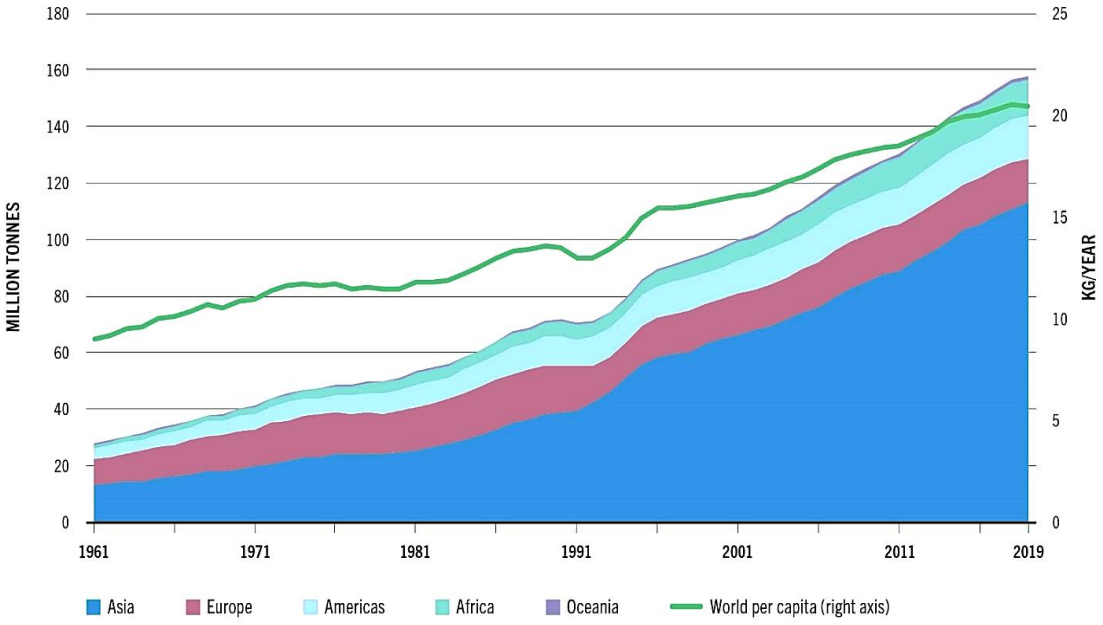


Figure 2.4: Global consumption of aquaculture products (FAO, 2022)

Figure 2.5 illustrates the comprehensive value of aquatic products traded internationally, categorised by prominent species groups, for 2020. In 2020, finfish accounted for the largest proportion of the international value of aquatic product exports (exclusive of algae), accounting for approximately 66.5% of the total. Crustaceans followed closely behind, representing approximately 22.8% of the total value, whereas molluscs and other aquatic invertebrates accounted for approximately 10.7% of the value of exports. Salmonids have consistently been the most valuable

commodities traded since 2013, representing approximately 18 % of the overall value in 2020. The following year, shrimp and prawns accounted for approximately 16 % of the total, while tuna, bonitos, and billfish accounted for 9.7 %. Cods, hakes, and haddocks were responsible for an additional 9.6 %, and squids, cuttlefish, and octopuses represented 6.8 % of the total value.

Figure 2.5: Species quota of aquatic species (FAO, 2022)

Aquatic crustaceans (shrimp and prawns) are the most profoundly exported products. Currently, large scale shrimp farming operations in Latin America, East Asia, and Southeast Asia predominantly generate these goods, which are mainly favoured by markets in North America, Europe, and Japan. The major producers of warm-water species, including India, Indonesia, Thailand, and Vietnam, primarily supply the consumer demands in the United States of America and Japan (FAO, 2022). The European Union primarily sources warm-water species from Asian and Latin American producers, while largely obtaining cold-water species from capture fisheries located in the polar regions. In recent years, economies in Asia, particularly China, have been absorbing a growing share of shrimp supply globally. There are however,

restricted prospects for enhancing per capita consumption within the conventional markets. Despite this, the exports of shrimp and prawns have risen significantly, albeit maintaining a comparatively stable proportion of the entire aquatic product exports globally. The international trade of shrimp and prawns in 1976 was estimated to be USD 1.2 billion, which represented 15.4% of the global worth of seafood products. On the contrariwise, in 2020, the value of this trade was USD 24.7 billion representing for 16.4% of the total value of the aquatic products.

2.2 The Pacific Whiteleg shrimp*, Litopeneaus vannamei*

Whiteleg shrimp, *Litopeneaus vannamei* is indigenous to the Eastern Pacific coast, encompassing the region from Sonora, Mexico in the north, to Tumbes in Peru in the south (FAO, 2022). This species thrives in tropical marine environments and is characterised by year-round water temperatures exceeding 20°C. After successful outcomes were achieved through unilateral ablation and appropriate nourishment in Panama in 1976, commercial cultivation of *L. vannamei* commenced in South and Central America. Subsequent advancements in intensive breeding and rearing methods allowed for its cultivation in Hawaii, mainland United States, and various parts of Central and South America by the early 1980's.

Whiteleg shrimp has emerged as a pre-eminent species because of its widespread acceptance following its introduction to China, as well as its capacity to thrive in a range of temperatures and salinity levels, from brackish water of 1-2 ppt to hypersaline water of 40 ppt (Lin et al., 2015). *L. vannamei* is a member of the decapoda order or group of crustaceans. One of the distinguishing characteristics of shrimp compared to other decapods is the similarity in size between the front-most section of their abdomen and the rest of the sections, as well as the five pairs of abdominal appendages adapted for swimming (Wolfe et al., 2019). *L. vannamei*, which is characterised by its greyish-white colour and omnivorous scavenging nature, is

generally recognised to be less aggressive and carnivorous than *Penaeus monodon*. According to FAO (2022), a total of 9.4 million tonnes of crustaceans were reared globally in 2018, with a value of USD 69.3 billion. Shrimps, which have become one of the most valuable globally traded crustaceans in recent years, have contributed more than 4.5 billion tonnes to global consumption.

L. vannamei is a widely cultivated species of shrimp that has gained global prominence, with annual production exceeding four million tonnes (FAO, 2018). The farming of this species not only serves in ensuring food security but is also responsible for the economies and livelihoods of several nations in Asia, Africa, and South America (FAO, 2022). The breeding techniques for the shrimp were first established in the 1980's and have since been adopted in the United States, where the species originates, and Asia, the receiving continent. Captive breeding and genetic selection have been made possible by the completion of the life cycle of this species. According to an FAO (2019) report, *L. vannamei*, commonly known as whiteleg shrimp, now accounts for a significant majority of all shrimp produced worldwide, with a total production volume of 81%. The leading countries in the production of this species are Ecuador, China, Indonesia, India, Vietnam, and Thailand, with production volumes of 510,000, 1,760,000, 708,680, 620,000, 475,000, and 347,258 tons, respectively.

2.3 Biofloc technology

The introduction of BFT has effectively addressed the challenge of toxic waste accumulation in closed aquaculture systems by introducing a microbial community that coexists in a complex, multifunctional interaction to create a culture system that promotes optimal growth, supported by auto-synthesised food sources, and enhanced health conditions. The formation of lethal nitrogenous waste caused by organic residues under intensive culture conditions is a major hindrance to sustainable aquaculture (Boyd et al., 2020; Nenciu et al., 2022). BFT is an environmentally

friendly aquaculture method that relies on the in-system cultivation of microorganisms such as bacteria, algae, protozoa, and nematodes (Emerenciano et al., 2021), the majority of which are heterotrophic bacteria. Biofloc is an accumulation of detrimental metabolic by-products produced during the rearing of aquatic animals in the BFT system. Bacteria typically release a range of inorganic compounds into their surroundings as a result of metabolic processes. These bacteria excrete extracellular enzymes that can break down a variety of organic materials such as keratin and lignin, which are typically difficult to degrade (Dave & Das, 2020; Gupta et al., 2023; Narayanan et al., 2023). The utilisation of a closed biofloc system is useful in the preservation of water quality and promotion of the growth and well-being of cultured animals. This system presents a viable solution for the issue of environmental pollution (Nisar et al., 2022). Heterotrophic bacteria in BFT play a vital role in transforming inorganic nitrogen into energy-rich biomaterials, which are used as a source of nutrition for cultured animals (Minaz et al., 2023; Tanay et al., 2020). This process not only detoxifies the culture system by breaking down waste generated by faecal pellets and unconsumed feeds, but also recycles waste as a resource, promoting the sustainability of the aquaculture system (Kumar et al., 2023). BFT serves as a self-sufficient system that effectively converts harmful waste into valuable resources, supporting the principles of circular economy in aquaculture.

Biofloc-based aquaculture systems have demonstrated superior benefits over traditional methods in promoting faster growth of aquatic animals and enhancing water quality. These advantages are attributed to the presence of biofloc, which is a complex microbial community (Hosain et al., 2021). Unlike certain recirculating aquaculture systems that depend on external biological filtration to decompose waste, biofloc-based systems leverage a massive microbial community in the water column to carry out this process (Lakra & Krishnani, 2021). BFT is a system for raising animals that significantly reduces cost in several ways. In addition, biofloc systems offer several benefits in terms of ventilation. By constantly providing oxygen, the microbial community facilitates the metabolic operations of both the microbial community and organisms being reared. The use of biofloc systems in aquaculture provides a substantial advantage over untreated water because the microbial communities in these systems require oxygen to carry out their metabolic processes (Padeniya et al., 2022).

In addition, the gut microbiota in biofloc systems promotes the growth and well-being of aquatic animals, unlike in clear-water systems (Khanjani et al., 2023; Yuvarajan, 2021).

The waste generated from shrimp metabolic processes stimulates, sustains, and fosters the development of a microbial community dominated by bacterial species in biofloc systems. Research has demonstrated that a relatively small proportion of the nitrogen provided in the diet of aquatic organisms is absorbed by the time of harvest, as previously established (Yuvarajan, 2021; Yu et al., 2019; Zablon et al., 2022). All aquaculture systems must contend with nitrogen balance, as the residual by-products from these systems can have detrimental effects on the health of cultivated animals. Accumulation of NH_4^+-N and NO_2^- is a characteristic feature of closed systems (Du et al., 2021). The use of BFT in shrimp farming, often referred to as the blue revolution, allows for intensive production in a limited growing space, which in turn leads to significant waste discharge. Heterotrophic bacteria in BFT process wastes promote nutrient and water reuse and enhance the overall conditions of the system (El-Sayed, 2021; Goswami et al., 2022). The nutrient supply of the system comes from organic decomposers operating at an optimal C/N ratio, with carbon often augmented by added carbohydrate sources (Tinh et al., 2021). Microbial protein synthesis results from the trophic activities of microorganisms on nitrate, which is obtained from the oxidation of ammonia derived from organic waste in the system. The introduction of carbon aids in the utilisation of nitrate and carbon to produce biomass containing proteins, carbohydrates, lipids, and nucleic acids (Vargas-Estrada et al., 2022), which serves as a food source for the growth of aquatic animals (Ray et al., 2022; Tibebu, 2020).

Farmers primarily employ biofloc systems to harness the immunity-boosting capabilities and nutrient synthesis abilities of the microbial community to meet the dietary needs of farmed animals. The system is specifically designed to promote the growth and well-being of aquatic creatures, such as fish and shrimp. Biofloc systems achieve this through photosynthesis and the organic growth of macro-aggregates, which helps to balance carbon and nitrogen levels and encourages self-nitrification in the culture water (Ogello et al., 2021). Establishing the microbial composition of a

profitable aquaculture operation in BFT presents a considerable challenge. Understanding the individual roles of each microbial species is crucial to the overall functionality of the BFT system. The absence of such knowledge may result in farmers encountering greater difficulties, particularly regarding the pathogenic bacteria present in the biofloc microbial community. This can lead to disease outbreaks, shrimp mortality, and consequent economic loss.

2.3.1 BFT vs Clearwater shrimp culture

Several studies from research repositories have investigated different aspects of the comparison between BFT and clear water in shrimp. Bakhshi et al., (2018) examined sugar beet molasses, refined sugar, and corn starch in the grow-out culture of *Cyprinus carpio* and discovered that corn starch had the lowest total ammonia nitrogen concentration and yielded the highest fish yield. Huang et al., (2022) investigated the growth response of *L. vannamei* upon administration of glucose, molasses, and starch, resulting in the discovery that glucose and molasses were the most effective in both growth performance and water quality. Other studies on carbon sources include Abakari et al., (2021), Kokkuar et al., (2021) and Miao et al., (2020). Similarly, some researchers have examined the impact of specific bacterial species to on performance in BFT. Based on the study by Che Hashim et al (2022), *Bacillus infantis* was added to the production system of *L. vannamei*, resulting in improved water quality and decreased *Vibrio* sp. populations compared with the control (clearwater) system. Similarly, Jiménez-Ordaz et al (2021) found that adding microalgae and probiotic bacteria to the biofloc culture of *L. vannamei* led to better growth and lower *Vibrio* sp. infestation in the treatment units than in the control without these additives. Kasan et al (2017) isolated 125 bacteria in BFT and emphasized the importance of *Bacillus* sp. and *Halomonas* sp. in biofloc formation. Other studies that have conducted extensive research on the microbial composition of BFT include those by (Chen et al (2020), Sun et al (2024) and Zhang et al (2020).

Furthermore, research has been conducted on stocking density in the BFT. da Silva et al (2022) tested water quality, growth performance, and microbial community of *L. vannamei* at the stocking densities of 2000, 4000, and 6000m^3 and recorded the highest yield of 0.133 $kg.m^{-3}$ from the highest stocking density with the corresponding lowest survival rate (71.66%). Majhi et al (2023) stocked butter catfish (*Ompok bimaculatus*) at stocking densities of 0.5 gL^{-1}, 1.0 gL^{-1}, 1.5 gL^{-1}, and 2.0 gL^{-1}, and better water quality conditions were observed in treatments with lower stocking densities along with better growth performances. Other studies that investigated stocking density in BFT include Adineh et al., (2022), Battisti et al., (2020), Irani et al (2023) and Zaki et al., 2020). Most of these studies have primarily focused on the water quality conditions and growth indices of various cichlid and shrimp species. **Table 2.1** and **Table 2.2** provide a compilation of additional research findings on shrimp growth parameters and water quality, respectively. The variations in the previous results obtained are due to the type of carbon source used and the species of animal cultured in the biofloc and clearwater culture systems.

The evaluation of water quality and zootechnical parameters demonstrated that BFT presents more favourable figures, which is supported by the presence of microorganisms. In closed-system aquaculture, it is crucial to maintain minimal ammonia, nitrite, and nitrate concentrations (Deng et al. 2020). Detoxification of ammonia and its intermediate products is essential in BFT and is indicative of a productive microbial composition in flocs (Hossain et al., 2023; Yu et al., 2023). Probiotics have been shown to accelerate the immobilisation of ammonia more rapidly than traditional nitrifying bacteria do. The accumulation of flocs is a typical cause of the high turbidity in BFT systems. However, the impact of turbidity on BFT is generally minimal, as biosynthesis does not depend heavily on photosynthesis, which requires sunlight penetration in the water column.

Table 2.1: Water quality conditions in biofloc and clearwater systems

Culture system		References
Biofloc	**Clearwater**	
Temperature (ºC)		
29.10	29.10	Ray et al., 2017
27.20	29.00	Kasan et al., 2021
27.90	*28.00*	Tierney & Ray, 2018
27.00	*27.80*	AftabUddin et al., 2020
26.20	*26.50*	Zhao et al., 2016
26.20	*26.40*	Zhao et al., 2012
26.50	*26.60*	Valenzuela-Jiménez et al., 2021
23.12	*23.39*	Rajkumar et al., 2016
32.30	*32.00*	Effendy et al., 2016
Dissolved Oxygen (mg/L)		
6.40	6.30	Ray & Lotz, 2017
5.04	5.37	Kasan et al., 2021
5.70	*6.00*	Tierney & Ray, 2018
5.02	6.12	AftabUddin et al., 2020
7.60	8.10	Zhao et al., 2016
5.60	7.80	Zhao et al., 2012
7.10	7.20	Valenzuela-Jiménez et al., 2021
5.99	6.57	Rajkumar et al., 2016
4.13	4.20	Effendy et al., 2016
pH		
7.70	7.90	Ray & Lotz, 2017
6.61	6.77	Kasan et al., 2021
7.59	*8.02*	AftabUddin et al., 2020
7.87	*8.03*	Zhao et al., 2016
7.80	*8.30*	Zhao et al., 2012
7.40	*8.20*	Valenzuela-Jiménez et al., 2021
7.80	*7.80*	Rajkumar et al., 2016
8.22	*8.23*	Effendy et al., 2016
Salinity ($g.L^{-1}$)		
28.40	28.80	Ray & Lotz, 2017
25.00	*25.00*	Tierney & Ray, 2018
31.60	31.90	Kasan et al., 2021
16.85	*16.94*	AftabUddin et al., 2020
10.50	*10.36*	Fleckenstein et al., 2018
8.91	*8.55*	Khanjani et al., 2021
31.80	*31.50*	Zhao et al., 2016
22.70	*22.90*	Zhao et al., 2012
34.70	*35.00*	Valenzuela-Jiménez et al., 2021
41.20	*39.90*	Effendy et al., 2016
Total Ammonia- Nitrogen, TAN (mg/L)		
0.10	0.30	Ray & Lotz, 2017
1.50	*0.30*	Tierney & Ray, 2018
0.43	1.15	AftabUddin et al., 2020
0.13	0.09	Zhao et al., 2016

0.26	0.42	Luis-Villaseñor et al., 2015
0.57	0.78	Rajkumar et al., 2016
Nitrite (mg/L)		
2.20	0.90	Ray & Lotz, 2017
9.20	*2.50*	Tierney & Ray, 2018
0.58	*1.15*	AftabUddin et al., 2020
0.43	*0.13*	Zhao et al., 2016
0.15	*0.45*	Luis-Villaseñor et al., 2015
0.93	*1.89*	Rajkumar et al., 2016
0.52	*0.03*	Effendy et al., 2016
Nitrate (mg/L)		
39.30	20.50	Ray & Lotz, 2017
21.40	*11.30*	Tierney & Ray, 2018
1.89	*3.02*	AftabUddin et al., 2020
0.949	*1.617*	Luis-Villaseñor et al., 2015
2.42	*3.21*	Rajkumar et al., 2016
0.08	*0.03*	Effendy et al., 2016
Turbidity (NTU)		
90.10	6.10	Ray & Lotz, 2017
15.10	*3.80*	Tierney & Ray, 2018
18.20	58.20	Luis-Villaseñor et al., 2015

Table 2.2: Zootechnical variables in the biofloc and clearwater systems

Culture system		**References**
Biofloc	**Clearwater**	
Final weight (g)		
11.10	11.60	Ray & Lotz, 2017
9.55	7.06	Kasan et al., 2021
6.70	5.90	Tierney & Ray, 2018
17.97	12.95	AftabUddin et al., 2020
0.62	1.26	Esparza-Leal et al., 2015
0.73	0.70	Okomoda et al., 2022
10.78	9.52	Zhao et al., 2016
4.00	3.60	Luis-Villaseñor et al., 2015
11.33	9.98	Zhao et al., 2012
9.28	8.08	Valenzuela-Jiménez et al., 2021
6.64	5.97	Rajkumar et al., 2016
20.50	18.00	Effendy et al., 2016
Net yield (kg/m^3)		
1.60	1.90	Ray & Lotz, 2017
3.66[a]	2.36 [a]	Kasan et al., 2021
1.96[a]	1.13 [a]	AftabUddin et al., 2020
1.30	0.92	Zhao et al., 2012
0.683[b]	0.613[b]	Effendy et al., 2016
SGR (%/day)		
2.85[c]	3.05[c]	Ray & Lotz, 2017
0.12[c]	0.08[c]	Kasan et al., 2021
2.08[c]	2.06[c]	Tierney & Ray, 2018
0.14	0.10	AftabUddin et al., 2020
1.06	0.46	Zhao et al., 201
0.09	0.07	Okomoda et al., 2022
1.48	1.08	D. Zhao et al., 2016
1.40	0.92	Luis-Villaseñor et al., 2015
0.07	0.06	Rajkumar et al., 2016
1.09	1.03	Effendy et al., 2016
FCR		
1.80	1.50	Ray & Lotz, 2017
1.00	1.50	Kasan et al., 2021
1.10	*1.40*	Tierney & Ray, 2018
1.42	*2.30*	AftabUddin et al., 2020
1.60	*1.10*	Esparza-Leal et al., 2015
0.90	*0.80*	Fleckenstein et al., 2018
1.67	*1.80*	Zhao et al., 2012
1.41	*1.86*	Luis-Villaseñor et al., 2015
2.60	*2.90*	Effendy et al., 2016
Survival (%)		
69.00	78.00	Ray & Lotz, 2017
46.07	40.22	Kasan et al., 2021
86.20	*80.20*	Tierney & Ray, 2018
81.87	*65.73*	AftabUddin et al., 2020

98.40	*85.00*	Esparza-Leal et al., 201
96.40	*95.50*	Fleckenstein et al., 2018
97.94	*95.38*	Khanjani et al., 2021
65.70	*52.30*	Zhao et al., 2012
56.67	*60.00*	Valenzuela-Jiménez et al., 2021
82.20	*86.90*	Rajkumar et al., 2016
81.00	*83.00*	Effendy et al., 2016

*units converted from: a = kg.3m^{-3} to kg.m^{-3}; b = kg.ha^{-1} to kg.m^{-3}; c = g.wk^{-1}to %.day^{-1}

2.3.2 Biochemical processes by microorganisms in BFT

Biofloc is a system that involves the purposeful introduction of a microbial community and organic residue into a culture system to harness the metabolic activities of microorganisms for beneficial purposes. The metabolic processes of heterotrophic bacteria in the breakdown of carbohydrates generate energy in the form of BFT, which also contains a significant amount of protein (Chakrapani et al., 2021). The oxidation of faecal materials and unconsumed feed initiates the glycolytic pathway, which ultimately leads to the synthesis of high-energy compounds, such as ADP, ATP, and thioester bonds, including acetyl-CoA and succinyl-SCoA, through a catabolic reaction.

Heterotrophic (chemoorganotrophic) bacteria undergo amphibolic degradation, which simultaneously generates energy and precursors for the synthesis of new cellular components (Artigas et al., 2020). This reaction chain converts organic waste products in BFT into fatty acids, glucose derivatives, and proteins to meet the nutritional needs of cultured animals, while also preventing the build-up of these by-products (**Figure 2.6**). Organic residues in aquaculture systems contain carbon and nitrogen that are biosynthesized into nicotinamide adenine dinucleotide ($NADH^{+}$), which provides the chemical energy necessary for the breakdown of additional organic sources. Pyruvate is produced from glucose through two-stage phosphorylation of the glycolytic pathway, and it serves as a crucial intermediary product in the biosynthetic process in the biofloc system. Pyruvate oxidation in prokaryotic heterotrophic bacteria is enhanced by the pyruvate dehydrogenase complex, resulting in the production of

acetyl-CoEnzyme A (acetyl-CoA). Pyruvate formate-lyase (PFL) plays a role in the breakdown of pyruvate to acetyl-CoA in prokaryotic bacteria (Yuan et al., 2022). The formation of acetyl-CoEnzyme A is formed through the decarboxylation of pyruvate and covalent connection of a thioester linkage to Co-enzyme A, resulting in the formation of a molecule known as acetyl-CoA.

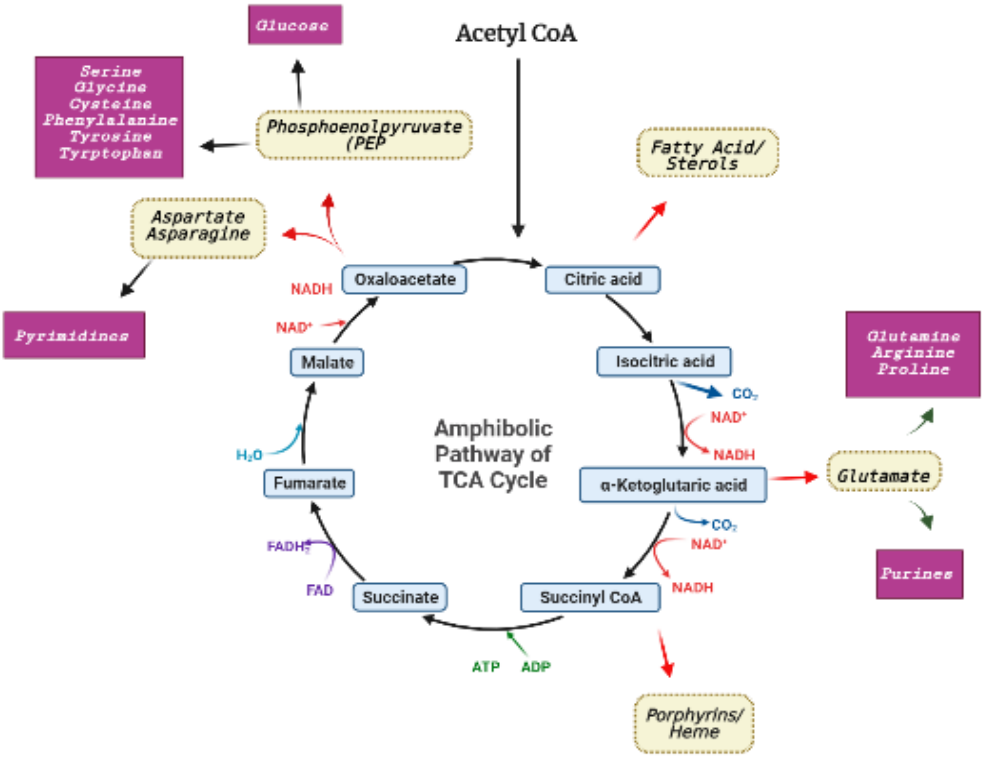


Figure 2.6: Amphibolic pathway of TCA cycle by heterotrophic bacteria in BFT system (modified from Gupta & Gupta, 2021)

Acetyl-CoA serves as the foundation for entry into the Tricarboxylic Acid (TCA) cycle. As an electron acceptor, it reacts with oxaloacetate to form citrate. Within prokaryotic heterotrophic bacteria in the BFT system, the NADP-dependent enzyme isocitrate dehydrogenase (NADP+) facilitates the dehydrogenation of D-threo-isocitrate to oxoglutarate. This process differs from that of other eukaryotes, which utilise NAD+-dependent enzymes, such as isocitrate dehydrogenase (NAD+), to catalyse the same reaction (Bian et al., 2023). The synthesis of fatty acids catalysed by acetyl-CoA carboxylase begins with the carboxylation of acetyl-CoA to malonyl-

CoA. The production of protein-bound acyl carrier protein (ACP) occurs through the transfer of an acetyl group from acetyl-CoA to a thiol group, leading to a series of reactions involving carboxylation, hydrogenation, condensation, and dehydrogenation, ultimately resulting in the formation of β-hydroxydecanoyl-ACP dehydratase. This enzyme produces cis-b, y (or Δ3)-decanoyl ACP in anaerobic heterotrophic bacteria (Zhang et al., 2021). Additionally, amino acids are generated from α-ketoglutarate (AKG). Glutamate, a precursor compound, is converted to glutamine, proline, arginine, and purines. It is also known as 2-oxoglutarate, and is an essential metabolite required to regulate all metabolic reactions that influence physiological and genetic modifications in animals (Legendre et al., 2020). During amphibolic reactions, AKG biosynthesis plays a crucial role in antioxidative defense and other critical cellular functions (Mailloux et al., 2019), which are essential for proper cellular performance. In addition, AKG serves in the immunological functions of heterotrophic bacteria in BFT. Furthermore, succinyl-CoA synthesizes porphyrin and hemes, whereas oxaloacetate synthesizes phosphoenolpyruvate (PEP), which is an intermediate product for the synthesis of glucose, serine, glycine, cysteine, phenylalanine, tyrosine, and tryptophan, as well as aspartate and asparagine, which form pyrimidines.

2.3.3 Microbial community in biofloc system

BFT culture systems are sustained by biofloc, a complex community of microorganisms including bacteria, algae, fungi, protozoa, rotifers, micro-invertebrates, and detritus (Rahman et al., 2022). These microorganisms perform various functions, such as those of saprophytes, algae grazers, pathogenic bacteria, nitrifying bacteria, and floc-farming organisms (Kasan et al., 2019; Kumar et al., 2021). In an ideal biofloc system, all the microorganisms that make up the system must be suspended in the water column and interact with one another in a sustainable manner. This interaction is facilitated by the presence of chemo-phototrophic and autotrophic microbes, which help to break down and reuse chemical waste (Liu et al., 2023). The population of heterotrophic beneficial microorganisms in a biofloc system is immense, consisting of species such as *Bacillus* sp., *Acinetobacter* sp.,

Sphingomonas sp., *Pseudomonas* sp., *Rhodopseudomonas* sp., *Micrococcus* sp., *Nitrosomonas* sp., *Nitrospira* sp., *Nitrobacter* sp., *Cellulomonas* sp., and yeast. The well-being and growth of adult animals depend on the microbial community in biofloc, which provides essential nutrients in the system. The role of biofloc is closely related to the coexistence of microorganisms in their trophic interactions for acquiring nutrients and undergoing metabolic processes (Khanjani & Sharifinia, 2022). Various species of microorganisms in floc exploit different organic substrates and perform varying metabolic actions, resulting in the production of different amounts and types of protein products, as well as their ability to detoxify in the biofloc system. The activities of this diverse group of bacteria are known to exhibit antioxidant properties and offer health benefits (Chandra et al., 2020), and they also play a role in competing with pathogenic bacteria (Khanjani & Sharifinia, 2022).

Closed biofloc systems provide numerous advantages in aquaculture, including improved water quality, enhanced growth performance, and improved disease resistance for aquatic animals. The natural process of bioremediation is facilitated by the accumulation of microorganisms in BFT systems (Kumar et al., 2021). Beneficial bacteria in biofloc systems contribute by suppressing the activities of harmful bacteria and strengthening the resistance of aquatic animals to diseases. Enhancing the diet with these productive bacteria can further improve water quality, disease immunity, and growth output (Betanzo-Torres et al., 2020; Gautam et al., 2020). Additionally, the implementation of biofloc technology reduces input costs, enhance biosecurity, and regulate ammonia concentration in aquaculture ponds (Vyas, 2020).

Heterotrophic bacteria depend on carbon for the metabolic process of breaking down ammonia, followed by its absorption. A suitable C/N ratio encourages the growth of beneficial bacteria and inhibits harmful ones, leading to better water quality, disease control, increased nitrogen uptake, and improved biotoxin breakdown (El-Sayed, 2021; Guo et al., 2020). Additionally, maintaining the appropriate C/N ratios can optimize biofloc production, waste decomposition, and nitrogen uptake, as illustrated in Kumar et al (2021). Ensuring these ratios is essential for the success of biofloc technology in shrimp farming. In BFT, microbes play a vital role in the food

chain by converting organic matter into food sources for cultured animals, during which harmful compounds and toxins are eliminated from the system. The utilization of poly-β-hydroxybutyrate (PHB), a bacterial compound accumulated in biofloc, has been reported to improve various aspects of cultured animals. Specifically, PHB has been shown to enhance the growth responses and digestibility of food for cultured animals, as well as provide immunity against bacterial contaminations (Asiri et al., 2022). Biofloc serves a dual purpose, functioning as both a nutrition source and bio-accessible compound, thus promoting aquatic growth and health (Padeniya et al., 2022). However, it is crucial to monitor the water quality and microbial community within the system to ensure optimal conditions for aquatic life. This is particularly important as signs of stress or disease in organisms may indicate the presence of harmful bacteria. By maintaining a robust microbial community and ensuring excellent water quality, biofloc systems can contribute to effective and sustainable aquaculture.

Aquatic organisms reared in BFT environments exhibit a more robust immune capacity than their counterparts. This enhanced immunity is attributed to the presence of microorganisms that stimulate the immune system and enhance enzyme activities in floc, leading to improved immune responses (Becerril-Cortés et al., 2017). The potential of *Bacillus* sp as a bioagent for improving the health and productivity of aquatic cultured organisms has been demonstrated (Jamal et al., 2020; Panigrahi et al., 2020; Paul et al., 2020). This distinguishes the Bacillus-based Floating Trash system from other recirculating aquaculture systems (RAS), as the performance of the BFT is promoted by the probiotic bacterial community. In the BFT system, an aggregation of heterotrophic bacteria plays a role in the bioremediation of harmful waste generated in the system. Considered as a complex community of bacteria and other microbes, BFT contains abundant bioactive compounds that boost shrimp tolerance to stress and stimulate their antioxidant activity. Kumar et al (2021) emphasized the cohabitation of several classes of microorganisms in biofloc systems, which creates a symbiotic relationship with cultured aquatic animals and other microbial species. The composition of bacterial populations in biofloc systems is influenced by several factors, including the type of aquatic animal being cultured, the quality of feed used, and environmental conditions such as water quality.

Certain microorganisms, such as *Vibrio* sp., can have detrimental effects on biofloc technology, especially in shrimp culture systems (Baker-Austin et al., 2018; Tepaamorndech et al., 2020). However, managing the C/N ratio can reduce the risk of acute hepatopancreatic necrosis disease (AHPND), which is triggered by the presence of *Vibrio parahaemolyticus* in the biofloc system (Hostins et al., 2019). It is essential to identify the specific symptoms associated with different vibrio diseases, as they can be distinguishable from one another. Generally, shrimps can suffer from bacterial septicemia caused by *Vibrio alginolyticus*, *V. anguillarium*, and *V. parahaemolyticus*; necrosis with *Vibrio* sp. as the pathogen; brown spot disease caused by *Aeromonas* sp. and *Flavobacterium* sp.; and filamentous bacterial disease caused by *Leucothrix mucor* (Baker-Austin et al., 2018; Tepaamorndech et al., 2020).

Aquaculture may encounter various challenges when using a biofloc system, such as high ammonia levels, increased turbidity, slow growth, and poor animal health. The goal of the BFT system is to transform ammonia into nitrate, with probiotic bacteria playing a key role in this process (Putra et al., 2020). However, if the system experiences a sudden rise in ammonia or nitrite levels, it may indicate a bacterial malfunction. Maintaining appropriate ammonium levels is crucial in aquaculture, and chemoautotrophic bacterial nitrification (CBN) is a vital process in achieving these concentrations (Luo et al., 2020). The biofloc system provides the ideal environment for nitrifying bacteria to thrive by accumulating flocculated matter, ammonia, and nitrite.

2.4 Aquaculture systems and technology

Aquaculture systems and technology (AST) encompasses various methods and techniques for raising aquatic animals in an organised setting that facilitates routine management practices for improved production (Vo et al., 2021). The achievement of sustainable and profitable aquaculture largely depends on the optimal conditions of the

culture systems, which are inherent in the various components that enable efficient production. AST specializes in closed aquaculture systems, which are designed to create a controlled environment for farmed organisms, allowing for greater efficiency and sustainability as well as reduced environmental impact (Sankhla et al., 2020). The metabolic processes of fish and other poikilotherms are directly regulated by water quality conditions in the aquaculture system, which are influenced by the nature of the aquaculture system and technology. The deployment of technology in aquaculture is becoming increasingly crucial due to the unsustainability of capture fisheries caused by overfishing, prevailing distorted climate, and increasing demand for fish products (Mejaes, 2021).

Recent advancements in the aquaculture industry have led to improved routine farm practices, mainly owing to the integration of modern technologies that promote automation in various procedures (Saad et al., 2022). These technological advancements have increased the efficiency and sustainability of fish farming, which is crucial to satisfy the growing global demand for fish and other aquatic products. Improvements in aquaculture systems and technology have been made in areas such as breeding and production systems, feed production technology, animal health, breeding and species selection, propagative control, aeration and water exchange (Obiero et al., 2019). In addition, advancements in genome-based technologies, selection of single-sex and sterile populations, and recirculating aquaculture systems (RAS) are among the significant improvements in aquaculture system technology (Yue & Shen, 2022). RAS currently account for less than 5% of the world's farmed fish, but this figure is expected to increase to 40% by 2030 owing to the increasing demand for more space and other critical production factors, as reported by Goddard & Delghandi, (2020). In addition to RAS, other cutting-edge aquaculture systems, such as BFT, neo-female technology (NFT), aquaponics, and compensatory growth technology (CGT), were identified by Jena et al., (2017) as systems controlled by technology to support high productivity under adverse conditions. To optimize the results, these technologies were subjected to systematic optimisation to determine the control factors that lead to the maximum yield.

Aquaculture system optimisation aims to enhance productivity, resource utilization, environmental protection, and disease prevention. This involves the strategic use of by-products, application of big data analysis, development of sustainable processes, improvement of technology, and diversification of species. The strategic use of aquaculture by-products is becoming increasingly recognized as a way to improve economic efficiency, environmental sustainability and food security (Papáček et al., 2019). Additionally, there is growing awareness of the importance of developing green technology and sustainable processes, which has led to a greater interest in using unwanted marine resources, such as the large amounts of waste obtained from fishing and aquaculture, for high market value (Coppola et al., 2021). Aquaculture has experienced significant advancements in technology and resource management in inland areas, leading to improved productivity and green development, emphasizing environmental sustainability and innovation in fishery technology (Kim et al., 2022). The green development of aquaculture focuses on protecting fishery resources, preserving the ecological environment, and adhering to legal management standards. This approach ensures a sustainable and healthy aquaculture system that incorporates environment-friendly practices, good agricultural practices, and the health and safety of human and food (Cavalli et al., 2021). The integration of Fourth Industrial Revolution Technologies, such as artificial intelligence, is increasingly being explored in marine aquaculture to enhance sustainable food production in the future, highlighting the benefits of technological adaptation for sustainable development (Mustafa et al., 2021).

Improving aquaculture is crucial to increasing production, ensuring sustainability, and enhancing profitability. Aquaculture optimisation aids efficient and sustainable in feed utilization, wastewater treatment, and production strategies for improved health of cultured shrimp (Ahmad et al., 2022; Chauhan & Mishra, 2022; Sun & Kainz, 2023). Optimizing aquaculture production strategies has resulted in improved production efficiency and yield through resource utilization (Pathan et al., 2022). Dong & Dong (2023) emphasized the importance of sustainable intensification in aquaculture systems, which involves integrating anthropogenic inputs with aquaculture ecosystem services. By adopting system optimisation, aquaculture production has the potential to catch up with the rising demand for aquatic food

products. An optimized aquaculture system typically features technological advancements, such as automation, precision feeding, and data analysis in its production process. Real-time monitoring systems that combine IoT technology, cloud computing, and chatbot assistants are used in monitoring water quality in aquaculture. Additionally, intelligent auto-feeders, smart aerators, and connected sensors are available to improve the efficiency and sustainability of the industry (Antonucci & Costa, 2020; Dhal et al., 2022).

2.4.1 Taguchi system optimisation

The Taguchi method is a system design technique that aims to improve operational efficiency by minimising counterproductive or noise factors in the final outputs and procedures (Acherjee, 2020). The ultimate goal of this method is to create systems and processes that can operate efficiently regardless of several factors that may impede their optimality. Therefore, the Taguchi method focuses on the variability rather than the means of the responses to certain differences tested in an experiment. The popularity of this method is owing to its practical approach to designing high-quality systems with reduced variation and optimum process control parameters (Lin et al., 2020). The Taguchi Design of Experiments (DOE) is a critical tool for selecting the best resources from a range of options and efficiently achieving optimal output. The significance of these factors depends on whether their variability causes unpredictable random effects or systematic effects unrelated to the operation of the system (nonrandom effects) (Pundir et al., 2018).

The Taguchi robust design methodology is often utilized to optimize control factors designated as *'x'* and minimize variations caused by noise factors referred to as *'z'* in **Figure 2.7**. When the design objective involves achieving a performance that is closely aligned with the target value *'M'*, both designs at different levels are considered acceptable, because their means coincide with the target *'M'*. To achieve robustness, it is evident that when *'x'* is set to *'i'*, the performance exhibits significant variability in response to deviations in noise factor *'z'*. When assigning the value *'ii'* to *'x'*, the

deviation in performance decreases significantly in the presence of the noise factor 'z'. Consequently, the design solution characterized by *'x' = 'ii'* is inherently more robust than that of *'x' = 'i'*. This is because *'x' = 'ii'* is more effective than *'x' = 'i'* at mitigating the impact of noise factors. The difference in robustness between *'x' = 'i'* and *'x' = 'ii'* becomes particularly evident when comparing their influence on performance relative to the target *'M'*. While both designs satisfy the acceptable criteria in terms of mean performance aligned with the target *'M'*, the resilience of *'x' = 'ii'* to variations in the noise factor *'z'* makes it a more robust design solution than *'x' = 'i'*. The use of the Taguchi robust design method helps in selecting control factors that not only optimize the mean performance, but also demonstrate resilience to variations induced by noise factors.

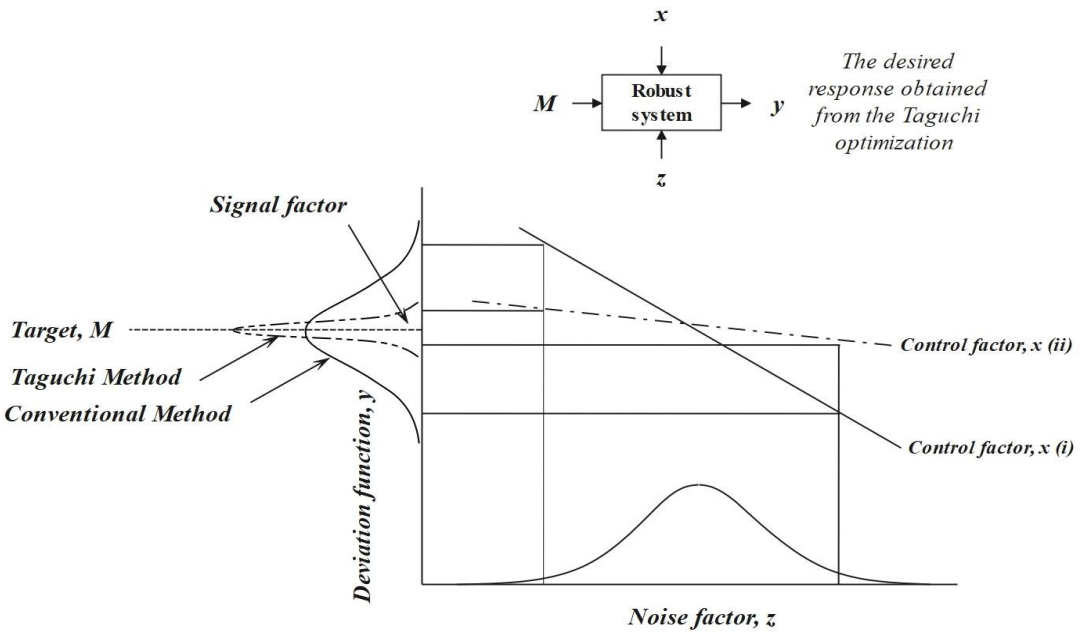


Figure 2.7: Taguchi Robust Design (modified from Chen et al., 1995)

In conventional scientific methodology, a full factorial design is typically considered the most appropriate approach in examining the various levels of parameters and their interactions to achieve anticipated outcomes. However, this approach is not always feasible due to constraints such as limited resources of space, time, and finances (Kurniawan et al., 2022). Additionally, the sequential testing

approach is ineffective, as it fails to consider interactions often overlooked in experimental procedures (Garofalo et al., 2022). The Taguchi method is an incomplete factorial design characterized by the systematic combination of specific test parameters through established protocols. The substantial variance in factor levels represents the variation in process output attributed to changes in these levels. Through the measurement of significant variance, Taguchi distinguishes the factors with the most marked impact on process output. The Taguchi method simplifies experimental procedures by prioritizing the performance sensitivity of a system to parameters (Sao et al., 2022). This approach enables a more focused assessment of the target, thereby reducing the number of factors under consideration. As a result, Taguchi optimizes experimental efficiency by minimizing the difficulties associated with multiple experimental units, ultimately enhancing the precision and applicability of a study.

The Taguchi method involves a deliberate selection of target factors, which facilitates the construction of orthogonal arrays - matrices with distinct attributes in each column. Notably, within these arrays, every pair of elements in the columns occurs an equal number of times. Orthogonal arrays serve as blueprints for multifactor trials, where columns represent factors, entries indicate test levels, and rows denote test runs (Freddi & Salmon, 2019). In planning a Taguchi Design of Experiments (DOE), treatment units (runs) systematically combine control factors, defining the type of design employed. **Table 2.3** provides a comprehensive overview of the various orthogonal arrays employed in the Taguchi method obtained from **Equation 2.1**.

$$L_x(n^f) \qquad \textbf{(Equation 2.1)}$$

Where;

L_x is orthogonal array containing n elements; f, the maximum number of factors whose effect can be estimated without any interaction and $x.f$ is a matrix of the factors and level.

Table 2.3: Taguchi orthogonal array

No. of Runs	**Factor Levels**			
Single level Design				
	2	**3**	**4**	**5**
4	L4 (2^3)			
8	L8 (2^7)			
9		L9 (3^4)		
12	L12 (2^{11})			
16	L16 (2^{15})		L16 (4^5)	
25				L25 (5^8)
27		L27 (3^{13}) L27 (3^{22})		
32	L32 (2^{31})			
Mixed level Design				
	2 and 3	**2 and 4**	**2 and 8**	**3 and 6**
8	L8 (2^4 X 4^1)			
16	L16 (2^9 X 4^2) L16 (2^6 X 4^3) L16 (2^3 X 4^4) L16 (2^{12} X 4^1)	L16 (2^8 X 8^1)		
18	L18 (2^{11} X 3^{12}) L18 (2^2 X 3^6)			L18 (3^6 X 6^1)
32		L32 (2^1 X 4^9)		
36	L36 (2^{11} X 3^{12}) L36 (2^3 X 3^{13})			
54	L54 (2^1 X 3^{25})			

*Modified from Cimbala, (2014)

For illustration, the L9 Taguchi design with 9 experimental units is distinguished from a full factorial design of 27 experimental units for the evaluation of 3 factors at 3 levels each (**Figure 2.8**).

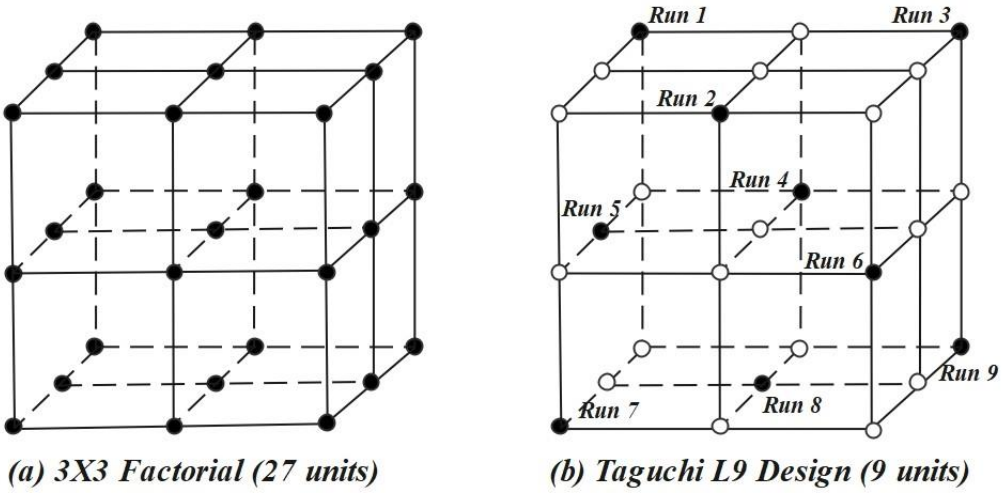


Figure 2.8: Layout of (a) 3 X 3 factorial Design and (b) Taguchi L9 design (Zhang et al., 2019)

The marked nodes in **Figure 2.8 (b)** represent the factor/level combination, as presented in **Table 2.4**. This layout ensures that each factor is studied at every level across the experimental runs, allowing for a methodical examination of the main effects and interactions between factors that may not be apparent when studying factors in isolation. This design minimizes the number of experimental runs required, while maximizing the information obtained.

Table 2.4: Factor/level combination in L9 Taguchi design

Run (Experimental Units)	**Levels in Factor**		
	Factor 1	**Factor 2**	**Factor 3**
Run 1	1	1	1
Run 2	1	2	2
Run 3	1	3	3
Run 4	2	1	2
Run 5	2	2	3
Run 6	2	3	1
Run 7	3	1	3
Run 8	3	2	1
Run 9	3	3	2

2.4.1.1 Loss function

Designing aquaculture systems requires identifying control factors, ensuring efficiency, and enhancing quality by minimising interferences, often referred to as noise effects. A robust system is characterized by its ability to function effectively under adverse conditions impeding performance to achieve the desired outcomes. The main goal of the Taguchi method is to discover and eliminate any control factors that contribute to variability (Hamzaçebi, 2021). According to Mongomery (2017), robust design involves selecting control factor levels that help achieve the desired output targets while minimising any fluctuations around those targets to ensure optimal product creation.

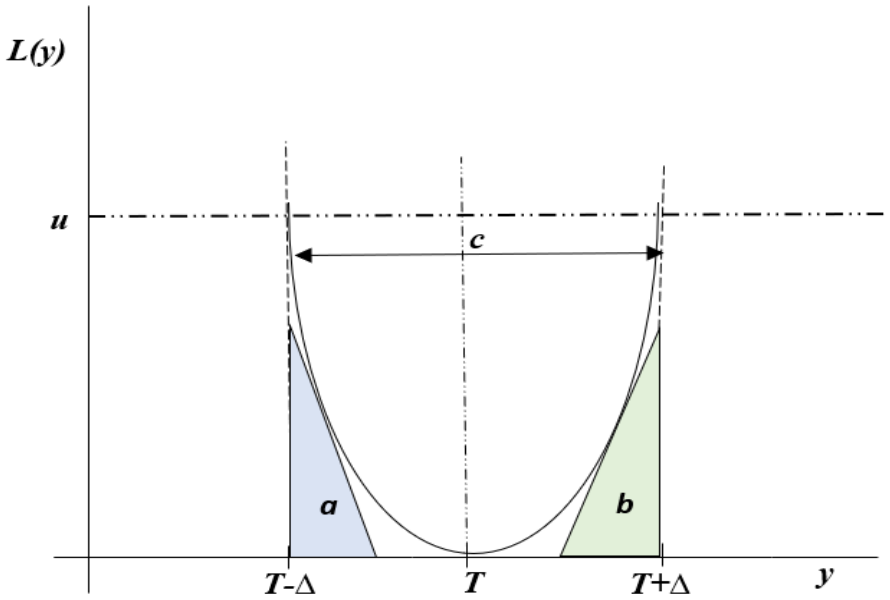


Figure 2.9: Noise factors in Taguchi method

All noise factors have their specific minimizations namely: larger-the-better, smaller-the-better and nominal-the-better (on-target). The Taguchi loss function of a system whose performance is represented by *(T)* has a possible output of T-Δ and T+Δ as the lower and upper limits respectively computed from the relationship

$$L(y) = k(y - T)^2$$ **(Equation 2.2)**

The output curve is usually a quadratic curve because of its possible fluctuation between the lower and upper limits and because of this, equation (ii) is converted to a quadratic function as;

$$L(y) = \frac{u}{\Delta^2}(y - T)^2$$ **(Equation 2.3)**

Where *u* is the useful life of the system which connotes cost of replacement or repairs.

In reference to **Equation 2.2** and **Equation 2.3**, three relationships represented as *'a'*, *'b'* and *'c'* indicated in **Figure 2.9** are possible

$$L(y) = k(\frac{1}{y^2});$$ y≥0 **(Equation 2.4)**

$$L(y) = ky^2;$$ y≥0 **(Equation 2.5)**

and

$$L(y) = k(y - T)^2;$$ *k* = constant **(Equation 2.6)**

Equation 2.4, **Equation 2.5** and **Equation 2.6** are referred to as larger-the-better; smaller-the-better and nominal-the-better respectively.

According to Hamzaçebi (2021), signal factors are essential for every optimisation effort. The optimisation process is evaluated using the signal-to-noise ratio, which is calculated as the ratio of the expected optimal values to interferences, known as noise factors. A robust system shows resilience against both internal and external factors that may impede the achievement of its goals (Sojobi & Liew, 2022). This includes factors such as non-conformity with set objectives and unfavourable environmental conditions. The robustness of a system can be assessed through its signal-to-noise ratio, which reflects its ability to maintain optimal performance despite various challenges. The S:N ratio is used to forecast the optimum values and evaluate the response variation relative to the nominal or target values under different noise conditions (Ajmal et al., 2020; Nobrega et al., 2021). The computation of the signal-to-noise ratio is used to defined noise factors, as outlined in **Equation 2.7**, **Equation**

2.8 and **Equation 2.9**, which correspond to larger-the-better, smaller-the-better, and nominal-the-better scenarios, respectively.

$$S{:}\,N\ Ratio\ =\ -10\ log[\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}] \qquad \textbf{(Equation 2.7)}$$

$$S{:}\,N\ Ratio\ =\ -10\ log[\frac{1}{n}\sum_{i=1}^{n}y_i^2] \qquad \textbf{(Equation 2.8)}$$

$$S{:}\,N\ Ratio\ =\ 10log[\frac{\bar{y}^2-{s_i^y}/{n}}{s_i^2}] = 10\ [log(\frac{\bar{y}}{s_y})^2-\frac{1}{n}] \qquad \textbf{(Equation 2.9)}$$

Where;

$\bar{y}$ and s_i are the mean and variance of the considered parameters respectively.

2.4.1.2 Application of Taguchi Method in the design of aquaculture systems

The utilization of the Taguchi method for system optimisation in fisheries and aquaculture is relatively low despite its critical role in promoting resource efficiency. Research conducted by Pradana & Sulistiyowati, 2022) demonstrated 1.3% adoption of the Taguchi method within the agricultural and biological science sectors (**Figure 2.10**). A thorough examination of Scopus index data, encompassing 2000 research reports, has revealed its predominant usage in disciplines such as engineering (Eng), material science (M.sc), physics and astronomy (P&A), and chemical engineering (C.Eng), which collectively constitute 65.3%. On the other hand, other disciplines, including computer science (Cmp), chemistry (Chm), environmental science (Envt), energy (Engr), mathematics (Mth), and business management (B/Mgt), contribute to 34.7%. A variety of fields, including biochemistry, decision science, earth and planetary sciences, multidisciplinary studies, medicine, social sciences, pharmacology, toxicology, pharmaceutics, immunology and microbiology, economics, econometrics, and finance, had a 10.3% adoption rate. Notably, aquaculture, particularly in system optimisation, registers negligible utilization of the Taguchi method.

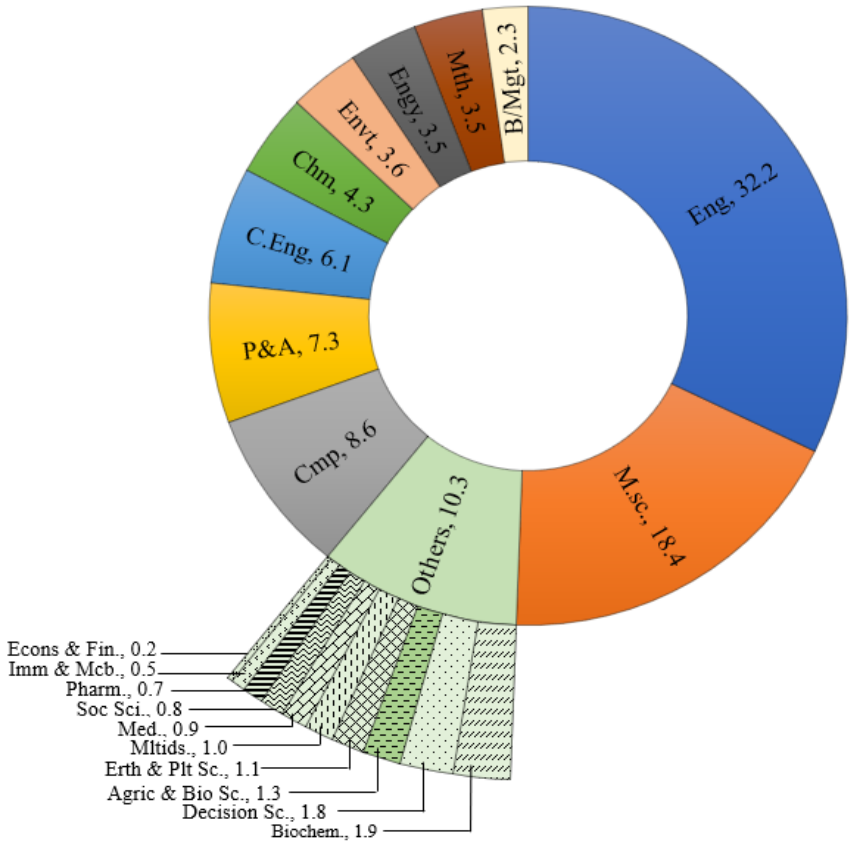


Figure 2.10: Application of Taguchi method
(Modified from Pradana & Sulistiyowati, 2022)

The application of the Taguchi method within the aquaculture subsector is primarily observed in specific fields, such as Aquaculture Economics, Aquaculture Systems and Technology, and Toxicology and Risk Management (**Table 2.5**). Given the complex nature of aquaculture production, which relies significantly on diverse production resources, it is evident from this analysis that the utilization of the Taguchi method is insufficient within the aquaculture discipline.

Table 2.5: Adoption of Taguchi method in aquaculture

Research	**Reference**	**Specific area**
Optimizing a bi-objective multi-period fish closed-loop supply chain network design by three multi-objective meta-heuristic algorithms	Fasihi et al., 2021	Aquaculture Economics
DMTIOLA: decision making tool for identification of optimal location for aquaculture farming development	Mahalakshmi et al., 2012	Aquaculture Economics
The economic feasibility of adoption of a new in-pond mechanical grader for food-sized channel catfish (*Ictalurus punctatus*)	Trimpey & Engle, 2005	Aquaculture Economics
Modelling Single-Screw Extrusion Processing Parameters and Resulting Extrudate Properties of DDGS-Based Nile Tilapia (*Oreochromis niloticus*) Feeds	Ayadi et al., 2013	Aquaculture Engineering
Optimization of multiple performance responses of a fish feed pelletizer machine	Ighodalo et al., 2020	Aquaculture Engineering
Quantitative analysis of temperature, salinity and pH on WSSV proliferation in Chinese shrimp *Fenneropenaeus chinensis* by real-time PCR	Gao et al., 2011	Aquaculture systems technology
Decreased resistance to bacterial cold-water disease and excessive inflammatory response in ayu (*Plecoglossus altivelis*) reared at high water temperature.	Kato et al., 2023	Aquaculture systems technology
Taguchi System based criteria selection for shrimp aquaculture development.	Mahalakshmi & Ganesan, 2009	Aquaculture systems technology
Experiment test analysis of hydram flow rate using L9 Taguchi at fish pond aquaculture	(Prihatin et al., 2023a)	Aquaculture systems technology
Optimization of the culture medium and characterization of antioxidant compounds of a marine isolated microalga as a promising source in aquaculture feed	Safavi et al., 2021	Aquaculture systems technology
Quality evaluation for scale-up of moss (*Sphagnum* sp.) rooftop greening panel using Taguchi method	Ushada et al., 2015	Aquaculture systems technology
Effect of combined photoperiod, water calcium concentration and pH on survival, growth, and moulting of juvenile crayfish (*Procambarus clarkii*) cultured under laboratory conditions	Yue et al., 2009	Aquaculture systems technology

Graphene oxide/Mg-Zn-Al layered double hydroxide for efficient removal of doxycycline from water: Taguchi approach for optimization	Rahman & Raheem, 2022	Aquaculture Toxicology and Risk Management
Synthesis and characterisation of MCM-41@ SiO2-NH-pydc as a new nano mesoporous sorbent: application for the simultaneous preconcentration of cationic dyes previous spectrophotometric determination, using Taguchi experimental design	Sharifi et al., 2021	Aquaculture Toxicology and Risk Management
The exposure of OPFRs in fish from aquaculture area: Backward tracing of the ecological risk regulation	Yang et al., 2022	Aquaculture Toxicology and Risk Management

2.5 Metagenomic analysis of aquaculture species cultured in BFT

Metagenomic analysis has emerged as a powerful tool in studying microbial communities in aquaculture systems to evaluate microbial diversity, ecosystem functioning, and host-microbe interactions (Diwan et al 2022; Nam et al 2023). Understanding the microbial composition and functionality within these systems is crucial for optimizing shrimp culture practices and mitigating disease risks. Recent metagenomic studies have reported the dominance of Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, and Actinobacteria in biofloc environments (Baliga et al 2021, Martinez-Porchas et al 2023, Huang et al 2020). Proteobacteria, predominantly members of the genera Vibrio and Pseudoalteromonas, are both beneficial and pathogenic organisms. *Vibrio harveyi* is prevalent in many aquaculture systems, including biofloc environments where its abundance is significantly reduced under optimized biofloc conditions (Guo et al., 2023). Moreover, the presence of beneficial microbes such as Planctomycetes, known for their role in nitrogen cycling, suggests the efficiency of biofloc system in bioremediation and nutrient recycling (Yun et al., 2022).

Metagenomic analysis provides insights into the functional capabilities of microbial communities in biofloc systems (Ntakirutimana et al., 2023). Functional profiling also indicates that Proteobacteria contribute to critical ecosystem services, such as ammonia oxidation and organic matter degradation, which are essential for maintaining water quality in high-intensity shrimp farming (Loiola et al., 2023). The microbial communities in biofloc systems influence shrimp health by modulating gut microbiota composition and immune responses. Studies have shown a strong correlation between the intestinal microbiota and the surrounding water environment, with biofloc reducing the prevalence of opportunistic pathogens such as Vibrio parahaemolyticus and *Vibrio vulnificus* (Chen et al, 2017). The introduction of probiotic strains in biofloc systems is also reported to enhance microbial diversity and resilience against diseases (Huang et al 2023). High-throughput sequencing has identified probiotic species, such as *Bacillus* sp., which contribute to disease prevention.

2.6 Transcriptomic analysis of aquaculture species cultured in BFT

Recent advances in transcriptomic analysis have provided an understanding of the physiological and genetic adaptations of aquaculture species reared in biofloc systems (Kuang et al 2025). RNA sequencing (RNA-Seq) is currently an important tool for investigating immune and stress responses, metabolism, and growth regulation in fish and crustaceans cultured in biofloc environments (Natnan et al 2021). Several studies have reported significant transcriptomic changes in aquaculture species cultured in BFT. Genes related to immune system modulation, such as pattern recognition receptors (PRRs), antimicrobial peptides, and cytokine signalling pathways, have been reported to be upregulated in *Litopenaeus vannamei* and *Oreochromis niloticus* under biofloc conditions (Pushparaj et al., 2022). This suggests an improved innate immune response which potentially contribute to increased resistance against opportunistic pathogens present in biofloc environments. Also, transcriptome analysis shows remarkable changes in metabolic pathways associated

with nitrogen assimilation, lipid metabolism, and carbohydrate utilization (Yu et al 2017). The upregulation of genes encoding enzymes concerned with nitrogen cycling such as glutamine synthetase and ammonium transporters, prove the adaptive capacity of cultured species to the nutrient-rich biofloc environment (Ghosh et al., 2024). Heat shock proteins (HSPs), oxidative stress markers, and genes related to cellular homeostasis are commonly upregulated in species reared under biofloc conditions (Yu et al., 2021). This proves that shrimp and fish develop physiological mechanisms to counter oxidative stress and fluctuating microbial loads common in biofloc environments. On the other hand, growth-related genes such as insulin-like growth factors (IGFs) and myogenic regulatory factors (MRFs) exhibit variable expression depending on biofloc composition and stocking densities.

2.7 Biofloc meal – towards waste recovery in biofloc aquaculture system

Biofloc technology has gained prominence because of its capacity to enhance the quality of water, waste management, and disease prevention in intensive aquaculture systems (Liu, et al 2022). The inclusion of unconventional feed additives, such as algae as a replacement for fish meal has yielded significant advantages in the aquaculture industry (Michalak & Mahrose, 2020). Recently, the utilization of unconventional aquaculture feed such as biofloc meal has garnered significant attention owing to its potential to promote sustainable aquaculture practices. BFT is a system that maximizes aquaculture productivity using microbial biotechnology to improve the efficacy and utilization of fish feed (Jamal et al., 2020). The usage of biofloc meal contribute to sustainable aquaculture development (McCusker et al., 2023). As the cost and availability of conventional protein sources, such as fishmeal, continue to increase, there is a growing need for alternative and sustainable sources (Lunda et al., 2020). Biofloc has been found to contain adequate levels of proteins, lipids, carbohydrates, and ash, making it a promising alternative for use in aquaculture feed (Khanjani et al., 2023). The aquaculture industry is increasingly turning to unconventional protein meals to create sustainable and economically feasible diets

(Colombo et al., 2023). The implementation of biofloc technology within the realm of aquaculture has demonstrated enhanced productivity, reduced feed conversion ratios, and an optimal culture environment (Jusoh et al., 2020).

Although the nutrient content of biofloc meal vary, it generally contains a substantial amount of crude protein and relatively low lipid levels (Khanjani et al., 2023). Biofloc is made up of rich bioactive compounds (carotenoids, bromophenols, amino sugars, chlorophylls, phytosterols, vitamins, and essential amino acids) (Glencross et al., 2020). Research has confirmed that Biofloc meal serves as a suitable alternative ingredient in aquafeeds, with crude protein levels ranging from 120 to 490 g/kg and lipid levels below 20 g/kg (Jones et al., 2020; Paul et al., 2018; Pereira et al., 2022). The use of biofloc in aquaculture diets has been effective in meeting the nutritional requirements of various species, including shrimp and fish. Conventional fish feed typically contains fishmeal, soybean meal, and other plant-based ingredients. Fishmeal is a protein-rich ingredient between 60% and 75% crude protein, while soybean meal has lower levels of essential amino acids than fishmeal.

Binalshikh-Abubkr et al (2021) evaluated different sources of biofloc waste and drying procedures for producing biofloc with optimal nutrient content. The study found that the protein, ash, fiber, lipid, total nitrogen-free extract (NFE), and energy content of the dried biofloc varied significantly, with values ranging from 12.12 to 24.09 g/100 g for protein, 0.35 - 0.92 g/100 g for lipid, 42.45 - 61.01 g/100 g for ash, 7.43 - 17.11 g/100 g for fiber, 16.45 - 18.59 g/100 g for NFE, and 0.99 - 1.94 Kcal/g for energy. Amino acids were significantly higher in freeze-dried tilapia biofloc (FDTBF). According to Nethaji et al (2022), *P. vannamei* is fed five different diets with varying inclusion levels of biofloc meal (0%, 10%, 20%, 30%, and 40%). The results revealed that shrimp fed with 30% biofloc meal showed significantly higher growth and survival rates, as well as improved digestive enzyme activities and immune responses compared to the other treatments. Additionally, the study observed better water quality, reduced vibrio count, and enhanced hepatopancreas structure in the biofloc treatment groups. Research conducted by Ekasari et al (2019) examined the nutritional value and growth performance of African catfish juveniles fed two different

types of biofloc meals produced from tapioca and molasses carbon sources. The addition of biofloc meal in the diets at 10% and 20% levels resulted in increased fish growth, feed intake, protein efficiency ratio, lower FCR, higher red blood cell counts, phagocytic and lysozyme activities, and antioxidative capacity. These results confirm that biofloc meal can be used as a feed ingredient for African catfish juveniles, and that it can improve the growth performance and health of fish. Some reports on the proximate composition and amino acid and fatty acid of biofloc meal are presented in Table 2.6.

Table 2.6: Proximate composition (% wt) of biofloc meal

	(i)	**(ii)**	**(iii)**	**(iv)**	**(v)**	**(vi)**	**(vii)**	**(viii)**	**(ix)**	**(X)**	**(x1)**
Proximate profile											
Ash	33.80	11.16	1.41	11.8	32.20	48.86	31.20				
CF	4.80	16.92	5.73								
CL	3.50	3.35	5.02	7.26	4.40	0.33	4.20				
CP	23.50	16.48	47.94	38.70	24.10	13.97	30.40				
Fatty acids											
18:2n-6			0.19	3.8		1.34		0.23	3.40	7.6	
18:3n-3			0.10	1.35		7.29		0.07	0.59		
20:4n-6			0.02			1.73		0.65	1.62	16.04	
20:5n-3			0.25			29.29		4.41	1.94	6.65	
22:6n-3			0.30	2.32		3.12		0.70	1.40	2.3	
SFAs								5.44	54.11		
MFAs								*3.54*	*36.92*		
PUFAs								5.90	3.92		
PBHs								0.91	5.02		
Amino acids											
Arg		2.08	0.60			0.66			1.40	1.10	3.62
Lys		1.66	1.00			0.34			1.09	1.05	1.45
Threo		1.30	1.00			0.34			1.54	1.55	1.66
Hist		0.80	1.00			0.41			0.33	1.22	0.93
Iso		1.53	1.00			0.48			0.86	1.21	1.46
Leu		2.90	0.71			0.64			1.85	2.01	2.28
Meth		0.55	1.00			0.23			0.16	0.37	0.46

Phenyl		1.76	1.00			0.46			1.15	1.58	1.98
Tryp			1.00			0.17			1.54	1.20	0.54
Val		1.85	1.00			0.52			1.72	1.91	2.38
Carbon source	Molasses	Molasses	Molasses	Molasses	Rice bran	Molasses	Molasses	Molasses	Wheat bran	Molasses	Molasses

(i) Caldini et al., 2018 (ii) Ekasari et al., 2019 (iii) Promthale et al., 2019 (iv) de Sousa et al., 2019

(v) Hasan-Nataj-Niazi et al., 2022 (vi) Uawisetwathana et al., 2021 (vii) Khatoon et al., 2016 (viii) Cardona et al., 2016

(ix) Kuhn et al., 2016 (x) Li et al., 2021 (xi) Luo et al., 2022

CF Crude fibre CL Crude lipids CP Crude protein

Arg Arginine Lys Lysine Thr Threonine

Hist Histidine Iso Isoleucine Leu Leucine

Meth Methionine Phen Phenylalanine Tryp Tryptophan

Val Valine

Linoleic acid (18:2n-6) Eicosapentaenoic acid (20:5n-3)

Linolenic acid (18:3n-3) Docosahexaenoic acid (22:6n-3)

Arachidonic acid (20:4n-6) Saturated fatty acids (SFAs)

Monosaturated fatty acids (MFAs) Polyunsaturated fatty acids (PUFAs)

Poly-β-hydroxybutyrate (PBHs)

2.8 Phytoremediation of aquaculture wastewater using seaweed, *Caulerpa lentilifera*

Phytoremediation is a sustainable and eco-friendly approach utilised in the treatment of aquaculture wastewater, which relies on the natural ability of plants to absorb and accumulate toxins (Vithalani et al., 2022; Yuliasni et al., 2023). This process involves the use of plants to purify and treat wastewater, and has been increasingly recognised as an effective method for addressing water pollution and recycling water in aquaculture systems (Abubakar et al., 2023; Van Tung et al., 2021). The process comprises the recirculation of wastewater after biological treatment and/or filtration, making aquaculture almost independent of the available water resources. Aquaculture wastewater frequently contains suspended particles that can negatively impact water quality and certain biochemical processes. Phytoremediation refers to the treatment of wastewater and recovery of contaminated systems through the use of plants. Suspended particles in the water are filtered by the roots and foliage of phytoremediation agents, leading to a reduction in the concentration of total suspended solids. This method is both eco-friendly and cost-effective (Kafle et al., 2022; Kristanti & Hadibarata, 2023; Vance & Jacobs, 2019). Microalgae-based phytoremediation produces high-value biomass useful for aquaculture feeds in addition to reducing the amount of nutrient in water bodies, (Cardoso et al., 2022). Improving water quality by reducing the total suspended solids, ammonia, and phosphate is essential for promoting healthy and reliable aquaculture systems. Total suspended solids (TSS) can cause turbidity, which interferes with the photosynthetic process of organisms in the water column (Akhrianti et al., 2023).

Ammonia, produced through the microbial degradation of organic waste, is a major component of aquaculture waste. Aquaculture waste contains significant amounts of nitrogenous compounds, mostly in the form of ammonia (NH_3^+), that can have detrimental effects on aquatic ecosystems (Ashour et al., 2021). Ammonia accumulation in aquaculture systems is exacerbated by decomposition of organic waste (Kokkuar et al., 2021). The build-up of nitrogenous waste, including ammonia, is a significant obstacle to sustainable aquaculture production, particularly under zero-

water discharge conditions (Preena et al., 2021). Nitrifying biofilters are commonly installed in RAS to reduce the concentration of ammonia and to transform it into a less toxic form of nitrate through a two-phase process. The initial phase comprises the conversion of ammonia to nitrite, while the second phase is the conversion of nitrite to nitrate (Godoy-Olmos et al., 2019). In the same vein, Handajani et al., (2021) investigated the potential of *Echinodorus palaefolius* as a phytoremediation agent for reducing nitrogen and phosphate wastes in intensive culture systems. Additionally, research has shown that the use of heterotrophic bacteria in biofloc systems can effectively reduce ammonia levels in aquaculture systems (Ferreira et al., 2020; Luo et al., 2023; Robles-Porchas et al., 2020).

Aquaculture wastewater contains phosphates from faeces and uneaten feed (Beheary et al., 2019). High phosphate levels can lead to eutrophication and algal blooms in aquatic systems (Gao et al., 2022), which can be harmful. Phytoremediation, which involves using aquatic plants to absorb and store phosphates is used to control phosphate levels in aquaculture wastewater (Nizam et al., 2020). Research has shown that plants such as *Ceratophyllum demersum*, *Lemna minor*, *Eichhornia crassipes,* and *Pistia stratiotes* can remove pollutants, including phosphates, from aquaculture wastewater (Amyati et al., 2020). The application of phytoremediation to control eutrophication has been proven effective in removing excessive amounts of nitrogen and phosphorus from water sources. Furthermore, physiological tracer experiments validated the importance of mycorrhizal phosphate uptake pathways in delivering acquired phosphate to plants (Xue et al., 2018). The reduction in total suspended solids, ammonia, and phosphate led to a significant improvement in water quality.

Phytoremediation agents in finfish/shrimp aquaculture primarily convert toxic metabolites and toxins into nutrients by taking them up and breaking them down. The capacity of seaweed to absorb substances serves as an indicator of water quality within a culture system owing to its sensitivity to environmental fluctuations and natural ecological disturbances. An investigation conducted by Rahardjo et al., (2017) demonstrated that *Gracilaria* sp. had the highest level of ammonia among various seaweed species, ranging from 16.7% to 50.0%. This followed *Caulerpa lentilifera*

(15.3% - 37.0%). Additionally, Adharini et al (2021) examined the effectiveness of certain seaweed species as biofilters to reduce wastewater nutrients and prevent water pollution from hybrid grouper cultures. Among these seaweed species, *Ulva* sp. was found to be the most effective in reducing nitrogen and phosphorus levels in wastewater, with nitrogen and phosphorus levels reduced by 80% and 88%, respectively.

CHAPTER 3

OPTIMIZATION OF TRADITIONAL BIOFLOC SYSTEM BASED ON WATER QUALITY CONDITIONS AND EVALUATION OF ITS PERFORMANCE USING THE GROWTH AND PHYSIOLOGY OF *L. VANNAMEI*.

3.1 Introduction

Sustainable shrimp farming relies on advanced culture systems that balance productivity with environmental concerns. BFT is a technology that harnesses microbial ecosystems to transform waste into resources where bacteria, algae, and other microorganisms thrive in suspended floc. The floc is identified to detoxify ammonia and nitrite which are common by-products of shrimp metabolism and feed degradation. In addition, the floc provides a nutrient-rich supplement for *Litopenaeus vannamei* thereby reducing reliance on external feeds (Raza et al., 2024). The zero-water exchange nature of BFT minimizes discharge to make produce an eco-friendly alternative for high-density shrimp culture (Taufik et al, 2023). However, achieving consistent biofloc performance requires precise control of operational parameters, which IMTAS seeks to addresses through its integrated design. IMTAS utilizes rapid biofloc for automated production, flocculation, and phytoremediation that leverages IoT technology in its operation of shrimp and other derived products.

Central to this effort is the rapid biofloc approach, which accelerates microbial aggregation to establish a robust nutrient-cycling network. The Taguchi L9 method is employed to determine the optimum levels of the control factors namely, stocking density, carbon-to-nitrogen (C/N) ratio, and probiotic dosage. Stocking density plays a key role in determining microbial load and shrimp competition in biofloc production (Mandal et al., 2024). The C/N ratio on the other hand is a driver of the growth of heterotrophic bacterial growth useful in creating floc nutritional value and water quality (Li et al., 2023). Probiotics enhance microbial diversity and shrimp immunity essential for mitigating pathogen risks in confined biofloc systems (Nurhafizah et al., 2021). These parameters are interdependent in a manner that requires a systematic approach to identify their ideal settings.

This optimization process aims to establish a design for IMTAS that ensures rapid biofloc formation and supports sustainable shrimp yields. Research shows that precise adjustment of C/N boosts protein content in biofloc which directly benefit shrimp growth (Ghosh et al., 2024). Similarly, probiotics have been linked to reduced Vibrio prevalence, a common threat in intensive setups (Zuo et al., 2023). The combination of these biofloc principles in IMTAS automated by IoT-driven monitoring will create a high-performance system that may be better than the traditional biofloc system. Water quality regulates almost all physiological reactions in cultured aquatic animals, as reflected in their growth performance and disease resistance (Abdel-Tawwab et al. 2019; Dauda 2020, Elayaraja et al. 2020). Inadequate knowledge of the optimum levels of control factors in biofloc aquaculture systems has resulted in poor yields and water quality challenges (Abd El-Hack et al. 2022). This study adopted the Taguchi methodology cum grey relational analysis and principal component analysis for the optimisation of IMTA. The experiment aimed at determining the optimum levels of stocking density, C/N ratio, and probiotic dosage for the best performance of the culture system.

3.2 Methodology

3.2.1 Experimental site

The study was conducted in two experiments of 12 weeks each to address four research objectives. The culture period was adopted from the reports of Poersch et al (2021) and Zhang et al (2022) for *L. vannamei* under biofloc culture system. Experiment 1 ran from March 14 – June 15, 2023, while Experiment 2 was conducted between August 02 and October 17, 2023. Both experiments were conducted at the indoor hatchery of the Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia, Terengganu. The first experiment was a preliminary examination that used the traditional biofloc system to establish optimum conditions for the establishment of IMTAS. Experiment 2 was conducted to test the working efficiency of IMTAS. The flowchart of the research framework is presented in **Figure 3.1**. The first study focused on optimising the control factors (stocking density, C/N ratio, and probiotic dosage) in a traditional biofloc system using the Taguchi L9 method, while the second study evaluated the performance of the IMTAS system. The collected data were subjected to Taguchi multi-response optimisation integrated with GRA to determine the optimal parameter levels. The first and second grey relational grades were used to set up the IMTAS in Experiment 2.

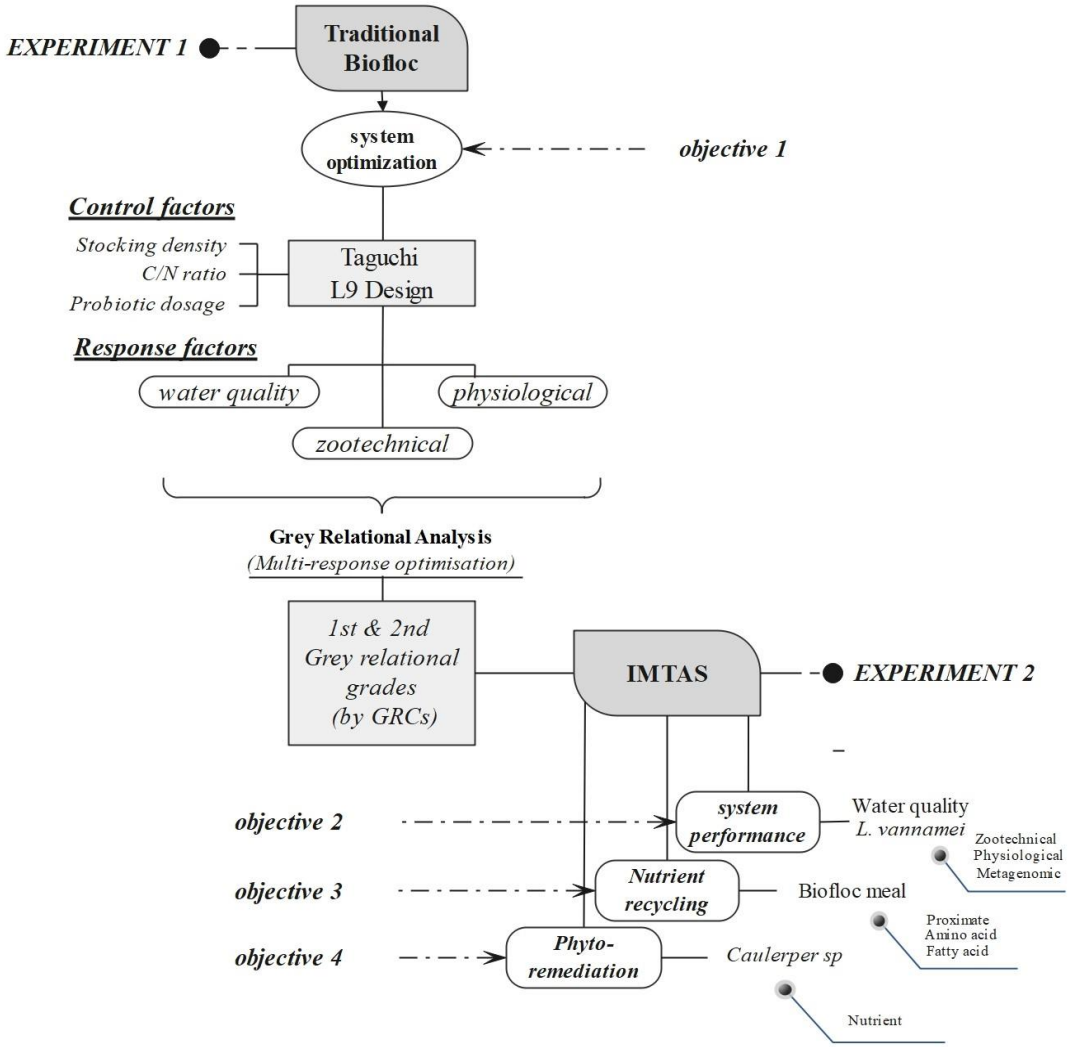
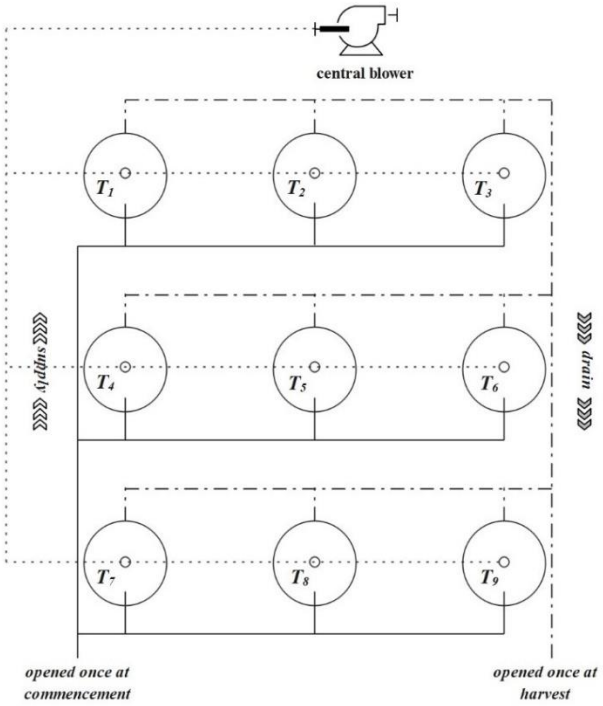


Figure 3.1: Flowchart of experimental design

3.2.2 Experimental set-up and design

The experiment was carried out using three parallel series of HDPE tanks, each with a volume of 3 m^3, connected by 3-inch PVC pipes, with no water discharged during the experiment. Each tank had an aerotube inserted in the centre, to provide aeration in a vertical current. Taguchi L9 design was used to determine the optimum stocking density, C/N ratio, and dosage of probiotic. The C/N ratio was maintained at 10, 15, and 20 with the addition of molasses, and the stocking densities were 100, 200, and 300 shrimp/m^3, respectively. Rapid BFT^{TM} was inoculated as a probiotic at daily dosages of 7, 14, and 21 ml/L until the floc matured at week 5. The levels for each of

these control factors were adopted from the findings of Arnold et al. (2009), Braga et al. (2016), Ferreira et al. (2016), Wasielesky et al. (2006) for stocking density; Asaduzzaman et al. (2008), Burford et al. (2003), Xu & Pan (2013) for C/N ratio and Amjad et al. (2022), Ekasari et al. (2014), Ferreira et al. (2015), Silva et al. (2012) for probiotic dosage. The L9 orthogonal array was generated by Minitab 19th edition based on orthogonal array function (**Equation 2.1**) and layout (**Table 2.3** and **2.4**) as presented in the nine experimental units (run) illustrated in **Figure 3.2**.



Factor Levels

Factors	Level 1	Level 2	Level 3
Stocking Density (PL/m^3)	100	200	300
C/N ratio (xc1)	10	15	20
Probiotic (ml/l)	7	14	21

Taguchi L9 orthogonal array

		Control factors		
Run	Tank	Stocking Density	C/N Ratio	Probiotic
Run 1	T_1	100	10	7
Run 2	T_2	100	15	14
Run 3	T_3	100	20	21
Run 4	T_4	200	10	14
Run 5	T_5	200	15	21
Run 6	T_6	200	20	7
Run 7	T_7	300	10	21
Run 8	T_8	300	15	7
Run 9	T_9	300	20	14

Figure 3.2: Experimental layout for the parameter setting of an Indoor Multi-Techno Aquaculture System (IMTAS)

3.2.3 Preparation of inoculum and molasses application

3.2.3.1 Preparation of probiotic inoculum

Rapid BFT™ was used as a bacteria inoculum in this study. It is a pure culture of *Bacillus tropicus* that triggers rapid flocculation and formation of a microbial community. Deionised water (1000 ml) was mixed with 40.25 g of Zobell marine broth 2216 to dissolve the pellets. The mixture was autoclaved for 15 min at a pressure of 15 lbs and temperature of 121ºC. After the broth had cooled to room temperature, a streak was made from a culture of *B. tropicus* into the broth and kept in an incubator shaker (Sartorius, Certomat® IS) for 24 h before use. The ready-to-use probiotic was a community of bred living microorganisms (**Figure 3.3**).

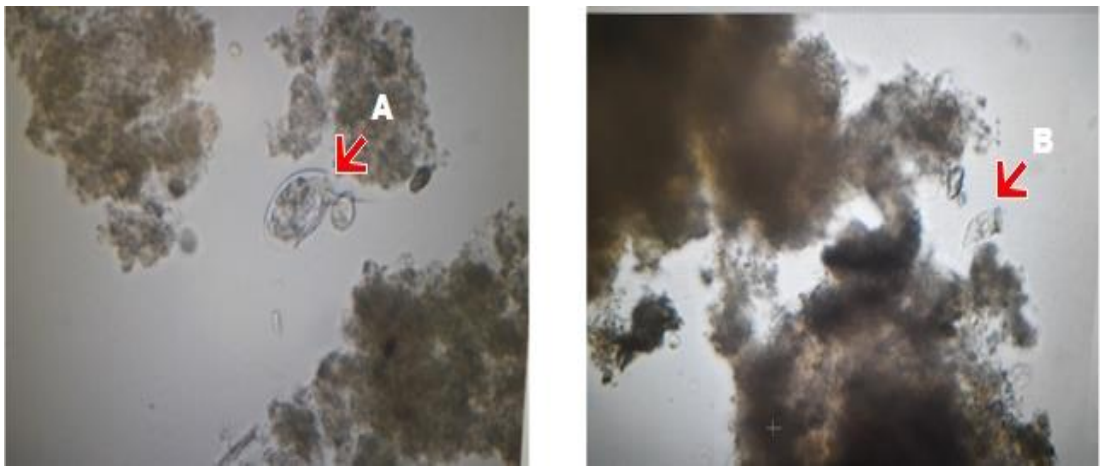


Figure 3.3: Micrograph of the prepared probiotic viewed under the microscope (X 40) showing (A) *Daphnia* sp. (B) *Vorticella* sp.

3.2.3.2 Quantification of molasses

Sugar beet molasses (carbon, approximately 50% on a dry matter basis) was used as the carbon source in the experiment. The amount of sugar beet molasses was calculated according to the method of Panigrahi (2018) as follows:

A. *Estimation of carbon and nitrogen contributions from feed*

The estimation of carbon (C) and nitrogen (N) contributions from feed was conducted based on dry matter content, feed assimilation efficiency, and crude protein composition. **Equations 2.10** – **2.16** were applied as follows:

i. Carbon estimation

$C = (F \times DM \times WF)/CF$ **(Equation 2.10)**

where:

C = Carbon contribution from feed (g); F = Feed input (kg); DM = Dry matter fraction; WF = Waste fraction (proportion of feed not assimilated by fish); CF = Carbon fraction in dry matter (~50%)

ii. Nitrogen Estimation

$N = (F \times DM \times WF \times CPF) / K$ **(Equation 2.11)**

where:

N = Nitrogen contribution from feed (g); CPF = Crude protein fraction of feed; K = Protein-to-nitrogen conversion factor (6.25).

B. Adjustment of the Carbon-to-Nitrogen Ratio

To achieve a target carbon-to-nitrogen (C:N) ratio, the required carbon supplementation was determined as follows:

$C_{req} = N \times R$ **(Equation 2.12)**

where:

Creq = Required carbon for the target C:N ratio (g); R = Desired C:N ratio

The additional carbon required from molasses was calculated as:

$C_{molasses} = C_{req} - C_{feed}$ **(Equation 2.13)**

where:

C_molasses = additional carbon required; Given that molasses typically contains ~50% carbon by dry weight, the required quantity of molasses was estimated using:

$M_{req} = C_{molasses} \times 0.5$ **(Equation 2.14)**

C *Carbon Supplementation to maintain specified C:N ratio*

The total ammonia nitrogen (TAN) present in each experimental unit was determined as follows:

$$TAN_{system} = TAN_{conc} \times Water\ Vol. \quad \textbf{(Equation 2.15)}$$

where:

TANsystem = Total ammonia nitrogen in the system (g); TANconc = TAN concentration in water (mg L^{-1}).

To maintain the biofloc system at a predefined C:N ratio:

$$M_{supplementation} = TAN_{system} \times M_{req} \quad \textbf{(Equation 2.16)}$$

The amount of molasses was applied periodically and monitored to ensure appropriate adjustments based on TAN levels within the system.

Sugar beet molasses application was adjusted weekly based on the rate of feed administered to the shrimp which were fed to satiation using autofeeders. The same procedure was followed in Experiment 2.

3.2.4 Stocking and feeding

Post larvae, PL of Pacific Whiteleg shrimp, *L. vannamei*, were obtained from a local supplier in Pahang, Malaysia. The stock passed the stress test performed on the PLs upon arrival (87%), which measured the mortality of 100 PLs placed in 1000ml of swirling water for 30 min. Then, using the Taguchi layout shown **Figure 3.1**, the PLs were counted and stocked into each tank. The shrimp were fed with commercial Blanca® shrimp feed (36% crude protein).

3.2.5 Water quality monitoring

Temperature (°C), dissolved oxygen (mg/L), electrical conductivity (µS/cm), total dissolved solids (mg/L), salinity (ppt), and pH were assessed using a YSI multiparameter analyser (Professional Plus). UV spectrophotometer (Shimadzu, UV-1800) was used to measure the total ammonia nitrogen (TAN), nitrite, and phosphate.

TAN was determined using the phenol-hypochlorite method described in Liang et al. (2016). The standard solution was prepared by dissolving 3.670 g ammonium sulphate in 1000 ml deionised water. A serial dilution was prepared from this stock solution to produce standards of 0.00 mg/l, 0.05 mg/l, 0.10 mg/l, 0.30 mg/l, 0.50 mg/l and 1.00 mg/l by adding 5 ml deionised water to each preceding unit of standards. The working solutions were phenol, sodium nitroprusside, alkaline citrate, and oxidising agent. The samples and standards (50 ml) were measured and placed in separate test tubes. In each tube, 0.2 ml of phenol and nitroprusside solution was added to each tube, followed by 0.5 ml of the oxidising agent. The solution was incubated for an hour. After incubation, the contents of the test tubes were transferred to cuvettes and read on a spectrophotometer at a wavelength of 640 nm.

Nitrite was measured using a spectrophotometer, as outlined by Imorwitz & Keliher (1984). Sodium nitrate of (1.432 g) of was dissolved in 1000 ml in deionised water as a stock solution. Serial dilutions of the stock solution were prepared in triplicate by adding 100 ml of deionised water to 0.5, 1, 3, 5, and 10 ml of the solution. The sulfanilamide solution was dissolved in 1 g of sulfanilamide in 60 ml of deionised water, and 10 ml of hydrochloric acid was added. The solution was then diluted to 100 ml volume. Ten millilitres of each standard solution and water sample were measured in a test tube, and 0.2 mL sulfanilamide solution was added to the test tube. The resulting mixture was vortexed and kept at room temperature for 8 min. After adding 2 ml of NED, the tubes were kept for another 1 h before measurement on the spectrophotometer at a wavelength of 543 nm.

Phosphate concentration was measured using the method described by Shyla et al. (2011). The standard was prepared by dissolving 1.432 g of potassium dihydrogen phosphate in 100 ml deionised water and further serial dilutions of 0.5, 1, 3, 5 and 10 ml, respectively. The orthophosphate solution was prepared from mixed reagents made of ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimonyl tartrate solution. 0.5 ml of the mixed solution obtained from the four reagents was added to the water samples and kept for 1 h at room temperature. The concentration was then read at 680nm wavelength on the spectrophotometer.

3.2.6 Statistical analysis (Parameter optimisation)

Taguchi analysis combined with grey relational analysis (GRA) and principal component analysis (PCA) were used to evaluate the optimum levels of some water quality variables as a guide to obtaining the optimum control factors for the best performance of IMTA. While Taguchi single response analysis evaluated the variability of each of the variables from the optimum standards, GRA was used to integrate the various water quality variables into a unit of evaluation. PCA on the other hand measured the multiple interactions among the response factors (water quality parameters). The analysis was conducted on Minitab 19th Edition for Windows. A schematic flow of the statistical analysis conducted in this study is presented in **Figure 3.4.**

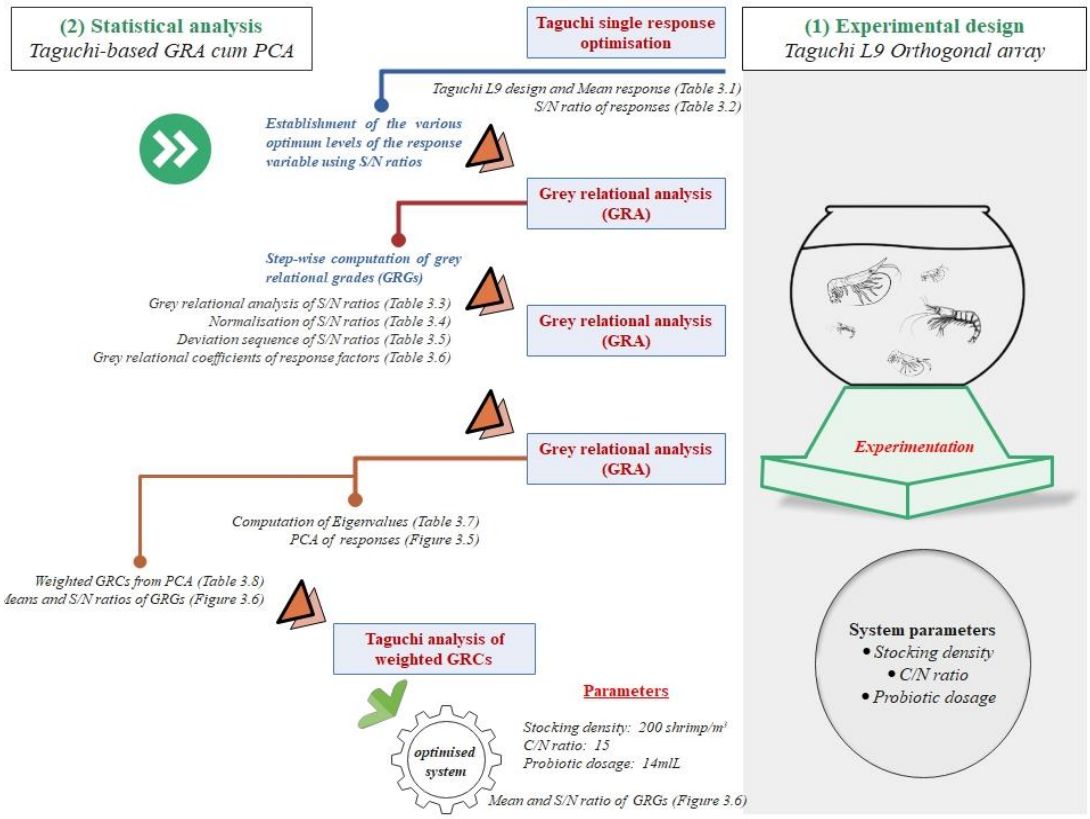


Figure 3.4: Schematic flow of Taguchi design and analysis for the parameter setting for IMTAS

3.3 Results

3.3.1 Single-response optimisation

Water quality data obtained during the experimental period were computed into means of response factors (**Table 3.1**), where signal-to-noise ratios (S/N ratio) were calculated (**Table 3.2**). The S/N ratio was set based on the impact and trend of desirability established for each response factor. *'small for the better'* was set for TAN (Addo et al. 2023, Chen et al. 2023, Chu & Brown 2022, Huang et al. 2022, Mansour et al. 2022); nitrite (Addo et al. 2023; Huang et al. 2023, Pérez-Velasco et al. 2023, Syaichudin et al. 2022) and phosphate (Khanjani et al. 2022, Krummenauer et al. 2022, Pérez-Velasco et al. 2023, Prates et al. 2023) while DO (Ayesha Jasmin et al. 2022, Guo et al. 2014, Holanda et al. 2022, Huang et al. 2023, Whangchai et al. 2022); EC (Kumar et al. 2019, Nayak et al. 2023); TDS (Carvalho et al. 2023, Das et al. 2023, Holanda et al. 2022, Huang et al. 2022, Khanjani & Sharifinia 2022), Sal (Chen et al. 2023, Holanda et al. 2022, Huang et al. 2022), pH (Brito et al. 2018, Holanda et al. 2022, Huang et al. 2022b) and Temp (Blancaflor & Baccay 2022, Carvalho et al. 2023, Catalani et al. 2023, Kim et al. 2022) were set at *'the larger the better'*.

The S/N ratio for the *'smaller was the better'* was computed from the formula

$$S/N\ ratio = (-10)^{*} log_{\,10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\chi_{ij}^{2} \qquad \textbf{(Equation 3.1)}$$

While the S/N ratio for the *'larger was the better'* was calculated from the relationship

$$S/N\ ratio = (-10)^{*} log_{\,10}\left(\frac{1}{n}\right)\sum_{1=1}^{n}\frac{1}{\chi_{ij}^{2}} \qquad \textbf{(Equation 3.2)}$$

Where;

n is the number of replicates, and

χ_{ij} is the response factor (water quality variables).

The phosphate levels showed minimal fluctuations across the runs. Run 2 showcased the highest phosphate concentration at 0.135 mg/L, while Run 1 recorded the lowest phosphate concentration at 0.126 mg/L. Among the runs, Run 6 featured the highest DO concentration of 5.797 mg/L, whereas the lowest DO level was observed in Run 5, with a reading of 5.317 mg/L. Run 5 displayed the highest EC value of 49321.939 $\mu S.cm^{-1}$, whereas Run 8 exhibited the lowest EC value of 46920.247 $\mu S.cm^{-1}$. The TDS values also varied, with Run 7 recording the highest TDS value at 30330.730 mg/L and Run 8 having the lowest TDS value at 27239.611mg/L. Sal levels varied, with the highest value recorded in Run 4 at 35.367 ppm and the lowest in Run 2 at 33.763 ppm. Run 5 featured the highest pH value at 7.608, while Run 8 had the lowest pH reading at 7.351. Run 5 recorded the highest Temp at 28.929°C, while the lowest was observed in Run 4 at 28.621°C.

A Single-Response Optimisation was conducted, and signal-to-noise (S/N) ratios were computed to assess the performance of the aquaculture system across various control factor configurations. Run 7 had the best performance by recording an optimum S/N ratio of 0.309 for TAN, whereas Run 9 displayed the lowest performance with an S/N ratio of 2.273. For nitrite, the optimum S/N ratio was observed in Run 1, with a value of 7.929, whereas the lowest S/N ratio for nitrite was found in Run 2, with an S/N ratio of 6.077. In the case of phosphate, Run 1 yielded the highest S/N ratio at 17.960, whereas Run 6 had the lowest S/N ratio for phosphate, (17.385). The optimised S/N ratio for DO was achieved in Run 6, resulting in a value of 15.265, whereas Run 5 showed the lowest S/N ratio for DO at 14.514. EC optimisation led to the highest S/N ratio in Run 5, as 93.861, whereas Run 9 had the lowest S/N ratio for EC at 93.157. For TDS, the optimised S/N ratio was found in Run 7, with a value of 89.638, while the lowest S/N ratio for TDS was recorded in Run 1 at 88.351. Run 4 achieved the highest S/N ratio for Sal at 30.972, whereas Run 2 displayed the lowest S/N ratio for Sal, which was 30.569. The optimum S/N ratio for pH was attained in Run 2, reaching 17.625, whereas the lowest S/N ratio for pH was observed in Run 8, with a value of 17.327. Run 5 had the highest S/N ratio for temp (29.227), whereas the lowest S/N ratio was observed in Run 9, with an S/N ratio of 29.145. Generally, the high S/N ratio recorded for DO and pH represented their importance as control parameters in minimising the system variability to enhance efficient performance of IMTAS.

Table 3.1: Taguchi L9 design and mean response factors obtained for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**
Run 1	100/m^3	10	7 ml/L	1.005	0.401	0.126	5.4	46302.6	26155.3	34.1	7.5	28.7
Run 2	100/m^3	15	14 ml/L	1.046	0.497	0.135	5.7	48949.9	28137.9	33.7	7.6	28.6
Run 3	100/m^3	20	21 ml/L	1.030	0.500	0.135	5.6	47792.6	27676.4	34.2	7.5	28.8
Run 4	200/m^3	10	14 ml/L	1.053	0.458	0.132	5.7	46721.7	28559.5	35.3	7.4	28.6
Run 5	200/m^3	15	21 ml/L	1.035	0.431	0.128	5.3	49321.9	28753.2	35.0	7.5	28.9
Run 6	200/m^3	20	7 ml/L	1.040	0.488	0.135	5.7	47193.5	29151.8	34.9	7.5	28.7
Run 7	300/m^3	10	21 ml/L	0.965	0.459	0.130	5.3	47666.8	30330.7	35.2	7.5	28.6
Run 8	300/m^3	15	7 ml/L	1.110	0.543	0.131	5.7	46920.2	27239.6	34.5	7.3	28.7
Run 9	300/m^3	20	14 ml/L	1.299	0.476	0.129	5.7	45485.3	28852.0	35.2	7.5	28.6

Table 3.2: S/N Ratio in the single response optimisation for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**
Run 1	100/m^3	10	7 ml/L	0.042	7.929*	17.960*	14.727	93.312	88.351	30.673	17.588	29.171
Run 2	100/m^3	15	14 ml/L	0.392	6.077	17.403	15.232	93.795	88.986	30.569	17.625*	29.145
Run 3	100/m^3	20	21 ml/L	0.257	6.029	17.408	14.977	93.587	88.842	30.687	17.518	29.205
Run 4	200/m^3	10	14 ml/L	0.447	6.788	17.564	15.176	93.390	89.115	30.972*	17.415	29.134
Run 5	200/m^3	15	21 ml/L	0.300	7.310	17.861	14.514	93.861*	89.174	30.891	17.569	29.227*
Run 6	200/m^3	20	7 ml/L	0.344	6.224	17.385	15.265*	93.478	89.293	30.879	17.532	29.162
Run 7	300/m^3	10	21 ml/L	0.309*	6.765	17.697	14.539	93.564	89.638*	30.948	17.532	29.155
Run 8	300/m^3	15	7 ml/L	0.909	5.298	17.644	15.234	93.427	88.704	30.769	17.327	29.161
Run 9	300/m^3	20	14 ml/L	2.273	6.442	17.813	15.206	93.157	89.204	30.944	17.529	29.148
Optimum				$S_{L3}C_{L1}A_{L3}$	$S_{L1}C_{L1}A_{L1}$	$S_{L1}C_{L1}A_{L1}$	$S_{L2}C_{L3}A_{L1}$	$S_{L2}C_{L2}A_{L3}$	$S_{L3}C_{L1}A_{L3}$	$S_{L2}C_{L1}A_{L2}$	$S_{L1}C_{L2}A_{L2}$	$S_{L2}C_{L3}A_{L3}$
Criteria				*Smaller*	*Smaller*	*Smaller*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*

Noted that; S = stocking density; C = C/N ratio; A = probiotic dosage and subscripts L1 – L3 are levels 1-3 respectively. * is the optimised S/N ratio in parameter

3.3.2 Grey Relational Analysis

Table 3.3 presents the Grey relational analysis (GRA) of the S/N ratio of the response factors. Accordingly, the GRA of TAN, nitrite and phosphate which are desired at low concentrations were calculated from the established S/N ratios in single response optimisation using the relationship

$$\chi_j^*(p) = \frac{\chi_j(p) - min\ \chi_j(p)}{max\ \chi(p) - min\ \chi_j(p)} \quad \textbf{(Equation 3.3)}$$

Dissolved oxygen, electrical conductivity, total dissolved solids, salinity, pH and temperature are preferred at higher values for the optimum performance of the biofloc aquaculture system and the GRA for these parameters was therefore computed from the equation

$$\chi_j^*(p) = \frac{max\ \chi(p) - \chi(p)}{max\ \chi_j(p) - \min \chi_i(p)} \quad \textbf{(Equation 3.4)}$$

Where;

$\chi_j^*(p)$ are the calculated grey relational values of each run,

p is the number of response factors (the nine water quality variables in this research) in 9 stepwise sequence $\chi_j(p)$,

j = 1,2,3...,9 represents the L9 orthogonal array.

$Max\ \chi(p)$ and $min\ \chi(p)$ represented the maximum and minimum values of the computed p^{th} S/N ratios.

The normalised grey relational values are presented in **Table 3.4**. Normalisation was performed to test the optimality in the performance of the grey relational values which were placed at a maximum of 1 (Peterson 2021).

The deviation sequence of the normalised values was computed as absolute values of the differences between a normalised value at a run $\chi_0^*(p)$ referential to the sequence $\chi_j^*(p)$ in the relationship (**Table 3.5**)

$$\Delta_{0j}(p) = |\chi_0^*(p) - \chi(p)| \quad \textbf{(Equation 3.5)}$$

The grey relational coefficients, GRCs (**Table 3.6**) were then calculated from the formula

$$\xi\left(\chi_j^*(p), \chi_0^*(p)\right) = \frac{\Delta_{min}(p) + \zeta\Delta_{max}(p)}{\Delta_{0j}(p) + \Delta_{max}(p)} \quad \textbf{(Equation 3.6)}$$

Although the distinguishing coefficient, ζ ranges between 0 and 1, this research set ζ at 0.5, based on the criteria considered by Qazi et al. (2020) and Sarikaya & Güllü (2015).

Table 3.3: Grey Relational Analysis of Signal-to-Noise ratios for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Amount of probiotic**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**
Run 1	100/m^3	10	7 ml/L	0.042	7.929	17.960	14.727	93.312	88.351	30.673	17.588	29.171
Run 2	100/m^3	15	14 ml/L	0.392	6.077	17.403	15.232	93.795	88.986	30.569	17.625	29.145
Run 3	100/m^3	20	21 ml/L	0.257	6.029	17.408	14.977	93.587	88.842	30.687	17.518	29.205
Run 4	200/m^3	10	14 ml/L	0.447	6.788	17.564	15.176	93.390	89.115	30.972	17.415	29.134
Run 5	200/m^3	15	21 ml/L	0.300	7.310	17.861	14.514	93.861	89.174	30.891	17.569	29.227
Run 6	200/m^3	20	7 ml/L	0.344	6.224	17.385	15.265	93.478	89.293	30.879	17.532	29.162
Run 7	300/m^3	10	21 ml/L	0.309	6.765	17.697	14.539	93.564	89.638	30.948	17.532	29.155
Run 8	300/m^3	15	7 ml/L	0.909	5.298	17.644	15.234	93.427	88.704	30.769	17.327	29.161
Run 9	300/m^3	20	14 ml/L	2.273	6.442	17.813	15.206	93.157	89.204	30.944	17.529	29.148
Criteria				*Smaller*	*Smaller*	*Smaller*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*	*Larger*
Min				0.042	*5.298*	*17.385*	*14.514*	*93.157*	*88.351*	30.569	17.327	29.134
Max				*2.273*	*7.929*	*17.960*	*15.265*	*93.861*	*89.638*	30.972	17.625	29.227
Range				*2.231*	*2.631*	*0.575*	*0.751*	*0.704*	*1.287*	*0.403*	*0.298*	*0.093*

Table 3.4: Normalisation of Signal-to-Noise ratios for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**
Run 1	100/m^3	10	7 ml/L	0.864	1.000	1.000	0.284	0.220	0.000	0.258	0.875	0.401
Run 2	100/m^3	15	14 ml/L	0.728	0.296	0.031	0.957	0.907	0.493	0.000	1.000	0.116
Run 3	100/m^3	20	21 ml/L	0.781	0.278	0.041	0.617	0.611	0.382	0.294	0.639	0.762
Run 4	200/m^3	10	14 ml/L	0.707	0.566	0.312	0.882	0.331	0.594	1.000	0.294	0.000
Run 5	200/m^3	15	21 ml/L	0.764	0.765	0.828	0.000	1.000	0.639	0.800	0.812	1.000
Run 6	200/m^3	20	7 ml/L	0.747	0.352	0.000	1.000	0.455	0.732	0.770	0.688	0.303
Run 7	300/m^3	10	21 ml/L	1.000	0.558	0.542	0.034	0.579	1.000	0.940	0.688	0.224
Run 8	300/m^3	15	7 ml/L	0.528	0.000	0.451	0.960	0.384	0.274	0.498	0.000	0.293
Run 9	300/m^3	20	14 ml/L	0.000	0.435	0.744	0.922	0.000	0.663	0.931	0.676	0.152

Table 3.5: Deviation Sequence of the normalised S/N ratios for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**
Run 1	100/m^3	10	7 ml/L	0.136	0.000	0.000	0.716	0.780	1.000	0.742	0.125	0.599
Run 2	100/m^3	15	14 ml/L	0.272	0.704	0.969	0.043	0.093	0.507	1.000	0.000	0.884
Run 3	100/m^3	20	21 ml/L	0.219	0.722	0.959	0.383	0.389	0.618	0.706	0.361	0.238
Run 4	200/m^3	10	14 ml/L	0.293	0.434	0.688	0.118	0.669	0.406	0.000	0.706	1.000
Run 5	200/m^3	15	21 ml/L	0.236	0.235	0.172	1.000	0.000	0.361	0.200	0.188	0.000
Run 6	200/m^3	20	7 ml/L	0.253	0.648	1.000	0.000	0.545	0.268	0.230	0.312	0.697
Run 7	300/m^3	10	21 ml/L	0.000	0.442	0.458	0.966	0.421	0.000	0.060	0.312	0.776
Run 8	300/m^3	15	7 ml/L	0.472	1.000	0.549	0.040	0.616	0.726	0.502	1.000	0.707
Run 9	300/m^3	20	14 ml/L	1.000	0.565	0.256	0.078	1.000	0.337	0.069	0.324	0.848

Table 3.6: Grey Relational Coefficients for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**								
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC**
Run 1	100/m^3	10	7 ml/L	0.786	1.000	1.000	0.411	0.391	0.333	0.403	0.800	0.455
Run 2	100/m^3	15	14 ml/L	0.648	0.415	0.340	0.921	0.842	0.497	0.333	1.000	0.361
Run 3	100/m^3	20	21 ml/L	0.695	0.409	0.343	0.566	0.562	0.447	0.415	0.580	0.678
Run 4	200/m^3	10	14 ml/L	0.631	0.536	0.421	0.809	0.428	0.552	1.000	0.414	0.333
Run 5	200/m^3	15	21 ml/L	0.679	0.680	0.744	0.333	1.000	0.581	0.714	0.727	1.000
Run 6	200/m^3	20	7 ml/L	0.664	0.435	0.333	1.000	0.479	0.651	0.685	0.616	0.418
Run 7	300/m^3	10	21 ml/L	1.000	0.531	0.522	0.341	0.543	1.000	0.893	0.616	0.392
Run 8	300/m^3	15	7 ml/L	0.515	0.333	0.477	0.925	0.448	0.408	0.499	0.333	0.414
Run 9	300/m^3	20	14 ml/L	0.333	0.469	0.661	0.866	0.333	0.597	0.879	0.607	0.371

3.3.3 Principal component analysis of GRCs

The GRCs computed from the response factors obtained in this research were subjected to principal component analysis, PCA in a response matrix (**Equation 3.7**)

$$\chi = \begin{vmatrix} \chi_1(1) & \chi_1(2) & \chi_1(k) \\ \chi_2(1) & \chi_2(2) & \chi_2(k) \\ \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots \\ \chi_j(1) & \chi_j(2) & \chi_j(k) \end{vmatrix} \quad \textbf{(Equation 3.7)}$$

Where;

$\chi_r(p)$ represents the GRC of each response factor is explained by r = 1,2,...

j is the experimental run and p = 1,2,…

k, is the number of response factors (water quality parameters).

A coefficient of correlation matrix was generated from this experiment with run (j) and parameters (k) as follows

$$\vartheta_{jl} = \left(\frac{Cov\,(\chi_r(p),\chi_r(l))}{\sigma_{\chi r}(p)*\sigma_{\chi r}(l)}\right) r = 1,2,...k;\ l = 1,2,...,k \quad \textbf{(Equation 3.8)}$$

Where the covariance of $\chi_r(p)$ and $\chi_r(l)$ is represented by $Cov\,(\chi_r(p),\chi_r(l))$ and their standard deviations are $\sigma_{\chi r}(p)$ and $\sigma_{\chi r}(l)$, respectively.

The eigenvalues and eigenvectors were then calculated from the resultant array from the formula

$$(\varrho - \lambda_k l_j)V_{pk} = 0 \quad \textbf{(Equation 3.9)}$$

From the matrix ϱ, the eigenvalues (λ_k) and eigenvectors V_{pk} were used to calculate the uncorrelated principal components from the relationship

$$Z_{jk} = \sum_{i=1}^{n} \chi(p) * V_{pk} \quad \textbf{(Equation 3.10)}$$

The Z_{jk} represents the kth principal component which is arranged in descending order with eigenvalues to indicate the level of variance among the response factors.

The eigenvalues and eigenvectors of the PCA for the response variables are presented in **Table 3.7**. The eigenvalues represent the variance explained by each principal component and capture the variability of the response factors. PC1 had the highest eigenvalue of 0.145, which explained 35.20% of the total variance in the system. PC2, PC3, and the subsequent components explained the decreasing amounts of variance. In total, PC1 to PC9 cumulatively explained 100% of the disparities in the response factors. DO was observed to have the highest variability recorded in PC1 (0.522), whereas TDS had the least variations (0.035), where the eigenvectors of Nitrite (-0.314), phosphate (0.290), EC (-0.231), salinity (0.388), pH (0.261) and Temp (-0.482) were within these limits.

Table 3.7: Eigenvectors of response factors for the parameter setting of an Indoor Multi-Techno Aquaculture System

Run	**Principal Components**								
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
Eigenvalue	0.145	0.077	0.075	0.047	0.034	0.028	0.003	0.001	0.000
Proportion	0.352*	0.187	0.182	0.115	0.084	0.068	0.009	0.003	0.000
Cumulative	0.352	0.538	0.720	0.835	0.919	0.987	0.997	1.000	1.000
Eigenvectors									
TAN	-0.197	0.155	-0.231	0.158	0.461	-0.655	-0.316	-0.271	0.208
Nitrite	-0.314	-0.139	0.103	0.551	0.456	0.155	0.534	0.213	0.069
Phosphate	0.290	-0.362	0.596	-0.022	0.160	0.075	-0.057	-0.442	0.446
DO	0.522	-0.001	-0.633	-0.137	0.229	0.176	0.352	-0.158	0.275
EC	-0.231	0.165	0.208	-0.755	0.487	0.026	0.220	0.050	-0.134
TDS	0.035	0.546	0.232	0.005	0.379	-0.355	0.516	-0.045	0.329
Salinity	0.388	0.504	0.209	0.145	0.301	0.206	-0.363	0.485	0.176
pH	0.261	-0.488	0.037	-0.156	-0.022	-0.529	0.098	0.608	0.091
Temp	-0.482	-0.089	-0.195	-0.192	-0.171	0.245	-0.179	0.227	0.716

*indicates the highest proportional contribution to variations in the system.

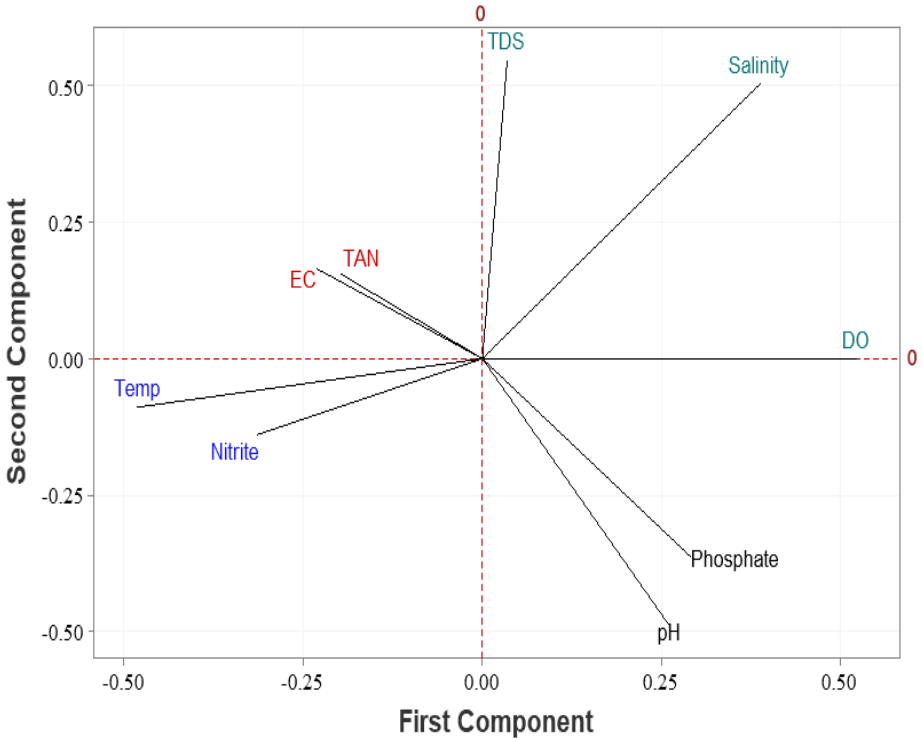


Figure 3.5: PCA Biplot of response factor for the control setting of an Indoor Multi-Techno Aquaculture System

The covariances of the response factors are shown in the PCA biplot in **Figure 3.5**. Temperature and nitrite had similar covariances and an inverse trend to DO, TDS and salinity. Similarly, EC and TAN had reverse covariances with phosphate and pH. EC and TAN showed the closest impacts in the aquaculture system.

GRGs, were calculated from the GRCs and eigenvectors using **equation 3.11**, with the values presented in **Table 3.8**. GRGs (between 0 and 1) are correlations of the response factors where higher GRGs indicate better results from the response factors in the respective runs.

$$\gamma_i\left(\chi_0^*, \chi_j^*\right) = \frac{1}{n}\sum_{p=1}^{n} \xi\left(\chi_j^*(p), \chi_0^*(p)\right) \qquad \textbf{(Equation 3.11)}$$

Where;

$\gamma_i\left(\chi_0^*, \chi_j^*\right)$ is the GRG of the j^{th} experimental run

N is the number of response factors.

Run 1 obtained a GRG of 0.534, which indicates a moderate level of correlation among the response factors. In the PCA ranking, it held the lowest position at 9, signifying that it captured a relatively smaller portion of the variability in the system compared to the other runs. Conversely, Run 2 had a comparatively higher GRG of 0.651 and held a PCA ranking of 4, suggesting a more effective capture of the variability in the system. Run 3 displayed a lower GRG of 0.483, and PCA ranking of 8, implying a weaker correlation among the response factors. Run 4 achieved the highest GRG of 0.762, signifying a strongest correlation among the response factors. It also achieved the top PCA ranking (1), representing exceptional performance in capturing the variability in the system. Run 5, with a GRG of 0.577, was ranked 6^{th}, suggesting a relatively lesser capacity to capture the variability of the system. Run 6 obtained the second largest GRG of 0.705, and ranked 2^{nd} PCA ranking. Run 7 had a moderate GRG of 0.490, signifying a reasonable correlation among response factors. In addition, its PCA ranking was 7, indicating comparatively weaker performance in capturing system variability. Run 8 achieved a high GRG of 0.680, showing a strong correlation among the response factors. It also earned a favourable PCA ranking of 3, demonstrating good performance in capturing system variability. Run 9 obtained a moderately high GRG of 0.648, suggesting a relatively strong correlation among response factors. It secured a PCA ranking of 5, indicating a fair performance in capturing system variability. The GRGs and PCA rankings provided an evaluation of the performance of each parameter setting within the system, where Run 4 recorded the best performance in both the GRG and PCA rankings.

Table 3.8: Weighted Grey Relational grades and PCA ranking for the control setting of an Indoor Multi-Techno Aquaculture System

Run	**Factors/Levels**			**Water Quality Parameters**									**PCA Ranking**		
	Stocking density	**C/N ratio**	**Probiotic dosage**	**TAN (mg/L)**	**Nitrite (mg/L)**	**Phosphate (mg/L)**	**DO (mg/L)**	**EC (µS/cm)**	**TDS (mg/L)**	**Salinity (ppm)**	**pH**	**Temp (ºC)**	**Eigen-vector**	**GRG**	**Rank**
Run 1	100/m^3	10	7 ml/L	0.786	1.000	0.536	0.411	0.391	0.333	0.400	0.552	0.856	0.039	0.534	9
Run 2	100/m^3	15	14 ml/L	0.648	0.415	0.340	0.921	0.842	0.497	0.586	0.552	0.842	0.099	0.651	4
Run 3	100/m^3	20	21 ml/L	0.695	0.409	0.343	0.566	0.562	0.447	0.333	0.526	1.000	0.084	0.483	8
Run 4	200/m^3	10	14 ml/L	0.631	0.536	0.421	0.809	0.428	0.552	1.000	0.333	0.652	0.272	0.762	1^{α}
Run 5	200/m^3	15	21 ml/L	0.679	0.680	0.744	0.333	1.000	0.581	0.815	0.354	0.741	0.053	0.577	6
Run 6	200/m^3	20	7 ml/L	1.000	0.531	0.333	1.000	0.479	0.651	0.885	0.483	0.499	0.001	0.705	2^{Ψ}
Run 7	300/m^3	10	21 ml/L	0.664	0.435	0.522	0.341	0.543	1.000	0.789	0.494	0.615	0.151	0.490	7
Run 8	300/m^3	15	7 ml/L	0.515	0.333	1.000	0.925	0.448	0.408	0.803	1.000	0.333	0.068	0.680	3
Run 9	300/m^3	20	14 ml/L	0.333	0.469	0.661	0.866	0.333	0.597	0.832	0.433	0.563	0.232	0.648	5

*Note that $^{\alpha}$ and $^{\Psi}$ indicate the first two run with the best performance

3.3.4 Taguchi analysis of Grey relational grades

The GRGs (**Table 3.8**) calculated from the GRA and PCA were further subjected to the conventional Taguchi analysis with *'larger-the-better'* as the option of S/N ratio to establish the respective levels of each control factor that produced the optimum GRGs as shown in **Figure 3.6**.

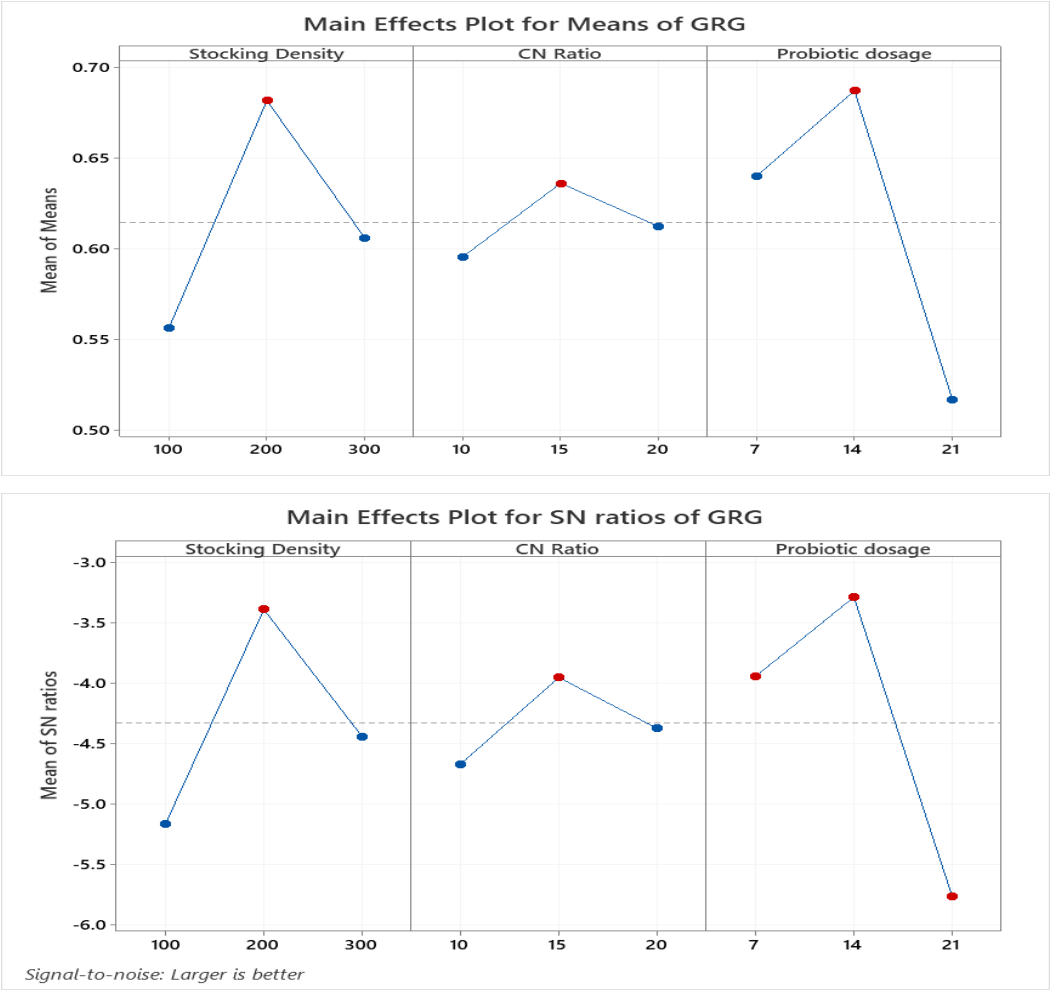


Figure 3.6: Means and S/N ratios of the GRGs of the response factors for the control setting of an Indoor Multi-Techno Aquaculture System

The response of the means of GRGs (**Table 3.9**) shows that the probiotic dosage ranked first at level 2 (GRG 0.686, 14 $ml.l^{-1}$); while stocking density ranked second at level 2 (GRG 0.6815, 200 $shrimp/m^3$) and C/N ratio third at level 2 (GRG 0.6359, C15N) respectively.

Table 3.9: Response of the means of Grey relational grades

Level	**Stocking density**	**C/N ratio**	**Probiotic dosage**
1	0.5562	0.5954	0.6398
2	0.6815	0.6359	0.6869
3	0.6068	0.6121	0.5168
Delta	0.1252	0.0405	0.1701
Rank	2	3	1

An analysis of variance of the GRG from the generalised linear model is presented in **Table 3.10**. Stocking density had an F-value of 5.17, and a p-value of 0.162. The F-value indicates that the variation due to stocking density was relatively high compared to the error, while the p-value (0.162) indicates that the effect of stocking density was not statistically significant ($p>0.05$). The C/N ratio also had an F-value of 0.54, and a p-value of 0.650, signifying that C/N ratio did not also have a statistically significant effect on the response variable. The probiotic dosage had an F-value of 10.03, and a p-value of 0.091. The F-value was comparatively higher, representing higher variation caused by the probiotic dosage. However, the probiotic dosage had the greatest impact on the variation in the response factors.

Table 3.10: Analysis of variance of the grey relational grades of response factors for the control setting of an Indoor Multi-Techno Aquaculture System

Source	df	Adj SS	Adj MS	F-value	P-value
Stocking density	2	0.023862	0.011931	5.17	0.162
C/N ratio	2	0.002486	0.001243	0.54	0.650
Probiotic dosage	2	0.046294	0.023147	10.03	0.091
Error	2	0.004615	0.002308		
Total	8	0.077257			

The findings from the optimisation protocol presented in terms of the GRGs (**Figure 3.6**) were transposed into the actual control factors and their respective levels. Based on the water quality conditions obtained from this experiment, 200 shrimp.m^{-3} was considered the optimum level of stocking density, while the C/N ratio and probiotic dosage were 15 and 14 ml/L, respectively.

3.4 Discussion

The high S/N ratio (>10.0) recorded in phosphate, electrical conductivity, total dissolved solids, salinity, DO, pH and temperature explains their low variability in from desirable levels with low which represents their robust against the control factors (stocking density, C/N ratio, or probiotic dosage). In this context, electrical conductivity, total dissolved solids were recorded to have the least variability in reference to the control factors. On the other hand, the low S/N ratio (<10.0) for TAN and nitrite suggests higher variability and sensitivity to control settings where their high fluctuations suggested instability and potential to constitute toxicity risks if not well managed in IMTAS. Phosphate levels in biofloc systems are managed through microbial processes that convert waste into beneficial proteins. Microbial communities in biofloc systems efficiently uptake phosphate, maintaining it within desirable ranges and contributing to a stable environment (Zuo et al., 2023). Electrical conductivity and TDS are often stable due to the zero-water exchange and efficient waste management

in biofloc systems. This stable condition help maintain consistent ionic levels, ensuring a high S/N ratio and optimum conditions for aquatic life (Podder et al., 2023). Biofloc systems plays a key role in maintaining stable salinity because there is often no ion exchange during water renewal. This stability contributes to the high S/N ratio in salinity levels (Manan et al., 2024). Through aeration and microbial activities in biofloc systems DO are usually maintained at consistent DO levels (Lal et al., 2024). These mechanisms ensure a high S/N ratio, providing a stable oxygen environment essential for fish health. pH levels in biofloc systems are maintained within safe ranges through microbial processes and minimal water exchange. Therefore, BFT does not significantly affect pH, contributing to its stability and high S/N ratio (Malpartida et al., 2023). Temperature is a critical factor in aquaculture, influencing metabolic rates and microbial activity. Biofloc systems maintain optimum temperatures through aeration to reduce variability and increasing the S/N ratio (Podder et al., 2023). The composition of dissolved ions in water significantly impacted EC and TDS. The presence of ions such as sodium, calcium, magnesium, and potassium in biofloc systems contributes to both EC and TDS by reducing variability in these parameters (Khan et al., 2025). Nitrite, as an intermediate in the nitrogen cycle, is a naturally occurring compound that can accumulate under certain conditions, posing toxicity risks to aquatic organisms. Studies have shown that sustained organic loading disturbances, particularly variations in food-to-biomass (F:M) and carbon-to-nitrogen (C:N) ratios, can lead to stable nitrite accumulation in bioreactors (Santillan et al., 2020). TAN loading rates in biofloc systems must be carefully managed to avoid toxicity thresholds. The variability and sensitivity of TAN and nitrite in biofloc systems pose significant challenges for system stability and toxicity management. Promoting bacterial diversity through appropriate system management can enhance the overall stability and efficiency of biofloc systems.

DO and salinity aligned closely on the positive side of the first component to indicate their influence on the system. Salinity levels have direct influence on the oxygen solubility in aquatic systems (Spelta et. al, 2021) which could have impacted DO levels in the system. TAN and EC showed moderate influence on both components with some variability. This is because high TAN and EC levels typically result from accumulation of waste, uneaten feed, and metabolic by-products, which release

nitrogenous compounds and dissolved ions biofloc system. The negative similar trend of nitrite and temperature indicates a disruption in the balance of beneficial heterotrophic and nitrifying bacteria in the biofloc system which may have impaired nitrogen conversion to increase nitrite levels. Phosphate and pH showed distinct variation from TDS and salinity to imply DO and salinity are key major drivers of IMTAS.

The main effects plots for the means and S/N ratios of GRG in IMTAS showed probiotic dosage had the highest influence on the GRG performance followed by stocking density while C/N ratio was the least. This indicates IMTAS means the nature of probiotic inoculation is very important in the success of biofloc culture. Aparna et al (2024) has established that the addition of probiotics can reduce total nitrogen concentration, ammonia levels, and other nitrogenous compounds, thereby maintaining optimal water quality for aquatic organisms. Also, Hassan et al (2024) reported that the inoculation of probiotics in biofloc systems has been consistently associated with improved growth performance in aquatic organisms. Stocking density on the hand is responsible for mitigating water quality issues by reducing the accumulation of harmful nitrogenous compounds. Stocking density also impacts the immune and physiological responses of aquatic organisms in biofloc systems. Higher stocking densities are associated with increased stress levels, as indicated by elevated cortisol concentrations (Nazarpour et al, 2023). C/N ratio is generally adjusted in accordance to feeding rate which is regulated by the stocking density. It is therefore noted that this control factor would be regulated by the ammonia level generated in the system. The conversion of ammonia in biofloc systems is primarily mediated by heterotrophic bacteria, which thrive in environments with an optimal C/N ratio. These bacteria assimilate ammonia and convert it into bacterial biomass, which is then consumed by the cultured organisms or removed from the system (Srivastava et al., 2024).

3.5 Conclusions

According to the findings in the experiments, the IMTAS would function best at a stocking density of 200 shrimp/m^3, C/N ratio of 15, and a probiotic dosage of 14 ml/L based on the water quality variables measured in this research. These optimum levels of the control factors were obtained from a conventional indoor biofloc culture system to guide the setting of IMTAS using the Taguchi-based GRA and PCA methods.

CHAPTER 4

DEVELOPMENT OF IMTAS AND EVALUATION OF ITS PERFORMANCE USING THE GROWTH, PHYSIOLOGY AND GENETIC RESPONSE OF *L. VANNAMEI*

4.1 Introduction

With the shift towards intensive shrimp culture, the effects of environmental conditions on the performance of the culture system have become more apparent. These environmental changes are evident in the physiological indices that govern the overall biological functions of shrimp. To develop effective management techniques for the cultivation of shrimp and other aquatic animals, it is essential to closely monitor and comprehend their physiological processes (Berry et al., 2019). Environmental changes can have a significant impact on the health and productivity of these animals, as they often interact in complex ways (Iber & Kasan, 2021; Kumar et al., 2022).

The biofloc system requires a series of conditions that trigger advanced physiological modifications in shrimp for improved performance. It is crucial to maintain an appropriate balance of nutrients within a biofloc system for optimal system performance. This equilibrium is attained by adjusting the carbon-to-nitrogen (C/N) ratio because an imbalance can result in the accumulation of toxic metabolites or

nutrient deficiencies, which can negatively affect microbial community and shrimp growth (Chu & Brown, 2021). An unbalanced C/N ratio increases the equilibrium of the microbial community, allowing for the proliferation of harmful bacteria and fungi that can harm shrimp. Biofloc systems promote disease prevention through the microbial community by offering a broad range of immunostimulatory effects (Kheti et al., 2017). Enhanced physiological activity and disease resistance have been demonstrated in biofloc systems by improving haematological characteristics, antioxidant capacity, and immune responses (Kim et al., 2021; Martn Ros et al., 2023). The dynamic nature of water quality and microbial composition in a biofloc system necessitates the continuous monitoring of physiological indices for sustainable shrimp cultivation.

The study of the microbiome encompasses the taxonomy and functional characterisation of microbial taxa as well as their roles in the cycling of nutrients, variations in water quality, and potential impacts on the growth conditions of cultured animals (Gutiérrez-Pérez et al., 2022). Advanced DNA sequencing techniques, such as metagenomic or 16SrRNA gene sequencing, have been used to identify and quantify the composition and diversity of microbial communities within biofloc. This information is critical for understanding and managing important biological processes, including nitrogen metabolism, carbon cycling dynamics, and the mechanisms that regulate disease resistance and growth (Ranallo et al., 2021). Transcriptomic analysis involves systematic RNA sequencing across the different developmental stages of organisms in various environments (Manan et al., 2022).

The information obtained from the quantified complete set of RNA transcripts provides insight into the active genes and pathways that explain the physiological responses of organisms, which is crucial for understanding their environmental conditions. Transcriptome analysis involves determining gene expression patterns associated with various factors, such as nutrient utilisation, immune system modulation, stress responses, and growth regulation within culture systems (Hassan et al., 2022; Said et al., 2022). By analysing transcriptomic data along with physiological parameters, such as growth rates, survival rates, and water quality conditions,

researchers can gain a better understanding of how organisms genetically respond to their external environments (Wu et al., 2023; Zhao et al., 2021). The utilization of transcriptomic analysis to evaluate the tissue of shrimp reared in biofloc systems has greatly enhanced understanding of gene expression patterns, microbial interactions, and environmental adaptations to the alterations that occur in aquaculture systems (Yun et al., 2022). This method has advanced the knowledge of the molecular mechanisms governing shrimp health, growth, and disease resistance. Transcriptomic studies have facilitated the optimisation of biofloc technology for sustainable shrimp production and effective environmental management in aquaculture.

4.2 Methodology

4.2.1 Setup of Indoor Multi-Techno Aquaculture System

The schematic diagram illustrating the components of IMTAS in experiment 2 is presented in **Figure 4.1**. IMTAS is composed of three fundamental components: production, flocculation, and phytoremediation. The production unit was made of culture tanks where the shrimp were raised while the flocculation had cyclone tanks with a fine filter to sieve the floc. Ultraviolet light was placed above the cyclone tanks for disinfection of potential contaminants from the production unit. The phytoremediation unit was grown with *Caulerpa lentilifera* to absorb the nutrients generated by the production unit. The treated water was then returned to the reservoir tank, where it was pumped back to the production unit.

The operation of IMTAS is automated by Internet of Things (IoT). IoT is a network of smart devices comprising sensors and enabling technologies for collecting, storing and sharing of data for efficient operations. Water circulation across the three units was controlled by an electric valves and sensors connected to a timer. The system

also regulated the continuous monitoring of water quality data as well as its storage in a virtual database. Feeding was carried out using autofeeders, which were managed by the IoT. This automation ensured that the floc and nutrient concentrations remained within acceptable levels for optimum system performance. The harvested floc, a by-product of the system, was recycled as aquafeeds while the seaweed absorbed nutrients in the culture system.

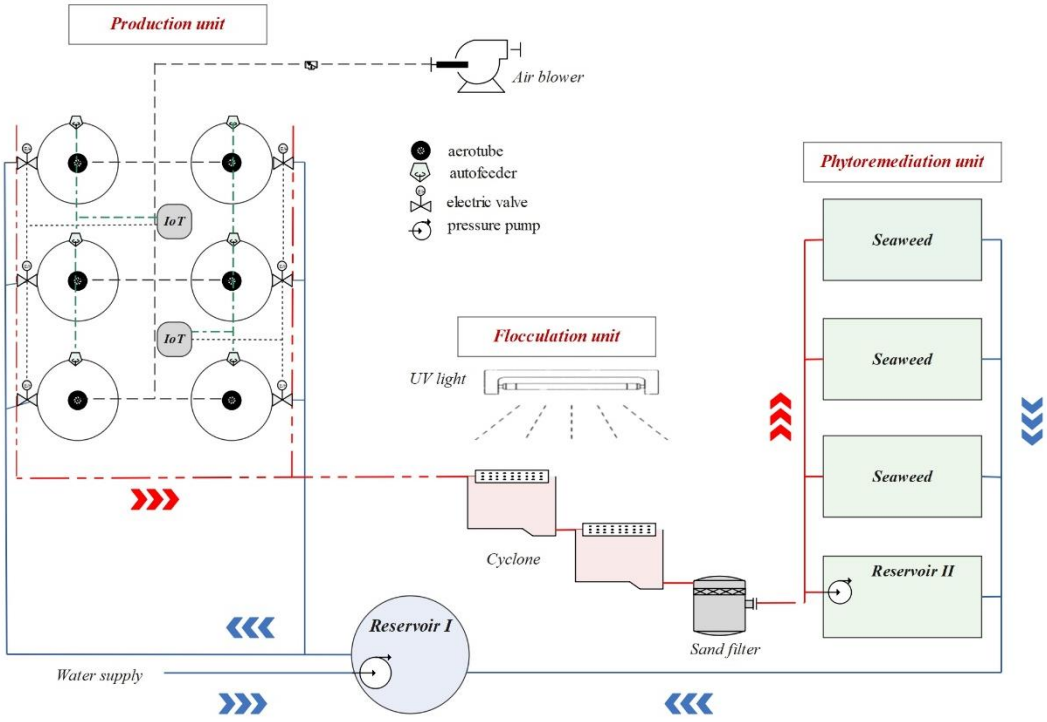


Figure 4.1: System layout of the Indoor Multi-Techno Aquaculture System (IMTAS)

The details of the system components are presented in **Figure 4.1.** In addition, **Figures 4.2** and **Figure 4.3** captured the production unit and showed the various components of the water recirculation system. **Figure 4.4** showed the IoT system while **Figure 4.5** focused on the phytoremediation tanks and mechanical filter.

Figure 4.2: The production unit showing water recirculation channels and control mechanisms of IMTAS.

Note that DP = drain pipe; SP = supply pipe; WS = water sampling pipe; MB = monitoring board; V_1 = electric valve (drain); V_2 = electric valve (supply); LP = level pipe; HT = holding tank

The supply of water to the production tanks was facilitated through supply pipes (SP) from the primary reservoir by opening the inlet electric valve (V_2) controlled by the Internet of Things (IoT) weekly. The water level in the holding tank (HT) was maintained at a height of 3 m by using a level pipe (LP). The electric valve (V_1) positioned at the outlet was activated by the Internet of Things (IoT) when the water level reached the designated level. The released water was then directed towards the flocculation unit via a discharge pipe (DP). The water sampling pipe (WS) automatically delivered water samples to the sample chambers at regular 8-h intervals for water quality assessment. The monitoring board (MB) regulated the automated processes.

Figure 4.3: The production unit showing the airpipe and autofeeder of IMTAS Note that MR= main reservoir; AP = airpipe; AF = autofeeder; AT = aerotube; SP = stand pipe; PP = pressure pump

The central air blower, positioned outdoor, supplied air to each holding tank via air-pipes. These air-pipes were connected to round aerotubes, constructed from ¼" PVC pipe and standing on short perforated standpipes. A pump was installed within the holding tanks to facilitate the uptake of water into the sampling chamber of the IoT for reading. The auto feeders were programmed to release pellets at 8-h intervals, with estimated quantities equivalent to 5% of the body weight of the cultured shrimp. The first ranking treatment in experiment 1, GRG 1 (defined by a stocking density of 200 shrimp/m^3, a C/N ratio of 10 and probiotic dosage of 14ml/L) and the second ranking treatment, GRG 2 (200 shrimp/m^3, C/N ratio 20 and probiotic dosage of 7 ml/L) were adopted in experiment 2 as described in the setup. The sampling chamber (SC) was

installed with sensors to measure all the water quality variables (**Figure 4.4**). The IOT controlled the automation of all operations in the system.

Figure 4.4: The production unit showing the water sampling chamber with sensors and the IoT of IMTAS

Note that SC = sampling chamber; IOT = Internet of Things

Figure 4.5: The phytoremediation unit showing the mechanical filters and *Caulerpa* tanks of IMTAS.

Note that CT = Caulerpa tank; SP = supply pipe; SF = sand filter; PR = Phytoremediation reservoir; RP = return pipe; CY = cyclone tank

Wastewater from the production unit was conveyed to the cyclone tanks (CY) in the flocculation unit, where the floc residue was collected. The resultant water was passed over a mechanical sand filter (SF) and left to settle in the phytoremediation reservoir (PR). After 36 h, the stored water was released through the supply pipe (SP) to the Caulerpa tanks (CT) containing seaweed grown on floating crates. Water from the Caulerpa tanks in the previous recirculation cycle was conducted to the main reservoir (MR) through the return pipe (RP) for onwards recirculation back to the production unit at timed intervals.

4.2.2 Water quality monitoring in the IMTAS

Water quality parameter measurements were automated using specialised sensors controlled by the IoT. The data were retrieved weekly from the IoT application database using an electronic device.

4.2.3 Studies on the physiology of *L. vannamei*

Haemolymph was collected to evaluate the impact of the various biofloc treatments on the biological functions of the cultured shrimp. Haemolymph is a blue-green fluid rich in haemocyanin that transports oxygen, nutrients and hormones as well as play the role of immune defence in crustaceans. The measurement of immune and antioxidant parameters from haemolymph provided information on the health, growth and survival of shrimp in the various biofloc conditions.

4.2.3.1 Haemolymph collection

A volume of 200 µL of haemolymph was extracted from each shrimp using a sterile syringe (25-gauge needle) containing an equal volume of anticoagulant solution. The anticoagulant solution consisted of 30 mM trisodium citrate, 0.34 M sodium chloride, and 10 mM Ethylenediaminetetraacetic Acid Sodium salt (EDTA-Na), adjusted to a pH of 7.55 and an osmolality of 780 mosM kg^{-1} using 0.115 M glucose. The haemolymph samples from each treatment were pooled and mixed gently in a sterile Eppendorf tube immediately after extraction. Subsequently, a small portion of the sample was reserved to quantify the haemocyte count, while the remaining portion was centrifuged using an Eppendorf centrifuge (5430 R) at 800 ×g for 10 min at 4 °C. The resulting supernatant fluid was then transferred into 2.0 mL Eppendorf tubes as plasma samples and stored at -80 °C for analysis of additional immune and antioxidant parameters. Concurrently, the hepatopancreas of the sampled shrimp was

removed, washed with cold normal saline (0.8%, w/v), patted dry, and the pancreas of ten shrimp was pooled, ground, weighed, and then placed in 2.0 mL Eppendorf tubes for storage at -80 °C. Prior to analysis, the samples were thawed at 4 °C and then homogenised using an electric homogeniser at 800 rpm in 9 volumes of chilled buffer, composed of 50 mM Tris-HCl, 1 mM EDTA-Na_2, and 0.25 mM sucrose at pH 8.2. The resulting crude homogenates were centrifuged at 3000 ×g for 10 min at 4 °C, and the resulting supernatants were collected for immediate analysis of antioxidant parameters.

4.2.3.2 Measurement of physiological indices

The hepatosomatic index (HSI) was calculated as the ratio of the weight of hepatopancreas to shrimp body weight. The total haemocyte count (THC) was determined using a Neubauer haemocytometer, and haemocytes were counted by observing an anticoagulant haemolymph drop under an Olympus light microscope. The count was expressed as cells mL^{-1} haemolymph. Haemocyanin concentration was determined from 10 µL of haemolymph diluted in 990 µL of distilled water in a quartz cuvette by measuring the absorbance at 350 nm using a spectrophotometer (Bio-Rad) that was manually calibrated with distilled water. To determine the haemolymph glucose concentration, plasma was extracted from the haemolymph that had been previously diluted in an isotonic solution (SICEDTA at 2 - 8^{o}C) with a complex to avoid coagulation of the haemolymph (Vargas-Albores et al., 2019), considering a haemolymph:anticoagulant ratio of 1:2. The haemolymph diluted in the anticoagulant was centrifuged at 2500 rpm for 3 min at 4 °C, and the supernatant was collected in Eppendorf tubes.

Glucose was determined in plasma aliquots of 20 µL with 200 µL of the reactive solution (Kit Bayer- Sera-Pak Plus B01 4509-01; Hall and van Ham, 1998) which were placed in microplates and read in an ELISA lector (Bio-Rad model 550) at 540 nm. SIC-EDTA was used as the blank. The concentration of the metabolites

was determined using a calibration curve, wherein the standard substrate acted as the reagent in the kit.

Glycogen in the hepatopancreas was extracted in the presence of sulfuric acid and phenol, as described by Dubois et al., (1956). First, the hepatopancreas was homogenised in 5% trichloroacetic acid for 2 min at 3340 g. The supernatant was quantified and 200 µL was transferred to a tube and mixed with 5 volumes of 95% ethanol. The tubes were then placed in an oven at 37 - 40°C for 3 h. After precipitation, the tubes were centrifuged at 7000 × g for 15 min using an Eppendorf centrifuge (5430 R). The glycogen pellets were dissolved by adding 0.5 µl of boiling water, followed by the addition of 1 µL of concentrated sulfuric acid and phenol (5%). The tube contents were then transferred to microplates in duplicate and the absorbance was read at 490 nm using a Bio-Rad 550 microplate reader. An aliquot of haemolymph was further diluted 1:25 with SSS before the measurement of total protein using a Pierce BCA Protein Assay Kit (Thermo Scientific, 23225). The haemolymph was freeze-dried and reconstituted with ultrapure water to match the original sample volume for each individual. Subsequently, the samples were analysed using lactate (Randox LC2389) and triglyceride (Randox TR1697) assays adapted to a microplate format.

Phenoloxidase activity was determined spectrophotometrically by measuring the formation of dopachrome from L-dihydroxyphenylalanine (L-DOPA) as described by Leonard et al., (1985). A cell suspension containing 100 cells/mL was incubated with 50 µL laminarin (1 mg/ml in cacodylate buffer) or cacodylate buffer alone for one hour at 20°C. Subsequently, 50 µL of L-DOPA (3 mg/ml in cacodylate buffer) was added, and the reaction was allowed to proceed for ten minutes at 20°C. After the reaction, 800 µL of cacodylate buffer was added and the absorbance at 490 nm was measured. Phenoloxidase activity was expressed as the change in spectrophotometric absorbance at 490 nm per minute per mg of protein.

Superoxide dismutase (SOD) was assayed according to the method described by Misra & Fridovich, (1972), which involves the oxidation of epinephrine-adrenochrome transition by the enzyme, and the absorbance was measured at 480 nm for 3 min. The activity of one unit of SOD was expressed as the amount of protein required for 50% inhibition of epinephrine autooxidation.

4.2.4 Studies on the growth performance of *L. vannamei*

The weight of the shrimp in each treatment replicate tank was measured to a uniform weight not exceeding <0.01 g after counting. The mean weight of the shrimp at stocking was 0.37 g in all treatments and replicates. The shrimp were fed for 12 weeks (84 days) during which time, a representative sample of 10% was taken to monitor progressive growth, while the entire harvest was performed at the end of the experiment. Survival rate was calculated at the end of each culture period as a proportion of live shrimp as a proportion of live shrimp to the initial stock using the relationship in **Equation 4.6**. At the end of the experiment, samples from each tank were counted and weighed alongside their respective body length and weight to calculate the following growth indices:

$$WG = \left(\frac{MFW - MIW}{MIW}\right) X100 \quad \textbf{(Equation 4.1)}$$

$$RWG = \left(\frac{MWG}{MFW}\right) X\ 100 \quad \textbf{(Equation 4.2)}$$

$$SGR = \left(\frac{Ln\ (MFW) - Ln\ (MIW)}{Culture\ period\ (84\ days)}\right) X\ 100 \quad \textbf{(Equation 4.3)}$$

$$FCR = \frac{total\ feed\ intake}{total\ weight\ gain} \quad \textbf{(Equation 4.4)}$$

$$Condition\ factor, CF = \left(\frac{final\ wt}{l^3}\right) X\ 100 \quad \textbf{(Equation 4.5)}$$

$$Survival\ rate = \left(\frac{final\ stock}{initial\ stock}\right) X\ 100 \quad \textbf{(Equation 4.6)}$$

4.2.5 Studies on the gut microbiome of *L. vannamei*

Ten gut samples were collected from each treatment group for sequencing analysis at the time of harvest. To prepare the samples, the body surface of the shrimp was wiped with 70% ethanol and the mid gut was removed using sterile forceps. The midgut was placed in a 2 ml sterile lyophilization tube and stored in liquid nitrogen before being transferred to a refrigerator at -80°C for analysis. Genomic DNA was extracted from the gut samples using PowerFecal® DNA isolation kits (QIAGEN Sciences, Germantown, MD, USA) according to the manufacturer's instructions. The extracted genomic DNA was checked for quality and purity using 1% agarose gel electrophoresis and a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The V3-V4 region of the 16SrRNA gene from bacteria was amplified using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using an ABI GeneAmp® 9700 PCR thermocycler (ABI, Foster City, CA, USA). The PCR product was subsequently extracted from a 2% agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. The purified product was quantified using a Quantus fluorometer (Promega, Madison, WI, USA). The purified amplicons were combined in equal amounts and then sequenced in paired-end mode on either the Illumina MiSeq PE300 platform or the NovaSeq PE250 platform (Illumina, San Diego, CA, USA), using standard protocols provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Sequencing libraries were constructed using the Majorbio Cloud Platform (www.majorbio.com), which facilitates high-throughput sequencing (Shanghai Majorbio Bio-Pharm Technology Co. Ltd.).

4.2.6 Studies on the tissue transcriptome of *L. vannamei*

4.2.6.1 RNA extraction and quality control

RNA extraction was performed following the protocol for next-generation sequencing, as detailed in Masoodi et al., (2020). Tissue samples of *L. vannamei* were isolated under sterile conditions and stored at -80 °C. Each of the three treatments had three replicates, with nine treatments in all. Subsequently, the tissues were thawed, and 50-100 mg of the samples were homogenised, after which RNA lysis buffer was carefully added to the samples at 4°C under aseptic conditions. This was followed by centrifugation to separate the RNA from other cellular debris and chemical traces. To obtain high-quality and high-purity RNA, DNase treatment was added to remove DNA from the sample and incubated for 5-8 minutes to disrupt the nucleoprotein complex. RNA was dissolved in RNase-free water and the quality and presence of RNA were determined using conventional agarose gel electrophoresis at a concentration of 0.8 - 1%. The 260/280 spectrophotometric ratio was used to assess RNA purity, which should be between 1.9 and 2.1. Additionally, a fluorochrome that binds to single-stranded RNA was used to quantify the RNA. Finally, RNA integrity analysis was performed to evaluate the stability of RNA, which was measured using a unit called RIN.

4.2.6.2 Library construction

Messenger RNA was purified from total RNA using magnetic beads coated with poly-T oligo. The RNA was then fragmented and first-strand cDNA was synthesised using random hexamer primers. Second-strand cDNA synthesis was carried out using dUTP for a directional library and dTTP for a non-directional library. The library was then checked using Qubit and real-time PCR for quantification and a bioanalyzer for size distribution detection. The quantified libraries were pooled and sequenced on an Illumina platform based on the effective library concentration and

amount of data. The original image data file from the high-throughput sequencing platforms was transformed into sequenced reads (Raw Data/Raw Reads) using CASAVA base recognition (Base Calling). Raw data were stored in FASTQ(fq) format files, which included the sequences of the reads and the corresponding base quality.

4.2.6.3 Examination of sequencing error rate

The error rate for each base was transformed to a Phred using the relationship

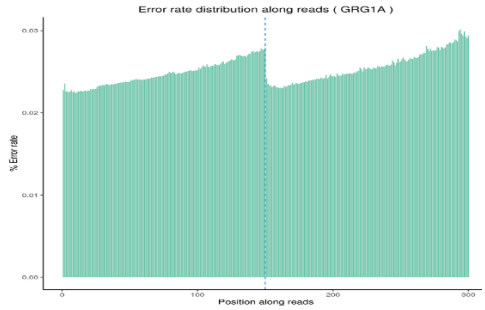
$$Qphred = -10log10(e)$$ (**Equation 4.7**)

Where,

"e" represents sequencing error rate,

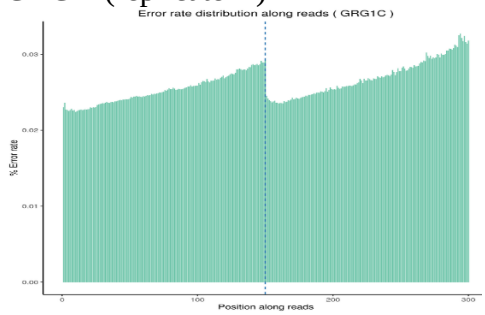
"Qphred" represents base quality values of Illumina platforms.

The first six nucleotides in the cDNA synthesis process have a relatively high error rate, which is attributable to the incomplete binding of random hexamers used as primers. This error rate should ideally be less than 1% for a single base. In this research, all the samples considered for sequencing had an error rate of <0.04% (**Figure 4.6**).

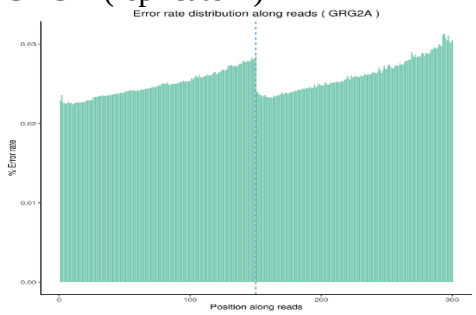


GRG 1 (replicate A)

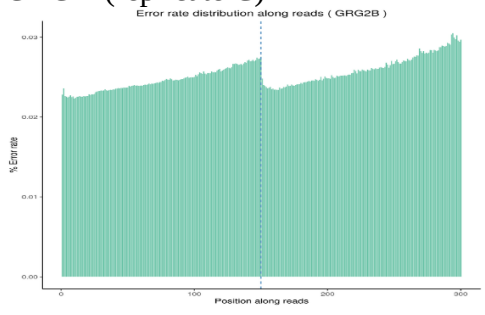
GRG 1 (replicate B)



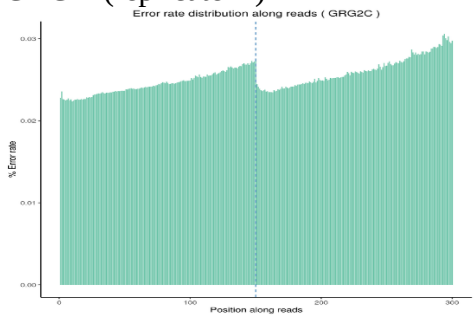
GRG 1 (replicate C)



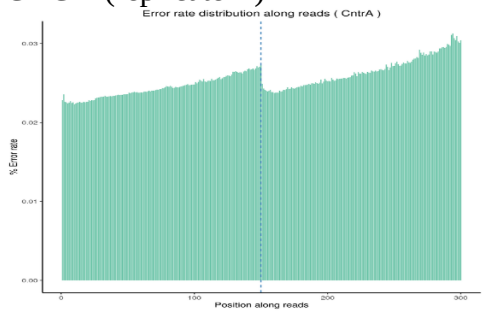
GRG 2 (replicate A)



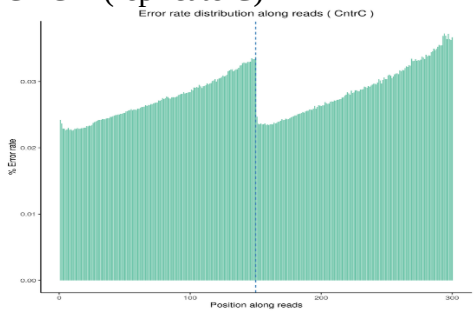
GRG 2 (replicate B)



GRG 2 (replicate C)



Control (replicate A)

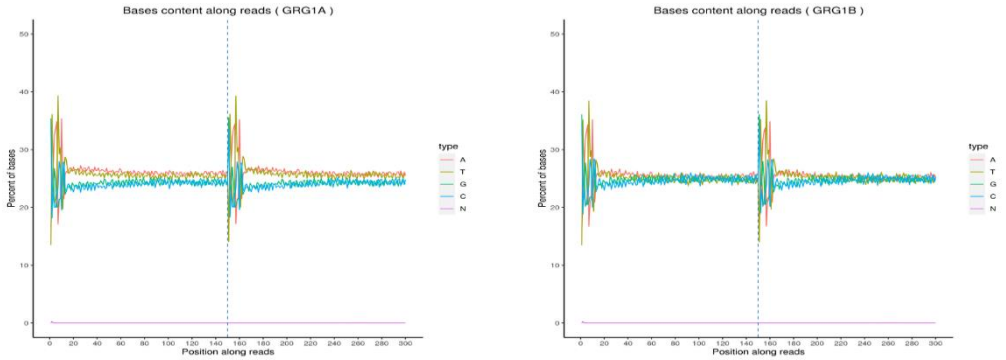


Control (replicate C)

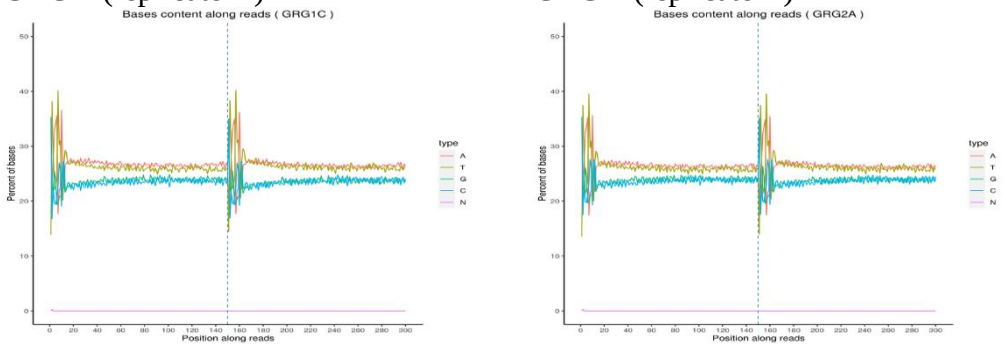
Figure 4.6: Sequencing data error rate distribution of the samples. Note that Control (replicate B) did not pass quality control test

4.2.6.4 GC content distribution

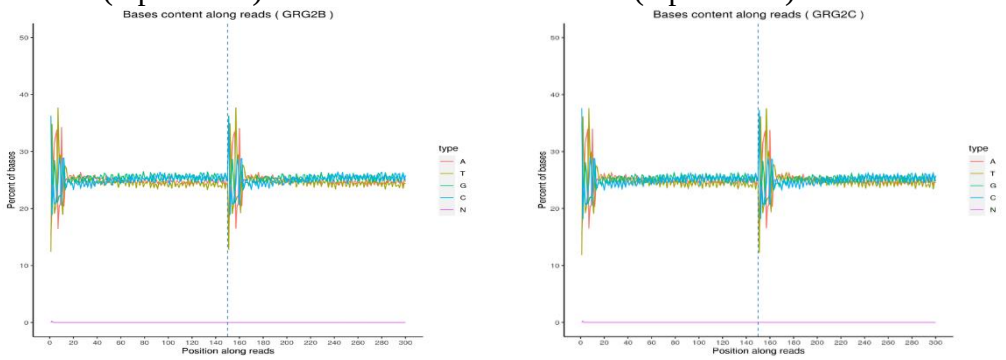
The distribution of GC content shows potential AT/GC separation which may impact subsequent gene expression quantification. This is based on the random fragmentation and biological law of G/C-A/T content, which states that G and C, as well as A and T, should be equal and the content should remain stable throughout the entire sequencing process for non-stranded libraries (Parkhomchuk et al., 2009). The graph illustrating the distribution of GC content is presented in **Figure 4.7**. The x-axis displays the base position within a read, while the y-axis represents the percentage of each base, with each base distinguished by a unique colour. The left side of the vertical dashed line corresponds to the GC-content of read 1, while the right side represents the GC-content of read 2.



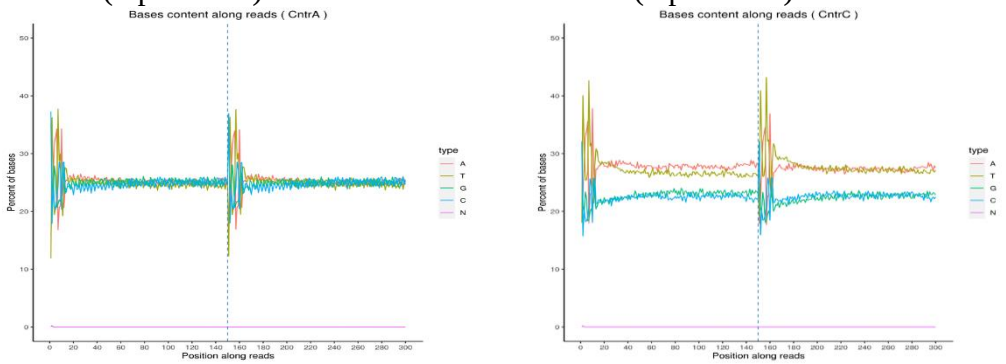
GRG 1 (replicate A) GRG 1 (replicate B)



GRG 1 (replicate C) GRG 2 (replicate A)



GRG 2 (replicate B) GRG 2 (replicate C)



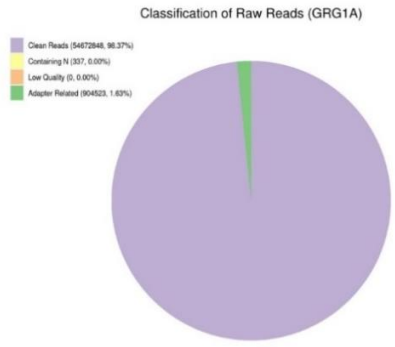
Control (replicate A) Control (replicate C)

Figure 4.7: GC content distribution of the samples.

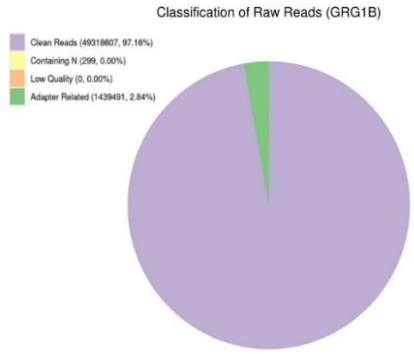
Note that Control (replicate B) did not pass quality control test

4.2.6.5 Sequencing data filtering

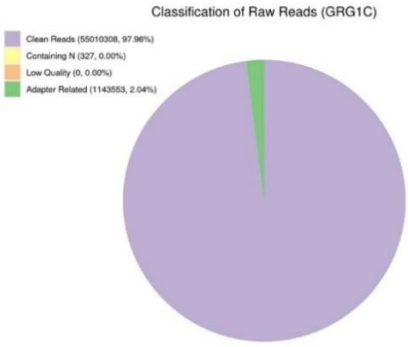
The clean reads obtained from the filtering process were >97% for all the samples, as shown in **Figure 4.8**, after removing reads with adapter contamination and those with more than 10% uncertain nucleotides (N > 10%). Raw reads often contain low-quality reads and reads with adapters, which can negatively affect downstream analysis.



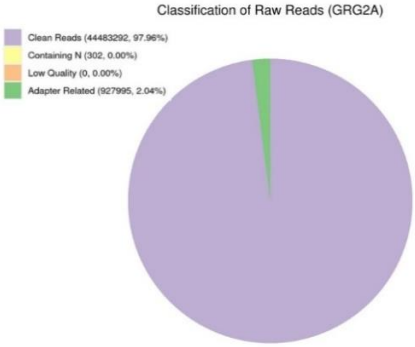
GRG 1 (replicate A)



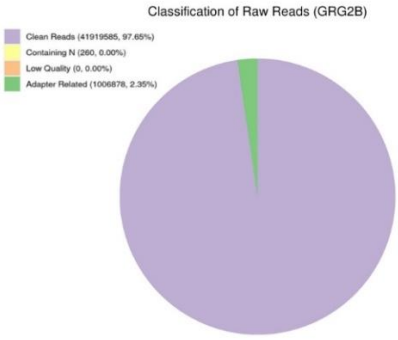
GRG 1 (replicate B)



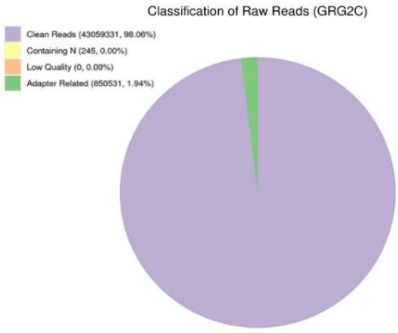
GRG 1 (replicate C)



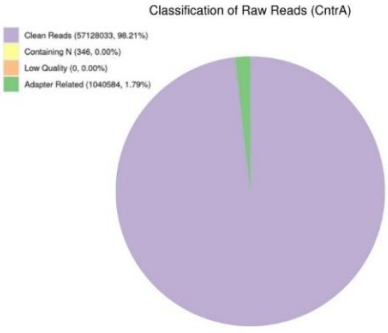
GRG 2 (replicate A)



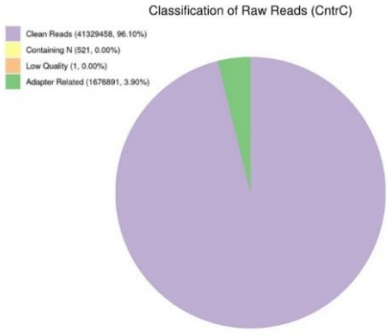
GRG 2 (replicate B)



GRG 2 (replicate C)



Control (replicate A)



Control (replicate C)

Figure 4.8: Sample Sequencing Data Filtering

Note that Control (replicate B) did not pass quality control test

4.2.6.6 Statistics of sequencing quality

The summary of the sequencing data as presented in **Table 4.1** shows the raw bases, which were calculated by multiplying the number of raw reads and the sequence length, with values expressed in G units. The clean reads were obtained by filtering the raw data and calculating the sequence length by multiplying the clean reads by 150 bp and storing the result in G unit. The sequencing error rate was calculated as the average of the number of bases with a Q Phred value > 20, divided by the total number of bases, and represented as Q20 and Q30 based on Q Phred values > 20 and 30, respectively. The GC percentage was calculated as the percentage of G and C base numbers out of the total number of bases.

Raw sequencing reads were taken through quality control using FastQC and MultiQC to evaluate Phred scores, adapter contamination, and sequence duplication. Low-quality bases and adapter sequences were then removed using Cutadapt to retain high-quality reads. SortMeRNA was used to filter out ribosomal RNA while PRINSEQ and Picard were used to remove low-complexity sequences and PCR duplicates. The cleaned reads were then aligned to the reference genome using HISAT2 to aid precise mapping. Unmapped and multi-mapped reads were excluded based on quality thresholds set by Samtools to retain only high-confidence sequences. Gene expression quantification was thereafter performed using HTSeq with low-abundance transcripts filtered out to enhance statistical significance. Finally, normalization was performed using using DESeq2 to correct sequencing depth variations and ensure data comparability. Data obtained in this process were transformed raw sequencing dataset suitable for differential expression analysis and functional analysis.

Table 4.1: Sample sampling data quality summary

Sample	Library	Raw reads	Raw bases	Clean reads	Clean bases	Error rate	Q20	Q30	GC pct
GRG1A	DRRA240001340-1a	111155416	16.67G	109345696	16.4G	0.03	97.85	94.16	48.28
GRG1B	DRRA240001341-1a	101516794	15.23G	98637214	14.8G	0.03	97.75	93.96	49.44
GRG1C	DRRA240001342-1a	112308376	16.85G	110020616	16.5G	0.03	97.45	93.37	47.13
GRG2A	DRRA240001343-1a	90823178	13.62G	88966584	13.34G	0.03	97.71	93.83	47.49
GRG2B	DRRA240001344-1a	85853446	12.88G	83839170	12.58G	0.03	97.79	94.07	50.50
GRG2C	DRRA240001345-1a	87820214	13.17G	86118662	12.92G	0.03	97.79	94.07	50.29
ControlA	DRRA240001346-1a	116337926	17.45G	114256066	17.14G	0.03	97.74	93.91	49.82
ControlC	DRRA240001348-1a	86013742	12.9G	82658916	12.4G	0.03	96.56	91.97	45.10

The alignment of the data to the reference was carried out using HISAT2 by employing multiple alignment strategies and integrating these small indices (local indices), HISAT2 was able to effectively align RNA-seq reads, particularly those crossing multiple exons. Mapping of the data was performed after aligning it into exons, introns, or intergenic regions, and was saved in BAM format for visualisation using Integrative Genomics Viewer (IGV) software.

4.2.6.7 Novel gene prediction

The integration of information from all the samples was achieved by combining them and inputting them into the Cufflinks assembler. The assembled transfragments were subsequently compared with reference transcripts to assess their novelty in terms of differences from existing data. This process was used to detect novel genes, identify novel exons of established genes, and refine the start and end information of known transcripts. The results of this mapping procedure were stored in Gene Transfer Format (GTF) files. The assembled genes were annotated using the Kyoto Encyclopaedia of Genes and Genomes (KEGG).

4.2.7.8 Gene expression level analysis

Gene expression level is typically determined by the number of mapped reads, which directly reflects the abundance of transcripts. In RNA-seq experiments, the expression level of a gene is estimated by counting the number of sequencing reads aligned to the genome or exon (Goldstein et al., 2016). The relationship between read counts and gene expression levels is proportional to the gene length and sequencing depth, as demonstrated by (Liao et al., 2014). To estimate gene expression levels, Fragments Per Kilobase of transcript sequence per million base pairs sequenced (FPKM) were utilised. The distribution of gene expression levels and FPKM among various samples is presented in **Figure 4.9**.

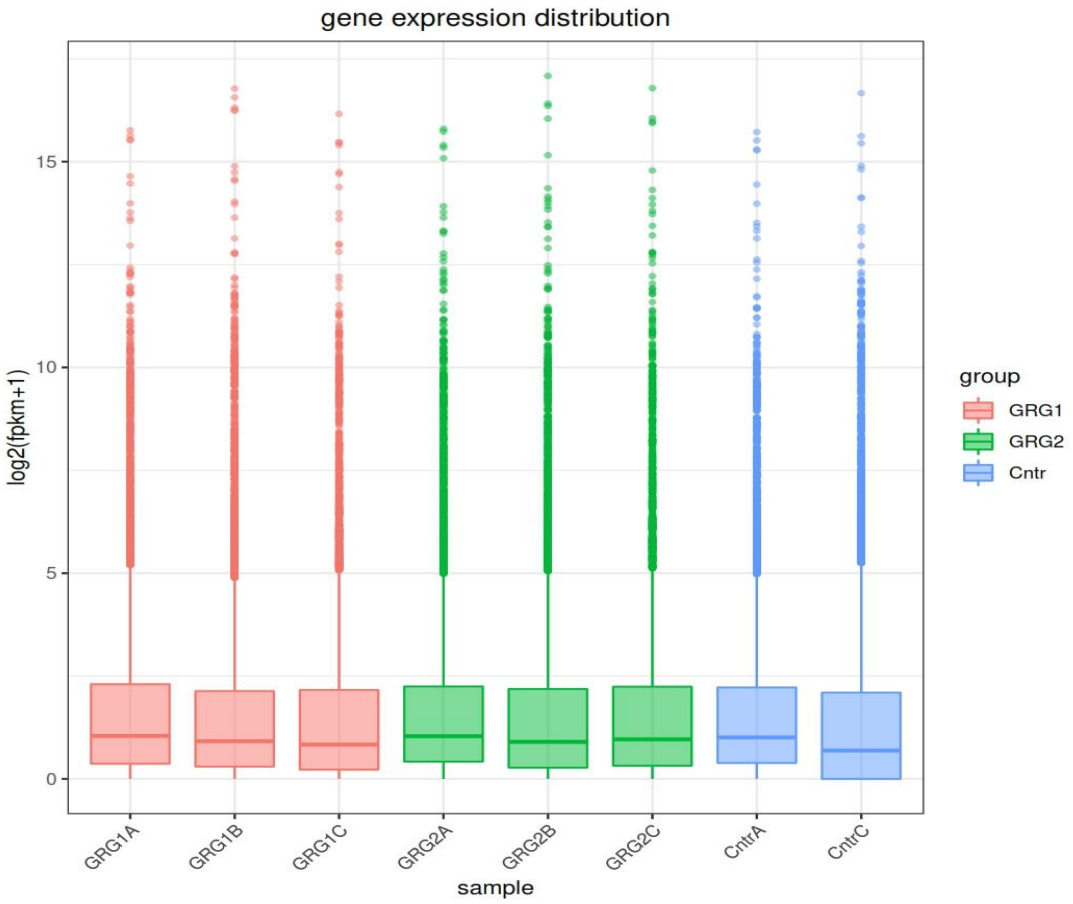


Figure 4.9: Boxplot of gene expression distribution

The correlations between the samples (**Figure 4.10**) was performed to verify sample reliability and for estimating differential gene expression. All samples showed strong positive correlations, with GRG 1 and control having the lowest correlation coefficient of 0.733 (R^2 = 0.75%). The highest correlation coefficient was observed between GRG2B and GRG 2C, at 0.926 (R^2 = 0.95%). A higher correlation coefficient indicates a closer expression pattern.

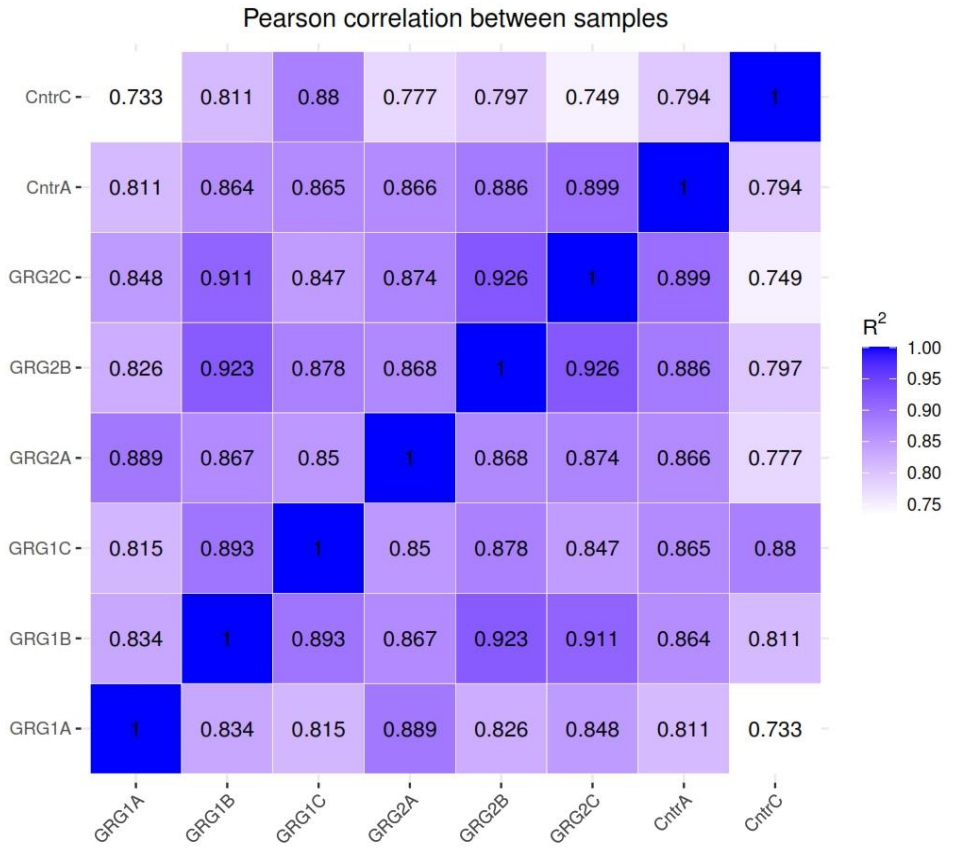


Figure 4.10: Correlation matrix of the gene expression levels between samples

4.2.6.9 Principal component analysis of treatment groups

Principal component analysis (PCA) was applied to evaluate the intergroup variations and intragroup sample replication. PCA employs the linear algebra calculation technique to lessen the dimension and obtain principal components from numerous gene variables, as shown in **Figure 4.11**. This analysis was carried out on the gene expression values (FPKM) of all samples.

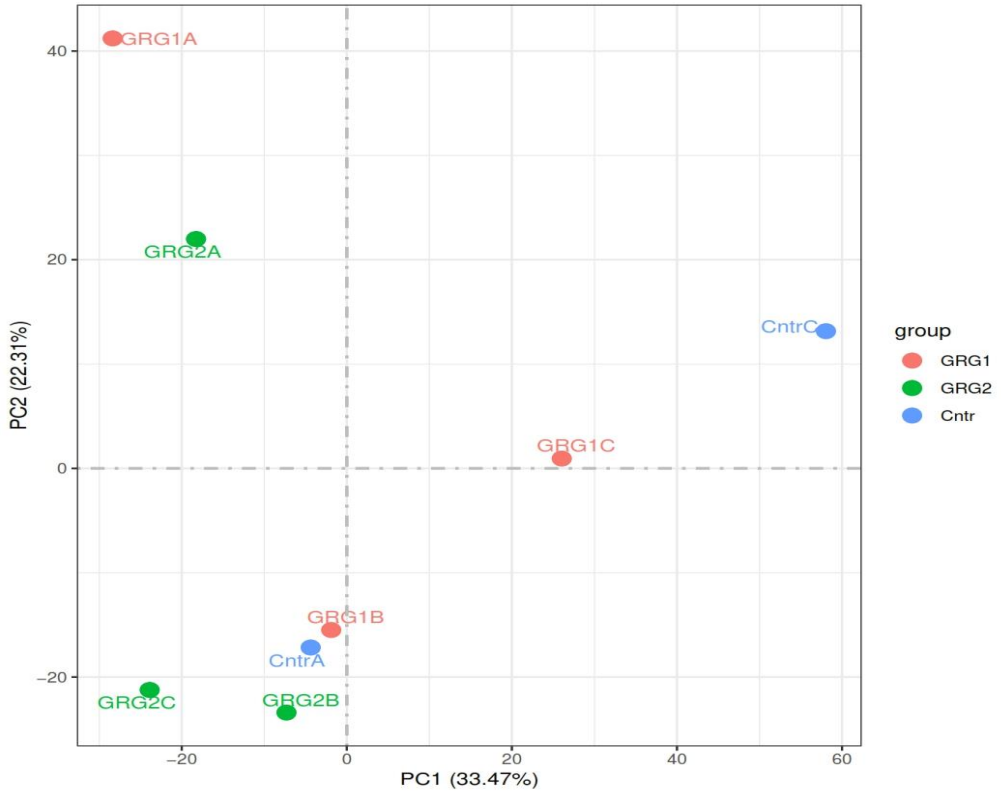


Figure 4.11: Principal component analysis of the treatments showing the intergroup differences

4.2.6.10 Co-expression Venn diagram

The co-expression Venn diagram in **Figure 4.12** shows the number of genes that are specifically expressed within each group or sample. The overlapping regions illustrates the number of genes that were co-expressed in two or more groups or samples. GRG 1 and GRG 2 shared more genes in common than in the control, with 530 overlapping genes. Specifically, GRG 1 had 250 genes in common with control, whereas GRG 2 had 271 genes. Furthermore, the three treatments shared a significant number of genes, totalling 4393.

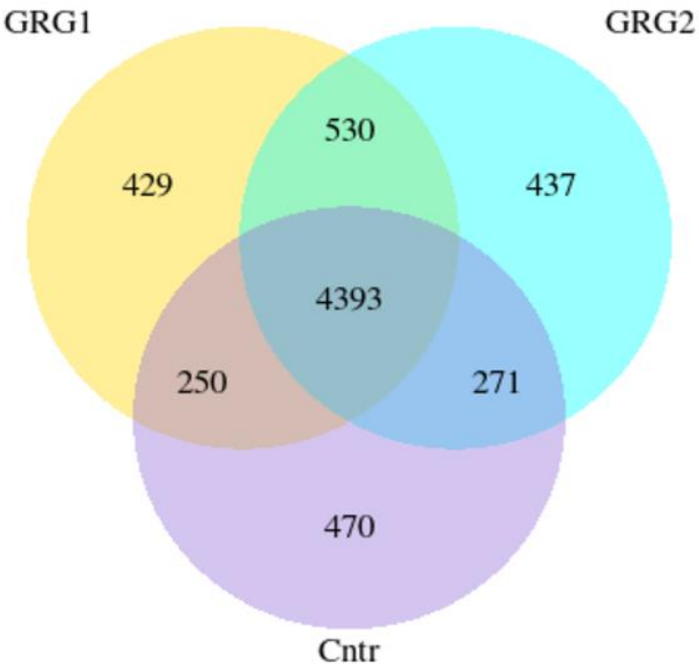


Figure 4.12: Co-expression Venn diagram of genes

4.3 Results

4.3.1 Water quality conditions of IMTAS

Table 4.2 presents the results of the water quality assessment of the Indoor Multi-Techno Aquaculture System. The research examined the water quality parameters in two separate experiments (Experiment 1 and Experiment 2), specifically focusing on contrasting the optimum treatment from Experiment 1 with three treatments from Experiment 2 (GRG 1, GRG 2, and the Control). The optimal conditions established during the system optimisation in Experiment 1 were derived from the Taguchi-based Grey Relational Analysis (GRA) using the control factors (stocking density, C/N ratio, and probiotic dosage) based on Taguchi L9 orthogonal design detailed in Chapter 3. The four treatments were considered equivalent for comparison.

The levels of total ammonia-nitrogen were considerably lower in the Optimum of Experiment 1 (0.98±0.03 mg/L) compared to Control (1.95±0.16 mg/L). GRG 1 (0.90±0.01 mg/L) and GRG 2 (0.87±0.03 mg/L) were, however, lower than the concentration in the optimum of Experiment 1 ($p < 0.01$). Nitrite levels also showed significant differences, with Control (0.57±0.04 mg/L) being higher than GRG 1 (0.42±0.01 mg/L) and GRG 2 (0.44±0.00 mg/L) treatments as well as the optimum in experiment 1 ($p = 0.03$). Dissolved oxygen level was highest in GRG 1 (6.92±0.08 mg/L) and the optimum in Experiment 1 (6.11±0.24 mg/L), while GRG 2 and Control recorded 6.01±0.24 mg/L and 5.06±0.07 mg/L, respectively ($p < 0.01$). The differences in electrical conductivity were statistically significant, with GRG 1 (44926±784 $\mu S.cm^{-1}$) displaying lower conductivity than both the optimum (49234±424 $\mu S.cm^{-1}$) and Control (49342±168 $\mu S.cm^{-1}$) treatment groups ($p < 0.01$).

Total dissolved solids showed significant differences among treatments. The optimum (29290±377 mg/L) and Control (2994±200 mg/L) treatments had higher levels compared to GRG 1 (27411±141 mg/L) and GRG 2 (27821±280 mg/L) treatments ($p < 0.01$). Salinity levels also varied significantly, with Optimum (32.89±1.98 ppm) and Control (33.31±0.10 ppm) treatments having higher salinity than GRG 1 (28.06±0.45 ppm) and GRG 2 (29.15±0.01 ppm) treatments ($p = 0.04$). pH values showed significant differences, with optimum (7.67±0.03) treatments consistently displaying higher pH than the Control (6.40±0.17) and GRG treatments (6.96±0.05 and 7.24±0.03) ($p < 0.01$). Although temperature was highest in the optimum treatment in Experiment 1 (29.00±0.11 ^{o}C), there was no statistically significant difference among the treatments.

Table 4.2: Water Quality conditions during Taguchi optimisation and in IMTAS

Water quality variables	**Experiment 1**		**Experiment 2**			**p-value**
	Optimum		**Control**	**GRG 1**	**GRG 2**	
Total Ammonia-Nitrogen (mg/L)	0.98 ± 0.03^{b}		1.95 ± 0.16^{a}	0.90 ± 0.01^{b}	0.87 ± 0.03^{b}	<0.01
Nitrite (mg/L)	0.46 ± 0.00^{b}		0.57 ± 0.04^{a}	0.42 ± 0.01^{ab}	0.44 ± 0.00^{ab}	0.03
Phosphate (mg/L)	0.13 ± 0.00^{b}		0.27 ± 0.03^{a}	0.12 ± 0.00^{b}	0.13 ± 0.00^{b}	0.01
Dissolved oxygen (mg/L)	6.11 ± 0.24^{b}		5.06 ± 0.07^{c}	6.92 ± 0.08^{a}	6.01 ± 0.24^{b}	<0.01
Electrical conductivity ($\mu S.cm^{-1}$)	49234 ± 424^{ab}		49342 ± 168^{ab}	44926 ± 784^{c}	46206 ± 535^{bc}	<0.01
Total dissolved solids (mg/L)	29290 ± 377^{a}		2994 ± 200^{a}	27411 ± 141^{b}	27821 ± 280^{b}	<0.01
Salinity (ppm)	32.89 ± 1.98^{ab}		33.31 ± 0.10^{a}	28.06 ± 0.45^{b}	29.15 ± 0.01^{b}	0.04
pH	7.67 ± 0.03^{a}		6.40 ± 0.17^{c}	6.96 ± 0.05^{b}	$7.24\pm0.03b^{ab}$	<0.01
Temperature (^{o}C)	29.00±0.11		27.06±0.08	27.80±0.66	28.62±0.75	0.16^{ns}

**Note that means on the same row with different superscript are statistically significant ($p<0.05$); ns = not significant*

4.3.2 Evaluation of the physiological responses of *L. vannamei* raised in IMTAS

The performance of IMTAS was assessed by determining the optimal physiological conditions for cultured shrimp in both the traditional biofloc system (used for control parameter setting and system optimisation, Experiment 1) and those in IMTAS (Experiment 2). Optimum physiological indices were obtained using a Taguchi-based regression approach during the parameter settings.

4.3.2.1 Taguchi single-response optimisation of the physiological indices of *L. vannamei* raised in IMTAS

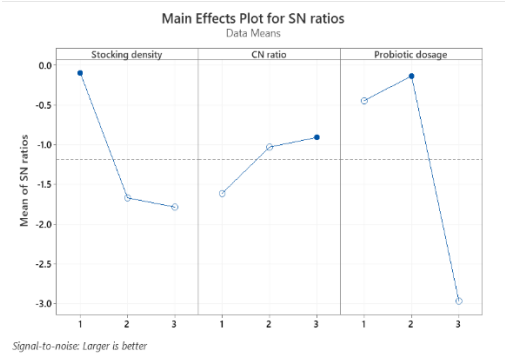
The results of the Taguchi analysis of the physiological responses of the cultured shrimp are presented in **Table 4.3**. This study showed different optimal levels of the control factors that affected the physiological indices. The optimal value of 1.00 for protein was observed at Level 1 of the stocking density. The C/N ratio was optimal at Level 3 with a value of 0.91, whereas the probiotic dosage was optimal at Level 3 with a value of 0.99. Probiotic dosage was found to be the most significant factor affecting the protein content. Glycogen levels were found to be optimum at Level 2 for stocking density, with a value of 1.41, a C/N ratio of 1.36 at Level 3, and a probiotic dosage of 1.39 Level 3. Optimal glucose concentration was achieved at stocking density level 1 (0.72), C/N ratio level 1 (0.64), and probiotic dosage level 3 (0.64). THC levels were optimum at stocking density level 1 (23.00), C/N ratio level 3 (21.00), and probiotic dosage level 3 (20.67). The hepatosomatic index (HSI) attained optimum values at stocking density level 1 (3.34), C/N ratio level 2 (3.00), and probiotic dosage level 3 (3.03). Prophenoloxidase activity (ProPO) exhibited optimal values at stocking density level 1 (64.33), C/N ratio level 3 (52.10), and probiotic dosage level 3 (51.33). Superoxide dismutase activity (SOD) was highest for stocking density at level 1 (2.36), C/N ratio at level 3 (2.20), and probiotic dosage at level 2 (2.05). Haemocyanin levels were maximized at stocking density level 1 (3.62), C/N ratio level 3 (3.23), and probiotic dosage level 3 (3.11).

Table 4.3: Optimum values of physiological indices of *L. vannamei* cultured in IMTAS

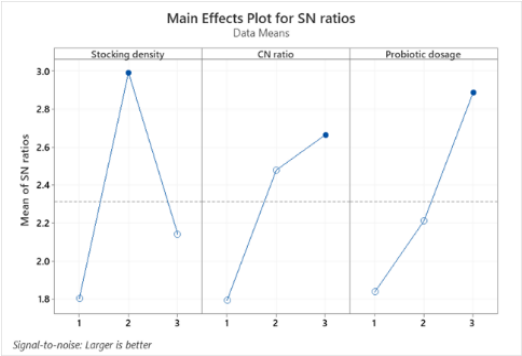
Physiological indices		**Factors**		
	Levels	**Stocking density**	**C/N ratio**	**Probiotic dosage**
Protein	1	1.00*	0.85	0.95
	2	0.82	0.90	0.99*
	3	0.83	0.91*	0.71
	Delta	*0.17*	*0.05*	*0.28*
	Rank	*2*	*3*	*1*
Glycogen	1	1.23	1.23	1.24
	2	1.41*	1.33	1.29
	3	1.28	1.36*	1.39*
	Delta	*0.17*	*0.12*	*0.15*
	Rank	*1*	*3*	*2*
Glucose	1	0.72*	0.64*	0.63
	2	0.52	0.61	0.56
	3	0.59	0.57	0.64*
	Delta	*0.20*	*0.07*	*0.08*
	Rank	*1*	*3*	*2*
THC	1	23.00*	19.67	20.00
	2	17.33	19.67	19.67
	3	20.00	21.00*	20.67*
	Delta	*5.67*	*1.33*	*1.00*
	Rank	*1*	*2*	*3*
HSI	1	3.16	2.47	2.62
	2	1.79	2.82	2.64
	3	3.34*	3.00*	3.03*
	Delta	*1.55*	*0.53*	*0.41*
	Rank	*1*	*2*	*3*
ProPO	1	64.33*	52.00	50.67
	2	40.00	48.67	50.67
	3	48.33	52.10*	51.33*
	Delta	*24.33*	*3.33*	*0.67*
	Rank	*1*	*2*	*3*
SOD	1	2.36*	1.87	2.05*
	2	1.50	2.20*	2.04
	3	2.12	1.90	1.88
	Delta	*0.86*	*0.33*	*0.16*
	Rank	*1*	*2*	*3*
Haemocyanin	1	3.62*	2.46	2.72
	2	1.99	2.52	2.38
	3	2.59	3.23*	3.11*
	Delta	*1.63*	*0.76*	*0.71*
	Rank	*1*	*2*	*3*

**indicates optimum values as described by the Signal-noise ratio*

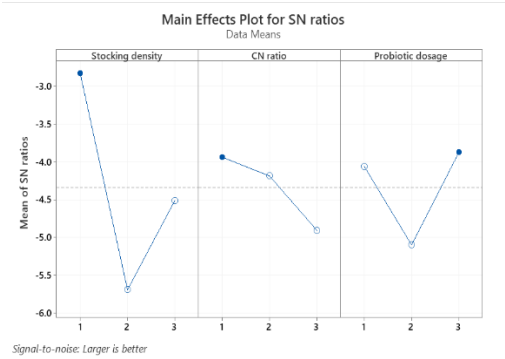
The S/N ratios of the physiological indices are presented in **Figure 4.13**. The signal-to-noise ratio was classified as '*larger is better*' based on the physiological indices measured, where a positive trend has been noted to explain the good conditions of whiteleg shrimp, *L. vannamei*.



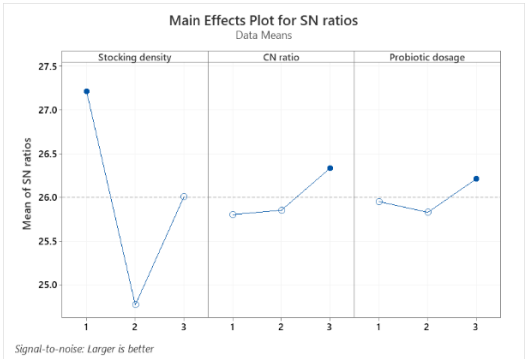
S/N Ratio of protein



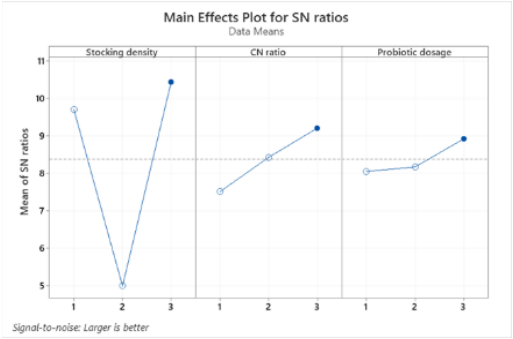
S/N Ratio of glycogen



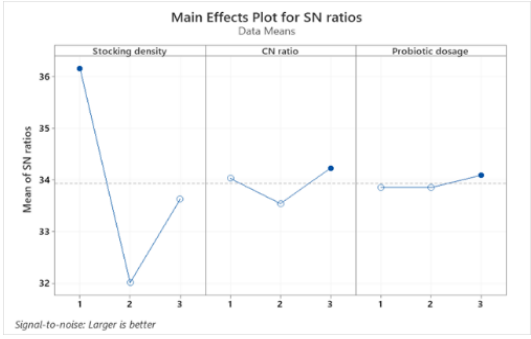
S/N Ratio of glucose



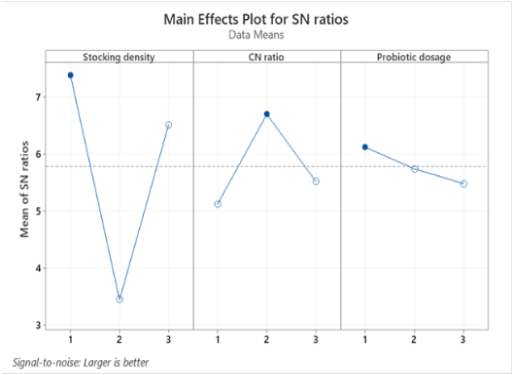
S/N Ratio of THC



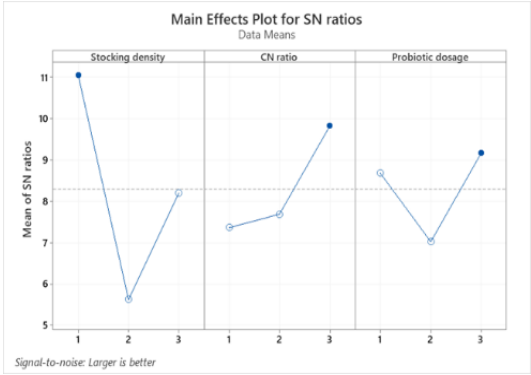
S/N Ratio of HSI



S/N Ratio of ProPO



S/N Ratio of SOD



S/N Ratio of Haemocyanin

Figure 4.13: Signal-to-noise ratio of the physiological indices of *L. vannamei* cultured in IMTAS

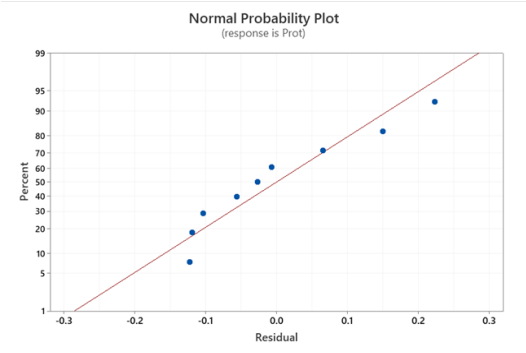
4.3.2.2 Taguchi-based regression analysis of the physiological indices *L. vannamei* raised in IMTAS

A fit regression model was obtained from the Taguchi layout using the values of the physiological indices obtained during Experiment 1. A summary of the models and their regression coefficients (R^2) are presented in **Table 4.4**. 25% of the measured indices had weak regression (<30%), 62.5% had moderate regression (30 -70%), and 12.5% had strong regression of the control factors. The lowest regression coefficient (28.13%) was recorded for SOD, while the highest (70.77%) was recorded for HSI. The normal probability plots of the regression analysis are presented in **Figure 4.14**.

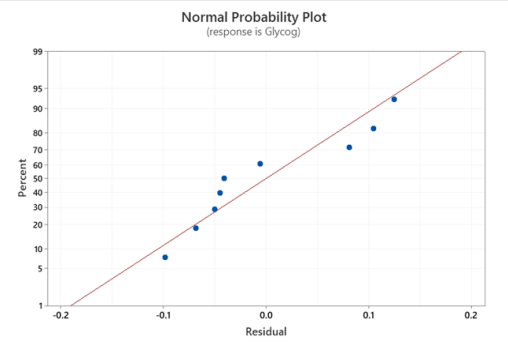
Table 4.4: Prediction models of the physiological indices of *Litopeneaus vannamei* cultured in an IMTAS

Physiological indices		**Const**		**Stocking density**		**CN ratio**		**Probiotic dosage**	**R^2**
Protein	=	1.25	-	0.087	+	0.026	-	0.123	53.72 %
Glycogen	=	0.98	+	0.021	+	0.063	+	0.077	53.75 %
Glucose	=	0.80	-	0.062	-	0.036	+	0.003	37.26 %
THC	=	21.11	-	1.500	+	0.670	+	0.330	68.58 %
HSI	=	1.64	+	0.090	+	0.267	+	0.205	70.77 %
ProPO	=	66.20	-	8.000	+	0.000	+	0.330	66.67 %
SOD	=	2.36	-	0.120	+	0.017	-	0.083	28.13 %
Haemocyanin	=	2.64	-	0.519	+	0.382	+	0.187	59.08 %

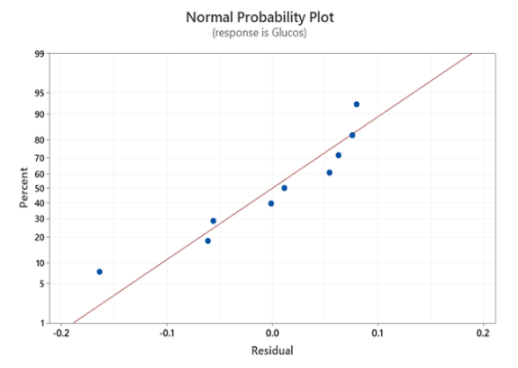
*note that THC = Total Haemocyte Count; HSI = Hepatosomatic Index; ProPO = Prophenoloxidase; SOD = Superoxide Dismutase



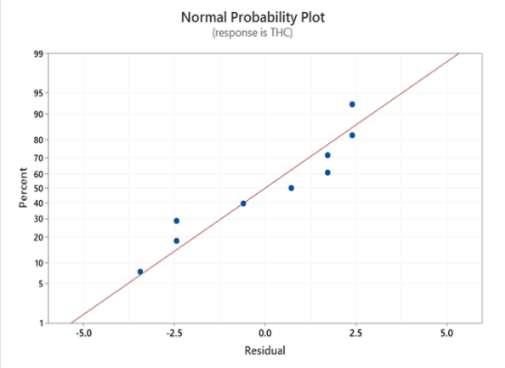
Probability plot of protein



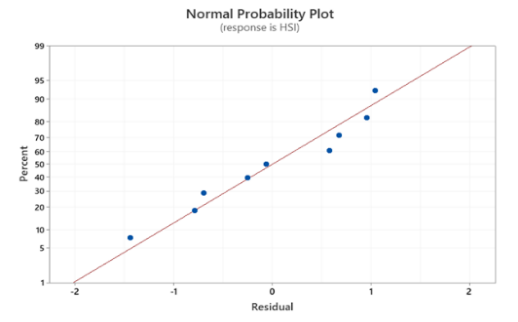
Probability plot of glycogen



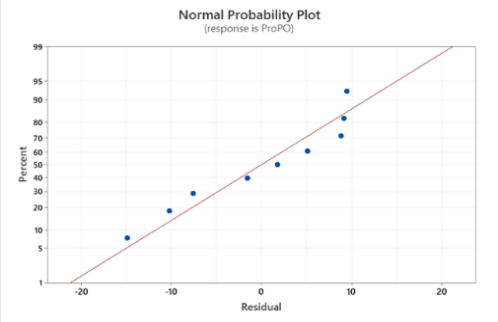
Probability plot o of glucose



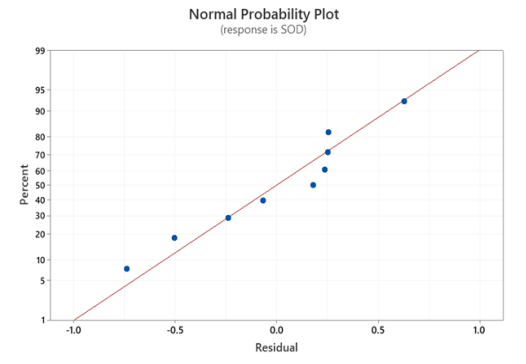
Probability plot of THC



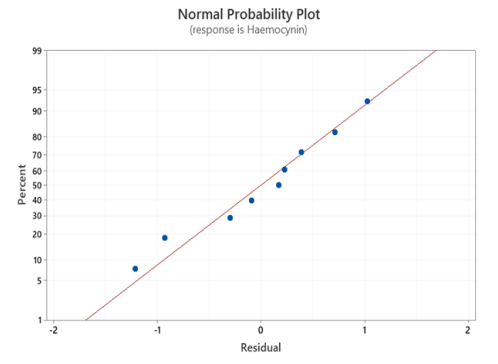
Probability plot of HSI



Probability plot of ProPO



Probability plot of SOD



Probability plot of Haemocyanin

Figure 4.14: Normal probability plots of the physiological indices of *L. vannamei* cultured in IMTAS

The optimum values obtained are presented in **Table 4.5**. The variation within the methods were within the reliability limits of < 20% set by Cetin et al., (2011).

Table 4.5: Comparison of the optimum values of the physiological indices of *L. vannamei* cultured in IMTAS

Parameters	**Regression Predicted**	**Taguchi Predicted**	**Observed from Experiment**	**Difference**
Protein	1.24	1.13	1.26	0.12
Glycogen	1.24	1.55	2.80	1.56
Glucose	0.73	0.79	0.81	0.07
THC	27.70	26.44	27.70	1.25
HSI	3.36	3.85	3.36	0.48
ProPO	66.05	65.88	66.20	0.32
SOD	1.95	2.63	2.37	0.68
Haemocyanin	4.42	4.48	4.42	0.06

4.3.2.3 Performance of IMTAS based on the physiological indices of *L. vannamei* in IMTAS

The performance of IMTAS was assessed based on the physiological indices of *L. vannamei*, as presented in **Table 4.6**. The protein level was significantly higher in GRG 1 of Experiment 2 (2.00±0.03) in contrast to other treatments ($p < 0.01$). In contrast, the control in Experiment 2 had the lowest protein level at 1.03±0.07. The glycogen level was also greater in GRG 1 of Experiment 2 (3.17±0.14) when compared to the Control and GRG 2 treatments ($p < 0.01$), with the optimum level in Experiment 1 (1.55±0.03) being the least. Furthermore, glucose levels recorded significant variation, with GRG 1 in Experiment 2 showing the highest values (1.16±0.06) ($p < 0.01$). Similarly, the Total Haemocyte Count significantly increased in GRG 1 of Expt 2 (34.21±0.65) compared to the Optimum and Control treatments ($p < 0.01$). The Hepatosomatic Index showed significant differences as well, with GRG 1 in Experiment 2 showing the highest index (4.23±0.11) ($p < 0.01$). Prophenoloxidase (62.18±0.03), Superoxide dismutase (3.33±0.08), and haemocyanin levels (5.17±0.05) were highest in GRG 1 of Experiment 2 and varied significantly across treatments.

Table 4.6: Performance of IMTAS based on the physiological indices of *L. vannamei* based on physiological indices

Physiological indices	Experiment 1		Experiment 2 (IMTAS)			p-value
	Optimum		Control	GRG 1	GRG 2	
Protein	1.13±0.04^{bc}		1.03±0.07^{c}	2.00±0.03^{a}	1.58±0.05^{b}	<0.01
Glycogen	1.55±0.03^{b}		1.94±0.07^{b}	3.17±0.14^{a}	2.83±0.14^{a}	<0.01
Glucose	0.79±0.01^{b}		0.71±0.02^{c}	1.16±0.06^{a}	0.99±0.00^{ab}	<0.01
THC	26.44±0.61^{c}		24.93±0.30^{c}	34.21±0.65^{a}	29.91±0.23^{b}	<0.01
HSI	3.85±0.04^{b}		2.99±0.12^{b}	4.23±0.11^{a}	4.01±0.03^{a}	<0.01
ProPO	65.88±0.16^{a}		57.51±0.23^{b}	62.18±0.03^{ab}	61.37±0.08^{ab}	0.01
SOD	2.63±0.06^{b}		3.00±0.01^{a}	3.33±0.08^{a}	3.06±0.04^{a}	<0.01
Haemocyanin	4.48±0.04^{ab}		3.97±0.03^{b}	5.17±0.05^{a}	4.744±0.26^{b}	0.01

**Note that means on the same row with different superscript are statistically significant ($p<0.05$)*

4.3.3 Evaluation of the growth responses of shrimp raised in IMTAS

The growth responses of the cultured shrimp were measured to evaluate the performance of the IMTAS compared with the traditional biofloc system. The optimum growth values obtained during system optimisation in Experiment 1 were compared with the best two control settings and a control.

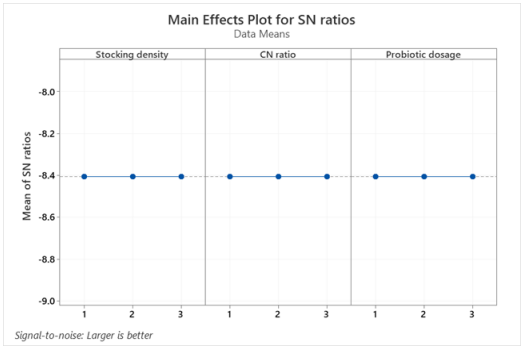
4.3.3.1 Taguchi single-response optimisation of the growth indices of *L. vannamei* raised in IMTAS

The single-response optimisation of the growth indices of *L. vannamei* raised in the indoor multi-techno aquaculture system is presented in **Table 4.7**. The signal-to-noise ratios of the growth indices are shown in **Figure 4.15**. While the signal-to-noise ratio of feed conversion ratio was set at '*smaller is better*', mean final weight, mean weight gain, relative weight gain, specific growth rate condition factor and percentage survival were set at '*larger is better*'.

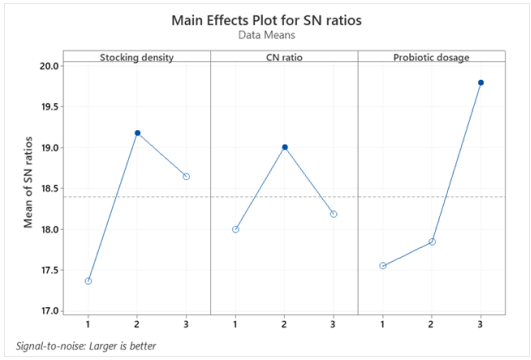
Table 4.7: Optimum Values of growth indices of *L. vannamei* cultured in IMTAS

Growth indices		**Control factors**		
	Levels	**Stocking density**	**CN ratio**	**Probiotic dosage**
Mean initial weight (g)	1	0.38	0.38	0.38
	2	0.38	0.38	0.38
	3	0.38	0.38	0.38
	Delta	*0.00*	*0.00*	*0.00*
	Rank	-	-	-
Mean final weight (g)	1	7.43	8.13	7.62
	2	9.20*	9.03*	7.80
	3	8.66	8.13	9.87*
	Delta	*1.77*	*0.90*	*2.24*
	Rank	*2*	*3*	*1*
Mean weight gain (g)	1	7.05	7.75	7.24
	2	8.82*	8.65*	7.42
	3	8.28	7.75	9.49*
	Delta	*1.77*	*0.90*	*2.24*
	Rank	*2*	*3*	*1*
Relative weight gain (g)	1	36.78	42.07	38.54
	2	46.14*	45.61*	38.36
	3	44.15	39.39	50.17*
	Delta	*9.36*	*6.22*	*11.80*
	Rank	*2*	*3*	*1*
Specific growth rate (g/day)	1	4.22	4.39	4.28
	2	4.52*	4.49*	4.32
	3	4.48	4.34	4.63*
	Delta	*0.30*	*0.15*	*0.35*
	Rank	*2*	*3*	*1*
Feed conversion rate	1	1.41	1.29	1.30
	2	1.13	1.16*	1.22
	3	1.11*	1.21	1.15*
	Delta	*0.30*	*0.13*	*0.15*
	Rank	*1*	*3*	*2*
Condition factor	1	0.85	0.93	0.89
	2	0.96	0.90	0.91
	3	0.98*	0.95*	0.98*
	Delta	*0.13*	*0.04*	*0.08*
	Rank	*1*	*3*	*2*
% Survival	1	49.30	51.00	52.51
	2	59.78*	52.79*	43.19
	3	44.70	49.99	58.08*
	Delta	*15.08*	*2.81*	*14.89*
	Rank	*1*	*3*	*2*

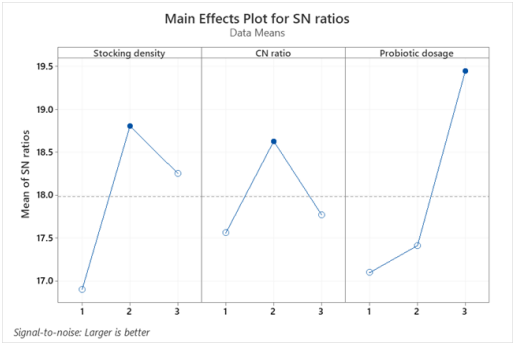
**indicates optimum values as described by the Signal-noise ratio*



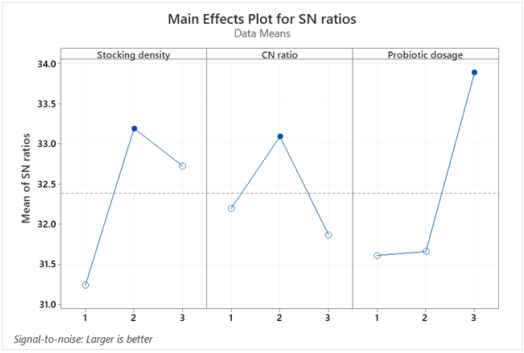
S/N Ratio of MIW



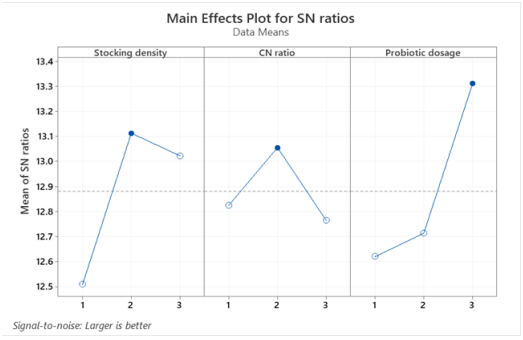
S/N Ratio of MFW



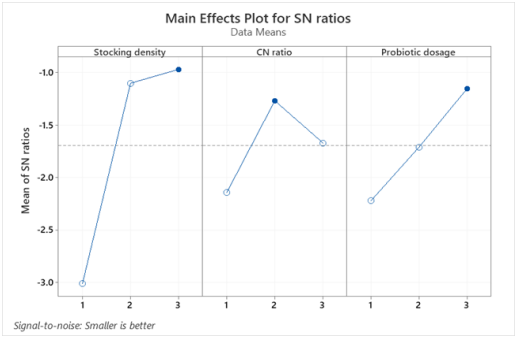
S/N Ratio of MWG



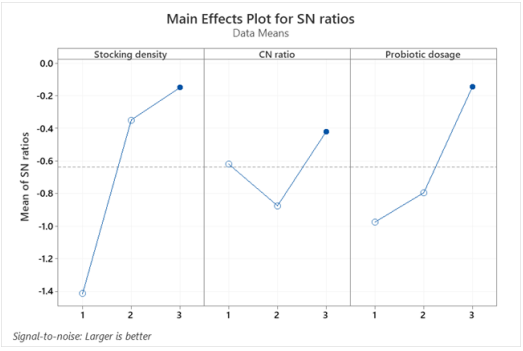
S/N Ratio of RWG



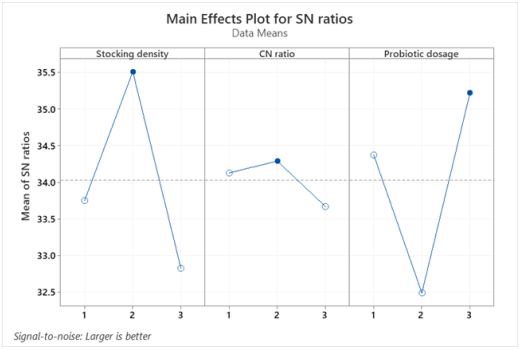
S/N Ratio of SGR



S/N Ratio of FCR



S/N Ratio of CF



S/N Ratio of % Survival

Figure 4.15: Signal-to-noise ratio of the growth indices of *L. vannamei* cultured in IMTAS

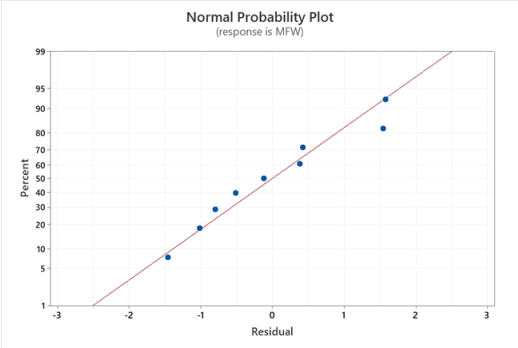
4.3.3.2 Taguchi-based regression analysis of the growth indices of *L. vannamei* raised in IMTAS

The coefficients of the control factors and regression coefficients in the predictive models of the growth indices of *L. vannamei* cultured in an Indoor Multi-Techno Aquaculture System is presented in **Table 4.8**. Five (71.43%) of the growth indices had moderate regression coefficients, while two (28.57%) had strong regression coefficients. The normal probability plots of these regression models are presented in **Figure 4.16**.

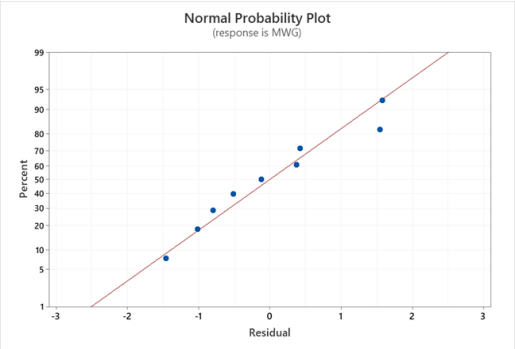
Table 4.8: Prediction models of the growth indices of *Litopeneaus vannamei* cultured in IMTAS

Growth indices		**Const**		**Stocking density**		**CN ratio**		**Probiotic dosage**	**R^2**
MIW (g)	=	NULL							
MFW (g)	=	4.970	-	0.611	+	0.320	+	1.124	51.43 %
MWG (g)	=	4.590	-	0.611	+	0.385	+	1.123	51.42 %
RWG (g)	=	26.00	+	0.169	-	0.380	+	0.810	50.82 %
SGR ($g.day^{-1}$)	=	3.842	+	0.129	-	0.021	+	0.176	58.84 %
FCR	=	1.758	-	0.150	-	0.039	-	0.076	76.33 %
CF	=	0.692	+	0.067	+	0.008	+	0.045	73.51 %
% Surv	=	51.300	-	2.300	-	0.510	+	2.790	59.88 %

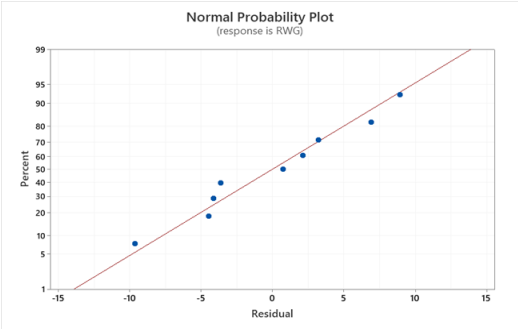
* Note that MIW = Mean initial weight; MFW = Mean final weight; MWG = Mean weight gain; RWG = Relative weight gain; SGR = Specific growth rate; FCR = Feed conversion ratio; CF = Condition factor; % Surv = Percentage survival



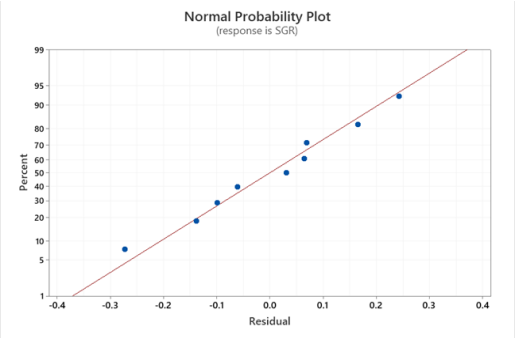
Probability plot of MFW



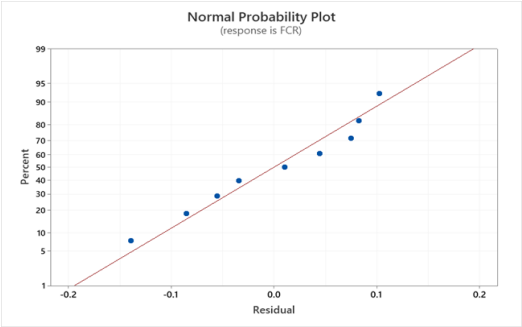
Probability plot of MWG



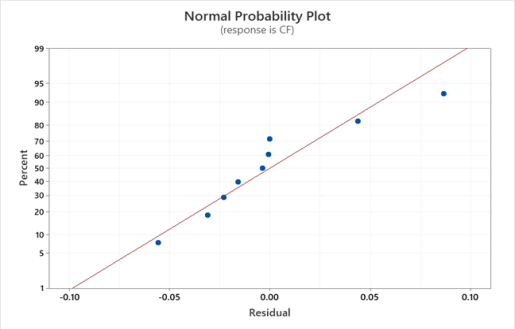
Probability plot o of RWG



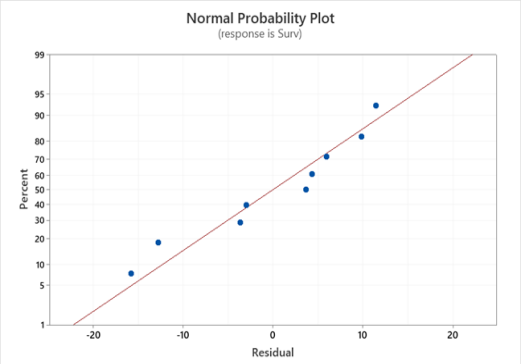
Probability plot of SGR



Probability plot of FCR



Probability plot of CF



Probability plot of % Surv

Figure 4.16: Normal probability plots of the growth indices of *L. vannamei* cultured in IMTAS

The optimum values of the measured growth indices as presented in **Table 4.9** were also within the reliability criteria of < 20% set by Cetin et al., (2011).

Table 4.9: Optimum values of the growth indices of *L. vannamei* cultured in IMTAS

Parameters	**Regression Predicted**	**Taguchi Predicted**	**Observed from Experiment**	**Difference**
MIW (g)	Null	0.38	0.38	-
MFW (g)	12.38	11.24	13.89	2.65
MWG (g)	13.19	10.87	13.51	2.64
RWG (g)	57.10	57.19	56.42	0.77
SGR (g/day)	5.15	4.84	4.21	0.94
FCR	1.46	0.98	1.21	0.47
CF	0.81	1.06	1.08	0.27
% Surv	66.15	68.14	65.45	2.69

Null = not predicted by regression model due to non-variation of treatment and replicate readings

4.3.3.3 Performance of IMTAS based on the growth indices of *L. vannamei*

The performance of IMTAS based on the growth indices is presented in **Table 4.10**. The mean Initial Weight (MIW) were statistically similar across all treatments. However, mean final Weight (MFW) and mean weight Gain (MWG) were significantly higher in GRG 1 (MFW: 21.45 g, MWG: 21.07 g) and GRG 2 (MFW: 18.10 g, MWG: 17.72 g) compared to Control (MFW: 10.50 g, MWG: 10.12 g) and Optimum (MFW: 11.05 g, MWG: 10.76 g) ($p<0.01$). Relative Weight Gain (RWG) showed a similar trend, with GRG 1 (98.22 g) and GRG 2 (97.92 g) indicating significantly higher values than the control (96.42 g) and optimum (56.71 g) ($p<0.01$). Specific Growth Rate (SGR) was higher in GRG 1 (4.79g/day) compared to Control (3.96g/day) and Optimum (4.17g./day) groups ($p<0.01$). Additionally, the Feed Conversion Ratio (FCR) was significantly lower in GRG 1 (1.05) than in the Control (1.39) and Optimum (1.24), indicating greater feeding efficiency. The condition Factor (CF) was also higher in GRG 1 (1.50) than in the control (1.03) and optimum (1.17) groups ($p<0.01$), accounting for the better well-being of the cultured shrimp. Furthermore, the Percentage Survival (% Surv) rates were significantly higher in GRG

1 (75.91%) and GRG 2 (67.96%) than in the control (49.08%) and optimum (65.29%) groups ($p<0.01$).

Table 4.10: Performance of IMTAS based on the physiological indices of *L. vannamei* based on growth indices

Growth indices	Experiment 1		Experiment 2			p-value
	Optimum		Control	GRG 1	GRG 2	
MIW (g)	0.37±0.00		0.37±0.01	0.37±0.00	0.37±0.00	0.80^{ns}
MFW (g)	$11.05±0.05^{b}$		$10.50±0.20^{b}$	$21.45±1.35^{a}$	$18.10±0.20^{a}$	<0.01
MWG (g)	$10.76±0.04^{b}$		$10.12±0.19^{b}$	$21.07±0.35^{a}$	$17.72±0.19^{a}$	<0.01
RWG (g)	$56.71±0.29^{c}$		$96.42±0.02^{b}$	$98.22±0.11^{a}$	$97.92±0.00^{a}$	<0.01
SGR ($g.day^{-1}$)	$4.17±0.02^{b}$		$3.96±0.00^{b}$	$4.79±0.07^{a}$	$4.61±0.00^{a}$	<0.01
FCR	$1.24±0.03^{ab}$		$1.39±0.00^{a}$	$1.05±0.03^{c}$	$1.17±0.02^{bc}$	<0.01
CF	$1.17±0.00^{bc}$		$1.03±0.00^{c}$	$1.50±0.05^{a}$	$1.30±0.00^{ab}$	<0.01
% Surv	$65.29±0.15^{b}$		$49.08±2.06^{c}$	$75.91±0.33^{a}$	$67.96±0.15^{b}$	<0.01

Means on the same row with different superscript are statistically significant ($p<0.05$)

The growth trend graph presented in **Figure 4.17** illustrates the growth dynamics of *L. vannamei* over a 12-week period. During weeks 1-3, all treatments showed a lag phase characterised by slow growth as the shrimp acclimatised to the experimental conditions. Weeks 4-9 had an exponential growth phase marked by rapid weight gain across all treatments, with GRG 1 showing the highest growth rates, followed by GRG 2. Optimum and Control however had comparatively lower growth rates. Stationary growth was recorded in weeks 10-12, however, GRG 1 and GRG 2 maintained comparatively higher growth rates than the Optimum and Control groups during this phase.

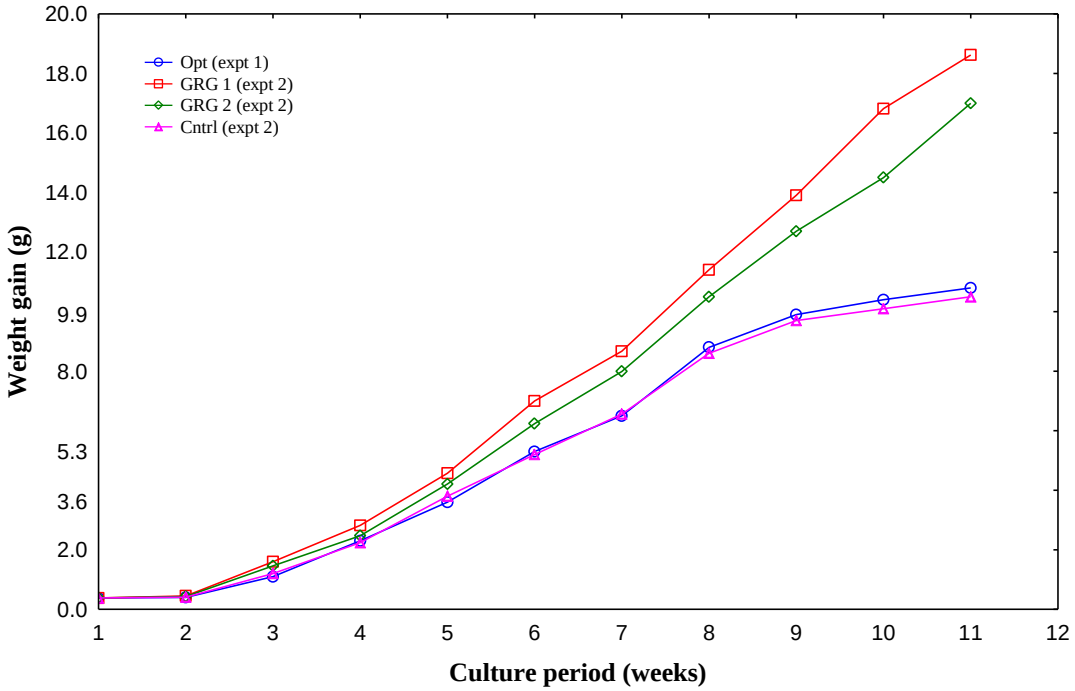


Figure 4.17: Growth trend of *L. vannamei* grown in the various culture systems

4.3.4 Overall performance of IMTAS based on water quality of the system and the physiological and growth performance of the cultured *L. vannamei*

The percentage contributions of the measured parameters to each dimension are presented in **Figures 4.18** and **Figure 4.19**. Based on the composition of the measured parameters in each dimension, DIM 1 was classified as a growth and well-being indicator, while DIM 2 was known for Environmental and Stress Factors. DIM 1 was largely influenced by parameters related to growth performance, physiological indices, and the overall condition of shrimp. The high contribution of specific growth rate, feed conversion ratio, total haemocyte count, percentage survival, hepatosomatic index, mean weight gain, and protein levels suggests that this dimension represents factors associated with the growth and health status of shrimp. In contrast, DIM 2 was dominated by parameters related to the environmental conditions and stress responses. The comparatively high contributions of relative weight gain, pH, prophenoloxidase, superoxide dismutase, and temperature suggest that this dimension captures factors

that might be associated with environmental stress and water quality issues affecting the shrimp culture system. Accordingly, the PCA biplot designated the treatment that supported the identified physiological functions in shrimp. The development of IMTAS need to make these crucial considerations to create a system that supports the growth, stress response and immunity in shrimp.

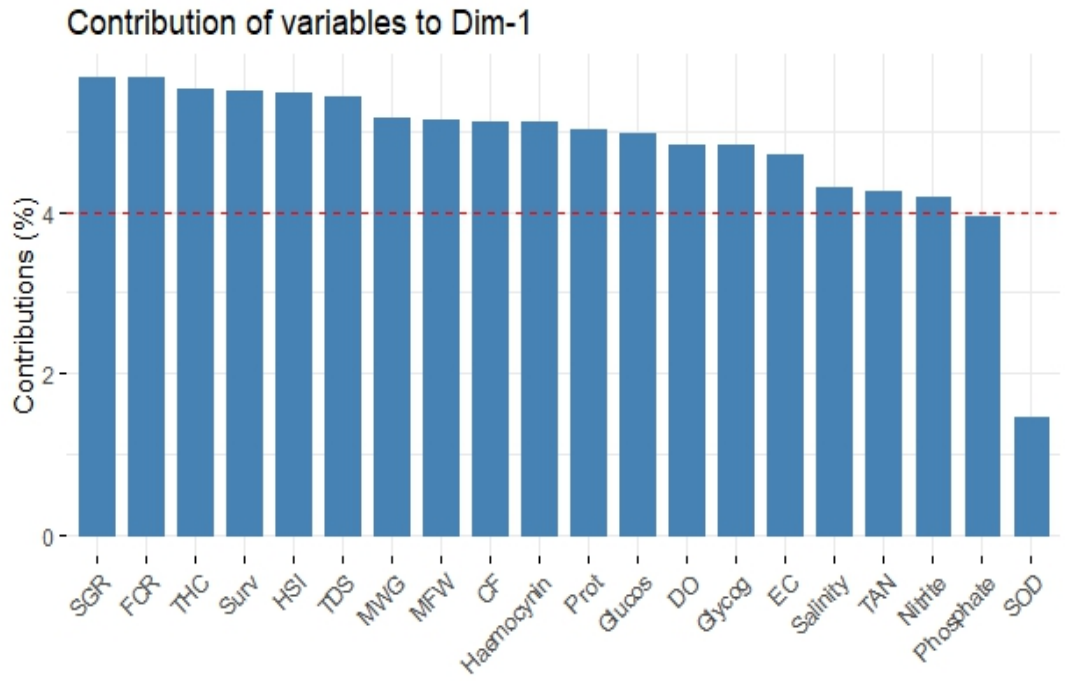


Figure 4.18: Percentage Contributions of parameters to the PCA on Dimension 1

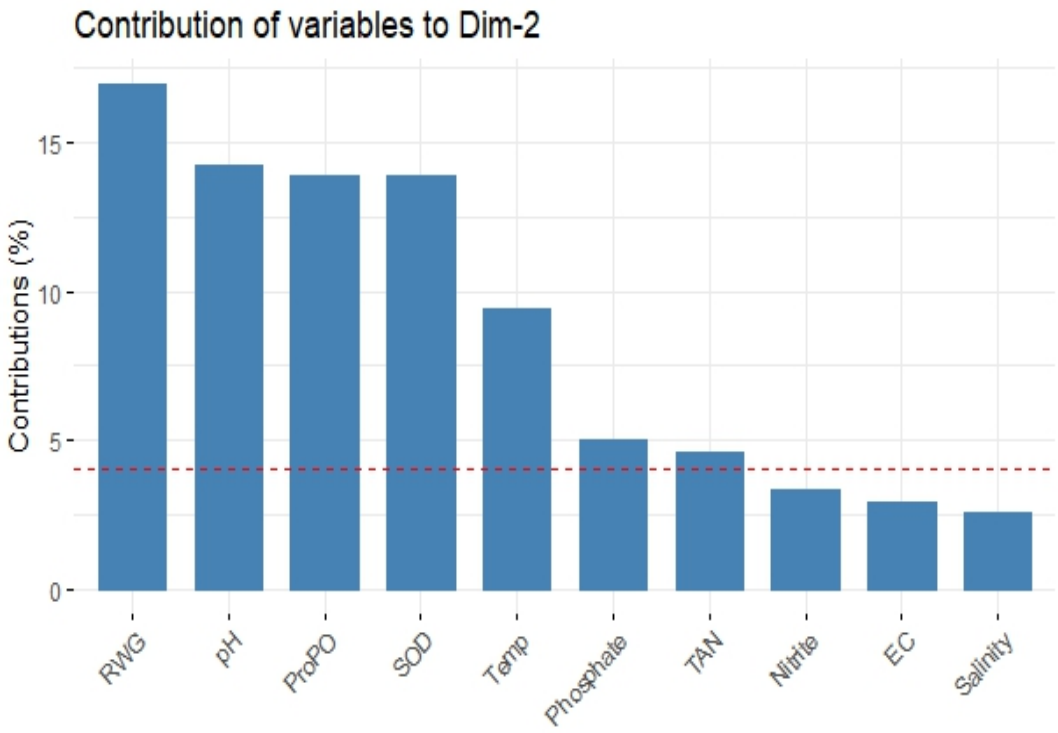


Figure 4.19: Percentage Contributions of parameters to the PCA on Dimension 2

The positions of the treatments in the PCA plot (**Figure 4.20**) provided information on their performance based on the two principal dimensions (growth and well-being indicators for DIM 1 and Environmental and Stress Factors for DIM 2). GRG 1 had a high positive value, indicating strong performance in growth and health indicators on DIM 1 associated with better growth rates, higher survival rates, better feed conversion, and overall good physiological health. The relative position of DIM 2 indicated a moderate influence of environmental and stress factors. GRG 2 also had a positive value on DIM 1, which was lower than GRG 1 but indicated good performance in growth and health indicators. The comparatively lower positive for DIM 2 showed marginal performance in terms of environmental and stress factors. The control group had a negative value for DIM 1, representing poor performance in growth and health indicators. The positive value of DIM 2 suggests appreciable performance in terms of the environmental and stress factors. The control group also experienced higher levels of stress and environmental issues. The negative value for the Opt treatment on DIM 1 indicates poor performance in growth and health indicators. Opt treatment performed comparatively less than GRG 1 or GRG 2 in terms

of growth and health. Similarly, DIM 2 showed poor performance for environmental and stress factors. GRG 1 was confirmed to have the best performance in terms of growth and health indicators as well as a considerable level of performance in terms of stress and environmental factors.

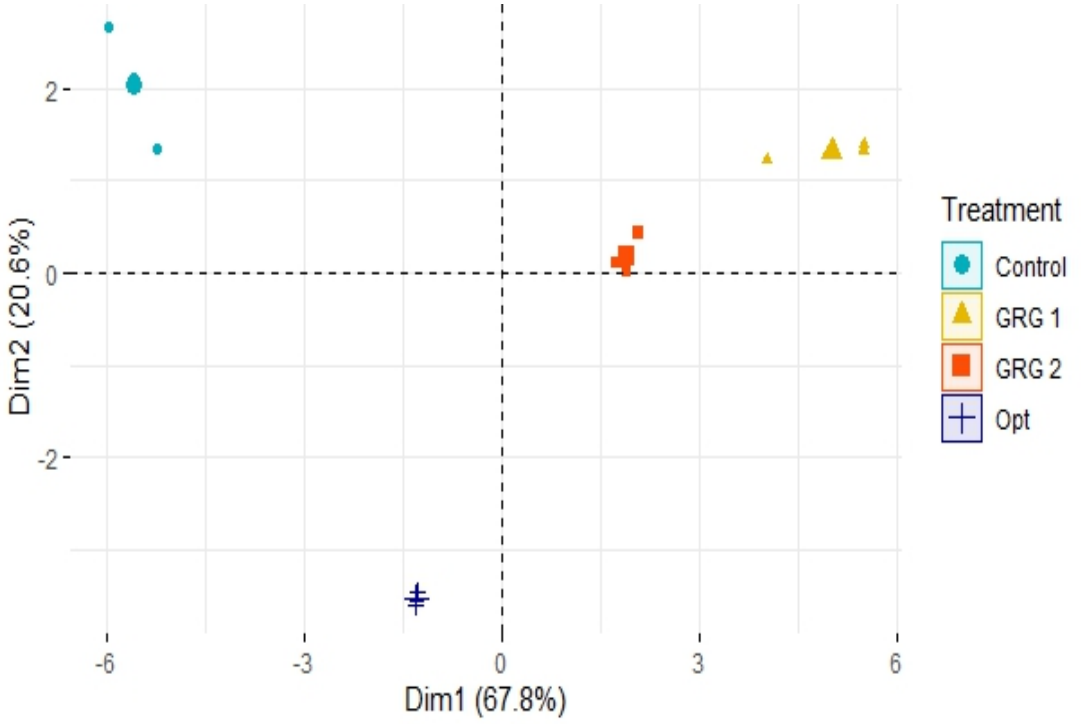


Figure 4.20: PCA plot of the treatments

4.3.5 Evaluation of the gut microbial community of *Litopeneaus vannamei* raised in IMTAS

The microbial community structure of shrimp raised in the biofloc system under the three treatments is shown in **Figure 4.21**. The microbiome analysis was performed using a hierarchical clustering approach with three layers (specific microbial taxa, groupings of similar taxa, and broader community patterns).

Cluster 1A, which grouped the predominant microorganisms, consistently featured *Planctomycetales*, *Vibrionaceae*, and *Gammaproteobacteria* across all treatments, with little variation in their presence within the shrimp gut. However, *Silicimonas* showed a slight increase in GRG2 compared to that in the Control and GRG1. *Nodosilineaceae* and related taxa were not present in GRG 1 and 2 treatments compared to the Control in cluster 1B. Clusters 1A and 1 B formed cluster 2A in the second hierarchy. Cluster 1C, comprising *Photobacterium* and *Vibrio*, showed an increase in abundance for GRG 1 and 2 compared to Control. *Spongiimonas* in cluster 1D increased under GRG2 compared to GRG1 and the control. 1E included *Alkalimarinus* and *Rhodobacteraceae*, which generally increased in GRG 1 and 2 treatments, indicating a shift towards a more beneficial microbial community. Additionally, opportunistic microbes such as *Tenacibaculum* and *Candidatus Bacilloplasma* were the most abundant in GRG 1 and 2. Cluster 1G had *Colwelliaceae*, *Marinicella*, and *Arcobacter*, which were significantly increased in GRG treatments. Cluster 1H comprised *Rubinisphaeraceae*, *Roseovarius*, and *Rhizobiaceae*, consistently increased in GRG 1 and 2.

Cluster 3A, which comprised the most dominant microorganisms from clusters 2A to 2C, was found to have a higher abundance of microbes than cluster 3B. The analysis showed that certain microbial species were present in both biofloc treatments (GRG 1 and GRG 2), which represents a diverse and abundant population of beneficial microbes compared to the control.

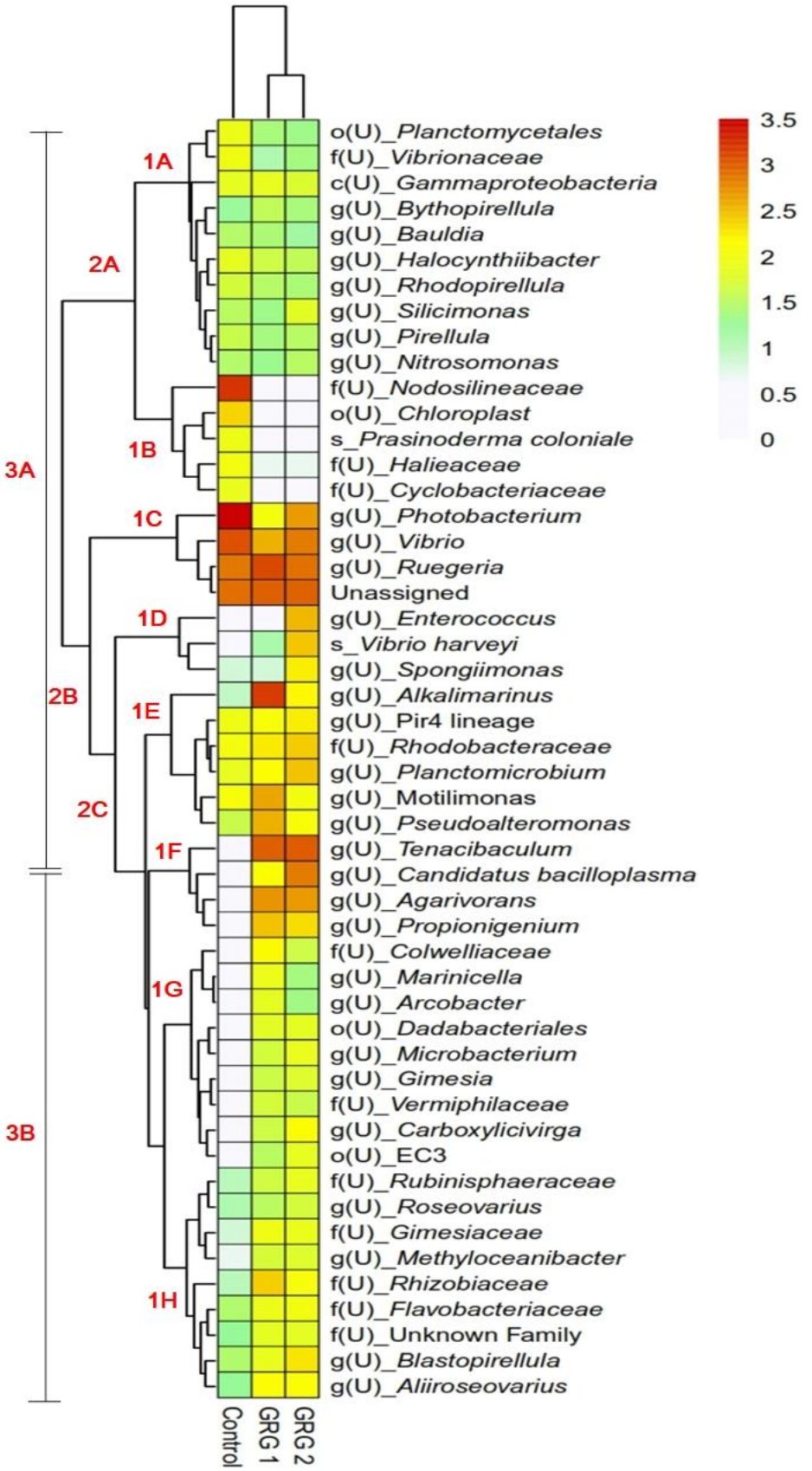


Figure 4.21: Heatmap of the distribution of microbial community in the gut of *L. vannamei* raised in IMTAS

The structure of the microbial community in the gut of *L. vannamei* at the phylum level under various rearing conditions in an indoor multi-techno aquaculture system is illustrated in **Figure 4.22**. The study involved three groups of treatments (GRG 1, GRG 2, and the control group). The relative abundance of the major microbial phyla differed among the groups. Proteobacteria was the most prevalent phylum in all groups, with the Control group having the highest mean relative abundance of 0.79, followed by GRG 2 at 0.71, and GRG 1 at 0.52. Fusobacteriota had significant differences, with GRG 1 having a relatively high mean relative abundance of 0.31 compared to 0.02 in GRG 2 and 0.13 in the Control group. In contrast, Firmicutes were relatively similar across the groups, ranging from 0.05 in the Control group to 0.09 in GRG 2, while Firmicutes A was present in GRG 1 and GRG 2 but absent in the Control group. Bacteroidota was recorded in GRG 2 at a mean relative abundance of 0.05, and was absent in GRG 1 and the control group. The 'others' category, comprising less abundant phyla, varied among the groups, with GRG 2 showing the highest mean relative abundance at 0.11, followed by GRG 1 at 0.06, while the Control group had 0.03. These results suggest that different rearing conditions had an impact on the gut microbiota composition of *L. vannamei* raised in various culture systems.

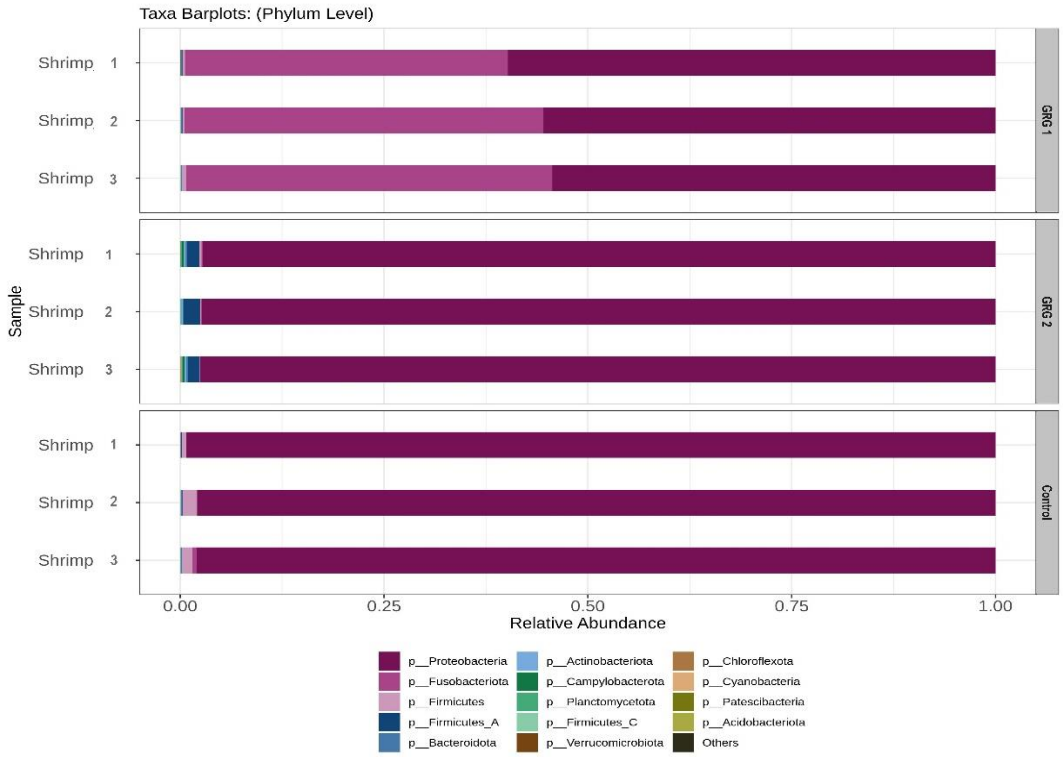


Figure 4.22: Phyla relative abundance of microbial community in the gut of *L. vannamei* raised in IMTAS

The composition of the microbial community within the gut of *L. vannamei* at the family level is illustrated in **Figure 4.23**. Fusobacteriaceae was found to be the most abundant in GRG 2 at 0.79, followed by GRG 1 at 0.32, while the least abundant was observed in the Control group at 0.03. Enterobacteriaceae had relatively higher levels in GRG 1 compared to GRG 2 and the Control. Shewanellaceae showed similar mean relative abundances across all three groups, with values of 0.05, 0.02, and 0.02 for GRG 1, GRG 2, and the Control, respectively. Aeromonadaceae was observed at low levels in GRG 2 and the Control, and was completely absent in GRG 1. Burkholderiaceae recorded a higher mean relative abundance in GRG 1 (0.08) compared to GRG 2 (0.04) and the Control (0.01). Peptostreptococcaceae was present in both GRG 1 and GRG 2 at 0.01, but was not detected in the Control group. Enterococcaceae were only observed in GRG 2 and the Control at 0.02 and 0.01, respectively, while Clostridiaceae were found in GRG 2 (0.01) but not in GRG 1 and Control. Alteromonadaceae were present in GRG 2 (0.01) and the Control (0.03) but were absent in GRG 1. The 'others' category, comprising less abundant families, had varying relative abundances among the groups, with GRG 1 showing an average

relative abundance of 0.14, GRG 2, 0.06, and the Control, 0.03. The variation in the microbial community structure suggests that the biofloc condition selectively promoted the growth of specific microbial families.

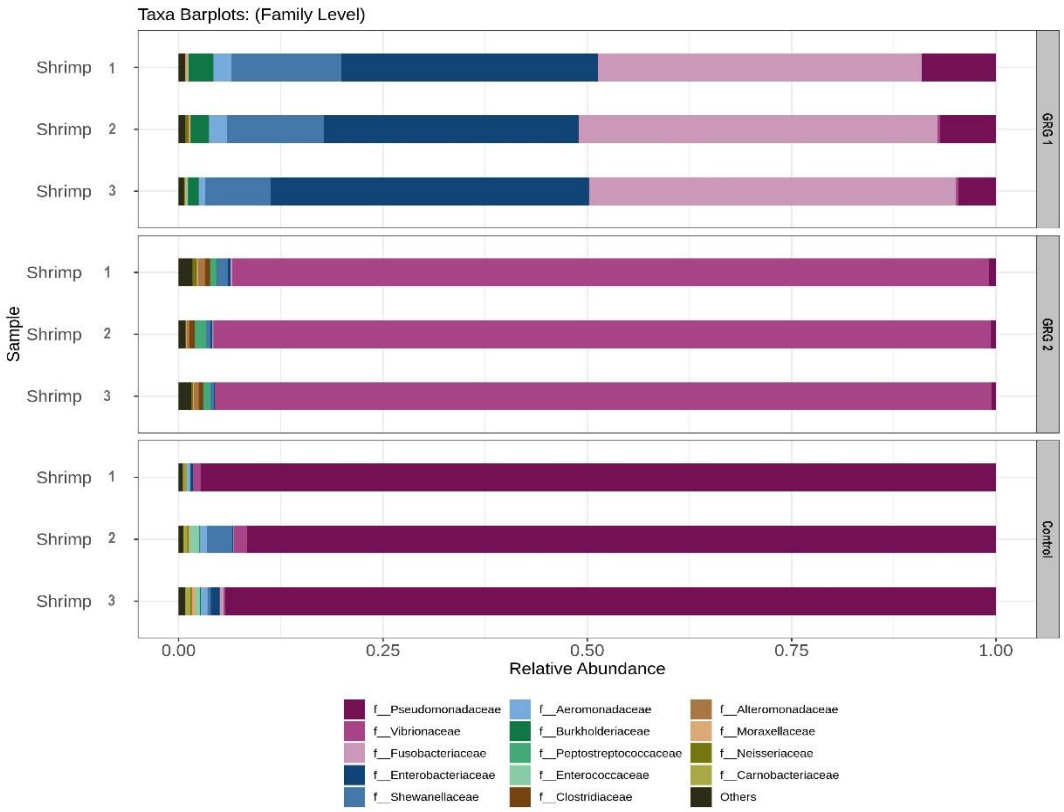


Figure 4.23: Family relative abundance of microbial community in the gut of *L. vannamei* raised in IMTAS

The microbial community structure in the gut of *L. vannamei*, presented at the species level, is presented in **Figure 4.24**. *Pseudomonas* sp. was most abundant in the Control (0.90) followed by GRG 1 (0.14) and GRG 2 (0.01). *Vibrio* sp. showed a high abundance in GRG 2 (0.64) but was comparably absent in GRG 1 and Control (0 and 0.01 respectively). *Fusobacterium* sp. was prevalent in GRG 1 (0.35), followed by GRG 2 (0.08), and was least abundant in the control (0.01). *Rahnella* sp. was present in GRG 1 (0.21) and GRG 2 (0.03) but was absent in the control. *Shewanella baltica* was found in GRG 1 (0.07) but was absent in both GRG 2 and the Control. *Photobacterium marinum* had a relative abundance of 0.04 in GRG 2 and 0.03 in GRG

1 but was not detected in the Control. *Enterobacteria* sp. were present in GRG 1 (0.02) and absent in GRG 2 and the control. *Aeromonas* sp. was detected in the control (0.02) and GRG 1 (0.01) but was absent in GRG 2. *Janthinobacterium* sp. was found in GRG 1 and the Control at 0.01 but was not detected in GRG 2. *Shewanella baltica* had a mean relative abundance of 0.02 GRG 2 and 0.01 both GRG 1. Another species of *Shewanella* sp. was present in both GRG 1 and GRG 2 at 0.01 but was absent in the control. *Vibrio mytili* was found in GRG 2 at 0.02. The 'others' category, which includes less abundant species, varied across the groups, with GRG 2 showing the highest mean relative abundance of 0.17, followed by GRG 1 (0.14), and the Control (0.04).

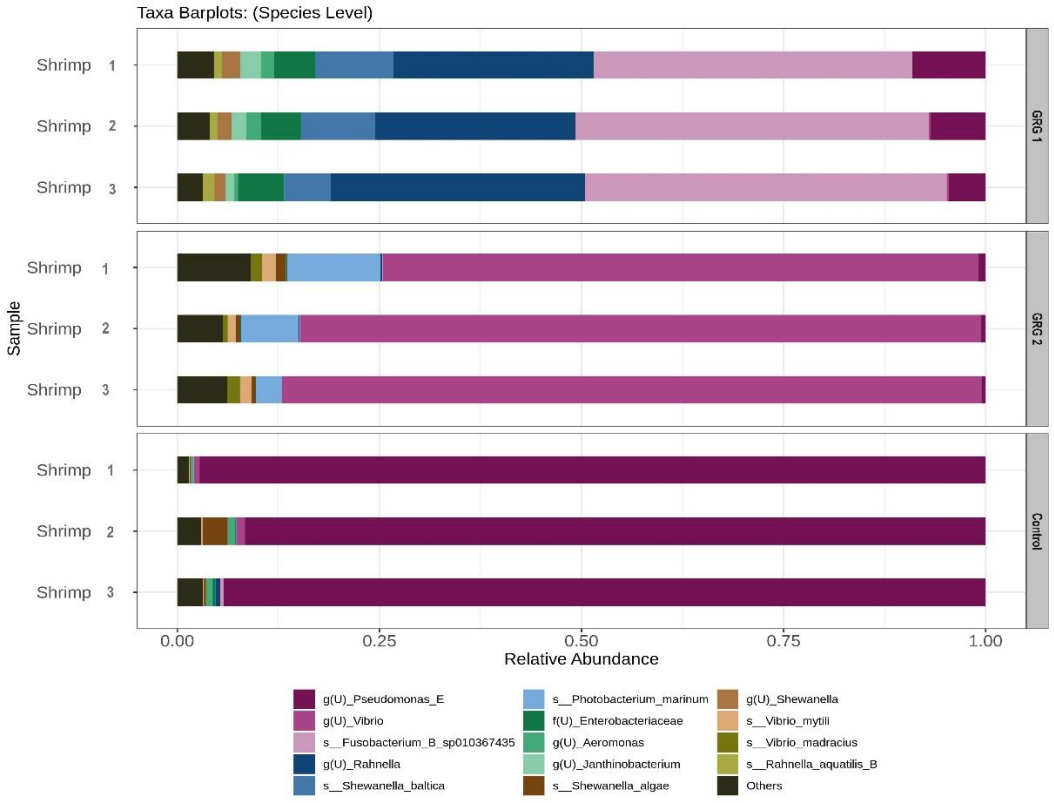


Figure 4.24: Species relative abundance of microbial community in the gut of *L. vannamei* raised in IMTAS

4.3.6 Transcriptomic analysis of the tissue of *L. vannamei* raised in IMTAS

4.3.6.1 Differential gene expression analysis

Gene expression data were subjected to statistical analysis of gene expression levels among the samples. DESeq2 software was used to perform the analysis using DESeq normalisation method with negative binomial distribution model for calculating the p-value at a differential gene screening threshold of

|log2(FoldChange)| >= 1 & padj <= 0.05. (**Equation 4.8**)

Genes were considered differentially expressed when they differed more than twice in expression in both sets of samples. The statistics of the number of differential genes (including upregulation and downregulation) for each comparison group and the threshold for screening are shown in **Figure 4.25**. There was a significant gene count in the differentially expressed genes (DEG) for GRG 1 versus Control (1245) and GRG 2 versus Control (1145), while the comparison between GRG 1 and GRG 2 had 681 up- and down-regulation gene counts.

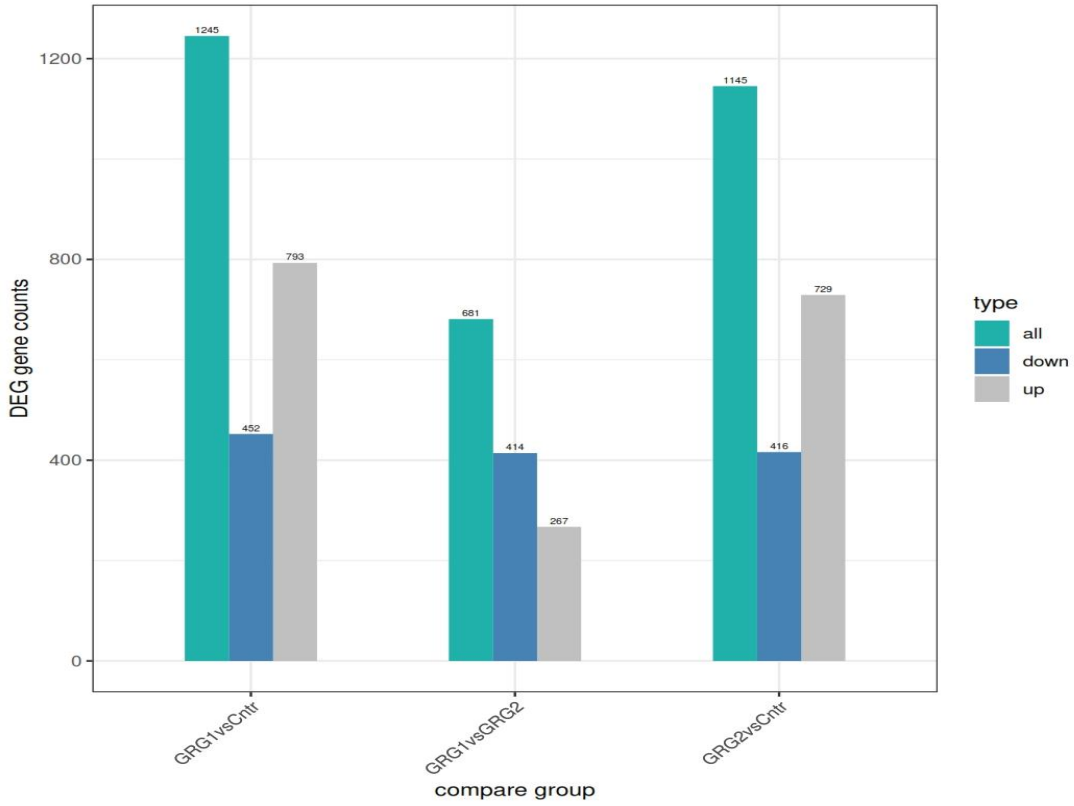


Figure 4.25: Differentially expressed genes (DEG) count of the treatments

4.3.6.2 Cluster analysis

All differentially expressed genes in the comparison group were pooled as differential gene sets. Mainstream hierarchical clustering was used to cluster the FPKM values of genes and homogenise the row (Z-score), as presented in **Figure 4.26**. The colour of each grid was obtained after homogenising the expression data rows.

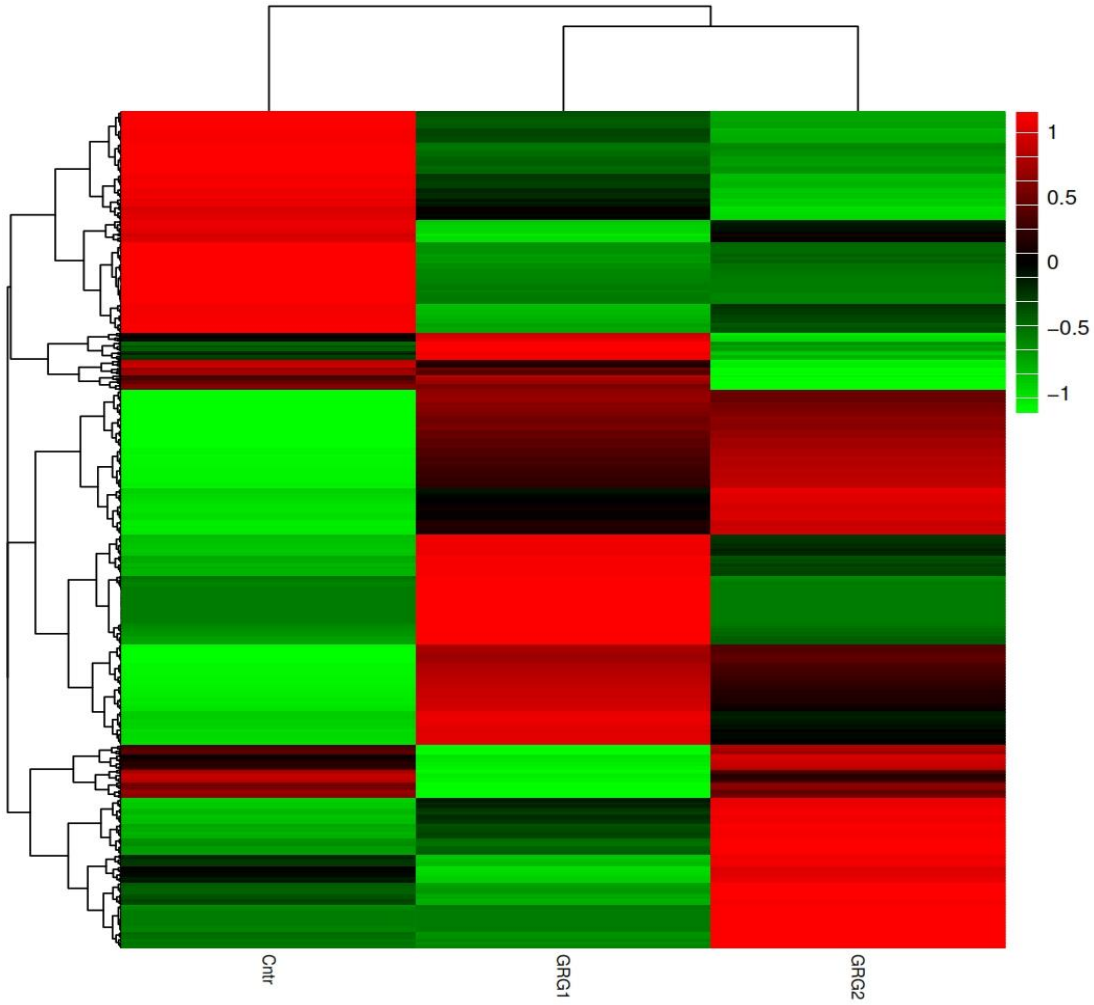


Figure 4.26: Cluster diagram showing the differentially expressed genes in each treatment

4.3.6.3 Functional analysis

Enrichment analysis of differentially expressed genes was conducted to identify the biological functions or pathways significantly associated with them. ClusterProfiler software (Yu et al., 2012) was used for this purpose. The transcriptome data analysis is presented in **Figure 4.27, Figure 4.28** and **Figure 4.29**, where the pathways are presented on the vertical axis and the gene ratio on the horizontal axis. The size and colour of each point indicate the adjusted p-value (padj) and number of differentially expressed genes (count), respectively. Pathways with larger points had more differentially expressed genes, and those coloured red were statistically

significant. Furthermore, pathways with points farther to the right had higher gene ratios, explaining the higher proportion of differentially expressed genes. In this research, pathways with a gene count >10, a gene ratio above 50%, and a padj between 0.00 – 0.25 were set as criteria for consideration of biological responses significantly affecting the physiology of the grown shrimps in various treatment conditions.

Using this established benchmark, the key pathways that significantly affect the biological responses of shrimp under various treatment conditions were identified. For GRG 1 vs. control, important pathways included proteasomes, autophagy-animals, and phagosomes. In GRG 2 vs. Control, the significant pathways were aminoacyl-tRNA biosynthesis and protein processing in the endoplasmic reticulum. GRG 1 vs. GRG 2 only had oxidative phosphorylation, which emerged as a significant pathway.

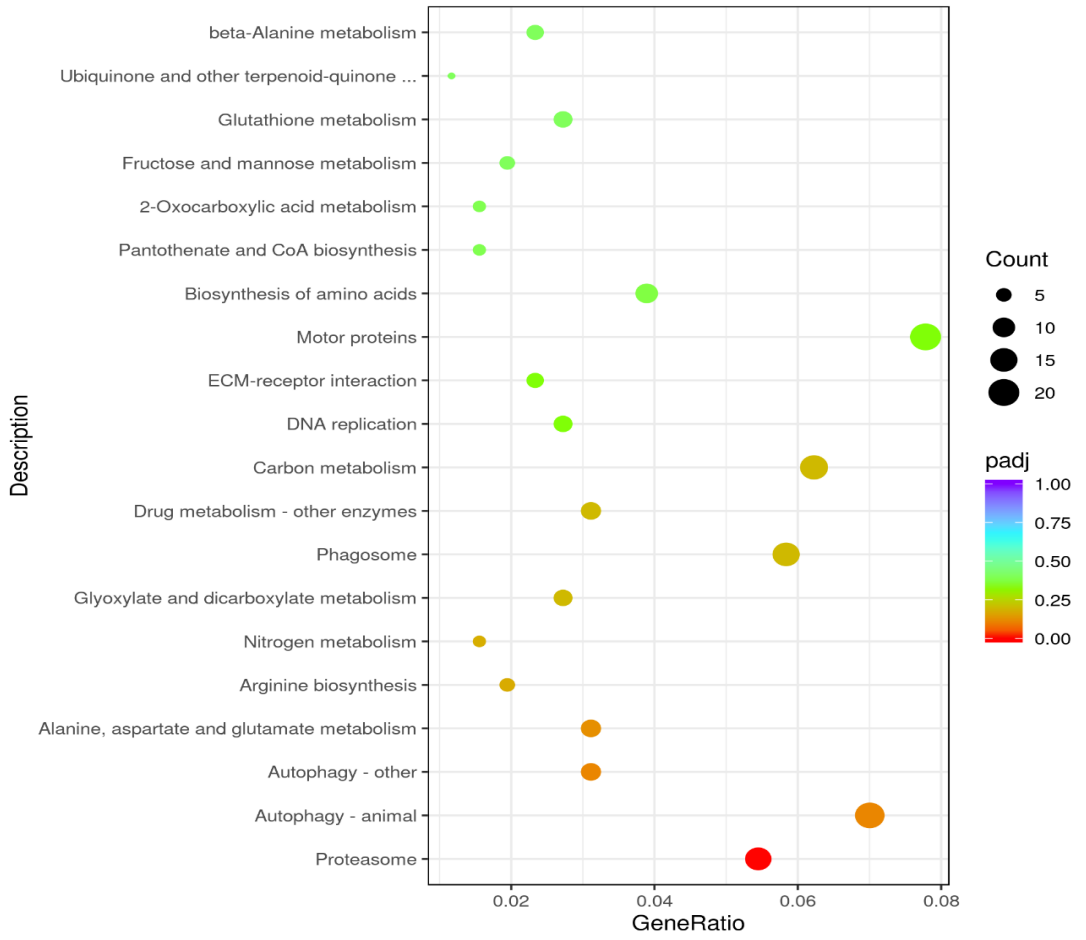


Figure 4.27: Functional analysis of GRG 1 vs. Control comparison of DEG

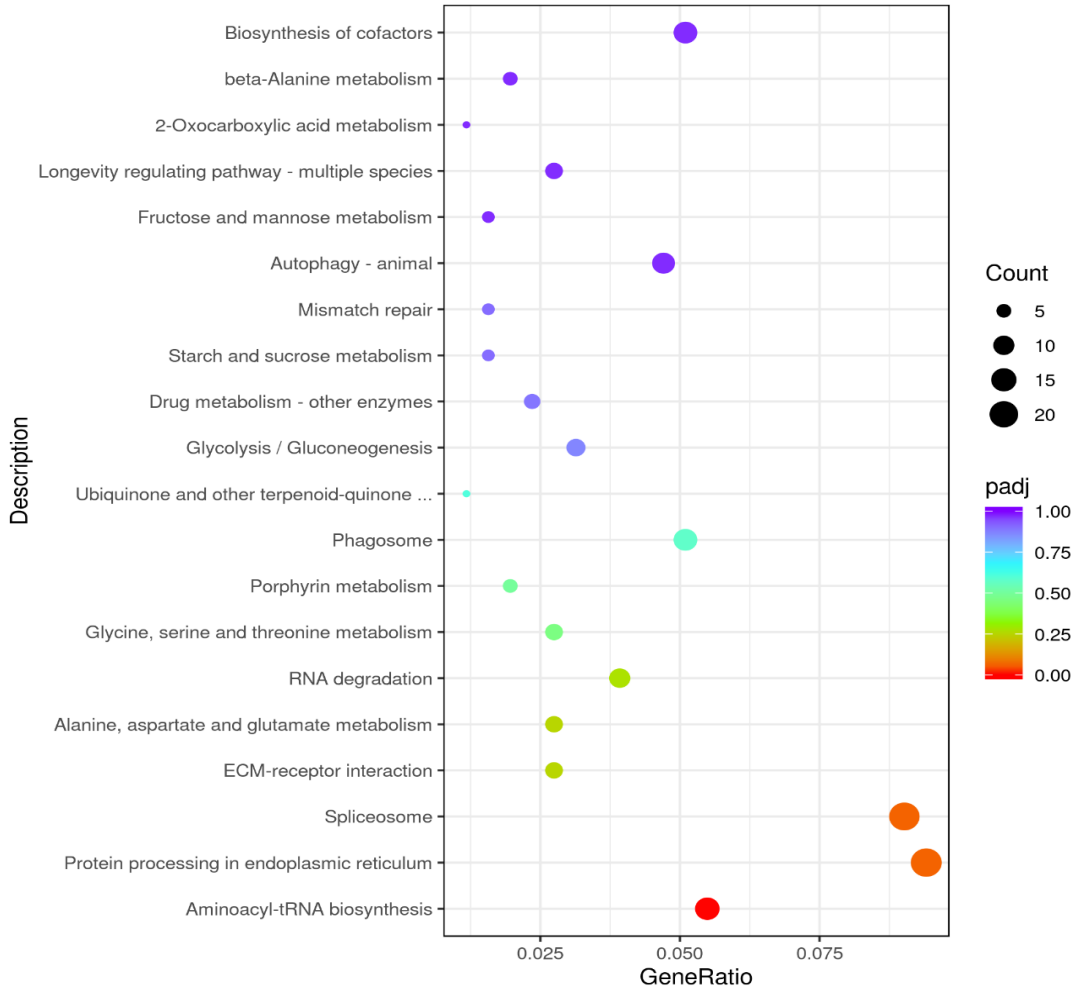


Figure 4.28: Functional analysis of GRG 2 vs. Control comparison of DEG

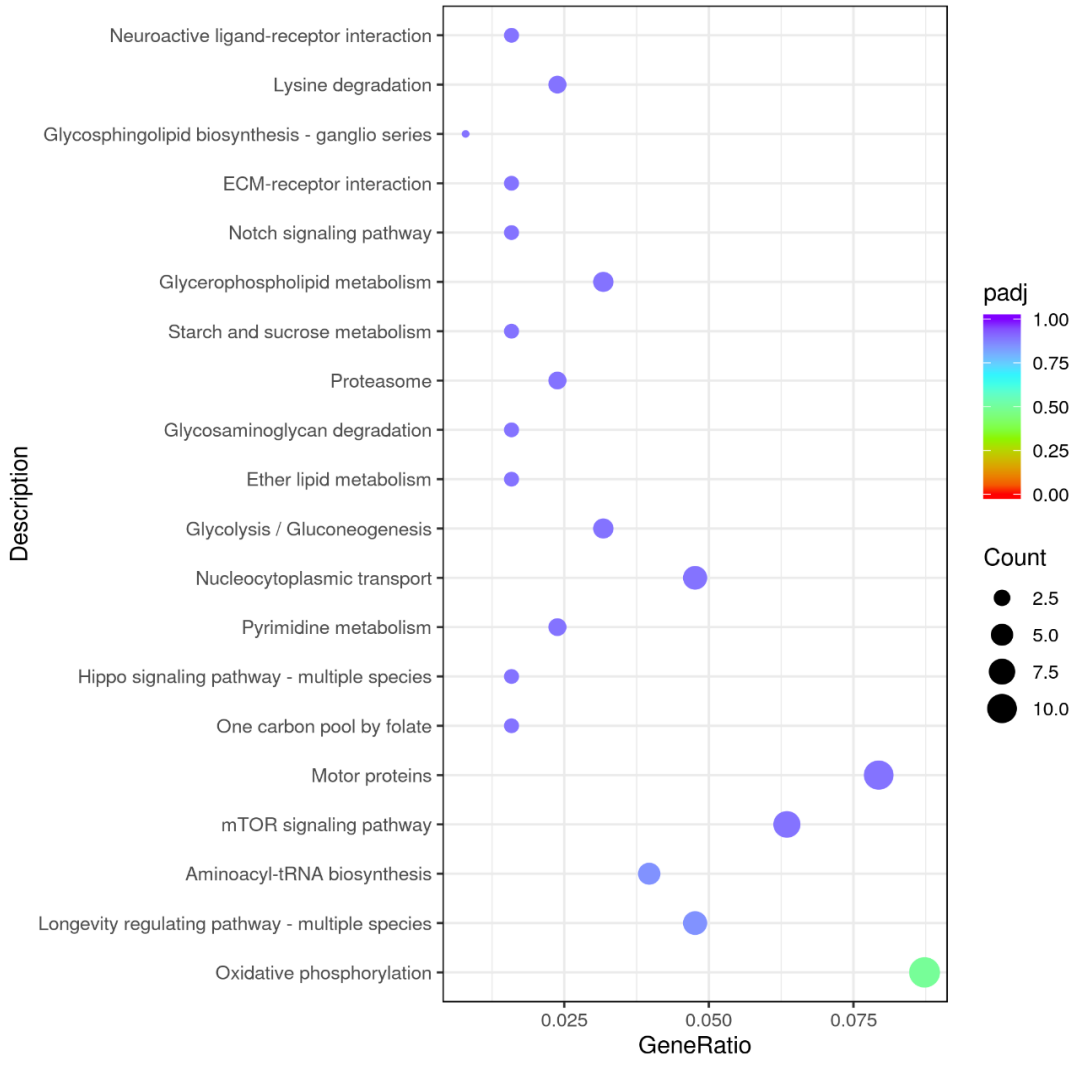


Figure 4.29: Functional analysis of GRG 1 vs. GRG 2 comparison of DEG

4.3.6.4 Pathways of DEGs observed in the treatment comparisons

The pathways of the DEGs confirmed by the functional analysis are presented for each comparing pairs of the treatments as follows.

4.3.6.4.1 GRG1 versus Control

4.3.6.4.1.1 Proteosome

Figure 4.30 presents the proteasome pathway (pvm03050) responsible for the regulation of intracellular protein degradation, which is essential for maintaining cellular homeostasis and regulating various cellular processes. Several key components of the proteasome pathway were identified in the differential gene expression (DEG) analysis of GRG 1 versus Control. The regulatory particles, which are part of the 26S proteasome, include subunits from both the PA700 (Lid) and PA (Base) complexes. The PA700 (Lid) complex includes Rpn5, Rpn6, Rpn7, Rpn8, Rpn10, and Rpn11, all of which function in the recognition and unfolding of ubiquitinated proteins with a function score of 3. The PA (base) complex comprised Rpn2, Rpt1, Rpn13, and Rpt3, with Rpn13 and Rpt3 having a function score of 2 and Rpn2 and Rpt1 having a score of 3. Additionally, the core particles of the 20S proteasome, responsible for the proteolytic degradation of proteins, included the standard subunits α2, α4, β2, and β4. α2, α4, and β2 had function scores of 2, whereas β4 had a function score of 4.

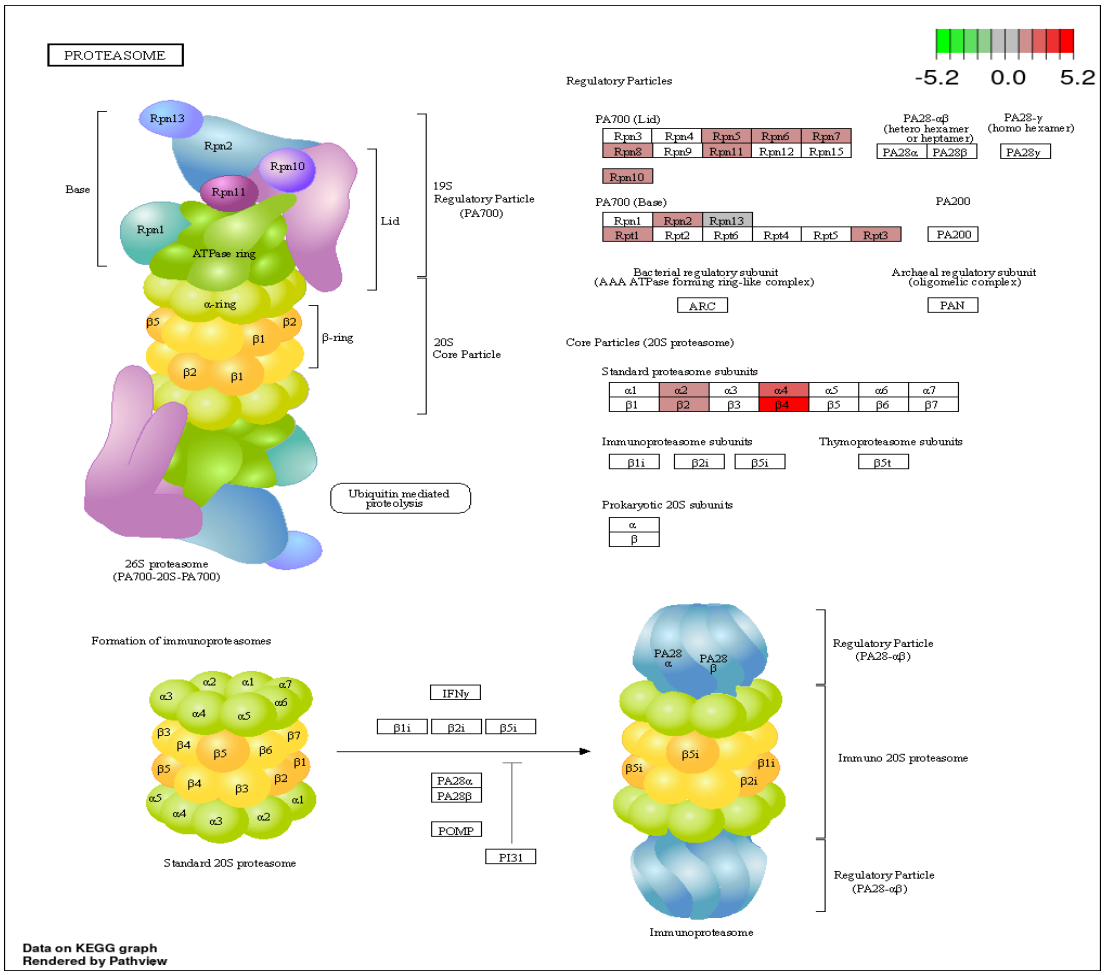


Figure 4.30: Proteosome pathway of the DEG in GRG1 vs. control

4.3.5.4.1.2 Autophagy-animal

Figure 4.31 shows the gene expression of the autophagy and signal transduction pathways (pvm04140) in GRG 1 compared to the control group. MEK1/2 (Ras-related protein M-Ras), involved in growth and differentiation signal transduction pathways, is downregulated with an expression ratio of -3.5, indicating a potential suppression of these pathways in GRG 1. In contrast, BNIP3 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 3), which responds to cellular stress such as hypoxia, was upregulated by 3.2-fold, indicating an enhanced stress response. Genes involved in autophagy regulation were significantly upregulated. TSC1 (tuberous sclerosis 1) and AMPK (5'-AMP-activated protein

kinase, catalytic alpha subunit), both crucial for inhibiting mTORC1 activity and maintaining cellular energy homeostasis, were increased by 4-fold. LC3 (GABA(A) receptor-associated protein), which is essential for the expansion and maturation of autophagosomes, showed a 5-fold increase in autophagic activity. Similarly, mTOR (serine/threonine-protein kinase mTOR), a central regulator of cell growth and autophagy, was upregulated by 3.3-fold. The initiation of autophagy was further supported by a 4.3-fold upregulation of ULK1/2 (serine/threonine protein kinase ULK2). Rab1a (Ras-related protein Rab-1A), a regulator of intracellular vesicle trafficking, showed a 2.5-fold increase, indicating enhanced vesicle trafficking activities. ATG2 and ATG9L (autophagy-related proteins) were upregulated 4-fold, reflecting their critical roles in autophagosome formation and the maintenance of cellular homeostasis. VMP1 (vacuole membrane protein 1), which plays a role in autophagosome formation and membrane dynamics, was downregulated by -2.5-fold, suggesting potential alterations in membrane dynamics in GRG 1. Beclin1, VPS15, and VPS34, which are involved in autophagosome maturation, showed low upregulation (1-to 1.5-fold), indicating active autophagy. Jummy (myotubularin-related protein 14), essential for autophagosome formation, maturation, and lysosomal function, increased by 4.3-fold, indicating active autophagy. Lastly, cathepsin L (CTSL) and cathepsin B (CTSB), key proteases in the autophagic degradation phase, were upregulated (1.5-to 2-fold), representing enhanced autophagic degradation activity in GRG 1. The upregulation of genes involved in autophagy and the cellular stress response in GRG 1, indicates significant alterations in cellular homeostasis and stress management pathways compared to the control.

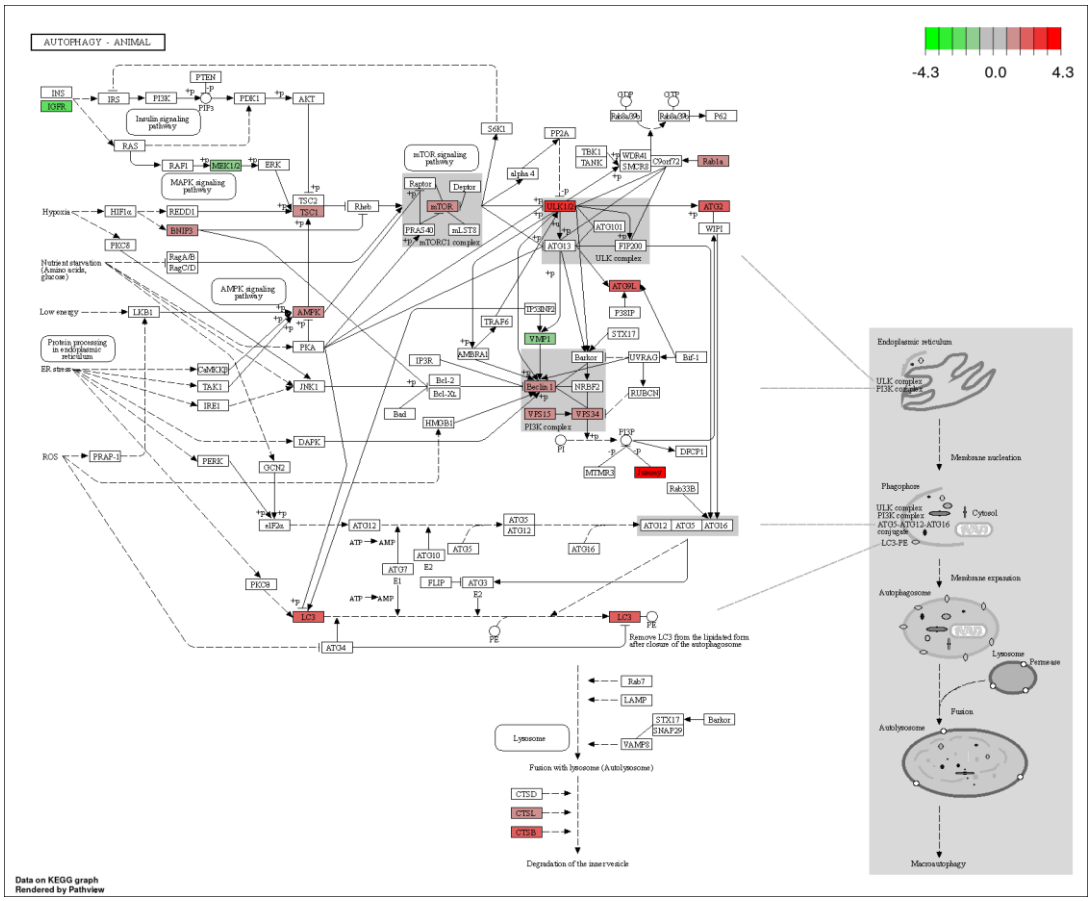


Figure 4.31: Autophagy – animal pathway of the DEG in GRG1 vs. control

4.3.6.4.1.3 Phagosome

Figure 4.32 shows a significant upregulation of genes involved in the cytoskeletal pathway (pvm04145) in the GRG 1 group and the control group. Tubulin α (TUBA) showed a ten-fold increase, indicating enhanced microtubule formation and activity in mRNA biogenesis and transport useful in cellular structural integrity and communication. Tubulinβ (TUBB), upregulated five-fold, supported increased microtubule dynamics and stability useful for chromosomal segregation during cell division. Similarly, Actinα/γ1 (F-actin), Phosphatidylinositol 3-Kinase (VPS34), Cathepsin L, and various integrins (α2β1, α5β1, β1) showed five-fold increases, explaining their roles in cell motility, signal transduction, protein degradation, and cell adhesion. Thrombospondin (TSP) increased in twenty-two-fold signifying an enhanced cell motility and extracellular matrix interactions. These findings showed

substantial alterations in cytoskeletal organisation and function in GRG 1 (biofloc treatment), with enhanced cellular dynamics, signalling processes, and structural integrity compared to the control.

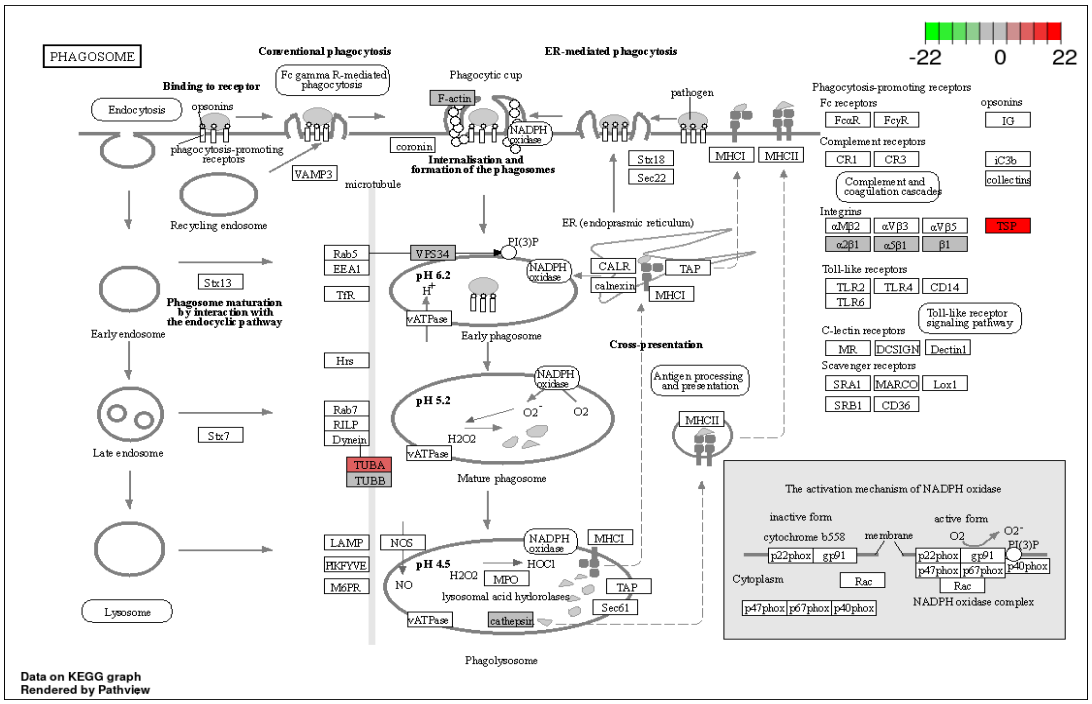


Figure 4.32: Phagosome pathway of the DEG in GRG1 vs. Control

4.3.6.4.2 GRG 2 versus Control

4.3.6.4.2.1 Aminoacyl-tRNA biosynthesis

The pathway for the differential expression of aminoacyl-tRNA synthetases (pvm00970) in GRG1 versus Control is presented in **Figure 4.33**. Alanyl-tRNA synthetase (EC: 6.1.1.7), which catalyzes the attachment of alanine to its cognate tRNA, exhibited upregulation (fold-change = 1), representing an increase in alanine incorporation into proteins. Conversely, several other aminoacyl-tRNA synthetases were downregulated. Glutaminyl-tRNA synthetase (EC: 6.1.1.18), responsible for attaching glutamine to its corresponding tRNA, had a fold change of -3; aspartyl-tRNA

synthetase (EC: 6.1.1.12), involved in aspartate-tRNA charging, showed a fold change of -3; and glycyl-tRNA synthetase (EC: 6.1.1.14), which attaches glycine to its cognate tRNA, recorded a fold change of -2. These findings indicate alterations in amino acid availability and metabolic processes. The upregulation of alanyl-tRNA synthetase suggests a possible increase in alanine utilisation for protein synthesis, whereas the downregulation of glutaminyl-tRNA synthetase, aspartyl-tRNA synthetase, and glycyl-tRNA synthetase may reflect decreased incorporation of glutamine, aspartate, and glycine into proteins, respectively, in GRG1.

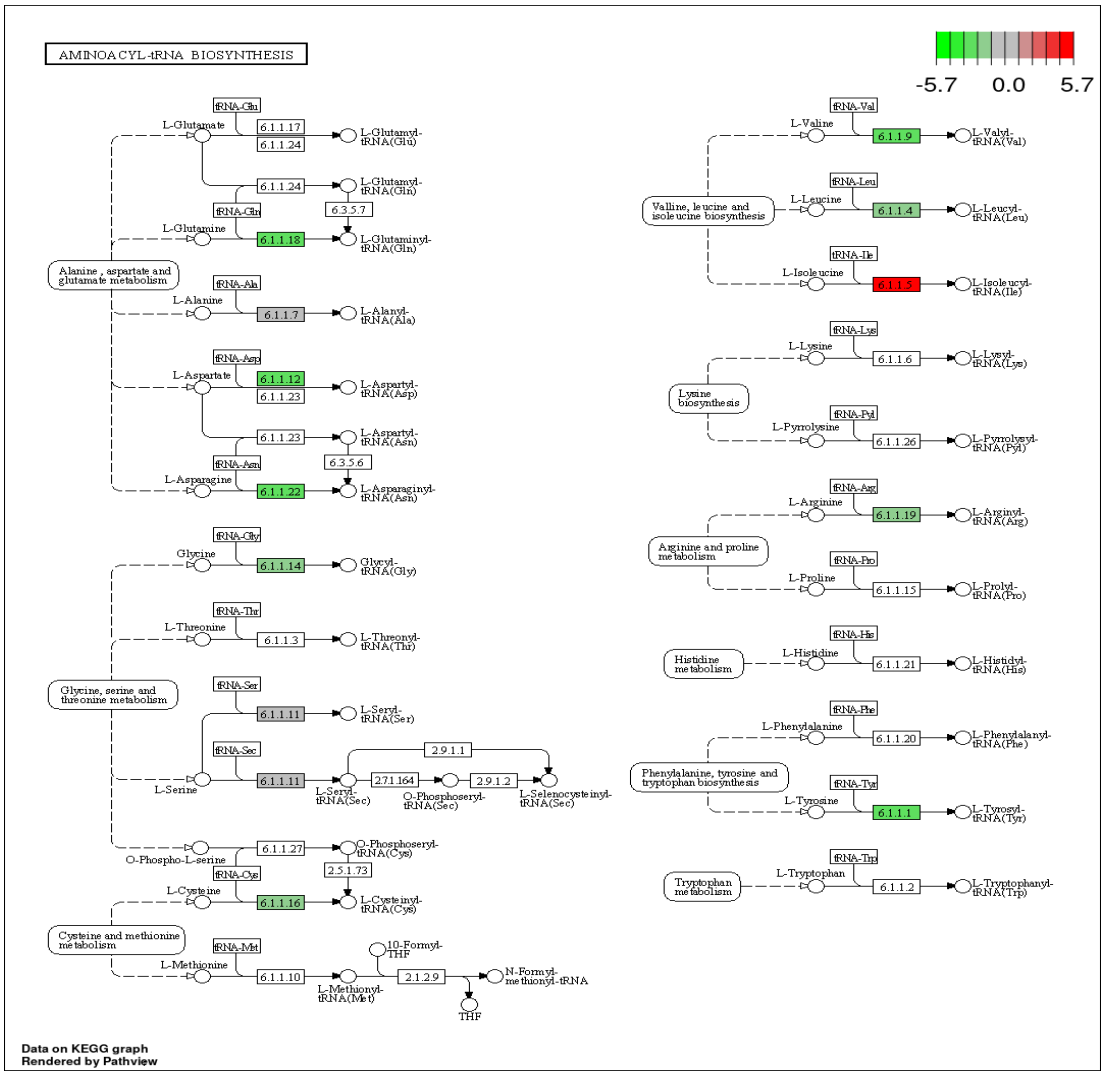


Figure 4.33: Aminoacyl-tRNA biosynthesis pathway of the DEG in GRG 2 vs. Control.

4.3.6.4.2.2 Protein processing in endoplasmic reticulum

Figure 4.34 presents the differential expression of key proteins involved in the endoplasmic reticulum (ER) protein processing pathway in GRG 2 versus the control treatment. Calnexin (CNX) and the molecular chaperone NEF, both essential for protein folding and quality control, were downregulated by 2-fold. BiP, which is essential for protein processing, was significantly downregulated by 4-fold, while Hsp40, which supports protein folding and disulfide bond formation, was downregulated by 4-fold. eIF2α, which regulates translation initiation, was significantly downregulated by 5-fold. In contrast, ASK1, which coordinates cellular responses to ER stress, was upregulated by 2-fold. SEC23/24, which is involved in protein transport from the ER to the Golgi apparatus, was upregulated by 5-fold. TRAP, which is involved in the co-translational targeting and translocation of proteins into the ER, was downregulated 5-fold. Heat shock proteins Hsp70 and Hsp40, crucial for protein folding and quality control, showed differential expression, with Hsp70 upregulated by 4-fold and Hsp40 upregulated by 2-fold. UBX domain-containing protein (ubx), which is involved in protein quality control and ER stress response, was upregulated 2-fold. The transitional ER ATPase p97 and ubiquitin fusion degradation protein ufd1, vital for maintaining ER homeostasis and proper protein folding, were upregulated by 3-fold. Png1, which catalyzes the removal of N-glycans from glycoproteins, showed a marginal 1-fold upregulation.

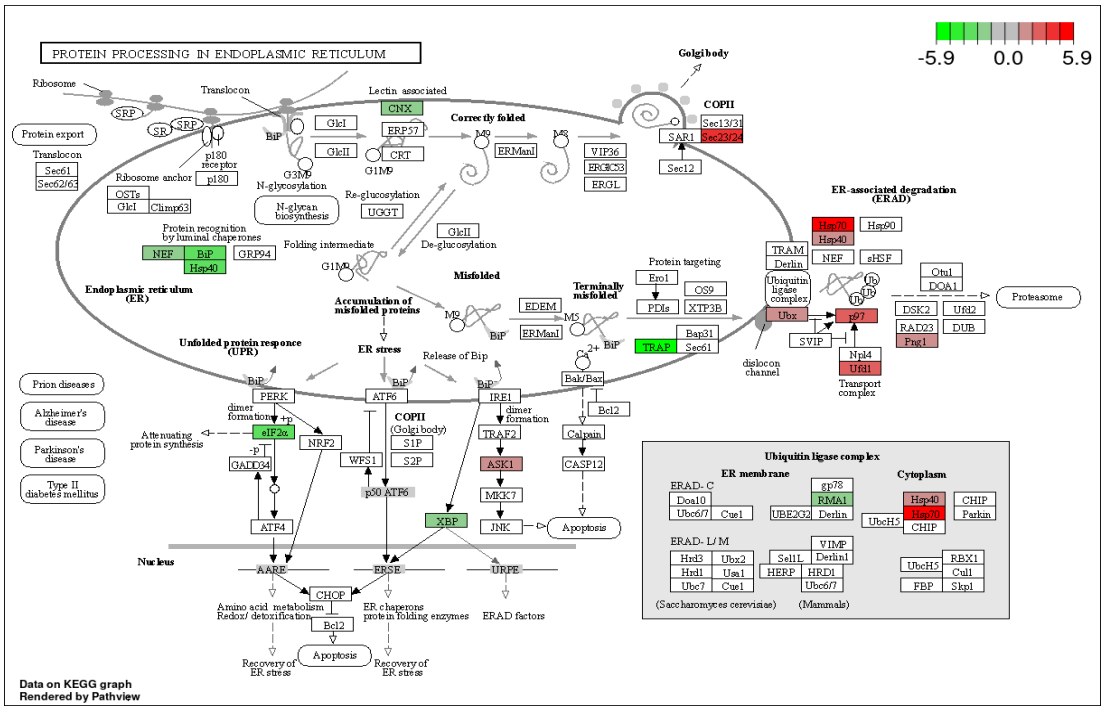


Figure 4.34: Protein processing in endoplasmic reticulum pathway of the DEG in GRG 2 vs. Control.

4.3.6.4.2.3 GRG 1 versus GRG 2

4.3.6.4.2.2.1 Oxidative phosphorylation

Figure 4.35 presents the pathway for oxidative phosphorylation in the comparison of GRG 1 and GRG 2. NADH dehydrogenase, a vital component of the electron transport chain (ETC), includes several subunits, such as ND1, ND3, ND4, ND4L, and ND5, all of which contribute to electron transfer, proton pumping, and the generation of the proton motive force crucial for ATP synthesis. These subunits showed varying gene expression ratios, with ND3, ND4, ND4L, and ND5 showing upregulation ratios of 2, 1, 1, and 1, respectively. This explains the increased electron transfer activity and proton pumping that are essential for efficient energy production. Additionally, Ndufs3 and Ndufv2, which are components of NADH dehydrogenase, showed upregulated gene expression ratios of 2 and 4, respectively, indicating their significant roles in oxidative phosphorylation and ATP synthesis. In contrast,

cytochrome C oxidase (COX) subunits, such as COX5B and CYC, which are involved in the final step of electron transfer to oxygen, showed downregulated gene expression ratios of -2 and -1, respectively, indicating a potential decrease in energy production efficiency. The V-type ATPase subunit D, responsible for pH regulation and ion homeostasis, showed an upregulated gene expression ratio of 2 owing to its enhancement of ATP synthesis by maintaining optimal cellular conditions.

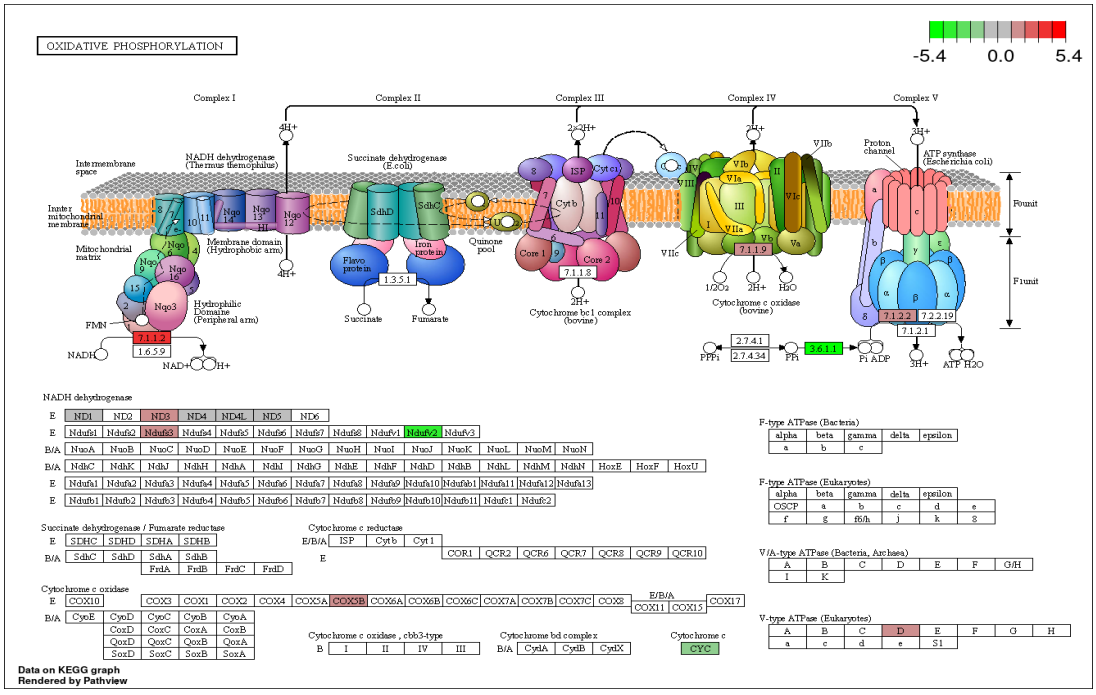


Figure 4.35: Oxidative phosphorylation pathway of the DEG in GRG1 vs. GRG 2

4.4 Discussion

The analysis of water quality parameters across different treatments revealed significant variations in electrical conductivity (EC), total dissolved solids (TDS), salinity, total ammonia nitrogen, nitrite levels, and dissolved oxygen (DO). These differences indicated the impact of varying stocking densities, C/N ratios, and probiotic concentrations on the performance of the biofloc system. GRG 1 treatment,

defined by a stocking density of 200 shrimp.m^{-3}, C/N ratio of 15, and a probiotic dosage of 14 ml/L. This finding agrees with that of Minabi et al., (2020), who noted that EC levels tended to increase with higher C/N ratios. Tasnim et al., (2022) emphasized that higher conductivity has a negative impact on most water quality parameters. The Optimum treatment in Experiment 1 and the Control treatment in Experiment 2 were significantly higher than those in GRG 1 and GRG 2. Minabi et al., (2020) also reported that TDS levels increased as the carbon to nitrogen (CN) ratio in the biofloc system rose from 11:1 to 23:1. This increase in TDS was observed alongside an increase in water electrical conductivity (EC) and floc volume (FV) in the experimental treatments. The electrical conductivity level is linked to the concentration of dissolved ions essential for the growth and activities of beneficial microbial community in biofloc system. Electrical conductivity relates closely with total dissolved solids and salinity because their concentrations are controlled by similar ions.

The variations in TDS levels were in line with the changes observed in other water quality parameters, such as EC, floc volume, and total suspended solids, as the C/N ratio was adjusted in the biofloc system. Mohammadi et al., (2023) noted that a TDS concentration of 400-600 mg/L is suitable for shrimp species. Research indicates that while TDS levels below 600 mg/L are generally recommended for *Litopenaeus vannamei*, concentrations in the range of 400-600 mg/L also support shrimp growth and system performance. Rajkumar et al (2016) reported TDS as 500 mg/L in biofloc system which is within the range in this experiment. TDS concentrations above 600 mg/L is known to obstruct gills, leading to increased mortality and reduced growth rates (Schveitzer et al, 2023). However, these values were considerably lower than those reported in the present study. Khanjani et al., (2020) subjected juveniles of Pacific whiteleg shrimp to salinity levels of 10 - 32 ppm and noted the highest increase in growth performance and survival rate was observed in the salinity level of 32 ppm. The findings from this research also recorded the salinity within this range, with the best performance recorded at 28.06 ppm. The high TDS, salinity and low pH (acidic) could indicate an accumulation of dissolved organic matter from unconsumed feed which caused the release of acidic-by-product such as ammonia. Nutrient breakdown of organic waste by microbes in biofloc system affect ion concentration. Accordingly,

variations in the control factors (stocking density, C/N ratio and probiotic dosage) affected the microbial composition in each biofloc treatment. This also affected the salinity as noted in the results. The Optimum treatment in Experiment 1 showed significantly lower total ammonia-nitrogen levels compared to the Control, GRG 1, and GRG 2 treatments. Chen et al., (2022) tested the impact of stocking density on total ammonia nitrogen (TAN) and reported 0.55 mg/L from the highest stocking density. The experiment showed that the total ammonia nitrogen levels were influenced by the stocking density of shrimp larvae, with the highest density (D400) leading to elevated concentrations of this parameter. In addition, Khanjani et al., (2022) found that total ammonia nitrogen levels were significantly higher in the control group (density of 1000 shrimp m^3 with 50 daily water exchanges) than in the biofloc treatments with limited water exchange at various stocking densities (T1:1000 shrimps, T2:2000 shrimps, T3:3000 shrimps, T4:4000 shrimps/m^3). The results indicated that the biofloc system helped reduce the total ammonia nitrogen levels.

The control treatment had higher nitrite levels than GRG 1, GRG 2, and optimum treatments. Huang et al., (2022) reported that nitrite levels were significantly influenced by the type of carbon source used in the biofloc system. Specifically, the highest nitrite levels were observed in the molasses treatment, indicating a potential impact of the carbon source on nitrite concentrations in the system, which were within acceptable limits for *L. vannamei* culture. Dissolved oxygen levels were the highest in GRG 1 and the Optimum treatment in Experiment 1. Kaya et al., (2020) reported that DO levels below 3 mg/L can lead to poor feed intake and negatively affect the health of fish and other aquaculture organisms in biofloc systems. DO values obtained in this study were above this threshold. de Morais et al., (2020) explained the importance of maintaining adequate dissolved oxygen levels for nitrifying bacteria activity, growth, and reproduction such as Ammonia-Oxidizing Bacteria (AOB) and Nitrite-Oxidizing Bacteria (NOB) which require oxygen for cellular functions and nitrification processes. DO levels were highest in GRG 1 and the optimum treatments in experiment 1. Maintaining adequate DO levels is essential for maintaining the performance of aquatic organisms. The high DO levels in GRG 1 and the optimum treatment suggests an enhanced environment for both shrimp and beneficial microbial activity, supporting better growth and health outcomes. In summary, GRG 1 treatment consistently showed

better performance in terms of water quality parameters, probably because of its balanced C/N ratio, optimal stocking density, and probiotic concentration.

Evaluation of the IMTAS system for *L. vannamei* culture was conducted based on several physiological parameters. The highest plasma protein levels were observed at the stocking density of 200 shrimp/m^3, C/N ratio, and probiotic dosage level 3. Chuang et al., (2016) reported a decrease in total plasma protein in shrimp challenged with *Vibrio harveyi*, while Sánchez-Paz et al., (2007) indicated that protein concentration in the plasma is significantly affected by short-term starvation, demonstrating its role as a primary energy source during food shortages. Sadat et al., (2018) reported that specific probiotic dosages significantly increased plasma protein levels in Whiteleg shrimp while Selven & Philip, (2013) observed a higher haemolymph protein in shrimp exposed to increased salinity levels post-infection with *V. harveyi*, suggesting a stress response. The recorded glucose levels were mostly within ranges previously reported by Su et al (2022) and Pascual et al (2003). Haemocyanin had the highest concentration at stocking density level 1, C/N ratio level 3, and probiotic dosage level 3. Mendoza-Porras et al., (2020) described the role of haemocyanin in immune responses, including antibacterial and antiviral activities. Zhao et al., (2020) also noted that high ammonia levels impair the oxygen transport function of haemocyanin, thereby affecting disease susceptibility in shrimp. Jiménez-Ordaz et al., (2021) reported that exposure to nitrite and nitrate significantly decreases oxyhaemocyanin levels, which are responsible for oxygen transport.

Glycogen levels were the highest at GRG 1. Zhang et al., (2022) reported significant increases in glycogen levels in the hepatopancreas and muscle of shrimp post-ammonia-N exposure, with the highest levels at 12 and 6 hours respectively. Bao et al., (2020) observed a decrease in glycogen level in the liver of Chinese mitten crabs under hypoxia, indicating energy store depletion during stress. Mendoza-Porras et al., (2020) also noted significant glycogen and glucose level decreases in *Penaeus vannamei* after prolonged fasting. The glucose concentration was also at GRG 1. Chakrapani et al., (2021) linked higher glucose metabolism and growth performance in shrimp reared in biofloc systems to the upregulation of the hexokinase and pyruvate

kinase genes. Yong et al., (2020) also suggested that diet-modulated haemolymph metabolites contribute to the stress response in shrimp. Mahasri et al., (2022) found that high stocking density increase the blood glucose levels in shrimp. The Hepatosomatic Index (HSI) was optimal at GRG 1. Lu et al., (2020) reported an inverse relationship between HSI and dietary protein levels. Prophenoloxidase (ProPO) and Superoxide Dismutase (SOD) activities also showed optimum levels for specific combinations of control factors. Boonchuen et al., (2021) reported that the ProPO system is essential for antibacterial immunity in shrimp. Additionally, activation of ProPO system leads to melanization in neutralising pathogens Paul et al., (2020) and plays a dual role in the activation of ProPO for antimicrobial activity Thongsoi et al., (2024). GRG1 and GRG 2 were experimental units in experiment 2 (IMTAS) while 'optimum' was the best performance in experiment 1 (the traditional biofloc system). This indicates that IMTAS performed better than the traditional biofloc system. The survival rates reported in biofloc system generally range between 23.69 – 97.20%. While Manan et al (2020) reported the least survival rate (23.69%) in biofloc system, Said, (2024) recorded a survival of 97.20% for whiteleg shrimp in biofloc condition. The moderate survival rate recorded in this research (43.19 – 59.78%) could have been due to handling during sampling.

The assessment of gut microbial communities in *L. vannamei* under different rearing conditions revealed significant variations in microbial composition and diversity. GRG1 and GRG2 treatments were particularly effective in enhancing the diversity and abundance of beneficial microbes compared with the Control. This was enhanced by the addition of probiotics to the culture medium. Vázquez-Euan et al., (2022) reported that probiotics enhance microbial ecosystem diversity and functionality and improve the gut health of shrimp. Distinct differences in microbial taxa were observed among treatments. Bacteroidetes was present in GRG2, but absent in GRG1 and the Control. de Souza Valente et al., (2020) reported that Bacteroidota present in the gut of Pacific Whiteleg shrimp influenced the gut microbiota composition in shrimp. Additionally, microbial families such as Shewanellaceae, Aeromonadaceae, and Burkholderiaceae showed varying mean relative abundances across treatments, indicating the impact of different rearing conditions on microbial composition, as previously reported by Padeniya et al., (2022). The absence of

Aeromonadaceae in GRG 1, despite its presence at low levels in GRG 2 and the Control, could be due to differences in microbial community dynamics in the culture systems. GRG 1 might have had conditions such as higher competition from other microbial taxa, specific water quality parameters, or lower organic matter availability that were unfavourable for Aeromonadaceae proliferation. Conversely, GRG 2 and the Control may have provided niches or conditions that supported their survival, albeit at low levels. The microbial community structure varied significantly across different rearing conditions, as evidenced by differences in the relative abundances of various phyla and families. These results demonstrate the capacity of biofloc treatments (GRG1 and GRG2) to promote a more diverse and functional microbial ecosystem in the shrimp gut, potentially leading to improved shrimp gut health compared to the control group. The higher abundance of *Fusobacteriaceae* in GRG 2, despite its suboptimal survival and growth performance, suggests that *Fusobacteriaceae* may not play a dominant role in enhancing shrimp performance. Its abundance might instead reflect differences in microbial community dynamics or environmental conditions specific to GRG 2. In contrast, GRG 1, with the best growth performance, likely benefited from a more favorable microbial composition or other combined factors that supported shrimp health and growth more effectively. The "others" category in GRG 1 likely includes microbial families such as Rhodobacteraceae, which are known for their roles in nutrient cycling, water quality maintenance, and probiotic effects. These microbes may enhance system functionality by breaking down organic matter, removing toxic compounds, and supporting shrimp health through improved gut microbiota and immunity. The higher abundance of these diverse microbes in GRG 1 suggests a more balanced and beneficial microbial community, potentially contributing to the superior growth and survival rates observed in this group.

GRG 1 (biofloc treatment stocked at 200 shrimp/m3, C/N ratio of 10, and probiotic dosage of 14 ml/L) versus Control had the most expressed gene differentials. The significant variation between the biofloc conditions in GRG1 and the Control resulted in a high gene count. There is a significant influence of the proteasome pathway and protein modulation through enhanced degradation of misfolded proteins and promotion of autophagy and ER stress response mechanisms. The significant upregulation of Rpn5, Rpn6, Rpn7, Rpn8, Rpn10, Rpn11, Rpn2, Rpt1, Rpn13, Rpt3,

α2, α4, β2, and β4 resulted in improved protein conversion and cellular regulation in GRG 1. This explains the improved cellular homeostasis, stress management, and growth of the shrimp. Said et al., (2022) reported that the upregulation of these components enhanced protein utilisation and cellular regulation. Furthermore, Dong et al., (2021) confirmed the positive impact of biofloc on gene expression, stress response, and growth in shrimp. Biofloc treatment impacts gene expression, stress, and growth in shrimp, especially at high stocking densities, which negatively affects their performance and gene expression. Biofloc treatment significantly modulated the autophagy pathway by influencing key regulatory genes. Upregulation of TSC1, AMPK, and ULK1/2 triggers the initiation of autophagy in response to stress (Nguyen et al., 2024) as observed in the DEG comparing GRG 1 and control. Higher levels of LC3, ATG2, and ATG9L indicate active autophagosome formation, essential for autophagic degradation Broadbent et al., (2023) as confirmed in the gene expression.

Increased Rab1a levels support the movement and maturation of autophagic vesicles, ensuring proper execution of autophagy. The upregulation of mTOR, alongside other autophagy-promoting genes, suggests a balance between cell growth and autophagy, which is useful in the adoption of shrimp reared in the biofloc environment. Biofloc technology has prompted autophagy in shrimp which enhanced their ability to cope with stress and maintain cellular homeostasis in the culture system. The presence of beneficial microbial communities in BFT enhances the phagosome pathway responsible for the control of genes and proteins involved in immune responses and cellular functions in cultured shrimp. An increased in phagocytic activity of immune cells stimulates the activities of genes involved in pathogen processing (Asiri, 2024). Phagosome pathway has been reported to influence the expression and organization of cytoskeletal components such as Tubulin α/β and Actin α/γ1, so as to improve cell motility and phagosome maturation (Mao et al., 2020).

BFT enhanced the activity of VPS34, which is responsible for phagosome-lysosome fusion and pathogen degradation. Upregulation of Cathepsin L further enhanced proteolytic activity within phagosomes, whereas an increase in integrins improved immune cell adhesion, migration, and activation. BFT improves the immune

response of cultured shrimp through the activities of genes and proteins. Biofloc technology (BFT) significantly affects the aminoacyl-tRNA biosynthesis pathway required for protein synthesis. Gupta et al., (2023) reported that BFT upregulated alanyl-tRNA synthetase, which improved the incorporation of alanine into proteins for protein synthesis and cellular function. Conversely, BFT downregulates glutaminyl-, aspartyl-, and glycyl-tRNA synthetases to reduce their availability in the aminoacyl-tRNA pathway. This selective downregulation directs protein synthesis towards alanine-rich proteins to stimulate the adoption of shrimp in the biofloc environment. This modulation of aminoacyl-tRNA synthetases supported the efficient protein synthesis required for the growth and survival of cultured shrimp.

BFT downregulates calnexin, BiP, Hsp40, and eIF2α during protein processing in the endoplasmic reticulum (ER) pathway to reduce the need for the unfolded protein response (UPR) for efficient utilisation in BFT. Sgnaulin et al., (2021) reported that BFT downregulated Calnexin, BiP, Hsp40, and eIF2α to reduce unfolded protein response (UPR). Concurrently, it upregulated SEC23/24, Hsp70, UBX, p97, and ufd1 to improve protein trafficking, folding, and the ER-associated degradation (ERAD) pathway. Belenichev et al., (2023) also stated that SEC23/24, UBX, p97, and ufd1, which are linked to protein trafficking, are upregulated under stressed conditions. These changes improved the efficiency of protein processing and reduced the cellular tolerance relevant for the management of stress in cultured shrimp in the biofloc system. BFT upregulates NADH dehydrogenase subunits in the oxidative phosphorylation pathway required for cellular energy generation in the mitochondria to improve ATP synthesis. On the other hand, cytochrome C oxidase subunits were downregulated to adjust cellular metabolism, energy production, and oxidative stress responses required for metabolic processes in cultured shrimp biofloc systems.

4.5 Conclusion

This study assessed the effects of biofloc treatments on water quality, physiological responses, gut microbial communities, and gene expression in *L. vannamei*. GRG 1 treatment, characterised by a stocking density of 200 shrimp/m^3, C/N ratio of 10, and probiotic concentration of 14 ml/L, had the most favourable water quality conditions. Assessment of some physiological indices also suggested that the combination of ideal levels of stocking density, C/N ratio, and probiotic dosage combinations had a significant impact on plasma protein, haemocyanin, glycogen, glucose levels, and Hepatosomatic Index (HSI). The highest levels of plasma protein and haemocyanin were observed in shrimps reared at 200 shrimp/m^3 stocking density, C/N ratio of 20 and 7 ml/L probiotic dosages. The biofloc treatment also improved glucose metabolism and immune responses of the cultured shrimp as shown by increased prophenoloxidase (ProPO) and superoxide dismutase (SOD) activity.

Analysis of gut microbial communities indicated that GRG1 and GRG2 treatments promoted a more diverse and beneficial microbial ecosystem than the control. Notably, the presence of specific microbial families, such as Bacteroidetes, in GRG 1 proved the positive impact of biofloc systems on microbial diversity and gut health. This suggests that biofloc systems significantly enhance digestive efficiency in shrimps. The gene expression analysis revealed significant upregulation of genes involved in proteasome activity, autophagy, phagosome formation, aminoacyl-tRNA biosynthesis, and oxidative phosphorylation pathways in shrimp treated with biofloc. The enhanced expression of proteasome components and autophagy-related genes in GRG 1 indicated improved protein utilisation, immune responses, cellular functions and stress management needed for the enhanced growth and survival of the cultured shrimps. This research could establish that biofloc technology, particularly GRG 1 treatment, significantly improved water quality, physiological health, gut microbial diversity, and gene expression in *L. vannamei*.

CHAPTER 5

NUTRIENT EVALUATION OF BIOFLOC MEAL HARVESTED FROM THE INDOOR MULTI-TECHNO AQUACULTURE SYSTEM

5.1 Introduction

Aquaculture has encountered production difficulties owing to the increasing scarcity of conventional feed ingredients such as fishmeal and fish oil (Boyd et al., 2022). Consequently, as the industry continues to expand globally, the cost of aquafeeds has risen, necessitating the exploration of alternative, cost-effective options to replace traditional feed ingredients (Gule & Geremew, 2022). To address this challenge, the use of unconventional feed materials has become more widespread in the aquaculture sector to ensure long-term viability (Xie et al., 2021). Alternative sources of proteins and lipids have emerged as feasible replacements for fishmeal and fish oil in aquafeeds (Kokou et al., 2022). These unconventional feedstuffs offer a potential solution to decrease reliance on marine-sourced resources and improve the ecological sustainability of aquaculture (Bélanger et al., 2021). Unconventional aquaculture feedstuffs include plant-based ingredients (soybean meal, corn gluten meal, pea protein, lupin, and canola meal) and algal and microbial sources (microalgae such as Spirulina and Chlorella, single-cell proteins). Through the integration of alternative ingredients, including plant-based materials, microbial proteins, insect

meal, and by-products from diverse industries, aquafeed formulations can be made eco-friendly and cost-effective.

Biofloc technology is acknowledged for its contribution to the enhancement of water quality, alleviation of stress in aquaculture systems, and promotion of feed conversion efficiency (El-Sayed, 2021). The utilisation of biofloc meal in aquaculture feeds has been linked to benefits, such as increased productivity, reduced feed conversion ratios, and improved culture environments for farmed species (Manan et al., 2022; Wiyono et al., 2023). Recent research has explored the potential of integrating biofloc-based farming with other aquatic species, representing the advantages of biofloc meal in promoting the growth performance, feed efficiency, and overall health of farmed organisms (Pérez-Velasco et al., 2023). The inclusion of biofloc meal into aquafeeds has demonstrated the capacity to partially or entirely replace fishmeal to meet the need for sustainable protein sources in aquaculture diets. The adoption of biofloc technology along with cutting-edge methods such as aquaponics and IoT-based monitoring systems, has significantly improved aquaculture practices (Rashid et al., 2021; Wijayanti et al., 2021). Furthermore, some culture systems have devised methods for harvesting floc residue for feed production, which reduces floc accumulation and enhances the water quality of the system.

The application of biofloc meal in aquaculture feeds has been linked to several advantages, including increased production levels, reduced feed conversion ratios, and improved living conditions for farmed species (Manan et al., 2020). Recent studies have explored the possibility of incorporating biofloc-based farming with other aquatic organisms, highlighting the advantages of biofloc meal in enhancing the development, feed utilisation, and general health of farmed organisms (El-Sayed, 2021). Also, some culture systems have devised a way to harvest floc residue for feed production while simultaneously reducing floc build-up and enhancing the water quality of the system. In this study, floc residues were collected from the flocculation unit of the IMTAS to produce aquafeeds. The nutrient profiles of the floc were analysed by determining the proximate composition and amino acid and fatty acid content.

5.2 Methodology

5.2.1 Harvesting of floc residue

High-quality jute material was placed over cyclone tanks for the filtration of the wastewater from the production unit. Each of the three replicate tanks in GRG 1 and GRG 2 treatments was connected to two separate cyclone tanks from which they were connected to a sand filter. The floc was collected in a weekly cycle between weeks 7 - 12 when the floc was matured (**Figure 5.1**).

Figure 5.1: Matured floc in the IMTAS culture tank at week 9

5.2.2 Sample preparation of biofloc meal

Two methods were used for the drying of the harvested floc. The floc (**Figure 5.2**) were divided into two portions for the purpose of drying.

Figure 5.2: Harvested floc in the IMTAS culture tank.
Note that T1 – T3 are replicates tanks of GRG 1; T4 – T6 replicates tanks of GRG 2

5.2.2.1 Freeze-drying of biofloc meal

The part meant for freeze-drying was preserved at -80°C for 48 h to prevent microbial degradation. This portion was then transferred to a vacuum freeze-dryer (Alpha 1–2 LDplus, Osterode am Harz, Germany) and dried for 72 h following the procedure of Lee et al., (2017). Subsequently, the samples were ground to powder and stored in an airtight plastic container.

5.2.2.2 Oven-drying of biofloc meal

The collected floc residue was filled in aluminium foil cups and placed in an oven (Memmert UFP 400, Schwabach, DE, USA) at 60°C for 72 h. The dried ground biofloc was kept in -18^{o}C refrigerator for further analysis.

5.2.3 Nutrient profile of biofloc meal

The proximate composition, amino acid and fatty acid profile analysis was conducted at the laboratory of UNIPEQ, Universiti Kebangsaan Malaysia (UKM), Malaysia.

5.2.3.1 Proximate composition of biofloc meal harvested from the IMTAS

5.2.3.1.1 Ash

The ash content of the biofloc samples was analysed according to the AOAC 20th Edition (923.03). Up to 3 g of each sample were placed inside a porcelain cup and dried using a burner. After drying, the sample was placed in a high-temperature furnace at temperature of 500 - 550 °C for 5 h. The ash content was then determined by the equation.

$$Ash\ (\%) = \frac{ash\ wt}{sample\ wt}\ X\ 100 \qquad \textbf{(Equation 5.1)}$$

5.2.3.1.2 Crude fibre (CFr)

The crude fibre content in the biofloc was assessed using the AOAC, 20th Edition 978.10 method, which involves digestion, filtration, washing, drying, and combustion. Up to 100 g of sample was refluxed with 1.25% H_2SO_4 and 28% KOH. Refluxing was performed by adding 1.6 g of the sample to a beaker with 200 ml of 1.25% H_2SO_4, boiling for 30 min, mixing with 20 ml of 28% KOH, and boiling for another 30 min. The mixture was filtered through a sintered glass crucible covered with a sand layer, rinsed with hot distilled water, and filtered repeatedly with 1% H_2SO_4 and 1% NaOH, followed by a final wash with acetone. The remaining residue was dried at 130°C for 2 h, cooled, weighed, and then incinerated at 550°C before being weighed again to determine the crude fibre content.

5.2.3.1.3 Crude protein (CP)

The analysis of the crude protein content in the samples was carried out using the modified Kjeldahl method according to the guidelines in the 20th Edition of the AOAC, 988.05. To accomplish this, 10 g of the sample was dried in an oven at 105°C overnight in a Kjeldahl flask. The samples were hydrolysed with concentrated H_2SO_4 at a ratio of 1:10 to the total weight of the sample residue. This was performed by adding 1 g of $CuSO_4$ and heating the mixture at ±420°C for 2-3 h. Subsequently, distilled water was added to the samples before neutralisation and titration. The protein content was determined using the following equation:

$$Protein\ (\%) = \frac{[vol\ NaOH\ (blank - sample)\ X\ N\ NaOH\ X\ 14.007\ X\ 6.25\ X\ 100]}{sample\ wt}\ X\ 1000 \qquad \textbf{(Equation 5.2)}$$

5.2.3.1.4 Dry matter (DM)

The dry matter (DM) content of the biofloc samples was determined according to a method outlined in the AOAC, 20th Edition. Firstly, an empty crucible was placed in an oven at 105°C for 3 h and allowed to cool in a desiccator. Approximately 10 g of the sample was placed in a crucible and dried in an oven at 105°C. Following this, the samples were transferred to a desiccator for cooling. Dry matter was then calculated using the equation:

$$DM = 100 - [\left(\frac{w_1 - w_2}{w_1}\right) X\ 100] \quad \textbf{(Equation 5.3)}$$

Where:

W_1 = sample before drying (g);

W_2 = sample after drying (g).

5.2.3.1.5 Ether Extract (EE)

The ether extract of the samples was analysed by crude fat analysis in accordance with AOAC 945.16. Equal amount of the sample (10 g) was placed on a petri dish coated with filter paper, which was then dried in an oven at 105°C for 2 h. The filter paper was then wrapped in fresh filter paper and secured in place. The sample was subsequently placed in a Soxhlet apparatus for an extraction process that lasted 4 h. The fat content of the samples was determined using a predetermined equation.

$$EE\ (\%) = \frac{wt\ of\ fat}{wt\ of\ dry\ sample}\ X\ 100 \quad \textbf{(Equation 5.4)}$$

5.2.3.1.6 Metabolizable energy

Metabolizable energy was determined using predictive equation as described in AOAC, 20th Edition as

$ME = 0.08\ X\ DE$ (**Equation 5.5**)

here:

ME = ME is the Metabolizable Energy in g/100g

DE=DE is the Digestible Energy in g/100g

The digestible energy was determined from the relationship

$DE = (4.0\ X\ CP) + (9.0\ X\ EE) + (4.0\ NFE)$ (**Equation 5.6**)

Using the values of the CP (g/100 g), EE (g/100) and NFE (g/100) obtained from the samples

5.2.3.1.7 Nitrogen Free Extract (NFE)

NFE was calculated from the formula

NFE (%) = % DM – (%EE + % CP + % Ash + % CF) (**Equation 5.7**)

5.2.3.2 Amino acid profile of biofloc meal harvested from the IMTAS

5.2.3.2.1 Acid hydrolysis

Acid hydrolysis method was used to measure the levels of aspartic acid, serine, glutamic acid, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, lysine, isoleucine, leucine, and phenylalanine. Acid hydrolysis was carried out using the ACCQ Tag Waters Method, also referred to as the High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD) method outlined in Spackman et al., (1958). 100 g of the dried biofloc samples were weighed and placed in a reaction vessel for acid hydrolysis. 6 ml concentrated hydrochloric acid (HCl) solution was added to the sample, which was then heated to 110 °C for 24 h to break down the proteins into their constituent amino acids. The resulting acidic solution was neutralised by slowly adding 6 ml sodium hydroxide (NaOH) solution to prevent the

loss of volatile amino acids. The free amino acids were then subjected to derivatisation to improve their detectability. 10 µl of AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) was used to react with the amino acids to form stable, highly fluorescent derivatives. The derivatised amino acids were then injected into the HPLC system to separate the amino acids based on their hydrophobicity and interactions with the stationary phase of the column. The mobile phase comprising a gradient of water and acetonitrile, was accompanied by a buffer that enabled the separation of amino acids at different times, referred to as the retention times. The eluted amino acids were then directed through a fluorescence detector (FLD) with specific excitation (250 nm) and emission (395 nm) wavelengths, which corresponded to the fluorescent derivatives of the amino acids. The detector assessed fluorescence intensity, which was directly proportional to the concentration of each amino acid in the feed sample. The area under each peak on the chromatogram generated by FLD was measured and compared to a calibration curve established using known amino acid standards.

5.2.3.2.2 Alkaline hydrolysis

The alkaline hydrolysis procedure was used to evaluate tryptophan, which would otherwise be degraded under acidic hydrolysis conditions (AOAC International, 2005). 100 g of sample were weighed and transferred to a reaction vessel for alkaline hydrolysis to safeguard tryptophan from degradation. 4 ml of 4 M NaOH was added to the sample in the reaction vessel, which was then sealed and heated to 110°C for 16–24 h. Subsequently, 4 m of 4 M HCl was added dropwise to neutralise the mixture and prevent rapid reactions that could cause the loss of amino acids. 10 µL of AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) was added to form a stable fluorescent derivative. The derivatised amino acids were then injected into the HPLC system equipped with a reverse-phase column. A reversed-phase Hypersil BDS C18 column was used for the separation of amino acids, with a gradient elution mobile phase in the high-performance liquid chromatography (HPLC) process, with detection achieved using a fluorescent detector and 2-[2-(7H-dibenzo[a,g]carbazol-7-yl)-ethoxy] ethyl chloroformate (DBCEEC) as the fluorescent labelling reagent. The

detector was set to an excitation wavelength of 250 nm and an emission wavelength of 395 nm, which is optimal for detecting AQC-derivatised tryptophan. A stock solution of 1 mg/mL was diluted to 10 µg/mL, 5 µg/mL, and 1 µg/mL, and these standards were injected into the HPLC system. The detector response was recorded and compared with calibration curves generated from known amino acid standards to determine the concentration of tryptophan.

5.2.3.2.3 Performic acid hydrolysis

The performic acid hydrolysis was employed to quantify cysteine and methionine, which are sulphur-containing amino acids that require oxidation for precise measurement. 2 mL of performic acid was added to the samples to oxidise cysteine to cysteic acid, and methionine to methionine sulfone, which are stable forms that do not undergo further degradation during hydrolysis. The oxidation of these amino acids ensures that they remain intact throughout the hydrolysis process. The oxidised forms of cysteine (cysteic acid) and methionine (methionine sulfone) were derivatised by adding 10µL AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate to each sample. The peaks of the chromatogram corresponding to cysteine and methionine were identified (at 10 and 12 minutes respectively) and compared with calibration curves generated using known amino acid standards to determine their respective concentrations.

5.2.3.3 Fatty acid profile of biofloc meal harvested from the IMTAS

Prior to lipid extraction, 1 ml of the internal standard solution in chloroform was added to 100 g of feed sample. Thereafter, the samples were subjected to ultrasonic extraction for 20 min using a mixture of distilled water, methanol, and chloroform (1:2:1; 20 ml; v:v:v). The lower chloroform phase was then separated by centrifugation at 2500 rpm for 5 min (650 g), and the process was repeated three times. The extracted lipids were saponified under reflux for 2 h at 100 °C with 1 ml of 2 M

NaOH and 2 ml of methanol solution. Finally, the lipids were recovered using 2 × 2 ml of chloroform after acidification with an ultra-pure HCI solution (37.5%). The fatty acids of the total lipids were transformed into fatty acid methyl esters (FAME) by refluxing with 1 ml of a 14% BF3-methanol solution for 10 min under a nitrogen stream. The lipid extract containing FAME was then extracted with 2 ml of hexane, and 2 ml of distilled water was added. The solution was vigorously shaken for 1 min and centrifuged for 3 min at 2500 rpm (650 g). The upper hexane layer containing FAMEs was transferred to a clean sample vial (approximately 1-2 ml) for GC analysis.

Gas chromatograph (GC-14B Shimadzu) was used to separate and quantify the FAMEs. Separation was carried out using an FFAP-polar capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film thickness) where hydrogen was used as the carrier gas. The injection temperature was set at 60 °C, and the oven temperature was increased to 150°C at a rate of 40 °C/min, then to 230°C at 3 °C/min, and finally held constant for 30 min. The flame ionisation detector was set to 240°C. The retention times of the FAME peaks were compared to those of authentic standards (Supelco Inc) to identify them. The retention times used for the various fatty acid methyl esters (FAMEs) were 5.5 min for C6:0, 7.5 min for C8:0, 9.5 min for C10:0, 11.5 min for C12:0, 13.5 min for C14:0, 16.5 min for C16:0, 19.5 min for C18:0, 18.0 min for C18:1, 17.5 min for C18:2, 20.0 min for C18:3, 22.0 min for C20:0, 25.5 min for C22:0, and 28.0 min for C24:0. The fatty acids were designated as n:pωx, where n is the number of carbon atoms in the aliphatic chain, p is the number of double bonds, and x is the position of the first double bond from the terminal methyl group.

5.2.4 Statistical analysis

Analysis of the proximate composition, amino and fatty acid profile of the biofloc meal samples from the two treatments (GRG 1 and GRG 2) was conducted using a 2-way ANOVA to evaluate the effect of treatment, drying method, and their interaction. Data were analysed using Minitab Express (version 1.5.2; 169254v12) for Windows. The mean and their standard errors were calculated while mean differences

were determined at $p<0.05$ significance level. Tukey's Honest Significant Difference (HSD) test was employed as the mean separation techniques.

5.3 Results

5.3.1 Proximate composition of biofloc meal collected from IMTAS

The composition of biofloc meal in terms of proximate components in the two treatments (GRG 1 and GRG 2) and two drying methods (freeze-drying, FD, and oven-drying, OD) is shown in **Table 5.1**. A comparison of treatments revealed significant differences in ether extract (EE) and nitrogen-free extract (NFE). EE was higher in GRG 1 (1.50±0.03%) while GRG 2 had a higher NFE (13.20±0.15%). The drying method had a significant impact on all proximate components, except for dry matter (DM). Freeze-drying resulted in higher crude protein (CP) (32.12±0.20%), EE (1.30±0.03%), metabolizable energy (ME) (1725.25±2.45 kcal/kg), and NFE (13.45±0.17%), but lower ash (39.56±0.29%) and crude fibre (CFr) (8.69±0.39%) compared to oven-drying. Interaction effects were observed for all proximate components except for DM and ME, with specific combinations producing varying results. Ash was the highest in GRG 2 × OD (44.95±0.40%), while the lowest was observed in GRG 2 × FD (38.00±0.04%). The CF amount showed GRG 2 x OD as the highest interaction (12.35±0.75%), while the lowest interaction was 7.22±0.17% in GRG 1 x FD. The highest and lowest crude protein contents (CP) were observed in the GRG 2 × FD (32.80±0.20%) and GRG 2 × OD (27.35±0.32%) samples, respectively. For ether extract (EE), the highest interaction effect was recorded in the GRG 1 x FD (1.85±0.04%) sample, while the lowest interaction effects were observed in the GRG 2 x FD (0.75±0.04%) sample. The best performance was shown by the GRG 1 × FD (13.55±0.25%) sample in the nitrogen-free extract (NFE) and the least interaction effects were observed in the same sample (11.24±0.20%). These interactions indicate

that FD improved crude protein, ether extract and nitrogen-free extract content. However, oven drying (OD) favoured higher ash and crude fibre content.

Table 5.1: Proximate composition of the biofloc meal harvested from IMTAS

Proximate	Treatment			Drying method			Interactions				
	GRG 1	**GRG 2**	**p-value**	**Freeze-dried (FD)**	**Oven-dried (OD)**	**p-value**	**GRG 1 X FD**	**GRG 1 X OD**	**GRG 2 X FD**	**GRG 2 X OD**	**p-value**
Ash	42.41±0.29	41.47±0.29	0.09^{ns}	39.56±0.29^{b}	44.32±0.24^{a}	<0.01	41.12±0.42^{b}	43.70±0.40^{ab}	38.00±0.04^{c}	44.95±0.40^{a}	<0.01
CF	8.52±0.39	12.25±0.12	0.36^{ns}	8.69±0.39^{b}	16.09±0.34^{a}	0.04	7.22±0.17^{d}	9.83±0.25^{c}	10.15±0.53^{b}	12.35±0.75^{a}	0.03
CP	30.15±0.22	30.07±0.20	0.82^{ns}	32.12±0.20^{a}	28.10±0.22^{b}	<0.01	31.45±0.32^{a}	28.85±0.30^{b}	32.80±0.20^{a}	27.35±0.32^{b}	0.01
DM	95.07±0.31	94.82±0.30	0.60^{ns}	95.17±0.30	94.72±0.30	0.37^{ns}	95.55±0.45	94.60±0.32	94.80±0.52	94.85±0.45	0.33^{ns}
EE	1.50±0.03^{a}	0.77±0.03^{b}	<0.01	1.30±0.03^{a}	0.98±0.02^{b}	<0.01	1.85±0.04^{a}	1.16±0.05^{b}	0.75±0.04^{c}	0.80±0.04^{c}	<0.01
ME	1646.75±2.15	1650.75±1.50	0.90^{ns}	1725.25±2.45^{a}	1572.25±2.15^{b}	<0.01	1743.00±3.45	1550.50±3.48	1707.50±3.50	1594.00±2.41	0.26^{ns}
NFE	12.39±0.18^{b}	13.20±0.15^{a}	0.03	13.45±0.17^{a}	12.14±0.15^{b}	<0.01	13.55±0.25^{a}	11.24±0.20^{c}	13.35±0.25^{ab}	13.05±0.25^{b}	0.01

Means on the same row (in each section) with different superscript are statistically significant (p<0.05); ns = not significant

CF = crude fibre; CP = crude protein; DM = dry matter; EE = ether extract; ME = metabolizable energy; NFE = nitrogen free extract

5.3.2 Amino acid profile of biofloc meal collected from IMTAS

5.3.2.1 Variations of amino acid in treatments and drying methods of biofloc meal

Table 5.2 shows the variations in amino acid composition of biofloc meal under different treatments (GRG 1 and GRG 2) and drying methods (freeze-dried, FD and oven-dried, OD). Several amino acids had statistically significant variations between GRG 1 and GRG 2. Aspartic acid, glutamic acid, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, lysine, isoleucine and leucine were higher in GRG 1 compared to GRG 2, except for methionine and thryosine which was higher in GRG 2. On the other hand, serine, phenylalanine, cysteine, and tryptophan did not show significant differences between the two treatments.

The drying method also had a significant impact on the amino acid composition. Most amino acids were significantly higher in the FD method compared to the OD method. Aspartic acid, glutamic acid, histidine, arginine, threonine, alanine, proline, tyrosine, valine, lysine, isoleucine, leucine, phenylalanine, cysteine, and tryptophan were higher in FD than in OD. However, Methionine was significantly higher in OD. Glycine, however, did not show significant differences between FD and OD methods.

The results revealed substantial variations in amino acid levels resulting from the interaction between treatment (GRG 1 and GRG 2) and drying method (Freeze-dried (FD) and Oven-dried (OD)). Aspartic Acid was found to be highest in GRG 2 X FD (3.63), showing significant interaction effects. Serine showed the highest level in GRG 1 X FD (1.73), with significant interaction. Glutamic Acid was highest in GRG 1 X FD (5.01) and was statistically significant ($p < 0.01$). GRG 2 X OD recorded the highest interaction for glycine (1.77), which was statistically significant ($p < 0.01$). Histidine had the highest level in GRG 2 X OD ($p < 0.01$). Arginine showed the highest

interaction in GRG 1 X FD (1.06). Threonine was found to have the highest level in GRG 1 X FD (1.97) and its lowest in GRG 1 X OD (1.44) ($p < 0.01$). Alanine had the highest levels in both GRG 1 X FD and GRG 2 X FD (2.16), with the lowest in GRG 1 X OD (1.18) ($p < 0.01$). Tyrosine showed significant interaction effects ($p = 0.05$) with the highest level in GRG 1 X FD (0.67). Valine was highest in GRG 1 X FD (1.95) and lowest in GRG 1 X OD (1.16) ($p < 0.01$). Lysine peaked in GRG 1 X FD (1.82) and was lowest in GRG 1 X OD (0.89) ($p < 0.01$). Isoleucine reached its highest level in GRG 2 X FD (1.49) and its lowest in GRG 1 X OD (1.03) ($p < 0.01$). Leucine had the highest levels in both GRG 1 X FD and GRG 2 X FD (2.17 and 2.20), and the lowest in GRG 1 X OD (1.48) ($p < 0.01$). Cysteine displayed significant interaction effects ($p < 0.01$), exhibiting the highest level in GRG 2 X FD (2.31) and the lowest in both GRG 1 X OD and GRG 2 X OD (1.78 and 1.68). Methionine had its highest level in GRG 1 X OD (6.70) and its lowest in GRG 1 X FD (4.64) ($p < 0.01$). Tryptophan showed the highest level in GRG 2 X FD (0.36) and the lowest in GRG 2 X OD (0.24) ($p = 0.05$). Proline, however, did not display significant interaction effects ($p = 0.13$).

Table 5.2: Amino acid composition of the biofloc meal harvested from IMTAS

Amino acids	Treatment			Drying method			Interactions				
	GRG 1	GRG 2	p-value	Freeze-dried (FD)	Oven-dried (OD)	p-value	GRG 1 X FD	GRG 1 X OD	GRG 2 X FD	GRG 2 X OD	p-value
Aspartic acid	3.46±0.01^{a}	2.66±0.01^{b}	<0.01	3.45±0.01^{a}	2.67±0.01^{b}	<0.01	3.61±0.01^{a}	1.71±0.01^{c}	3.28±0.01^{b}	3.63±0.01^{a}	<0.01
Serine	1.44±0.02	1.40±0.02	0.27^{ns}	1.56±0.02^{a}	1.28±0.02^{b}	<0.01	1.73±0.03^{a}	1.07±0.03^{d}	1.39±0.03^{c}	1.50±0.03^{b}	<0.01
Glutamic acid	4.65±0.03^{a}	3.73±0.03^{b}	<0.01	4.78±0.03^{a}	3.61±0.03^{b}	<0.01	5.01±0.04^{a}	2.46±0.04^{c}	4.55±0.04^{b}	4.76±0.04^{bc}	<0.01
Glycine	1.63±0.03^{a}	1.49±0.03^{b}	0.03	1.59±0.03	1.53±0.03	0.26^{ns}	1.68±0.04^{b}	1.30±0.04^{d}	1.50±0.04^{c}	1.77±0.04^{a}	<0.01
Histidine	0.57±0.01^{a}	0.48±0.01^{b}	<0.01	0.55±0.01^{a}	0.49±0.01^{b}	0.02	0.60±0.01^{a}	0.35±0.01^{c}	0.50±0.01^{b}	0.64±0.01^{a}	<0.01
Arginine	0.95±0.01^{a}	0.80±0.01^{b}	<0.01	1.07±0.01^{a}	0.68±0.01^{b}	<0.01	1.06±0.02^{a}	0.55±0.02^{b}	1.07±0.02^{a}	0.82±0.02^{bc}	<0.01
Threonine	1.89±0.01^{a}	1.71±0.01^{b}	<0.01	1.94±0.01^{a}	1.65±0.01^{b}	<0.01	1.97±0.02^{a}	1.44±0.02^{c}	1.91±0.02^{a}	1.86±0.02^{b}	<0.01
Alanine	1.96±0.03^{a}	1.67±0.03^{b}	<0.01	2.16±0.03^{a}	1.47±0.03^{b}	<0.01	2.16±0.04^{a}	1.18±0.04^{c}	2.16±0.04^{a}	1.76±0.04^{b}	<0.01
Proline	0.98±0.01^{a}	0.90±0.01^{b}	0.02	1.04±0.01^{a}	0.84±0.01^{b}	<0.01	1.02±0.02	0.78±0.02	1.06±0.02	0.91±0.02	0.13^{ns}
Thyrosine	0.54±0.00^{b}	0.58±0.00^{a}	0.03	0.63±0.00^{a}	0.49±0.00^{b}	<0.01	0.67±0.01^{a}	0.49±0.01^{c}	0.59±0.01^{b}	0.49±0.01^{c}	0.05
Valine	1.75±0.02^{a}	1.56±0.02^{b}	<0.01	1.81±0.02^{a}	1.50±0.02^{b}	<0.01	1.95±0.03^{a}	1.16±0.03^{c}	1.66±0.03^{b}	1.83±0.03^{ab}	<0.01
Lysine	1.50±0.01^{a}	1.35±0.01^{b}	<0.01	1.76±0.01^{a}	1.10±0.01^{b}	<0.01	1.82±0.02^{a}	0.89±0.02^{d}	1.70±0.02^{b}	1.30±0.02^{c}	<0.01
Isoleucine	1.42±0.02^{a}	1.24±0.02^{b}	<0.01	1.48±0.02^{a}	1.19±0.02^{b}	<0.01	1.46±0.02^{ab}	1.03±0.02^{c}	1.49±0.02^{a}	1.35±0.02^{b}	<0.01
Leucine	2.03±0.01^{a}	1.82±0.01^{b}	<0.01	2.18±0.01^{a}	1.67±0.01^{b}	<0.01	2.17±0.02^{a}	1.48±0.02^{c}	2.20±0.02^{a}	1.86±0.02^{b}	<0.01
Phenylalanine	1.26±0.01	1.23±0.01	0.37^{ns}	1.31±0.01^{a}	1.18±0.01^{b}	<0.01	1.28±0.02^{b}	1.18±0.02^{c}	1.33±0.02^{a}	1.18±0.02^{c}	0.04
Cysteine	2.00±0.02	1.94±0.02	0.12^{ns}	2.20±0.02^{a}	1.73±0.02^{b}	<0.01	2.10±0.03^{b}	1.78±0.03^{c}	2.31±0.03^{a}	1.68±0.03^{c}	<0.01
Methionine	5.17±0.02^{b}	5.67±0.02^{a}	<0.01	4.91±0.02^{b}	5.93±0.02^{a}	<0.01	4.64±0.03^{c}	6.70±0.03^{a}	5.18±0.03^{b}	5.16±0.03^{b}	<0.01
Tryptophan	0.30±0.01	0.29±0.01	0.52^{ns}	0.34±0.01^{a}	0.25±0.01^{b}	<0.01	0.31±0.01^{a}	0.26±0.01^{b}	0.36±0.01^{a}	0.24±0.01^{b}	0.05

Means on the same row (in each section) with different superscript are statistically significant ($p<0.05$); ns = not significant

5.3.2.2 Percentage composition of amino acids in biofloc meal

The percentage composition of amino acids in the biofloc meal of GRG 1 is illustrated in **Figure 5.3**. Glutamic acid, methionine, aspartic acid, leucine, and alanine were the amino acids with the highest percentages, accounting for a cumulative total of 50.37%. Methionine had the highest percentage, amounting to 15.09% in the oven-dried sample and 13.14% in the freeze-dried sample. Glutamic acid was 14.18% in the freeze-dried samples and 13.27% in the oven-dried samples. Subsequently, aspartic acid was present in 10.24% and 9.57% of the freeze-dried and oven-dried samples, respectively. Leucine was observed at 6.41% and 6.15% in oven-dried and freeze-dried samples, respectively. Tryptophan had the lowest percentages (0.89% and 1.07 %) in the freeze-dried and oven-dried samples, respectively.

The percentage composition of amino acids in the biofloc meal of GRG 2 is shown in **Figure 5.4**. Methionine, glutamic acid, cysteine, aspartic acid, and leucine constituted 53.38% of the amino acids measured in the biofloc meal of the GRG 2. Methionine had the highest percentage, accounting for 25.87% of the freeze-dried samples and 15.73% of the oven-dried ones. Glutamic acid comprised 9.52% of the amino acids in the freeze-dried biofloc samples, while the oven-dried samples comprised 14.51%. Cysteine accounted for 6.88% and 5.14% of the freeze-dried and oven-dried samples, respectively. Similarly, aspartic acid comprised 11.09% of the total amino acid content in the oven-dried samples and 6.63% in the freeze-dried samples. Leucine was 5.72% in the freeze-dried method and 5.68% in the oven-dried method. Conversely, tryptophan had the lowest percentage composition, which was 1.03% in the freeze-dried samples and 0.73% in the oven-dried samples. The asterisk indicates the method with the highest percentage of each amino acid.

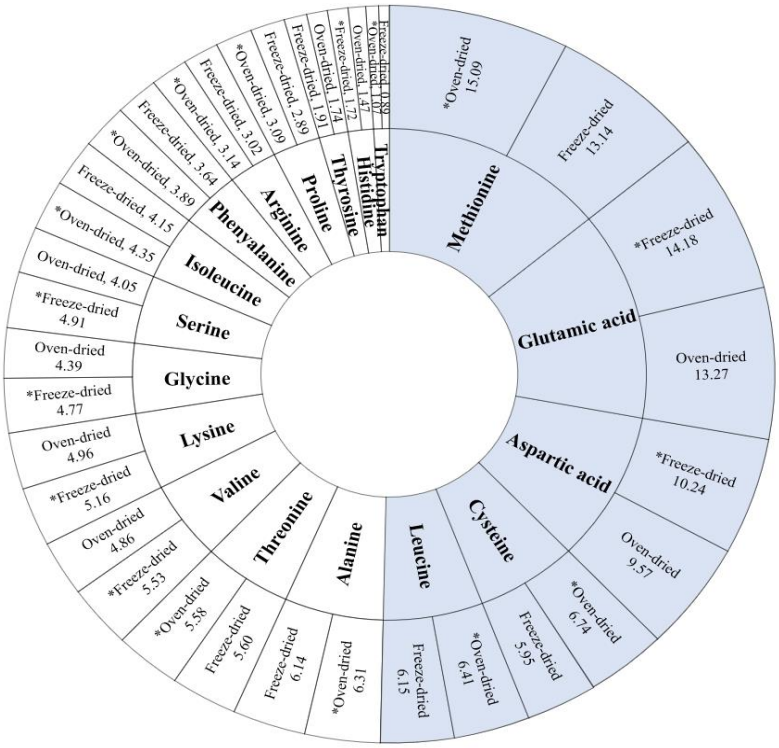


Figure 5.3: Percentage composition of amino acids in the biofloc meal of GRG 1

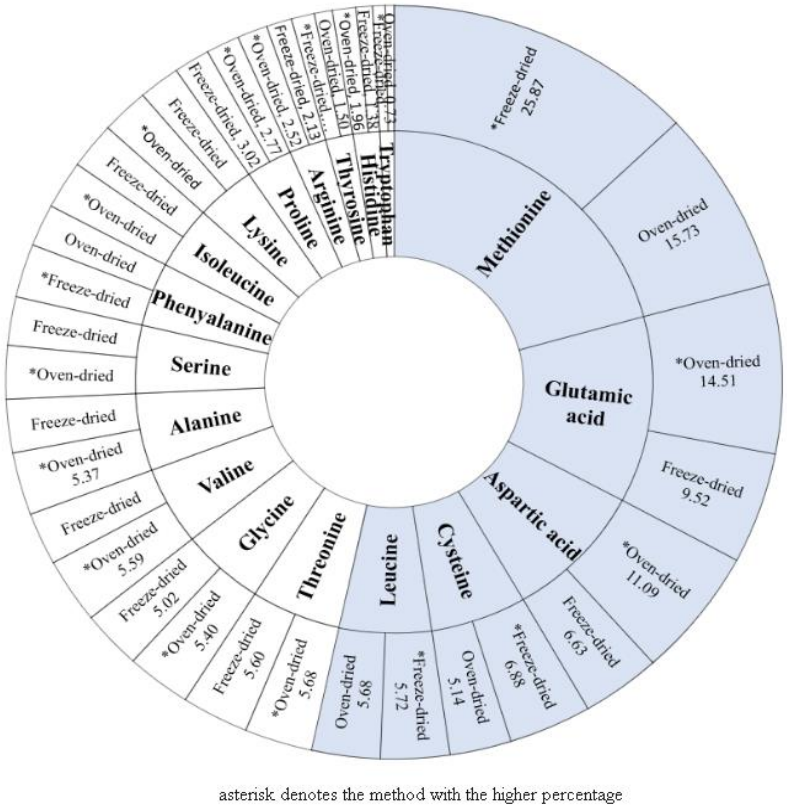


Figure 5.4: Percentage composition of amino acids in the biofloc meal of GRG 2

5.3.3 Fatty acid profile of biofloc meal collected from IMTAS

5.3.3.1 Variations of saturated fatty acid in treatments and drying methods of biofloc meal

Table 5.3 presents the saturated fatty acid profile of biofloc meal harvested from IMTAS. Treatments GRG 1 and GRG 2 showed notable differences in fatty acid concentrations, where GRG 1 generally had higher levels of C16:0 (palmitic acid) at 39.14±0.23 compared to 35.01±0.01 for GRG 2 and C24:0 (lignoceric acid) at 3.79±0.01 compared to 1.04±0.01 for GRG 2. The drying method also impacted the fatty acid profile, with freeze-dried (FD) and oven-dried (OD) samples showing significant differences for C12:0 (lauric acid) 0.35±0.02 in FD and 1.36±0.02 in OD and C18:0 (stearic acid) 4.74±0.01 in FD and 5.10±0.01 in OD. The interaction between treatment and drying method also showed significant variation in C14:0 (myristic acid) with GRG 2 X FD at 3.30±0.03 and GRG 1 X OD at 2.30±0.03.

Table 5.3: Saturated fatty acid profile of the biofloc meal harvested from IMTAS

Fatty acids	Treatment			Drying method			Interactions				
	GRG 1	GRG 2	p-value	Freeze-dried (FD)	Oven-dried (OD)	p-value	GRG 1 X FD	GRG 1 X OD	GRG 2 X FD	GRG 2 X OD	p-value
C4:0	0.70±0.03	0.35±0.03	0.53^{ns}	0.56±0.03	0.79±0.03	0.36^{ns}	$0.14±0.05^{c}$	$1.26±0.05^{a}$	$0.38±0.05^{b}$	$0.32±0.05^{b}$	0.04
C6:0	ND	ND		-	-		-	-	-	-	
C8:0	ND	ND		-	-		-	-	-	-	
C10:0	ND	ND		-	-		-	-	-	-	
C11:0	ND	ND		-	-		-	-	-	-	
C12:0	$1.00±0.02^{a}$	$0.70±0.02^{b}$	<0.01	$0.35±0.02^{b}$	$1.36±0.02^{a}$	<0.01	$0.13±0.03^{d}$	$1.88±0.03^{a}$	$0.58±0.03^{c}$	$0.83±0.03^{b}$	<0.01
C13:0	ND	ND		-	-		-	-	-	-	
C14:0	$1.80±0.02^{b}$	$2.90±0.02^{a}$	<0.01	$2.30±0.02^{b}$	$2.40±0.02^{a}$	0.05	$1.31±0.03^{c}$	$2.30±0.03^{b}$	$3.30±0.03^{a}$	$2.50±0.03^{b}$	<0.01
C15:0	$0.05±0.01^{b}$	$1.03±0.01^{a}$	<0.01	$0.74±0.01^{a}$	$0.33±0.01^{b}$	0.05	$0.10±0.01^{c}$	$0.00±0.00^{c}$	$1.39±0.05^{a}$	$0.67±0.15^{b}$	0.05
C16:0	$39.14±0.23^{a}$	$35.01±0.01^{b}$	<0.01	$37.58±0.23^{a}$	$36.57±0.22^{b}$	0.03	$49.27±0.32^{a}$	$29.02±0.32^{c}$	$25.89±0.32^{d}$	$44.12±0.30^{b}$	<0.01
C17:0	$0.07±0.00^{b}$	$0.33±0.00^{a}$	<0.01	$0.40±0.00^{a}$	$0.00±0.00^{b}$	<0.01	$0.14±0.01^{b}$	$0.00±0.00^{c}$	$0.66±0.01^{a}$	$0.00±0.00^{c}$	<0.01
C18:0	$4.35±0.01^{b}$	$5.49±0.01^{b}$	<0.01	$4.74±0.01^{b}$	$5.10±0.01^{a}$	<0.01	$4.40±0.02^{c}$	$4.30±0.02^{c}$	$5.08±0.02^{b}$	$5.90±0.02^{a}$	<0.01
C20:0	ND	ND		-	-		-	-	-	-	
C21:0	ND	ND		-	-		-	-	-	-	
C22:0	$0.06±0.01^{b}$	$0.39±0.01^{a}$	<0.01	$0.45±0.01^{a}$	$0.00±0.00^{b}$	<0.01	$0.12±0.02^{b}$	$0.00±0.00^{c}$	$0.78±0.02^{a}$	$0.00±0.00^{c}$	<0.01
C23:0	$0.59±0.01^{b}$	$0.98±0.01^{a}$	<0.01	$0.49±0.01^{b}$	$1.07±0.01^{a}$	<0.01	$0.12±0.02^{c}$	$1.06±0.02^{ab}$	$0.87±0.02^{b}$	$1.09±0.02^{a}$	<0.01
C24:0	$3.79±0.01^{a}$	$1.04±0.01^{b}$	<0.01	$0.69±0.01^{b}$	$4.15±0.01^{a}$	<0.01	$0.15±0.01^{d}$	$7.43±0.01^{a}$	$1.22±0.01^{b}$	$0.86±0.01^{c}$	<0.01

Means on the same row (in each section) with different superscript are statistically significant ($p<0.05$); ns = not significant

5.3.3.2 Variations of monosaturated fatty acid in treatments and drying methods of biofloc meal collected from IMTAS

Table 5.4 shows the differences in monosaturated fatty acid concentrations in the biofloc meal. Myristoleic acid (C14:1) varied significantly between GRG 1 and GRG 2 ($p < 0.01$). GRG 2 (3.58±0.04) had higher levels than GRG 1 (1.00±0.04). The results also indicated that the drying method had a significant impact, with freeze-dried (FD) samples (2.45±0.04) having higher levels than oven-dried (OD) samples (2.13±0.04). The interaction between the treatment and drying method was also significant, with GRG 2 × FD having the highest concentration (4.66±0.06) and GRG 1 × FD having the lowest (0.25±0.06). The differences between treatments for cis-10-pentadecenoic acid (C15:1) were also significant, with GRG 2 (2.29±0.01) being higher than GRG 1 (0.86±0.01). Additionally, the drying method significantly affected the levels, with OD samples (1.91±0.01) being higher than FD samples (1.23±0.01). The interactions between treatment and drying method were also significant, with GRG 2 × FD having the highest interaction (2.38±0.02) and GRG 1 × FD having the lowest (0.09±0.02). Finally, the results showed that palmitoleic acid (C16:1) varied significantly between GRG 1 (1.87±0.02) and GRG 2 (5.43±0.02), and the drying method significantly affected the levels, with FD (3.99±0.02) being higher than OD (3.31±0.02).

The results showed that the interaction between the factors was statistically significant ($p < 0.01$), with GRG 2 × FD exhibiting the highest concentration (7.44±0.03), and GRG 1 × FD displaying the lowest (0.54±0.03). The levels of Oleic Acid (C18:1) varied significantly between treatments, with GRG 1 (26.38±0.14) having higher levels than GRG 2 (20.43±0.14). The drying method also displayed a significant difference, with FD (25.87±0.14) having higher concentrations than OD (20.94±0.14). The interactions between the factors were significant, with GRG 1 × FD showing the highest concentration (33.47±0.21) and GRG 2 × FD the lowest (18.27±0.21). There was a significant difference in gadoleic acid (C20:1(11c)) levels between GRG 1 (0.05±0.00) and GRG 2 (0.00±0.00). The drying method also had a significant effect, with FD (0.05±0.00) having higher values than OD (0.00±0.00). The

interaction between the variables was significant, with the highest concentration of GRG 1 × FD being 0.11±0.00. In contrast, other interaction effects were not detectable. Nervonic Acid (C24:1) did not show any significant differences between GRG 1 and GRG 2. However, the drying method demonstrated a significant discrepancy, with OD (1.22±0.03) being higher than FD (0.43±0.03). The interactions were significant, with GRG 1 × OD exhibiting the highest level at 1.60±0.05, while GRG 1 × FD was the lowest at 0.05±0.01. Notably, Heptadecenoic acid (C17:1), elaidic acid (C18:1n9t), Cis-11-Eicosenoic acid (C20:1n9), and erucic acid (C22:1n9) were not detected (ND) in any sample.

Table 5.4: Monosaturated fatty acid profile of the biofloc meal harvested from IMTAS

Fatty acids	Treatment			Drying method			Interactions				
	GRG 1	GRG 2	p-value	Freeze-dried (FD)	Oven-dried (OD)	p-value	GRG 1 X FD	GRG 1 X OD	GRG 2 X FD	GRG 2 X OD	p-value
C14:1	1.00±0.04^{b}	3.58±0.04^{a}	<0.01	2.45±0.04^{a}	2.13±0.04^{b}	<0.01	0.25±0.06^{d}	1.76±0.06^{c}	4.66±0.06^{a}	2.49±0.06^{b}	<0.01
C15:1	0.86±0.01^{b}	2.29±0.01^{a}	<0.01	1.23±0.01^{b}	1.91±0.01^{a}	<0.01	0.09±0.02^{c}	1.63±0.02^{b}	2.38±0.02^{a}	2.20±0.02^{a}	<0.01
C16:1	1.87±0.02^{b}	5.43±0.02^{a}	<0.01	3.99±0.02^{a}	3.31±0.02^{b}	<0.01	0.54±0.03^{c}	3.19±0.03^{b}	7.44±0.03^{a}	3.42±0.03^{b}	<0.01
C17:1	ND	ND		-	-		-	-	-	-	
C18:1n9t	ND	ND		-	-		-	-	-	-	
C18:1n9c	26.38±0.14^{a}	20.43±0.14^{b}	<0.01	25.87±0.14^{a}	20.94±0.14^{b}	<0.01	33.47±0.21^{a}	19.29±0.21^{c}	18.27±0.21^{c}	22.59±0.21^{b}	<0.01
C20:1n9	0.05±0.00^{a}	0.00±0.00^{b}	<0.01	0.05±0.00^{a}	0.00±0.00^{b}	<0.01	0.11±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	<0.01
C22:1n9	ND	ND		-	-		-	-	-	-	
C24:1	0.82±0.03	0.82±0.03	0.99^{ns}	0.43±0.03	1.22±0.03	<0.01	0.05±0.01^{c}	1.60±0.05^{a}	0.82±0.05^{b}	0.83±0.05^{b}	<0.01

Means on the same row (in each section) with different superscript are statistically significant ($p<0.05$); ns = not significant

5.3.3.3 Variations of polyunsaturated fatty acid in treatments and drying methods of biofloc meal collected from IMTAS

Variations in the concentrations of polyunsaturated fatty acids in the biofloc meal are presented in **Table 5.5**. Linoleic acid (C18:2n6c) showed significant differences between the treatments, with GRG 2 having higher levels (9.39±0.04) than GRG 1 (8.04±0.04). Similarly, the drying method also showed significant variation, with freeze-dried (FD) samples (9.06±0.04) having higher levels than oven-dried (OD) samples (8.37±0.04). The interaction between treatment and drying method was also found to be significant, with the highest concentration observed in GRG 2 × FD (10.20±0.06) and the lowest in GRG 1 × FD (7.90±0.06). A significant difference in the variation of α-linolenic acid (C18:3n3) was noted, with GRG 2 (0.63±0.01) having higher levels than GRG 1 (0.08±0.00). The drying method also had a significant impact on the levels, with FD samples (0.71±0.00) being higher than OD samples (0.00±0.00). The interaction was found to be significant, with GRG 2 × FD having the highest concentration (1.26±0.00), while neither GRG 1 × OD nor GRG 2 × OD had detectable levels. Arachidonic acid (C20:4n6) showed a significant difference between the treatments, with GRG 2 (1.14±0.00) displaying higher levels than GRG 1 (0.04±0.00). The FD samples had higher levels of the compounds than the OD samples, with the exception of Cis-13,16-Docosadienoic acid (C22:2), where GRG 1 had higher levels than GRG 2. The interaction between the drying method and granulation method had a significant impact on the levels of the compounds, with GRG 2 × FD having the highest concentration of Cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3) and Cis-13,16-Docosadienoic acid (C22:2), and GRG 2 × OD having the lowest concentration of Cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3). The effect of the drying method on the levels of Cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3) was significant, with OD samples having higher levels than FD samples. The interaction was significant, with GRG 1 × FD as the highest concentration (10.89±0.04) and GRG 2 × OD having the lowest (1.60±0.04). Linolelaidic (Trans) acid (C18:2n6t), Cis-11,14-Eicosadienoic Acid (C20:2), γ-linolenic acid (C18:3n6), Cis-8,11,14-Eicosatrienoic Acid (C20:3n6), and Cis-11,14,17-Eicosatrienoic Acid (C20:3n3) were not detected (ND) in any of the samples measured.

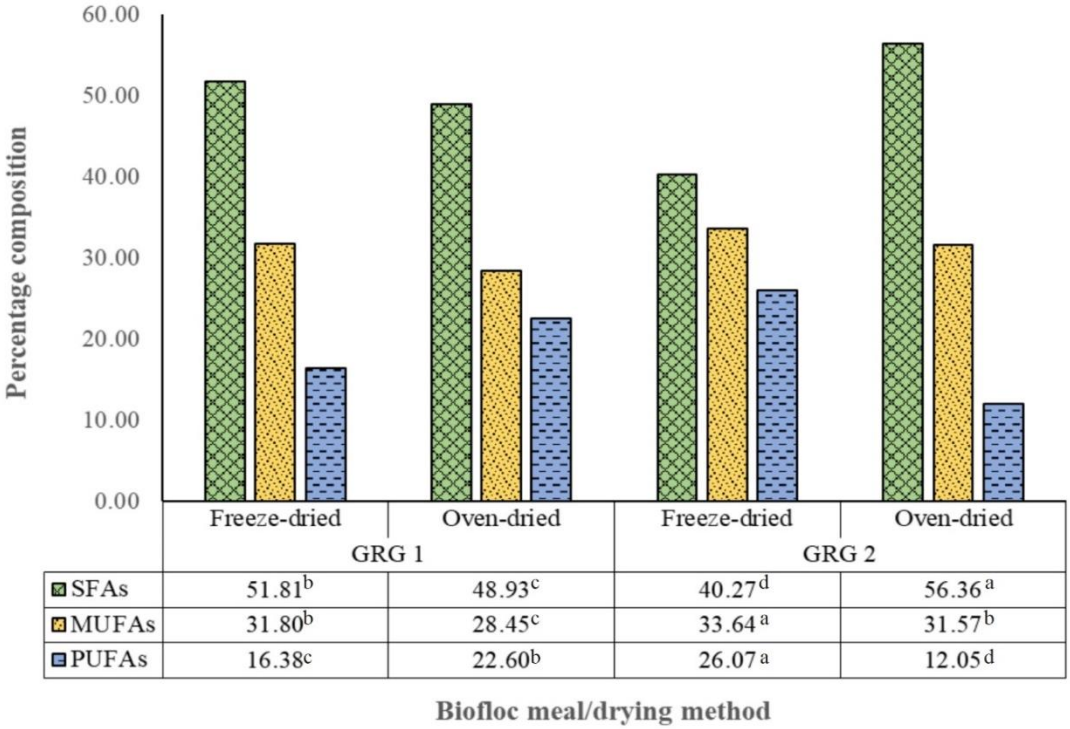
Table 5.5: Polyunsaturated fatty acid profile of the biofloc meal harvested from IMTAS

Fatty acids	Treatment			Drying method			Interactions				
	GRG 1	GRG 2	p-value	Freeze-dried (FD)	Oven-dried (OD)	p-value	GRG 1 X FD	GRG 1 X OD	GRG 2 X FD	GRG 2 X OD	p-value
C18:2n6t	ND	ND		-	-		-	-	-	-	
C18:2n6c	8.04±0.04^{b}	9.39±0.04^{a}	<0.01	9.06±0.04^{a}	8.37±0.04^{b}	<0.01	7.90±0.06^{c}	8.17±0.06^{bc}	10.20±0.06^{a}	8.57±0.06^{b}	<0.01
C18:3n6	0.08±0.00^{b}	0.63±0.01^{a}	<0.01	0.71±0.00^{a}	0.00±0.00^{b}	<0.01	0.17±0.01^{b}	0.00±0.00^{c}	1.26±0.00^{a}	0.00±0.00^{c}	<0.01
C20:2	ND	ND		-	-		-	-	-	-	
C20:3n6	ND	ND		-	-		-	-	-	-	
C20:3n3	ND	ND		-	-		-	-	-	-	
C20:4n6	0.04±0.00^{b}	1.14±0.00^{a}	<0.01	1.19±0.00^{a}	0.00±0.00^{b}	<0.01	0.09±0.00^{b}	0.00±0.00^{c}	2.28±0.00^{a}	0.00±0.00^{c}	<0.01
C20:5n3	1.39±0.07^{b}	5.33±0.07^{a}	<0.01	5.62±0.04^{a}	1.10±0.07^{b}	<0.01	0.58±0.10^{c}	2.20±0.10^{b}	10.66±0.10^{a}	0.00±0.00^{d}	<0.01
C22:2	0.28±0.01^{a}	0.00±0.00^{b}	<0.01	0.00±0.00^{b}	0.28±0.01^{a}	<0.01	0.00±0.00^{b}	0.56±0.02^{a}	0.00±0.00^{b}	0.00±0.00^{b}	<0.01
C22:6n3	9.95±0.03^{a}	2.50±0.03^{b}	<0.01	5.31±0.03^{b}	7.18±0.03^{a}	<0.01	9.02±0.04^{b}	10.89±0.04^{a}	1.60±0.04^{d}	3.47±0.04^{c}	0.02

Means on the same row (in each section) with different superscript are statistically significant ($p<0.05$)

5.3.3.4 Percentage composition of saturated monosaturated and polyunsaturated fatty acids in biofloc meal collected from IMTAS

The percentage composition of saturated monosaturated and polyunsaturated fatty acids in the biofloc meal are presented in **Figure 5.3**.



	GRG 1		GRG 2	
	Freeze-dried	Oven-dried	Freeze-dried	Oven-dried
SFAs	51.81[b]	48.93[c]	40.27[d]	56.36[a]
MUFAs	31.80[b]	28.45[c]	33.64[a]	31.57[b]
PUFAs	16.38[c]	22.60[b]	26.07[a]	12.05[d]

Figure 5.5: Percentage composition of Saturated monosaturated and polyunsaturated fatty acids in the biofloc meal

Figure 5.5 illustrates the composition of fatty acids in biofloc meal when subjected to two different processing techniques for saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). It was observed that GRG 1 freeze-dried samples contain a higher percentage (51.81%) of saturated fatty acids compared to oven-dried samples (48.93%). This finding suggests that freeze-drying retained more saturated fats in GRG 1 than oven-drying. In contrast, GRG 2 showed a significantly higher percentage of saturated fatty acids (SFAs) in oven-dried samples (56.36%) compared to freeze-dried samples (40.27%).

Overall, the SFAs in the oven-dried samples of GRG 2 (56.36%) were significantly higher for an aggregate of SFAs. GRG 1 freeze-dried samples of monounsaturated fatty acids (MUFAs) had a higher percentage (31.80%) compared to oven-dried samples (28.45%). Records from GRG 2 showed that the freeze-dried samples had a slightly higher percentage of MUFAs (33.64%) compared to oven-dried samples (31.57%). The percentage composition of MUFAs was highest in GRG 2, freeze-dried samples (33.64%). GRG 1 recorded a higher percentage of polyunsaturated fatty acids (PUFAs) in oven-dried samples (22.60%) compared to freeze-dried samples (16.38%). In contrast, the percentage of PUFAs in freeze-dried samples of GRG 2 was found to be higher (26.07%) than in oven-dried samples (12.05%). It is noteworthy that the percentage composition of PUFAs in the former (freeze-dried samples) was significantly higher than SFAs and MUFAs.

5.3.4 Nutrient evaluation of biofloc meal collected from IMTAS

Figures 5.6 and **Figure 5.7** illustrate the contributions of the variables to dimensions 1 and 2, respectively. PCA revealed that dimension 1, which explained proximate composition and fatty acids, was influenced by a wide range of amino acids and fatty acids. Only three variables, phenylalanine, ether extract, and crude fibre, did not significantly contribute to this dimension. Dimension 2, which is characterised by amino and fatty acid composition, was significantly affected by amino acids and fatty acids, as well as some proximate components. Five variables, leucine, cis-10-pentadecenoic acid, heptadecenoic acid, ether extract, and crude fibre, did not contribute significantly to this dimension.

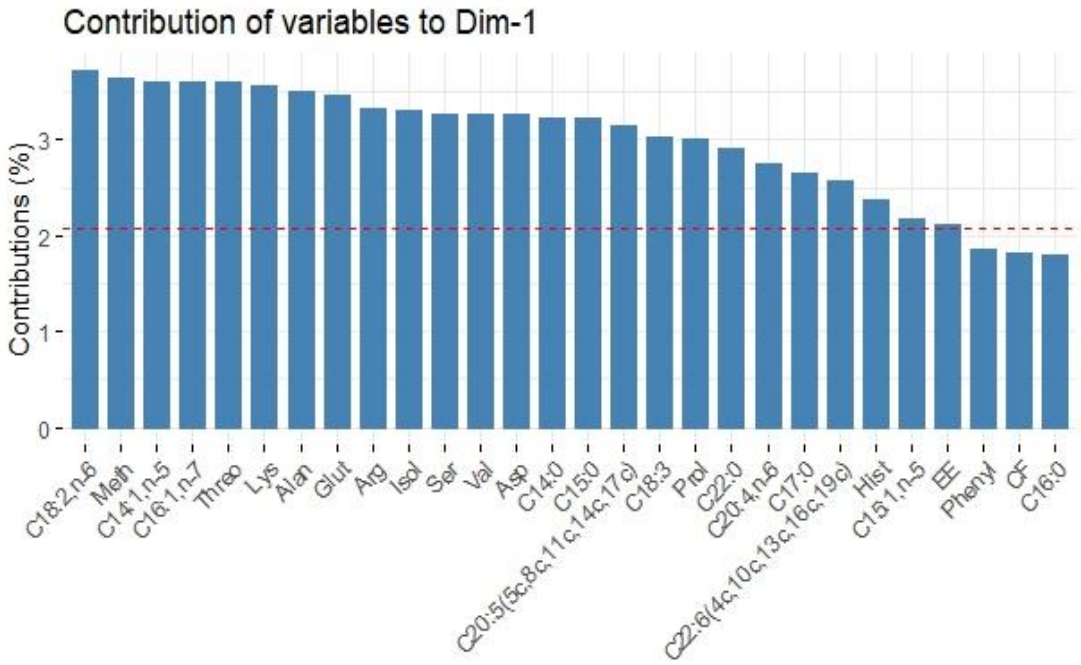


Figure 5.6: Contributions of PCA variables in dimension 1 (Dim 1)

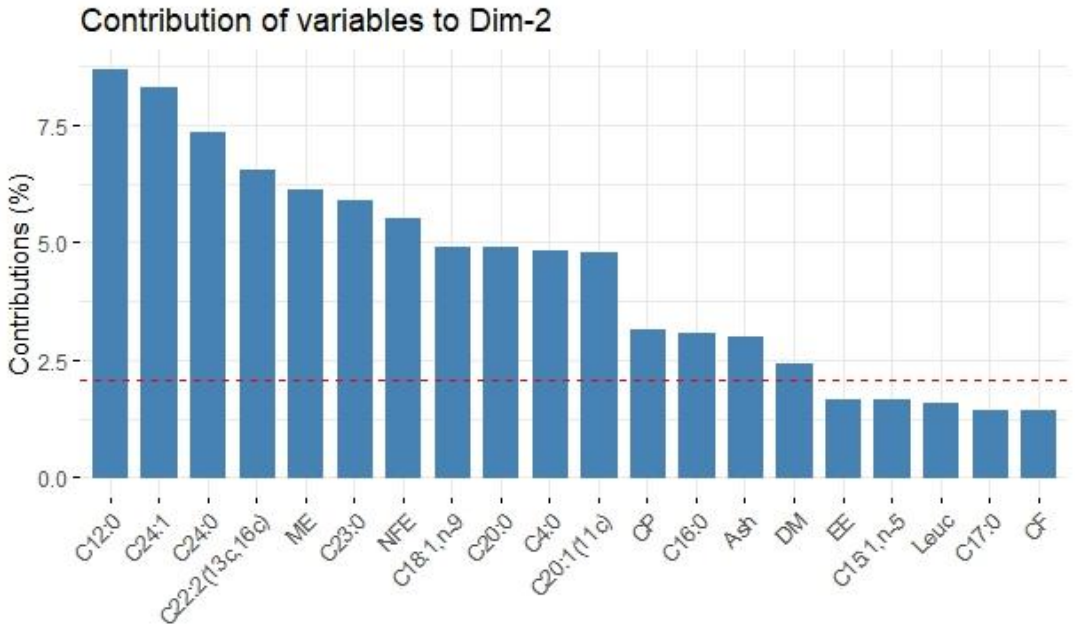


Figure 5.7: Contributions of PCA variables in dimension 2 (Dim 2)

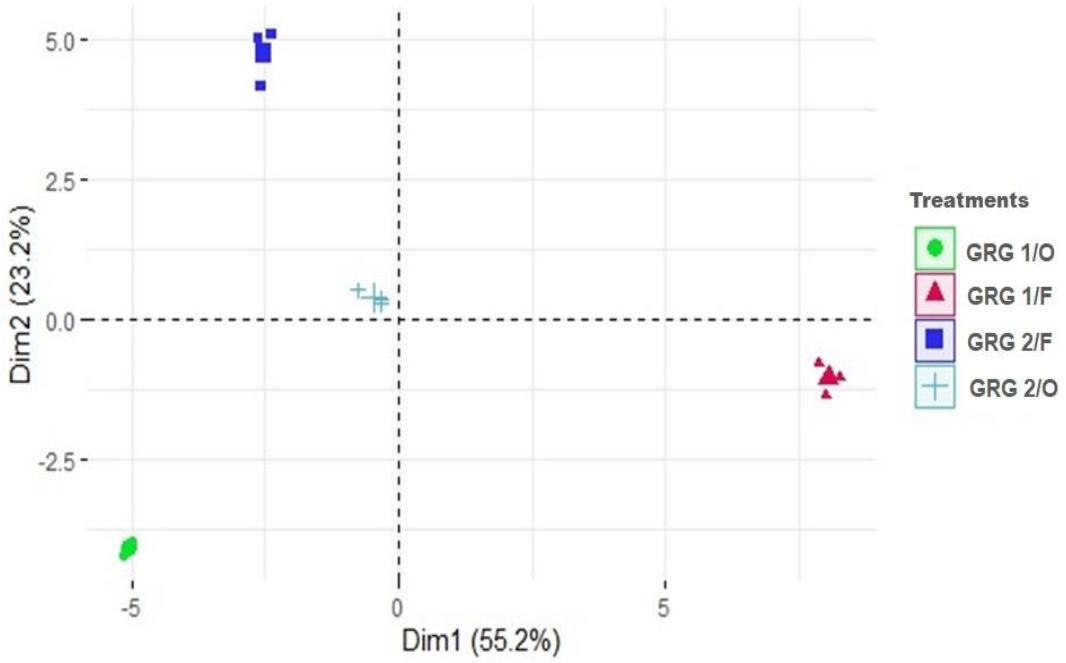


Figure 5.8: PCA of the compositional function of the feed samples

The PCA of the compositional functions of the feed samples is shown in **Figure 5.8**. GRG 1/F, with dimensions of 8 for dim 1 and -1.23 for dim 2, was deemed the best feed sample because of its high levels of proximate composition and fatty acids, supported by a moderate amino acid profile. In contrast, GRG 1/O, with dimensions of -5 for dim 1 and -3.8 for dim 2, was the least nutrient-dense feed sample, exhibiting low levels of both proximate components and amino acids. GRG 2/F, with dimensions of -2.5 for dim 1 and 5 for dim 2, had a high amino acid content but low levels of proximate composition and fatty acids. On the other hand, GRG 2/O, with dimensions of -0.5 for dim 1 and 0.5 for dim 2, displayed a balanced composition of proximate components, fatty acids, and amino acids. However, their concentrations were too low for proximate composition and amino acid and fatty acid profiles.

5.4 Discussion

The results indicated that GRG 1 possessed a greater amount of ether extract (EE) content than GRG 2. Generally, a higher ether extract (EE) content in feedstuff is an indication of a higher fat content, which is essential for providing energy and improving diet palatability for poultry (Kerr et al., 2024). High ether extract represents increased energy density, which can be beneficial for meeting the energy requirements of animals, especially those with high energy needs, such as lactating or growing animals (Noblet et al., 2022). Diet energy density can be modified by including ingredients that either reduce or increase the energy concentration, such as fibre-rich ingredients that decrease energy concentration or fat fibre-rich ingredients that increase energy concentration. Animals are known to prefer fatty feeds for increased feed intake. A study by Palomar et al., (2020) showed the preference of hens for high-fat diets over low-fat ones, demonstrating that the fat content significantly affects feed palatability and intake. Similarly, Lauridsen et al., (2021) found that the presence of fat in the diet enhances the absorption of fat-soluble vitamins (A, D, E, and K) due to fats facilitating the formation of micelles, which are crucial for the absorption of these vitamins in the gut.

The nitrogen-free extract (NFE) content of GRG 2 was found to be higher compared to the other feedstuffs. This increase in NFE content is indicative of a higher concentration of easily digestible carbohydrates, such as sugars and starches. According to Amrullah et al., (2019), feedstuffs with high NFE content are particularly important in the diets of young and growing animals, as they require more energy for development and maintenance of bodily functions. Furthermore, a higher NFE content improves feed efficiency. A study by Bowyer et al., (2020) on the growth performance, feed efficiency, and gut morphology of juvenile Nile tilapia (*Oreochromis niloticus*) fed different UK lupin meal cultivars found higher growth in fish diets with a higher nitrogen-Free Extract (NFE), which they inferred to be due to the improved feed efficiency provided by the readily digestible carbohydrates. They also noted that the NFE content in feedstuff enhances the energy density and digestibility of the feed, supporting growth and production. Attia et al., (2022) also reported that nitrogen-free

extract, including starch, improves nutrient digestibility and energy utilisation in poultry feed.

Freeze-drying resulted in increased crude protein (CP) content in the freeze-dried samples. Unlike the oven-drying method, which can cause denatured proteins or nutrient loss, freeze-drying effectively preserves the original nutritional profile. By maintaining the physical structure and bioavailability of nutrients, freeze-drying enhances digestibility, thereby improving nutrient absorption and metabolism, which contributes to a higher metabolisable energy (ME) content. High-temperature drying methods have been shown to cause degradation of some carbohydrates or chemical changes, resulting in a decrease in the neutral detergent fibre (NDF) content (Minorova et al., 2022). Consequently, the high organic fraction of the dry matter in the freeze-dried feed reduced the relative percentage of ash. The increased concentration of non-fibre components (proteins, fats, and soluble carbohydrates) in the freeze-dried samples of GRG 1 and GRG 2 resulted in a smaller fraction of crude fibre, which constituted a smaller proportion of the total dry matter. Oven-drying at high temperatures results in the loss of volatile organic compounds, which would otherwise contribute to the total dry matter thereby increasing the relative proportion of inorganic minerals, and leading to a higher measured ash content, as observed in the oven-dried samples of GRG 1 and GRG 2. On the other hand, freeze-drying (FD) is an effective method for preserving higher crude protein (CP), ether extract (EE), and nitrogen-free extract (NFE) in feedstuff due to its low-temperature, non-thermal process. This prevents nutrient degradation, volatilisation, and loss by removing water through sublimation, thereby preserving the nutritional quality of the feed. The low temperatures used in the freeze-dried samples of GRG 1 and GRG 2 samples improved the nutrient profile by preventing protein denaturation, lipid oxidation, and carbohydrate degradation.

The GRG 1 feed sample contained a substantial amount of both essential and non-essential amino acids, which are known to have a substantial impact on the growth of animals. A study conducted by Maiti et al., (2022), reported that the optimal dietary level of lysine is 23.46 g/kg for shrimp for muscle growth and enlargement in

metabolic pathways that lead to hyperplasia and hypertrophy. Lysine is also a precursor of carnitine, which is essential for mitochondrial beta-oxidation and transport of fatty acyl groups. McLean et al., (2022) also reported that lysine and arginine are necessary for protein synthesis and muscle development of aquaculture animals. Li et al., (2021) also confirmed that these essential amino acids play a significant role in enhancing the immune function of crustaceans in resisting diseases and stress, thereby promoting their survival and health in aquaculture environments. These essential amino acids contribute to the efficient utilisation of energy in the diet, which is essential for the maintenance and growth of aquatic animals. Wang & Liu, (2023) reported that arginine aids in nitric oxide synthesis for antimicrobial action and immune modulation in shrimp, whereas glutamic acid helps in the production of glutathione, protecting against oxidative stress, and enhancing shrimp resilience to environmental stressors. In contrast, Kombat et al., (2023) reported that non-essential amino acids (NEAAs) such as aspartic acid, glycine, alanine, and proline are vital for efficient metabolism and energy production and play roles in various metabolic pathways, cellular structure maintenance, and tissue repair, while aspartic and glutamic acids are key components of the krebs cycle, essential for energy metabolism in aquatic animals. On the other hand, GRG 2 was higher in methionine. Higher C/N ratio in GRG 2 (C20N) promoted more heterotrophic microbial activities to enhance synthesis of methionine and tyrosine than GRG 1 (C10N) with lower C/N ratio. Machado et al., (2023) reported that graded increase of methionine dipeptide up to 0.24 enhances growth performance and feed efficiency in shrimp.

Based on these result, GRG 1 was considered a better feed sample for shrimp culture because of its wide-ranging amino acid profile essential for growth, protein synthesis, and immune function. The drying method significantly affected the amino acid composition of the feed samples. Freeze-dried (FD) samples showed higher levels of essential amino acids, such as histidine, arginine, threonine, valine, lysine, isoleucine, leucine, phenylalanine, tryptophan, and cysteine, as well as non-essential amino acids (aspartic acid, glutamic acid, alanine, proline, and tyrosine) in contrast to oven-dried samples that had methionine concentrations.

The results of saturated fatty acids (SFAs) in biofloc meal showed significant variations influenced by treatments (GRG 1 and GRG 2) and drying methods (freeze-drying, D, and oven-drying, OD). GRG 1 contained significantly higher levels of lauric, myristic, pentadecanoic, heptadecanoic, stearic, behenic, tricosanoic, and lignoceric acids, whereas GRG 2 contained higher levels of lauric and palmitic acids. Baharuddin et al., (2024) measured various saturated fatty acids (SFAs) in the marine benthic diatom *Halamphora* sp. and enriched harpacticoid copepod (EHC) and reported their role in the growth and health of shrimp postlarvae. FD often retained more SFAs, except for lauric and tricosanoic acids, which were higher in OD samples. Although GRG 2 had a broader range of long-chain saturated fatty acids (SFAs), such as myristic, pentadecanoic, heptadecanoic, stearic, behenic, and tricosanoic acids, which are beneficial for energy storage and membrane structure, it lacked the antimicrobial advantage of lauric acid. Additionally, freeze-drying (FD) retained more SFAs, further enhancing the nutritional value of the GRG 1. The high oleic acid content in GRG 1 made it a better feed sample for shrimp culture because of its ability to provide essential energy and anti-inflammatory benefits. Additionally, the presence of gadoleic and nervonic acids is essential for supporting cellular and neural well-being. This resulted in GRG 1, especially in freeze-dried samples possessing enhanced energy supply for improved functioning of the cell membrane. Although GRG 2 had higher levels of myristoleic and palmitoleic acids, which are essential for antimicrobial and metabolic functions, GRG 1 had a broader range of beneficial monounsaturated fatty acids (MUFAs) needed for the growth, energy, and immunity of animals. The presence of cis-13,16-docosadienoic acid in GRG 1 is reported to support cell membrane performance. Lu et al., (2024) confirmed that the upregulation of cis-13,16-docosadienoic acid was linked to its role in metabolic processes or stress responses of organisms. In addition, high levels of docosahexaenoic acid are essential for neural and visual development. GRG 2, on the other hand, had beneficial PUFAs such as linoleic acid, α-linolenic acid, arachidonic acid, and eicosapentaenoic acid, which support immune function and reduce inflammation. Freeze drying enhances these benefits by retaining more essential PUFAs. The high content of oleic acid in GRG 1 made it a better feed sample for animal culture due to its ability to provide essential energy and anti-inflammatory benefits. In addition, gadoleic and nervonic acids are essential for supporting cellular and neural health. Phung et al., (2023) noted that

nervonic acid is essential for cellular structure, signaling, anti-inflammation, lipid mobilization.

In summary, freeze-drying of GRG 1 was noted to retain more saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), which are essential for energy provision, cellular health, and metabolic function. In contrast, oven-dried GRG 1 retained more polyunsaturated fatty acids (PUFAs). The inclusive nutritional balance of SFAs and MUFAs in freeze-dried GRG 1 samples offered a balanced profile to support the growth and health required in aquafeeds. On the other hand, GRG 2 had higher SFA retention when oven-dried and more MUFAs under freeze-drying conditions. The balanced fatty acid profile in freeze-dried GRG 1 provides the necessary energy, supports cellular performance, and promotes optimum growth of animals.

5.5 Conclusion

Freeze-drying effectively preserved the nutritional quality of the feed samples by preventing nutrient degradation, lipid oxidation, and protein denaturation, as shown by the higher levels of crude protein, ether extract, and nitrogen-free extract in the freeze-dried samples. GRG 1 had a reliable fatty acid profile, with higher levels of lauric, myristic, pentadecanoic, heptadecanoic, stearic, behenic, tricosanoic, and lignoceric acids, which are useful for the provision of energy, immune function, and the structural role of cell membranes. Lauric acid has antimicrobial properties that enhance the health of cultured animals. Additionally, beneficial monounsaturated fatty acids such as oleic, gadoleic, and nervonic acids which support cellular health and energy metabolism, were found in the GRG 1 samples. GRG 1 also contained higher levels of essential and non-essential amino acids, which are crucial for protein synthesis, muscle development, immune function, and efficient metabolism.

CHAPTER 6

PHYTOREMEDIATION OF WASTERWATER IN THE INDOOR MULTI-TECHNO AQUACULTURE SYSTEM USING *Caulerpa lentilifera*

6.1 Introduction

Discharge of aquaculture wastewater into the environment can have severe consequences, leading to water pollution and ecosystem degradation. Research has shown that the release of untreated or poorly treated aquaculture effluents can contaminate both surface and underground water sources, create air pollution, and disrupt aquatic ecosystems (Adetunji & Olaniran, 2021; Razman et al., 2023). Aquaculture effluents are characterised by an abundance of nutrients, organic matter, and pollutants, which can have deleterious effects on water quality and surrounding ecosystems when not treated properly (Cargnin & João, 2021; Esteves et al., 2022). Discharging aquaculture effluents that contain high levels of pollutants without treatment can result in eutrophication, water body pollution, and endangering marine environments (Shao et al., 2021). The release of untreated biofloc wastewater can result in eutrophication, which occurs when excess nutrients stimulate algal growth, deplete oxygen levels, and harm aquatic organisms (Selvarajan et al., 2021). Moreover, untreated biofloc wastewater has been linked to the spread of antibiotic resistance genes, affecting microbial communities, and potentially posing a threat to public health (Gvozdić et al., 2023; Nazifa et al., 2021).

Bioremediation is a cutting-edge, eco-friendly technology for pollution control that employs natural biological processes to transform harmful pollutants into less toxic or non-toxic substances. Utilising microorganisms such as bacteria, fungi, and plants, this process involves the degradation or metabolism of pollutants present in soil, water, and air, as demonstrated in studies by Ganguly et al., (2023) and Mohamed et al., (2024). Certain bacterial and fungal strains can transform toxic chemicals into harmless byproducts, such as carbon dioxide, water, and biomass, whereas specific plants, referred to as hyperaccumulators, absorb and accumulate pollutants through their root systems and tissues. The primary methods of bioremediation involve biodegradation, where microorganisms use enzymes to break down organic pollutants; bioaugmentation, which entails adding specific strains of microorganisms to contaminated sites to accelerate degradation; and biostimulation (Ayilara & Babalola, 2023).

Bioremediation involves modifying the environment to stimulate existing microorganisms through the addition of nutrients, oxygen, or other amendments. This approach is often more cost-effective than traditional physical and chemical methods, as it reduces the need for contaminant removal and promotes a sustainable approach by utilising natural processes. However, excessive nutrients (nitrogen and phosphorus) in aquaculture systems, which are generated from fish feed and waste, can lead to eutrophication, causing algal blooms and oxygen depletion (Sahu et al., 2024). Phytoremediation involves intentional introduction of specific plant species, such as seaweed, water hyacinth, and duckweed, to absorb excess nutrients through their tissues, thus reducing their concentrations in the water. The biomass generated by these plants is utilised as feed, fertiliser, or bioenergy. In a study conducted by Fernando et al., (2024), the biomass from the Giant reed (*Arundo donax* L.) was found to be utilized for the production of various bioenergy and biofuel products, including paper pulp, building materials, and nanocellulose.

Caulerpa lentilifera is a highly effective phytoremediation agent in aquaculture systems due to its ability to absorb and accumulate a variety of pollutants, including excess nutrients like nitrogen and phosphorus from aquaculture wastewater. The

biomass produced by *Caulerpa lentilifera* is also utilised for animal feed, as fertiliser in agriculture, and as a raw material for bioenergy production. In this study, *Caulerpa lentilifera* was applied for the purpose of removing nutrients from wastewater meant for reuse in the Indoor Multi-Techno Aquaculture System.

6.2 Methodology

6.2.1 Description of the phytoremediation unit

The design of the phytoremediation unit was intended to promote nutrient absorption by *Caulerpa lentilifera* (**Figure 6.1**). Prior to entering the phytoremediation unit, wastewater from the flocculation unit was passed through a sand filter chamber for mechanical filtration. The phytoremediation unit consisted of four PVC tanks, each measuring 2 X 4 X 1m in size, with one serving as the reservoir of the unit and the other three tanks used to grow *Caulerpa lentilifera* fixed on hung crates. Three 120-watt flood lamps were positioned 0.5 m above the tanks and managed by a timer that provided light from 7 AM - 6 PM daily to simulate natural diurnal lighting conditions indoors. In addition, an air blower was installed to ensure efficient water circulation and nutrient distribution within tanks. The stock was supported by crates constructed using PVC ¼" pipes and mesh, measuring 0.5 X 0.5 m, which were suspended on a frame situated midway in the water column. The system operated on a recirculation cycle that lasted for seven days, during which water was periodically moved back to the main reservoir to replenish the production unit.

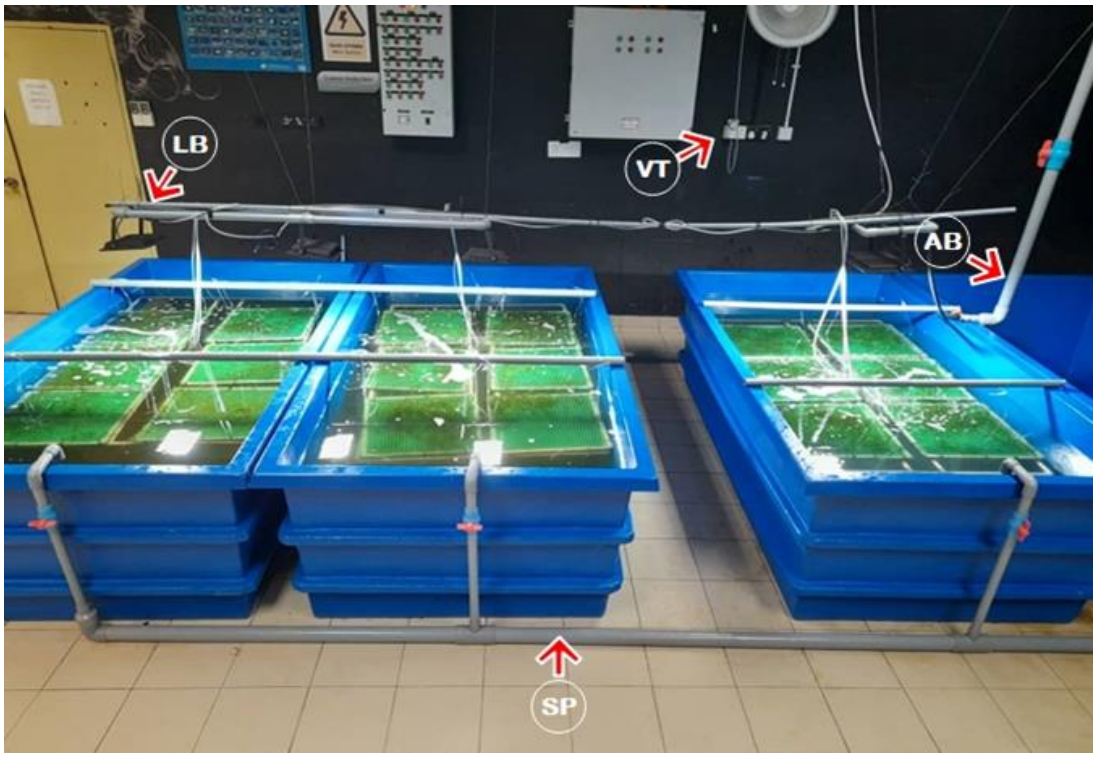


Figure 6.1: The phytoremediation unit.
Note that LP=lighting bulb; VT=voltage timer; AB=airblower; SP=supply pipe

6.2.2 Stocking of *Caulerpa lentilifera* in the phytoremediation unit

During the stocking of the phytoremediation unit, 50 g of *Caulerpa lentilifera* was measured and placed in a mesh. Each crate was then covered with the same type of mesh and securely fastened using cable-tie clips to ensure that the *Caulerpa lentilifera* was held in position within the crate. These loaded crates were subsequently positioned on the holding frames, which were set to suspend them midway within the water column. This strategic placement was essential to facilitate optimal exposure to the light source and aid the interaction between *Caulerpa lentilifera* and water for efficient phytoremediation.

6.2.3 Monitoring of water quality in the phytoremediation unit

Water quality parameters were measured following the methods earlier described in Chapter 3 (**Section 4.2.5**). Water samples were collected from the phytoremediation reservoir after mechanical filtration as the entry point and the return point in the main reservoir as the exit point.

6.2.4 Determination of tissue nutrient of *Caulerpa lentilifera* in phytoremediation unit

6.2.4.1 Determination of phosphate concentration

Phosphate levels were determined using the Malachite Green (MG) technique, as described by D'Angelo et al., (2001). This process involved the preparation of two different reagents. Reagent 1 comprised 14.2 mmol L^{-1} ammonium molybdate in 3.1 M H_2SO_4. Reagent 2, on the other hand, comprised 3.5 g L^{-1} aqueous polyvinyl alcohol (PVA) and 0.35 g L−1 malachite green. The addition of PVA to Reagent 2 enhanced its stability, thereby maintaining the stability of the reagents at room temperature. To estimate the phosphorus (P) concentration in the extract, a sample of the supernatant was transferred to a 25-mL volumetric flask, and the pH was adjusted with NaOH/HCl, similar to the procedure employed in the MB-AA method (excluding the use of 4-nitrophenol as an indicator). The flask was subsequently filled with deionised water to a volume that ensured a pH above 5.4 and the dilution factor. Two hundred microliters of the diluted supernatant (200 µL) was then transferred to a well in a 96-well microplate using an Eppendorf pipette to measure the absorbance. Subsequently, 40 µL of reagent 1 was added to the sample and mixed for 30 min. Forty microliters of Reagent 2 was then added, followed by a sixty-minute centrifugation period at a speed of 100 revolutions per minute on a rotator (Eppendorf 5810). Absorbance was measured at 630 nm using a universal microplate reader (Bio-Rad model 550).

The conversion from phosphate to phosphorus concentrations was carried with the conversion formula:

$$PO_4^{2-}\ (\text{g}) = \frac{3.066\ X\ P}{1000}$$ (**Equation 6.1**)

6.2.4.2 Determination of ammonia, nitrite and nitrate

The assessment of the ammonia, nitrite, and nitrate levels was based on the methodology outlined by Hachiya & Okamoto, (2017). Using a portion of *Caulerpa lentilifera* tissue weighing 10 g, samples were collected and stored at -80°C for analysis. To extract nitrate from the samples, 10 volumes of ultrapure water were added, followed by heating the sample at a temperature of 100°C for 20 minutes, while simultaneously shaking the samples periodically. The samples were subsequently subjected to centrifugation at a velocity of 20,400 × g for 10 min using a rotator (Eppendorf 5810) at room temperature. The concentration of nitrate in the supernatant was determined by adding salicylic acid in sulfuric acid (0.05% w/v) to a microtube containing the supernatant (10 µL) and incubating it at room temperature for 20 min. Thereafter, a solution of NaOH in ultrapure water (8% w/v) was added and the mixture was vigorously shaken until it became transparent. The absorbance of the resulting solution was determined using a spectrophotometer (Shimadzu UV-1800 UV-Vis) at a wavelength of 410 nm. A series of potassium nitrate dilutions was used to create the standard curve for nitrate.

Five volumes of extraction Reagent 1 were added to 10 g of the powdered sample, which was then subjected to centrifugation at 20,400 × g for 10 min at 4°C. The resulting supernatant was used for nitrite analysis. The concentration of nitrite was determined by combining 150 µL of the supernatant with 150 µL of freshly prepared sulfanilamide in 1 mol/L hydrochloric acid (1% w/v) in a 1.5 mL microtube, followed by centrifugation to remove any precipitates. An additional 100 µL of freshly prepared 0.02% (w/v) N-1-naphthylethylenediamine dihydrochloride in ultrapure water was added to 200 µL of the supernatant, mixed thoroughly, and incubated at room

temperature for 15 min. Absorbance was measured at 540 nm using a spectrophotometer. A standard curve for nitrite concentration was prepared using potassium nitrite dilutions.

Ammonium-nitrogen was measured using an ammonia test kit (Ammonia Test Wako, Wako Pure Chemical Industries, catalogue number: 277-14401), according to the manufacturer's instructions. One millilitre of 0.1 mol/L hydrochloric acid was added, followed by 500 µL of chloroform. The mixture was gently shaken at 4ºC for 15 min and centrifuged at 12,000 X g for 10 min at 8ºC. The aqueous phase was transferred to a 1.5 millilitre microtube containing acid-washed activated charcoal, shaken, and centrifuged at 12,000 X g for 5 min at 8ºC. The supernatants were collected for ammonium determination. The supernatant (200 µL) was combined with 800 µL of the deproteinisation reagent from the ammonia test kit and centrifuged at 2,300 × g for 5 min at 4 °C. Subsequently, 200 µL of the supernatant was mixed with 200 µL of reagent A, 100 µL of reagent B, and 200 µL of reagent C from the ammonia test kit, combined thoroughly, and incubated at 37°C for 20 min. The absorbance was measured at 630 nm using a spectrophotometer. A standard curve for ammonium was established using ammonium chloride dilutions.

6.2.5 Evaluation of growth and bio-filtration efficiency of *Caulerpa lentilifera* in the phytoremediation unit

The initial biomass per crate (50g) taken for each crate was noted and considered as ω_{d0}. The weight at harvest on day 7 was considered as the final weight marked ω_{d7}. The average daily growth (ADG) of *Caulerpa lentilifera* was calculated as described in Rahardjo et al., (2017).

$$ADG\ (\%) = \frac{(\omega_{d7} - \omega_{d0})}{7days} X\ 100 \qquad \textbf{(Equation 6.2)}$$

Where;

ω_{d0} = initial biomass of *Caulerpa* (day 0) and

ω_{d7} = final biomass of *Caulerpa* at harvest (day 7).

The percentage biofiltration efficiency (BE) of *Caulerpa lentilifera* was calculated based on the method described in Mansour, et al., (2022) as;

$$BE\ (\%) = \frac{(\alpha - \gamma)}{\alpha} X\ 100 \qquad \textbf{(Equation 6.3)}$$

Where;

α = concentration of wastewater at entry point into the phytoremediation unit and

γ = concentration of filtrate leaving the unit.

6.3 Results

6.3.1 Water quality in the phytoremediation unit

The mean daily variations in the measured water quality parameters are presented in **Figure 6.2, Figure 6.3, Figure 6.4, Figure 6.5, Figure 6.6, Figure 6.7, Figure6.8 and Figure 6.9**. **Figure 6.2** shows a trend graph depicting the mean variations in total ammonia nitrogen over a 7-day culture period. The initial values (entry points) ranged from 0.93 mg/L on Day 1 to 0.78 mg/L on Day 7, while the final values (exit points) ranged from 0.14 mg/L on Day 1 to 0.05 mg/L on Day 7. The variations, which indicate the differences between the exit and entry points, were highest on day 1 (0.79 mg/L) and lowest on day 5 (0.73 mg/L). In general, there was a decreasing trend in the total ammonia nitrogen measured during the culture period.

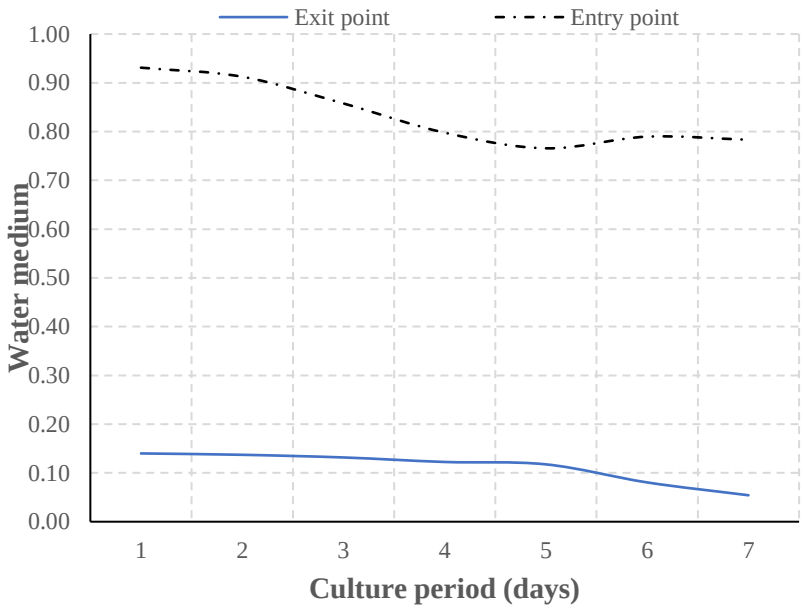


Figure 6.2: Mean daily variation in total ammonia-nitrogen of the water in the phytoremediation unit

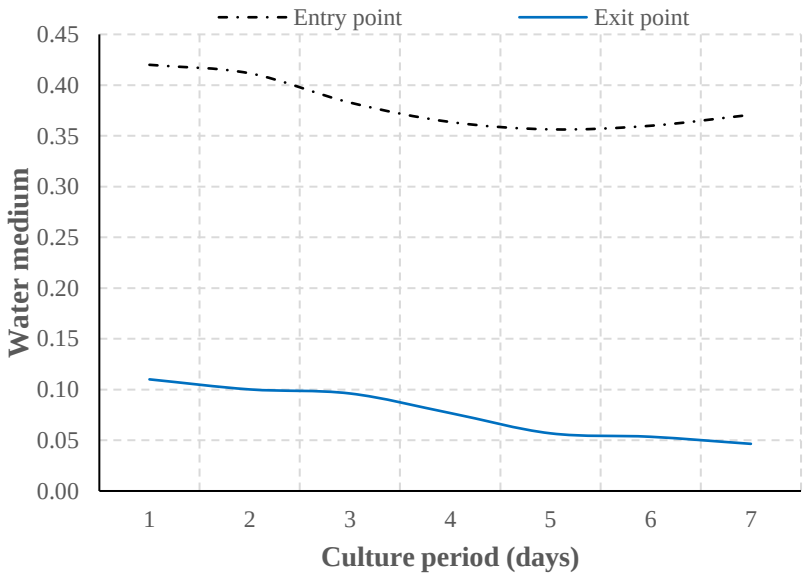


Figure 6.3: Mean daily variation in nitrite of the water in the phytoremediation unit

The mean daily variations in nitrite levels over the 7-day culture period are shown in **Figure 6.3**. There was a steady decline in the concentration of nitrite during

the culture period from 0.11 mg/L in day 1 to 0.05 mg/L in day 7. The mean difference between the entry and exit points was calculated to be 0.30 mg/L.

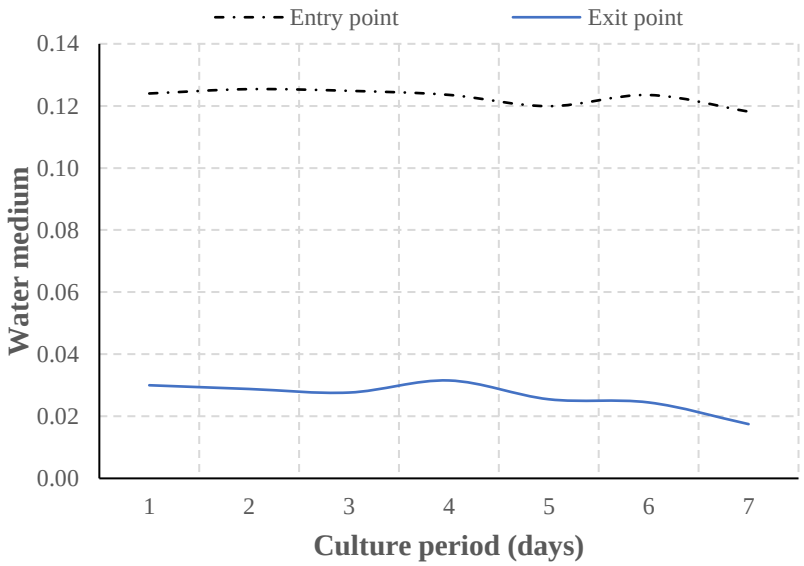


Figure 6.4: Mean daily variation in phosphate of the water in the phytoremediation unit

The phosphate concentration showed a slight reduction during the culture period, as shown in **Figure 6.4**. On day 1, the concentration was 0.028 mg/L, and by day 7, it had decreased to 0.017 mg/L. A noticeable discrepancy existed between the concentrations at the entry and exit points.

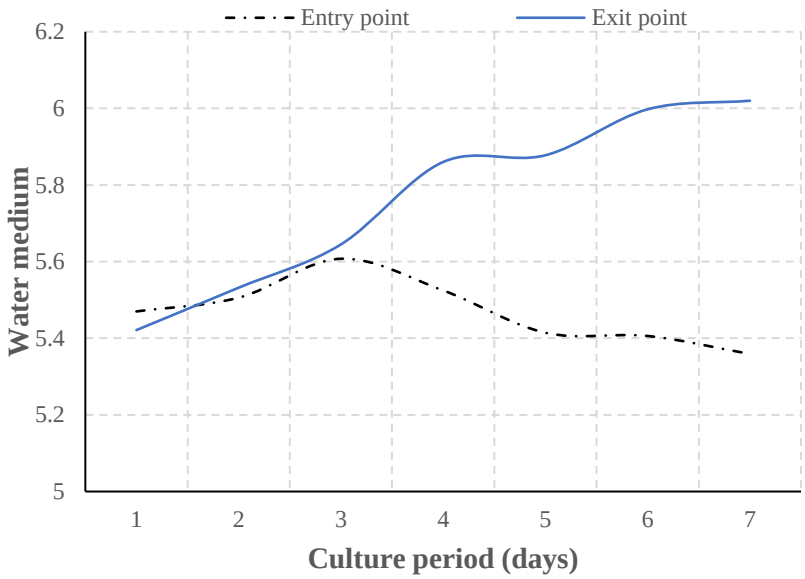


Figure 6.5: Mean daily variation in dissolved oxygen of the water in the phytoremediation unit

The graph in **Figure 6.5** illustrates the average fluctuations in dissolved oxygen levels throughout the cultivation period. On day 3, the concentration of dissolved oxygen at the entry point, which is the phytoremediation reservoir, was highest at 5.64 mg/L, but it decreased to 5.35 mg/L by day 7. In contrast, the concentration at the exit point consistently increased from 5.42 mg/L on days 1 to 6.02 mg/L on day 7.

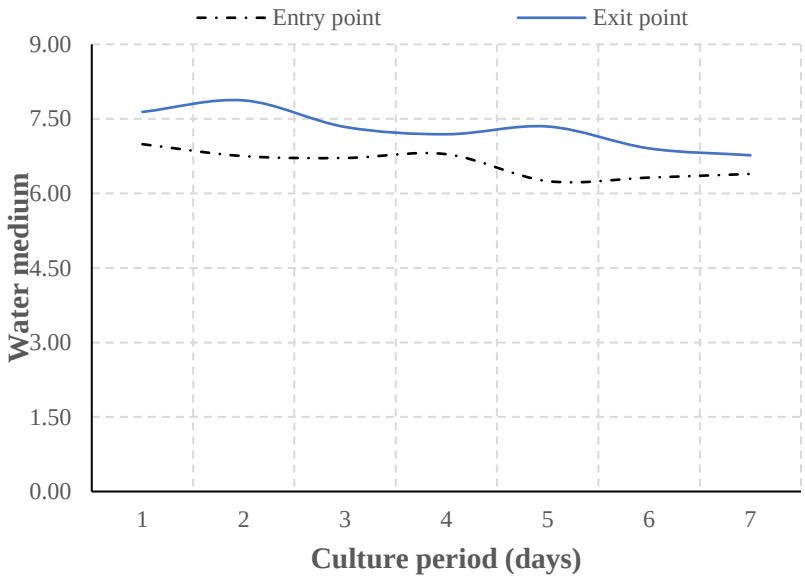


Figure 6.6: Mean daily variation in pH of the water in the phytoremediation unit

pH showed negligible variation during the culture period (**Figure 6.6**). The lowest pH was recorded on day 5 (6.25) at the entry point, whereas the highest pH (7.87) was observed on day 2 at the exit point. The variation in pH between entry and exit was also insignificant.

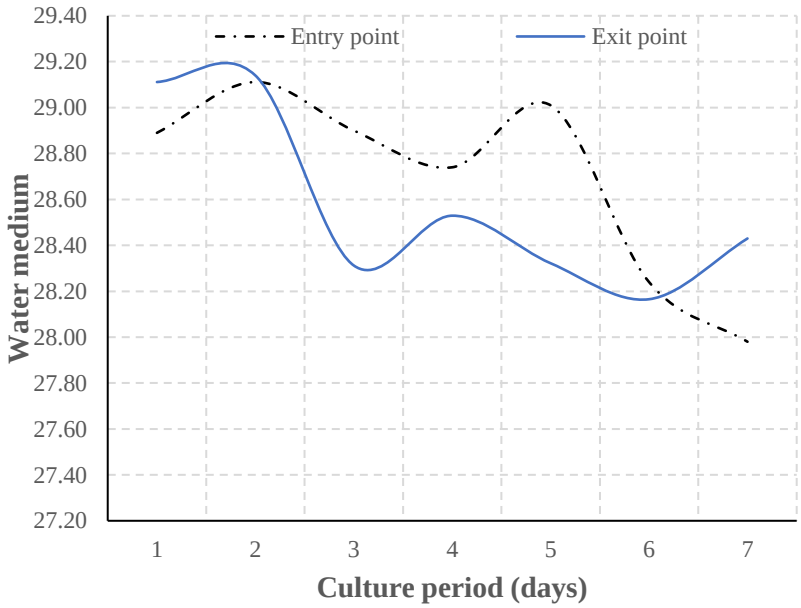


Figure 6.7: Mean daily variation in temperature of the water in the phytoremediation unit

The temperature generally showed a downward trend in the highly fluctuating trend (**Figure 6.7**). The highest temperature (29.09^{o}C) was observed on day 5 at the entry point, while the least (28.53 ^{o}C) was recorded on day 3. Generally, there was a decline in both entry and exit points during the culture period.

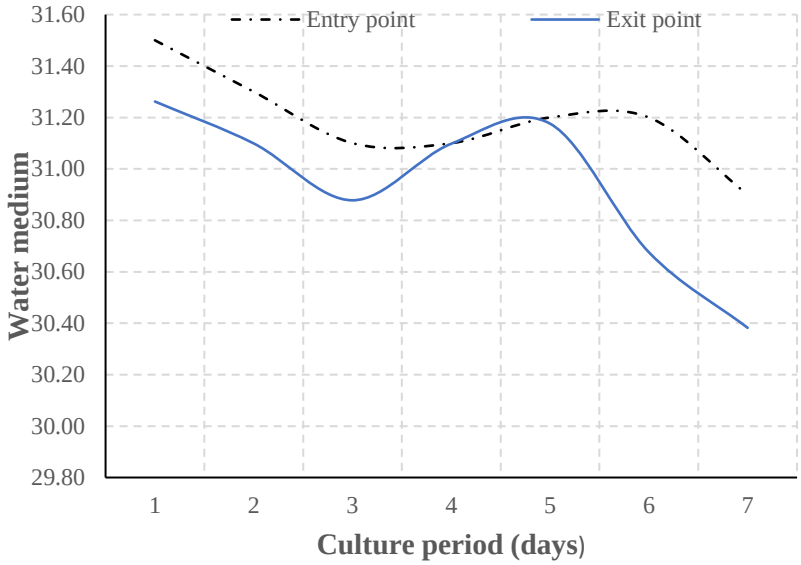


Figure 6.8: Mean daily variation in salinity of the water in the phytoremediation unit

The graph in **Figure 6.8** illustrates the salinity trend during the culture period. The lowest salinity value was recorded on day 3 (30.88 ppm) at the exit point, whereas the highest value (31.18 ppm) was recorded on day 5 at the same exit point. The salinity level at the exit point on Day 7 (30.38 ppm) was significantly lower than that at the entry point (30.90 ppm).

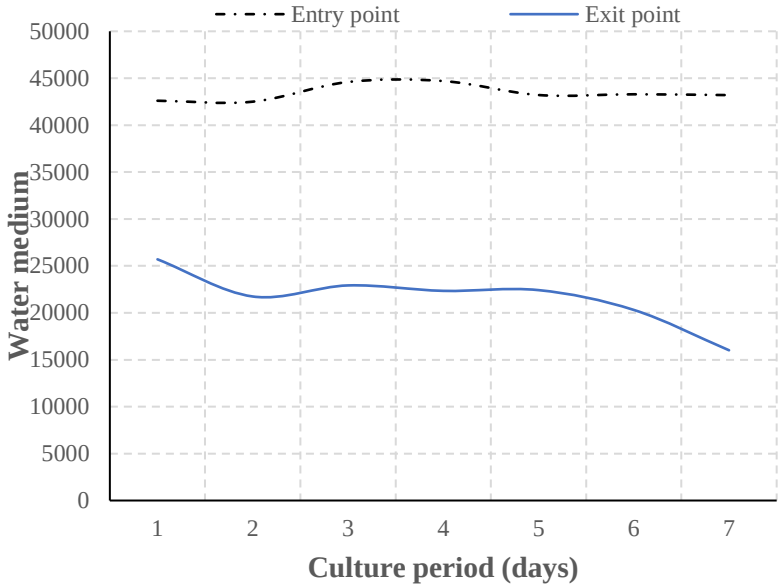


Figure 6.9: Mean daily variation in electrical conductivity of the water in the phytoremediation unit

The changes in the electrical conductivity throughout the culture period are shown in **Figure 6.9**. On the first day, there was a slight variation in electrical conductivity, which stood at 25,700 µS/cm, and it decreased to 16,010.60 µS/cm by the seventh day at the exit point. There was a noticeable difference in the electrical conductivity between the entry and exit points.

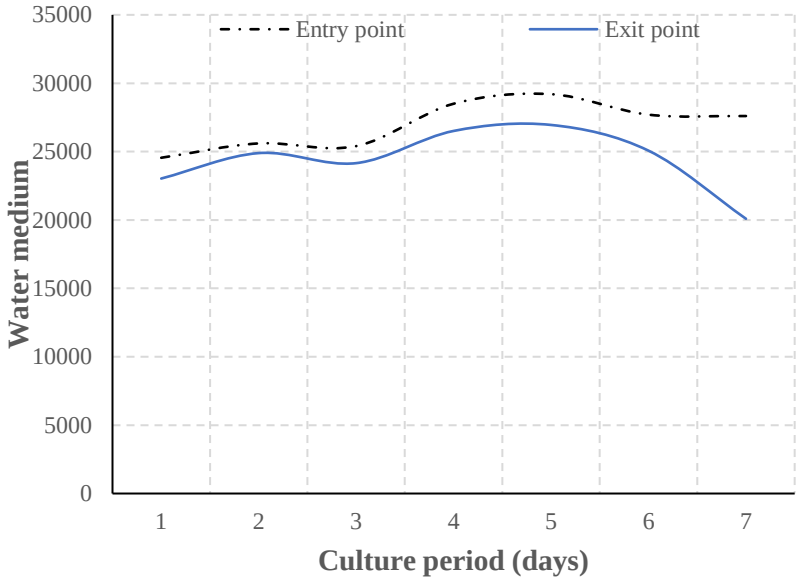


Figure 6.10: Mean daily variation in total dissolved solids of the water in the phytoremediation unit

Figure 6.10 represents the trend in the changes in the concentration of total dissolved solids (TDS) throughout the culture period. For the most part, there was a minimal variation between the entry and exit points. On the first day, the TDS level at the exit point was the lowest, amounting to 24892 mg/L, which decreased to 20088 mg/L on the seventh day. The entry point generally had a higher concentration.

6.3.2 Nutrient profile of *Caulerpa lentilifera* tissue harvested from the phytoremediation unit

Table 6.1 presented the nutrient profile of Caulerpa tissue. Ammonia-nitrogen (NH_3-N) was measured as 1.25 ± 0.07 µmol/g, indicating while nitrite (NO_2^-) showed a concentration of 0.28 ± 0.01 µmol/g. Nitrate (NO_3^-) on the other hand was markedly higher in concentration (4.70 ± 0.27 µmol/g). Phosphate (PO_4^{3-}) recorded a concentration of 0.32 ± 0.05 µmol/g in the tissue of Caulerpa.

Table 6.1: Nutrient profile of Caulerpa tissue

Nutrient	**Concentration (µmol/g)**
NH_3-N	1.25±0.07
NO_2^-	0.28±0.01
NO_3^-	4.70±0.27
PO_4^{3-}	0.32±0.05

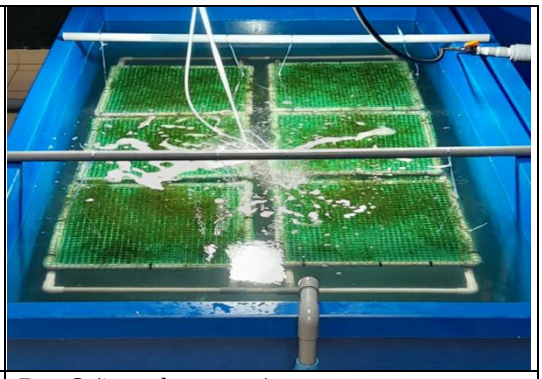
6.3.3 Growth and bio-filtration efficiency of *Caulerpa lentilifera* in the phytoremediation unit

Table 6.2 presents the average daily growth (ADG) rates of *Caulerpa lentilifera* in three phytoremediation tanks. Tank 1 recorded the highest ADG at 15.64 ± 1.02 g/day, followed by Tank 3 with 15.83 ± 0.59 g/day while Tank 2 was the least (13.95 ± 1.81 g/day). The pooled mean ADG across all tanks was 14.88 ± 0.76 g/day. In addition, **Figure 6.11** shows the pictorial view of the growth of *Caulerpa lentilifera* in the phytoremediation unit.

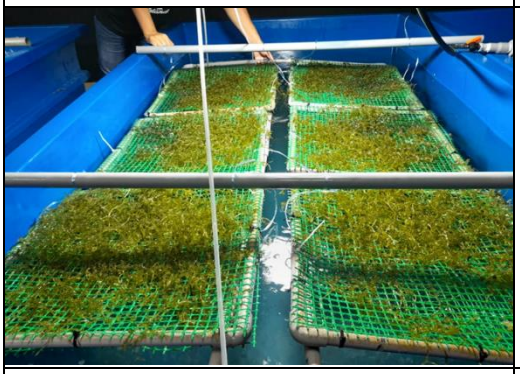
Table 6.2: Average daily growth (ADG) of *Caulerpa lentilifera*

Tank	**ADG (g/day)**
Tank 1	15.64±1.02
Tank 2	13.95±1.81
Tank 3	15.83±0.59
Mean	*14.88±0.76*

Day 1 (50g of *Caulerper* sp set in crates)



Day 3 (in early sprouts)



Day 5 (luxuriant growth)

Day 7 (Harvest)

Figure 6.11: Progressive growth of *Caulerpa lentilifera* in the phytoremediation unit.

Table 6.3 presents the biofiltration efficiency (% BE) of *Caulerpa lentilifera* in the phytoremediation tanks. Tank 3 had the highest efficiency for NH_3-N removal at 87.28 ± 2.02%, followed by Tank 1 at 85.06 ± 0.19%. Tank 1 showed the highest efficiency for NO_2^- removal, (80.02 ± 1.30%), while Tank 3 recorded the highest efficiency for NO_3^{2-} (86.20 ± 0.53%) and PO_4^{3-} (83.90 ± 0.70%) removal. The pooled mean BE values were computed as 83.45 ± 1.89% for NH_3-N; 77.52 ± 1.27% for NO_2^-; 84.42 ± 0.46% for NO_3^{2-} and 86.87 ± 0.42%) for PO_4^{3-} .

Table 6.3: Biofiltration efficiency (BE) of *Caulerpa lentilifera*

Tank	**NH_3-N**	$\boldsymbol{NO_2^-}$	$\boldsymbol{NO_3^-}$	$\boldsymbol{PO_4^{3-}}$
Tank 1 (%)	85.06±0.19	80.02±1.30	86.50±0.79	85.52±0.90
Tank 2 (%)	78.00±1.57	74.04±1.81	87.91±0.41	83.84±0.25
Tank 3 (%)	87.28±2.02	78.51±0.21	86.20±0.53	83.90±0.70
Mean (%)	83.45±1.89	77.52±1.27	84.42±0.46	86.87±0.42

6.3.4 Phytoremediation capacity of *Caulerpa lentilifera*

Nutrient concentrations at the entry and exit points of the phytoremediation system were assessed to determine the phytoremediation capacity of the *Caulerpa lentilifera* Standard least square regression was executed using ADG as the dependent variable and differentials in nutrients as the independent variable on JMP Pro 17.0.0. **Figure 6.12** shows the association between the observed and estimated average daily gains (ADG). The root means square error (RMSE) indicated a standard deviation of the prediction error of 0.352. The coefficient of determination (R.Sq) indicated a strong regression relationship of 0.90, which was statistically significant ($p < 0.01$).

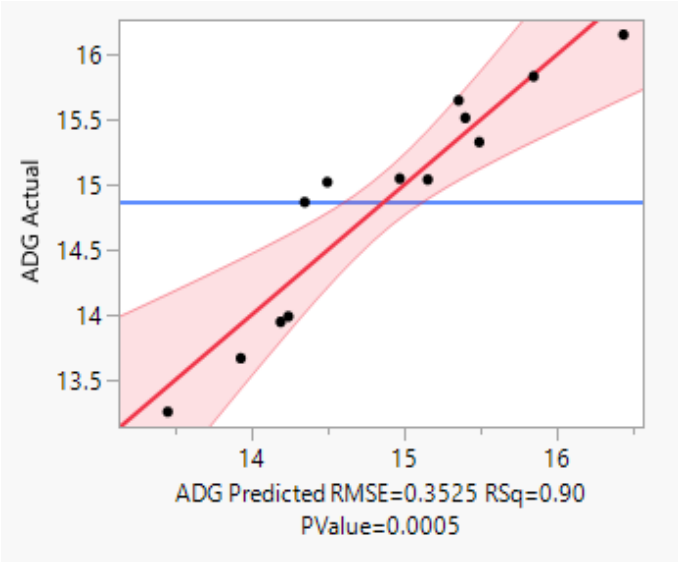


Figure 6.12: Predicted versus actual plot of average daily growth (ADG) of *Caulerpa lentilifera*

Table 6.4 shows the logworth and statistical significance (p-value) of the response variables (nutrients) in the multiple regression analysis with average daily growth (ADG). The nitrate had a logworth value of 1.646, which was statistically significant (p = 0.022). In contrast, nitrite, ammonia-nitrogen, and phosphate had logworth values of 0.523, 0.445, and 0.322, respectively, which were not statistically significant.

Table 6.4: Logworth and statistical significance of response variables

Source	Logworth		P-value
NO_3^-	1.646		0.022
NO_2^-	0.523		0.299
NH_3-N	0.445		0.358
PO_4^{3-}	0.322		0.476

Table 6.5 shows the estimated values of the response variables. The intercept term had a value of 2.075, which was not statistically significant (p-value = 0.519). The measurement of ammonia nitrogen was 3.334, with a non-significant p-value of 0.358. The estimated rate for nitrite was -4.946, which was not statistically significant (p = 0.300). The nitrate term had an estimate of 2.883, which was statistically significant (p-value = 0.022). The measurement of phosphate was 2.822, with a non-significant p-value of 0.476.

Table 6.5: Parameter estimates of the response variables

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.075	3.078	-0.67	0.519
NH_3-N	3.334	3.424	0.97	0.358
NO_2^-	-4.946	4.463	-1.11	0.300
NO_3^-	2.883	1.023	2.82	0.022*
PO_4^{3-}	2.822	3.778	0.75	0.476

**indicates statistical significance at 95% CL*

The graph in **Figure 6.13** illustrates the optimal prediction of ADG, which is based on the values of the response variables. According to the profiler, the ideal ADG was 14.835 g/day, with lower and upper confidence limits of 12.791 g/day and 16.878 g/day respectively. To achieve this optimal ADG, the required response variable values were 1.141 µmol/g for ammonia-nitrogen, 0.420 µmol/g for nitrite, 4.754 µmol/g for nitrate, and 0.523 µmol/g for phosphate. It is worth noting that ammonia-nitrogen was set to the lowest concentration owing to its toxic nature.

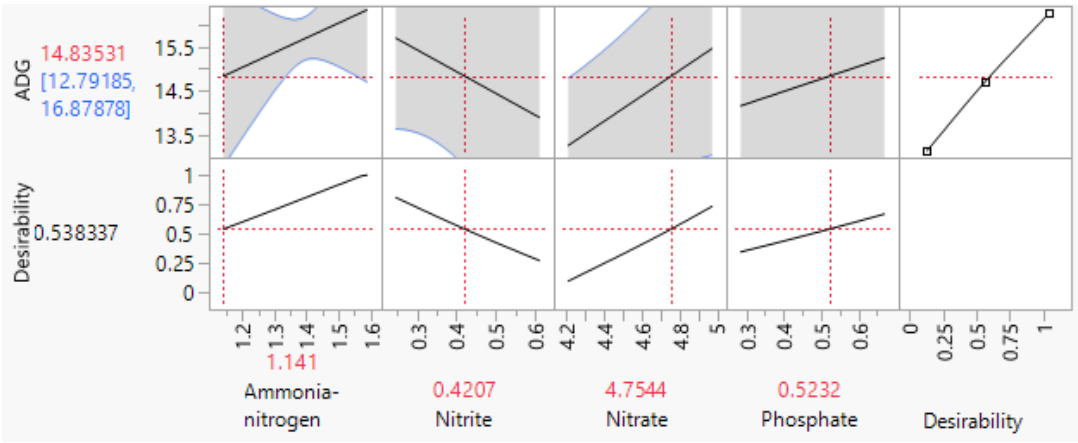


Figure 6.13: Optimisation prediction of ADG based on the response variables

6.4 Discussion

The observed trend in NH_3-N agrees with the findings of Huang et al., (2023), who reported that *Caulerpa taxifolia* achieved a maximum NH_4-N removal efficiency of 71.4% during the light period of the circadian rhythm, representing its strong ability to absorb ammonia nitrogen from artificial seawater. According to Putra et al., (2020), it is crucial to maintain nitrite levels below 0.07 mg/L for the health and growth of aquatic organisms. In this research, the phytoremediation process resulted in a reduction of nitrite concentration to 0.05 mg/L by Day 7, indicating effective removal of nitrite. Phosphate concentrations showed a slight decline throughout the culture period, decreasing from 0.028 mg/L on day 1 to 0.017 mg/L on day 7. This pattern

agrees with the findings of Fitria & Dhokhikah, (2021), who reported a significant decrease in phosphate levels using *Typha* sp. and *Echinodorus palaefolius*. Furthermore, Pang et al., (2021) reported that nutrient-replete microalgae could decrease the phosphate levels in low-strength aquaculture effluent to 0.05 mg/L, rendering it suitable for discharge.

The trend of dissolved oxygen levels at the entry point was descendant, reaching a peak of 5.64 mg/L on Day 3 and declining to 5.35 mg/L on Day 7. At the exit point, an upward trend was observed, with levels rising steadily from 5.42 mg/L on day 1 to 6.02 mg/L on Day 7. Maintaining dissolved oxygen levels above 5 mg/L is crucial for the well-being and growth of fish in Recirculatory Aquaculture Systems (RAS), as reported by Mnyoro et al., (2022). This explains why the phytoremediation process can enhance oxygen levels in water, thereby benefiting aquatic life. The pH levels in the culture system remained stable throughout the study period, with only minor fluctuations. The lowest pH was observed on day 5 (6.25) at the entry point, while the highest pH was recorded on day 2 (7.87) at the exit point. The addition of microalgae to wastewater can alter the pH from acidic to alkaline, promoting the growth of algae, as reported by Shahid et al., (2020). However, in the present study, the difference in pH between the entry and exit points was not significant, indicating stable pH levels during the culture period.

The temperature tended to decrease with variations, with the highest reading of 29.09°C recorded on Day 5 at the entry point and the lowest at 28.53 °C on Day 3. Similar temperature fluctuations were reported by Amyati et al., (2020) during the process of phytoremediation of catfish aquaculture wastewater using diverse aquatic plants. These fluctuations were likely due to interactions between aquatic plants, wastewater, and environmental conditions during the remediation process. Salinity also displayed fluctuations, with a downward trend observed at the exit point, starting at 30.88 ppm on Day 3 and peaking at 31.18 ppm on Day 5. On Day 7, the salinity level at the exit point was significantly lower (30.38 ppm) than at the entry point (30.90 ppm). Differences in salinity can have a significant impact on the growth properties of *Caulerpa lentilifera* According to Mosquera-Murillo & Pena-Salamanca, (2016),

Caulerpa sertularioides showed the highest growth rates at 25 ppt, whereas lower salinity levels resulted in decreased growth. Bambaranda et al., (2019) also emphasised the importance of maintaining salinity at 30 ppt for optimal growth and nutrient uptake in *Caulerpa lentillifera*.

The analysis of the nutrient content in the *Caulerpa lentilifera*, tissue collected from the phytoremediation system revealed that the nitrate concentration (NO_3^-) was significantly higher (4.70 ± 0.27 µmol/g) than the other measured nutrients. The ammonia-nitrogen (NH_3-N) level was measured at 1.25 ± 0.07 µmol/g, while the nitrite (NO_2^-) level was 0.28 ± 0.01 µmol/g, and the phosphate (PO_4^{3-}) level was 0.32 ± 0.05 µmol/g. The high nitrate concentration in *Caulerpa lentilifera* tissue is in agreement with previous research, which reported its high efficiency in absorbing nitrate. Bambaranda et al., (2019) found that the highest Nitrogen Use Efficiency (NUE) for nitrate was observed at a *Caulerpa lentilifera* density of 30 g/L, demonstrating its proficiency in nitrate assimilation. According to Alexandre & Santos, (2020), the total nitrogen acquisition rate for *Caulerpa prolifera* is 689 mmol m^{-2} h^{-1}, which explains its substantial nitrogen uptake from the environment. The measurement of ammonia-nitrogen (1.25 ± 0.07 µmol/g), although lower than nitrate, still demonstrated significant absorption. The phosphate concentration in *Caulerpa lentilifera* tissue was found to be 0.32 ± 0.05 µmol/g, representing the importance of the ability of the plant to absorb and store phosphate for its role in phytoremediation. Studies by Fitria & Dhokhikah, (2021) confirmed that other aquatic plants can also achieve significant phosphate reduction. The efficiency of *Caulerpa lentilifera* in mitigating eutrophication in nutrient-rich waters was demonstrated by its high phosphate uptake. The nutrient profile of the *Caulerpa lentilifera* tissue harvested from the phytoremediation unit revealed its ability to absorb and store essential nutrients, particularly nitrate, ammonia-nitrogen, nitrite, and phosphate, with high efficiency.

The regression analysis showed a strong relationship between the observed and predicted Average Daily Gain (ADG) values, with an R-squared (RSq) value of 0.90. This shows that 90% of the variability in ADG was explained by the model, indicating good predictive capability. The statistical significance (p-value < 0.01) further

supported the reliability of the model. High R-squared values have also been reported in phytoremediation studies involving *Lemna minor*, which showed an R-squared of 0.92 for nitrate removal prediction (Can-Terzi et al., 2021). The RMSE of 0.352 signifies the standard deviation of the prediction errors, indicating a relatively small difference between the observed values and the predicted values. This low RMSE value supports the reliability of the model in predicting ADG. The logworth values and p-values for various parameters suggest their statistical significance in affecting ADG. Nitrate had a logworth value of 1.646 and a statistically significant p-value of 0.022, indicating its substantial impact on ADG. On the other hand, nitrite, ammonia-nitrogen, and phosphate had lower logworth values and were not statistically significant, suggesting a lesser influence on ADG. This finding agrees with the study conducted by Shakouri & Balouch, (2020), who identified nitrate as the most significant factor influencing the growth rate of macro algae, *Ulva rigida*.

The analyst calculated the optimal daily weight gain (ADG) to be 14.835 g/day, with a lower limit of 12.791 g/day and an upper limit of 16.878 g/day. The specific nutrient concentrations required to reach this optimal ADG were 1.141 µmol/g for ammonia-nitrogen, 0.420 µmol/g for nitrite, 4.754 µmol/g for nitrate, and 0.523 µmol/g for phosphate. These values indicate that managing nitrate levels is essential for maximizing ADG, while minimizing ammonia-nitrogen levels due to their toxicity. Research by Pozzobon et al., (2021) on *Chlorella vulgaris* similarly emphasizes the significance of nitrate management for optimal growth. A study conducted by Ting et al., (2020) investigated the function of *Eichhornia crassipes* in eliminating ammonia-nitrogen. Their results confirmed the detrimental effects of elevated ammonia concentrations as observed in the present study. The influence of nitrate on ADG emphasises its important role in the development of *Caulerpa lentilifera* and other aquatic plant observations. The insignificant effect of nutrients such as phosphate and nitrite suggest that targeting nitrate levels would yield optimal growth outcomes. The necessity of controlling ammonia-nitrogen levels due to its toxicity is supported by multiple studies, indicating the importance of maintaining safe concentrations for effective phytoremediation (Shilpha et al., 2023). The optimized average daily gain values indicate specific nutrient concentrations that promote improved growth, emphasizing the critical role of ammonia-nitrogen management. These findings

contribute to the emerging evidence supporting the effectiveness of *Caulerpa lentilifera* in phytoremediation.

6.5 Conclusion

Caulerpa lentilifera demonstrated its substantial ability in enhancing water quality and nutrient absorption in the phytoremediation system. During the 7-day period, notable reductions were observed in total ammonia nitrogen, nitrite, and phosphate levels, consistent with prior research on the effective nutrient uptake of *Caulerpa lentilifera* Furthermore, dissolved oxygen levels improved, promoting aquatic health, while pH levels remained stable to ensure a balanced environment. The temperature and salinity in *Caulerpa lentilifera* tissue fluctuated, necessitating monitoring for optimal results. The nutrient profile analysis showed high levels of nitrate, ammonia-nitrogen, nitrite, and phosphate in the tissue, indicating the proficiency of *Caulerpa lentilifera* in nutrient storage. Furthermore, the strong regression relationship between the average daily growth of *Caulerpa lentilifera* and the nutrient composition in water in the phytoremediation unit emphasised the importance of managing nutrient levels for water reuse in the Indoor Multi-Techno Aquaculture System.

CHAPTER 7

CONCLUSION AND RECOMMENDATION

7.1 Conclusion

This research aimed at optimizing the Indoor Multi-Techno Aquaculture System (IMTAS) for the production of Pacific whiteleg shrimp (*Litopenaeus vannamei*), incorporating rapid biofloc technology and phytoremediation using *Caulerpa lentilifera* The findings provide strong evidence that procedural optimization of control factors of aquaculture systems such as stocking density, carbon-to-nitrogen (C/N) ratio, and probiotic dosage can lead to significant improvements in the production of shrimp, the water quality management and nutrient recycling in the culture system. The application of Taguchi L9 design and Grey Relational Analysis (GRA) identified the optimum conditions as 200 shrimp/m^3 for stocking density, a C/N ratio of 15, and a probiotic dosage of 14 ml/L. This research also reported remarkable improvement in water quality, particularly nitrogenous waste management. Shrimp aquaculture systems are highly susceptible to the accumulation of ammonia-nitrogen, nitrite, and other nitrogenous compounds, which are toxic to aquatic organisms under poor management. The adjustment of the C/N ratio in the optimized biofloc system promoted the growth of heterotrophic bacteria that efficiently utilized nitrogen compounds by converting them into biomass useful as natural food material for the reared shrimp.

The research also confirmed that optimizing the dosage of application of probiotic has crucial effect in the enhancement of shrimp health. Probiotics contribute to the growth of beneficial microbial communities in the gut, which are used for nutrient absorption and support of immune system. The optimum probiotic dosage of 14 ml/L markedly improved the microbial diversity of the shrimp gut which contributed to better feed conversion ratios and overall growth performance. These findings proved that probiotics form an integral component of sustainable aquaculture systems for improved shrimp health and maintenance of water quality due to its ability to inhibit the activities of harmful bacteria.

The second stage of the study introduced Internet of Things (IoT) technology into the IMTAS for enhanced system management and control. IoT systems was useful for real-time monitoring of critical water quality parameters, such as dissolved oxygen, pH, and temperature for precise recording of data. The IoT-based system also facilitated optimal usage of resources essential for the reduction of operational costs. The incorporation of a phytoremediation unit grown with *Caulerpa lentilifera* was another key novelty of this research. Nutrient absorption of excess nitrogen and phosphate was highly efficient with the use of *Caulerpa lentilifera* for the prevention of the accumulation of these nutrients to harmful levels. The successful integration of *Caulerpa lentilifera* in the IMTA system improved the water quality at zero-cost for nutrient management in aquaculture systems. The positive correlation between the growth of *Caulerpa lentilifera* and nutrient concentrations suggests that the productivity of the system could be enhanced by further optimizing the conditions for macroalgal growth.

One of the significant contributions of this study was the investigation of biofloc meal as an alternative feed for shrimp diet. The freeze-drying process preserved essential nutrients, including proteins, fatty acids, and amino acids, making biofloc meal a sustainable and nutritionally valuable feed supplement. The GRG 1 treatment, freeze-dried biofloc meal had the most reliable nutrient profile. This treatment confirmed the potential for biofloc meal to replace conventional fishmeal, which is often expensive and especially in regions where fishmeal availability is

limited. The findings suggest that biofloc meal could serve as an alternative feed source to conventional feeds for high growth performance in aquaculture systems.

7.2 Recommendation

Aquaculture practitioners should adopt the identified optimal IMTAS parameters (stocking density of 200 shrimp/m³, C/N ratio of 15, and probiotic dosage of 14 ml/L) to achieve the best performance in indoor biofloc culture systems for the culture of *L. vannamei*. Further research is recommended to explore the scalability of these parameters in different aquaculture settings.

The significant benefits of GRG 1 biofloc treatment should be leveraged on so as to improve the growth and productivity of *L. vannamei*. It is also recommended that there should be continued investigation of the long-term effects of biofloc treatments on shrimp physiology and microbial communities. The adoption of freeze-drying methods for feed processing should be encouraged so as to maintain the nutritional quality of the aquaculture feed. The nutritional profile of GRG 1 suggests that similar approaches may be beneficial for other aquaculture feedstuffs. Further research should be focused on digestibility and other investigations on biofloc meal to ascertain its suitability as aquaculture feed.

The use of *Caulerpa lentilifera* in phytoremediation systems should be expanded to improve the water quality and nutrient management in aquaculture systems. Further research should focus on optimising phytoremediation systems for different aquaculture environments and on exploring the potential of other species of macroalgae.

The findings in this study provide useful details relevant for the development and optimisation of IMTAS for the sustainable production of *L. vannamei*. To promote

practical application of these research outcomes, it is recommended that a cost analysis of the system should be studied to determine its profitable application.

REFERENCES

Abakari, G., Luo, G., & Kombat, E. O. (2021). Dynamics of nitrogenous compounds and their control in biofloc technology (BFT) systems: A review. *Aquaculture and Fisheries*.6(5): 441 - 447. https://doi.org/10.1016/j.aaf.2020.05.005

Abd El-Hack, M. E., El-Saadony, M. T., Nader, M. M., Salem, H. M., El-Tahan, A. M., Soliman, S. M., & Khafaga, A. F. (2022). Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *International Journal of Biometeorology*. 66 (11):2183 - 2194. https://doi.org/10.1007/s00484-022-02347-6

Abdel-Tawwab, M., Monier, M. N., Hoseinifar, S. H., & Faggio, C. (2019). Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. In *Fish Physiology and Biochemistry*. 45 (3):997 - 1013. https://doi.org/10.1007/s10695-019-00614-9

Abubakar FB, Ibrahim S, & Moruf RO. (2023). Phytoremediation of aquaculture wastewater: a review of microalgae bioremediation. *Science World Journal*, 18(1): 83 - 89.

Acherjee, B. (2020). Laser transmission welding of dissimilar plastics: analyses of parametric effects and process optimization using grey-based Taguchi method. *Modern Manufacturing Processes*, 131–144. https://doi.org/10.1016/B978-0-12-819496-6.00006-3

Addo, F. G., Zhang, S., Manirakiza, B., Ma, Y., Yuan, S., Alklaf, S. A., Guo, S., & Abakari, G. (2023). Brown sugar addition enhanced nutrient removal rates, growth performance, and bacterial community in a rice straw-based biofloc shrimp culture system. *Aquaculture*, *567*, 739274. https://doi.org/10.1016/J.AQUACULTURE.2023.739274

Adegboye, M. A., Aibinu, A. M., Kolo, J. G., Aliyu, I., Folorunso, T. A., & Lee, S. H. (2020). Incorporating Intelligence in Fish Feeding System for Dispensing Feed Based on Fish Feeding Intensity. *IEEE Access*, *8*. https://doi.org/10.1109/ACCESS.2020.2994442

Adetunji, A. I., & Olaniran, A. O. (2021). Treatment of industrial oily wastewater by advanced technologies: a review. *Applied Water Science* (Vol. 11, Issue 6). https://doi.org/10.1007/s13201-021-01430-4

Adharini, R. I., Murwantoko, Probosunu, N., Setiawan, R. Y., & Satriyo, T. B. (2021). The effectiveness of seaweeds as biofilter for reducing wastewater nutrient and preventing water pollution from hybrid grouper culture. *Jurnal Ilmiah Perikanan Dan Kelautan*, *13*(2). https://doi.org/10.20473/jipk.v13i2.28105

Adineh, H., Naderi, M., Jafaryan, H., Khademi Hamidi, M., Yousefi, M., & Ahmadifar, E. (2022). Effect of Stocking Density and Dietary Protein Level in Biofloc System on the Growth, Digestive and Antioxidant Enzyme Activities, Health, and Resistance to Acute Crowding Stress in Juvenile Common Carp (*Cyprinus carpio*). *Aquaculture Nutrition*, *2022*. https://doi.org/10.1155/2022/9344478

AftabUddin, S., Siddique, M. A. M., Sein, A., Dey, P. K., Rashed-Un-Nabi, M., & Haque, M. A. (2020a). First use of biofloc technology for Penaeus monodon culture in Bangladesh: Effects of stocking density on growth performance of shrimp, water quality and bacterial growth. *Aquaculture Reports*, *18*. https://doi.org/10.1016/j.aqrep.2020.100518

Ahmad, I., Babitha Rani, A. M., Verma, A. K., & Maqsood, M. (2017). Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture International*, *25*, 1215-1226.

Ahmad, A. L., Chin, J. Y., Mohd Harun, M. H. Z., & Low, S. C. (2022). Environmental impacts and imperative technologies towards sustainable treatment of aquaculture wastewater: A review. *Journal of Water Process Engineering* (Vol. 46). https://doi.org/10.1016/j.jwpe.2021.102553

Ajmal, M., Aiping, S., Awais, M., Ullah, M. S., Saeed, R., Uddin, S., Ahmad, I., Zhou, B., & Zihao, X. (2020). Optimization of pilot-scale in-vessel composting process for various agricultural wastes on elevated temperature by using Taguchi technique and compost quality assessment. *Process Safety and Environmental Protection*, *140*. https://doi.org/10.1016/j.psep.2020.05.001

Akhrianti, I., Nugraha, M. A., Pamungkas, A., & Gustomi, A. (2023). Total suspended solid distribution as impact tin mining at surrounded Pangkalpinang seawater Bangka Island. *IOP Conference Series: Earth and Environmental Science*, *1207*(1). https://doi.org/10.1088/1755-1315/1207/1/012029

Alexandre, A., & Santos, R. (2020). High Nitrogen and Phosphorous Acquisition by Belowground Parts of *Caulerpa prolifera* (Chlorophyta) Contribute to the Species' Rapid Spread in Ria Formosa Lagoon, Southern Portugal. *Journal of Phycology*, *56*(3). https://doi.org/10.1111/jpy.12988

Alfeus, A., & Gabriel, N. N. (2023). *Applications of Aquatic Plants in the Remediation of Aquaculture Wastewater: An Opportunity for African Aquaculture*. https://doi.org/10.1007/978-981-19-7451-9_13

Amjad, K., Dahms, H. U., Ho, C. H., Wu, Y. C., Lin, F. Y., & Lai, H. T. (2022). Probiotic additions affect the biofloc nursery culture of white shrimp (*Litopenaeus vannamei*). *Aquaculture*, *560*. https://doi.org/10.1016/j.aquaculture.2022.738475

Amrullah, M., Iskandar Tampoebolon, B. M., & Waluyo Prasetyono, B. (2019). Kajian Pengaruh Proses Fermentasi Sekam Padi Amoniasi Menggunakan *Aspergillus niger* Terhadap Serat Kasar, Protein Kasar, dan Total Digestible Nutrients. *Jurnal Pengembangan Penyuluhan Pertanian*, *16* (29). https://doi.org/10.36626/jppp.v16i29.64

Amyati, Hasan, Z., Supriadi, D., & Hamdani, H. (2020). The Effectiveness of the Use of Aquatic Plants (*Lemna perpusilla, Landoltia punctata and Azolla pinnata*) in the Phytoremediation Process of Catfish Aquaculture Wastewater. *Asian Journal of Fisheries and Aquatic Research*. https://doi.org/10.9734/ajfar/2020/v8i430144

Antonucci, F., & Costa, C. (2020). Precision aquaculture: a short review on engineering innovations. *Aquaculture International* (Vol. 28, Issue 1). https://doi.org/10.1007/s10499-019-00443-w

Antony, J., Sandeep, K. P., Aravind, R., Panigrahi, A., & Balasubramanian, C. P. (2019). Growth, Survival, and Osmoregulation of Indian White Shrimp, *Penaeus indicus* Juveniles Reared in Low Salinity Amended Inland Saline Groundwater and Seawater. *Journal of Coastal Research*, *86*(sp1). https://doi.org/10.2112/SI86-004.1

Aparna, Y., Banafsha, S. H., & Reddy, M. S. (2024). Monoculture and Mixed Culture of Pacific White Shrimp Litopenaeus vannamei and Tiger Shrimp *Penaeus monodon* in Biofloc System: A Comparative Study. *Asian Journal of Fisheries and Aquatic Research*, *26*(3), 87-106.

Arambul-Muñoz, E., Ponce-Palafox, J. T., Santos, R. C. D. L., Aragón-Noriega, E. A., Rodríguez-Domínguez, G., & Castillo-Vargasmachuca, S. G. (2019). Influence of stocking density on production and water quality of a photoheterotrophic intensive system of white shrimp (*Penaeus vannamei*) in circular lined grow-out ponds, with minimal water replacement. *Latin American Journal of Aquatic Research*, *47*(3). https://doi.org/10.3856/vol47-issue3-fulltext-7

Arnold, S. J., Coman, F. E., Jackson, C. J., & Groves, S. A. (2009). High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. *Aquaculture*, *293*(1–2). https://doi.org/10.1016/j.aquaculture.2009.03.049

Artigas, J., Batisson, I., & Carles, L. (2020). Dissolved organic matter does not promote glyphosate degradation in auto-heterotrophic aquatic microbial communities. *Environmental Pollution*, *259*. https://doi.org/10.1016/j.envpol.2020.113951

Asaduzzaman, M., Wahab, M. A., Verdegem, M. C. J., Huque, S., Salam, M. A., & Azim, M. E. (2008). C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*, *280*(1–4). https://doi.org/10.1016/j.aquaculture.2008.04.019

Asche, F., Pincinato, R. B. M., & Tveteras, R. (2022). Productivity in Global Aquaculture. In *Handbook of Production Economics*. https://doi.org/10.1007/978-981-10-3455-8_41

Ashour, M., Alprol, A. E., Heneash, A. M. M., Saleh, H., Abualnaja, K. M., Alhashmialameer, D., & Mansour, A. T. (2021). Ammonia bioremediation from aquaculture wastewater effluents using *Arthrospira platensis* niof17/003: Impact of biodiesel residue and potential of ammonia-loaded biomass as rotifer feed. *Materials*, *14*(18). https://doi.org/10.3390/ma14185460

Asiri, F. (2024). Polyhydroxyalkanoates for Sustainable Aquaculture: A Review of Recent Advancements, Challenges, and Future Directions. In *Journal of Agricultural and Food Chemistry* (Vol. 72, Issue 4). https://doi.org/10.1021/acs.jafc.3c06488

Asiri, F., Kim, J., & Chu, K. H. (2022). Chitosan-harvested polyhydroxybutyrate-rich *Zobellella denitrificans* ZD1 as a multifunction feed for aquaculture. *Aquaculture*, *561*. https://doi.org/10.1016/j.aquaculture.2022.738655

Attia, Y. A., Al-Khalaifah, H. S., Alqhtani, A. H., Abd El-Hamid, H. S., Alyileili, S. R., El-Hamid, A. E. H. E. A., Bovera, F., & El-Shafey, A. A. (2022). The impact of multi-enzyme fortification on growth performance, intestinal morphology, nutrient digestibility, and meat quality of broiler chickens fed a standard or low-density diet. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.1012462

Ayadi, F. Y., Fallahi, P., Rosentrater, K. A., & Muthukumarappan, K. (2013). Modeling Single-Screw Extrusion Processing Parameters and Resulting Extrudate Properties of DDGS-Based Nile Tilapia (*Oreochromis niloticus*) Feeds. *Journal of Food Research*, *2*(2), 11. https://doi.org/10.5539/JFR.V2N2P11

Ayesha Jasmin, S., Ramesh, P., & Tanveer, M. (2022). An intelligent framework for prediction and forecasting of dissolved oxygen level and biofloc amount in a shrimp culture system using machine learning techniques. *Expert Systems with Applications*, *199*, 117160. https://doi.org/10.1016/J.ESWA.2022.117160

Ayilara, M. S., & Babalola, O. O. (2023). Bioremediation of environmental wastes: the role of microorganisms. *Frontiers in Agronomy* (Vol. 5). https://doi.org/10.3389/fagro.2023.1183691

Baharuddin, N. D., Muthukhrisnan, S., de Cruz, C. R., Kassim, Z., Hasnan, H. H., Abdullah, M. I., Khaw, Y. S., Tan, H. T., Nazarudin, M. F., & Natrah, I. (2024). Evaluation of diatom Halamphora sp. and harpacticoid copepod Amphiascoides neglectus as live food for black tiger shrimp Penaeus monodon postlarvae. *Aquaculture*, *586*. https://doi.org/10.1016/j.aquaculture.2024.740773

Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). Vibrio spp. infections. *Nature Reviews Disease Primers*, *4*(1). https://doi.org/10.1038/s41572-018-0005-8

Bakhshi, F., H Najdegerami, E., Manaffar, R., Tokmechi, A., Rahmani Farah, K., & Shalizar Jalali, A. (2018). Growth performance, haematology, antioxidant status, immune response and histology of common carp (*Cyprinus carpio* L.) fed biofloc grown on different carbon sources. *Aquaculture Research*, *49*(1). https://doi.org/10.1111/are.13469

Bakhshi, F., Najdegerami, E. H., Manaffar, R., Tukmechi, A., & Farah, K. R. (2018). Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio* L.) fingerlings. *Aquaculture*, *484*. https://doi.org/10.1016/j.aquaculture.2017.11.036

Baliga, P. A. L. L. A. V. I., Goolappa, P. T., Shekar, M. A. L. A. T. H. I., Girisha, S. K., Ramesh, K. S., Udyavara, V. I. L. A. S. I. N. I., & Venugopal, M. N. (2021). Bacterial communities associated with *Penaeus vannamei* Boone, 1931 surface and its rearing water in biofloc culture system. *Asian Fish. Sci*, *34*, 34-46.

Bambaranda, B. V. A. S. M., Sasaki, N., Chirapart, A., Salin, K. R., & Tsusaka, T. W. (2019). Optimization of macroalgal density and salinity for nutrient removal by *Caulerpa lentillifera* from aquaculture effluent. *Processes*, *7*(5). https://doi.org/10.3390/pr7050303

Bao, J., Li, X., Xing, Y., Feng, C., & Jiang, H. (2020). Effects of hypoxia on immune responses and carbohydrate metabolism in the Chinese mitten crab, *Eriocheir sinensis*. *Aquaculture Research*, *51*(7). https://doi.org/10.1111/are.14612

Barajas-Sandoval, D., Escobedo-Fregoso, C., & Peña-Rodríguez, A. (2023). Temperature and fasting effect on compensatory growth and transcriptomic response in the Pacific white shrimp *Penaeus vannamei*. *Aquaculture*, *574*, 739711.

Battisti, E. K., Rabaioli, A., Uczay, J., Sutili, F. J., & Lazzari, R. (2020). Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. *Aquaculture*, *524*. https://doi.org/10.1016/j.aquaculture.2020.735213

Becerril-Cortés, D., Monroy-Dosta, M. del C., Coelho-Emerenciano, M. G., Castro-Mejía, G., Cienfuegos-Martínez, K., & de Lara-Andrade, R. (2017). Nutritional importance for aquaculture and ecological function of microorganisms that make up Biofloc, a review. *Int. J. of Aquatic Science*, *8*(2).

Beheary, M., Sheta, B. M., Hussein, M., Nawareg, M., El-Matary, F. A., & Hyder, A. (2019). Environmental remediation of tilapia aquaculture wastewater using *Ceratophyllum demersum* and *Lemna minor*. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(2). https://doi.org/10.21608/ejabf.2019.31974

Bélanger, A., Sarker, P. K., Bureau, D. P., Chouinard, Y., & Vandenberg, G. W. (2021). Apparent digestibility of macronutrients and fatty acids from microalgae (*Schizochytrium* sp.) fed to rainbow trout (*Oncorhynchus mykiss*): A potential candidate for fish oil substitution. *Animals*, *11*(2). https://doi.org/10.3390/ani11020456

Belenichev, I. F., Aliyeva, O. G., Popazova, O. O., & Bukhtiyarova, N. V. (2023). Involvement of heat shock proteins HSP70 in the mechanisms of endogenous neuroprotection: the prospect of using HSP70 modulators. *Frontiers in Cellular Neuroscience* (Vol. 17). https://doi.org/10.3389/fncel.2023.1131683

Berry, S. E., Simon, C. J., Foote, A. R., Jerry, D. R., & Wade, N. M. (2019). Evaluation of baseline haemolymph biochemistry, volume and total body energetics to determine an accurate condition index in the black tiger shrimp, *Penaeus monodon*. *Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology*, *228*. https://doi.org/10.1016/j.cbpb.2018.10.003

Betanzo-Torres, E. A., Piñar-álvarez, M. de los Á., Sandoval-Herazo, L. C., Molina-Navarro, A., Rodríguez-Montoro, I., & González-Moreno, R. H. (2020). Factors that limit the adoption of biofloc technology in aquaculture production in mexico. *Water (Switzerland)*, *12*(10). https://doi.org/10.3390/w12102775

Bian, M., Zhao, J., Xu, W., Han, X., Chen, X., Wang, P., & Zhu, G. (2023). Enzymatic Characterization of the Isocitrate Dehydrogenase with Dual Coenzyme Specificity from the Marine Bacterium Umbonibacter marinipuiceus. *International Journal of Molecular Sciences*, *24*(14). https://doi.org/10.3390/ijms241411428

Binalshikh-Abubkr, T., & Hanafiah, M. M. (2022). Effect of Supplementation of Dried Bioflocs Produced by Freeze-Drying and Oven-Drying Methods on Water

Quality, Growth Performance and Proximate Composition of Red Hybrid Tilapia. *Journal of Marine Science and Engineering*, *10* (1). https://doi.org/10.3390/jmse10010061

Binalshikh-Abubkr, T., Hanafiah, M. M., & Das, S. K. (2021). Proximate chemical composition of dried shrimp and tilapia waste bioflocs produced by two drying methods. *Journal of Marine Science and Engineering*, *9*(2). https://doi.org/10.3390/jmse9020193

Blancaflor, E. B., & Baccay, M. (2022). Assessment of an automated IoT-biofloc water quality management system in the *Litopenaeus vannamei*'s mortality and growth rate. *Automatika : Časopis Za Automatiku, Mjerenje, Elektroniku, Računarstvo i Komunikacije*, *63*(2), 259–274. https://doi.org/10.1080/00051144.2022.2031540

Bohnes, F. A., Hauschild, M. Z., Schlundt, J., Nielsen, M., & Laurent, A. (2022). Environmental sustainability of future aquaculture production: Analysis of Singaporean and Norwegian policies. *Aquaculture*, *549*. https://doi.org/10.1016/j.aquaculture.2021.737717

Boonchuen, P., Jaree, P., Somboonviwat, K., & Somboonwiwat, K. (2021). Regulation of shrimp prophenoloxidase activating system by lva-miR-4850 during bacterial infection. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-82881-2

Bossier, P., & Ekasari, J. (2017). Biofloc technology application in aquaculture to support sustainable development goals. *Microbial Biotechnology*, *10*(5). https://doi.org/10.1111/1751-7915.12836

Bowyer, P. H., El-Haroun, E. R., Salim, H. S., & Davies, S. J. (2020). Benefits of a commercial solid-state fermentation (SSF) product on growth performance, feed efficiency and gut morphology of juvenile Nile tilapia (*Oreochromis niloticus*) fed different UK lupin meal cultivars. *Aquaculture*, *523*. https://doi.org/10.1016/j.aquaculture.2020.735192

Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society* (Vol. 51, Issue 3). https://doi.org/10.1111/jwas.12714

Boyd, C. E., McNevin, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food Security*, *14*(3). https://doi.org/10.1007/s12571-021-01246-9

Braga, A., Magalhães, V., Hanson, T., Morris, T. C., & Samocha, T. M. (2016). The effects of feeding commercial feed formulated for semi-intensive systems on *Litopenaeus vannamei* production and its profitability in a hyper-intensive biofloc-dominated system. *Aquaculture Reports*, *3*. https://doi.org/10.1016/j.aqrep.2016.03.002

Brito, L. O., Junior, L. C., Abreu, J. L., Severi, W., Moraes, L. B. S., & Galvez, A. O. (2018). Effects of two commercial feeds with high and low crude protein content on the performance of white shrimp *Litopenaeus vannamei* raised in an integrated

biofloc system with the seaweed *Gracilaria birdiae*. *Spanish Journal of Agricultural Research*, *16*(1). https://doi.org/10.5424/sjar/2018161-11451

Broadbent, D. G., Barnaba, C., Perez, G. I., & Schmidt, J. C. (2023). Quantitative analysis of autophagy reveals the role of ATG9 and ATG2 in autophagosome formation. *Journal of Cell Biology*, *222*(7). https://doi.org/10.1083/jcb.202210078

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2003). Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, *219*(1–4). https://doi.org/10.1016/S0044-8486(02)00575-6

Butucel, E., Balta, I., McCleery, D., Marcu, A., Stef, D., Pet, I., Callaway, T., Stef, L., & Corcionivoschi, N. (2023). The Prebiotic Effect of an Organic Acid Mixture on *Faecalibacterium prausnitzii* Metabolism and Its Anti-Pathogenic Role against *Vibrio parahaemolyticus* in Shrimp. *Biology*, *12*(1). https://doi.org/10.3390/biology12010057

Caldini, N. N., Capistrano, H. H. de A., Filho, P. R. N. R., & E Sá, M. V. D. C. (2018). Partial replacement of artificial diets by wet bioflocs biomass in nile tilapia culture tanks. *Acta Scientiarum - Animal Sciences*, *40*. https://doi.org/10.4025/actascianimsci.v40i1.42426

Can-Terzi, B., Goren, A. Y., Okten, H. E., & Sofuoglu, S. C. (2021). Biosorption of methylene blue from water by live *Lemna minor*. *Environmental Technology and Innovation*, *22*. https://doi.org/10.1016/j.eti.2021.101432

Cardona, E., Lorgeoux, B., Chim, L., Goguenheim, J., Le Delliou, H., & Cahu, C. (2016). Biofloc contribution to antioxidant defence status, lipid nutrition and reproductive performance of broodstock of the shrimp *Litopenaeus stylirostris*: Consequences for the quality of eggs and larvae. *Aquaculture*, *452*. https://doi.org/10.1016/j.aquaculture.2015.08.003

Cardoso, L. G., Lemos, P. V. F., de Souza, C. O., Oliveira, M. B. P. P., & Chinalia, F. A. (2022). Current advances in phytoremediation and biochemical composition of Arthrospira (*Spirulina*) grown in aquaculture wastewater. In *Aquaculture Research* (Vol. 53, Issue 14). https://doi.org/10.1111/are.15996

Cargnin, J. M. R., & João, J. J. (2021). Removal of nutrients from aquaculture residual water: A review. *Revista Ambiente e Agua* (Vol. 16, Issue 6). https://doi.org/10.4136/AMBI-AGUA.2747

Carvalho, A., Costa, L. C. de O., Holanda, M., Poersch, L. H., & Turan, G. (2023). Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuc*a Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. *Fishes 2023, Vol. 8, Page 163*, *8*(3), 163. https://doi.org/10.3390/FISHES8030163

Catalani, K., Zuñiga, R., de Souza, M. S., Wasielesky, W., & Fóes, G. (2023). Comparison between biofloc technology system and aquamimicry in the cultivation of *Litopenaeus vannamei* in lined ponds in Southern Brazil. *Boletim Do Instituto de Pesca*, *49*, 49. https://doi.org/10.20950/1678-2305/BIP.2023.49.E787

Cavalli, L. S., Da Rocha, A. F., De Brito, B. G., De Brito, K. C. T., & Rotta, M. A. (2021). Major Sustainable Development Goals applied to Aquaculture. *Pesquisa Agropecuária Gaúcha*, *27*(1). https://doi.org/10.36812/pag.2021271110-126

Cetin, M. H., Ozcelik, B., Kuram, E., & Demirbas, E. (2011). Evaluation of vegetable based cutting fluids with extreme pressure and cutting parameters in turning of AISI 304L by Taguchi method. *Journal of Cleaner Production*, *19*(17–18). https://doi.org/10.1016/j.jclepro.2011.07.013

Chakraborty, B. K. (2022). Effect of stocking density on growth and survival of endangered *Mystus bleekeri* (day, 1877) in nursery system. *International Journal of Biological Innovations*, *04*(02). https://doi.org/10.46505/ijbi.2022.4206

Chakrapani, S., Panigrahi, A., Sundaresan, J., Mani, S., Palanichamy, E., Rameshbabu, V. S., & Krishna, A. (2021). Utilization of Complex Carbon Sources on Biofloc System and Its Influence on the Microbial Composition, Growth, Digestive Enzyme Activity of Pacific White Shrimp, *Penaeus vannamei* Culture. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(4). https://doi.org/10.4194/trjfas18813

Chandra, P., Sharma, R. K., & Arora, D. S. (2020). Antioxidant compounds from microbial sources: A review. *Food Research International* (Vol. 129). https://doi.org/10.1016/j.foodres.2019.108849

Chauhan, R. S., & Mishra, A. (2022). New innovative technologies for sustainable aqua production. *Biodiversity: Threats and Conservation*. https://doi.org/10.1201/9781003220398-8

Che Hashim, N. F., Manan, H., Okomoda, V. T., Ikhwanuddin, M., Khor, W., Abdullah, S. R. S., & Kasan, N. A. (2022). Inoculation of bioflocculant-producing bacteria for enhanced biofloc formation and pond preparation: Effect on water quality and bacterial community. *Aquaculture Research*, *53*(4). https://doi.org/10.1111/are.15678

Chen, F. L., & Ou, T. Y. (2011). Sales forecasting system based on Gray extreme learning machine with Taguchi method in retail industry. *Expert Systems with Applications*, *38*(3). https://doi.org/10.1016/j.eswa.2010.07.014

Chen, H. wei, Sun, D. chuan, Liu, W. chang, Li, S., & Tan, H. xin. (2023). Stocking Density Effects on Pacific White Shrimp *Litopenaeus vannamei* Hatchery Performance in Algal-Bacterial Biofloc Systems. *North American Journal of Aquaculture*, *85*(1), 3–12. https://doi.org/10.1002/NAAQ.10264

Chen, W. H., Carrera Uribe, M., Kwon, E. E., Lin, K. Y. A., Park, Y. K., Ding, L., & Saw, L. H. (2022). A comprehensive review of thermoelectric generation optimization by statistical approach: Taguchi method, analysis of variance (ANOVA), and response surface methodology (RSM). *Renewable and Sustainable Energy Reviews* (Vol. 169). https://doi.org/10.1016/j.rser.2022.112917

Chen, W., Tsui, K. L., Allen, J. K., & Mistree, F. (1995). Integration of the response surface methodology with the compromise decision support problem in developing a general robust design procedure. *American Society of Mechanical Engineers, Design Engineering Division (Publication) DE* (Vol. 82, Issue 1).

Chen, W. Y., Ng, T. H., Wu, J. H., Chen, J. W., & Wang, H. C. (2017). Microbiome dynamics in a shrimp grow-out pond with possible outbreak of acute hepatopancreatic necrosis disease. *Scientific reports*, *7*(1), 9395.

Chen, X., He, Z., Zhao, J., Liao, M., Xue, Y., Zhou, J., Hoare, R., Monaghan, S. J., Wang, N., Pang, H., & Sun, C. (2022). Metagenomic Analysis of Bacterial Communities and Antibiotic Resistance Genes in Penaeus monodon Biofloc-Based Aquaculture Environments. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.762345

Chen, X., Shao, T., & Long, X. (2018). Evaluation of the effects of different stocking densities on the sediment microbial community of juvenile hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) in recirculating aquaculture systems. *PLoS ONE*, *13*(12). https://doi.org/10.1371/journal.pone.0208544

Chen, Z., Chang, Z., Zhang, L., Wang, J., Qiao, L., Song, X., & Li, J. (2020). Effects of carbon source addition on microbial community and water quality in recirculating aquaculture systems for *Litopenaeus vannamei*. *Fisheries Science*, *86*(3). https://doi.org/10.1007/s12562-020-01423-3

Chua, A., Imperial, E. D., Mababangloob, G. R., Tinhay, J. B., & Concepcion, R. (2023). Intelligent Water Quality Management in Maturation Tanks for *Penaeus monodon* Using Fuzzy Logic. *2023 8th International Conference on Business and Industrial Research, ICBIR 2023 - Proceedings*. https://doi.org/10.1109/ICBIR57571.2023.10147655

Chuang, W. H., Liu, P. C., Chien, Y. C., Wu, Y. C., & Lee, K. K. (2016). Effects of *Vibrio harveyi* on plasma clotting protein (coagulogen) of white shrimp *Litopenaeus vannamei*. *Aquaculture Research*, *47*(11). https://doi.org/10.1111/are.12796

Chu, S. O., Lee, C., Noh, J., Song, S. J., Hong, S., Ryu, J., Lee, J. S., Nam, J., Kwon, B. O., & Khim, J. S. (2020). Effects of polluted and non-polluted suspended sediments on the oxygen consumption rate of olive flounder, *Paralichthys olivaceus*. *Marine Pollution Bulletin*, *154*. https://doi.org/10.1016/j.marpolbul.2020.111113

Chu, Y. T., & Brown, P. B. (2021). Sustainable Marine Aquaponics: Effects of Shrimp to Plant Ratios and C/N Ratios. *Frontiers in Marine Science*, *8*, 771630. https://doi.org/10.3389/FMARS.2021.771630/BIBTEX

Chu, Y. T., & Brown, P. B. (2022). Optimal Dietary Crude Protein in Commercial Feeds for Shrimp and Halophytes in Marine Aquaponic Biofloc Systems. *Frontiers in Marine Science*, *9*, 824973. https://doi.org/10.3389/FMARS.2022.824973/BIBTEX

Cimbala, M. J. (2014). Taguchi Orthogonal Arrays. *Instrumentation, Measurements, and Statistics*, *September*.

Colombo, S. M., Roy, K., Mraz, J., Wan, A. H. L., Davies, S. J., Tibbetts, S. M., Øverland, M., Francis, D. S., Rocker, M. M., Gasco, L., Spencer, E., Metian, M., Trushenski, J. T., & Turchini, G. M. (2023). Towards achieving circularity and

sustainability in feeds for farmed blue foods. *Reviews in Aquaculture* (Vol. 15, Issue 3). https://doi.org/10.1111/raq.12766

Coppola, D., Lauritano, C., Esposito, F. P., Riccio, G., Rizzo, C., & de Pascale, D. (2021). Fish Waste: From Problem to Valuable Resource. In *Marine Drugs* (Vol. 19, Issue 2). https://doi.org/10.3390/MD19020116

Coyne, L., Arief, R., Benigno, C., Giang, V. N., Huong, L. Q., Jeamsripong, S., Kalpravidh, W., McGrane, J., Padungtod, P., Patrick, I., Schoonman, L., Setyawan, E., Sukarno, A. H., Srisamran, J., Ngoc, P. T., & Rushton, J. (2019). Characterizing antimicrobial use in the livestock sector in three South east Asian countries (Indonesia, Thailand, and Vietnam). *Antibiotics*, *8*(1). https://doi.org/10.3390/antibiotics8010033

D'Angelo, E., Crutchfield, J., & Vandiviere, M. (2001). Rapid, Sensitive, Microscale Determination of Phosphate in Water and Soil. *Journal of Environmental Quality*, *30*(6). https://doi.org/10.2134/jeq2001.2206

Da Silva, J. L. S., Carneiro, A. P. C., Brito, A. L. C., Oliveira, A. V. S., Vieira, J. L., Soares, R. C., De Freitas, R. M., & De Sousa, O. V. (2023). In vitro manipulation of the bacterial community to improve the performance of bioflocs in aquaculture systems. *Anais Da Academia Brasileira de Ciencias*, *95*(1). https://doi.org/10.1590/0001-3765202320220311

da Silva, W. A., da Silva, J. L., Oliveira, C. Y. B., de Morais, A. P. M., Shinozaki-Mendes, R. A., & Silva, U. L. (2022). Effect of stocking density on water quality, plankton community structure, and growth performance of *Litopenaeus vannamei* post-larvae cultured in low-salinity biofloc system. *International Aquatic Research*, *14*(2). https://doi.org/10.22034/IAR.2022.1936674.1176

Das, R. R., Panigrahi, A., Sarkar, S., Saravanan, A., Biju, I. F., Ambikanandham, K., Jayanthi, M., & Kannappan, S. (2023). Growth, survival, and immune potential of post larvae of Indian white shrimp, *Penaeus indicus* (H. Milne Edwards, 1937) in different salinities with biofloc system (BFT) during nursery phase. *Aquaculture International*, *31*(1), 273–293. https://doi.org/10.1007/S10499-022-00979-4/METRICS

Das, S., De, D., Biswas, G., Kumar, P., Lalramchhani, C., Kumar, S., & Ghoshal, T. K. (2023). Effect of molasses supplementation on growth performance, water quality and microbial dynamics during indoor rearing of pacific white shrimp *Penaeus vannamei* Boone, 1931. *Indian Journal of Fisheries*, *70*(1). https://doi.org/10.21077/ijf.2023.70.1.115891-11

Dauda, A. B. (2020). Biofloc technology: a review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals. *Reviews in Aquaculture* (Vol. 12, Issue 2). https://doi.org/10.1111/raq.12379

Dave, S., & Das, J. (2020). Role of microbial enzymes for biodegradation and bioremediation of environmental pollutants: Challenges and future prospects. In *Bioremediation for Environmental Sustainability: Toxicity, Mechanisms of Contaminants Degradation, Detoxification and Challenges*. https://doi.org/10.1016/B978-0-12-820524-2.00013-4

de Lima Vieira, J., dos Santos Nunes, L., Rodrigues de Menezes, F. G., Vieira de Mendonça, K., & Viana de Sousa, O. (2021). An integrated approach to analyzing the effect of biofloc and probiotic technologies on sustainability and food safety in shrimp farming systems. *Journal of Cleaner Production*, *318*. https://doi.org/10.1016/j.jclepro.2021.128618

de Morais, A. P. M., Abreu, P. C., Wasielesky, W., & Krummenauer, D. (2020). Effect of aeration intensity on the biofilm nitrification process during the production of the white shrimp *Litopenaeus vannamei* (Boone, 1931) in Biofloc and clear water systems. *Aquaculture*, *514*. https://doi.org/10.1016/j.aquaculture.2019.734516

Deng, Y., Xu, X., Yin, X., Lu, H., Chen, G., Yu, J., & Ruan, Y. (2019). Effect of stock density on the microbial community in biofloc water and Pacific white shrimp (*Litopenaeus vannamei*) gut microbiota. *Applied Microbiology and Biotechnology*, *103*(10), 4241–4252. https://doi.org/10.1007/S00253-019-09773-4/FIGURES/5

De Schouwer, F., Claes, L., Vandekerkhove, A., Verduyckt, J., & De Vos, D. E. (2019). Protein-Rich Biomass Waste as a Resource for Future Biorefineries: State of the Art, Challenges, and Opportunities. *ChemSusChem* (Vol. 12, Issue 7). https://doi.org/10.1002/cssc.201802418

de Sousa, A. A., Pinho, S. M., Rombenso, A. N., de Mello, G. L., & Emerenciano, M. G. C. (2019). Pizzeria by-product: A complementary feed source for Nile tilapia (*Oreochromis niloticus*) raised in biofloc technology? *Aquaculture*, *501*. https://doi.org/10.1016/j.aquaculture.2018.11.055

de Souza Valente, C., Rodiles, A., Freire Marques, M. R., & Merrifield, D. L. (2020). White spot syndrome virus (WSSV) disturbs the intestinal microbiota of shrimp (*Penaeus vannamei*) reared in biofloc and clear seawater. *Applied Microbiology and Biotechnology*, *104*(18). https://doi.org/10.1007/s00253-020-10816-4

Dhal, S. B., Jungbluth, K., Lin, R., Sabahi, S. P., Bagavathiannan, M., Braga-Neto, U., & Kalafatis, S. (2022). A Machine-Learning-Based IoT System for Optimizing Nutrient Supply in Commercial Aquaponic Operations. *Sensors*, *22*(9). https://doi.org/10.3390/s22093510

Diwan, A. D., Harke, S. N., Gopalkrishna, & Panche, A. N. (2022). Aquaculture industry prospective from gut microbiome of fish and shellfish: An overview. *Journal of Animal Physiology and Animal Nutrition*, *106* (2), 441-469.

Dong, K. T. P., Saito, Y., Hoa, N. T. N., Dan, T. Y., & Matsuishi, T. (2019). Pressure–State–Response of traceability implementation in seafood-exporting countries: evidence from Vietnamese shrimp products. *Aquaculture International*, *27*(5). https://doi.org/10.1007/s10499-019-00378-2

Dong, S., Chen, H., Zhou, Q., & Liao, N. (2021). Protein degradation control and regulation of bacterial survival and pathogenicity: the role of protein degradation systems in bacteria. *Molecular Biology Reports* (Vol. 48, Issue 11). https://doi.org/10.1007/s11033-021-06744-9

Dong, S.-L., & Dong, Y.-W. (2023). Sustainability of Aquaculture Production Systems. In *Aquaculture Ecology*. https://doi.org/10.1007/978-981-19-5486-3_15

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3). https://doi.org/10.1021/ac60111a017

Du, K., Han, Y., Hao, J., Qin, X. X., Liu, C., & Fan, S. (2021). Effects of different NO_3^-: NH_4^+ ratios on the ultrastructure and ion flux rate of lettuce roots. *Journal of Plant Nutrition*, *44*(17). https://doi.org/10.1080/01904167.2021.1913184

Effendy, I., Al Deen, S., & Chithambaran, S. (2016). Semi Intensive and Semi Biofloc Methods for the Culture of Indian White Prawn, *Fenneropenaeus indicus* in High-density Polyethylene Liner Ponds. *HAYATI Journal of Biosciences*, *23*(3). https://doi.org/10.1016/j.hjb.2016.06.004

Einarsson, Á., & Óladóttir, Á. D. (2020). Fisheries and Aquaculture: The Food Security of the Future. *Fisheries and Aquaculture: The Food Security of the Future*. https://doi.org/10.1016/C2019-0-02984-6

Ekasari, J., Hanif Azhar, M., Surawidjaja, E. H., Nuryati, S., De Schryver, P., & Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish & Shellfish Immunology*, *41*(2), 332–339. https://doi.org/10.1016/J.FSI.2014.09.004

Ekasari, J., Setiawati, R., Ritonga, F. R., Setiawati, M., & Suprayudi, M. A. (2019). Growth and health performance of African catfish *Clarias gariepinus* (Burchell 1822) juvenile fed with graded levels of biofloc meal. *Aquaculture Research*, *50*(7). https://doi.org/10.1111/are.14059

Elayaraja, S., Mabrok, M., Algammal, A., Sabitha, E., Rajeswari, M. V., Zágoršek, K., Ye, Z., Zhu, S., & Rodkhum, C. (2020). Potential influence of jaggery-based biofloc technology at different C:N ratios on water quality, growth performance, innate immunity, immune-related genes expression profiles, and disease resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology*, *107*. https://doi.org/10.1016/j.fsi.2020.09.023

El-Saadony, M. T., Shehata, A. M., Alagawany, M., Abdel-Moneim, A. M. E., Selim, D. A., Abdo, M., Khafaga, A. F., El-Tarabily, K. A., El-Shall, N. A., & Abd El-Hack, M. E. (2022). A review of shrimp aquaculture and factors affecting the gut microbiome. *Aquaculture International*, *30*(6), 2847–2869. https://doi.org/10.1007/S10499-022-00936-1/METRICS

El-Sayed, A. F. M. (2021). Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. *Reviews in Aquaculture* (Vol. 13, Issue 1). https://doi.org/10.1111/raq.12494

Emerenciano, M. G. C., Miranda-Baeza, A., Martínez-Porchas, M., Poli, M. A., & Vieira, F. do N. (2021). Biofloc Technology (BFT) in Shrimp Farming: Past and Present Shaping the Future. *Frontiers in Marine Science*, *8*, 813091. https://doi.org/10.3389/FMARS.2021.813091/BIBTEX

Emerenciano, M. G., Rombenso, A. N., Vieira, F. D. N., Martins, M. A., Coman, G. J., Truong, H. H., ... & Simon, C. J. (2022). Intensification of penaeid shrimp culture: an applied review of advances in production systems, nutrition and breeding. *Animals*, *12*(3), 236.

Esparza-Leal, H. M., Cardozo, A. P., & Wasielesky, W. (2015). Performance of *Litopenaeus vannamei* postlarvae reared in indoor nursery tanks at high stocking density in clear-water versus biofloc system. *Aquacultural Engineering*, *68*. https://doi.org/10.1016/j.aquaeng.2015.07.004

Esteves, A. F., Soares, S. M., Salgado, E. M., Boaventura, R. A. R., & Pires, J. C. M. (2022). Microalgal Growth in Aquaculture Effluent: Coupling Biomass Valorisation with Nutrients Removal. *Applied Sciences (Switzerland)*, *12*(24). https://doi.org/10.3390/app122412608

Faizullah, M., Rajagopalsamy, C., Ahilan, B., & Daniel, N. (2019). Application of biofloc technology (BFT) in the aquaculture system. *~ 204 ~ Journal of Entomology and Zoology Studies*, *7*(4).

FAO. (2019). The State of Food and Agriculture 2019. *The State of Food and Agriculture 2019*. https://doi.org/10.4060/ca6030en

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. In *FAO*.

Fasihi, M., Tavakkoli-Moghaddam, R., Najafi, S. E., & Hajiaghaei, M. (2021). Optimizing a bi-objective multi-period fish closed-loop supply chain network design by three multi-objective meta-heuristic algorithms. *Scientia Iranica*, *0* (0). https://doi.org/10.24200/sci.2021.57930.5477

Fernando, A. L., Barbosa, B., Gomes, L. A., Costa, J., & Papazoglou, E. G. (2024). Giant reed (Arundo donax L.)—A multi-purpose crop bridging phytoremediation with sustainable bioeconomy. *Bioremediation and Bioeconomy: a Circular Economy Approach, Second Edition*. https://doi.org/10.1016/B978-0-443-16120-9.00029-7

Ferreira, G. S., Bolívar, N. C., Pereira, S. A., Guertler, C., Vieira, F. do N., Mouriño, J. L. P., & Seiffert, W. Q. (2015). Microbial biofloc as source of probiotic bacteria for the culture of Litopenaeus vannamei. *Aquaculture*, *448*, 273–279. https://doi.org/10.1016/J.AQUACULTURE.2015.06.006

Ferreira, G. S., Silva, V. F., Martins, M. A., da Silva, A. C. C. P., Machado, C., Seiffert, W. Q., & do Nascimento Vieira, F. (2020). Strategies for ammonium and nitrite control in Litopenaeus vannamei nursery systems with bioflocs. *Aquacultural Engineering*, *88*. https://doi.org/10.1016/j.aquaeng.2019.102040

Ferreira, L. M. H., Lara, G., Wasielesky, W., & Abreu, P. C. (2016). Biofilm versus biofloc: Are artificial substrates for biofilm production necessary in the BFT system? *Aquaculture International*, *24*(4). https://doi.org/10.1007/s10499-015-9961-0

Fitria, F. L., & Dhokhikah, Y. (2021). Phytoremediation of phosphate using *Typha* sp. and *Echinodorus palaefolius*. *Journal of Physics: Conference Series*, *1832*(1). https://doi.org/10.1088/1742-6596/1832/1/012010

Fleckenstein, L. J., Tierney, T. W., & Ray, A. J. (2018). Comparing biofloc, clear-water, and hybrid recirculating nursery systems (Part II): Tilapia (*Oreochromis niloticus*) production and water quality dynamics. *Aquacultural Engineering*, *82*, 80–85. https://doi.org/10.1016/J.AQUAENG.2018.06.006

Fraga-Corral, M., Ronza, P., Garcia-Oliveira, P., Pereira, A. G., Losada, A. P., Prieto, M. A., Quiroga, M. I., & Simal-Gandara, J. (2022). Aquaculture as a circular bio-economy model with Galicia as a study case: How to transform waste into revalorized by-products. *Trends in Food Science and Technology* (Vol. 119). https://doi.org/10.1016/j.tifs.2021.11.026

Freddi, A., & Salmon, M. (2019). Introduction to the Taguchi method. *Springer Tracts in Mechanical Engineering*. https://doi.org/10.1007/978-3-319-95342-7_7

Ganguly, D., Prasanna, K. L. V., Neelapu, S., & Goswami, G. (2023). Role of Microbes in Bioremediation. *Industrial Microbiology and Biotechnology: Emerging Concepts in Microbial Technology* (Vol. 3). https://doi.org/10.1007/978-981-99-2816-3_19

Gao, C., Yang, J., Hao, T., Li, J., & Sun, J. (2021). Reconstruction of Litopenaeus vannamei Genome-Scale Metabolic Network Model and Nutritional Requirements Analysis of Different Shrimp Commercial Varieties. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.658109

Gao, H., Kong, J., Li, Z., Xiao, G., & Meng, X. (2011). Quantitative analysis of temperature, salinity and pH on WSSV proliferation in Chinese shrimp *Fenneropenaeus chinensis* by real-time PCR. *Aquaculture*, *312*(1–4). https://doi.org/10.1016/j.aquaculture.2010.12.022

Gao, S., Gong, W., Zhang, K., Li, Z., Wang, G., Yu, E., Xia, Y., Tian, J., Li, H., & Xie, J. (2022). Effectiveness of agricultural waste in the enhancement of biological denitrification of aquaculture wastewater. *PeerJ*, *10*. https://doi.org/10.7717/peerj.13339

Garofalo, S., Giovagnoli, S., Orsoni, M., Starita, F., & Benassi, M. (2022). Interaction effect: Are you doing the right thing? *PLoS ONE*, *17*(7 July). https://doi.org/10.1371/journal.pone.0271668

Gautam, R. K., Samal, S. K., & Prasad, L. (2020). Biofloc technology for sustainable aquaculture production. *Food and Scientific Reports*, *1*(6).

Ghimire, R. R., Ghimire, A., Karki, D., Basyal, D., & Rai, K. B. (2023). Determination of Ammonia Level and Its Protein Conversion in the Water of Biofloc Fish Farming Technology. *Prithvi Academic Journal*. https://doi.org/10.3126/paj.v6i1.54572

Ghosh, A. K., Hasanuzzaman, A. F. M., Sarower, M. G., Islam, M. R., & Huq, K. A. (2024). Unveiling the biofloc culture potential: Harnessing immune functions for resilience of shrimp and resistance against AHPND-causing *Vibrio parahaemolyticus* infection. *Fish & Shellfish Immunology*, 109710.

Glencross, B. D., Huyben, D., & Schrama, J. W. (2020). The application of single-cell ingredients in aquaculture feeds—a review. In *Fishes* (Vol. 5, Issue 3). https://doi.org/10.3390/fishes5030022

Goddard, S., & Delghandi, M. (2020). Importance of the Conservation and Management of Freshwater to Aquaculture. *Encyclopedia of the World's Biomes*, *4–5*, 35–44. https://doi.org/10.1016/B978-0-12-409548-9.11954-2

Godoy-Olmos, S., Martínez-Llorens, S., Tomás-Vidal, A., Monge-Ortiz, R., Estruch, G., & Jover-Cerdá, M. (2019). Influence of temperature, ammonia load and hydraulic loading on the performance of nitrifying trickling filters for recirculating aquaculture systems. *Journal of Environmental Chemical Engineering*, *7*(4). https://doi.org/10.1016/j.jece.2019.103257

Goldstein, L. D., Cao, Y., Pau, G., Lawrence, M., Wu, T. D., Seshagiri, S., & Gentleman, R. (2016). Prediction and quantification of splice events from RNA-seq data. *PLoS ONE*, *11*(5). https://doi.org/10.1371/journal.pone.0156132

Goswami, M., Trudeau, V. L., & Lakra, W. S. (2022). Biotechnology in modern aquaculture: Innovations, advancements, and challenges. *Frontiers in Aquaculture Biotechnology*. https://doi.org/10.1016/B978-0-323-91240-2.00003-8

Gule, T. T., & Geremew, A. (2022). Dietary Strategies for Better Utilization of Aquafeeds in Tilapia Farming. *Aquaculture Nutrition*, *2022*. https://doi.org/10.1155/2022/9463307

Guo, H., Fu, X., He, J., Wang, R., Yan, M., Wang, J. & Zhang, D. (2023). Gut bacterial consortium enriched in a biofloc system protects shrimp against *Vibrio parahaemolyticus* infection. *Microbiome*, *11*(1), 230.

Guo, H., Huang, L., Hu, S., Chen, C., Huang, X., Liu, W., Wang, S., Zhu, Y., Zhao, Y., & Zhang, D. (2020). Effects of Carbon/Nitrogen Ratio on Growth, Intestinal Microbiota and Metabolome of Shrimp (*Litopenaeus vannamei*). *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00652

Guo, J., Wang, S., Wang, Z., & Peng, Y. (2014). Effects of feeding pattern and dissolved oxygen concentration on microbial morphology and community structure: The competition between floc-forming bacteria and filamentous bacteria. *Journal of Water Process Engineering*, *1*. https://doi.org/10.1016/j.jwpe.2014.03.011

Gupta, T., Chakraborty, D., & Sarkar, A. (2023). Microbial enzymes and role in bioremediation of environmental pollutants: Prospects and challenges. In *Microbial Degradation and Detoxification of Pollutants*. https://doi.org/10.1515/9783110743623-011

Gupta, R. & Gupta, N. (2021). Alternate Tricarboxylic Acid Cycle. In: Fundamentals of Bacterial Physiology and Metabolism. Springer, Singapore. https://doi.org/10.1007/978-981-16-0723-3_13

Gutiérrez-Pérez, E. D., Vázquez-Juárez, R., Magallón-Barajas, F. J., Martínez-Mercado, M., Escobar-Zepeda, A., & Magallón-Servín, P. (2022). How a holobiome perspective could promote intensification, biosecurity and eco-efficiency in the shrimp aquaculture industry. *Frontiers in Marine Science* (Vol. 9). https://doi.org/10.3389/fmars.2022.975042

Gvozdić, E., Bujagić, I. M., Đurkić, T., & Grujić, S. (2023). Untreated wastewater impact and environmental risk assessment of artificial sweeteners in river water and sediments of the Danube River Basin in Serbia. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-023-28348-5

Gyan, R. W., Yang, Q., Tan, B., Dong, X., Chi, S., Liu, H., & Zhang, S. (2022). Effects of Replacing Fish Meal with Distillers' Dried Grains with Soluble on the Growth Performance and Gut Microbiota in Juvenile Pacific Whiteleg Shrimp *Litopenaeus vannamei*. *North American Journal of Aquaculture*, *84*(2). https://doi.org/10.1002/naaq.10236

Hachiya, T., & Okamoto, Y. (2017). Simple Spectroscopic Determination of Nitrate, Nitrite, and Ammonium in *Arabidopsis thaliana*. *BIO-PROTOCOL*, *7*(10). https://doi.org/10.21769/bioprotoc.2280

Hamzaçebi, C. (2021). Taguchi Method as a Robust Design Tool. *Quality Control - Intelligent Manufacturing, Robust Design and Charts*. https://doi.org/10.5772/intechopen.94908

Hamza, F., & Zinjarde, S. (2023). Use of marine microorganisms in designing anti-infective strategies for sustainable aquaculture production. *Journal of applied microbiology* (Vol. 134, Issue 7). https://doi.org/10.1093/jambio/lxad128

Handajani, H., Widanarni, W., Budiardi, T., & Setiawati, M. (2021). Phytoremediation by *Echinodorus palaefolius* to Reduce Nitrogen and Phosphate Waste of Intensive Culture *Anguilla bicolor* in Recirculation Aquaculture Systems. *International Journal on Advanced Science, Engineering and Information Technology*, *11*(2). https://doi.org/10.18517/ijaseit.11.2.9521

Hanumantha Rao, M., Ghule, V. D., & Muralidharan, K. (2021). Nitrogen-rich compounds: s-triazine and tri-s-triazine derivatives as high energy materials. *Journal of Chemical Sciences*, *133*(1). https://doi.org/10.1007/s12039-020-01865-3

Hardi, E. H., Susmiyati, H. R., Diana, R., Palupi, N. P., Agriandini, M., Saptiani, G., Asikin, A. N., & Agustina. (2023). Traditional polyculture as a mangrove restoration solution in Delta Mahakam, East Kalimantan Indonesia. *IOP Conference Series: Earth and Environmental Science*, *1208*(1). https://doi.org/10.1088/1755-1315/1208/1/012056

Hasan-Nataj-Niazi, E., Agh, N., Noori, F., Atashbar, B., & Van Stappen, G. (2022). Substitution of microalgae by bioflocs as a food source for the brine shrimp *Artemia franciscana*. *Aquaculture Research*, *53*(12). https://doi.org/10.1111/are.15936

Hassan, S. A. H., Sharawy, Z. Z., El Nahas, A. F., Hemeda, S. A., El-Haroun, E., & Abbas, E. M. (2022). Modulatory effects of various carbon sources on growth indices, digestive enzymes activity and expression of growth-related genes in Whiteleg shrimp, *Litopenaeus vannamei* reared under an outdoor zero-exchange system. *Aquaculture Research*, *53*(16). https://doi.org/10.1111/are.16041

Hassan, M. S. A., Elias, N. A., Hassan, M., Mocktar, N. A., & Harun, N. A. (2024). Integrated overview on status, diagnosis and disease management of Acute Hepatopancreatic Necrosis Disease (AHPND) in shrimp aquaculture through metallic nanoparticles (MNPs) application–A review. *Aquaculture*, 741649.

Henriksson, P. J. G., Troell, M., Banks, L. K., Belton, B., Beveridge, M. C. M., Klinger, D. H., Pelletier, N., Phillips, M. J., & Tran, N. (2021). Interventions for

improving the productivity and environmental performance of global aquaculture for future food security. *One Earth* (Vol. 4, Issue 9). https://doi.org/10.1016/j.oneear.2021.08.009

Hernández, M., Londoño, I., Hernández, A., & Torres, C. (2019). The biofloc systems: an efficient strategy in the aquaculture production. *CES Med. Zootec*, *14*(1).

Holanda, M., Wasielesky, W., de Lara, G. R., & Poersch, L. H. (2022a). Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. *Fishes 2022, Vol. 7, Page 283*, *7*(5), 283. https://doi.org/10.3390/FISHES7050283

Holanda, M., Wasielesky, W., de Lara, G. R., & Poersch, L. H. (2022b). Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. *Fishes 2022, Vol. 7, Page 283*, *7*(5), 283. https://doi.org/10.3390/FISHES7050283

Hosain, M. E., Nurul Amin, S. M., Kamarudin, M. S., Arshad, A., & Romano, N. (2021). Effects of C-N ratio on growth, survival and proximate composition of *Macrobrachium rosenbergii* post larvae reared under a corn starch based zero-exchange brackish water biofloc system. *Aquaculture Research*, *52*(7). https://doi.org/10.1111/are.15146

Hossain, S., Manan, H., Shukri, Z. N. A., Othman, R., Kamaruzzan, A. S., Rahim, A. I. A., Khatoon, H., Minhaz, T. M., Islam, Z., & Kasan, N. A. (2023). Microplastics biodegradation by biofloc-producing bacteria: An inventive biofloc technology approach. *Microbiological Research* (Vol. 266). https://doi.org/10.1016/j.micres.2022.127239

Hostins, B., Wasielesky, W., Decamp, O., Bossier, P., & De Schryver, P. (2019). Managing input C/N ratio to reduce the risk of Acute Hepatopancreatic Necrosis Disease (AHPND) outbreaks in biofloc systems – A laboratory study. *Aquaculture*, *508*. https://doi.org/10.1016/j.aquaculture.2019.04.055

Huang, B., Wang, Z., Chu, Y., & Ding, L. (2023). Investigating the Role of Terminal Stolon of Marine Invasive Green Macroalga *Caulerpa taxifolia* in the Removal of Inorganic Nitrogen from Seawater. *Journal of Marine Science and Engineering*, *11*(1). https://doi.org/10.3390/jmse11010043

Huang, H. H., Li, C. Y., Lei, Y. J., Zhou, B. L., Kuang, W. Q., Zou, W. S., & Yang, P. H. (2023). Effects of Bacillus strain added as initial indigenous species into the biofloc system rearing *Litopenaeus vannamei* juveniles on biofloc preformation, water quality and shrimp growth. *Aquaculture*, *569*, 739375. https://doi.org/10.1016/J.AQUACULTURE.2023.739375

Huang, H. H., Li, C. Y., Liang, T., Lei, Y. J., Yang, P. H., & Wu, M. X. (2022). Effects of carbon-to-nitrogen ratio (C:N) on water quality and growth performance of *Litopenaeus vannamei* (Boone, 1931) in the biofloc system with a salinity of 5‰. *Aquaculture Research*, *53*(15). https://doi.org/10.1111/are.16013

Huang, H. H., & Li, C. Y. (2024). Adaptability of commercial probiotics to biofloc system: Influences on autochthonal bacterial community, water quality and growth performance of shrimp (*Litopenaeus vannamei*). *Aquaculture*, *590*, 740992.

Hu, J., Zhang, H., Wu, L., Zhu, F., Zhang, X., Gui, F., Qu, X., & Feng, D. (2022). Investigation of the inlet layout effect on the solid waste removal in an octagonal aquaculture tank. *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.1035794

Iber, B. T., & Kasan, N. A. (2021). Recent advances in Shrimp aquaculture wastewater management. *Heliyon*, *7*(11), e08283. https://doi.org/10.1016/J.HELIYON.2021.E08283

Ighodalo, O., Amiebenomo, S. O., & Esabunor, T. (2020). Optimization of multiple performance responses of a fish feed pelletizer machine. *Journal of Advances in Science and Engineering*, *3*(1). https://doi.org/10.37121/jase.v3i1.71

Imorwitz, G., & Keliher, P. I. M. (1984). Spectrophotometric determination of nitrite with composite reagents containing sulphanilamide, sulphanilic acid or 4-nitroaniline as the diazotisable aromatic amine and N-(1-Naphthyl) ethylenediamine as the coupling agent. *The Analyst*, *109*. https://doi.org/10.1039/AN9840901281

Irani, M., Rajabi Islami, H., Nafisi Bahabadi, M., & Hosseini Shekarabi, S. P. (2023). Production of Pacific white shrimp under different stocking density in a zero-water exchange biofloc system: Effects on water quality, zootechnical performance, and body composition. *Aquacultural Engineering*, *100*. https://doi.org/10.1016/j.aquaeng.2022.102313

Irshath, A. A., Rajan, A. P., Vimal, S., Prabhakaran, V. S., & Ganesan, R. (2023). Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development. *Vaccines* (Vol. 11, Issue 2). https://doi.org/10.3390/vaccines11020470

Jamal, M. T., Broom, M., AL-Mur, B. A., AL Harbi, M., Ghandourah, M., AL Otaibi, A., & Haque, M. F. (2020). Biofloc technology: Emerging microbial biotechnology for the improvement of aquaculture productivity. In *Polish Journal of Microbiology* (Vol. 69, Issue 4). https://doi.org/10.33073/PJM-2020-049

Jena, A. K., Biswas, P., & Saha, H. (2017). Advanced Farming Systems in Aquaculture: Strategies to Enhance the Production. *Innovative Farming*, *1*(1).

Jiménez-Ordaz, F. J., Cadena-Roa, M. A., Pacheco-Vega, J. M., Rojas-Contreras, M., Tovar-Ramírez, D., & Arce-Amezquita, P. M. (2021). Microalgae and probiotic bacteria as biofloc inducers in a hyper-intensive pacific white shrimp (*Penaeus vannamei*) culture. *Latin American Journal of Aquatic Research*, *49*(1). https://doi.org/10.3856/vol49-issue1-fulltext-2442

Joffre, O., Bush, S., & Belton, B. (2021). Aquaculture will be key to global food and nutrition security. *ID4D*.

Johnson, T. R., Beard, K., Brady, D. C., Byron, C. J., Cleaver, C., Duffy, K., Keeney, N., Kimble, M., Miller, M., Moeykens, S., Teisl, M., van Walsum, G. P., & Yuan, J. (2019). A social-ecological system framework for marine aquaculture research. *Sustainability (Switzerland)*, *11*(9). https://doi.org/10.3390/su11092522

Jones, S. W., Karpol, A., Friedman, S., Maru, B. T., & Tracy, B. P. (2020). Recent advances in single cell protein use as a feed ingredient in aquaculture. In *Current*

Opinion in Biotechnology (Vol. 61). https://doi.org/10.1016/j.copbio.2019.12.026

Jusoh, A., Nasir, N. M., Yunos, F. H. M., Jusoh, H. H. W., & Lam, S. S. (2020). Green technology in treating aquaculture wastewater. *AIP Conference Proceedings*, *2197*. https://doi.org/10.1063/1.5140892

Kafle, A., Timilsina, A., Gautam, A., Adhikari, K., Bhattarai, A., & Aryal, N. (2022). Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances* (Vol. 8). https://doi.org/10.1016/j.envadv.2022.100203

Kasan, N. A., Ghazali, N. A., Ikhwanuddin, M., & Ibrahim, Z. (2017). Isolation of potential bacteria as inoculum for biofloc formation in pacific whiteleg shrimp, *Litopenaeus vannamei* culture ponds. *Pakistan Journal of Biological Sciences*, *20*(6). https://doi.org/10.3923/pjbs.2017.306.313

Kasan, N. A., Kamaruzzan, A. S., Rahim, A. I. A., Ishak, A. N., Jauhari, I., & Ikhwanuddin, M. (2019). Production of Pacific whiteleg shrimp, *Litopenaeus vannamei* through implementation of rapid biofloc technology. *IOP Conference Series: Earth and Environmental Science*, *370*(1), 012005. https://doi.org/10.1088/1755-1315/370/1/012005

Kasan, N. A., Manan, H., Tuan Ismail, T. I., Abdul Salam, A. I., Abdul Rahim, A. I., Kamarruzan, A. S., Ishak, A. N., Deraman, S., Nasrin, Z., Che Engku Chik, C. E. N., Che Hashim, N. F., & Terkula Iber, B. (2021). Effect of Biofloc product-Rapid BFT^{TM} vs. clear water system in improving the water quality and growth performances of Pacific Whiteleg shrimp, *P. vannamei*, cultured in indoor aquaculture system. *Aquaculture Research*, *52*(12), 6504–6513. https://doi.org/10.1111/ARE.15519

Kato, G., Nakajima, H., Suzuki, K., Kanzawa, Y., Nakayasu, C., Taguchi, K., Kurata, O., & Sano, M. (2023). Decreased resistance to bacterial cold-water disease and excessive inflammatory response in ayu (*Plecoglossus altivelis*) reared at high water temperature. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1101491

Kuang, H., Xiao, D., Liu, W., Guo, Y., Li, J., Luo, G., & Tan, H. (2025). The short-term biological effects of different particle sizes of bioflocs on *Litopenaeus vannamei*. *Aquaculture International*, *33*(1), 31.

Kavitha, K., & Krishna, P. V. (2020). Sustainable Growth and Survival of *Litopenaeus Vannamei* Through Wastewater Recycling. *Recent Trends in Waste Water Treatment and Water Resource Management*. https://doi.org/10.1007/978-981-15-0706-9_8

Kaya, D., Genc, E., Genc, M. A., Aktas, M., Eroldogan, O. T., & Guroy, D. (2020). Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: Effects of different feeding rates and stocking densities. *Aquaculture*, *528*. https://doi.org/10.1016/j.aquaculture.2020.735526

Kemal, R., Fauzi, I. A., Nuryati, S., Wardani, W. W., & Suprayudi, M. A. (2023). Evaluation of Selenoprotein Supplementation on Digestibility, Growth, and

Health Performance of Pacific White Shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, *2023*. https://doi.org/10.1155/2023/2008517

Kerr, B. J., Dozier, W. A., & Lee, D. T. (2024). Energy and ether extract digestibility of commercially available lipids fed to broilers. *Journal of Applied Poultry Research*, *33*(2). https://doi.org/10.1016/j.japr.2024.100406

Khan, S. N., Anjum, L., Arshad, A., Ali, S., Aleem, M., & Nasir, A. (2025). Nature-Based Solution for Wastewater Treatment and Reuse Using Phytoremediation with Floating Plants. *Water*, *17*(7), 1080.

Khanjani, M. H., Alizadeh, M., Mohammadi, M., & Sarsangi Aliabad, H. (2021). Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity. *Iranian Journal of Fisheries Sciences*, *20*(2). https://doi.org/10.22092/ijfs.2021.123873

Khanjani, M. H., Alizadeh, M., & Sharifinia, M. (2020). Rearing of the Pacific white shrimp, *Litopenaeus vannamei* in a biofloc system: The effects of different food sources and salinity levels. *Aquaculture Nutrition*, *26*(2). https://doi.org/10.1111/anu.12994

Khanjani, M. H., Eslami, J., Ghaedi, G., & Sourinejad, I. (2022). The effects of different stocking densities on nursery performance of Banana shrimp (*Fenneropenaeus merguiensis*) reared under biofloc condition. *Annals of Animal Science*, *22*(4). https://doi.org/10.2478/aoas-2022-0027

Khanjani, M. H., Mohammadi, A., & Emerenciano, M. G. C. (2022). Microorganisms in biofloc aquaculture system. *Aquaculture Reports* (Vol. 26). https://doi.org/10.1016/j.aqrep.2022.101300

Khanjani, M. H., Mozanzadeh, M. T., Sharifinia, M., & Emerenciano, M. G. C. (2023). Biofloc: A sustainable dietary supplement, nutritional value and functional properties. *Aquaculture* (Vol. 562). https://doi.org/10.1016/j.aquaculture.2022.738757

Khanjani, M. H., & Sharifinia, M. (2022). Biofloc technology with addition molasses as carbon sources applied to *Litopenaeus vannamei* juvenile production under the effects of different C/N ratios. *Aquaculture International*, *30*(1). https://doi.org/10.1007/s10499-021-00803-5

Khanjani, M. H., Sharifinia, M., & Hajirezaee, S. (2023). Strategies for promoting sustainable aquaculture in arid and semi-arid areas. *Annals of Animal Science*. https://doi.org/10.2478/aoas-2023-0073

Khatoon, H., Banerjee, S., Guan Yuan, G. T., Haris, N., Ikhwanuddin, M., Ambak, M. A., & Endut, A. (2016). Biofloc as a potential natural feed for shrimp postlarvae. *International Biodeterioration and Biodegradation*, *113*. https://doi.org/10.1016/j.ibiod.2016.04.006

Kheti, B., Kamilya, D., Choudhury, J., Parhi, J., Debbarma, M., & Singh, S. T. (2017). Dietary microbial floc potentiates immune response, immune relevant gene expression and disease resistance in rohu, *Labeo rohita* (Hamilton, 1822) fingerlings. *Aquaculture*, *468*. https://doi.org/10.1016/j.aquaculture.2016.11.018

Kiani, S., Kujala, K., T. Pulkkinen, J., Aalto, S. L., Suurnäkki, S., Kiuru, T., Tiirola, M., Kløve, B., & Ronkanen, A. K. (2020). Enhanced nitrogen removal of low carbon wastewater in denitrification bioreactors by utilizing industrial waste toward circular economy. *Journal of Cleaner Production*, *254*. https://doi.org/10.1016/j.jclepro.2020.119973

Kim, J. H., Jeong, E. H., Jeon, Y. H., Kim, S. K., & Hur, Y. B. (2021). Salinity-mediated changes in hematological parameters, stress, antioxidant responses, and acetylcholinesterase of juvenile olive flounders (*Paralichthys olivaceus*). *Environmental Toxicology and Pharmacology*, *83*. https://doi.org/10.1016/j.etap.2021.103597

Kim, J. H., Kim, S. K., & Hur, Y. B. (2019). Hematological parameters and antioxidant responses in olive flounder *Paralichthys olivaceus* in biofloc depend on water temperature. *Journal of Thermal Biology*, *82*. https://doi.org/10.1016/j.jtherbio.2019.04.013

Kim, J. H., Sohn, S., Kim, S. K., & Hur, Y. B. (2020). Effects on hematological parameters, antioxidant and immune responses, AChE, and stress indicators of olive flounders, *Paralichthys olivaceus*, raised in bio-floc and seawater challenged by *Edwardsiella tarda*. *Fish and Shellfish Immunology*, *97*. https://doi.org/10.1016/j.fsi.2019.12.011

Kim, S. K., Song, J., Rajeev, M., Kim, S. K., Kang, I., Jang, I. K., & Cho, J. C. (2022). Exploring bacterioplankton communities and their temporal dynamics in the rearing water of a biofloc-based shrimp (Litopenaeus vannamei) aquaculture system. *Frontiers in Microbiology*, *13*, 995699. https://doi.org/10.3389/FMICB.2022.995699/BIBTEX

Kokkuar, N., Li, L., Srisapoome, P., Dong, S., & Tian, X. (2021). Application of biodegradable polymers as carbon sources in ex situ biofloc systems: Water quality and shift of microbial community. *Aquaculture Research*, *52*(8). https://doi.org/10.1111/are.15200

Kokou, F., Gupta, S., & Kumar, V. (2022). Editorial: Understanding the Interplay Between Diet, Feed Ingredients and Gut Microbiota for Sustainable Aquaculture. *Frontiers in Marine Science* (Vol. 9). https://doi.org/10.3389/fmars.2022.853548

Kombat, E. O., Abakari, G., Alhassan, E. H., & Zhao, J. L. (2023). Effect of acute and chronic salinity exposure on the amino acid composition in muscle, intestine and gill tissues of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *570*. https://doi.org/10.1016/j.aquaculture.2023.739406

Kristanti, R. A., & Hadibarata, T. (2023). Phytoremediation of contaminated water using aquatic plants, its mechanism and enhancement. *Current Opinion in Environmental Science and Health* (Vol. 32). https://doi.org/10.1016/j.coesh.2023.100451

Krummenauer, D., Freitas Silva, A., Xavier, M., Poersch, L. H., & Cardozo, A. (2022). Comparative analysis of the culture of pink shrimp *Farfantepenaeus brasiliensis* and Pacific white shrimp *Litopenaeus vannamei* in biofloc system. https://doi.org/10.21203/RS.3.RS-1646196/V1

Kuhn, D. D., Lawrence, A. L., Crockett, J., & Taylor, D. (2016). Evaluation of biofloc derived from confectionary food effluent water as a replacement feed ingredient for fishmeal or soy meal for shrimp. *Aquaculture*, *454*. https://doi.org/10.1016/j.aquaculture.2015.12.009

Kumar, P., Saurabh, S., Pal, A. K., Sahu, N. P., & Arasu, A. R. T. (2014). Stress mitigating and growth enhancing effect of dietary tryptophan in rohu (*Labeo rohita*, Hamilton, 1822) fingerlings. *Fish Physiology and Biochemistry*, *40*(5). https://doi.org/10.1007/s10695-014-9927-6

Kumar, S., Shyne Anand, P. S., De, D., Ghoshal, T. K., Alavandi, S. V., & Vijayan, K. K. (2019). Integration of substrate in biofloc based system: Effects on growth performance, water quality and immune responses in black tiger shrimp, *Penaeus monodon* culture. *Aquaculture Research*, *50*(10). https://doi.org/10.1111/are.14256

Kumar, S., Verma, A. K., Singh, S. P., & Awasthi, A. (2022). Immunostimulants for shrimp aquaculture: paving pathway towards shrimp sustainability. *Environmental Science and Pollution Research 2022 30:10*, *30*(10), 25325–25343. https://doi.org/10.1007/S11356-021-18433-Y

Kumar, V., Roy, S., Behera, B. K., Swain, H. S., & Das, B. K. (2021). Biofloc Microbiome with Bioremediation and Health Benefits. *Frontiers in Microbiology*, *12*, 741164. https://doi.org/10.3389/FMICB.2021.741164/BIBTEX

Kumar, V., Swain, H. S., Vuong, P., Roy, S., Upadhyay, A., Malick, R. C., Bisai, K., Kaur, P., & Das, B. K. (2023). Microbial inoculums improve growth and health of *Heteropneustes fossilis* via biofloc-driven aquaculture. *Microbial Cell Factories*, *22*(1). https://doi.org/10.1186/s12934-023-02107-0

Kurniawan, S. B., Imron, M. F., Abdullah, S. R. S., Othman, A. R., Purwanti, I. F., & Hasan, H. A. (2022). Treatment of real aquaculture effluent using bacteria-based bioflocculant produced by *Serratia marcescens*. *Journal of Water Process Engineering*, *47*. https://doi.org/10.1016/j.jwpe.2022.102708

Lakra, W. S., & Krishnani, K. K. (2021). Circular bioeconomy for stress-resilient fisheries and aquaculture. *Biomass, Biofuels, Biochemicals: Circular Bioeconomy: Technologies for Biofuels and Biochemicals*. https://doi.org/10.1016/B978-0-323-89855-3.00010-8

Lal, J., Vaishnav, A., & Brar, K. S. (2024). Biofloc Technology: Optimizing Aquaculture Through Microbial Innovation. *Journal of Advances in Microbiology*, *24*(7), 11-24.

Lauridsen, C., Matte, J. J., Lessard, M., Celi, P., & Litta, G. (2021). Role of vitamins for gastro-intestinal functionality and health of pigs. *Animal Feed Science and Technology* (Vol. 273). https://doi.org/10.1016/j.anifeedsci.2021.114823

Lavanya K, & Dayakar Y. (2022). Histopathological alterations in the white leg shrimp *L. Vennamei* supplemented with farm isolated probiotic (LAB) upon challenge with White Spot Syndrome Virus (WSSV). *International Journal of Science and Research Archive, 7*(2). https://doi.org/10.30574/ijsra.2022.7.2.0317

Lee, C., Kim, S., Lim, S. J., & Lee, K. J. (2017). Supplemental effects of biofloc powder on growth performance, innate immunity, and disease resistance of Pacific white shrimp *Litopenaeus vannamei*. *Fisheries and Aquatic Sciences*, *20*(1). https://doi.org/10.1186/s41240-017-0059-7

Lee, L. H., Mutalib, N. S. A., Law, J. W. F., Wong, S. H., & Letchumanan, V. (2018). Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in Selangor reveals carbapenemase producing *Vibrio parahaemolyticus* in marine and freshwater fish. *Frontiers in Microbiology*, *9*(OCT). https://doi.org/10.3389/fmicb.2018.02513

Legendre, F., MacLean, A., Appanna, V. P., & Appanna, V. D. (2020). Biochemical pathways to α-ketoglutarate, a multi-faceted metabolite. *World Journal of Microbiology and Biotechnology* (Vol. 36, Issue 8). https://doi.org/10.1007/s11274-020-02900-8

Leonard, C., Söderhäll, K., & Ratcliffe, N. A. (1985). Studies on prophenoloxidase and protease activity of *Blaberus craniifer* haemocytes. *Insect Biochemistry*, *15*(6). https://doi.org/10.1016/0020-1790(85)90109-X

Liang, Y., Yan, C., Guo, Q., Xu, J., & Hu, H. (2016). Spectrophotometric determination of ammonia nitrogen in water by flow injection analysis based on NH3- o-phthalaldehyde -Na2SO3 reaction. *Analytical Chemistry Research*, *10*. https://doi.org/10.1016/j.ancr.2016.10.001

Liao, Y., Smyth, G. K., & Shi, W. (2014). Feature Counts: An efficient general-purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7). https://doi.org/10.1093/bioinformatics/btt656

Li, C., Zhang, X., Chen, Y., Zhang, S., Dai, L., Zhu, W., & Chen, Y. (2023). Optimized utilization of organic carbon in aquaculture biofloc systems: A review. *Fishes*, *8*(9), 465.

Li, J., Chen, X., Townend, I., Shi, B., Du, J., Gao, J., Chuai, X., Gong, Z., & Wang, Y. P. (2021). A comparison study on the sediment flocculation process between a bare tidal flat and a clam aquaculture mudflat: The important role of sediment concentration and biological processes. *Marine Geology*, *434*. https://doi.org/10.1016/j.margeo.2021.106443

Li, J., Huang, J., Li, C., Zhang, Y., Wang, Y., Hou, S., Cheng, Y., & Li, J. (2021). Evaluation of the nutritional quality of edible tissues (muscle and hepatopancreas) of cultivated *Procambarus clarkii* using biofloc technology. *Aquaculture Reports*, *19*. https://doi.org/10.1016/j.aqrep.2021.100586

Lin, H., Chen, Y., Niu, J., Zhou, C., Huang, Z., Du, Q., & Zhang, J. (2015). Dietary methionine requirements of pacific white shrimp *Litopenaeus Vannamei*, of three different sizes. *Israeli Journal of Aquaculture - Bamidgeh*, *67*. https://doi.org/10.46989/001c.20713

Lin, H., Liang, X., Han, F., Luo, X., & Li, E. (2023). Growth, Biochemical Characteristics, Flesh Quality, and Gut Microbiota of the Pacific White Shrimp (*Penaeus vannamei*) Fed a Defatted Superworm (*Zophobas atratus*) Larvae Meal. *Aquaculture Nutrition*, *2023*. https://doi.org/10.1155/2023/8627246

Lin, M. C., Qiu, G. P., Zhou, X. H., & Chen, C. N. (2020). Using Taguchi and neural network approaches in the optimum design of product development process. *International Journal of Computer Integrated Manufacturing*, *33*(4). https://doi.org/10.1080/0951192X.2019.1639218

Lin, W., Luo, H., Wu, J., Hung, T. C., Cao, B., Liu, X., Yang, J., & Yang, P. (2023). A Review of the Emerging Risks of Acute Ammonia Nitrogen Toxicity to Aquatic Decapod Crustaceans. *Water (Switzerland)* (Vol. 15, Issue 1). https://doi.org/10.3390/w15010027

Liu, Y., Chen, J., Hu, H., Qu, K., & Cui, Z. (2022). A Low-Cost Electrochemical Method for the Determination of Sulfadiazine in Aquaculture Wastewater. *International Journal of Environmental Research and Public Health*, *19*(24). https://doi.org/10.3390/ijerph192416945

Liu, Y., Chen, L., Yu, L., Zhu, J., Wang, F., Jiang, F., Sun, C., Zheng, L., & Yang, Y. (2023). Benefited wastewater utilization via configurable, spatialized, and microorganisms-integrated biophotonic systems. *Chemical Engineering Journal*, *466*. https://doi.org/10.1016/j.cej.2023.143250

Loiola, M., Silva, A. E. T., Krull, M., Barbosa, F. A., Galvão, E. H., Patire, V. F., ... & Meirelles, P. M. (2023). Mangrove microbial community recovery and their role in early stages of forest recolonization within shrimp ponds. *Science of The Total Environment*, *855*, 158863.

Lou, F., Gao, T., & Han, Z. (2019). Transcriptome analyses reveal alterations in muscle metabolism, immune responses and reproductive behavior of Japanese mantis shrimp (*Oratosquilla oratoria*) at different cold temperature. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *32*. https://doi.org/10.1016/j.cbd.2019.100615

Lu, B. F., Kang, W. J., Shi, S. L., Guan, J., Jing, F., & Wu, B. (2024). Differences in Fatty Acid and Central Carbon Metabolite Distribution among Different Tissues of Alfalfa–Rhizobia Symbiotic System. *Agronomy*, *14*(3). https://doi.org/10.3390/agronomy14030511

Luis-Villaseñor, I. E., Voltolina, D., Audelo-Naranjo, J. M., Pacheco-Marges, M. R., Herrera-Espericueta, V. E., & Romero-Beltrán, E. (2015). Effects of biofloc promotion on water quality, growth, biomass yield and heterotrophic community in *Litopenaeus vannamei* (Boone, 1931) experimental intensive culture. *Italian Journal of Animal Science*, *14*(3). https://doi.org/10.4081/ijas.2015.3726

Luna, M., Llorente, I., & Cobo, A. (2020). Aquaculture production optimisation in multi-cage farms subject to commercial and operational constraints. *Biosystems Engineering*, *196*. https://doi.org/10.1016/j.biosystemseng.2020.05.012

Lunda, R., Roy, K., Dvorak, P., Kouba, A., & Mraz, J. (2020). Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-76692-0

Luo, G., Chen, X., Tan, J., Abakari, G., & Tan, H. (2020). Effects of carbohydrate addition strategy and biofloc levels on the establishment of nitrification in biofloc

technology aquaculture systems. *Aquaculture*, *514*. https://doi.org/10.1016/j.aquaculture.2019.734441

Luo, G., Jiayang, L., Jinxiang, X., Liu, W., & Tan, H. (2023). Effects of dissolved organic carbon and total ammonia nitrogen concentrations with the same DOC/TAN on biofloc performance. *Aquaculture*, *574*. https://doi.org/10.1016/j.aquaculture.2023.739713

Luo, G., Xu, J., Li, J., Zheng, H., Tan, H., & Liu, W. (2022). Rapid production bioflocs by inoculation and fertilized with different nitrogen and carbon sources. *Aquacultural Engineering*, *98*. https://doi.org/10.1016/j.aquaeng.2022.102262

Lu, X., Peng, D., Chen, X., Wu, F., Jiang, M., Tian, J., Liu, W., Yu, L., Wen, H., & Wei, K. (2020). Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (*Procambarus clarkii*). *Aquaculture Reports*, *18*. https://doi.org/10.1016/j.aqrep.2020.100542

Machado, M., Fernández-Boo, S., Teixeira, C., Viegas, M., Serradeiro, R., Dias, J., Costas Refojos, B., & Masagounder, K. (2023). DL-methionyl-DL-methionine as an efficient methionine source for promoting zootechnical performance and methionine-related pathways in the whiteleg shrimp (*Penaeus vannamei*). *British Journal of Nutrition*, *130*(4). https://doi.org/10.1017/S0007114522003579

Malpartida Pasco, J. J., Carvalho Filho, J. W., de Espirito Santo, C. M., & Vinatea, L. (2018). Production of Nile tilapia *Oreochromis niloticus* grown in BFT using two aeration systems. *Aquaculture Research*, *49*(1), 222-231.

Mahalakshmi, P., & Ganesan, K. (2009). Mahalanobis Taguchi System based criteria selection for shrimp aquaculture development. *Computers and Electronics in Agriculture*, *65*(2), 192–197. https://doi.org/10.1016/J.COMPAG.2008.09.003

Mahalakshmi, P., Ganesan, K., & Venkatasubramanian, V. (2012). DMTIOLA: Decision making tool for identification of optimal location for aquaculture farming development. *Aquaculture International*, *20*(5). https://doi.org/10.1007/s10499-012-9516-6

Mahasri, G., Satyantini, W. H., & Mukti, A. T. (2022). Analysis of Blood Glucose Levels and the Development of Ectoparasite Infestation on Pacific White Shrimp (*Litopenaeus vannamei*) which were given crude protein Zoothamnium penaei at High Stocking Densities. *IOP Conference Series: Earth and Environmental Science*, *1036*(1). https://doi.org/10.1088/1755-1315/1036/1/012013

Mahmudi, M., Musa, M., Bunga, A., Wati, N. A., Arsad, S., & Lusiana, E. D. (2022). A Water Quality Evaluation of Integrated Mangrove Aquaculture System for Water Treatment in Super-Intensive White Leg Shrimp Pond. *Journal of Ecological Engineering*, *23*(4). https://doi.org/10.12911/22998993/146746

Mailloux, R. J., Gill, R., & Young, A. (2019). Protein S-glutathionylation and the regulation of cellular functions. *Oxidative Stress: Eustress and Distress*. https://doi.org/10.1016/B978-0-12-818606-0.00013-4

Mair, G. C., Halwart, M., Derun, Y., & Costa-Pierce, B. A. (2023). A decadal outlook for global aquaculture. *Journal of the World Aquaculture Society* (Vol. 54, Issue 2). https://doi.org/10.1111/jwas.12977

Maiti, M. K., Sahu, N. P., Sardar, P., Garg, C. K., Varghese, T., Shamna, N., Deo, A. D., & Harikrishna, V. (2022). Dietary lysine requirement of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931) reared in inland saline water of 10 ppt salinity. *Animal Feed Science and Technology*, *291*. https://doi.org/10.1016/j.anifeedsci.2022.115378

Majhi, S. S., Singh, S. K., Biswas, P., Debbarma, R., Parhi, J., Ngasotter, S., Waikhom, G., Meena, D. K., Devi, A. G., Mahanand, S. S., Xavier, K. A. M., & Patel, A. B. (2023). Effect of stocking density on growth, water quality changes and cost efficiency of butter catfish (*Ompok bimaculatus*) during Seed Rearing in a Biofloc System. *Fishes*, *8*(2). https://doi.org/10.3390/fishes8020061

Manan, H., Amin-Safwan, A., Kasan, N. A., & Ikhwanuddin, M. (2020). Effects of biofloc application on survival rate, growth performance and specific growth rate of Pacific whiteleg shrimp, *Penaeus vannamei* culture in closed hatchery system. *Pakistan Journal of Biological Sciences*, *23*(12). https://doi.org/10.3923/pjbs.2020.1563.1571

Manan, H., Kasan, N. A., Ikhwanuddin, M., Kamaruzzan, A. S., Jalilah, M., Fauzan, F., ... & Amin-Safwan, A. (2024). Biofloc technology in improving shellfish aquaculture production–a review. *Annals of Animal Science*, *24*(4), 983-993.

Manan, H., Rosland, N. A., Mat Deris, Z., Che Hashim, N. F., Kasan, N. A., Ikhwanuddin, M., Suloma, A., & Fauzan, F. (2022). 16S rRNA sequences of *Exiguobacterium* spp. bacteria dominant in a biofloc pond cultured with whiteleg shrimp, *Penaeus vannamei*. *Aquaculture Research*, *53*(5). https://doi.org/10.1111/are.15731

Mandal, A., Holeyappa, S. A., Khairnar, S. O., Barik, S., Tyagi, A., & Surasani, V. K. R. (2024). Growth performance, health status and flesh quality of striped catfish (*Pangasianodon hypophthalmus*) reared in variable stocking densities in biofloc system. *Aquaculture*, *590*, 741047.

Mansour, A. T., Ashry, O. A., El-Neweshy, M. S., Alsaqufi, A. S., Dighiesh, H. S., Ashour, M., Kelany, M. S., El-Sawy, M. A., Mabrouk, M. M., Abbas, E. M., & Sharawy, Z. Z. (2022). Effect of Agricultural By-Products as a Carbon Source in a Biofloc-Based System on Growth Performance, Digestive Enzyme Activities, Hepatopancreas Histology, and Gut Bacterial Load of Litopenaeus vannamei Post Larvae. *Journal of Marine Science and Engineering*, *10*(10). https://doi.org/10.3390/jmse10101333

Mao, F., Mu, H., Wong, N. K., Liu, K., Song, J., Qiu, J., Lin, Y., Zhang, X., Xu, D., Xiang, Z., Li, J., Zhang, Y., & Yu, Z. (2020). Hemocyte phagosomal proteome is dynamically shaped by cytoskeleton remodeling and interorganellar communication with endoplasmic reticulum during phagocytosis in a marine invertebrate, *Crassostrea gigas*. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-63676-3

Marini Wijayanti, Tanbiyaskur, Jubaedah, D., Saputra, A. B., Genti, K. S., Agustina, Nabila Saraswati, Siti Yuliani, & Hary Widjajanti. (2020). Eukaryote microbes potential for bioflocs in the swamp aquaculture. *Jurnal Akuakultur Indonesia*, *19*(1). https://doi.org/10.19027/jai.19.1.19-29

Martinez-Cordova, L. R. (2020). A preliminary evaluation of an integrated aquaculture-agriculture systems (tilapia and peppers) at mesocosm scale. *Journal of Aquaculture & Marine Biology*, *9*(1). https://doi.org/10.15406/jamb.2020.09.00272

Martinez-Porchas, M., Preciado-Álvarez, A., Vargas-Albores, F., Gracia-Valenzuela, M. H., Cicala, F., Martinez-Cordova, L. R., ... & Garibay-Valdez, E. (2023). Microbiota plasticity in tilapia gut revealed by meta-analysis evaluating the effect of probiotics, prebiotics, and biofloc. *PeerJ*, *11*, e16213.

Martín Ríos, L. D., Betancourt Monteagudo, E., Corrales Barrios, Y., Leyva González, L., Valdes Vaillant, Y. de la C., Bossier, P., & Arenal, A. (2023). Biofloc technology and immune response of penaeid shrimp: A meta-analysis and meta-regression. *Fish & Shellfish Immunology*, *138*, 108805. https://doi.org/10.1016/J.FSI.2023.108805

Masoodi, K. Z., Lone, S. M., & Rasool, R. S. (2020). Advanced Methods in Molecular Biology and Biotechnology: A Practical Lab Manual. *Advanced Methods in Molecular Biology and Biotechnology: A Practical Lab Manual*. https://doi.org/10.1016/C2020-0-01818-9

McCusker, S., Warberg, M. B., Davies, S. J., Valente, C. de S., Johnson, M. P., Cooney, R., & Wan, A. H. L. (2023). Biofloc technology as part of a sustainable aquaculture system: A review on the status and innovations for its expansion. *Aquaculture, Fish and Fisheries*, *3*(4). https://doi.org/10.1002/aff2.108

McLean, E., Alfrey, K. B., Gatlin, D. M., Gibson Gaylord, T., & Barrows, F. T. (2022). Muscle amino acid profiles of eleven species of aquacultured animals and their potential value in feed formulation. *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2022.04.010

Mejaes B.A. (2021). Classifying and estimating aquaculture subsidies and their risks to the marine environment. *Angewandte Chemie International Edition, 6(11), 951–952.*, *May*.

Mendoza-Porras, O., Kamath, S., Harris, J. O., Colgrave, M. L., Huerlimann, R., Lopata, A. L., & Wade, N. M. (2020). Resolving hemocyanin isoform complexity in haemolymph of black tiger shrimp *Penaeus monodon* - implications in aquaculture, medicine and food safety. *Journal of Proteomics*, *218*. https://doi.org/10.1016/j.jprot.2020.103689

Miao, S., Hu, J., Wan, W., Han, B., Zhou, Y., Xin, Z., & Sun, L. (2020). Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. *Aquaculture*, *525*. https://doi.org/10.1016/j.aquaculture.2020.735280

Michalak, I., & Mahrose, K. (2020). Seaweeds, intact and processed, as a valuable component of poultry feeds. *Journal of Marine Science and Engineering* (Vol. 8, Issue 8). https://doi.org/10.3390/JMSE8080620

Minabi, K., Sourinejad, I., Alizadeh, M., Ghatrami, E. R., & Khanjani, M. H. (2020). Effects of different carbon-to-nitrogen ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio* L.)

fingerlings. *Aquaculture International*, *28*(5). https://doi.org/10.1007/s10499-020-00564-7

Minaz, M., Yazici, İ. S., Sevgili, H., & Aydln, İ. (2023). Biofloc technology in aquaculture: Advantages and disadvantages from social and applicability perspectives. *Annals of Animal Science*. https://doi.org/10.2478/aoas-2023-0043

Minorova, A., Romanchuk, I., Verbytskyi, S., Danylenko, S., Krushelnytska, N., Potemska, O., & Narizhnyi, S. (2022). Effect of protein and carbohydrate components upon quality parameters and viable probiotic bacteria content in milk mixtures during their drying and storage. *Journal of Microbiology, Biotechnology and Food Sciences*, *11*(6). https://doi.org/10.55251/jmbfs.3778

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, *247*(10). https://doi.org/10.1016/s0021-9258(19)45228-9

Mnyoro, M. S., Munubi, R. N., Pedersen, L. F., & Chenyambuga, S. W. (2022). Evaluation of biofilter performance with alternative local biomedia in pilot scale recirculating aquaculture systems. *Journal of Cleaner Production*, *366*. https://doi.org/10.1016/j.jclepro.2022.132929

Mohamed, H. I., Abdel Aal, M. H., & El-Mahdy, O. M. (2024). Fungal metabolites and their role in remediation of environmental pollution. In H. I. M. Kamel A. Abd-Elsalam (Ed.), *Fungal Secondary Metabolites: Synthesis and Applications in Agroecosystem* (pp. 281–315). Elsevier. https://doi.org/10.1016/B978-0-323-95241-5.00020-4

Mohammadi, G., Seddiq Mortazavi, M., & Hafezieh, M. (2023). Evaluation of High Stocking Densities on the Water Quality and Growth Performance of Pacific White Shrimp *Litopenaeus vannamei* Reared in a Mixotrophic Biofloc Nursery System. *Aquaculture Research*, *2023*. https://doi.org/10.1155/2023/1765366

Mongomery, D. C. (2017). Design and Analysis of Experiments. *John Willy & Sons*.

Mosquera-Murillo, Z., & Pena-Salamanca, E. J. (2016). Effect of salinity on growth of the green alga *Caulerpa sertularioides* (Bryopsidales, Chlorophyta) under laboratory conditions. *Hidrobiologica*, *26*(2). https://doi.org/10.24275/uam/izt/dcbs/hidro/2016v26n2/mosquera

Mugwanya, M., Dawood, M. A. O., Kimera, F., & Sewilam, H. (2021). Biofloc systems for sustainable production of economically important aquatic species: A review. *Sustainability (Switzerland)* (Vol. 13, Issue 13). https://doi.org/10.3390/su13137255

Mustafa, S., Estim, A., Shapawi, R., Shalehand, M. J., & Sidik, S. R. M. (2021). Technological applications and adaptations in aquaculture for progress towards sustainable development and seafood security. *IOP Conference Series: Earth and Environmental Science*, *718*(1). https://doi.org/10.1088/1755-1315/718/1/012041

Nácher-Mestre, J., Ballester-Lozano, G. F., Garlito, B., Portolés, T., Calduch-Giner, J., Serrano, R., Hernández, F., Berntssen, M. H. G., & Pérez-Sánchez, J. (2018). Comprehensive overview of feed-to-fillet transfer of new and traditional

contaminants in Atlantic salmon and gilthead sea bream fed plant-based diets. *Aquaculture Nutrition* (Vol. 24, Issue 6). https://doi.org/10.1111/anu.12817

Najdegerami, E. H. (2014). The application of biofloc technology in intensive culture of common carp (*Cyprinus carpio*) fingerlings. *Journal of Animal Environment*.

Nam, N. N., Do, H. D. K., Loan Trinh, K. T., & Lee, N. Y. (2023). Metagenomics: An effective approach for exploring microbial diversity and functions. *Foods*, *12*(11), 2140.

Narayanan, M., Ali, S. S., & El-Sheekh, M. (2023). A comprehensive review on the potential of microbial enzymes in multipollutant bioremediation: Mechanisms, challenges, and future prospects. *Journal of Environmental Management* (Vol. 334). https://doi.org/10.1016/j.jenvman.2023.117532

Natnan, M. E., Low, C. F., Chong, C. M., Bunawan, H., & Baharum, S. N. (2021). Integration of omics tools for understanding the fish immune response due to microbial challenge. *Frontiers in Marine Science*, *8*, 668771.

Nayak, S., Mantri, J. K., & Swain, P. K. (2019). Design and performance analysis of rural aquaculture ponds using IoT. *International Journal of Recent Technology and Engineering*, *8*(2). https://doi.org/10.35940/ijrte.B2086.078219

Nayak, S., Yogev, U., Kpordzaxor, Y., Zhu, Z., Gur, N., Gross, A., & Zilberg, D. (2023). From fish excretions to high-protein dietary ingredient: Feeding intensively cultured barramundi (*Lates calcarifer*) a diet containing microbial biomass (biofloc) from effluent of an aquaculture system. *Aquaculture*, *562*, 738780. https://doi.org/10.1016/J.Aquaculture.2022.738780

Naylor, R., Fang, S., & Fanzo, J. (2023). A global view of aquaculture policy. In *Food Policy* (Vol. 116). https://doi.org/10.1016/j.foodpol.2023.102422

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature* (Vol. 591, Issue 7851). https://doi.org/10.1038/s41586-021-03308-6

Nazarpour, S., & Mohammadiazarm, H. (2023). Optimizing stocking density in biofloc culture of juvenile common carp (*Cyprinus carpio*) using growth and immune-biochemical indices as indicators. *Aquaculture Studies*, *23*(6).

Nazifa, T. H., Cata Saady, N. M., Bazan, C., Zendehboudi, S., Aftab, A., & Albayati, T. M. (2021). Anaerobic digestion of blood from slaughtered livestock: A review. *Energies* (Vol. 14, Issue 18). https://doi.org/10.3390/en14185666

Nenciu, F., Voicea, I., Cocarta, D. M., Vladut, V. N., Matache, M. G., & Arsenoaia, V. N. (2022). "Zero-Waste" Food Production System Supporting the Synergic Interaction between Aquaculture and Horticulture. *Sustainability (Switzerland)*, *14*(20). https://doi.org/10.3390/su142013396

Nethaji, M., Ahilan, B., Kathirvelpandiyan, A., Felix, N., Uma, A., Mosses, T. L. S. S., & Lingam, R. S. S. (2022). Biofloc meal incorporated diet improves the growth and physiological responses of *Penaeus vannamei*. *Aquaculture International*, *30*(5). https://doi.org/10.1007/s10499-022-00929-0

Nguyen, H. T., Wiederkehr, A., Wollheim, C. B., & Park, K. S. (2024). Regulation of autophagy by perilysosomal calcium: a new player in β-cell lipotoxicity. In *Experimental and Molecular Medicine* (Vol. 56, Issue 2). https://doi.org/10.1038/s12276-024-01161-x

Nguyen, T. P. C. (2020). Influence of stocking density on water quality and growth performance of white leg shrimp (*Litopenaeus vannamei*) reared in fiberglass tanks, without water exchange. *The Journal of Agriculture and Development*, *18*(06). https://doi.org/10.52997/jad.3.06.2019

Nisar, U., Peng, D., Mu, Y., & Sun, Y. (2022). A Solution for Sustainable Utilization of Aquaculture Waste: A Comprehensive Review of Biofloc Technology and Aquamimicry. *Frontiers in Nutrition* (Vol. 8). https://doi.org/10.3389/fnut.2021.791738

Nizam, N. U. M., Hanafiah, M. M., Noor, I. M., & Karim, H. I. A. (2020). Efficiency of five selected aquatic plants in phytoremediation of aquaculture wastewater. *Applied Sciences (Switzerland)*, *10*(8). https://doi.org/10.3390/APP10082712

Noblet, J., Labussière, E., Renaudeau, D., & van Milgen, J. (2022). Energy and energy metabolism in swine. *Sustainable Swine Nutrition: Second Edition*. https://doi.org/10.1002/9781119583998.ch3

Nobrega, G., Souza, M. S., Rodríguez-Martín, M., Rodríguez-Gonzálvez, P., & Ribeiro, J. (2021). Parametric optimization of the gnaw welding process in thin thickness of austenitic stainless steel by Taguchi method. *Applied Sciences (Switzerland)*, *11*(18). https://doi.org/10.3390/app11188742

Ntakirutimana, R., Syanya, F. J., & Mwangi, P. (2023). Exploring the impact of probiotics on the gut ecosystem and morpho-histology in fish: current knowledge of tilapia. *Asian Journal of Fisheries and Aquatic Research*, *25*(3), 93-112.

Nurhafizah, W. W. I., Lee, K. L., Nadirah, M., Danish-Daniel, M., Zainathan, S. C., & Najiah, M. (2021). Virulence properties and pathogenicity of multidrug-resistant Vibrio harveyi associated with luminescent vibriosis in Pacific white shrimp, *Penaeus vannamei*. *Journal of Invertebrate Pathology*, *186*, 107594.

Obiero, K. O., Waidbacher, H., Nyawanda, B. O., Munguti, J. M., Manyala, J. O., & Kaunda-Arara, B. (2019). Predicting uptake of aquaculture technologies among smallholder fish farmers in Kenya. *Aquaculture International*, *27*(6). https://doi.org/10.1007/s10499-019-00423-0

Ogello, E. O., Outa, N. O., Obiero, K. O., Kyule, D. N., & Munguti, J. M. (2021). The prospects of biofloc technology (BFT) for sustainable aquaculture development. *Scientific African* (Vol. 14). https://doi.org/10.1016/j.sciaf.2021.e01053

Okomoda, V. T., Ikhwanuddin, M., Oladimeji, A. S., Najiah, M., Alabi, K. I., Salam, A. I. A., Jauhari, I., & Kasan, N. A. (2022). Rearing water quality and zootechnical parameters of *Litopenaeus vannamei* in rapid Biofloc® and conventional intensive culture system. *Journal of King Saud University - Science*, *34*(1). https://doi.org/10.1016/j.jksus.2021.101729

Pacheco-Vega, J. M., Cadena-Roa, M. A., Leyva-Flores, J. A., Zavala-Leal, O. I., Pérez-Bravo, E., & Ruiz-Velazco, J. M. J. (2018). Effect of isolated bacteria and

microalgae on the biofloc characteristics in the Pacific white shrimp culture. *Aquaculture Reports, 11*. https://doi.org/10.1016/j.aqrep.2018.05.003

Padeniya, U., Davis, D. A., Wells, D. E., & Bruce, T. J. (2022). Microbial Interactions, Growth, and Health of Aquatic Species in Biofloc Systems. In *Water (Switzerland)* (Vol. 14, Issue 24). https://doi.org/10.3390/w14244019

Palomar, M., Soler, M. D., Roura, E., Sala, R., Piquer, O., & Garcés-Narro, C. (2020). Article degree of saturation and free fatty acid content of fats determine dietary preferences in laying hens. *Animals, 10*(12). https://doi.org/10.3390/ani10122437

Pang, H., Wang, Y. N., Chi, Z. Y., Xu, Y. P., Li, S. Y., Che, J., & Wang, J. H. (2021). Enhanced aquaculture effluent polishing by once and repetitive nutrients deprived seawater Chlorella sp. *Algal Research* (Vol. 60). https://doi.org/10.1016/j.algal.2021.102555

Panigrahi, A., Das, R. R., Sivakumar, M. R., Saravanan, A., Saranya, C., Sudheer, N. S., Kumaraguru Vasagam, K. P., Mahalakshmi, P., Kannappan, S., & Gopikrishna, G. (2020). Bio-augmentation of heterotrophic bacteria in biofloc system improves growth, survival, and immunity of Indian white shrimp Penaeus indicus. *Fish and Shellfish Immunology*, *98*. https://doi.org/10.1016/j.fsi.2020.01.021

Papáček, Š., Petera, K., Masaló, I., & Oca, J. (2019). On the Optimization of Recirculated Aquaculture Systems. *EngOpt 2018 Proceedings of the 6th International Conference on Engineering Optimization*. https://doi.org/10.1007/978-3-319-97773-7_106

Parkhomchuk, D., Borodina, T., Amstislavskiy, V., Banaru, M., Hallen, L., Krobitsch, S., Lehrach, H., & Soldatov, A. (2009). Transcriptome analysis by strand-specific sequencing of complementary DNA. *Nucleic Acids Research*, *37*(18). https://doi.org/10.1093/nar/gkp596

Pascual, C., Gaxiola, G. & Rosas, C. (2003). Blood metabolites and hemocyanin of the white shrimp, *Litopenaeus vannamei*: the effect of culture conditions and a comparison with other crustacean species. *Marine Biology* 142, 735–745. https://doi.org/10.1007/s00227-002-0995-2

Pathan, J. G. K., Shelke, S. T., Relekar, S. S., Gore, S. B., Telvekar, P. A., Kulkarni, A. K., Sahoo, U., & Joshi, H. D. (2022). Maximizing the Potential of Stunted Fish Fingerlings through Innovative and Sustainable Aquaculture Practices for Enhanced Growth and Production. *Current Journal of Applied Science and Technology*. https://doi.org/10.9734/cjast/2022/v41i484102

Paul, B. N., Singh, P., & Giri, S. S. (2018). Potentiality of new feed ingredients for aquaculture: A review. *Agricultural Reviews, of*. https://doi.org/10.18805/ag.r-1819

Paul, I., Panigrahi, A. K., & Datta, S. (2020). Influence of nitrogen cycle bacteria on nitrogen mineralisation, water quality and productivity of freshwater fish pond: A review. *Asian Fisheries Science*, *33*(2). https://doi.org/10.33997/j.afs.2020.33.2.006

Pereira, A. G., Fraga-Corral, M., Garcia-Oliveira, P., Otero, P., Soria-Lopez, A., Cassani, L., Cao, H., Xiao, J., Prieto, M. A., & Simal-Gandara, J. (2022). Single-

Cell Proteins Obtained by Circular Economy Intended as a Feed Ingredient in Aquaculture. *Foods* (Vol. 11, Issue 18). https://doi.org/10.3390/foods11182831

Pérez-Velasco, R., Hernández-Vergara, M. P., Pérez-Rostro, C. I., Frías-Quintana, C. A., Pérez-Velasco, R., Hernández-Vergara, M. P., Pérez-Rostro, C. I., & Frías-Quintana, C. A. (2023). Variation of dietary protein/lipid levels used in postlarvae of freshwater prawn *Macrobrachium rosenbergii* cultured in a biofloc system. *Latin American Journal of Aquatic Research*, *51*(1), 12–22. https://doi.org/10.3856/VOL51-ISSUE1-FULLTEXT-2924

Peterson, R. A. (2021). Finding Optimal Normalizing Transformations via bestNormalize. *R Journal*, *13*(1), 310–329. https://doi.org/10.32614/RJ-2021-041/

Phung, N. Van, Rong, F., Xia, W. Y., Fan, Y., Li, X. Y., Wang, S. A., & Li, F. L. (2023). Nervonic acid and its sphingolipids: Biological functions and potential food applications. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2023.2203753

Podder, S., Anoy, M. F. S., Rafid, S. T. S., & Ajwad, A. J. (2023, December). Smart Biofloc System: Leveraging IoT for Enhanced Aquaculture Sustainability. In *2023 5th International Conference on Electrical, Control and Instrumentation Engineering (ICECIE)* (pp. 1-7). IEEE.

Pozzobon, V., Cui, N., Moreaud, A., Michiels, E., & Levasseur, W. (2021). Nitrate and nitrite as mixed source of nitrogen for *Chlorella vulgaris*: Growth, nitrogen uptake and pigment contents. *Bioresource Technology*, *330*. https://doi.org/10.1016/j.biortech.2021.124995

Prasad, K. S., Sumalatha, A., Chandrarao, J. V. K. S., & Prasad, V. V. V. (2023). An Integrated Wireless Aquaculture Monitoring and Feed Management System. *Proceedings - 7th International Conference on Computing Methodologies and Communication, ICCMC 2023*. https://doi.org/10.1109/ICCMC56507.2023.10084236

Prates, E., Holanda, M., Pedrosa, V. F., Monserrat, J. M., & Wasielesky, W. (2023). Compensatory growth and energy reserves changes in the Pacific white shrimp (*Litopenaeus vannamei*) reared in different temperatures and under feed restriction in biofloc technology system (BFT). *Aquaculture*, *562*, 738821. https://doi.org/10.1016/J.AQUACULTURE.2022.738821

Preena, P. G., Rejish Kumar, V. J., & Singh, I. S. B. (2021). Nitrification and denitrification in recirculating aquaculture systems: the processes and players. *Reviews in Aquaculture* (Vol. 13, Issue 4). https://doi.org/10.1111/raq.12558

Prihatin, J. Y., Pangestu, B. A., Kristiyono, R., & Sulistyadi, S. (2023a). Experimental test analysis of hydram pump flow rate using L9 Taguchi at fish pond aquaculture. *Journal of Engineering and Applied Technology*, *4*(1), 33–39. https://journal.uny.ac.id/index.php/jeatech/article/view/58352

Prihatin, J. Y., Pangestu, B. A., Kristiyono, R., & Sulistyadi, S. (2023b). Experimental test analysis of hydram pump flow rate using L9 Taguchi at fish pond aquaculture. *Journal of Engineering and Applied Technology*, *4*(1). https://doi.org/10.21831/jeatech.v4i1.58352

Promthale, P., Pongtippatee, P., Withyachumnarnkul, B., & Wongprasert, K. (2019). Bioflocs substituted fishmeal feed stimulates immune response and protects shrimp from Vibrio parahaemolyticus infection. *Fish and Shellfish Immunology*, *93*. https://doi.org/10.1016/j.fsi.2019.07.084

Pundir, R., Chary, G. H. V. C., & Dastidar, M. G. (2018). Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing Aspergillus sp. *Water Resources and Industry*, *20*. https://doi.org/10.1016/j.wri.2016.05.001

Pushparaj, K., Kuchi Bhotla, H., Pappuswamy, M., Issara, U., Balasubramanian, B., Al-Dhabi, N. A., ... & Meyyazhagan, A. (2022). Perspectives and implications of probiotics as beneficial mediators in aquaculture industry. In *Aquaculture science and engineering* (pp. 79-97). Singapore: Springer Nature Singapore.

Putra, I., Effendi, I., Lukistyowati, I., Tang, U. M., Fauzi, M., Suharman, I., & Muchlisin, Z. A. (2020). Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia Oreochromis niloticus system. *F1000Research*, *9*. https://doi.org/10.12688/f1000research.22977.1

Qazi, M. I., Akhtar, R., Abas, M., Khalid, Q. S., Babar, A. R., & Pruncu, C. I. (2020). An integrated approach of GRA coupled with principal component analysis for multi-optimization of shielded metal arc welding (SMAW) process. *Materials*, *13*(16). https://doi.org/10.3390/MA13163457

Qiu, Z., Xu, Q., Li, S., Zheng, D., Zhang, R., Zhao, J., & Wang, T. (2023). Effects of Probiotics on the Water Quality, Growth Performance, Immunity, Digestion, and Intestinal Flora of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) in the Biofloc Culture System. *Water (Switzerland)*, *15*(6). https://doi.org/10.3390/w15061211

Rahardjo, S., Soepardjo, A. H., Djokosetiyanto, D., & Alamsyah, A. T. (2017a). Seaweed utilization for phytoremediation of *Litopenaeus vannamei* shrimp farming waste in recirculation systems (environmentally friendly design of sustainable shrimp culture). *Sustainable Future for Human Security: Environment and Resources*. https://doi.org/10.1007/978-981-10-5430-3_12

Rahardjo, S., Soepardjo, A. H., Djokosetiyanto, D., & Alamsyah, A. T. (2017b). Seaweed utilization for phytoremediation of *Litopenaeus vannamei* shrimp farming waste in recirculation systems (environmentally friendly design of sustainable shrimp culture). *Sustainable Future for Human Security: Environment and Resources*. https://doi.org/10.1007/978-981-10-5430-3_12

Rahi, M. L., Azad, K. N., Tabassum, M., Irin, H. H., Hossain, K. S., Aziz, D., Moshtaghi, A., & Hurwood, D. A. (2021). Effects of salinity on physiological, biochemical and gene expression parameters of black tiger shrimp (*Penaeus monodon*): Potential for farming in low-salinity environments. *Biology*, *10*(12). https://doi.org/10.3390/biology10121220

Rahman, N., & Raheem, A. (2022). Graphene oxide/Mg-Zn-Al layered double hydroxide for efficient removal of doxycycline from water: Taguchi approach for optimization. *Journal of Molecular Liquids*, *354*. https://doi.org/10.1016/j.molliq.2022.118899

Rajkumar, M., Pandey, P. K., Aravind, R., Vennila, A., Bharti, V., & Purushothaman, C. S. (2016). Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, *47*(11), 3432–3444. https://doi.org/10.1111/ARE.12792

Ranallo, R. T., Mcdonald, L. C., Halpin, A. L., Hiltke, T., & Young, V. B. (2021). The state of microbiome science at the intersection of infectious diseases and antimicrobial resistance. *Journal of Infectious Diseases*, *223*. https://doi.org/10.1093/infdis/jiab020

Rashid, M. M., Nayan, A. A., Rahman, M. O., Simi, S. A., Saha, J., & Kibria, M. G. (2021). IoT based Smart Water Quality Prediction for Biofloc Aquaculture. *International Journal of Advanced Computer Science and Applications*, *12*(6). https://doi.org/10.14569/IJACSA.2021.0120608

Ray, A. J., Drury, T. H., & Cecil, A. (2017). Comparing clear-water RAS and biofloc systems: Shrimp (*Litopenaeus vannamei*) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. *Aquacultural Engineering*, *77*. https://doi.org/10.1016/j.aquaeng.2017.02.002

Ray, A., Nayak, M., & Ghosh, A. (2022). A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production. *Science of the Total Environment* (Vol. 802). https://doi.org/10.1016/j.scitotenv.2021.149765

Raza, B., Zheng, Z., & Yang, W. (2024). A review on biofloc system technology, history, types, and future economical perceptions in aquaculture. *Animals*, *14*(10), 1489.

Razman, K. K., Hanafiah, M. M., Ramli, A. N., & Harun, S. N. (2023). Industrial wastewater treatment methods employed in Southeast Asian countries. *IOP Conference Series: Earth and Environmental Science*, *1167*(1). https://doi.org/10.1088/1755-1315/1167/1/012020

Ren, X., Wang, Q., Shao, H., Xu, Y., Liu, P., & Li, J. (2021). Effects of Low Temperature on Shrimp and Crab Physiology, Behavior, and Growth: A Review. In *Frontiers in Marine Science* (Vol. 8). https://doi.org/10.3389/fmars.2021.746177

Robles-Porchas, G. R., Gollas-Galván, T., Martínez-Porchas, M., Martínez-Cordova, L. R., Miranda-Baeza, A., & Vargas-Albores, F. (2020). The nitrification process for nitrogen removal in biofloc system aquaculture. In *Reviews in Aquaculture* (Vol. 12, Issue 4). https://doi.org/10.1111/raq.12431

Romano, N. (2021). Probiotics, prebiotics, biofloc systems, and other biocontrol regimens in fish and shellfish aquaculture. *Aquaculture Pharmacology*. https://doi.org/10.1016/B978-0-12-821339-1.00003-9

Saad, A., Nissen, O., Eilertsen, E., Bjørnson, F. O., Hagtun, T. N., Aspaas, O. G., Baikas, A. A., & Ohrem, S. J. (2022). Towards Improved Visualization and Optimization of Aquaculture Production Process. *Procedia Computer Science*, *207*. https://doi.org/10.1016/j.procs.2022.09.531

Sadat Hoseini Madani, N., Adorian, T. J., Ghafari Farsani, H., & Hoseinifar, S. H. (2018). The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and

immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) postlarvae. *Aquaculture Research*, *49*(5). https://doi.org/10.1111/are.13648

Safavi, M., Jafari Olia, M. S., Abolhasani, M. H., Amini, M., & Kianirad, M. (2021). Optimization of the culture medium and characterization of antioxidant compounds of a marine isolated microalga as a promising source in aquaculture feed. *Biocatalysis and Agricultural Biotechnology*, *35*. https://doi.org/10.1016/j.bcab.2021.102098

Sahu, A. K., Mir, S., Nayak, B., & Baitharu, I. (2024). Sustainable management of eutrophication and problems associated with the algal toxin in ponds and lakes of rural areas. *Water Resources Management for Rural Development: Challenges and Mitigation*. https://doi.org/10.1016/B978-0-443-18778-0.00021-0

Said, M. M. (2024). Light Limitation Alters Water Quality, Biofloc Composition, Zooplankton Community, and Performance of the Whiteleg Shrimp (*Litopenaeus vannamei*) Reared with Intensive Biofloc. *Egyptian Journal of Aquatic Biology and Fisheries*. https://doi.org/10.21608/ejabf.2024.355970

Said, M. M., & Ahmed, O. M. (2022). Carbohydrate Supplement Impact on Growth Performance, Bacterial Community, and Bacterial Food Quality of Whiteleg Shrimp (*Litopenaeus vannamei*) under Biofloc System. *Aquaculture Nutrition*, *2022*. https://doi.org/10.1155/2022/8964714

Sakkaravarthi, K. (2015). Determination of Effective Microbial Community for Biofloc Shrimp Culture System. *International Journal of Fisheries and Aquatic Studies*.

Sampantamit, T., Ho, L., Lachat, C., Sutummawong, N., Sorgeloos, P., & Goethals, P. (2020). Aquaculture production and its environmental sustainability in Thailand: Challenges and potential solutions. *Sustainability (Switzerland)* (Vol. 12, Issue 5). https://doi.org/10.3390/su12052010

Sánchez-Paz, A., García-Carreño, F., Hernández-López, J., Muhlia-Almazán, A., & Yepiz-Plascencia, G. (2007). Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific white shrimp (*Litopenaeus vannamei*). *Journal of Experimental Marine Biology and Ecology*, *340*(2). https://doi.org/10.1016/j.jembe.2006.09.006

Sankhla, M. S., Kumar, R., & Shefali. (2020). *New and Advanced Technologies in Aquaculture to Support Environmentally Sustainable Development*. https://doi.org/10.1007/978-981-15-2817-0_11

Santillan, E., Phua, W. X., Constancias, F., & Wuertz, S. (2020). Sustained organic loading disturbance favors nitrite accumulation in bioreactors with variable resistance, recovery and resilience of nitrification and nitrifiers. *Scientific Reports*, *10*(1), 21388.

Santos, N. B. V. dos, Furtado, P. S., César, D. E., & Wasielesky Junior, W. (2019). Assessment of the nitrification process in a culture of pacific white shrimp, using artificial substrate and bacterial inoculum in a biofloc technology system (BFT). *Ciência Rural*, *49*(6). https://doi.org/10.1590/0103-8478cr20180306

Sao, J. K., Naayagi, R. T., Panda, G., Patidar, R. D., & Swain, S. D. (2022). SAPF Parameter Optimization with the Application of Taguchi SNR Method. *Electronics (Switzerland)*, *11*(3). https://doi.org/10.3390/electronics11030348

Sarikaya, M., & Güllü, A. (2015). Multi-response optimization of minimum quantity lubrication parameters using Taguchi-based grey relational analysis in turning of difficult-to-cut alloy Haynes 25. *Journal of Cleaner Production*, *91*. https://doi.org/10.1016/j.jclepro.2014.12.020

Schveitzer, R., Baccarat, R. F. C., Gaona, C., Wasielesky, W., & Arantes, R. (2023). Concentration of suspended solids in superintensive culture of the Pacific white shrimp *Litopenaeus vannamei* with biofloc technology (BFT): A review. *Reviews in Aquaculture*. https://doi.org/10.1111/raq.12867

Seliger, C., Haslauer, M., Unfer, G., & Schmutz, S. (2021). Aquazone: An integrative tool for sustainable fish farm zoning. *Sustainability (Switzerland)*, *13*(3). https://doi.org/10.3390/su13031470

Selvarajan, R., Sibanda, T., Pandian, J., & Mearns, K. (2021). Taxonomic and functional distribution of bacterial communities in domestic and hospital wastewater system: Implications for public and environmental health. *Antibiotics*, *10*(9). https://doi.org/10.3390/antibiotics10091059

Selven, S., & Philip, R. (2013). Salinity a significant environmental factor for Vibrio harveyi virulence in *Fenneropenaeus indicus*. *Aquaculture Research*, *44*(5). https://doi.org/10.1111/j.1365-2109.2011.03080.x

Setyo Pradana, E., & Sulistiyowati, W. (2022). Literature Review: Use of the Taguchi Method for Quality Improvement. *PROZIMA (Productivity, Optimization and Manufacturing System Engineering)*, *6*(2). https://doi.org/10.21070/prozima.v6i2.1575

Sgnaulin, T., Pinho, S. M., Durigon, E. G., Thomas, M. C., de Mello, G. L., & Emerenciano, M. G. C. (2021). Culture of Pacu, *Piaractus mesopotamicus* in biofloc technology (BFT): insights on dietary protein sparing and stomach content. *Aquaculture International*, *29*(5). https://doi.org/10.1007/s10499-021-00748-9

Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S., Asraful Alam, M., & Mehmood, M. A. (2020). Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, *704*. https://doi.org/10.1016/j.scitotenv.2019.135303

Shakouri, A., & Balouch, G. M. (2020). The effects of nitrate and phosphate on growth of algae, *Ulva rigida*. *Iranian Journal of Fisheries Sciences*, *19*(1). https://doi.org/10.22092/ijfs.2018.117843

Shao, Y., Zhong, H., Wang, L., & Elbashier, M. M. A. (2021). Use of Bacillus subtilis D9 to purify coastal aquaculture wastewater and improve grass carp resistance to Vibrio infection. *Aquaculture Environment Interactions*, *13*. https://doi.org/10.3354/aei00404

Sharifi, M., Shokrollahi, A., & Ebrahimi, F. (2021). Synthesis and characterisation of MCM-41@SiO2-NH-pydc as a new nano mesoporous sorbent: application for

the simultaneous preconcentration of cationic dyes previous spectrophotometric determination, using Taguchi experimental design. https://doi.org/10.1080/03067319.2021.1928098

Shilpha, J., Song, J., & Jeong, B. R. (2023). Ammonium Phytotoxicity and Tolerance: An Insight into Ammonium Nutrition to Improve Crop Productivity. In *Agronomy* (Vol. 13, Issue 6). https://doi.org/10.3390/agronomy13061487

Shyla, B., Mahadevaiah, & Nagendrappa, G. (2011). A simple spectrophotometric method for the determination of phosphate in soil, detergents, water, bone and food samples through the formation of phosphomolybdate complex followed by its reduction with thiourea. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *78*(1), 497–502. https://doi.org/10.1016/J.SAA.2010.11.017

Silva, E. F., Soares, M. A., Calazans, N. F., Vogeley, J. L., do Valle, B. C., Soares, R., & Peixoto, S. (2012). Effect of probiotic (*Bacillus* spp.) addition during larvae and postlarvae culture of the white shrimp *Litopenaeus vannamei*. *Aquaculture Research*, *44*(1). https://doi.org/10.1111/j.1365-2109.2011.03001.x

Sojobi, A. O., & Liew, K. M. (2022). Multi-objective optimization of high-performance bio-inspired prefabricated composites for sustainable and resilient construction. *Composite Structures*, *279*. https://doi.org/10.1016/j.compstruct.2021.114732

Spackman, D. H., Stein, W. H., & Moore, S. (1958). Automatic Recording Apparatus for Use in the Chromatography of Amino Acids. *Analytical Chemistry*, *30*(7). https://doi.org/10.1021/ac60139a006

Spelta, A. C. F., Lorenzini, J. P. S., Neves, L. D. C., Rodrigues, L. D. S., Lana, Â. M. Q., Nakayama, C. L., ... & Miranda-Filho, K. C. M. (2021). Dynamics of the water quality parameters in the super-intensive culture of *Litopenaeus vannamei* in BFT system on artificial brackish water. *Aquaculture International*, *29*(5), 2049-2063.

Srivastava, N., Ibrahim, S. A., & Nasr, M. H. A. (Eds.). (2024). *Microbiome Engineering: The New Dimension of Biotechnology*. CRC Press.

Su, M., Zhang, X., Yuan, J., Zhang, X., & Li, F. (2022). The Role of Insulin-like Peptide in Maintaining Hemolymph Glucose Homeostasis in the Pacific White Shrimp *Litopenaeus vannamei*. *International Journal of Molecular Science*s, 23(6), 3268. https://doi.org/10.3390/ijms23063268

Sun, Y., Zhang, J., Dong, D., Li, M., Yang, X., Song, X., & Li, X. (2024). Effects of carbon source addition strategies on water quality, growth performance, and microbial community in shrimp BFT aquaculture systems. *Aquaculture*, *578*. https://doi.org/10.1016/j.aquaculture.2023.740027

Syaichudin, M., Jumriadi, Gafur, A., Akmal, Rahmi, Lideman, Sadat, Juniyanto, N. M., & Sujaka, S. (2022). Low Salinity Application to Improve Biofloc System in Early Grow-Out Stage of White Shrimp (*Litopenaeus vannamei*). *IOP Conference Series: Earth and Environmental Science*, *1118*(1), 012012. https://doi.org/10.1088/1755-1315/1118/1/012012

Tanay, D. D., Abella, T. T., Cruz, E. M. V., Sace, C. F., Fajardo, L. J., Velasco, R. R., & Abucay, J. S. (2020). Microbial Community Response to Carbon-Nitrogen Ratio Manipulation in Biofloc Culture. *Philippine Journal of Fisheries*, *27*(2). https://doi.org/10.31398/tpjf/27.2.2019A0014

Taufik, M., Ismail, T. I. T., Manan, H., Ikhwanuddin, M., Salam, A. I. A., Rahim, A. I. A., ... & Kasan, N. A. (2023). Synergistic effects of Recirculating Aquaculture System (RAS) with combination of clear water, probiotic and biofloc technology: A review. *Aquaculture and Fisheries*.

Tasnim, R., Shaikat, A. S., Al Amin, A., Hussein, M. R., & Rahman, M. M. (2022). Design of a Smart Biofloc Monitoring and Controlling System using IoT. *Journal of Engineering Advancements*. https://doi.org/10.38032/jea.2022.04.003

Tepaamorndech, S., Nookaew, I., Higdon, S. M., Santiyanont, P., Phromson, M., Chantarasakha, K., Mhuantong, W., Plengvidhya, V., & Visessanguan, W. (2020). Metagenomics in bioflocs and their effects on gut microbiome and immune responses in Pacific white shrimp. *Fish and Shellfish Immunology*, *106*. https://doi.org/10.1016/j.fsi.2020.08.042

Thongsoi, R., Maskaew, S., Puechpon, P., Noppradit, B., Inaek, N., Utarabhand, P., & Runsaeng, P. (2024). Identification of an essential role against shrimp pathogens of prophenoloxidase activating enzyme 1 (PPAE1) from *Fenneropenaeus merguiensis* haemocytes. *Developmental and Comparative Immunology*, *151*. https://doi.org/10.1016/j.dci.2023.105088

Tibebu Bogale, T. (2020). Microbial Protein Production from Agro-industrial Wastes as Food and Feed. *American Journal of Life Sciences*, *8*(5). https://doi.org/10.11648/j.ajls.20200805.16

Tierney, T. W., & Ray, A. J. (2018). Comparing biofloc, clear-water, and hybrid nursery systems (Part I): Shrimp (*Litopenaeus vannamei*) production, water quality, and stable isotope dynamics. *Aquacultural Engineering*, *82*. https://doi.org/10.1016/j.aquaeng.2018.06.002

Ting, W. H. T., Tan, I. A. W., Salleh, S. F., & Abdul Wahab, N. (2020). Ammoniacal nitrogen removal by *Eichhornia crassipes*-based phytoremediation: process optimization using response surface methodology. *Applied Water Science*, *10*(3). https://doi.org/10.1007/s13201-020-1163-x

Tinh, T. H., Koppenol, T., Hai, T. N., Verreth, J. A. J., & Verdegem, M. C. J. (2021). Effects of carbohydrate sources on a biofloc nursery system for whiteleg shrimp (Litopenaeus vannamei). *Aquaculture*, *531*. https://doi.org/10.1016/j.aquaculture.2020.735795

Trimpey, J., & Engle, C. (2005). The economic feasibility of adoption of a new in-pond mechanical grader for food-sized channel catfish (*Ictalurus punctatus*). *Aquacultural Engineering*, *32*(3–4). https://doi.org/10.1016/j.aquaeng.2004.09.004

Uawisetwathana, U., Situmorang, M. L., Arayamethakorn, S., Haniswita, Suantika, G., Panya, A., Karoonuthaisiri, N., & Rungrassamee, W. (2021). Supplementation of ex-situ biofloc to improve growth performance and enhance

nutritional values of the pacific white shrimp rearing at low salinity conditions. *Applied Sciences (Switzerland)*, *11*(10). https://doi.org/10.3390/app11104598

Ubina, N. A., & Cheng, S. C. (2022). A Review of Unmanned System Technologies with Its Application to Aquaculture Farm Monitoring and Management. In *Drones* (Vol. 6, Issue 1). https://doi.org/10.3390/drones6010012

Ushada, M., Suryandono, A., Wicaksono, A., & Murase, H. (2015). Quality evaluation for scale-up of moss (Sphagnum sp.) rooftop greening panel using Taguchi method. *Engineering in Agriculture, Environment and Food*, *8*(3). https://doi.org/10.1016/j.eaef.2015.07.004

Valenzuela-Jiménez, M., Aguilera-Rivera, D., Wasielesky, W., Hernández-López, I., Rodríguez-Fuentes, G., Durruty-Lagunes, C., Cuzon, G., & Gaxiola, G. (2021). The effect of biofloc and clear water at low and high salinity concentration on growth performance and antioxidant response of wild juveniles of Atlantic white shrimp penaeus setiferus. *Latin American Journal of Aquatic Research*, *49*(3). https://doi.org/10.3856/vol49-issue3-fulltext-2513

Vance, D. B., & Jacobs, J. A. (2019). Phytoremediation Enhancement of Natural Attenuation Processes. *Encyclopedia of Water*. https://doi.org/10.1002/9781119300762.wsts0225

Van Tung, T., Tran, Q. B., Phuong Thao, N. T., Vi, L. Q., Hieu, T. T., Le, S., Tuan, N. Q., Sonne, C., Lam, S. S., Hai, L. T., & Le, Q. Van. (2021). Recycling of aquaculture wastewater and sediment for sustainable corn and water spinach production. *Chemosphere*, *268*. https://doi.org/10.1016/j.chemosphere.2020.129329

Vargas-Albores, F., Martínez-Córdova, L. R., Gollas-Galván, T., Garibay-Valdez, E., Emerenciano, M. G. C., Lago-Leston, A., Mazorra-Manzano, M., & Martínez-Porchas, M. (2019). Inferring the functional properties of bacterial communities in shrimp-culture bioflocs produced with amaranth and wheat seeds as fouler promoters. *Aquaculture*, *500*. https://doi.org/10.1016/j.aquaculture.2018.10.005

Vargas-Estrada, L., Longoria, A., Arenas, E., Moreira, J., Okoye, P. U., Bustos-Terrones, Y., & Sebastian, P. J. (2022). A Review on Current Trends in Biogas Production from Microalgae Biomass and Microalgae Waste by Anaerobic Digestion and Co-digestion. In *Bioenergy Research* (Vol. 15, Issue 1). https://doi.org/10.1007/s12155-021-10276-2

Vázquez-Euan, R., Garibay-Valdez, E., Martínez-Porchas, M., Martínez-Córdova, L. R., Enriquez-Ocaña, L. F., Vílchez-Vargas, R., & Calderón, K. (2022). Effect of different probiotic diets on microbial gut characterization and gene expression of Litopenaeus vannamei cultivated in BFT system. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(12). https://doi.org/10.4194/TRJFAS21358

Verma, R., Sharma, S., Kundu, L. M., & Pandey, L. M. (2020). Experimental investigation of molasses as a sole nutrient for the production of an alternative metabolite biosurfactant. *Journal of Water Process Engineering*, *38*. https://doi.org/10.1016/j.jwpe.2020.101632

Verreth, J. A. J., Roy, K., & Turchini, G. M. (2023). Circular bio-economy in aquaculture. In *Reviews in Aquaculture* (Vol. 15, Issue 3). https://doi.org/10.1111/raq.12812

Vianna, B. da S., Miyai, C. A., Augusto, A., & Costa, T. M. (2020). Effects of temperature increase on the physiology and behavior of fiddler crabs. *Physiology and Behavior*, *215*. https://doi.org/10.1016/j.physbeh.2019.112765

Vithalani, P., Murnal, A., Akheja, P., Yagnik, U., & Bhatt, N. (2022). Review on Recent Technologies for Industrial Wastewater Treatment. *International Journal for Research in Applied Science and Engineering Technology*, *10*(8). https://doi.org/10.22214/ijraset.2022.46495

Voicea, I., Moga, C. I., Biriș, S. Ștefan, Constantin, G. A., Boruz, S., & Matache, M. G. (2021). Experimental research on obtaining pelletized feed recipes with applicability in semi-intensive aquaculture systems. *E3S Web of Conferences*, *286*. https://doi.org/10.1051/e3sconf/202128603005

Vo, T. T. E., Ko, H., Huh, J. H., & Kim, Y. (2021). Overview of smart aquaculture system: Focusing on applications of machine learning and computer vision. In *Electronics (Switzerland)* (Vol. 10, Issue 22). https://doi.org/10.3390/electronics10222882

Vyas, A. (2020). Biofloc systems in aquaculture: Global status and trends. In *New and Future Developments in Microbial Biotechnology and Bioengineering: Trends of Microbial Biotechnology for Sustainable Agriculture and Biomedicine Systems: Perspectives for Human Health*. https://doi.org/10.1016/B978-0-12-820528-0.00004-1

Wang, J., & Liu, J. (2023). Investigating the differences in metabolism, and the unfolded protein and apoptosis gene responses in two strains of the Pacific white shrimp, *Penaeus vannamei* Boone, 1931 (Decapoda, Penaeoidea) under ammonia stress. *Crustaceana*, *96*(2). https://doi.org/10.1163/15685403-bja10275

Wang, W., Jia, Y., Cai, K., & Yu, W. (2020). An Aquaponics System Design for Computational Intelligence Teaching. *IEEE Access*, *8*. https://doi.org/10.1109/ACCESS.2020.2976956

Wang, Y., Liu, M., Wang, B., Jiang, K., Wang, M., & Wang, L. (2019). A global view of hepatopancreas and intestinal reveals the potential influencing mechanism of aflatoxin B1 on nutrition and metabolism in *Litopenaeus vannamei*. *Aquaculture Nutrition*, *25*(6). https://doi.org/10.1111/anu.12956

Wasielesky, W., Atwood, H., Stokes, A., & Browdy, C. L. (2006). Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*, *258*(1–4). https://doi.org/10.1016/j.aquaculture.2006.04.030

Whangchai, N., Klahan, R., Balakrishnan, D., Unpaprom, Y., Ramaraj, R., & Pimpimol, T. (2022). Development of aeration devices and feeding frequencies for oxygen concentration improvement in 60-tones freshwater recirculating aquaculture and biofloc ponds of Asian seabass (*Lates calcarifer*) rearing. *Chemosphere*, *307*, 135761. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135761

Wijayanti, M., Amin, M., Tanbiyaskur, T., Jubaedah, D., Jaya, K., Ziyad, M. A., & Marsi, M. (2021). Aquaponic Biofloc Technology by Swamp Bacteria Probiotic for Clarias Catfish Rearing. *Journal of Aquaculture and Fish Health*, *10*(3). https://doi.org/10.20473/jafh.v10i3.23549

Wiloso, E. I., Romli, M., Nugraha, B. A., Wiloso, A. R., Setiawan, A. A. R., & Henriksson, P. J. G. (2022). Life cycle assessment of Indonesian canned crab (*Portunus pelagicus*). *Journal of Industrial Ecology*, *26*(6). https://doi.org/10.1111/jiec.13276

Wiyono, R. R., Dewi, N. N., Amin, M., & Fauzan, A. L. (2023). Feed conversion ratio and feed efficiency of giant gourami (*Osphronemus goramy*) seed with different densities on biofloc media. *IOP Conference Series: Earth and Environmental Science*, *1273*(1). https://doi.org/10.1088/1755-1315/1273/1/012082

Wolfe, J. M., Breinholt, J. W., Crandall, K. A., Lemmon, A. R., Lemmon, E. M., Timm, L. E., Siddall, M. E., & Bracken-Grissom, H. D. (2019). A phylogenomic framework, evolutionary timeline and genomic resources for comparative studies of decapod crustaceans. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1901). https://doi.org/10.1098/rspb.2019.0079

Wu, Z., Chu, K. H., & Ma, K. Y. (2023). Transcriptome Analysis of Multiple Tissues in the Shrimp Penaeus vannamei Reveals the Typical Physiological Response to Three Pathogens. *Journal of Marine Science and Engineering*, *11*(2). https://doi.org/10.3390/jmse11020389

Xie, D., Dai, Q., Xu, C., & Li, Y. (2021). Dietary tributyrin modifies intestinal function by altering morphology, gene expression and microbiota profile in common carp (*Cyprinus carpio*) fed all-plant diets. *Aquaculture Nutrition*, *27*(2). https://doi.org/10.1111/anu.13197

Xiong, J., Zhu, J., Dai, W., Dong, C., Qiu, Q., & Li, C. (2017). Integrating gut microbiota immaturity and disease-discriminatory taxa to diagnose the initiation and severity of shrimp disease. *Environmental Microbiology*, *19*(4). https://doi.org/10.1111/1462-2920.13701

Xue, L., Klinnawee, L., Zhou, Y., Saridis, G., Vijayakumar, V., Brands, M., Dörmann, P., Gigolashvili, T., Turck, F., & Bucher, M. (2018). AP2 transcription factor CBX1 with a specific function in symbiotic exchange of nutrients in mycorrhizal Lotus japonicus. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(39). https://doi.org/10.1073/pnas.1812275115

Xu, W. J., & Pan, L. Q. (2013). Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*, *412–413*. https://doi.org/10.1016/j.aquaculture.2013.07.017

Xu, W., Wen, G., Su, H., Xu, Y., Hu, X., & Cao, Y. (2022). Effect of Input C/N Ratio on Bacterial Community of Water Biofloc and Shrimp Gut in a Commercial Zero-Exchange System with Intensive Production of Penaeus vannamei. *Microorganisms*, *10*(5). https://doi.org/10.3390/microorganisms10051060

Yang, J., Li, X., Zhao, Y., Yang, H., & Li, Y. (2022). The exposure of OPFRs in fish from aquaculture area: Backward tracing of the ecological risk regulation. *Environmental Pollution*, *293*. https://doi.org/10.1016/j.envpol.2021.118550

Yang, P., Lai, D. Y. F., Jin, B., Bastviken, D., Tan, L., & Tong, C. (2017). Dynamics of dissolved nutrients in the aquaculture shrimp ponds of the Min River estuary, China: Concentrations, fluxes and environmental loads. *Science of the Total Environment*, *603–604*. https://doi.org/10.1016/j.scitotenv.2017.06.074

Yang, P. Y., Liao, Y. C., & Chou, F. I. (2023). Artificial Intelligence in Internet of Things System for Predicting Water Quality in Aquaculture Fishponds. *Computer Systems Science and Engineering*, *46*(3). https://doi.org/10.32604/csse.2023.036810

Yong, A. S. K., Mok, W. Y., Tamrin, M. L. M., Shapawi, R., & Kim, Y. S. (2020). Effects of dietary nucleotides on growth, survival and metabolic response in whiteleg shrimp, *Litopenaeus vannamei* against ammonia stress condition. *Aquaculture Research*, *51*(6). https://doi.org/10.1111/are.14570

Yuan, W., Du, Y., Yu, K., Xu, S., Liu, M., Wang, S., Yang, Y., Zhang, Y., & Sun, J. (2022). The Production of Pyruvate in Biological Technology: A Critical Review. *Microorganisms* (Vol. 10, Issue 12). https://doi.org/10.3390/microorganisms10122454

Yu, D., Zhai, Y., He, P., & Jia, R. (2022). Comprehensive Transcriptomic and Metabolomic Analysis of the *Litopenaeus vannamei* Hepatopancreas After WSSV Challenge. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.826794

Yu, C., Zhao, X., Qi, G., Bai, Z., Wang, Y., Wang, S. & Zhou, G. (2017). Integrated analysis of transcriptome and metabolites reveals an essential role of metabolic flux in starch accumulation under nitrogen starvation in duckweed. *Biotechnology for biofuels*, *10*, 1-14.

Yue, C. F., Wang, T. T., Wang, Y. F., & Peng, Y. (2009). Effect of combined photoperiod, water calcium concentration and pH on survival, growth, and moulting of juvenile crayfish (*Procambarus clarkii*) cultured under laboratory conditions. *Aquaculture Research*, *40*(11). https://doi.org/10.1111/j.1365-2109.2009.02216.x

Yue, K., & Shen, Y. (2022). An overview of disruptive technologies for aquaculture. In *Aquaculture and Fisheries* (Vol. 7, Issue 2). https://doi.org/10.1016/j.aaf.2021.04.009

Yu, G., Wang, L. G., Han, Y., & He, Q. Y. (2012). ClusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS A Journal of Integrative Biology*, *16*(5). https://doi.org/10.1089/omi.2011.0118

Yuliasni, R., Kurniawan, S. B., Marlena, B., Hidayat, M. R., Kadier, A., Ma, P. C., & Imron, M. F. (2023). Recent Progress of Phytoremediation-Based Technologies for Industrial Wastewater Treatment. *Journal of Ecological Engineering*, *24*(2). https://doi.org/10.12911/22998993/156621

Yun, H. S., Kim, D. H., Kim, J. G., Kim, Y. S., & Yoon, H. S. (2022). The microbial communities (bacteria, algae, zooplankton, and fungi) improved biofloc

technology including the nitrogen-related material cycle in Litopenaeus vannamei farms. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.883522

Yuvarajan, P. (2021). Study on floc characteristics and bacterial count from biofloc-based genetically improved farmed tilapia culture system. *Aquaculture Research*, *52*(4). https://doi.org/10.1111/are.15030

Yu, W., Liu, W., Sang, Y., & Wang, X. (2019). Analysis of Endogenous Semicarbazide during the Whole Growth Cycle of Litopenaeus vannamei and Its Possible Biosynthetic Pathway. *Journal of Agricultural and Food Chemistry*, *67*(29). https://doi.org/10.1021/acs.jafc.9b01779

Yu, Y.-B., Choi, J.-H., Lee, J.-H., Jo, A.-H., Choi, C.-Y., Lee, D.-C., Kang, J.-C., & Kim, J.-H. (2023). The use, application and efficacy of biofloc technology (BFT) in shrimp aquaculture industry: A review. *Environmental Technology & Innovation*. https://doi.org/10.1016/j.eti.2023.103345

Yu, Z., Dai, Z. Y., Li, L., Qin, G. X., & Wu, L. F. (2021). Dietary supplementation with biofloc promotes growth, improves immune and antioxidant status, and upregulates NF-κB/Nrf2 signalling molecules and stress resistance in *Rhynchocypris lagowskii* Dybowski. *Aquaculture Nutrition*, *27*(1), 225-239.

Zablon, W. O., Ogello, E. O., Getabu, A., & Omondi, R. (2022). Biofloc system improves protein utilization efficiency and growth performance of Nile tilapia, *Oreochromis niloticus* fry: Experimental evidence. *Aquaculture, Fish and Fisheries*, *2*(2). https://doi.org/10.1002/aff2.32

Zaki, M. A. A., Alabssawy, A. N., Nour, A. E. A. M., El Basuini, M. F., Dawood, M. A. O., Alkahtani, S., & Abdel-Daim, M. M. (2020). The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. *Aquaculture Reports*, *16*. https://doi.org/10.1016/j.aqrep.2020.100282

Zhang, B., Lingga, C., Bowman, C., & Hackmann, T. J. (2021). A New Pathway for Forming Acetate and Synthesizing ATP during Fermentation in Bacteria. *Applied and Environmental Microbiology*, *87*(14). https://doi.org/10.1128/AEM.02959-20

Zhang, J., Zhang, X., Hu, T., Xu, X., Zhao, D., Wang, X., Li, L., Yuan, X., Song, C., & Zhao, S. (2022). Polycyclic aromatic hydrocarbons (PAHs) and antibiotics in oil-contaminated aquaculture areas: Bioaccumulation, influencing factors, and human health risks. *Journal of Hazardous Materials*, *437*. https://doi.org/10.1016/j.jhazmat.2022.129365

Zhang, Y., Wu, B., Wang, J., Liu, M., & Zhang, X. (2019). Reactive powder concrete mix ratio and steel fiber content optimization under different curing conditions. *Materials*, *12*(21). https://doi.org/10.3390/ma12213615

Zhang, Z., Yang, Z., Zheng, G., Lin, Q., Zhuo, X., & Zhang, G. (2020). Effects of Addition of Sucrose and Probiotics on Whiteleg Shrimp *Litopenaeus vannamei* Postlarvae Performance, Water Quality, and Microbial Community. *North American Journal of Aquaculture*, *82*(1). https://doi.org/10.1002/naaq.10116

Zhao, D., Pan, L., Huang, F., Wang, C., & Xu, W. (2016). Effects of Different Carbon Sources on Bioactive Compound Production of Biofloc, Immune Response, Antioxidant Level, and Growth Performance of *Litopenaeus vannamei* in Zero-water Exchange Culture Tanks. *Journal of the World Aquaculture Society*, *47*(4), 566–576. https://doi.org/10.1111/JWAS.12292

Zhao, J., Chen, X., He, Z., Chen, G., Lin, Z., Liu, Y., Sun, C., & Wang, W. (2021). Transcriptome Analysis Provides New Insights into Host Response to Hepatopancreatic Necrosis Disease in the Black Tiger Shrimp *Penaeus monodon*. *Journal of Ocean University of China*, *20*(5). https://doi.org/10.1007/s11802-021-4744-x

Zhao, M., Yao, D., Li, S., Zhang, Y., & Aweya, J. J. (2020). Effects of ammonia on shrimp physiology and immunity: a review. *Reviews in Aquaculture* (Vol. 12, Issue 4). https://doi.org/10.1111/raq.12429

Zhao, P., Huang, J., Wang, X. H., Song, X. L., Yang, C. H., Zhang, X. G., & Wang, G. C. (2012). The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*. *Aquaculture*, *354–355*, 97–106. https://doi.org/10.1016/J.AQUACULTURE.2012.03.034

Zhao, Z., Xu, Q., Luo, L., Wang, C., Li, J., & Wang, L. (2014). Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system. *Aquaculture*, *434*, 442–448. https://doi.org/10.1016/J.AQUACULTURE.2014.09.006

Zuo, Z., Shang, B., Liu, H., Sun, J., Li, W., Liu, Y., & Sun, J. (2023). Identification and evaluation of potential probiotics against skin-ulceration disease in the Chinese tongue sole (*Cynoglossus semilaevis*). *Fish & Shellfish Immunology*, *137*, 108769.

PUBLICATIONS

	Title	Authorship	Status
1	Swinging between the beneficial and harmful microbial community in biofloc technology: A paradox	*First*	Published
2	Determination of optimum probiotic dosage for the culture of whiteleg shrimp, *Litopenaeus vannamei* in an indoor system	*First*	Published
3	Bibliographic insight of biofloc technology (BFT): Global trend, research hotspots and prospects	*Eighth*	Published
4	Evaluation of nutrients in biofloc meal harvested from an indoor multi-techno aquaculture system	*First*	Published
5	Profiling of the gut microbiome of Pacific Whiteleg shrimp, *Litopeneaus vannamei* raised in an indoor biofloc aquaculture system	*First*	Published
6	Evaluating the contributory effect of water quality on the growth performance of *Litopenaeus vannamei* in an indoor biofloc aquaculture system using a deep learning approach	*First*	Under review
7	Predicting optimum settings of control factors for an environmental-friendly smart aquaculture system using the Taguchi-based Regression Approach	*First*	Under review
8	System optimisation for an indoor aquaculture system incorporating Rapid Biofloc^{TM} for the culture of *Litopenaeus vannamei*	*First*	Under review

CONFERENCES

	CONFERENCE	VENUE	PAPER PRESENTED
1	Food Sustainability and Security (FUSE 2023): An International conference	Universiti Malaysia Terengganu, Malaysia	Predicting optimum settings of control factors for a smart aquaculture system using Taguchi-based Regression Approach
2	10th International Conference on Fisheries and Aquaculture 2023 (ICFA 2023)	Bali, Indonesia	Application of Rapid Biofloc Technology for sustainable marine shrimp aquaculture production

BIODATA OF AUTHOR

1. PERSONAL DATA

Name:	**AKANGE, Edward Terhemen**
Date of Birth:	May 27, 1980
Place of Birth:	Makurdi
Nationality:	Nigerian
State of Origin:	Benue
Local Govt. Area:	Gwer-West
Home Address:	31, Vandeikya Street, Idye-1, High level, Makurdi, Benue State
Marital Status:	Married
GSM:	07037157305, 08125310311
Email:	akangeter@gmail.com

2. EDUCATIONAL INSTITUTIONS ATTENDED WITH DATE

- ABenue State University, Makurdi 2008 – 2012
- University of Agriculture, Makurdi 1998 – 2004
- Padopads Harmony Secondary School, Makurdi 1991 – 1996
- St .Theresa's Primary School, Makurdi 1984 – 1990
- Nigerian Institute of Management (NIM Chartered) 2005
- Canata Training Institute, Makurdi 2007

3. QUALIFICATIONS OBTAINED WITH DATES

- Master of Science (M.Sc) 2012
  Hydrobiology and Fisheries
- Bachelor of Fisheries (B. Fisheries) 2004
  Fisheries and Aquaculture
- Senior School Certificate Examination (SSCE) 1996
- First School Leaving Certificate (FSLC) 1990
- Proficiency Certificate in Management 2005
- Diploma Certificate in Computer (Word/Data Processing) 2007