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Effects of palm oil mill effluent media on cell growth and lipid content of *Nannochloropsis oculata* and *Tetraselmis suecica*

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ABSTRACT

In this study, palm oil mill effluent (POME) was used as an alternative medium for algal biomass and lipid production. The influence of different concentrations of filtered and centrifuged POME in sea water (1, 5, 10 and 15%) on microalgal cell growth and lipid yield were investigated. Both *Nannochloropsis oculata* and *Tetraselmis suecica* had enhanced cell growth and lipid accumulation at 10% POME with maximum specific growth rate (0.21 d^{-1} and 0.20 d^{-1}) and lipid content (39.1 ± 0.73% and 27.0 ± 0.61%), respectively, after 16 days of flask cultivation. The total Saturated Fatty Acid (SFA) (59.24%, 68.74%); Monounsaturated Fatty Acid (MUFA) (15.14%, 12.26%); and Polyunsaturated Fatty Acid (PUFA) (9.07%, 8.88%) were obtained for *N. oculata* and *T. suecica*, respectively, at 10% POME. Algal cultivation with POME media also enhanced the removal of Chemical Oxygen Demand (COD) (93.6–95%), Biological Oxygen Demand (BOD) (96–97%), Total Organic Compound (TOC) (71–75%), Total Nitrogen (TN) (78.8–90.8%) and oil and grease (92–94.9%) from POME.

KEYWORDS

Lipid content; microalgae; *Nannochloropsis oculata*; palm oil mill effluent; *Tetraselmis suecica*; wastewater

1. Introduction

A wide variety of terrestrial biomass feed stocks have been identified as suitable candidates for conversion into biofuels. These include agricultural and forestry residues, food crops such as soybeans and corn, energy crops such as oil-seeds, transgenic species, biosolids, municipal solid wastes and manures (Perlack et al. 2005). Microalgae has been seen as a future source of transportation fuels primarily because of its potential to produce up to 10 times more oil per acre than traditional biofuel crops (Chisti 2007). Touted as biotechnology's "green gold" due to important and valuable co-products such as biopolymers, proteins, and animal feeds (Waltz 2009; Cooney, Young and Nagle 2009), high photosynthetic efficiency and faster growth rates than the higher plants, the lipid-rich microalgae is therefore one of the most efficient biological oil producers (Li et al. 2008; Mata, Martins, and Caetano 2010; Wijffels and Barbosa 2010).

Yeast, fungi, and algae have the ability for accumulation of lipids in the form of triacylglycerol. Lipids can be categorized in three parts: crude lipids, neutral lipids, and total lipids. Crude lipids include neutral lipids and pigments. Neutral lipids comprise of triglycerides, free fatty acids, hydrocarbons, sterols, wax and sterol esters, and free alcohols. Total lipids comprise of pigments, phospholipids, glycolipids, and the neutral lipids (Sharma, Singh, and Korstad 2011). In comparison to cyanobacteria, eukaryotic algae contain more unsaturated fatty acids. The major fatty acid in algae are oleic acid, palmitic acid, stearic acid and linoleic acid (Li, Zhao, and Bai 2007).

It is estimated that algae could yield oil in the range 8093.71 to 32 374.85 L per hectare which is 7–31 times more than that which could be obtained from a high yield terrestrial crop such as palm (Demirbas and Demirbas 2011). The high volume of oil accumulated by microalgae is the result of their efficiency to capture solar energy, which is 10–50 times more than the terrestrial plants. There are also higher losses of water during photosynthesis in the terrestrial plants. Like other photoautotrophs, algal requirements for photosynthesis and growth include sunlight, carbon dioxide, inorganic nutrients, and water (Singh et al. 2011a; Singh, Nigam, and Murphy 2011b). Microalgae may not only be proven reliable as a large-scale alternative feedstock for production of biodiesel, it also has great potential to sequester CO_2 as part of its overall growth process. Up to half of microalgal dry weight biomass is principally comprised of carbon obtained from CO_2 with around 100 tonnes of algal biomass fixes approximately 183 tonnes of CO_2 during photosynthesis (Demirbas and Demirbas 2011). Through photosynthesis, the unicellular structure of microalgae allows easy conversion of solar energy into chemical energy and lipid synthesis.

Although the main factor for lipid synthesis can be modulated by manipulating the cultivation conditions (Shah et al. 2014a,b), a microalgae-based fuel production technology still has a long way to go as not all microalgae have good prospects as feedstocks for biodiesel (Shang et al. 2010). The main challenges are in the selection of high-producing strains which can grow and produce high quantities of lipids, and reducing the high energy requirements for pumping, mixing, harvesting and extracting the biomass and lipids (Hu et al. 2008; Rodolfi et al. 2009; Nayak et al. 2011; Norsker et al. 2011). Algal cultivation using municipal sewage, agricultural,

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industrial or artificial wastewater could reduce the cost of production (Pittman, Dean, and Osundeko 2011). Furthermore, conventional treatment of municipal wastewater that involves primary and secondary biological treatment only manage to remove a fraction of nitrogen and phosphorus (Orpez et al. 2009). A life-cycle analysis (LCA) of biodiesel from microalgae utilizing seawater and wastewater over freshwater suggests reduction of freshwater use by 90% in open pond systems. The recycling of the harvested water shows that 100% recycling results in 55% decrease in nutrient requirement. Without recycling, 3,726 kg of water, 0.33 kg of nitrogen, and 0.71 kg of phosphate is required to generate 1 kg of biodiesel. Using recycled water, the water and nutrient requirements can be reduced by 84% and 55%, respectively (Yang et al. 2011). In the waste stabilization ponds, phosphorus treatment of 10 mg L^{-1} level with the microalgal solid concentration from 0 to 300 mg L^{-1} has been reported (Powell et al. 2008). Cultivation of *Botryococcus braunii* LEM 14 in photobioreactor has reportedly removed 79.63% of nitrogen and 100% of phosphorous after 14 days of culture at 25°C (Sydney et al. 2011).

Palm oil mill effluent (POME) is considered one of the most polluting agro-industrial effluent due to its high values of Chemical Oxygen Demand (COD) and Biological Oxygen Demand concentrations ranging from 50,000 to 90,000 mg L^{-1} (Damayanti et al. 2010). Without effective treatment, considerable environmental problems such as the percolation of POME into the waterways and ecosystems, land and water pollution and destruction of aquatic biota (Foo and Hameed 2010; Cheng, Zhu, and Borthwick 2010; Singh et al. 2011a; Singh, Nigam, and Murphy 2011b). Microalgae could reduce the eutrophication potential with a more environmentally sound approach (Orpez et al. 2009). As sludge disposal of wastewater treatment plants is one of the major challenges of sustainable wastewater engineering (Buys et al. 2008), utilizing and producing algal biomass in excess not only remediate the pollutants which eliminate nitrogen and phosphorus without organic carbon requirement, but also become a source of biomethane and high-value products (Aslan and Kapdan 2006; Ahmad et al. 2014). An algal treatment replacing conventional tertiary treatment can offer an oxygenated effluent and an ecologically safe, less expensive, and more efficient means to remove nutrients and metals (Ruiz et al. 2011).

Oleaginous *Nannochloropsis oculata* has received much attention because of its high lipid content and high lipid production rate, particularly the reserve lipids (triacylglycerols) which are the best substrate for biodiesel (Lee, Lewis, and Ashman 2009; Yu et al. 2009; Gao et al. 2010). *Tetraselmis suecica* is a marine green flagellate, belonging to the class chlorophyceae. It is widely used in aquaculture facilities as feed for bivalve molluscs, penaeid shrimp larvae and rotifers (Harwood and Guschina 2009). From environmental and economic point of view, culturing of *N. oculata* and *T. suecica* in POME and sea water offers an inexpensive alternative to utilize the nutrients in wastewater and abundant sea water to generate microalgal biomass for biofuels production or high-value compounds, whilst remediating the effluents.

The aim of this work was to explore the use of filtered and centrifuged POME at different composition in sea water for culturing the green marine microalgae *N. oculata* and *T. suecica*. The biomass production and lipid content were evaluated together with the removal of the nutrients, BOD, COD and oil and grease from POME.

2. Material and methods

2.1. Culture of microalgae

Two species of marine microalgae (*N. oculata* and *T. suecica*) used in the present study, were collected from Fisheries Research Institute (FRI), Pulau Sayak, Sungai Petani, Kedah, Malaysia. All chemicals and solvents were obtained from Merck (Darmstadt, Germany). The instruments used were Stirring Hot Plate (Fisher Scientific, 1110250SH), Lux Meter (LX1330B), Ultrasonic Homogenizer (150/VT), Centrifuge, Rotary Evaporator, Microscope (10 × 40 MAG), Gas Chromatography (7890A GC System), COD Thermoreactor (DRB 200), Spectrophotometer (HACH DR 500), BOD track, Autoclave (75 X), Deionized Water System (Thermo Scientific, TK Japan).

The stock culture (with density of 50.6×10^6 cells mL^{-1}) was inoculated into each 250 mL Erlenmeyer culture flask to get 10% (v/v) inoculum density. Conway media was used for Control culture and maintenance. Filtered sea water was obtained from FRI. The standard conditions for control culture were 30 ppt NaCl and initial pH 8, under 24 h illumination from fluorescence white light (Phillips) of 90 μmol photons $m^{-2}s^{-1}$ intensity. For experiments, all the flasks were kept under the cycle of 12 h photoperiod and 12 h dark for 16 days. The culture flasks were grown on an orbital shaker at 80 rpm, at 28 ± 2 °C. All the glass-wares used in the experiment were sterilized by autoclaving at 121°C for 20 mins, and all media constituents were added aseptically a laminar flow cabinet. Three replications were used both for the culture and control media.

Algal growth and lipid content were recorded every alternate day. After harvesting, the algal samples were centrifuged (Avanti J-251 Centrifuge) at 3000 rpm for 10 mins. The pellet were analysed for cell growth, and the supernatant for chemical properties.

2.2. POME collection

Raw POME was collected from FELCRA Nasaruddin Palm Oil Mill, Bota Kanan, Perak, Malaysia. The characteristics were first determined before storage in plastic container at 4°C in order to avoid contamination and biodegradation.

2.3. Preparation of POME medium

POME was filtered to remove sand and dust particles and then centrifuged (Avanti J-251 Centrifuge). The supernatant of the effluent was used as algal culture medium and the pellet kept for future analyses. The supernatant was made up to 1, 5, 10, and 15% composition in sea water, and autoclaved at 121°C for 30 mins. pH level of POME medium was adjusted to pH 7–8 and filtered again before use.

2.4. Chemical analyses of POME

COD measurement was carried out by using spectrophotometer DR2800 and 5000-Reactor Digested Method according to the standard method provided by HACH. The DR5000-Reactor was preheated to 150°C. One milliliter of sample was diluted at ratio 1:50, 1:100, 1:250 of POME and distilled water, respectively. Two milliliter diluted POME of each standard was added to the corresponding high range COD Digestion Reagent vials. For "blank," 2 mL of distilled water was added. Each vial was mixed well and placed in the reactor block. After two hours, the vials were removed and kept in a cooling rack for 20 min before reading. The stored HACH program 435 COD HR was recalled for COD test. The reading of COD in mg L^{-1} was displayed on the screen (HACH, USA 1997).

Measurement of BOD with BOD track was carried out according to Standard Method provided by HACH. One milliliter of sample was diluted at ratio 1:100 and 1:250 of POME and distilled water, respectively. The sample (95 mL) was poured into the specialized 300 mL BOD trak designed to allow full filling with no air space and sample bottle provided with an airtight seal. Four samples were prepared and 3.8 cm magnetic stir bar was placed in each sample bottle. BOD Nutrient Buffer Pillow was added to each sample and lithium hydroxide powder was added to the seal cup of each sample bottle. The instrument was placed in the incubator at 20°C. The stored Hach program for 5.25 days and 0–700 mg L^{-1} was selected for the BOD test. The reading was taken after 5 days with the reading BOD in mg L^{-1} displayed on the screen for each sample bottle (HACH, USA 1997).

Measurement of TOC and TN was carried out by using TOC Analyzer (TOC-VCSH SHIMADZU) according to the APHA Standard Method. The sample was diluted at ratio 1:50, 1:100 and 1:250. Oil and grease was measured by oil and grease analyzer (InfraCal TOG Model HATR-T2). Samples of POME were analyzed by adding hexane into bottles containing POME and vigorously shaken for 2 min for complete mixing. After the two layers separated, 50 µL was extracted from the upper layer using a syringe and deposited in the center of the sample crystal. Oil concentration displayed was recorded.

Removal efficiencies of BOD, COD, TOC, TN and oil and grease were calculated using the following equation:

$$\text{Removal efficiency } (\%) = \frac{Ai - Af}{Ai} \times 100$$

where *Ai* and *Af* are the initial and final parameter concentrations, respectively.

2.5. Measurement of cell growth and dry weight (DW)

Cells were harvested in triplicates after 16 days by removing 100 mL samples and later centrifuged at 3,000 rpm (Avanti J-251 Centrifuge). The biomass were washed with deionized water, dried at 80 °C in an oven for 8 h, cooled in a desiccator and weighed. The cell number and density was also measured by haemocytometer (Hirschmann/Germany) by removing 10 µL of sample.

2.6. Lipid extraction

Lipid content was determined based on modified method adapted from Bligh and Dyer (1959). This method extracted lipids from the algal cells by using a mixture of methanol, chloroform, and water. Algal sample was centrifuged at 3,500 rpm for 10 mins and the pellet was mixed with water, methanol, and chloroform. After overnight stay, the mixture was re-centrifuged and the lower layer that contained lipid and chloroform was extracted and put into pre-weighed vials. All vials were placed in a water bath at 65 °C for 8 h or kept in an oven at 80 °C for 4 h to evaporate the chloroform and lipids, before weighing.

2.7. Fatty acids analysis

The extracted lipid was first transesterified into fatty acid methyl esters (Mbatia et al. 2010), where 20 mg of lipid sample was mixed with 2 mL of toluene, followed by addition of 2 mL of 1.5% sulphuric acid in dry methanol. After mixing well, the mixture was incubated at 55°C overnight. Four milliliter of saturated NaCl solution was added, vortexed and 2 mL of hexane (HPLC grade) added, followed by 3 mL of sodium hydrogen carbonate (2% $NaHCO_3$). The mixture was vortexed and 180 µL of the upper phase was taken for gas chromatography analysis. FAME were separated and quantified using gas chromatography (7890A GC System), and separation achieved by Supelcowax TM 10 fused silica capillary column (60 m × 0.32 mm × 0.25 µm film thickness; USA). The carrier gas was helium at 550 kPa. The temperature programme was as follows: initial column oven temperature of 100°C held for 3 min, and increased to 170°C at 20°C/min for 0 min and 10°C/min for 25 min. The detector temperature was kept constant at 280°C and run time was 40.5 min.

2.8. Statistical analysis

The data were analyzed through one-way Analysis of Variance (ANOVA) by using Statgraphic Version 5 (Rockville, USA) to determine the significant difference among the treatment means.

3. Results and discussion

3.1. Cell growth and lipid content

Figure 1 (a and b) show the effects of different composition of POME on cell growth and dry weight of *N. oculata and T. suecica*. The highest cell density of 66.2 × 10^6 cells mL^{-1} and dry weight of 0.84 g L^{-1} was obtained under 10% POME for *N. oculata*. As shown in Table 1, this corresponds to the maximum biomass formation rate of 0.151 g L^{-1} d^{-1}, doubling time of 3.3 day, μ_{max} of 0.21 d^{-1} and lipid content of 39.1 ± 0.73%. Although the cell growth was comparable to control at 68.9 × 10^6 cells mL^{-1} and 0.88 g L^{-1} dry weight, the lipid content was far superior with POME medium. Similar trend was observed in *T. suecica* though with lower cell density of 38.7 × 10^6 cells mL^{-1} and dry weight of 0.74 g L^{-1} under under 10% POME. There is a statistically significant difference between the mean dry weight and lipid content from one

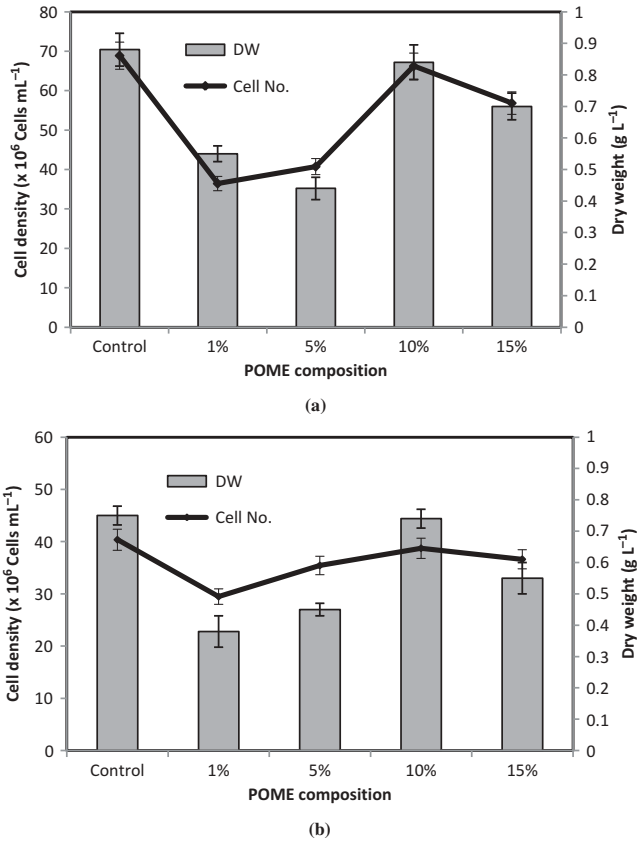

Figure 1. Effects of different POME compositions on cell density and dry weight of (a) *N. oculata*; (b) *T. suecica*.

level of POME dilution to another at 95% confidence level for both species ($P < 0.05$).

As shown in Table 2, raw POME had high concentrations of chemical and inorganic components which are suitable nutrients for microalgae. Due to higher and balanced nutrient concentrations despite the nature of POME and its dark color, the suitable composition in the formulation for optimum growth and lipid content of *N. oculata* and *T. suecica* was 10%. The biomass productivities (0.144–0.151 g L^{-1} d^{-1}) and specific growth rates (0.20–0.21 d^{-1}) of *T. suecica* and *N. oculata* were slightly lower than the reported values of 0.27 g $L^{-1}d^{-1}$ and μ_{max} of 0.49 d^{-1} with *Auxenochlorella protothecoides* UMN 280. However, the lipid accumulations at 27–39% were relatively higher than the reported 28.9% total lipid content for *A. protothecoides* UMN 280 cultured in

Table 2. Chemical characteristics of POME at different compositions in sea water before and after inoculation of *N. oculata* and *T. suecica*.

POME level	pH[a]	COD	BOD	TOC	TN	Oil and grease
1. Raw						
1%	6.5–7	627	183	33.4	5.43	30.3
5%	6.2	2974	843	153.1	28.6	144.5
10%	5.5	5839	1642	285.5	56.7	285.1
15%	4.7	8947	2448	456.8	80.3	364.5
2. *N. oculata*						
1%	7	63	21	12	0.5	5.3
5%	7.5	145	32	53	4	9.8
10%	7.8	375	47	87	7	15.6
15%	8.3	558	94	115	13	18.7
3. *T. suecica*						
1%	7	84	34	19	2	9.3
5%	6.5	196	47	67	7	16.7
10%	7.3	463	62	104	12	22.8
15%	7.2	638	114	132	18	31.7

[a]All values are in mg L^{-1} except for pH.

concentrated municipal wastewater (Zhou et al. 2012). Our study with *I. galbana* and *P. lutheri* strains however achieved the highest cell density of 15.4×10^6 cells mL^{-1} and 14.2×10^6 cells mL^{-1}, with corresponding lipid content of 26.3 ± 0.31% and 34.5 ± 0.82%, respectively, at 15% POME level (Shah et al., 2014b). A study on mixture of green algae and diatoms has the biomass concentrations increased from 0.5 g L^{-1} to 0.9 g L^{-1} and lipid content from 14% to 29% when the level of waste water is increased from 10% to 25% (Woertz et al. 2009). A study on marine *Isochrysis* sp. utilizing 5% POME-fortified medium achieves maximum biomass of 91.7 mg m^{-2} day^{-1} and lipid content of 52.8 ± 2.4% under 10 L outdoor culture system (Vairappan and Yen 2008). Another report suggests optimal level at 14% POME, followed by 10%, 20% and 30% (Anton, Kusnan, and Hussin 1994). High biomass accumulation has been reported for *Chlorella* sp. grown on concentrated municipal wastewater (Li et al. 2011) and another study on *Scenedesmus* sp. shows 98% removal of inorganic nutrients from municipal wastewater with the highest biomass density of 0.11 gL^{-1} and lipid content increased from 14% to 31% (Xin, Hong-ying, and Jia 2010).

3.2. Chemical analyses of POME

3.2.1. COD, BOD and TOC

Table 2 shows the decreasing trend of pH (7–4.7) with increasing POME composition from 1–15% before inoculation of microalgal species. On day 16, pH becoming more

Table 1. Kinetics of cell growth and lipid production of *N. oculata* and *T. suecica* cultivated under control and different POME compositions in sea water.

Media conditions		Maximum biomass formation rate, X'_{max} ($gL^{-1}d^{-1}$)	Maximum specific growth rate, μ_{max} (d^{-1})	Doubling time, t_d (day)	Lipid content (%)
N. oculata	Control	0.15	0.22	3.15	34.62 ± 0.62
	1%	0.13	0.17	4.07	30.91 ± 1.53
	5%	0.14	0.18	3.85	34.13 ± 1.20
	10%	0.15	0.21	3.30	39.14 ± 0.73
	15%	0.13	0.20	3.46	27.75 ± 1.12
T. suecica	Control	0.15	0.20	3.55	24.43 ± 0.28
	1%	0.12	0.17	4.07	20.71 ± 0.38
	5%	0.12	0.18	3.85	22.92 ± 0.42
	10%	0.14	0.20	3.62	27.04 ± 0.61
	15%	0.12	0.18	3.76	24.32 ± 0.46

basic around 7–8.3 with *N. oculata* and 6.5–7.3 with *T. Suecica*. These were most probably due to the absorption of nitrogen and lack of CO_2 sparging. Similarly, the highest growth rate of *Scenedesmus obliquus* is reportedly achieved at a constant pH of 7 (Hodaifa, Martinez, and Sanchez 2009). In microalgal cultivation, pH value usually increases because of the photosynthetic CO_2 assimilation and affects the availability of inorganic carbon. Some species such as *A. protothecoides* UMN280 tolerates high pH in concentrated municipal wastewater suggesting that pH variation may not be the major limiting factor for microalgae in wastewater (Zhou et al. 2011), as microalgae could well-adapt to varying growth conditions.

The highest average BOD of 2448 mg L^{-1} and lowest average BOD of 183.4 mg L^{-1} was recorded at 15% and 1% POME, respectively, before inoculation of *N. oculata* and *T. suecica* (Table 2). The BOD removal of 88.5–97% were achieved for 1–15% POME after the addition of *N. oculata*, while the BOD removal 81.5–96% were achieved after *T. suecica* addition. The COD and TOC removal efficiencies also varied with different POME levels. Higher removal of COD (90–95%) and TOC (64–75%) were achieved for 1–15% POME after addition of *N. oculata*, while lower removal of COD (86.6–93.6%) and TOC (43.1–71.1%) were achieved after addition of *T. suecica*. The COD removal was enhanced when the POME level was increased to 5% and 10%. In our previous study, the BOD removal was lower at 68.3–82.5% achieved with 1–20% POME after microalgal addition. The removal of COD (74.5–77.4%) and TOC (43.1–57.9%) were also lower, achieved with addition of *I. galbana*, while removal of COD (77.6–80.1%) and TOC (45.2–62.3%) were achieved with *P. lutheri* (Shah et al., 2014b). This was in agreement with the 76% COD removal from piggery wastewater associated with microbe in high rate algal ponds (Godos et al. 2009) and the removal efficiency of 88% COD and 96% TOC with *A. protothecoides* UMN280 when the algae is grown in concentrated municipal wastewater (Zhou et al. 2012). Lower COD removal of 41.8% has been reported in the axenic culture condition of *Desmodesmus* sp.CHX1 (Cheng, Tian, and Liu 2013). Different algal strain could utilize the different organic compounds as carbon sources at different efficiency depending on the nature or severity of the waste water conditions.

The algal strain could utilize the amount of dissolved oxygen to break down organic material. *Synechocystis* sp achieves the removal efficiency of 98% BOD from treated wastewater under hydraulic residence time of 24 h (Sekaran et al. 2013). The algae-based sewage treatment plant (STP) has reportedly achieved total BOD removal of 82% (Mahapatra, Chanakya, and Ramachandra 2013). A three-stage aquaculture of certain macrophytes and algae such as *Eichhornia crassipes, Microcystis aeruginosa, Scenedesmus falcatus, Chlorella vulgaris* and *Chlamydomonas mirabilis* involving a water hyacinth culture in the first stage, followed by an algal culture, and finally a second water hyacinth also achieve BOD reductions around 96.9% when tested in the laboratory conditions (Tripathi and Shukla 1991). Undigested dairy manure, diluted to 20 times, in a semi-continuous system increases *Chlorella vulgaris* biomass more than twice in 4 days and higher nutrient and COD removal efficiency, than when the algae grown in the digested dairy manure (Wang et al. 2010). The higher biological load, containing high amounts of organics, could enhance algal growth. However, if the loading rate had gone beyond a threshold level, the nutrient buildup could be lethal to the algae.

3.2.2. TN

The highest average TN of 80.3 mg L^{-1} and lowest average TN of 5.43 mg L^{-1} was recorded in 15% and 1% POME, respectively, before inoculation of *N. oculata and T. suecica*. After the addition of *N. oculata*, the highest removal of TN (90.8%) was achieved in 1% POME, while the highest removal of TN (78.8%) in 10% POME was achieved after the addition of *T. suecica* (Table 3). TN is the sum of organic nitrogen, ammonia (NH_3), and ammonium (NH_4^+) in the chemical analysis of wastewater. Nitrogen is an essential ingredient for cell growth. The relative constancy of uptake, irrespective of nitrogen source, is due to the saturation of the assimilator to the production of amino groupings for entry into nitrogenous metabolism. Nitrite is generated in the process of nitrate being reduced to ammonium and it is possible that part of the nitrite produced is excreted into the media (Burhenne and Tischner 2000). The assimilation of either NO_3^- or NH_4^+ affects pH of the growth media. When ammonia is utilized, pH could decrease because of the release of H^+ ions while nitrate uptake increases pH because of OH^- release (Chevalier et al. 2000).

Removal efficiencies of 36% Total Kjeldahl nitrogen (TKN), 18% ammonium N (NH_4 -N), 22% nitrate (NO_3 -N), and 57.8% nitrite (NO_2 -N) have been reported for algae-based sewage treatment plant (STP) where the predominant algae are euglenoides and chlorophycean members (Mahapatra, Chanakya, and Ramachandra 2013). Another study with *Chlorella* sp. achieves the removal efficiency of 100% NH_4^+, 75.7–82.5% of TN, 62.5–74.7% of total phosphorus, and 27.4–38.4% of COD when the algae is grown in different dilutions (10, 15, 20, and 25 times) of digested dairy manure (Wang et al. 2010). The TN removal of 50.8–82.8% is reported when the green *Chlorella* sp. Is grown in different wastewaters from municipal wastewater treatment plant (Wang et al. 2010). In our study, both marine species grew effectively in polluted water with 78.8–90.8% TN removal from POME. Despite no drastic improvement in the amount of biomass obtained as compared to that from a typical microalgae in the conventional conditions, the benefit of treatment of pollution water may make it a better alternative for economical large scale application.

Table 3. Removal efficiency of *N. oculata* and *T. suecica* at different compositions of POME.

	Removal efficiency (%)							
	N. oculata				*T. suecica*			
	1%	5%	10%	15%	1%	5%	10%	15%
COD	90	95	94	94	87	93	94	93
BOD	89	96	97	96	82	94	96	95
TOC	64	65	70	75	43	56	63	71
TN	91	86	88	84	62	76	79	78
Oil and grease	83	93	95	95	69	88	92	91

3.2.3. Oil and grease and other nutrients

POME contains 95–96% water, 0.6–0.7% oil and grease and 4–5% total solids. Typically, the oil and grease mean value is 6000 mg L^{-1} (Industrial Processes & The Environment 1999). The highest average oil and grease of 364.5 mg L^{-1} and the lowest average oil and grease of 30.3 mg L^{-1} was recorded at 15% and 1% POME, respectively, before inoculation of *N. oculata and T. suecica* (Table 2). After the addition of *N. oculata*, 94.9% oil and grease removal was achieved at 15% POME while the highest removal of 92% was obtained at 10% POME after the addition of *T. suecica* (Table 3). Oil and grease are poorly soluble in water due to their tendency to separate out from the aqueous phase. Although this characteristic is advantageous in facilitating the separation of oil and grease by the use of floatation devices, it does complicate the transportation of wastes through pipelines, their destruction in the biological treatment unit, and their disposal into receiving waters. The high removal of oil and grease content by microalgae in our study suggest good candidates for handling and treatment of the waste material for disposal. Free Gram-negative bacteria (*Pseudomonas* sp., *P. diminuta* and *P. pseudoalcaligenes*) for oil and grease removal from contaminated industrial effluents has been reported and all these bacteria are able to degrade the palm oil completely and utilized the free fatty acids (FFA) as a carbon source (El-Bestawy, El-Masry, and Nawal 2005).

Besides carbon, nitrogen and phosphorus, other macronutrients (e.g potassium, calcium, magnesium), micronutrients (manganese, molybdenum, copper iron, zinc, boron, chloride and nickel) and some trace elements are important for microalgal growth. Many of trace elements are important in enzyme reaction and for biosynthesis of many compounds (Cavet, Borrelly, and Robinson 2003). In the present study, both sea water and POME contain many natural macro and micro nutrients to fulfil microalgal growth requirements. Changes in all chemical parameters of the waste media after the culture of both microalgal species have paved the way for more environmentally-friendly approach to treat wastes whilst benefiting from harvesting the value-added products such as bioenergy and biocompounds.

3.2.4. Fatty acids composition

The fatty acids composition of lipid recovered from *N. oculata* and *T. suecica* cultivated in 10% POME composition with sea water is shown in Table 4. The major fatty acids were pentadecanoic acid (C15:0), palmitic acid (C16:0), stearic acid (C18:0) belonging to saturated fatty acids (SFA); and palmitolic acid (C16:1) and oleic acid (C18:1) belonging to monounsaturated fatty acids (MUFA) (Table 4). The total SFA (59.24%, 68.74%); MUFA (15.14%, 12.26%); and PUFA (9.07%, 8.88%) were obtained for *N. oculata* and *T. suecica*, respectively. *N. oculata* contained high palmitic acid (C16:0) at 28.22% and palmitolic (C16:1) at 9.37% while *T. suecica* contained high palmitic acid (C16:0) at 36.48% and pentadecanoic acid (C15:0) at 9.21%. This lipid profile is comparable to the main fatty acids present in the lipids of *Chlorella* sp. which are short-chain fatty acids (C14–C18) (Huang et al. 2010). A high level of SFA and low level of PUFA are common in bacteria and blue-green microalgae (Cyanophyceae), especially unicellular blue green microalgae (Pratoomyot, Srivilas, and Noiraksar 2005). *P. lutheri* undergoing different environmental stressors has reported the total SFA of 42%, MUFA of 38.51% and PUFA of 19.49% (Shah et al. 2014a). Fatty acid analysis of *P. lutheri* cultivated in semicontinuous mode suggests neutral lipids and glycolipids as the major constituents, accounting for 57 and 24%, respectively, of the total fatty acids residues (TFA) (Guedes et al. 2010).

Table 4. Fatty acids composition of lipid from *N. oculata* and *T. suecica* cultivated in 10% POME composition with sea water.

		Microalgae	
Fatty Acids (%)		*N. oculata*	*T. suecica*
Saturated Fatty Acid			
C12:0	Lauric acid	0.64	0.52
C14:0	Tetradecanoic acid	5.43	6.94
C15:0	Pentadecanoic acid	8.45	9.21
C16:0	Palmitic acid	28.22	36.48
C17:0	Heptadecanoic acid	2.31	3.62
C18:0	Stearic acid	7.44	8.33
C20:0	Eicosanoic acid	6.75	3.64
Total SFA		59.24	68.74
Monounsaturated Fatty Acid			
C16:1	Palmitoleic acid	9.37	5.81
C18:1	Oleic acid	5.77	6.45
Total MUFA		15.14	12.26
Polyunsaturated Fatty Acid			
C18:2	Linoleic acid	2.81	3.77
C18:3	Linolenic	4.56	5.11
C20:5	Eicosapentaenoic acid (EPA)	0.17	ND[a]
C22:6	Docosahexaenoic acid (DHA)	1.53	ND[a]
Total PUFA		9.07	8.88

[a]Not detected.

In PUFA profile, the highest percentage of linolenic acid (C18:3) was found in *N. oculata* (4.54%) and *T. suecica* (5.11%). This result agrees with the findings which indicate that some marine microalgae are producers of omega-3 long chain PUFA and are potential alternative live feed for marine organisms culture (Ryckebosch et al. 2012). The cultivation of *N. oculata* and *T. suecica* in 10% POME composition with sea water therefore is suitable for cell growth as well as PUFA production. This is similar to the report on marine *Isochrysis* sp. which not only has the cell density increased but also cell content of PUFA produced whilst treating POME in the photobioreactor and outdoor culture system (Vairappan and Yen 2008).

Both *N. oculata* and *T. suecica* investigated showed similar fatty acids profile, though different amount of fatty acids level. The percentage of fatty acids content of microalgae can be tuned based on the growth phases from which the cultures are harvested. During stationary phase, the limited nutrition reduces cell division and shift the focus of the cells towards storing the products in the form of fatty acids (Pratoomyot, Srivilas, and Noiraksar 2005). Additionally, limitation of nutrients such as nitrate, phosphate and silicate in the culture medium can increase Acetyl CoA carboxylase enzyme, which is a precursor for making lipid in microalgae (Schenk et al. 2008). With high saturated and monounsaturated fatty acids,

N. oculata and *T. suecica* are potential candidates for production of biodiesel.

4. Conclusions

The influences of different composition of POME in sea water on microalgal cell growth and lipid yield were investigated. High lipid content for *N. oculata* and *T. suecica* were observed at 10% POME. High removal efficiencies of COD, TOC, BOD, TN and oil and grease were achieved at different levels of POME. The fatty acid composition of both marine microalgae showed higher saturated fatty acids (SFA) as compared to monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) when cultivated in 10% POME composition with sea water. Cultivation of microalgae in POME may be a practical and economical alternative to efficiently enhance the nutrients removal from POME, coupled with biomass and fatty acids production.

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