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A study of coagulating protein of *Moringa oleifera* in microalgae bio-flocculation

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ABSTRACT

Moringa oleifera is characterized by high coagulation properties, low cost and low toxicity hence is very promising to be utilized as an alternative coagulant to recover microalgae biomass from its suspension system. Hence, this study was performed with the objective to investigate the potentiality of *M. oleifera* as coagulant agent in harvesting microalgae and to investigate the effect of zeta potential in its coagulation-flocculation activity. Bradford protein assay was applied for rapid and accurate determination of protein concentration in the *M. oleifera* seed powder and protein powder. The flocculation activities were determined at isoelectric pH by computing the flocculation efficiency in terms of microalgae biomass recovery and removal percentage at various coagulant dosages. It was observed that the protein concentration was 211.71 μg g^{-1} mL^{-1} in *M. oleifera* seed powder and 188.16 μg g^{-1} mL^{-1} in protein powder which yielded 97% and 78% of biomass recovery, respectively at the dosage of 10 mg L^{-1}. Result showed that *M. oleifera* seed derivatives supersede chemical coagulant, alum which yielded 34% of biomass recovery at the same dosage. *M. oleifera* seed powder and protein powder was proven to be highly promising bio-coagulant and suitable alternative to the chemical coagulant in environmentally-sustainable harvesting of microalgae biomass.

1. Introduction

Currently, the most commonly used separation methods are filtration and centrifugation (Teixeira et al., 2012). However, filtration is only effective for microalgal cells, which are relatively large, such as *Arthrospira* sp. but is unable to separate the biomass from the cultivation medium for cells of smaller dimensions (Papazi et al., 2010). The most successful techniques for microalgae biomass harvesting were centrifugation, filtration and flocculation. Commercial systems mainly use centrifugation for harvesting, but it is an expensive and energy intensive operation (Granados et al., 2012). Hence, it was suitable only for the harvesting of biomass with high-value products. For low-value products, a pre-concentration step was necessary. In practice, a combination of techniques was often performed to pre-concentrate the algae biomass.

There are two steps for the recovering of algal biomass in a commercial-scale processing before undergoing downstream processing (Lam and Lee, 2012; Molina Grima et al., 2003; Sharma et al., 2013). First, traditional harvesting method was the bulk harvesting which known as primary harvesting. The purpose of this primary harvesting is to separate microalgae from suspension via sedimentation, flocculation and floatation. The second step is the thickening processing which known as secondary dewatering to concentrate the microalgae slurry after bulk harvesting. Normally, the thickening process was performed by using centrifugation and filtration. An optimal harvesting technique should be independent of the cultured species, less energy consumption and few chemicals. It was also important that the harvesting technique was not cause any damage to the valuable products during extraction process. Brennan and Owende (2010) had summarized the advantages and disadvantages of conventional harvesting techniques from the

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previous studies (Table 1).

Microalgae harvesting technique were including centrifugation, filtration, sedimentation, chemical flocculation and floatation. In centrifugation, solid-liquid separation process was driving by a much greater force (gravity) to promote accelerated settling of microalgae cells. This technique can be used for almost all types of microalgae reliably and without difficulty (Pires et al., 2012). However, centrifugal recovery is only feasible if the metabolite content in the targeted biomass is a high-value product. This is due to the high energy consumption is required during the separation process. The large-scale biomass recovery become a problematic due to the high power consumption which increases the production costs. In addition, the coagulation-flocculation required to be followed by flotation-sedimentation and finally dewatering step by centrifugation were performed for low-cost biomass harvesting (Schenk et al., 2008).

Coagulation-flocculation was used to aggregate the microalgae cells and increase the effective "particle" size, thus enhancing biomass recovery. Coagulants such as aluminum sulfate (alum), ferric sulfate, ferric chloride, ferrous sulfate, sodium aluminate, iron salts and chitosan has been used for the recovery of microalgae via coagulation-flocculation and was demonstrated successfully to achieve the goal (Molina Grima et al., 2003). However, coagulation-flocculation by metal salts may be unacceptable if harvested biomass is to be used for aquaculture purposes, animal feed or organic fertilizer. High aluminum concentration does not caused effects only upon fish, but also birds and other higher animals in the food chain that consumed the contaminated fish and insects. According to De-Bashan and Bashan (2004), another negative environmental effect of aluminum is that its ions can react with phosphates, which causes total phosphate to be less available to water organisms. It was reported that the major component of alum and acrylamide could lead to human health implications, such as involvement in Alzheimer's disease and the cause of cancers (Ahmad et al., 2011; Hamid et al., 2014). Therefore, an alternative of environmentally friendly harvesting approach need to be developed completely not only to ease microalgae biomass recovery but also to preserve our natural environment.

Besides that, the treated medium was also not suitable to be reused because of the deterioration of water quality due to the addition of coagulants. Hence, the exploration of potential natural coagulant is crucial for sustainable wastewater treatment utilizing microalgae. The utilization of suspended microalgae culture in biological treatment would then lead to the requirement for the effective separation process. Releasing treated water to the water body without proper recovery of microalgae biomass could contribute to the environmental problem such as eutrophication and algal blooms. Recovery of the microalgae biomass from broth has been claimed to contribute 20–30% to the total cost of producing the biomass (Gudin and Thepenier, 1986; Molina Grima et al., 2003). Hence, the microalgae biomass harvesting issue was exclusively focused in order to promote effective aquaculture wastewater treatment for sustainable downstream processing of microalgae.

Harvesting of microalgae biomass requires the minimum of two solid-liquid separation steps. According to Molina Grima et al. (2003), microalgae biomass can be harvested through the process of centrifugation, filtration or gravity sedimentation. These processes may be preceded by a coagulation-flocculation step. But, centrifugal recovery of the biomass was only feasible for high-value products such as *Spirulina* sp. to reimburse the high maintenance cost. On the other hand, filtration recovery was unsatisfactory because it was relatively slow and unsuitable for the biomass recovery in large-scale volumes. Filtration recovery was unsatisfactory due to the exposure of the harvested biomass towards membrane fouling and its suitability to only large-cell-sized microalgae. Hence, it required regular membrane changing and maintenance which may contributed to the harvesting cost.

Among the aforementioned methods, coagulation-flocculation was considered to be an effective and convenient process, which allows rapid treatment of large quantities of microalgae (Oh et al., 2001). Various methods of coagulation-flocculation can be used to aggregate the microalgal cells to increase the effective particle size and hence ease the sedimentation process for biomass recovery. Microalgal cells carry a negative charge that prevents aggregation of cells in suspension (Molina Grima et al., 2003). Therefore, the surface charge can be neutralized or reduced by the addition of coagulants such as multivalent cations and cationic polymers to the culture. The use of biological based coagulant would assist sustainable aquaculture practices. The objectives of this study are (i) to investigate the potentiality of *Moringa oleifera* seed as coagulant agent for freshwater *Chlorella* sp. biomass harvesting and (ii) to study the effect of zeta potential and isoelectric of microalgae suspension toward coagulation-flocculation. As comparison to the efficiency of coagulation-flocculation process, alum was selected as control for the *M. oleifera* derivatives because it had been widely utilized as standard flocculation reagent in the water and wastewater treatment protocols. This study had successfully elucidated the potentiality of *M. oleifera* plant derivatives in harvesting *Chlorella* sp. from the water column which is absence in current research.

Table 1
Harvesting techniques and their respective advantages and disadvantages (Brennan and Owende, 2010).

Harvesting technique	Advantages	Disadvantages
Centrifugation	• High harvesting efficiency • Suitable for most algae types • Rapid separation process • Easy to operate	• High capital and operational costs • Cell damage • Difficult bulk harvest
Filtration	• Water and nutrition reuse • Wide variety of filter and membrane types available	• Fouling • Slow process • Suitable for large algal cells
Sedimentation	• Low power consumption • Low requirement for skilled operators • Useful as pre-concentration step	• Algal species specific • Slow sedimentation rates • Low cell recovery
Chemical flocculation	• Wide range of flocculants available • Ease to use	• Chemical contamination • Removal of flocculants • Highly sensitive to pH level • Flocculants may be algal specific species
Floatation	• Prone to harvest in mass culture	• Algal species specific

2. Methodology

2.1. Chlorella sp. cultivation

The microalgae culture used in this study was freshwater *Chlorella* sp., which was isolated from the African catfish, *Clarias gariepinus* pond of the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu. Freshwater suspended microalgae, *Chlorella* sp. was selected in this study due to its robustness to wide range of temperature, salinity and illumination conditions (Lananan et al., 2013). A water sample was collected using plankton nets with the mesh size of 20 μm. Water was sampled from fish pond and filtered through Whatman GF/C filter paper with 0.45 μm pore size to obtain concentrated algae paste. Then, the volume of concentrated algae paste was re-suspended in fresh culture media to promote growth of a particular species in isolation process. The water sample was left to bloom before further used in isolation process. The primary stock of monoculture was up-scaled gradually starting from 5 ml to 100 ml. After the culture blooms, the culture was transferred into 250 ml with the addition of culture media. It was cultivated for 8 days to produce secondary culture for further up-scaling. Normal air filtered with 40 μm air filter (Sartorius Stedim Midisart® 2000 In-Line, USA) was provided as sterile aeration to prevent any bacterial contamination. Microalgae cultures were maintained at room temperature (25 ± 2 °C), illuminated with white fluorescent light for 24 h photoperiod at 4100 lux (Hagner EC-1, Indonesia). The microalgae were cultivated until the early stationary phase of growth before undergoing coagulation-flocculation process.

2.2. Preparation of M. oleifera seed powder and oil extraction

The collection and processing of *M. oleifera* were prepared at Aquaculture Engineering and Water Quality Laboratory, Institute of Aquaculture Tropical, Universiti Malaysia Terengganu. Dry pods of *M. oleifera* were collected within the region of Kuala Terengganu, Malaysia. Dry pods were unshelled to obtain the seed. The bark enveloping the seeds in the pods was removed manually and only qualified seeds were selected to further extraction. Good quality seed were identified from those, which were not rotten, old, infected with diseases, brownish and dried once opened (Katayon et al., 2006). Prior to the preparation of coagulant, the dry pods of *M. oleifera* were dried in 24 h at controlled temperature of 40–50 °C. The temperature for drying process was ensure not exceed 60 °C to prevent the protein content from damage. *M. oleifera* seed powder was obtained from raw seed which were grounded using laboratory mill and sieved through 600 μm stainless steel sieve to ensure the distribution of fine powders. The powder form of these coagulants were stored in air-tight container and protected from light to avoid oxidation and degradation of its properties. Ethanol-based oil extraction procedure based on (Kwaambwa and Maikokera, 2007) was performed on the fine *M. oleifera* seed kernel powder. Ninety-five-percent of ethanol was added in 1:10 ratio (1 g of seed powder and 10 mL of ethanol) to form suspension. Then, the solution was mixed using magnetic stirrer for 10 min to extract the active ingredients. After that, supernatant from the extraction was separated by centrifugation (3000 rpm; 15 min) using bench-top centrifuge (Zentrifugen Universal 1200, Germany). The settled powder which are de-oiled seed was dried at room temperature for 24 h.

2.3. Purification of M. oleifera coagulation polymer as protein powder

The procedures for the purification of *M. oleifera* coagulation polymer were carried out based on Kwaambwa and Maikokera (2007). Dried de-oiled *M. oleifera* powder was used for the extraction of coagulant protein polymer. The extraction was performed by adding 3% (w/v) sodium chloride (NaCl) solution and this suspension was continuously agitated for 12 h in orbital shaker at controlled temperature of 25 ± 2 °C. The extract was filtered with Whatman filter No.44 (11 cm diameter, 0.4 μm) and brown colored of NaCl extract was collected. It was further heated by using hot-plate stirrer (IKA C-MAG HS7, Australia) in such a way that no white precipitation is formed at the bottom of solution. The heated crude protein extract solution was then poured into the dialysis tube and submerged completely for 12 h in beaker containing cold water kept in an ice bath to maintain constant temperature of 2 ± 2 °C. After completion of the dialysis procedure, the salt present in the crude brown protein was osmotically extracted into the surrounding water solution leaving white protein extract inside the dialysis tube. Subsequently, the extracted white protein was transferred from the tube into sterile glass petri plates by rinsing it with sterile deionized water. The isolated protein was then soaked with cold acetone in a homogenizer to remove lipid from the extracted protein polymer. This process is known as the delipidation procedure, where the protein was dried at room temperature to form fine protein powder.

2.4. Preparation of aluminum sulfate (alum) stock solution

Stock solution of alum (10 mg L^{-1} Al^{3+}) supplied by Merck was prepared by dissolving 1 g of dry solid in 99 mL of deionized water. Then, the solution was mixed by using magnetic stirrer to ensure all solids dissolve. A fresh solution was prepared every day for reliable results by ensuring constant flocculant activity and reducing oxidation degradation. In this study, alum was used as control coagulant for the comparison with natural coagulants of *M. oleifera* seed powder and protein powder in coagulation-flocculation of freshwater microalgae, *Chlorella* sp.

2.5. Zeta potential analysis

The analysis of ζ-potential was performed using Zeta-Meter System 3.0+ with unitron microscope and Type GT-2 cell (ZM3-D-G, USA) instrument. The ζ-potential measurement was performed using a technique of electrophoresis. A high quality stereoscopic microscope was used to observe colloidal particle inside a chamber called an electrophoresis cell. Electrodes placed in each end of the chamber were connected to a power supply which creates an electric field across the chamber. Charged colloids will move in the field and their velocity and direction were related to their ζ-potential. The electrophoresis cell holds the sample for viewing under the microscope. It consists of two electrode chambers connected by an optically polished electrophoresis tube, 10 cm long and 4 mm in diameter. Each cell was engraved with its relative cell size and *k* factor. The cell size is needed to exactly align the cell under the microscope while the *k* factor was used to preset the Zeta-Meter System 3.0+ for specific conductance measurement. The electrophoresis cell of this Zeta-Meter System 3.0+ was fabricated from fused quartz and Teflon that could handle both aqueous system and organic solvents. The Zeta-Meter System 3.0+ first measured the electrophoretic mobility (EM) of the particles, which was expressed as microns/second per volt/centimeter (μcm s^{-1} V^{-1}). The first term, microns per second was simply a velocity measurement, while the second term was the expression of electrical field strength.

2.6. Braford protein analysis

Prior to the coagulation-flocculation test, each of the *M. oleifera* seed derivatives prepared was subjected to the determination of crude protein content. Bradford protein assay was used for the rapid accurate determination of protein concentration. Among the several methods available, the spectroscopic Bradford assay was commonly used because it was considered as fast and sensitive (Bradford, 1976). The Bradford protein assay was a simple procedure for determination of total protein concentration in solutions that depends upon the change in absorbance based on the proportional binding of the dye Coomassie Blue G-250 to proteins. A set of standard was prepared from a stock solution of protein with known concentration. The Bradford values obtained from the standard was used to construct a standard curve. Then, this standard curve was used to determine the protein concentration in *M. oleifera* samples. The standard curve of Bovine Serum Albumin (BSA) was prepared within the range of 0–1 mg mL^{-1}. The Bradford reagent was a light brown in color. It was filtered through Whatman No.1 paper before use to rid of reagent of blue components. Stock solution with concentration of 1 mg mL^{-1} was prepared by dissolving 1 g of BSA in 1000 mL of 150 mM NaCl solution. 150 mM NaCl solution was prepared by dissolving 8.775 g crystalline NaCl in 1 L deionized water and stored frozen in 1 mL aliquots for quick use. NaCl solution was used to dissolve the standard and any unknown protein samples to be tested. Due to the tendency of protein to absorb water during storage, it was important to obtain the exact concentration of BSA by measuring its absorbance at 280 nm before use. Therefore, the measurement was carried out by using dual-beam spectrophotometer (Shimadzu UV-1800, Japan) to ensure that 1 mg mL^{-1} solution of BSA have an absorbance of 0.66 at wavelength 280 nm. After obtain the exact concentration, 100 μL mL^{-1} of BSA solution was of 595 nm. All measurements in this procedure was carried out by using the quartz cuvette to avoid color reagent stains on the cuvette.

2.7. Coagulation-flocculation assay

The Jar Test was performed to optimize the coagulation process and to determine the effect of adsorption on the coagulant mechanism. The test was performed in triplicates to ensure quality assurance and repeatability readings. Coagulation-flocculation and sedimentation experiments prepared were performed in glass beakers 0.12 m height by 0.09 m diameter (h/D = 1.33) using a 250 mL microalgae volume. Standard sedimentation procedure in jar and leaching test equipment (JLT-60, France) was used to determine the efficiency of coagulation with various coagulation parameters. The test rig has six space to accommodate 1000 mL flocculation beaker simultaneously. Each beaker was equipped with a paddle which the speed can be adjusted in the range of 20–400 rpm. A rapid stirring period of 5 min at 150 rpm followed by a slow stirring period of 15 min at 10, 20, 30, 40 and 50 rpm were carried out to initiate particle coagulation and flocculation. The rapid mixing procedure allowed the microalgae biomass to be mixed homogenously and in contact with the coagulant. However, slow mixing allowed the suspended microalgae cells to collide with sufficient energy to overcome the energy barrier and stick together to form larger colloid.

2.8. Statistical analysis

Two-way ANOVA analysis was selected initially for experiments with two interacting factors such as in flocculation assays which involved various flocculant inoculation dosages and various mixing rates. It could distinguish the significance within each factors and interaction between these factors. Although useful for multi-factorial statistical determination, two-way ANOVA lacked the determination capability to point treatment with the most significant reading and rank them to the least significant. One-way ANOVA was then employed to determine the significance within each factor based on the result of two-way ANOVA. OriginLab Pro 9.1™ was used as the main software for the purpose of graphical method of analysis.

3. Results and discussion

3.1. Effect of protein concentration in M. oleifera seed derivatives on biomass recovery

Fig. 1 shows the protein concentration and the biomass recovery percentage of *Moringa oleifera* seed derivatives from the preliminary coagulation-flocculation assay. The highest concentration of protein content was found in *M. oleifera* seed powder which is 211.71 μg g^{-1} mL^{-1} compared to seed coat (171.06 μg g^{-1} mL^{-1}) and cupule (183.97 μg g^{-1} mL^{-1}) for *M. oleifera* primary derivatives. In *M. oleifera* secondary derivatives, the protein concentration was 149.45 and 177.84 μg g^{-1} mL^{-1} for de-oiled seed and seed oil respectively. The protein concentration for *M. oleifera* tertiary seed derivatives were observed at 170.42 μg g^{-1} mL^{-1} in protein-lipid and 188.16 μg g^{-1} mL^{-1} in *M. oleifera* protein powder. The alum was not included in the determination of crude protein content. All

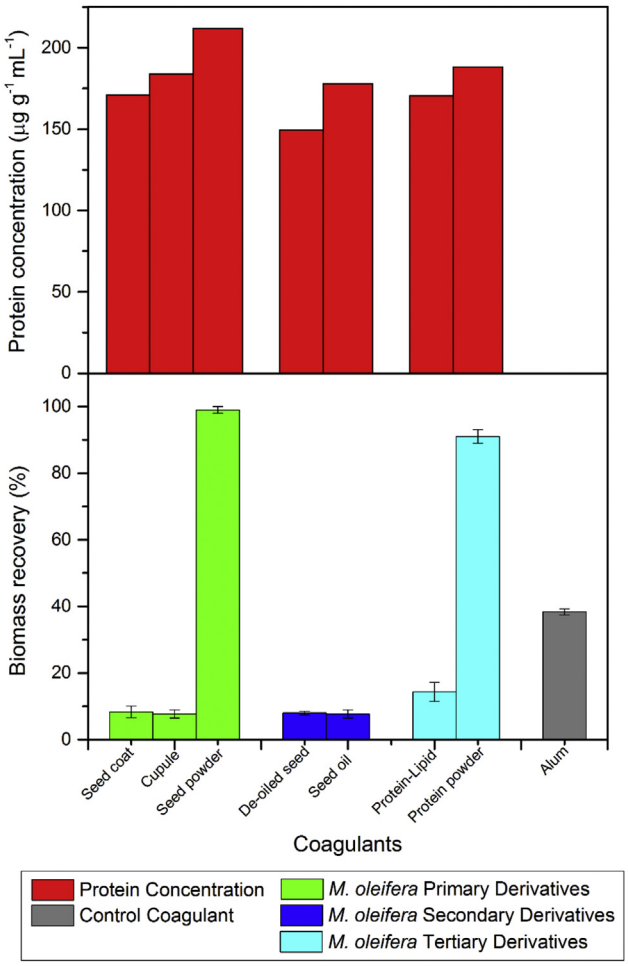

Fig. 1. Protein concentration and biomass recovery percentage of *M. oleifera* seed derivatives and alum (control).

of these protein concentration was found in 1 g of each coagulants. The preliminary coagulation-flocculation was performed in order to investigate the efficiency of each *M. oleifera* seed derivatives as coagulants.

In the coagulation-flocculation assay of primary *M. oleifera* derivatives (Table 2), it was observed that only the seed powder was able to recover microalgae biomass effectively with 97% biomass recovery. On top of that, cupule and seed coat had less than 5% and 10% biomass recovery, respectively. The presence of coagulating protein in the *M. oleifera* seed powder had contributed to the high microalgae biomass recovery as compared to cupule and seed coat. Although the protein concentration observed in both cupule and seed coat is quite high, the characteristic of its non-flocculating protein does not contributed to the coagulation-flocculation process. The positive charge of protein molecules neutralized the negatively charged microalgae cells once the *M. oleifera* seed powder were introduced to the microalgae suspension. The addition of *M. oleifera* seed powder as coagulant agent disturbs the stability of microalgae suspension system (Ndabigengesere and Narasiah, 1998; Teixeira et al., 2012). During the coagulation process, the energy barrier between the adjacent microalgae cells reduced and increased the attraction force. Hence, the aggregation of microalgae biomass occurred and become larger in floc size to ease sedimentation process. Furthermore, both coagulants in secondary derivatives were observed to have biomass recovery of less than 5%. For tertiary derivatives, it was found that only *M. oleifera* protein powder could recover the microalgae biomass for up to 78%. Less than 5% biomass recovery was observed in flocculation test using *M. oleifera* protein-lipid, which is the mixture of protein and lipid.

3.2. Determination of isoelectric point (i.e.p.) of coagulants

Fig. 2 shows the ζ-potential as a function of pH of the aqueous dispersions of alum, *M. oleifera* seed protein and *M. oleifera* protein powder. All these three coagulants can be characterized with an isoelectric point (i.e.p.) at the point ζ-potential is zero. In the neighborhood of the i.e.p., the dispersion is unstable and the ability of aggregation or flocculation to form larger particles is higher. If more alkali are added to the suspension, then the particles will tend to acquire a more negative charge. However, if acid is then added to the suspension, a point will be reached where the negative charge is neutralized. Any further addition of acid can cause a buildup of positive charge. Therefore, a ζ-potential versus pH curve will be positive at low pH and negative at high pH. The i.e.p. for all coagulant samples which are alum, *M. oleifera* seed powder and *M. oleifera* protein powder was indicated at the pH between pH 6.5 to pH 7.5. At this pH range, the value of ζ-potential was near to zero. Alum was very stable in acidic and alkali form due to the high value of positively (+64.38 mV at pH 3.38) and negatively ζ-potential (−38.40 mV at pH 10.83). Higher values of positively and negatively ζ-potential indicate the high stability level of that dispersion. This

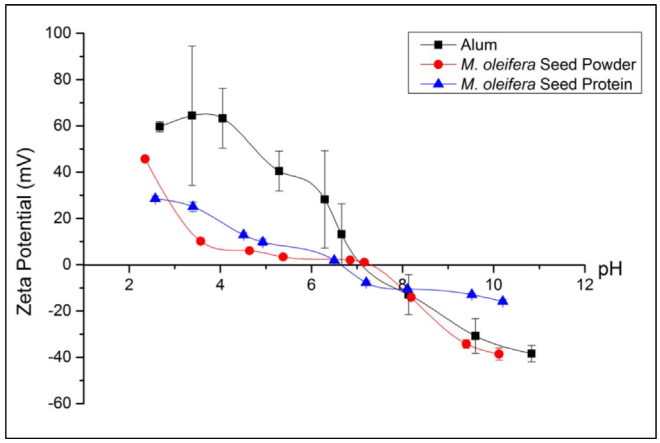

Fig. 2. Zeta potential as a function of pH of the aqueous dispersions of alum, *M. oleifera* seed protein and *M. oleifera* protein powder.

will lead to lower aggregation and flocculation of the particles. In *M. oleifera* seed powder dispersion, the value of positively and negatively ζ-potential were +45.69 mV and −38.60 mV both at pH 2.35 and pH 10.12, respectively. These values also indicate the high stability of the *M. oleifera* seed powder dispersion. However, the capability of aggregation of the particles is higher compared to alum. On the other hand, *M. oleifera* protein powder was exhibited the negative and positive ζ-potential values which are +28.51 mV at pH 2.57 and −15.93 mV at pH 10.2. Hence, *M. oleifera* protein powder has the highest capability of agglomeration and flocculation of particles compared to alum and *M. oleifera* seed powder.

3.3. Coagulation, flocculation, sedimentation and decantation

The stable suspension of microalgae biomass formed from aquaculture wastewater phytoremediation does affect the separation process. There are two factors which affect the stability of the microalgae in suspension. The first is an electric charge on the algal surface which cause the development of intercellular repulsion forces (Chen et al., 2011). While the second factor is the tiny cell dimension and cell density, which close to that of medium cause slow cell sinking rate. Both of the electric repulsion interactions between algal cells and cell interactions with the surrounding water contribute to the stability of the algal in suspension. Most of the planktonic algae is characterized as negatively charged surfaces. The intensity of that charge is a function of algal species, ionic strength of the medium, pH and other environmental conditions. Fig. 3 shows the coagulation (a), flocculation (b), sedimentation (c and d) and decantation (e and f) processes. A coagulation process occurs after the addition of coagulants following by the rapid mixing via agitation.

Table 2
The flocculation activity of *Chlorella* sp. culture using different *Moringa oleifera* seed derivatives and chemical coagulant, alum.

M. oleifera derivatives	Coagulants (10 mg L^{-1})	Flocculation activity	Flocculation efficiency
Primary	Cupule/wings	No	<5%
	Seed coat	No	<10%
	Seed powder	Yes	>97± 2%
Secondary	Seed oil	No	<5%
	De-oiled seed	No	<5%
Tertiary	Protein + lipid	No	<5%
	Protein powder	Yes	>78± 4%
Chemical	Aluminum sulfate,$Al_2(SO_4)_3$	>37± 4%	Yes

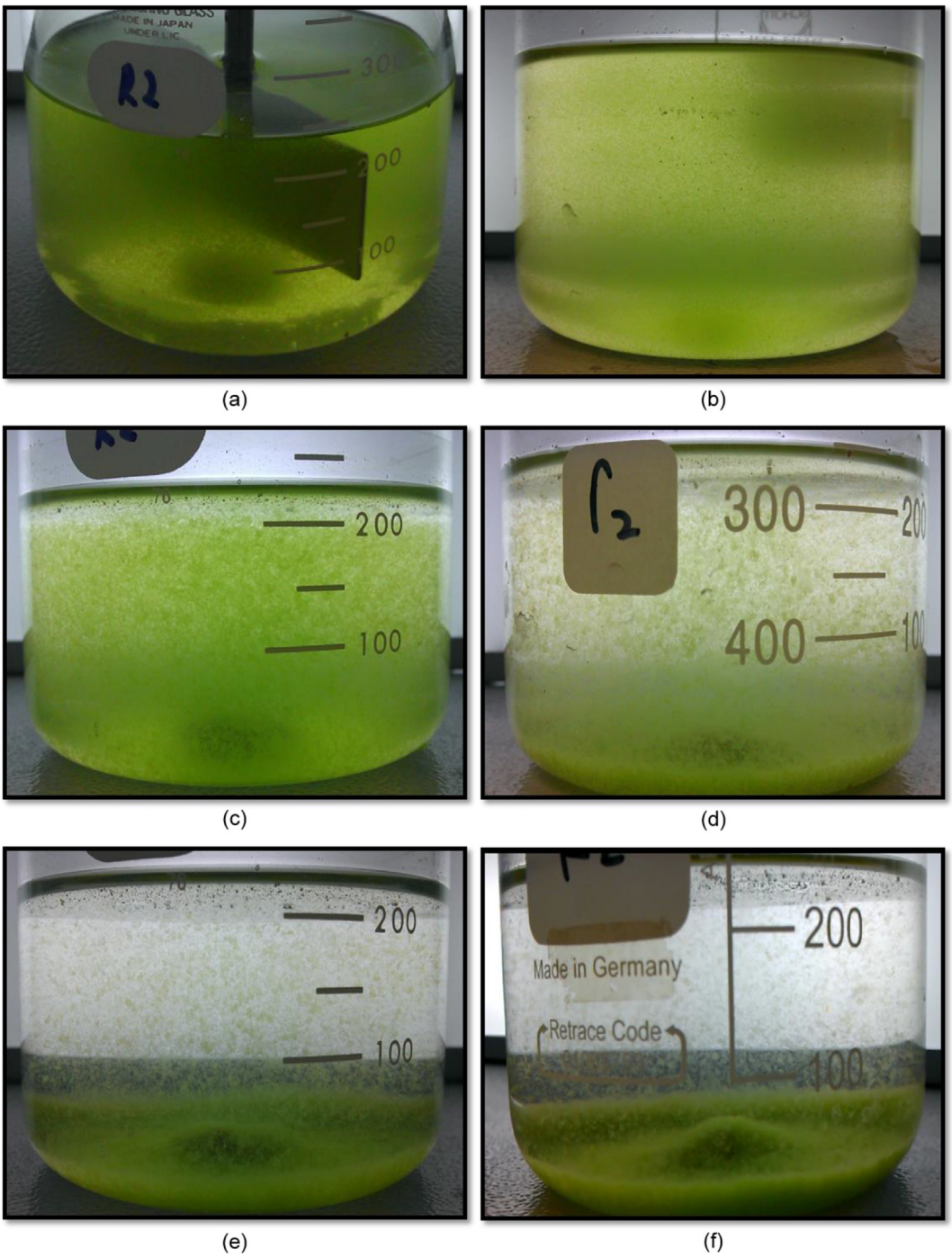

Fig. 3. Observation on *Chlorella* sp. sedimentation and decantation properties.

3.4. Optimization of coagulation-flocculation assay

The optimization of coagulation-flocculation for microalgae harvesting was done by manipulating the parameters such as coagulant dosage, the pH of the microalgal suspension and rapid-slow mixing protocol. The lab-scale harvesting process was performed using Jar Test in order to optimize the coagulation-flocculation process. In this study, coagulants used were *M. oleifera* seed powder and *M. oleifera* protein powder where these two derivatives show significant biomass recovery percentage from the preliminary coagulation-flocculation assay. The performance of these coagulants in terms of microalgae biomass recovery was compared with conventional coagulant, alum. In fact, alum was used commercially in treating wastewater for the removal of turbidity as well as microalgae harvesting.

The optimization of coagulation-flocculation assay was performed by defining the most important factor which is the ζ-potential of the suspended microalgae. The analysis of ζ-potential lead to the determination of i.e.p. of the colloidal system. Without the determination of ζ-potential value in the colloidal system, the optimization of coagulation-flocculation process could be problematic. Otherwise, the dosage used may be high and not economic.

Table 3
Current and reported harvesting methods for respective microalgae species.

Harvesting methods	Microalgae species	Types of microalgae	Harvesting efficiencies (%)	References
Flocculation with *M. oleifera* seed powder	*Chlorella* sp.	Freshwater	>97 ± 5	Current study
Flocculation with *M. oleifera* protein powder	*Chlorella* sp.	Freshwater	>78 ± 4	Current study
Flocculation with γ-PGA	*Chlorella protothecoides*, *Chlorella vulgaris* LIMCE 001 and *Botryococcus braunii* LIMCE 003	Freshwater	>95	Zheng et al. (2012)
Flocculation with γ-PGA	*Chlorella vulgaris*, *Nannochloropsis oculata* LICME 002 and *Phaeodactylum tricornutum*	Marine	>90	Zheng et al. (2012)
Flocculation with chitosan	*Thalassiosira pseudonana*	Marine	90	Heasman et al. (2000)
Flocculation with Aluminum Chloride (AlCl3)	*Chlorella minutissima*	Freshwater	>90	Papazi et al. (2010)
Centrifugation	*Phaeodactylum tricornutum*	Marine	94	Heasman et al. (2000)

This is due to the high energy barrier to be overwhelmed between the adjacent cells in a suspension. The determination of ζ-potential, however could reduce the consumption of coagulant and simplify the optimization process. The optimization of coagulation-flocculation process was considering the pH, mixing rate and coagulant dosage. In addition, the performance of harvesting process was determined by the biomass recovery percentage.

Table 3 shows the flocculation efficiency according to harvesting methods for respective microalgae species. Study conducted by (Zheng et al. (2012)) concluded that harvesting efficiency of freshwater *Chlorella protothecoides* flocculation with poly glutamic acid (γ-PGA) was over 95%. Marine *Chlorella vulgaris* flocculation utilizing γ-PGA also gives high efficiency, which exceeds 90%. This finding was similar with the flocculation of marine microalgae using chitosan which yield 90% efficiency (Heasman et al., 2000). The efficiency of biomass recovery via centrifugation method was 94%. Papazi et al. (2010) also reported that flocculation of freshwater *Chlorella minutissima* by using alum yield over 90% efficiency. The current study shows that the flocculation efficiency was over 97% using *M. oleifera* seed powder in low dosage. 78% flocculation

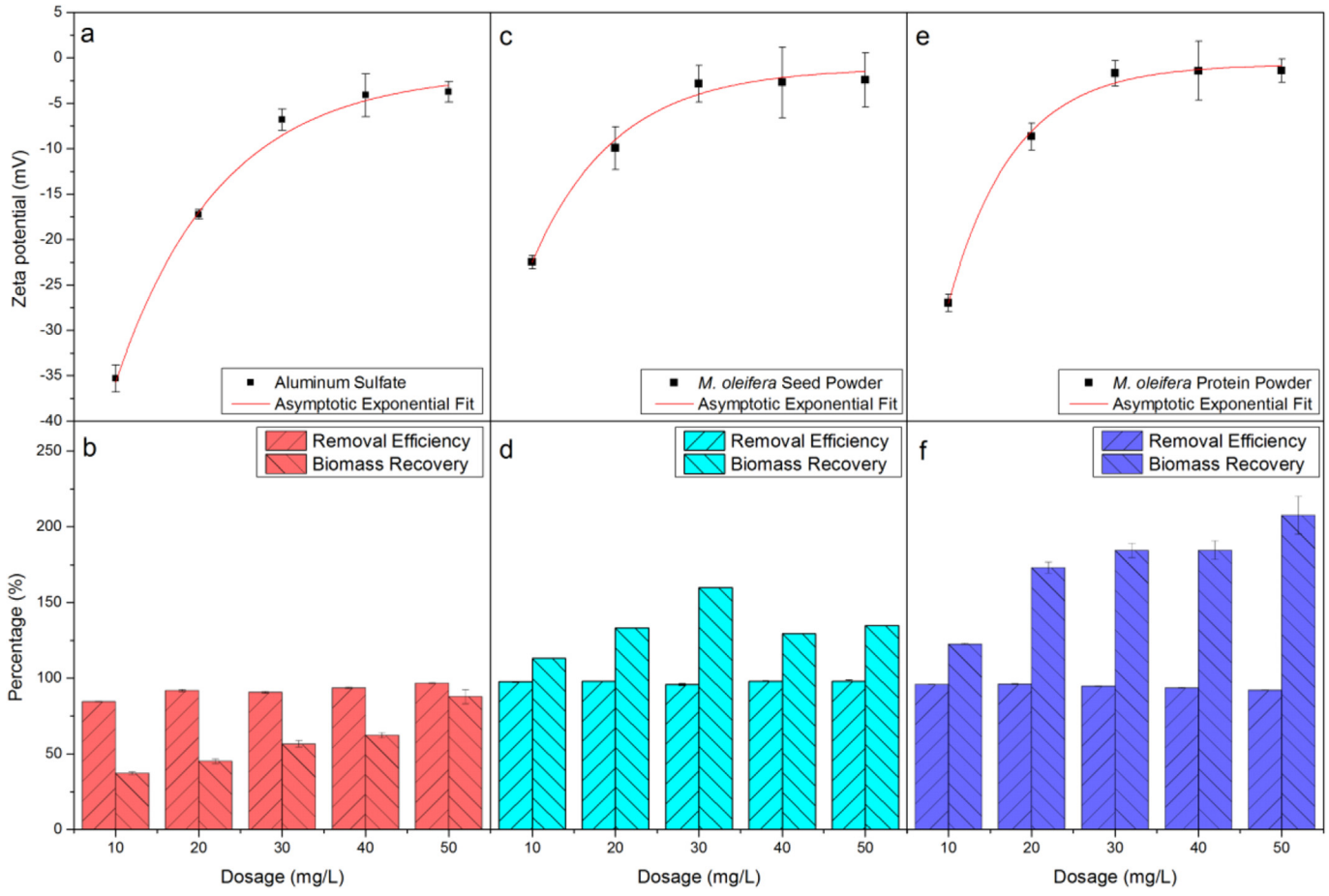

Fig. 4. The reduction of zeta potential of *Chlorella* sp. with respect to the coagulant dosage.

efficiency was achieved by the flocculation using *M. oleifera* protein powder. The major disadvantages of using ɣ-PGA as a coagulant purposes are its cytotoxicity and not biodegradable (Dubruel et al., 2003). Alum however, was known as a chemical coagulant that could remain in harvested biomass and cause the limitation of downstream processing for aquaculture feeds. Although chitosan are natural coagulant, the derivation process of chitosan from chitin consume high cost and not viable for large-scale biomass harvesting. *M. oleifera* seed powder and protein powder, however overcome all of the negative aspects from the various coagulants reported.

3.5. Effect of coagulant dosage on zeta potential of *Chlorella* sp.

The effect of coagulant dosage used during coagulation-flocculation assay was plotted through fitting with the Asymptotic Exponential Model as shown in Fig. 4. The purpose of the model fitting was to investigate the effect of coagulant dosage on ζ-potential of *Chlorella* sp. suspension. From the plotted curve, it was observed that all mixed suspension of *Chlorella* sp. and coagulants show the similar changes of ζ-potential values. The values of negatively ζ-potential of *Chlorella* sp. suspension decreased with the increasing of coagulant dosage from 10 to 50 mg L^{-1}. With the addition of 10 mg L^{-1} of alum, *M. oleifera* seed powder and *M. oleifera* protein powder into *Chlorella* sp. suspension had caused the reduction in zeta potential values to −35.31 mV, −22.49 mV and 26.99 mV respectively. Further addition of each coagulant into *Chlorella* sp. suspension shows the reduction, which causes the curve of ζ-potential near to asymptote, *a*. It was found that the mixed suspension of *Chlorella* sp. with *M. oleifera* protein powder yield the lowest i.e.p. of −0.74 mV as compared to −1.13 and −1.62 mV respectively for *M. oleifera* seed powder and alum at the dosage of 50 mg L^{-1}.

4. Conclusions

As compared to the traditional practice of conventional water treatment, *M. oleifera* seed and protein powder as bio-flocculant was proven to be a highly potential alternative to the chemical flocculant in harvesting microalgae *Chlorella* sp. biomass from the culture medium. In this study, the optimum flocculation mixing rate was found to be at 20 rpm for 20 min with optimum flocculant dosage of 10 mg L^{-1}. Indeed, both *M. oleifera* seed derivatives had a higher biomass removal efficiency of 97% and biomass recovery as compared to alum at 34%. *M. oleifera* seed derivatives were also found to concentrate the density of harvested microalgae to about two-fold the initial density. It was also found that the application of the zeta potential determination to predict the flocculation performance of coagulant was probable since both value was highly correlated. The use of *M. oleifera* seed derivatives as bio-flocculant could assist in lowering the economic cost and providing environmental-friendly approach for mass microalgae cultivation and the sustainable development of bio-based renewable energy in the future.

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