



Moringa Oleifera SEED DERIVATIVES AS POTENTIAL BIO-COAGULANT FOR MICROALGAE *Chlorella* Sp. HARVESTING

(Potensi Derivatif Biji *Moringa oleifera* sebagai Bio-Pengental untuk Penuaian Mikroalga *Chlorella* sp.)

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Abstract

Microalgae is an economical and potential raw material of biomass energy, which offer a wide range of commercial potential to produce valuable substances for applications in aquaculture feed, pharmaceutical purposes and biofuels production. However, lack of an economical, efficient and convenient method to harvest microalgae is a bottleneck to boost their full-scale application. Hence, this study was performed to investigate the potentialities of *Moringa oleifera* seed derivatives as an environmentally bio-coagulant to harvest microalgae *Chlorella* sp. biomass from the water column, which acts as a binder to coagulate particulate impurities to form larger aggregates. Results shown *M. oleifera* to have better biomass recovery of 122.51% as compared to 37.08% of alum at similar dosages of 10 mg·L⁻¹. In addition, it was found that the zeta potential values of mixed microalgae-coagulant suspension shows positive correlation on the flocculation parameters. For biomass recovery, the correlation for *M. oleifera* protein powder showed the R²-value of 0.9565 whereas the control chemical flocculant, alum with the R²-value of 0.7920. It was evidence that *M. oleifera* has a great potential in efficient and economical for environmentally microalgae harvesting and the adaptation of biological harvesting technology especially for the purpose of aquaculture feed in Malaysia.

Keywords: *Chlorella* sp., coagulation-flocculation, isoelectric point, *Moringa oleifera*, zeta potential

Abstrak

Mikroalga merupakan bahan mentah yang murah dan berpotensi untuk tenaga biojisim, yang menawarkan pelbagai peluang pengkomersialan untuk menghasilkan bahan bernilai untuk aplikasi dalam makanan akuakultur, farmaseutikal dan penghasilan bahan bakar bio. Walaubagaimanapun, kekurangan kaedah yang murah, cekap dan mudah untuk penuaian mikroalga merupakan halangan untuk meningkatkan aplikasi teknologi ini dalam skala yang lebih besar. Oleh itu, kajian ini telah dijalankan untuk menyiasat potensi derivatif biji *Moringa oleifera* sebagai bio-penggumpal biojisim mikroalga *Chlorella* sp., yang bertindak sebagai pengikat untuk menggumpalkan zarah enapcemar membentuk agregat yang lebih besar dari kolom air. Keputusan menunjukkan *M. oleifera* mempunyai pemulihan biojisim yang lebih baik iaitu 122.51% berbanding alum iaitu 37.08% pada dos yang sama sebanyak 10 mg·L⁻¹. Di samping itu, nilai keupayaan zeta campuran mikroalga dan penggumpal menunjukkan kolerasi positif terhadap parameter penggumpalan. Dalam pemulihan biojisim, kolerasi bagi serbuk protein *M. oleifera* menunjukkan nilai R² iaitu 0.9565 manakala penggumpal kimia kawalan, alum mempunyai nilai R² iaitu 0.7920. Ini membuktikan bahawa *M. oleifera* mempunyai potensi tinggi untuk penuaian mikroalga yang berkecekapan tinggi dan murah serta penyesuaian teknologi penuaian biologi terutamanya untuk tujuan makanan akuakultur di Malaysia.

Kata kunci: *Chlorella* sp., koagulasi-flokulasi, titik isoelektrik, *Moringa oleifera*, keupayaan zeta

Introduction

The exploration of potential natural coagulant is crucial for sustainability in mass microalgae harvesting. Microalgae harvesting in mass cultivation industry consisting of two stage process involving bulk harvesting and thickening. Bulk harvesting was aimed at the separation of biomass from the bulk suspension using flocculation techniques whereas thickening concentrated the slurry through energy intensive techniques such as centrifugation and filtration [1]. Chemical coagulants normally applied in the bulk harvesting stage. Thus, microalgae harvesting in massive scale would require high amount of chemical flocculants, which is not sustainable for long-term green technology application. Natural coagulant could be investigated for the desired flocculation characteristics similar to chemical flocculants. Plant-based natural coagulant is more environmental friendly and not pose health risks as chemical flocculants such as aluminum and ferric salts. The use of inorganic coagulants such as ferric chloride, FeCl_3 and aluminum sulfate, $\text{Al}_2(\text{SO}_4)_3$ shortly known as alum is proven to be effective for some microalgae flocculation, however it is certainly unacceptable if the harvested biomass is to be used for aquaculture purposes, animal feed or organic fertilizer. 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 [2]. According to Zheng et al. [3], these flocculants are required in high doses, both are more than $0.75 \text{ g}\cdot\text{L}^{-1}$ for microalgae harvesting.

In Malaysia, *M. oleifera*, commonly known as *kacang merunggai* is available locally and inexpensive hence making them a viable alternative to chemical coagulant in water and wastewater treatment, which is also possible as bio-flocculants in the microalgae biomass separation process and harvesting [4]. Bio-coagulant from *M. oleifera* was reported to have a high flocculation potential in water treatment, attaining 92 – 97 % flocculation efficiency [5, 6]. Previous study also concluded that *M. oleifera* seeds as a strong candidate as a bio-flocculant for microalgae harvesting at optimal pH range [7]. *M. oleifera* seed active compounds are known as the peptides of molecular weight ranging from 6 to 20 kDa, with an isoelectric pH value between 9 to 10 [6, 8].

Dispersion of microalgae cells in the water column leads to the difference of surface charges between microalgae biomass suspension and chemical ions of the cultivation media. According to Chen et al. [9], the proper reaction mechanisms existing at the colloid surfaces are differential loss ions from the crystal lattice because of the rupture of ionic or covalent bond, adsorption of charged species from the surrounding solution and ionization of chemical groups on the surface. The suspending microalgae cells in the water carried charge, which result in the generation of the electrical-double-layer. In solution, the presence of a net charge on a particle affects the distribution of ions surrounding it, resulting in an increase in the concentration of counter-ions. This region over which this influence extends was referred as electrical-double-layer. Conventionally, this layer is thought of as existing as two separate regions, which are inner region and outer layer. An inner region consists of strongly bound ions to the particle known as stern layer while the outer layer called diffuse layer associated with counter-ions. In the electrophoresis, an electric field is applied to a dispersion of particle, sometimes liquid droplets or even gas bubbles which gives rise to a motion of the charged particles with respect to the bulk liquid [10]. As the particle moves through solution, due to gravity or an applied voltage, the ions move with it. At some distance from the particle there exists a boundary, beyond which ions do not move with the particle. This is known as the surface of hydrodynamic shear, or the slipping plane which exists somewhere within the diffuse layer. During this phenomenon, the potential which is zeta potential do exists at the slipping plane.

The measurements of zeta potential is crucial in determining the stability of a colloidal suspension. Normally in a colloidal system, the dispersion are stable in which particles do not aggregate. The aggregation of particles in suspension depends on the magnitude of the zeta potential. In many instances stable dispersion are required. In other cases, such as water and wastewater treatment, paper manufacturing and oil recovery, unstable dispersion are desired. Stable or unstable dispersion could be produced in a variety of ways, which are by changing the pH of dispersion, addition of electrolyte and addition of coagulant. Generally, zeta potential study was correlated with coagulation-flocculation process. By measuring zeta potential, the isoelectric point (i.e.p.) in a colloidal system could be determined. Isoelectric point is very important from a practical consideration which normally indicated the least stable point in a colloidal system. At this point, the potential energy barrier opposing coagulation disappears,

and it is called the critical coagulation concentration [11]. Effective coagulation process could be performed in order to remove or recover the colloids from its suspended system.

The nature of suspended microalgae and surface charge could make the solid-liquid separation more difficult. However, the determination of zeta potential could lead to the understanding of isoelectric point of a colloidal system and eased explanation of the mechanism and interfacial phenomena occurring during coagulation-flocculation process of microalgae under various coagulants. The aim of this study was to explore the potential use of *M. oleifera* as a novel low cost bio-coagulant for microalgae *Chlorella* sp. **Harvesting**. The interaction of the zeta potential of commercial coagulant which are alum, natural *M. oleifera* seed powder and *M. oleifera* protein powder in harvesting locally isolated freshwater microalgae, *Chlorella* sp. were investigated. The correlation of zeta potential and flocculation ability was explored to explain the interaction between coagulants and microalgae cells in suspended. Selected conditions for coagulation-flocculation were presented, as well as removal and microalgae biomass recovery percentage for different dosage at optimum pH. In this study, the experimental strategy was focused on coagulation-flocculation process corresponding to the profiles of zeta potential with respect to pH for different coagulants used such as alum, *M. oleifera* seed powder and *M. oleifera* protein powder.

Materials and Methods

Study area

Microalgae maintenance and flocculation assays were carried out at the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Terengganu. The determination of zeta potential of microalgae suspension and *M. oleifera* seed derivatives were performed at the Civil Engineering Lab, Department of Civil Engineering, Universiti Putra Malaysia, Selangor.

Preparation of alum stock solution

Stock solution of aluminum sulfate ($10 \text{ mg} \cdot \text{L}^{-1} \text{ Al}^{3+}$) supplied by Sigma Aldrich was prepared by dissolving 1 g of dry solid in 99 mL of deionized water. The alum powder was totally soluble in the water. A fresh solution was prepared every day for reliable results. In this study, the zeta potential of alum was compared with natural coagulants of *M. oleifera* seed powder and protein powder in harvesting freshwater microalgae, *Chlorella* sp..

Cultivation of freshwater microalgae, *Chlorella* sp.

The inoculum of pure culture of freshwater microalgae, *Chlorella* sp. was obtained from the microalgae culture collections of the Live Feed Culture Lab, Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia. *Chlorella* sp. was cultivated in Bold's Basal Medium (BBM) with controlled temperature of $25 \pm 2 \text{ }^{\circ}\text{C}$ and 24 h illumination at 3350 lumen until late exponential phase in which cell density reached averagely $2.0 \times 10^7 \text{ cells} \cdot \text{mL}^{-1}$.

Spectrophotometric analysis of *Chlorella* sp. biomass

The *Chlorella* sp. biomass were measured using spectrophotometric colorimetric method to facilitate rapid measurement. Spectrum analysis was performed to determine the peak absorption wavelength for the spectrophotometric colorimetric technique which was at 686 nm. For the establishment of standard calibration curve, *Chlorella* sp. biomass was measured using haemocytometer (Marienfield Neubauer-improved 0.1 mm depth, Germany) and syringe liquid sampler particle system (PMS SLS-2000, USA).

Preparation of *M. oleifera* seed powder

M. oleifera seeds were obtained within the region of Kuala Terengganu, Malaysia. The plant were authenticated by the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (**UNISZA**) Tembil, Terengganu, Malaysia. The plant was deposited at the **University** herbarium. The collected dry pods were unshelled to obtain the seeds. Pods shells were removed manually and only qualified contaminant-free seeds were selected to be used as coagulant. Then, the seeds consisted of cupule, seed coat and seed kernel were grounded using laboratory mill and sieved through 600 μm stainless steel sieve to obtain homogenous fine powders from each seed structure.

Purification of *M. oleifera* seed protein

The procedures for the purification of *M. oleifera* coagulation polymer were carried out based on Kwaambwa, and Maikokera method [12]. Dried de-oiled *M. oleifera* powder was used for the extraction of coagulant protein polymer. The extraction was performed by adding 3% (w/v) 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 and further heated 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 with 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. Then, the protein was dried at room temperature to form fine protein powder.

Coagulation-flocculation and sedimentation experiments

Coagulants tested in this study included alum, *M. oleifera* seed powder and *M. oleifera* protein powder. Coagulation-flocculation and sedimentation experiments were performed in glass beakers 0.12 m height by 0.09 m diameter ($h/D = 1.33$) using a 250 mL microalgae volume. Flocculants, previously dissolved in water at concentration $10 \text{ mg} \cdot \text{L}^{-1}$ were added to the microalgae sample under intense stirring at 150 rpm for 3 minutes to ensure complete solubility of the flocculants. Following that, the stirring was reduced to gentle agitation at 20 rpm for 20 minutes, so as to initiate flocs formation. After that, the suspension was leaved for sedimentation in 20 minutes. When interphases appear, it was clear and 3 mL sample was taken at the top (clear supernatant) for removal efficiency and 3 mL from bottom (saturated *Chlorella* sp. biomass) for biomass recovery. The absorbance of the sample were measured using UV-vis spectrophotometer (Shimadzu UV-1800, Japan). All experiments were carried out in triplicates. Percentage of biomass removal was calculated from the absorbance ratio at clarified zone against the culture absorbance at the beginning of the experiment according to Equation 1:

$$\text{Biomass removal (\%)} = \frac{\text{Abs}_{668} \text{ initial culture} - \text{Abs}_{668} \text{ clarified zone}}{\text{Abs}_{668} \text{ initial culture}} \times 100 \quad (1)$$

Measurements of zeta potential

Methods of measuring zeta potential on a particles dependent both which are the particle and the suspension formulation. In general, the size and concentration of particles are the key parameters that determine which technique is applicable. In this study, zeta potential was measured based on the dispersion concentration of each coagulants which are alum, *M. oleifera* seed powder and *M. oleifera* protein powder in water at room temperature. Zeta potential was not measured directly. Perhaps the most widely used technique for measuring zeta potentials in electrophoresis. By applying an electric field across the sample, charged particles were induced to move. The velocity of particles known as electrophoretic mobility (EM). The electrophoresis measurements were carried out by injecting 25 mL of the aqueous dispersions of each coagulants into the cell of zeta potential instrument (Zeta-Meter System 3.0+, USA) at room temperature. The electrophoresis cell holds the sample for viewing under the microscope. It consists of two electrode chambers connected by an optically polished electrophoresis tube which is 10 cm long and 4 mm in diameter. Zeta potential measurements also was performed on suspending freshwater microalgae, *Chlorella* sp. culture before and after the addition of coagulants in order to investigate the effects of coagulants on its isoelectric point. By directly measuring the electrophoretic mobility of particle in dispersion, the zeta potential was determined using Henry's Equation 2 [13]:

$$\mu = \frac{2\varepsilon_0\varepsilon_r\zeta f(\kappa r)}{3\eta} \quad (2)$$

where,

μ	= electrophoretic mobility	η	= viscosity of medium
ε_0	= permittivity of a vacuum	$f(\kappa r)$	= Henry's function
ε_r	= medium dielectric constant (or permittivity)		
ζ	= zeta potential		
κ	= Debye-Hückle parameter		
r	= hydrodynamic radius of particle		

The Henry's function generally has value either 1.5 or 1.0. For the measurements of zeta potential in aqueous solutions of moderate electrolyte concentration, a value of 1.5 is used. In this study, zeta potential was calculated according to the Henry's equation, applying Smoluchowski approximation [14].

Statistical analysis

Zeta potential of *Chlorella* sp., alum, *M. oleifera* seed powder and *M. oleifera* protein powder with respect to pH were recorded in Microsoft Office Excel™ throughout the experimental period. Graphical analyses were performed using Originlab OriginPro 8.6™ whereas the statistical determination involving One-Way ANOVA and post-hoc analysis utilizing Tukey's HSD Test were implemented via Minitab 16™. A confidence level of 95% ($\alpha = 0.05$) was selected in order to strictly determine the significance of between the dosages and types of coagulant used with the dependent parameters on microalgae biomass recovery.

Results and Discussion

Zeta potential analysis

Figure 1 shows the zeta potential values of freshwater microalgae, *Chlorella* sp. biomass and *M. oleifera* seed derivatives suspension in the range of pH 2 to pH 12. Both *M. oleifera* seed powder and *M. oleifera* protein powder zeta potential values decreases from 45.68 and 28.51 mV at pH 2 to the zeta potential values of -38.59 and -15.93 mV at pH 11, respectively. Control coagulant, alum also had decreased zeta potential value as pH increased from pH 2 to pH 12 with the value of 59.64 to -38.40 mV. In addition, *Chlorella* sp. biomass exhibited moderate decrease of the zeta potential value from -24.15 to -49.99 mV at pH 2 to pH 11. As shown in Table 1, *Chlorella* sp., alum and *M. oleifera* protein powder had significant regression with the Boltzmann Sigmoidal Model with the R^2 -values of 0.9512, 0.9965 and 0.9549, respectively. Zeta potential values of *Chlorella* sp. and coagulants at various pH fitted with the model were shown in Figure 2.

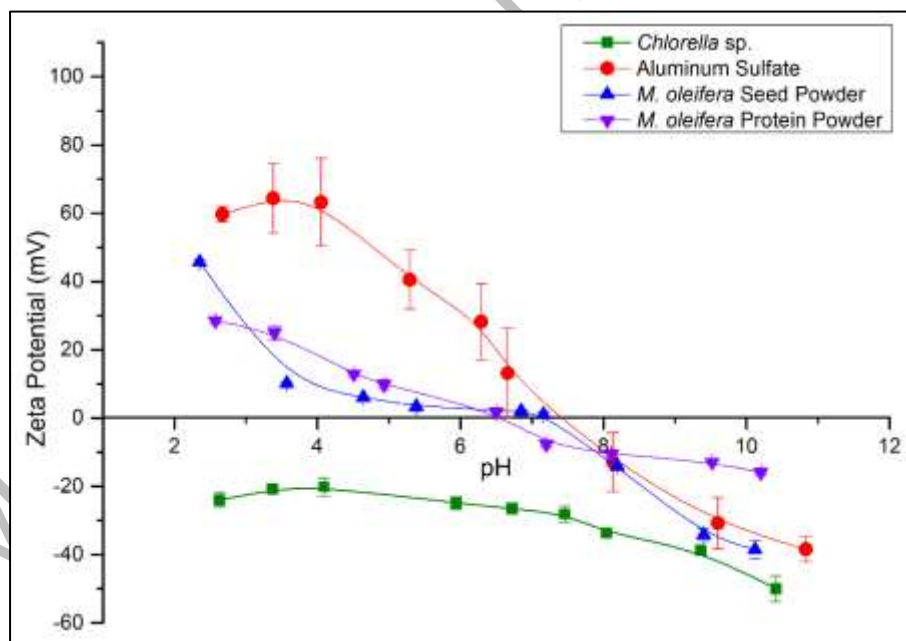


Figure 1. Spline curves of zeta potential values of *Chlorella* sp., aluminum sulfate, *M. oleifera* seed powder and *M. oleifera* protein powder at various pH.

Table 1. Boltzmann sigmoidal parameters of zeta potential at various pH for microalgae, *Chlorella* sp. and several coagulant suspensions

Suspension	Reduced Chi-Square	Adjusted R-Square	Boltzmann Sigmoidal Fit			
			Initial Value, A_1	Final Value, A_2	Centre, X_0	pH Constant, dx
<i>Chlorella</i> sp.	1.1477	0.9512	-19.7754 ± 2.6250	-83.0073 ± 110.0826	10.8864 ± 6.1201	2.0215 ± 1.4815
Aluminum Sulfate	0.1779	0.9965	62.6897 ± 2.2368	-41.5764 ± 3.4869	6.9651 ± 0.1999	1.1466 ± 0.2026
<i>M. oleifera</i> Seed Powder	112.9585	0.6962	411055.3 $\pm$ 1.2849×10^{10}	-1.7475 $\pm$ 7.0051	-8.5312 $\pm$ 37406.3888	1.1958 $\pm$ 2.5013
<i>M. oleifera</i> Protein Powder	8.8414	0.9549	32.8811 $\pm$ 11.2681	-18.9550 $\pm$ 5.3989	5.7435 $\pm$ 0.6186	1.6050 $\pm$ 0.6980

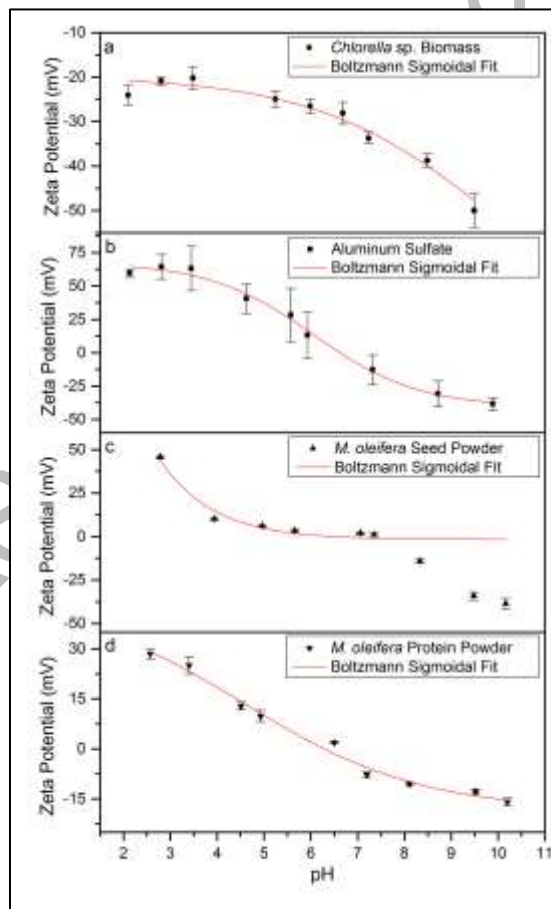


Figure 2. Zeta potential values of *Chlorella* sp. (a), aluminum sulfate (b), *M. oleifera* seed powder (c) and *M. oleifera* protein powder (d) at various pH fitted with Boltzmann Sigmoidal Model.

Through the model fittings, it was found that the initial zeta potential values were respectively -19.78, 62.69 and 32.88 mV for *Chlorella* sp., alum and *M. oleifera* protein powder. Whereas, the predicted final zeta potential values were respectively -83.01, -41.58 and -18.96 mV. The zeta potential of *M. oleifera* seed powder however could not be fitted with the sigmoidal model which yield R^2 -value of 0.6962. In order to harvest the *Chlorella* sp. biomass, the coagulants were mixed at various coagulant dosages with the microalgae biomass undergoing flocculation assay and also yielded the mixed suspension zeta potential values and flocculation performance which were removal efficiency and biomass recovery, which were shown in Figure 3 and Figure 4, respectively.

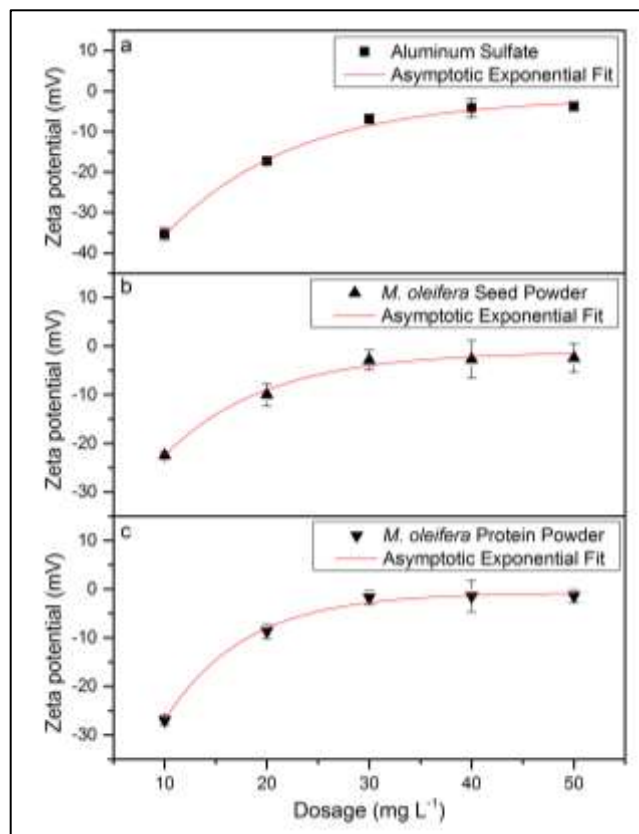


Figure 3. Zeta potential values of *Chlorella* sp. suspension mixed with aluminum sulfate (a), *M. oleifera* seed powder (b), *M. oleifera* protein powder (c) at various coagulant dosages fitted with Asymptotic Exponential Model.

The observed zeta potential values were further analyzed through fitting with the Asymptotic Exponential Model to optimize the coagulant dosage. It was found that the mixed suspension of *Chlorella* sp. with *M. oleifera* protein powder yield the highest 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. Higher i.e.p. values of *M. oleifera* seed protein suggested that it would have better flocculation rate as compared to *M. oleifera* seed powder and alum. In addition, *M. oleifera* protein powder also had the highest response range of 94.50 mV whereas *M. oleifera* seed powder had the lowest zeta potential response range of 58.23 mV as dosage increased from 0 mg·L⁻¹ to 50 mg·L⁻¹. Aluminum sulfate showed the highest rate of 0.92 mV per mg·L⁻¹ increase of coagulant followed by *M. oleifera* seed powder and seed protein, respectively 0.90 and 0.88 mV per mg·L⁻¹. This value shows that the ζ -potential value of *Chlorella* sp. was highly correlated to the dosage of coagulant used. The addition of dosage cause the value of ζ -potential increase. The

increasing of ζ -potential value indicate the disturbance on the stability of microalgal cells suspension. This is due to the charge neutralization of negatively charged cells with the addition of positively charged coagulants. Same trend was also observed by Muyibi et al. [15] which stated that the gradual decreased in biomass recovery was due to over dosage that led to restabilization of the destabilized suspended solids which previously had agglomerated into flocs.

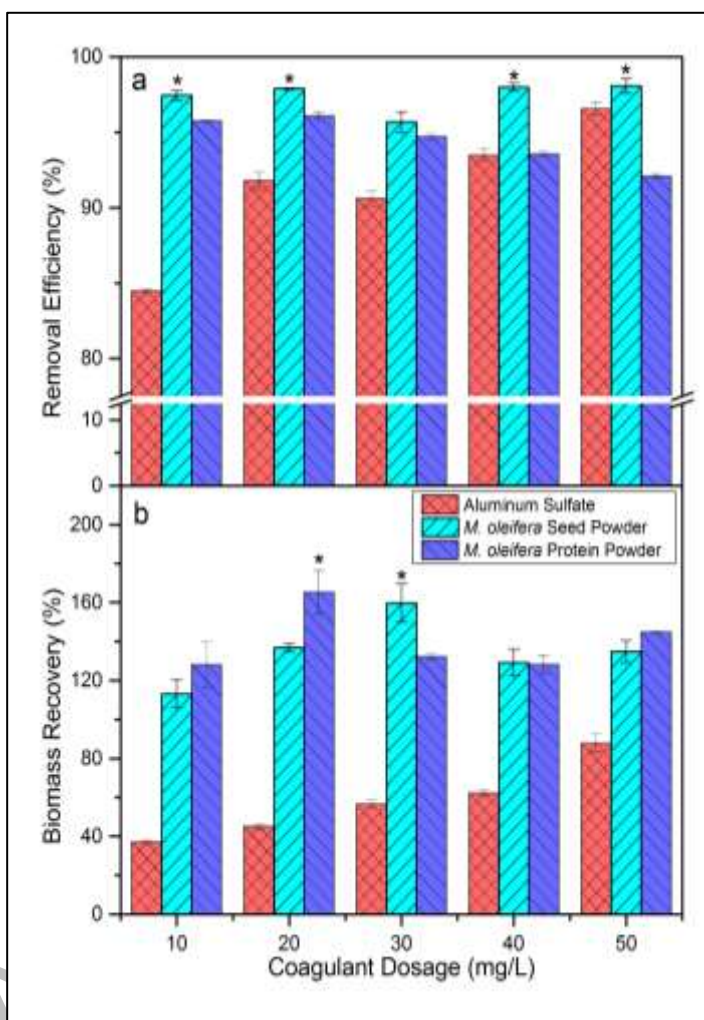


Figure 4. Removal efficiency (a) and biomass recovery (b) of aluminum sulfate, *M. oleifera* seed powder and *M. oleifera* protein powder at various coagulant dosages. Treatment with the significantly highest values was marked with asterisk (*).

Flocculation assays

Flocculation performance of coagulant consisted of removal efficiency and biomass recovery. Removal efficiency is the effectiveness of coagulant in removing the suspended particles from the water column producing a clear supernatant. Meanwhile, biomass recovery is its proficiency in aggregating, settling and compacting the suspended particles at the bottom of the jar test vessel. Figure 4 showed the removal efficiency and biomass recovery of alum, *M. oleifera* seed powder and *M. oleifera* seed derivatives with regards to various coagulant dosage of 10 to 50 mg·L⁻¹. *M. oleifera* seed powder achieved significantly highest (P < 0.05) removal efficiency of above 97% at coagulant dosage of 10, 20, 30, 40 and 50 mg·L⁻¹ as compared to other coagulants. The removal efficiency of alum

increased as dosage were increased but *M. oleifera* protein powder showed opposite trend. It could be observed that the maximum removal efficiency for *M. oleifera* protein powder was 96.09% at dosage of 20 mg·L⁻¹ and decreased to 92.08% as the dosage increased to 50 mg·L⁻¹. *M. oleifera* seed powder however showed consistent removal efficiency among various dosages. All coagulants showed increased biomass recovery as the dosage was increased up to 50 mg·L⁻¹. *M. oleifera* protein powder had the significantly highest biomass recovery of 207.46% as compared to other coagulants. *M. oleifera* seed powder, however only showed increased biomass recovery at the dosage of 10 to 30 mg·L⁻¹ with the maximum percentage of 159.78%. At the dosage of 40 mg·L⁻¹, the biomass recovery decreased at 30.48%. This negative relationship could be explained by the continuous addition of coagulant exceeding the optimum dosage which leads to the formation of excess coagulant residue since all of the available suspended microalgae already formed larger colloids [6].

Table 2 shows the coefficients for Asymptotic Exponential Model with the R²-value of 1.4443, 0.3026 and 0.4537 for *Chlorella* sp. suspension with aluminum sulfate, *M. oleifera* seed powder and *M. oleifera* protein powder, respectively. In addition, the output of the flocculation assays showed the ability of *M. oleifera* seed extracts not only in harvesting but also concentrate the recovered *Chlorella* sp. biomass for up to 159.78% and 207.46%, respectively for *M. oleifera* seed powder and *M. oleifera* protein powder. The performance of *M. oleifera* seed powder was confirmed by Teixeira et al. [4] which reported its performance in harvesting marine microalgae from cultivation medium with the removal efficiency of more than 90%. Nevertheless, other research involving the investigation on *M. oleifera* protein powder was still lacking.

Table 2. Asymptotic exponential parameters of zeta potential at various pH for mixed suspension of microalgae, *Chlorella* sp. with coagulants.

Microalgae + Coagulants	Reduced Chi-Square	Adjusted R-Square	Asymptotic Exponential Fit		
			Asymptote, a	Response Range, b	Rate, c
<i>Chlorella</i> sp. + Aluminum Sulfate	1.4443	0.9844	-1.6233 ± 1.7103	75.8976 ± 8.6003	0.9232 ± 0.0096
<i>Chlorella</i> sp. + <i>M. oleifera</i> Seed Powder	0.3026	0.9919	-1.1332 ± 1.6076	58.2269 ± 10.3332	0.9046 ± 0.0212
<i>Chlorella</i> sp. + <i>M. oleifera</i> Protein Powder	0.4537	0.9952	-0.7401 ± 0.8427	94.5035 ± 14.6837	0.8799 ± 0.0151

Correlation of zeta potential values and flocculation performance

Prior to the correlation analysis, the zeta potential values of mixed suspension of *Chlorella* sp. and coagulants were fitted with the Asymptotic Exponential Model yielding the i.e.p. value (asymptote), zeta potential response range and rate of zeta potential change regarding dosage. Figure 5 shown the correlation of zeta potential and biomass recovery. *M. oleifera* protein powder showed the highest correlation between the zeta potential values and mean biomass recovery with the R² value of 0.9565. This was followed by alum and *M. oleifera* seed powder with R²-value of 0.7920 and 0.7059, respectively. For alum, biomass recovery decreased beyond the dosage of 30 mg·L⁻¹ which was inconsistent with the measured zeta potential. Same trend was also observed by Muyibi et al. [5], which stated that the gradual decreased in biomass recovery was due to over dosage that led to restabilization of the destabilized suspended solids which previously had agglomerated into flocs.

Correlation of zeta potential values and flocculation performance involved the use of zeta meter and only jar tests in which only coagulation/flocculation and settling processes were employed. It is therefore recommended that investigations be carried out through pilot plant studies which involves continuous biomass harvesting and various unit operations and processes employed for mass microalgae cultivation. Some studies reported that flocculation of microalgae can occur spontaneously without the need for chemicals, referred to as autoflocculation which can be

induced by increasing the medium pH [16]. According to Harith et al. [17], harvesting efficiency of *Chaetoceros calcitrans* of higher than 90% was achieved by adjusting the culture pH to 10.2 using either NaOH or KOH. The alteration of pH of the culture medium lead to the changes in the zeta potential and hence reduce the colloidal stability. In this study, the addition of coagulant dosage reduce the zeta potential of the microalgae colloidal and increased the biomass recovery percentage. On the other hand, the usage of non-toxic organic polymer, such as polyacrylamide copolymers, chitosan, and cationic starch have been intensively investigated for large-scale applications [18,19]. Unfortunately, they are not economical for microalgae due to its higher price. The coagulant residuals in both algal biomass and harvested water are not only negative for downstream processing but also reduce the possibilities for culture medium recycling.

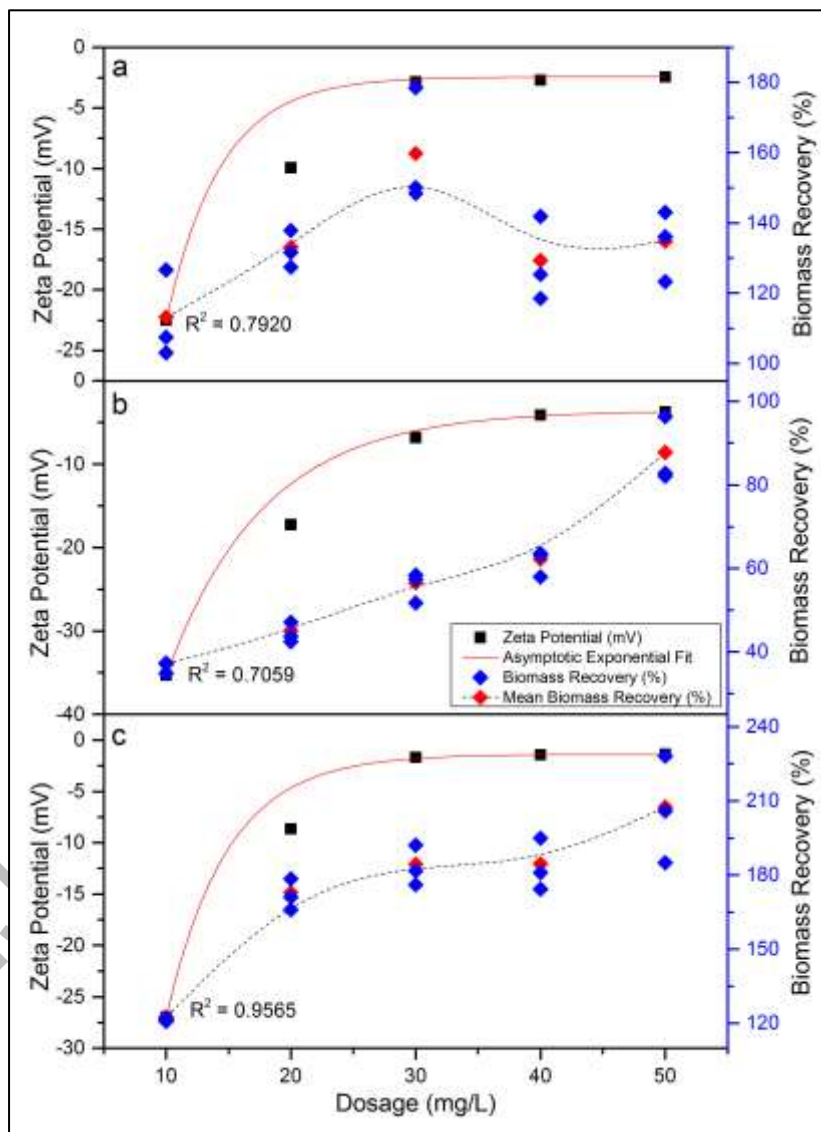


Figure 5. Correlation curves between zeta potential values and biomass recovery of aluminum sulfate (a), *M. oleifera* seed powder (b) and *M. oleifera* protein powder (c).

Conclusion

In addition 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. Indeed, both *M. oleifera* seed derivatives had a higher removal efficiency and biomass recovery as compared to alum even at low dosage. *M. oleifera* seed derivatives were also found to concentrate the density of harvested microalgae to about twofold 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. Further investigation on the interaction of the zeta potential of *M. oleifera* seed derivatives on the flocculation of different microalgae species is highly recommended. The use of *M. oleifera* seed derivatives as bio-flocculant could assist in lowering the economic cost and providing environmentally-friendly approach for mass microalgae cultivation and the sustainable development of bio-based renewable energy in the future.

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