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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