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Harvesting microalgae, *Chlorella* sp. by bio-flocculation of *Moringa oleifera* seed derivatives from aquaculture wastewater phytoremediation

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ABSTRACT

Moringa oleifera (MO) seed has been widely used for water treatment purposes due to their good flocculation, low cost and non-toxic characteristics. However, these advantages had not yet been utilized for the microalgae harvesting technology. Until today, the harvesting of microalgae biomass still depend on sophisticated and complex approaches such as hollow fiber filtration, chemical flocculants and Alfa Laval decanter technology. Thus, in this study the potential of natural plant-based coagulant was investigated. MO seed derivatives were determined for the harvesting of suspended freshwater microalgae, *Chlorella* sp.. Flocculation characteristics with various dosages were optimized. The output of this study proved that primary and tertiary MO derivatives yield excellent flocculation efficiency of more than 95% at 20 min sedimentation. In fact, MO derivatives even supersede chemical flocculants, Aluminum Sulfate in terms of flocculation efficiency and biomass recovery at a low dosage of 10 mg L^{-1} and normal pH (6.9–7.5). Seed powder had the highest removal efficiency whereas seed protein had the highest biomass recovery. Utilization of *M. oleifera* derivatives as bio-coagulant provides several advantages such as lower impact on the environment, lower cost for microalgae harvesting, allow rapid microalgae culture expansion and as chemical-free green technology.

1. Introduction

The utilization of microalgae for sustainable biofuel production is important because of the escalating price of petroleum fuel (Ahmad et al., 2011). Hence, many recent studies had focused on the harvesting and recovery of microalgae biomass from wide variety of cultivation medium and propagation scale (Granados et al., 2012; Zheng et al., 2012; Lee et al., 2013; Xu et al., 2013). Generally, commercial mass harvesting of microalgae biomass in majority relied on sophisticated and complex approaches such as hollow fiber filtration, high pressure membrane molecular sieving, chemical flocculation and Alfa Laval decanter technology (Christenson and Sims, 2011; Suali and Sarbatly, 2012). However, this approach had contributed to very high operational and maintenance cost due to the energy requirement for the machinery especially in massive scale (Bahadar and Khan, 2013; Razzak et al., 2013). As a result, mass production of microalgae only be justified in the case of production for expensive products such as drug precursors and pharmaceutical purposes (Gong et al., 2011; Lananan et al., 2013). Thus, the operational costs should be drastically decrease in order to make the commercial production feasible especially for low value, bulk biomass production such as for biofuel production (Quinn et al., 2012; Xu et al., 2013). Thus, to minimize the energy consumption of harvesting microalgae, an innovative and retrospective yet effective approach is required.

Coagulation–flocculation processes could provide mass microalgae biomass recoveries at a very reasonable costs (Ahmad et al., 2011). In this process, the selection of coagulant is crucial that the downstream processing of the biomass is not adversely affected by the coagulant contamination which then lead to the additional cost for biomass purification. Flocculation is one of the most convenient

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method for harvesting microalgae. Inorganic flocculants such as Ferric Chloride, $FeCl_3$ and Aluminum Sulfate, $Al_2(SO_4)_3$ including organic synthetic high polymer flocculants such as polyacrylamide derivatives and polyethylene amine have been used for the harvesting of microalgae biomass in the mass cultivation industry (Brennan and Owende, 2010; Şirin et al., 2011). Although Aluminum Sulfate commonly known as Alum, is proven to be an effective coagulant for some species of microalgae, coagulation-flocculation by metal chelate such as this is certainly unacceptable if the harvested biomass is used for aquaculture purpose, 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 (Ahmad et al., 2011). Hence, the use of natural coagulant would be an alternative in overcoming this possibility. The use of natural coagulants such as chitosan and starch in harvesting microalgae have been studied and shown to be effective in biomass recovery (Xu et al., 2013). However, the use of both natural coagulants is expensive and utilize potential food supply. In this study, the investigation was performed to explore the potential of cheaper natural coagulant which is *Moringa oleifera* (MO).

MO is known as a tropical plant which belongs to the family Moringaceae, a single family of shrubs. It is a tropical multipurpose tree that naturally grows in India, South Saharan Africa, South America and abundantly in Malaysia's climate. MO was reported to contain an active bio-coagulating compound. In addition, almost every part of the plant including leaves, flowers, seeds, roots and bark can be used as food or as medicinal and therapeutic purposes. Several studies have been done on the performance of MO seeds as an alternative coagulant and assisting coagulant to the conventional chemical coagulant in water treatment. In the underdeveloped country especially Africa, MO has been shown to be the most promising natural coagulant for raw water treatment with the potential usage on a large scale without adopting expensive technology. In Malaysia, MO is available locally and inexpensive hence making them a viable alternative in water and wastewater treatment. Thus, also possible as bio-flocculants in the microalgae biomass separation process and harvesting (Teixeira et al., 2012).

MO seed active compounds are known as the peptides of molecular weight ranging from 6 to 20 kDa, with an isoelectric pH value between 9 and 10 (Gassenschmidt et al., 1995; Ndabigengesere et al., 1995; Ndabigengesere and Narasiah, 1998; Lam and Lee, 2012). Bahadar and Khan (2013) reported that MO active compound is a polyelectrolyte in saline extract with a molecular weight around 3.0 kDa. In addition, Ghebremichael et al. (2005) had correlated the flocculation effect observed with a peptide obtained in saline extract whereas Lam and Lee (2012) purified and characterized a new lectin extracted in 0.15 M NaCl solution from MO seeds which was claimed to have flocculating activity. In this study, a laboratory investigation was carried out to determine the effects of physical parameters of rapid and slow mixing rate, flocculation dosage and pH on flocculation of suspended freshwater microalgae, *Chlorella* sp. with MO seed powder derivatives.

2. Material and methods

2.1. Preparation of MO primary seed derivatives

MO seeds were obtained within the region of Kuala Terengganu, Malaysia. 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 collected 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. The obtained MO seed powder was then stored in air-tight container and protected from moisture and light to avoid oxidation and light-degradation of its active properties. Cupule, seed coat and seed kernel powder were tested for its coagulation activity on the suspended freshwater microalgae, *Chlorella* sp. at various parameters such as dosage, pH, rapid mixing rate, slow mixing rate, mixing period and settling time.

2.2. Oil extraction from MO seed powder as secondary seed derivatives

Ethanol-based oil extraction procedure based on Kwaambwa and Maikokera (2007) was performed on the fine MO seed kernel powder. Ninety-five-percent ethanol were added in 1:10 ratio (1 g of seed powder and 10 mL of ethanol) to form suspension. Then, it was mixed using magnetic stirrer for 10 min to produce homogenous mixture. Supernatant was separated by centrifugation (300 rpm, 45 min) and the settled powder which was the de-oiled seed was dried at room temperature for 24 h. Both MO seed oil and de-oiled seed were subjected for the determination of coagulation efficiency on *Chlorella* sp..

2.3. Purification of MO coagulation polymer as tertiary seed derivatives

The procedures for the purification of MO coagulation polymer were carried out based on Kwaambwa and Maikokera (2007). Dried de-oiled MO 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 brown colored NaCl extract was collected. It was 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 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. This step is also known as protein desalination. 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. Following the delipidation procedure, the protein was dried at room temperature to form fine protein powder. Brown seed protein, white seed protein and coagulation protein polymer were tested for coagulation efficiency on *Chlorella* sp. with similar coagulation parameters.

2.4. Cultivation of freshwater microalgae, Chlorella sp.

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). Pure culture of *Chlorella* sp. was obtained from the microalgae culture collections of the Life Feed Culture Lab, Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia. Bold's Basal Medium was used for the growth and maintenance of the microalgae before inoculation into the culture vessel (5 L working volume, temperature 25 ± 2 °C, 24 h illumination at 3350 lumen). Cell density was sampled daily and measured using haemocytometer (Marienfield, Germany) to form calibration curve for the absorbance spectrophotometry. Two-hundred-milliliters sample from