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# Chitosan Flocculation-sedimentation for Harvesting Selected Microalgae Species Grown in Monoculture and Mixed Cultures

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Microalgae is an attractive feedstock for sustainable biodiesel production. The harvesting step of microalgae needs technology, which saves energy and time. One of the low cost strategies for addressing this problem is the use of flocculation-sedimentation process as an initial step. The aim of the present study is to evaluate the flocculation-sedimentation of selected microalgae species grown in monoculture (Nannochloropsis sp.) and mixed cultures (South Coast of Yogyakarta) using modified chitosan. The effect of flocculant dosage and sedimentation time that might affect the percentage of microalgae cell removal was investigated. Chitosan has proved to be highly effective for dewatering of the microalgae, Nannochloropsis sp. and Yogyakarta mixed cultures, with the optimum flocculation efficiency reaching over 72.09 % (25 ppm of chitosan dosage; 10 min of sedimentation time) and 87.25 % (25 ppm of chitosan dosage; 30 min of sedimentation time) of biomass removal. The characteristics of chitosan in term of high positive charge density and long chains allow the microalgae to aggregate to form flocs and settle to the bottom due to gravitational effect.

## 1. Introduction

The development of renewable energy is gaining attention due to the increasing concern over the depletion of petroleum resources (Sathish and Sims, 2012) and the increase in greenhouse gas emissions (Pradana et al., 2015). Biodiesel, one of the renewable liquid fuels (Huang et al., 2010), has been explored to meet the increasing demand for cleaner fuels (Xu et al., 2013). Other than its potential as renewable fuel, It also emits less carbon dioxide, sulphur and gaseous pollutants than petroleum diesel (Miao and Wu, 2006). Conventional biodiesel is derived from various feedstocks such as palm oil, which uses carbon-based catalyst (Raharningrum et al., 2013), rice husk, which uses ash-based catalyst (Chen et al., 2015), jatropha oil (Kusumaningtyas et al., 2016), soybean oil, which uses microwave (Encinar et al., 2012) and ultrasound systems (Brito et al., 2012), used cooking oil (Kawentar and Budiman, 2013), and palm fatty acid distillate, which uses char-based (Hidayat et al., 2015) and sulphated zirconia catalysts (Sawitri et al., 2016). However, large plantation area is required to produce biodiesel from conventional biomasses.
Recently, the utilisation of microalgae for sustainable biofuels production is of interest because of the escalating price of fossil fuels (Lei et al., 2015). Microalgae are photosynthetic microorganisms which require light, sugar, $CO_2$, nitrogen (N), phosphorus (P), and potassium (K) to grow to produce lipids, proteins and carbohydrates in large amounts over short periods of time (Demirbas, 2011). These photosynthetic microorganisms have been considered as a potential renewable fuel source (Nafis et al., 2015). They can be used as raw material for the production of biodiesel, biomethane, bioethanol, biohydrogen and biobutanol. These biofuels are viewed as the most promising alternatives to fossil fuels (Rawat et al., 2011) and are able to provide up to 25 % of global energy demand (Christenson and Sims, 2011). Microalgae have a number of advantages including faster growth rate (Minowa et al., 1995), and higher photosynthetic efficiency (Dote et

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al., 1994) and biomass production compared to other energy crops (Milne et al., 1990). Its cultivation process can be integrated with $CO_2$ removal from industrial flue gases and wastewater treatment process. Microalgae have the ability to assimilate nutrients and metals from wastewater, which plays an important remediation role during tertiary wastewater treatment phase (Barros et al., 2015). Nannochloropsis sp., one of prospective microalgae species grown in monoculture, is a widely available strain for commercial application. It exhibits great potentials as the future biodiesel feedstock due to its high growth rate and oil contents (Mitra et al., 2015). The other prospective microalgae species for biodiesel production is mixed cultures strain, such as mixed cultures microalgae from South Coast of Yogyakarta. The identified microalgae species of mixed cultures from South Coast of Yogyakarta are Cyclotella polymorpha and Chlamydomonas sp., which produce high yield from oil extraction process.

Several studies have been done on to recover microalgae from the growth medium during the harvesting step. Most microalgae species have a cell diameter of 1 - 30 μm and a low biomass concentration in culture of between 0.5 - 2 g/L (Xu et al., 2013). Microalgae slurry from the harvesting process must be dewatered prior to the oil extraction process. Efficient dewatering of biomass from cultivation medium is essential for the mass production of biofuels from microalgae. Traditional dewatering methods, such as centrifugation (Gerardo et al., 2015), filtration (Danquah et al., 2009) and electrocoagulation (Lee et al., 2013), require high energy input. A case study conducted by Chisti (2007) reported that the cost of the recovery process contributed about 50 % to the total cost of the biofuels production. These methods are too expensive for producing low-cost biodiesel. Flocculation, followed by sedimentation step, is a well-established method for removing suspended solids in water (Bratby, 2006), but is less developed for harvesting microalgae. The cell surface of microalgae has negative charge, which prevents them from adhering to each other by van der Waals forces (Ndikubwimana et al., 2015). The strategy is to use cationic flocculants to form large aggregates of microalgae that are heavy enough for sedimentation. Commonly used cationic flocculants are salts of multivalent metal ions (Gorin et al., 2015), such as ferric chloride and aluminium sulphate. The use of these inorganic flocculants requires low energy but can contaminate the harvested microalgae (Farid et al., 2013). The wastewater from the harvesting process cannot be recycled back to the cultivation stage. Cationic polymers are other widely used but are generally more expensive than metal salts. Bioflocculation is another alternative method for making flocs using chemicals produced by acceptable co-culture microorganism (Chatsungnoen and Chisti, 2016).

Chitosan is biopolymer obtained by deacetylation of chitin. It has been proven as an effective cationic flocculant for microalgae (Divakaran and Pillai, 2002). The properties of chitosan, including its cationic behaviour and molecular weight (Li et al., 2013), can assist in coagulation (particle aggregation induced by electrolyte addition) and flocculation (aggregation resulting from the linking of several particles by a polymer chain) processes. Unlike metal salts, chitosan is non-toxic, biodegradable and renewable (Renault et al., 2009). It causes little problem in the downstream processes for producing biofuels and recycling back the growth medium (Ahmad et al., 2011). The effectiveness of the flocculation step can be improved by modifying chitosan into modified chitosan, to have larger surface area and higher adsorption capacity (Zhang et el., 2016).

The aim of the study is to evaluate the flocculation-sedimentation of selected microalgae species grown in monoculture (Nannochloropsis sp.) and mixed cultures (South Coast of Yogyakarta) using chitosan. The effects of flocculants dosage and sedimentation time on the percentage of microalgae cell removal were investigated.

## 2. Methods

### 2.1 Materials

Monoculture-grown microalga used in this study was Nannochloropsis sp. obtained from Situbondo, East Java, Indonesia. Stock of mixed cultures microalgae was isolated from South Coast of Yogyakarta, Glagah, Yogyakarta, Indonesia. They were then cultivated in Biotechnology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta. The flocculants used in this study was chitosan.

### 2.2 Experimental

The chitosan was solubilised in a 0.1 M HCL solution and mixed with a stirrer at 100 rpm for 30 min to obtain 1 mg/mL of stock solution. The jar test was used to optimise the flocculation-sedimentation process and study the effect of flocculants dosage and sedimentation time. This study consisted of batch experiments, which used beaker glass, heater and stirrer. The beaker was filled with 50 mL of 3.36 g/L microalgae culture for each test run. The contents of each flask were simultaneously stirred at the same speed. The samples were mixed at 250 rpm for 1 min and then flocculated at 100 rpm for 5 min with different concentrations of chitosan (0 – 125 ppm) at room temperature. After flocculation, the samples were left to settle at varying sedimentation time (3 - 60 min). After sedimentation, 1 mL of each sample was collected from the middle of each respective

beaker. The concentration of biomass was measured using spectrophotometer at a wavelength of 682 nm (Farid et al., 2013). The percentage of cell removed was calculated using Eq(1).

$$\%\ \text{cell removed} = \frac{(I_{blank} - I_{sample})}{I_{blank}} \times 100\ \% \quad (1)$$

where $I_{blank}$ is the absorbance intensity of the reference culture without chitosan and $I_{sample}$ is the absorbance intensity of the sample after the flocculation-sedimentation process.

## 3. Results and discussion

### 3.1 Effect of chitosan dosage

Figure 1 shows the effect of chitosan dosage on the removal of microalgae cells at sedimentation time of 60 min. The highest percentage of mixed cultures microalgae cell removal was obtained at low concentration of chitosan. 88.79 % of cell removal was obtained at chitosan concentration of 25 ppm. After this point, the increase in chitosan concentration resulted in the percentage reduction of cell removal. The highest percentage of Nannochloropsis sp. cell removal was 73.03 %, which was obtained at chitosan concentration of 62.5 ppm. However, at chitosan concentration of 25 ppm, the resulted Nannochloropsis sp. cell removal of 72.40 %, is considered as an optimum removal percentage due to the insignificant increase in cell removal percentage as chitosan dosage increases.

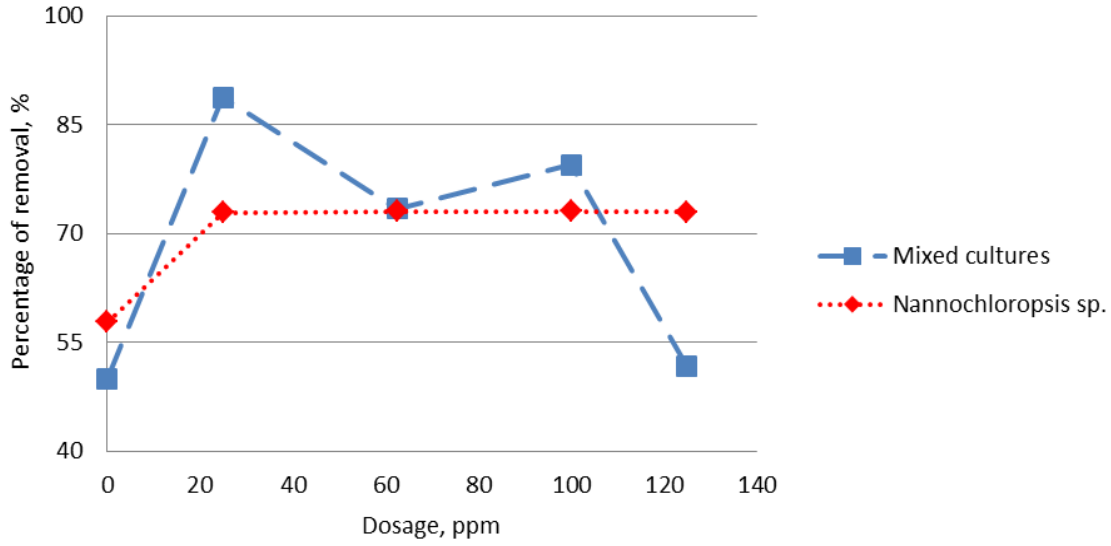


*Figure 1: Effect of chitosan dosage on the removal of microalgae cells*

The decrease in the cell removal of mixed cultures microalgae and constant cell removal of Nannochloropsis sp. during the harvesting process as chitosan concentration increased to more than the optimum point can be explained based on its properties mechanisms. The likely mechanisms involved in this flocculation-sedimentation process are adsorption and charge neutralisation. The positively charged chitosan is strongly attracted the negatively charged microalgae cells, which promotes flocculation by neutralising the charges (Ahmad et al., 2011). Chitosan continually attracted and destabilised the cells to form large aggregates at lower concentrations of chitosan. When the chitosan concentrations became higher than the optimum concentration, the excess cationic charges led to the restabilisation of the cells, which reduced the efficiency of the mechanism and hindered the formation of flocs.

### 3.2 Effect of sedimentation time

Generally, the sedimentation time depends on the floc size. An increase in floc size increases of free settling velocity driven by gravitational force which in turn, decreases the sedimentation time. Chitosan promotes faster aggregation of cells through charge neutralisation, which allows aggregated particle formation for faster settling.

Figure 2 shows the effect of sedimentation time on the removal of microalgae cells by chitosan at concentrations of 0 ppm (no flocculant), 25 ppm (low concentration) and 100 ppm (high concentration). The

flocculation-sedimentation of mixed cultures microalgae using chitosan interacted significantly with microalgae to form flocs for sedimentation time of less than 30 min. After 30 min, the liquid and solid had become nearly neutrally charged, causing only few flocs to form, resulting in fairly constant cell removal percentage. The optimum cell removal obtained was 87.25 % at the sedimentation time of 30 min and chitosan concentration of 25 ppm. Nannochloropsis sp. flocculation needed shorter time for sedimentation than mixed cultures. At 10 min of sedimentation time, the cell has been removed optimally due to the insignificant floc formation after this time. The optimum percentage of Nannochloropsis sp. cell removed was 72.09 % and obtained at 10 min of sedimentation time and 25 ppm of chitosan concentration.

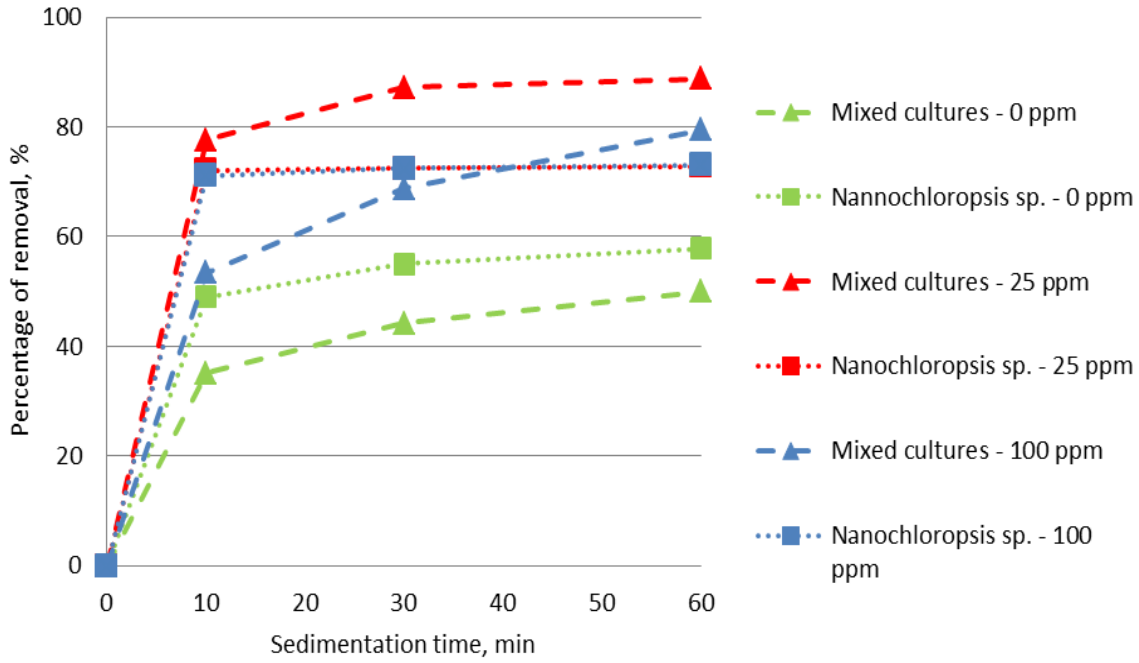


*Figure 2: Effect of sedimentation time on the removal of microalgae cells*

## 4. Conclusions

Chitosan has proved to be highly effective for dewatering of the microalgae, Nannochloropsis sp. and Yogyakarta mixed cultures, with the optimum flocculation efficiency reaching over 72.09 % (25 ppm of chitosan dosage; 10 min of sedimentation time) and 87.25 % (25 ppm of chitosan dosage; 30 min of sedimentation time) of cell removal. The characteristics of chitosan in term of high positive charge density and long chains allow the microalgae to aggregate to form flocs and settle to the bottom due to gravitational effect. This flocculation-sedimentation process saves energy and time for dewatering processes.

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