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UTILIZATION OF AUTO-FLOCCULATE OF MICROALGAE, *Scedesmus sp.* FOR HARVESTING OF FRESHWATER MICROALGAE, *Chlorella sp.* BIOMASS

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Abstract: Currently, Microalgae has contributed to the sustainable development of industries includes biofuel industry, the pharmaceutical industry, wastewater treatment industry and others. With increasing knowledge regarding a variety of microalgae uses results in high interest among people in the world to harvest microalgae. Harvesting of commercial microalgae is normally done by centrifugation, gravity filtration, ultrafiltration and other methods which require high costs. Therefore, the harvesting of *Chlorella sp* by bioflocculation method which involves autoflocculating microalgae, *Scenedemus sp* with lower cost was introduced. The removal efficiency >80 % and biomass recovery >75% were achieved for coagulation-flocculation of *Scenedesmus sp* to harvest *Chlorella sp*. Results of this study showed that the utilization of auto-flocculate microalgae in microalgae biomass harvesting could abridge downstream processing, redeemable resources and would reduce the production cost for future microalgae-based.

KEYWORDS: Sustainable microalgae, harvesting, chlorella, scenedesmus, aquaculture

Introduction

Microalgae become valuable products for the industries include biofuel industry, the pharmaceutical industry, wastewater treatment industry and others. This is due to the availability in producing numerous high-value products such as natural pigments, health food products, live feed for fish larvae, or a source of polyunsaturated fatty acids (Raja *et al.,* 2008; Spolaore *et al.,* 2006). Therefore, the harvesting of microalgae promotes high attention around the world. Mostly, the existing commercial methods use centrifugation for harvesting microalgae, but it requires high energy sources (Bleeke *et al.,* 2015). Gravity filtration method also one of the harvesting methods used to harvest microalgae but only suitable for the large size of microalgae such as *Arthrospira* (Becker, 1994). Smaller microalgae can theoretically be harvested using ultrafiltration. Nevertheless, it reduced the performance of membranes due to the extracellular organic matter contribute to the rapid fouling of membranes (Zhang *et al.,* 2010). Besides, microalgae can also be harvested using standing ultrasound waves, but need high energy and costly for the cooling systems of large-scale harvesting system (Bosma *et al.,* 2003). An integrated approach is required to diminish the energy consumption of harvesting microalgae. Therefore, an invention method has been recommended in harvesting microalgae, they called flocculation method. Flocculation is an extensively used process for microalgae harvesting, induced by inorganic or polymeric flocculants. Regardless of versatile applications of such flocculants, there are some restrictions with their use. Inorganic flocculants (e.g., alum and *ferric chloride*) are toxic and produce a significant amount of sludge, while polymeric flocculants are expensive to use.

Scenedesmus sp. is a genus of green algae whose coenobia are a flat plate of ellipsoidal to spindle-shaped cells that are arranged in a single, alternating or double series with their long axis parallel to one another. In contrast, *Chlorella sp*. is a single-cell green alga with spherical-shaped. *Scenedesmus sp*. has been identified as autoflocculation microalgae by Liu *et al.,* 2013 with the value of zeta potential that could be able to coagulate *Chlorella sp*. The identification of Scenedsmus sp. zeta potential possibly will fortify the proficiency

of the bio-flocculant in harvesting microalgae. Zeta potential can be defined as a magnitude measurement of the surface electrical charges of particles across phase boundaries. The value of zeta potential supports in predicting long-term colloidal stability and helps the researcher to produce trial formulations in a short period.

This study focused on the harvesting of *Chlorella sp*. by bioflocculation method which involves auto flocculating microalgae, *Scenedemus sp*. based on the removal efficiency and biomass recovery performance. *Chlorella sp*. was categorised as a biological tool in wastewater treatment for nutrient content reduction (Sydney *et al.,* 2010). Nutrient removal is crucial for aquaculture wastewater treatment to avoid eutrophication and for potential reuse of the treated water (Lam *et al.,* 2014). The subsequent biomass of *Chlorella sp*. as the by-product of the treatment process could be promoted as high-value products (Brennan and Owende, 2010). The potential of bio-flocculant microalgae, *Scenedesmus sp*. in harvesting microalgae *Chlorella sp*. discovered by determination of the zeta potential value. Zeta potential of *Chlorella sp*. and *Scenedesmus sp*. were determined at various pH to support the study of the flocculation performance of *Scenedesmus sp*. as bio-flocculant in harvesting *Chlorella sp*. biomass.

Materials and Methods

Cultivation of Freshwater Microalgae, Chlorella sp. and Scenedesmus sp.

Cultivation of microbial especially microalgae sp. is needed to observe the cells growth and to ensure the stationary and maturity phase of the cultured sp. In order to identify the cells growth of microalgae used in this study, *Chlorella sp*. and *Scenedesmus sp*. were cultured at Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu. Microalgae cultures maintained at a room temperature of about 25 ± 20C under the standard light intensity of 4100 lux from white fluorescent for 24 h in Bold's Basal Medium (BBM) solution (Nasir *et al.,* 2015). For upscaling purpose, the cultures were upscale in clear cylinder Perspex with a working volume of 20 L with a sterile condition to prevent any bacterial contamination. *Chlorella sp*. and *Scenedesmus sp*. were cultivated until stationary and maturity phases. Analyses of microalgae biomass were monitored daily by determination of the optical density at 686 nm using Dual-Beam UV-Vis Spectrophotometer (Shimadzu UV-1800, Japan).

Preparation of Alum Solution

Aluminium sulphate as an established coagulant was prepared and inoculated in microalgae culture. A stock solution of aluminium sulphate, supplied by Sigma Aldrich prepared by dissolving 10 g of dry solid in 1 L of deionized water. Then, the solution was stir-mixed until all solids dissolved. A fresh solution was prepared every day to obtain reliable results. The harvesting performance of alum was compared with the bioflocculant, *Scenedesmus sp*.

Zeta Potential of Chlorella sp. and Scenedesmus sp. Analysis

Zeta potential is a magnitude measurement of the electrostatic or charge attraction between particles and is one of the fundamental parameters known to affect stability. In order to investigate the interaction between *Chlorella sp*. and *Scenedesmus sp*., the surface charges were determined based on measurement of zeta-potential using Zeta Potential Analyzer (Zeta-Meter System 3.0þ) utilizing electrophoretic light scattering (ELS) (Brookhaven Instruments Corporation, USA). The experiment conducted at the Bio-medical Molecule Laboratory, Faculty of Civil Engineering, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The appropriate cell size for zeta potential analysis should be in the range of 0.5-100 mm. In fact, it is in line with this study where the size of microalgae was in between 5 and 50 mm. The different surface charges between these two types of microalgae could be the main reason for the agglomeration. It is widely known that *Chlorella sp*. carries a

negative charge in their cells (Henderson *et al.,* 2008). This circumstance shows that flocculation is employed to harvest microalgae when a positively charged component is applied. The microalgae cells can easily be harvested through the attraction forces derived from the opposite charges.

Flocculation of Microalgae Culture of Chlorella sp. and Scenedesmus sp.

Jar testing is a pilot-scale test of the treatment chemicals used in a particular water plant. It simulates the coagulation/flocculation process in a water treatment plant thus, helps operators determine the right amount of treatment chemicals to improve the plant's performance. The jar test was carried out to determine the appropriate flocculant concentration. The beakers were filled up with 400 mL water contained with microalgae, *Chlorella sp*. culture for each test run. Standard sedimentation jar test equipment will be used in this experiment to determine the amount of flocculant, which occurred under the different test conditions. The flocculant which contains *Scenedesmus sp*. will be applied at various pH. A rapid mixing period of 5 min at 150 rpm was then followed by a slow mixing period of 15 min at 30 rpm to allow flocculation to occur. The flocs were allowed to settle for 30 min before adsorption measurement of each sample will be taken. Colorimetric determination of *Chlorella sp*. cells will be measured at 686 nm using Dual-Beam UVeVis Spectrophotometer (Shimadzu UV-1800, Japan).

Results and Discussion

Growth Curve of Bioflocculant (Scenedesmus sp.)

Identification of the cells growth of *Scenedesmus sp*. was based on the growth curve plotting. This plotting was used to observe the maturity phase of the microalgae. Figure 1 illustrated the growth curve of *Scenedesmus sp*. in Bold Baisal Medium (BBM) within 18 days of cultivation. From the results, the duration of the lag phase was about two days, and the exponential phase was started at day 3rd until day 7th and takes about five days. It shows that the *Scenedesmus sp*. able to grow within a short period. Stationary phase started from day 7th and the cells showed no obvious decrement until day 18th. The maximum cells count and biovolume of *Scenedesmus sp*. cells in BBM are 4.54 x 10^5 cell/mL on day 11th. This circumstance is due to the limited availability of nutrients in the culture media to be consumed by the bio-flocculant. Therefore, *Scenedesmus sp*. on day 11th of cultivation was utilized in harvesting *Chlorella sp*. for the coagulation-flocculation assays.

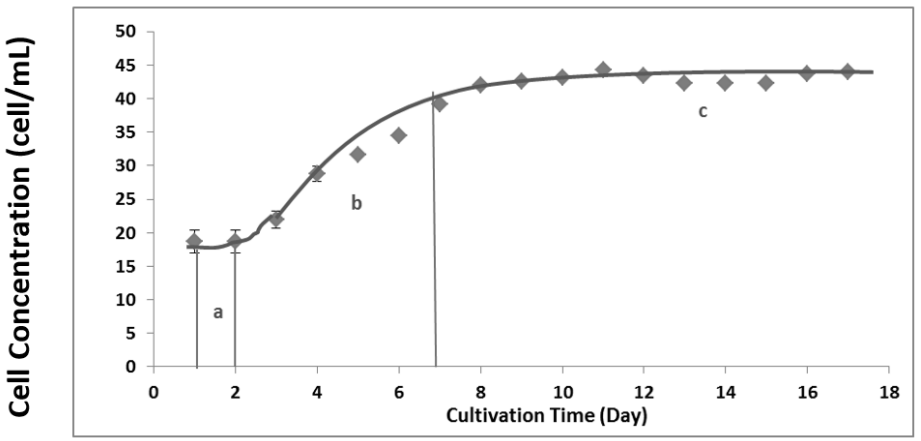

Figure 1: Growth curve based on cell number (x1000)/mL of *Scenedesmus* grown for 18 days culture.

Zeta Potential of Freshwater Microalgae, Chlorella sp. and Scenedesmus sp.

Zeta potential is a magnitude measurement of the electrostatic or charge attraction between particles, which used to evaluate the most appropriate range of pH in resulting high performance of attraction during the flocculation process. The zeta potential of the *Chlorella sp*. culture showed the isoelectric point (IEP) of the sample occurred in the range of pH 4 to 6.5 with the value of 20.00 to 20.90 mV. While, *Scenedesmus sp*. showed the isoelectric point (IEP) at pH 8.09 with the value of zeta potential 32.3 mV as shown in Figure 2. At this condition, the zeta potential of the *Chlorella sp*. and *Scenedesmus sp*. biomass were nearest to 0 mV. The value of pH beyond the range of pH 4.0 to 6.5 lead to the decreasing of zeta potential value less than -23 mV for *Chlorella sp*. contrast with the *Scenedesmus sp*. which will give the negative value with the increase of pH. The zeta potential high negative value indicates that the microalgae cells were well dispersed and very stable against aggregation. According to Abdul Hamid *et al.* (2014), isoelectric point indicates the point in which the colloidal stability was at its least steady state and had the maximum tendency to agglomerate. Almost all sources of surface water contain noticeable turbidity. Turbidity caused by the presence of particle within the size of $0.1e^{1}$ x 10^{6} mm which suspended within the water column. Particle sizes in the colloidal size range possess certain properties that prevent agglomeration which was known as zeta potential. In fact, pH becomes the most critical factor affected zeta potential value.

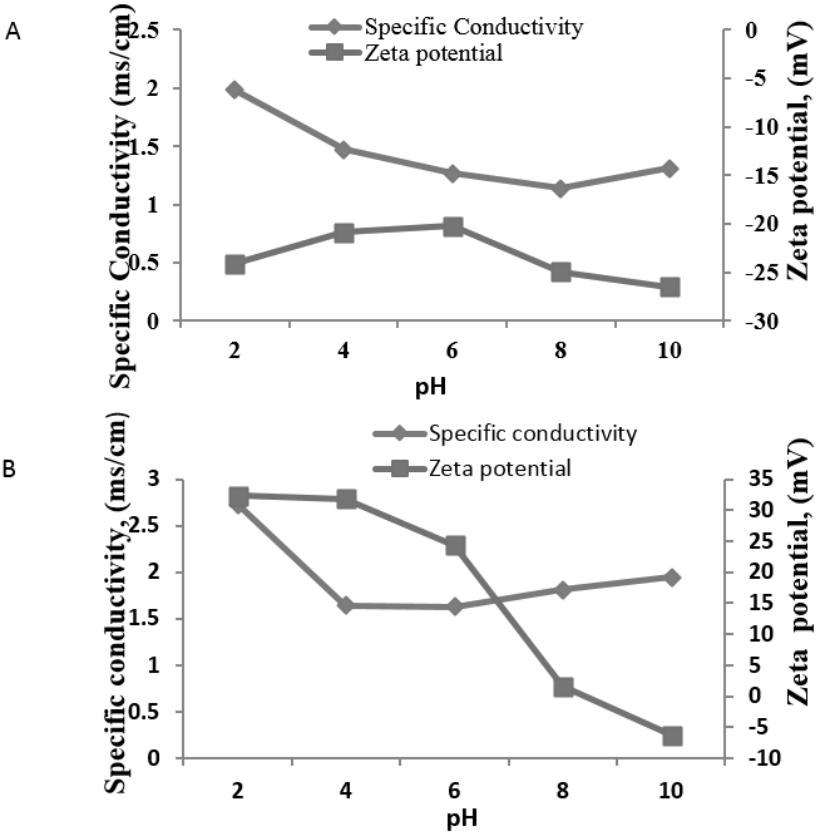

Figure 2: Zeta potential of microalgae culture corresponding to pH. (A) *Chlorella sp*. (B) *Scenedesmus sp*.

The concept of Coagulation-Flocculation Assay

Coagulation involves the neutralized negative surface charges of colloidal particles, while flocculation is the aggregation of neutralized particles followed by floc formation (Gutierrez *et al.,* 2015). Both types of cultured microalgae (as previously mentioned) used for this assay have different characteristics that allow them to coagulate together. *Scenedesmus sp*. has been observed to perform charge neutralization flocculation during the coagulation-flocculation assay in which any unoccupied surface of the positively charged bioflocculant. Naturally, *Chlorella sp*. is negatively charged and *Scenedesmus sp*. is positively charge (with zeta potential value 1.88mV and -28.1mV respectively). From the floc, jar testing showed that at pH 8-10 flocculation efficiency increased with the value >80%. This value was comparable with the chemical flocculant, alum solution which shows 80% flocculation efficiency. However, there are some restrictions with the chemical flocculant (alum solution) use due to the toxic content and produce a significant amount of sludge. Charge neutralization is the phenomenon in which charged ions, polymers or colloids strongly absorb on the oppositely charged surface of a particle, followed by destabilization, coagulation and flocculation. The electrostatic patch mechanism is the phenomenon in which a charged polymer binds to a particle with opposite charge. The polymer locally reverses the charge of the particle surface, resulting in patches of opposite charge on the particle surface. Particles subsequently connect with each other through patches of opposite charge, causing flocculation. Figure 3 shows the possible mechanism allows the attraction between the cells of *Chlorella sp*. and *Scenedesmus sp*. resulting in floc formation.

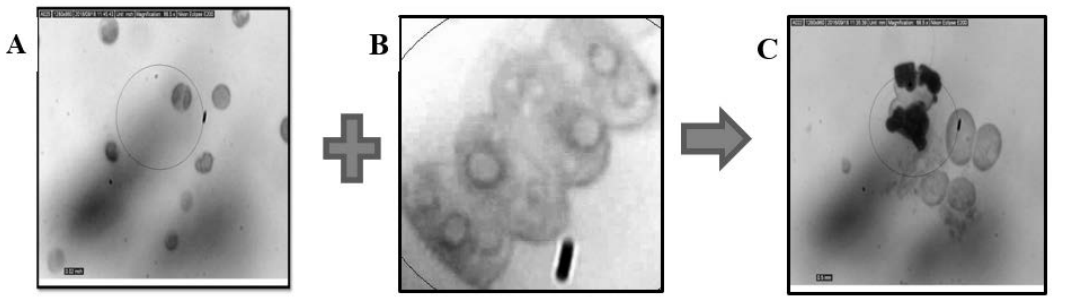

Figure 3: The mechanism likely to occur during coagulation flocculation assay of (A) *Chlorella sp*. and B) *Scenedesmus sp*. (C) Flocculation of *Chlorella sp*. and *Scenedsmus sp*.

Effects of Initial pH on Bioflocculation Efficiency of Scenedesmus sp. in Harvesting Chlorella sp.

The flocculation performance of *Scenedesmus sp*. cells increased as the pH of *Scenedesmus sp*. culture decreased. In this study, *Scenedesmus sp*. began to flocculate with the *Chlorella sp*. when pH decreased from 6 to 4, and about 51% flocculation efficiency recorded at pH 4 that has been illustrated in Figure 4. Further decrease in pH to 4.5, significantly increased the flocculation efficiency to 65%. However, the increase of pH between 8 to 10 give a high flocculation efficiency of greater than 80%. In fact, based on the result of zeta potential, *Scenedesmus sp*. showed the isoelectric point (IEP) at pH 8.09 which occur at this region of high-performance flocculation efficiency (pH 8 to 10). It is in accordance with the findings reported by Spilling *et al.* (2011) that autoflocculation commonly occurs in microalgae harvesting at pH above 9. The flocculation mechanism of microalgal cells mainly depends on the physical-chemical properties of a microalgal cell wall. Therefore, zeta potential measurement of microalgae during the flocculation process will significantly help to determine the flocculation mechanism. When the zeta potential is close to zero, particles can approach each other to a point where they

will be attracted by Van der Waals forces. When that happens, particles will aggregate and flocculation or coagulation will occur. These results demonstrate that the initial culture pH has a significant effect on *Scenedesmus sp*. autoflocculation efficiency. Biomass recovery increased with the increasing of settling time and pH as well. This is due to the relation to the degree of protonation at the chosen pH (pH 10). As a result, *scenedesmus sp*. showed an effective bioflocculant in harvesting *Chlorella sp*.

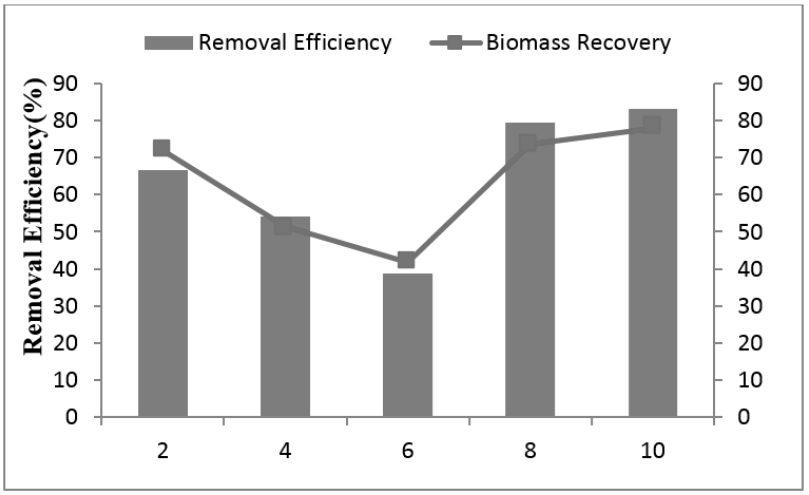

Figure 4: Flocculation performance sp. at 40% (v/v) *Scenedesmus sp*. Inoculation.

Conclusion

Auto-flocculating microalgae *Scenedesmus sp*. can be a promising media in harvesting microalgae. In this study, *Scenedesmus sp*. as bioflocculant was explored can give high performance in harvesting microalgae *Chlorella sp*. from the culture medium. The zeta potential of both microalgae and bio-flocculant were found affected by pH. The best performance flocculation was determined to be at the range of pH 8 to 10. The optimum pH was pH 10 at which the highest removal efficiency >80% and biomass recovery >75% achieved.

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