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Optimization of biomass harvesting of microalgae, *Chlorella* sp. utilizing auto-flocculating microalgae, *Ankistrodesmus* sp. as bio-flocculant

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ABSTRACT

Current microalgae harvesting technology depends on sophisticated and complex approaches such as hollow fiber filtration, chemical flocculants and centrifugation, which are deemed feasible if high value products were obtained. In this study the potential of auto-flocculating microalgae *Ankistrodesmus* sp. was examined for its potential as bio-flocculant to harvest *Chlorella* sp. Zeta Potential Analysis was performed on the microalgae in the pH range of 4.16–9.55 before subjecting the microalgae to the coagulation – flocculation assay. The isoelectric point of microalgae suspension was observed in the range of pH 5–8 and the magnitude of zeta potentials ranged between −10 mv to −35 mV. *Ankistrodesmus* sp. was inoculated at a dosage of 50% (v/v) into a batch of *Chlorella* sp. culture during the coagulation – flocculation assay. The removal efficiency shown by *Ankistrodesmus* sp. was 82% at pH 7.1 followed by 55% at pH 6.2. Utilizing *Ankistrodesmus* sp. for flocculation was efficient since it did not require any changes of pH because isoelectric point was observed within the normal pH of *Chlorella* sp. growth condition. Development of innovative microalgae treatment technology incorporating continuous bio-harvesting with microalgae-microalgae flocculation could provide a low-cost and sustainable wastewater treatment approach. In addition, the use of microalgae itself to harvest microalgae biomass harvesting could simplify downstream processing, saving resources and would reduce the production cost for future microalgae-based technology.

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1. Introduction

Modern aquaculture mainly consists of the rearing of aquaculture species intensively which allows the production of high density aquaculture species within very limited space (Lam et al., 2015). However, this leads to the release of highly concentrated wastewater to the environment as compared the traditional extensive aquaculture and this has led to higher risks of environmental pollution (Lam et al., 2008). Cao et al. (2007) reported that aquaculture is the major contributor to the increasing levels of organic waste and toxic compounds. Without proper treatment and management, aquaculture waste could potentially cause newly emerging diseases due to antibiotic resistance and algal bloom (Hegaret, 2008; Rubert, 2008).

The release of untreated wastewater poses serious environmental challenges to the receiving water bodies (Arora and Saxena, 2005; de-Bashan and Bashan, 2010). The major effect of releasing wastewater rich in organic compounds and inorganic chemicals is mainly eutrophication (de-Bashan et al., 2002; Godos et al., 2009; Mulbry et al., 2008; Pizarro et al., 2006). Denitrification plays a role in removing unwanted nitrates from the wastewater effluent, thus reducing the changes of the water discharged from treatment plants that can cause undesirable consequences such as algal bloom. The occurring of eutrophication in lakes and reservoir produces unsightly scums of algae on water surface and it can occasionally result in increased fish mortality by depriving it of oxygen as they use dissolved oxygen in their decomposition process.

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(Castine et al., 2013). This global problem could possibly be tackled by utilizing microalgae whereby the wastewater is utilized as feed for the maintenance of microalgae growth (Rawat et al., 2011).

It is crucial to develop an innovative and sustainable wastewater treatment system which is low-cost, effective and sustainable. There are several methods used in microalgae harvesting which includes centrifugation, filtration and coagulation – flocculation. Commercial systems mainly use centrifugation and filtration for harvesting but it is an expensive and energy intensive operation (Granados et al., 2012). Thus an alternative environmental-friendly approach is needed for low-cost microalgae biomass harvesting. Choosing an appropriate coagulant plays an important role in order to achieve an efficient harvesting of microalgae biomass. Coagulants that is widely used in water and wastewater treatment are inorganic coagulant (e.g. alum-based coagulant and iron salts) (Ndabigengesere and Subba Narasiah, 1998). Despite the capability of these inorganic coagulant to harvest microalgae with low cost, they could be harmful to human health and the environment and this has raised the interest to use organic coagulants to replace them (Ghebremichael et al., 2005). Therefore, the use of natural coagulant can be an alternative to overcome this problem. Salim et al. (2011) has reported the bio-flocculant activity of microalgae *Ankistrodesmus* sp. in treating water and wastewater hence the potential of this bio-flocculant to harvest *Chlorella* sp. could well be investigated.

Ankistrodesmus sp. is a green phototrophic microalgae that has a long crescent shape with slight curve at both ends. In contrast, *Chlorella* sp. is a spherical-shaped single-cell green algae. *Ankistrodesmus* sp. is known as an auto-flocculating microalgae with a shape and zeta potential that could have the ability to coagulate *Chlorella* sp. The determination of *Ankistrodesmus* sp. zeta potential may strengthen the competency of the bio-flocculant in harvesting microalgae. Zeta potential by definition is a measure of the surface electrical charges of particles across phase boundaries between solids and liquids. Knowledge on zeta potential aids in predicting long-term colloidal stability and helps in reducing time needed to produce trial formulations.

This research was motivated by the increasing interest in the application of biotechnology and the implementation of environmentally friendly tools in treating wastewater (Concas et al., 2010; Sato et al., 2010; Yoshimoto et al., 2005). *Chlorella* sp. was classified as a biological tool in wastewater treatment to reduce the presence of nutrient content (Sydney et al., 2010). Nutrient removal is essential for aquaculture wastewater treatment to protect natural waters from eutrophication and for potential reuse of the treated water (Lam et al., 2014). The resulting biomass of *Chlorella* sp. as the by-product of the treatment process could be marketed as high-value products (Brennan and Owende, 2010). The potential of bio-flocculant microalgae, *Ankistrodesmus* sp. in harvesting microalgae biomass of *Chlorella* sp. was explored by determination of the zeta potential value. Zeta potential of *Chlorella* sp. and *Ankistrodesmus* sp. were determined at various pH to support the investigation of the flocculation performance of *Ankistrodesmus* sp. as bio-flocculant in harvesting *Chlorella* sp. biomass.

2. Material and methods

2.1. Cultivation of freshwater microalgae, Chlorella sp. and Ankistrodesmus sp.

The microalgae culture used in this study consisted of freshwater *Chlorella* sp. and *Ankistrodesmus* sp. and they were obtained from the culture stock available at the Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu. Microalgae cultures were maintained at a room temperature of about 25 ± 2 °C under the standard light intensity of 4100 lux from white fluorescent for 24 h. Bold's Basal Medium (BBM) was selected for cultivation of freshwater microalgae, *Chlorella* sp. and *Ankistrodesmus* sp. (Nasir et al., 2015). For upscaling purpose, the culture were upscaled in clear cylinder Perspex with a working volume of 20 L. The cultures were continuously aerated with sterile-filtered air to prevent any bacterial contamination. *Chlorella* sp. and *Ankistrodesmus* sp. were cultivated until stationary and maturity phases. Analysis 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).

2.2. 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 was prepared by dissolving 10 g of dry solid in 1 L of deionized water. Then, the solution was stir-mixed to ensure all solids were fully dissolved. A fresh solution was prepared every day to ensure reliable results were obtained. The harvesting efficiency of alum was compared with the bio-flocculant, *Ankistrodesmus* sp.

2.3. Zeta-potential of Chlorella sp. and Ankistrodesmus sp. analysis

In order to investigate the interaction between *Chlorella* sp. and *Ankistrodesmus* sp., the surface charges were determined based on measurement of zeta-potential using Zeta Potential Analyzer (Zeta-Meter System 3.0+) utilizing electrophoretic light scattering (ELS) (Brookhaven Instruments Corporation, USA). The experiment was conducted at the Civil Engineering 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 μm. In fact, it is in line with this study where the size of microalgae was in between 5 and 50 μm.

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). Because of this characteristics, flocculation is employed to harvest microalgae by applying a positively charged component. The microalgae cells can easily be harvested through the attraction forces derived from the opposite charges.

2.4. Flocs jar test of Chlorella sp. biomass with Ankistrodesmus sp.

The jar test were carried out to determine the appropriate coagulant 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 testing program to determine the amount of coagulation, which occurred under the different test conditions. The coagulant which contains of *Ankistrodesmus* sp. will be applied at various pH. A rapid stirring period of 5 min at 150 rpm was then followed by a slow stirring 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. Colour will be measured at 686 nm using Dual-Beam UV–Vis Spectrophotometer (Shimadzu UV-1800, Japan).

3. Results and discussion

3.1. Concept of coagulation-flocculation assay

As previously mentioned, the two types of microalgae used for this assay have different characteristics that allow them to

coagulate together. Coagulation consists of neutralizing negative surface charges of colloidal particles, while flocculation is the aggregation of neutralized particles followed by floc formation (Gutiérrez et al., 2015). *Ankistrodesmus* sp. has been observed to perform bridging flocculation during the coagulation – flocculation assay in which any unoccupied surface of the positive charged bio-flocculant combines partly with the negative charged *Chlorella* sp. and thus bridging them and initiating clumping of bio-flocculant and microalgae. This is supported by Salim et al. (2011), who reported that positively charged polymers may bind partly or completely to microalgal cells and this can be termed as bridging flocculation and patching flocculation. A diagram of the possible mechanism as shown in Fig. 1 allows the attraction between the cells and resulting in floc formation.

In this research, *Chlorella* sp. proved to have negative surface charges in various pH by zeta potential analysis. However, the zeta potential for *Ankistrodesmus* sp. indicated that the positive surface charges at pH of 6.10–7.10. In this case, too alkaline or too acidic conditions have effect on the surface charges of the bio-flocculant. Therefore, it was shown from the result that *Ankistrodesmus* sp. has the ability to coagulate microalgae *Chlorella* sp. at pH ranging from 6.10 to 7.10. Further investigation on the capability of this bio-flocculant to neutralize negative surface charges and floc formation of *Chlorella* sp. was performed in the coagulation – flocculation assay.

3.2. Growth curve of bio-flocculant (*Ankistrodesmus* sp.)

Fig. 2 shows the growth pattern of *Ankistrodesmus* sp. within 20 days of cultivation. The two growth peaks indicates the maximum cell density of 1.11×10^6 cells mL^{-1} and 8.91×10^5 cells mL^{-1} at Day 7 and Day 16 respectively. This could be explained by the limited availability of nutrients in the culture media to be utilized by the bio-flocculant. The growth of the bio-flocculant started to decrease after 16th day of cultivation which indicated the occurrence of death phase. There was no obvious stationary phase exhibited in the growth curve of the bio-flocculant because the phase lasted in less than 24 h (Salim, 2013). Therefore, *Ankistrodesmus* sp. on Day 7 of cultivation were utilized in harvesting *Chlorella* sp. for the coagulation-flocculation assay.

3.3. Optimum dosage of *Ankistrodesmus* sp. in harvesting *Chlorella* sp.

Table 1 shows the performance in flocculating *Chlorella* sp. with seven different dosage inoculations of *Ankistrodesmus* sp. i. e 0, 10, 20, 30, 40, 50, 60 and 70% (v/v). Based on this table, there was no observable reduction of *Chlorella* sp. and turbidity at a low inoculation dosage ranging from 0 to 20% (v/v). However, the water sample was turbid and green which indicated the presence of suspended microalgae biomass. The highest removal efficiency of suspended *Chlorella* sp. biomass (74.1%) have occurred at inoculation dosage of 50% (v/v). The recovery of biomass (64.3%) was also determined based on the increase of microalgae biomass that reached the bottom of sedimentation tube (see Table 2).

3.4. Zeta potential of *Chlorella* sp. and *Ankistrodesmus* sp at various pH

One of the major factor affecting the harvesting of microalgae is the pH. In order to determine the effects of pH on the surface charges of microalgae, six different pH values were studied and monitored. The measurements of zeta potential is important to determine its isoelectric point of the suspension (Abdul Hamid et al., 2014). Fig. 3(a) shows the behaviour of the zeta potential of *Chlorella* sp. and *Ankistrodesmus* sp. In the range of pH 4.16 to 9.55, *Chlorella* sp. suspension exhibit negatively charged zeta potential value whereas *Ankistrodesmus* sp. shows positively charged zeta potential in the range of pH 6.10 to 7.10. The zeta potential of the *Chlorella* sp. culture showed that the isoelectric point occurred in the range of pH 4.16 to 9.55 in which the magnitude of zeta potentials were in the range of −10 mv to −35 mv (Henderson et al., 2008). According to Felder and Rousseau (2005), colloidal particles diameter ranging from 10^{-4} to 10^{-6} mm generally carries a negative electrical charge. The zeta potential of the *Chlorella* sp. biomass was near to 0 mV. Further decrease and increase of pH beyond the range of pH 4.16 to 9.55 led to the increase of zeta potential value exceeding −50 mV. The high negative zeta potential implies that

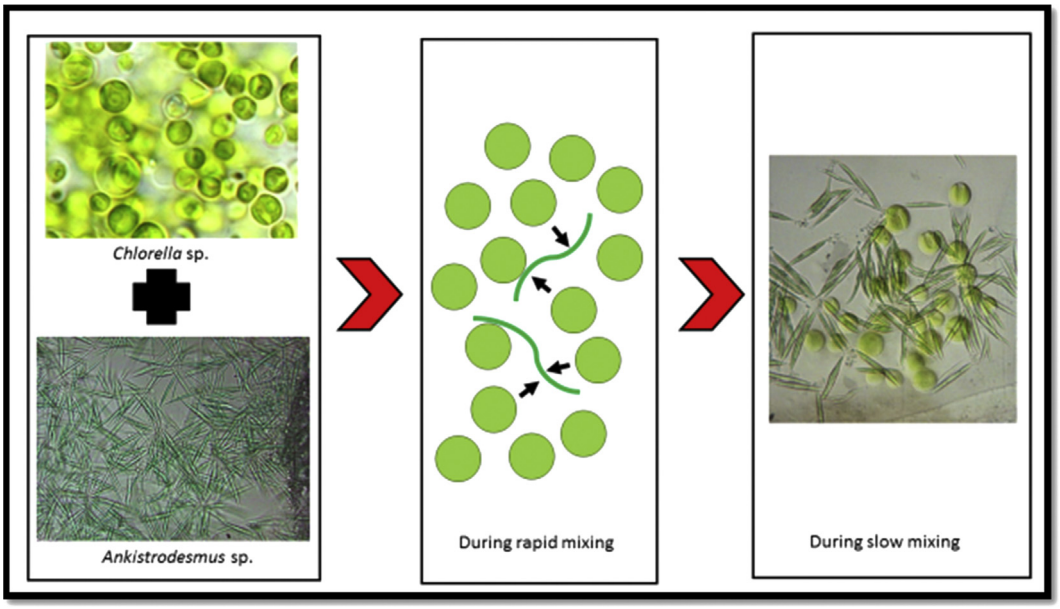

Fig. 1. The mechanism likely to occur during coagulation – flocculation assay of *Ankistrodesmus* sp. and *Chlorella* sp.

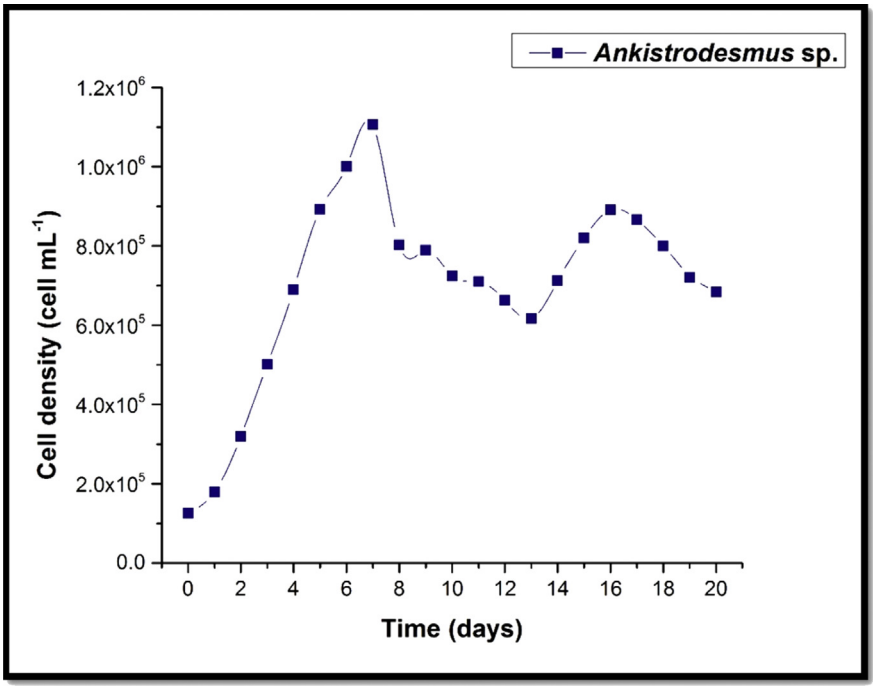

Fig. 2. Growth of bio-flocculant (*Ankistrodesmus* sp) cultured in 20 days.

Table 1
Cell density flocculated at various inoculation dosages of bio-flocculant (*Ankistrodesmus* sp.).

Inoculations dosage (v/v) %	Cell density flocculated by *Ankistrodesmus* sp.	
	Removal efficiency (%)	Biomass recovery (%)
0	2.10	0.04
10	16.09	5.3
20	32.12	10.7
30	42.19	14.1
40	56.76	44.34
50	74.18	64.26
60	69.50	60.15
70	60.10	53.19

the microalgae cells was well dispersed and very stable against aggregation.

According to Abdul Hamid et al. (2014), isoelectric point indicated the point in which the colloidal stability was at its least stable state and had the maximum tendency to agglomerate. In addition, *Ankistrodesmus* sp. exhibit positive zeta potential value within the *Chlorella* sp. isoelectric point range. Thus, mixing of both microalgae cultures could potentially leads to spontaneous coagulation – flocculation. The pH beyond the range of 4.16–9.55 had led to the increased of zeta potential and the stability of the suspended *Chlorella* sp. culture and finally the reduction in the probability for coagulation.

3.5. Flocculation assay of microalgae Chlorella sp. and bio-flocculant, Ankistrodesmus sp.

A dosage of 50% (v/v) bio-flocculant *Ankistrodesmus* sp. were inoculated into the microalgae suspension in order to harvest *Chlorella* sp. biomass. The dosage was adopted due to the colloidal stability between two types of microalgae occur at neutral pH. A variation of pH was performed to determine the optimum pH in order to produce the maximum flocculation efficiency. The coagulation – flocculation assay consists of rapid and slow mixing mode. The purpose of rapid mixing is to homogeneously mix both microalgae and bio-flocculant to ensure maximum contact incidents between them. Slow mixing provided sufficient energy and time for the formation of flocs. Unsuitable mixing mode would cause the breakage of the formed flocs and released the aggregated microalgae biomass back into the suspended forms.

As shown in Table 1, the highest removal efficiency and biomass recovery of the coagulation - flocculation assay were about 82% and 78% respectively that occurred at pH 7.10. Fig. 3 (a) and 3 (b), clearly indicate that the zeta potential played a major role in producing the highest removal efficiency and biomass recovery at the same range of pH (6.10–7.10). Removal efficiency and biomass recovery is the

Table 2
Cell density, zeta potential and flocculation of microalgae *Chlorella* sp. by *Ankistrodesmus* sp. as bio-flocculant.

pH	Microalgae, *Chlorella* sp.		Bio-flocculant, *Ankistrodesmus* sp.		Flocculation performance	
	Cell density (cell mL^{-1})	Zeta potential (mv)	Cell density (cell mL^{-1})	Zeta potential (mv)	Removal efficiency (%)	Biomass recovery (%)
4.16	$3.5 \times 10^7 \pm 4.0 \times 10^5$	−20.2 ± 2.6	$5.6 \times 10^6 \pm 2.7 \times 10^5$	−11.7 ± 2.4	–	0.02 ± 0.01
5.46	$3.2 \times 10^7 \pm 1.0 \times 10^6$	−24.9 ± 1.8	$5.4 \times 10^6 \pm 3.0 \times 10^4$	−0.2 ± 2.7	12.2 ± 3.0	4.1 ± 0.8
6.19	$2.9 \times 10^7 \pm 2.7 \times 10^6$	−26.5 ± 1.6	$5.3 \times 10^6 \pm 4.7 \times 10^5$	4.1 ± 1.9	55.0 ± 7.8	43.8 ± 7.0
7.10	$3.7 \times 10^7 \pm 1.6 \times 10^6$	−28.1 ± 2.5	$5.3 \times 10^6 \pm 1.5 \times 10^4$	5.1 ± 2.2	82.2 ± 7.6	77.8 ± 5.4
8.14	$3.2 \times 10^7 \pm 2.3 \times 10^5$	−33.7 ± 1.3	$5.9 \times 10^6 \pm 5.3 \times 10^4$	−5.7 ± 5.4	1.8 ± 0.2	0.4 ± 0.1
9.55	$3.3 \times 10^7 \pm 4.1 \times 10^5$	−38.8 ± 1.5	$5.0 \times 10^6 \pm 7.3 \times 10^4$	−15.3 ± 2.7	–	0.03 ± 0.02

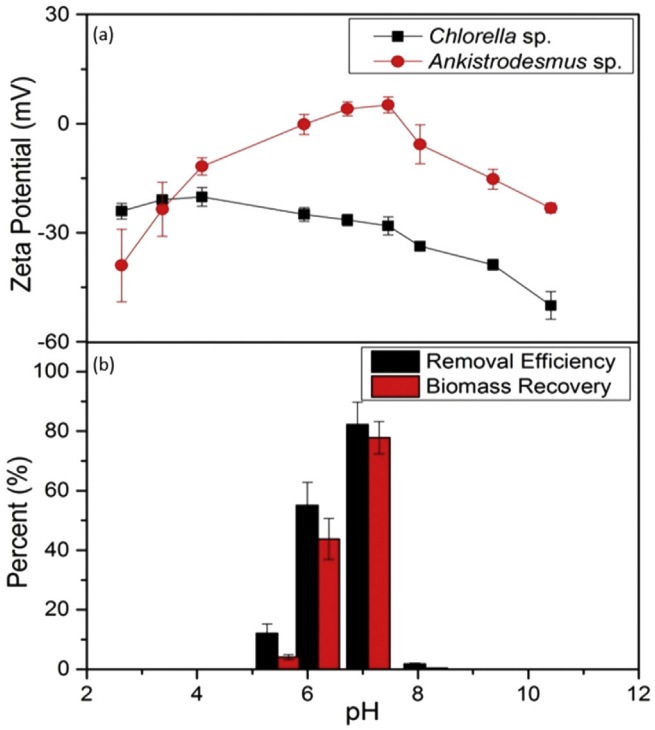

Fig. 3. Zeta potential (a), and flocculation performance (b) at 50% (v/v) *Ankistrodesmus* sp. inoculation.

measurement of flocculation performance in the assay. It was observed that the flocculation performance decreased as the pH shifted towards acidic and alkaline range.

It was previously mentioned that alum-based coagulant has been widely used in treating water and wastewater and thus could be applicable in harvesting microalgae. In this study, aluminium sulphate was used as a comparison to the bio-flocculant in harvesting *Chlorella* sp. biomass. At the same condition applied to the bio-flocculant, the coagulation - flocculation assay performed on alum showed a lower percentage in removal efficiency and biomass recovery compared to the use of the bio-flocculant. Therefore, the bio-flocculant is comparable with alum in terms of harvesting *Chlorella* sp. It was reported that the drawback of using alum is that it can cause cell lysis by means of rapid cell aggregation or cell membrane deterioration (Barros et al., 2015; Udom et al., 2013). These results suggest that the bio-flocculant could be a suitable candidate to replace chemical coagulant in harvesting microalgae biomass and also to increase the quality of the microalgal biomass harvested. Furthermore, it is possible to decrease the risk to human health and the environment using bio-flocculant.

Nowadays, the majority of mass microalgae cultivation relies on chemical flocculants where the process required additional steps and cost to separate the chemical flocculants from the harvested microalgae (Abdul Hamid et al., 2014; Barros et al., 2015). The addition of flocculants should not affect the final effluent quality and biomass reuse. However, chemical coagulants may have influence on both effluent and biomass by consuming alkalinity and decreasing pH (Gutiérrez et al., 2015). The use of bio-flocculant microalgae could simplify the downstream processing of microalgae biomass after harvesting since it could be processed directly with the same procedure as the harvested microalgae of interest. The utilization of *Ankistrodesmus* sp. could be costly at the initial stage but the mass cultivation of this microalgae could reduce the production cost and increase the viability of this approach for use in water and wastewater treatment, or as an efficient flocculant in microalgae biomass separation process.

4. Conclusion

An innovative harvesting mechanism was explored in this study utilizing auto-flocculating microalgae *Ankistrodesmus* sp. as bio-flocculant in harvesting microalgae *Chlorella* sp. from culture medium. The zeta potential of both microalgae and bio-flocculant were found affected by pH. The most suitable point to induce flocculation were determined to be in the range of pH 6.0 to 7.0. Coagulation - flocculation assay was performed to determine the flocculation performance of *Ankistrodesmus* sp. in harvesting *Chlorella* sp. *Ankistrodesmus* sp. was inoculated into *Chlorella* sp. culture at an inoculation dosage of 50% (v/v). The optimum pH was pH 7.10 at which the highest removal efficiency (82%) and biomass recovery (78%) were observed.

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