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Harvesting of microalgae *Scenedesmus obliquus* using chitosan-alginate dual flocculation system

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Microalgal biomass production cost at pilot scale could be reduced via reduction of harvesting cost. Among different microalgal downstream methods which include centrifugation, filtration gravity sedimentation, flotation and screening, flocculation is considered as an effective low-cost harvesting technique. Dual flocculants system is an advanced flocculation technique where two oppositely charged polymers mixed in microalgal culture to form polyelectrolyte complexes (PECs) leading to increase the harvesting efficiency. In this study, chitosan-alginate dual system was used as flocculation agent for the microalgae *Scenedesmus obliquus* at different pH levels. Dual harvesting system enhanced the flocculation efficiency. The highest flocculation efficiency (about 90%) was obtained due to addition of 30 ppm chitosan followed by 40 ppm sodium alginate to algal culture at pH 8. While more than 86% efficiency was achieved using only 10 ppm chitosan followed by 10 ppm sodium alginate for culture at pH6.

Keywords: Dual flocculation; Chitosan-alginate; Microalgae; *Scenedesmus obliquus*

INTRODUCTION

Microalgal biomass is considered as an important source for various bioproducts for medical, nutritional and biofuel applications (Vandamme et al.,, 2013; Atiku et al., 2016). The importance of pilot scale microalgae cultivation increased as well due to the elevated demand on its biomass. The main obstacle for the commercialization of microalgal biomass production is the high production and harvesting costs. Harvesting microalgal biomass is a costly process that needs to be reduced (Liu et al., 2013; Wan et al., 2015).

Among different microalgal downstream methods which include centrifugation, filtration gravity sedimentation, flotation and screening, flocculation is considered as an effective low-cost harvesting method (Anthony et al., 2013; Vandamme et al., 2013; Vermuë et al., 2014). Many flocculants either organic or inorganic substances are used to flocculate microalgal cells. This action depends on neutralization of cell surface charges or bridging cells (Li et al., 2016; Ummalyma et al., 2016). Chitosan is one of the most abundant biopolymers on the earth (Renault et al., 2009) and have tremendous applications in the fields of biotechnology, biomedicine, food processing and wastewater treatment (Yang et al., 2016). Application of chitosan was demonstrated as flocculent for many microalgal species, with different flocculation efficiency that depends mainly on the pH of the culture as well as application dose (Beach et al., 2012; Matter et al., 2016; Yang et al., 2016). The anionic polymer, sodium alginate, is a natural water soluble, low cost, polysaccharide that extracted from the cell walls of brown seaweed (Tripathy et al., 2001; Zhao et al., 2012). It can be cross-linked by ionic interactions (Patil 2008) and suggested to have coagulation behavior. Sodium alginate could increase the floc size when used in combination with polyaluminum chloride for wastewater treatment (Zhao et al., 2012; Vijayaraghavan et al., 2015). Despite the wide uses of sodium alginate in wastewater treatment either as flocculent or as adsorbent, few reports mention it for microalgae flocculation (Kumar et al., 2009; Zhu et al., 2014).

The so-called dual flocculation system consists of two or more oppositely charged polymers that added in sequence as flocculants, producing synergistic flocculation effects (Petzold and Schwarz 2014). Mixing two oppositely charged polymers (such as chitosan and sodium alginate) in an aqueous solution spontaneously form Polyelectrolyte Complexes (PEC) which could be used in flocculation systems (Petzold and Schwarz 2014; Sibaja et al., 2015). Produced polyelectrolyte complexes (PECs) properties affected by the properties of used polymers (chemical composition, ionic strength, flexibility and concentration) and mixing conditions i.e. pH, temperature and mixing order (Kulig et al., 2016). Few studies undertake the influence of combined flocculants for microalgae harvesting. Ma et al., (2016) developed a novel harvesting strategy for *C. vulgaris* using combined two flocculants, poly (γ-glutamic acid) and calcium oxide. They found increase in flocculation efficacy (up to 95%) following the dual flocculation system. Additionally, the order of flocculants addition affects the flocculation efficiency of the microalgae.

No previous reports were found for utilization of dual chitosan-sodium alginate as a combined flocculants for harvesting the fresh water *Scenedesmus obliquus*. The current study aimed to evaluate the dual application of chitosan and sodium alginate biopolymers for harvesting *Scenedesmus obliquus.* The effect of cultural pH on flocculation efficiency for the microalgae in dual flocculation system has been studied.

MATERIALS AND METHODS

Algal strain and cultivation condition

Scenedesmus obliquus NRCIbr1 (KY621475) was previously isolated, identified and characterized. Bold Basal Medium (BBM) (Barsanti and Gualtieri 2006) was used for cultivation of *S. obliquus* under continues illumination in an air bubble column photobioreactor. Culture used in flocculation study was collected during the late logarithmic phase at optical density (OD) of 1.24 at $Abs_{680\ nm}$. The OD before and after application of flocculants was measured using a spectrophotometer (SHIMADZU UV-2401PC, Japan) according to Lee (2008).

Preparation of flocculants solutions

Both flocculants, Chitosan and sodium alginate, were bought from Sigma-Aldrich (Sigma-Aldrich, Germany). A 1% solution from each flocculants in 1% acetic acid solution was freshly prepared at the same day of flocculation experiments.

Flocculation experimental design

The application of chitosan and sodium alginate individually or in sequence combination (Dual system) was evaluated as flocculants for harvesting *Scenedesmus obliquus* NRCIbr1 strain. The flocculation efficacy of different 6 concentrations (0, 10, 20, 30, 40 and 50 ppm) of each biopolymer was examined. All flocculation experiments were performed in test tubes contains 10 ml of *S. obliquus* culture in triplicates.

In dual treatments, the designated chitosan concentration was added then the tube vortexed vigorously for 5 sec before addition of sodium alginate. Finally, the tubes were vortexed for 5 sec then allowed to stand for an hour before sample collection. Sampling and calculation of flocculation efficiency were performed according to Matter et al., (2016).

All flocculation experiments performed at three different pH values i.e. 6, 8 and 10 frequently founds in microalgal cultures. Table (1) shows the different treatments at each pH level.

RESULTS AND DISCUSSION

Effect of pH on microalgae auto-flocculation

Auto-flocculation is a phenomenon that occurs in microalgae spontaneously in different degrees depending on different factors includes cultural age, pH, and densities (Becker 1994; Wan et al., 2015). To study the possibility of auto-flocculation of microalgae *Scenedesmus obliquus*, three different pH values were tested, *i.e.* pH 6, pH 8 and pH 10. Despite auto-flocculation could recover 75% of algal biomass within 10 min in case of *Chlorococcum sp.* under certain conditions (Ummalyma et al., 2016), the effects of different pH values on sedimentation of *S. obliquus* microalgal cells were very low under the current investigation conditions (Fig. 1). The maximum harvesting value reached 14.1% in case of pH 6 which was significantly higher than its value in culture with pH 8 (4.3%). Furthermore, the sedimentation after 1 hour in case of pH 10 did not exceed 8.2%. Similar results were recorded in a similar experiment on *Scenedesmus* sp. (Matter et al., 2016).

Table 1. Different treatments of chitosan and alginate separately or in combination

Treatment		Chitosan					
		0 ppm	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
Na- Alginate	0 ppm	0:0	10:0	20:0	30:0	40:0	50:0
	10 ppm	0:10	10:10	20:10	30:10	40:10	50:10
	20 ppm	0:20	10:20	20:20	30:20	40:20	50:20
	30 ppm	0:30	10:30	20:30	30:30	40:30	50:30
	40 ppm	0:40	10:40	20:40	30:40	40:40	50:40
	50 ppm	0:50	10:50	20:50	30:50	40:50	50:50

The first digit refers to Chitosan concentration (in ppm) while the second indicates the Na- Alginate.

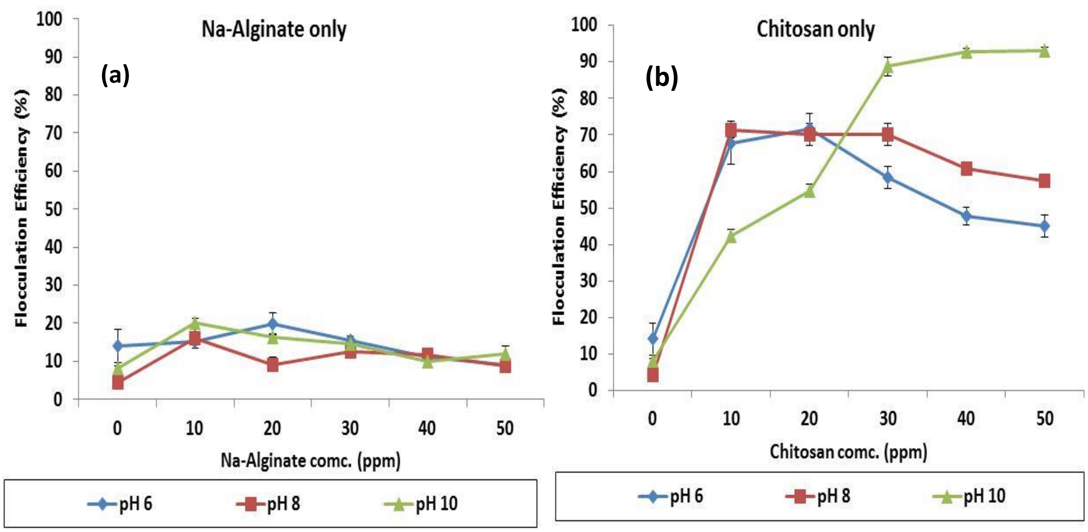

Figure.1. Effect of different chitosan and Na-Alginate concentrations on flocculation efficiency of *S. obliquus* at different pH levels.

In accordance with the obtained results, Zhu et al., (2014) revealed that the pH dependent flocculation efficiency did not exceeded 10% with increasing the culture pH up to 10. The auto-flocculation process could differ according to the type of microalgae and culture condition such as culture age, density, pH, etc (Morales et al., 1985). Generally, the lowest percentages of Auto-flocculation of microalgal cells in this case could be attributed to the negative charge on algal cells surfaces (Wyatt et al., 2012).

Harvesting of *S. obliquus* with individual flocculants (one step flocculation)

Chitosan is known as a flocculent that applied in the field of microalgae harvesting as well as wastewater treatment. Similarly, the anionic polymer sodium alginate is usually utilized to form a matrix for the encapsulation of living cells (Patil, 2008). Both polymers were used separately or in dual system to increase the floc size that improve flocculation efficacy. The results of the current study revealed that application of sodium alginate at concentrations of 10, 20, 30, 40 and 50 ppm to *S. obliquus* cultures at different pH values exhibited no effect on flocculation efficiency of the microalgal cultures (Fig. 1 a). Similarly, Zhu et al., (2014) studied the flocculation of microalgae *Picochlorum oklahomensis* using sodium alginate polymer which recorded the lowest flocculation efficiency compared to the other flocculants (chitosan and cationic starch) under the same investigation conditions. Flocculation efficiency of *S. obliquus* at pH 6, pH8 and pH 10 using different chitosan concentrations as a sole flocculent is presented in Figure (1 b). In the culture with pH 6, the concentration of 10 ppm chitosan displayed a 67.6% flocculation efficiency of algal biomass. This flocculation percentage slightly increased (to reach 71.5%) due to doubling chitosan concentration then decreased sharply reaching the level of 45% at 50 ppm chitosan treatment. In a similar study conducted by Xu et al., (2013) on flocculation of the microalgae *C. sorokiniana,* the concentration of 10 ppm chitosan per gram algal biomass resulted in a 99% flocculation percentage at pH 6. On the other hands, another investigators recommended 100 ppm chitosan to obtain the highest flocculation efficiency at the same pH values for different microalgal species (Danquah et al., 2009; Beach et al., 2012).

In case of pH 8, the maximum flocculation (71.5%) occurred at 10 ppm chitosan and this percentage slightly decreased to 70.2% when chitosan concentrations increased to 20 and 30 ppm. A further decrease in flocculation efficacy to reach 60.8% and 57.3% was documented with rising chitosan levels to 40 and 50 ppm, respectively. This results supported by those of Gerchman et al., (2017) who concluded the decline in flocculation efficiency as a flocculent with increasing its concentration over the 10 ppm level especially at pH lower than 10. Furthermore, elevating the culture pH to 10 was required higher chitosan concentrations to improve sedimentation effectiveness. The lowest harvesting level (42.3%) of cells was due to addition of 10 ppm chitosan and this percentage of harvesting increased contentiously with increasing the chitosan dose to peak (93%) at 50 ppm chitosan.

Flocculation efficiency using ionic flocculants was mainly affected by cultural pH which affects charge neutralization and bridging effects (Yang et al., 2016). Similar observation recorded in a study conducted by Zhu et al., (2014) on *Picochlorum oklahomensis* who indicated the maximum flocculation (70%) occurred at 40 ppm chitosan while no significant increasing observed when chitosan concentration raised up to 60 ppm. The varied flocculation effect of chitosan responding to changes in pH level could be clarified as a result of variance the characteristic of algal strain including surface charge and cell morphology, type of culture medium and growth conditions (Chen et al., 2014).

Effect of dual application of chitosan and alginate on flocculation efficiency of *S. obliquus* at different pH values

The application of the two biopolymers chitosan and alginate were studied to evaluate their harvesting efficacy in a dual flocculation system. The preliminary experiments showed that no positive flocculation was observed when a mixture of chitosan and sodium alginate was used as a flocculent or when sodium alginate added first followed by the addition of chitosan. Furthermore, the results were promising when alginate was added after chitosan. Thus, the current study was focused only on the flocculation due to sequential addition of chitosan followed by alginate. The obtained data reveals better results for the dual flocculation system compared to the individual sedimentation by either chitosan or Na-alginate. The flocculation efficacy varied depending on the pH level of the culture as well as the concentration of each flocculent.

Cultures at pH 6

The flocculation efficacy of dual application of Na-alginate and chitosan with different concentrations at pH 6 is presented in Figure (2). The addition of 10 ppm Na-alginate to algal culture after addition of 10 ppm chitosan was caused a high flocculation ratio (86.4%). The flocculation efficiency percentage decreased with increasing the chitosan concentration to reach the lowest value (25.2%) in case of 50 ppm chitosan. The same trend was observed when 20 ppm sodium alginate was added after chitosan application. Where the highest flocculation percentage (75.3%) was recorded for 10 ppm chitosan treatment, the precipitation percentages continuously decreased with rising chitosan concentration until it reached 19.6% in case of 50 ppm chitosan. Moreover, the addition of 30 ppm Na-Alginate to algal culture after addition of 20 ppm chitosan displayed 88.2% flocculation efficiency while it was 62% and 76.9% with 10 ppm and 30 ppm chitosan, respectively. The harvesting percentages were sharply decline when 30 ppm Na-Alginate was applied to cultures received 40 and 50 ppm chitosan (34.5 and 25.8 %, respectively). A similar trend was observed for 40 ppm sodium alginate treatment when applied the different chitosan concentrations under study condition. Whereas the highest flocculation efficiency (87.2%) was recorded with 20 ppm chitosan followed by 85.4% at 30 ppm level, the lowest percentage of sedimentation was observed with 50 ppm chitosan. The highest sodium alginate concentration under this investigation (50 ppm) following 30 and 40 ppm chitosan produced 87.9 and 80.4% flocculation, while the percentage were lower in other chitosan concentrations.

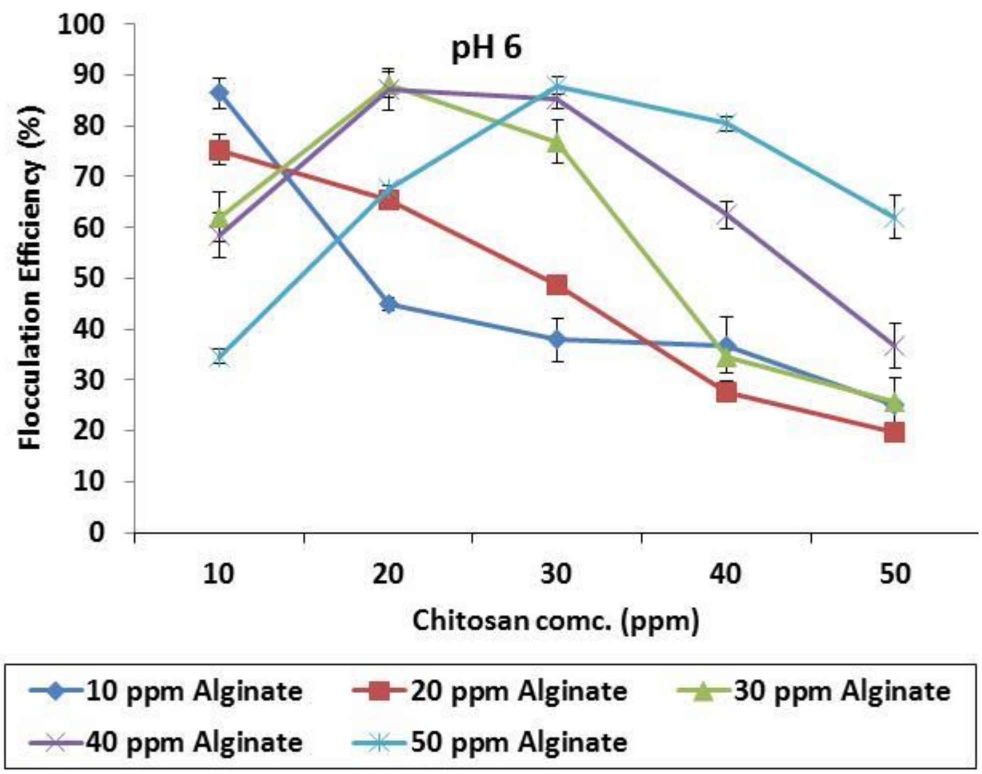

Fig. 2. Flocculation efficacy of *S. obliquus* using dual chitosan-alginate system at pH 6.

As a general trend, the combination of both alginate and chitosan improved the flocculation efficiency at pH 6 over the single flocculent application with more than 20%. The main flocculation trends of algal cultures with pH 6 due to addition of chitosan followed by sodium alginate revealed that the highest flocculation ratios occurs when relatively equal quantities of chitosan and alginate were used. The highest flocculation efficiency due to dual application of 10 ppm of both chitosan and alginate could attribute to the formation of polyelectrolyte complexes (PECs) that have unique characteristics aid to efficient flocculation. Myrnes (2016) stated that acidic conditions (pH range 3.5 to 6.5) could be ideal for alginate-chitosan polyelectrolyte complexes formation, where both polymers are soluble. Sæther et al., (2008) reported that the particle size of produced PECs affected mainly by charge ratio and the molecular weight in the mixture. At charge ratios close to one (neutral), the sizes of the largest particles were less reproducible. The smallest particles were obtained by adding one of the two polymer solutions into an excess amount of the other solution and by using forms of the component polymers with low molecular weights.

Cultures at pH 8

The pH of microalgal cultures usually tended to be alkaline, and the pH 8 is a normal pH value for many algal species. Increasing the culture pH from 6 to 8 improved the flocculation efficiency of the dual sedimentation system (Fig. 3)

The addition of 10 ppm chitosan to *S. obliquus* culture at pH 8 followed by addition of 10 ppm sodium alginate resulted in a flocculation rate of 82.8%. As the percentage of chitosan increased, the percentage of flocculation decreased until it reached 48.3% when 50 ppm chitosan was used.

In case of 20 ppm alginate added to cultures after addition of chitosan, 10 ppm chitosan cause flocculation in a percentage of 68.7% and this percentage slightly increased (reached 72%) due to increasing in chitosan concentration to 20 ppm and recorded the highest flocculation ratio (86.6%) with 30 ppm chitosan.

Increasing chitosan dose from 10 ppm to 20 ppm after addition of 30 ppm alginate cause an increase in flocculation of algal culture from 69.5 % to 86.9 %. The increment in flocculation efficacy was slightly lower (to reach 88.2%) when chitosan dose elevated to 30 ppm.

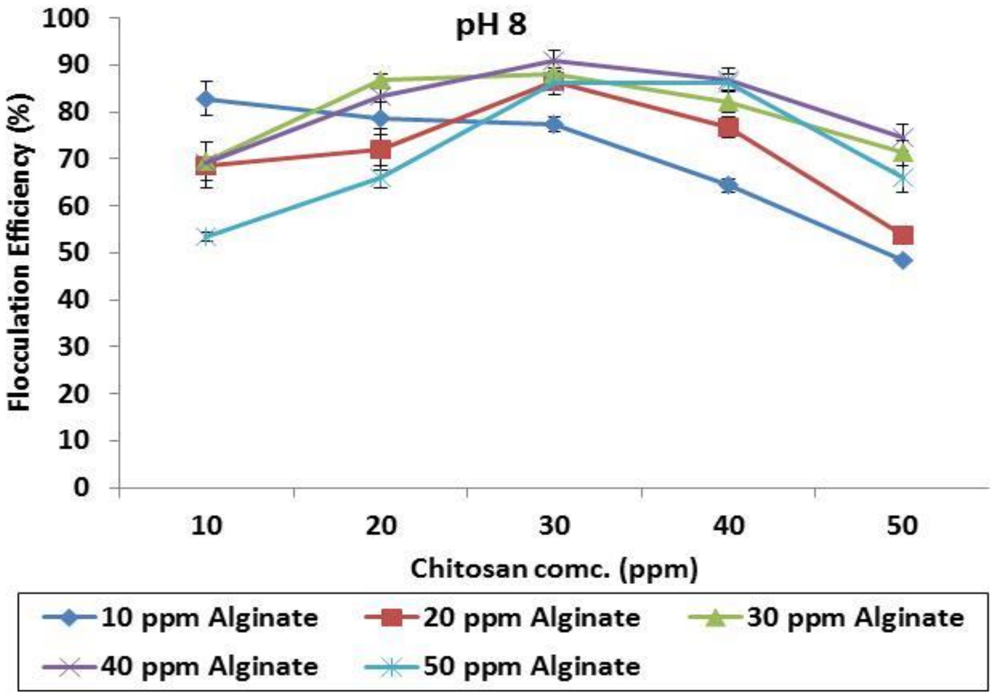

Fig. 3. Flocculation efficacy of *S. obliquus* using dual chitosan-alginate system at pH 8.

On the other hand, applications of chitosan at higher concentrations (40 and 50 ppm) decrease the flocculation percentage of algal culture when combined with 30 ppm alginate at pH 8 (82.2% and 71.2% respectively).

A similar trend was recorded in case of 40 ppm alginate, a 69.2% flocculation efficiency occurred in case of 10 ppm chitosan which increased to 83.3% and 90.9% with applying 20 and 30 ppm chitosan, respectively. The percentage of flocculation decreased again to 86.8% and 74.6% in case of 40 and 50 ppm chitosan, respectively.

The Addition of 50 ppm alginate after 10 ppm chitosan caused a flocculation percentage of 53.4%, this percentage increased with increasing chitosan concentrations to reach 86.4% when 40 ppm chitosan was applied. On the other hand, the percentage of flocculation reduced to 66.2% in case of 50 ppm of both polymers was utilized.

Changing the flocculation trend of the same microalgal cultures using the dual system of chitosan-alginate flocculants due the increases of pH from 6 to 8 could attributed to the changes in the size and surface charges surrounded the formed complexes (Hamman 2010). Sæther et al., (2008) founded that increasing pH of the PEC mixture above 7 leads to a significant increase in particle size, while the PECs were very stable over a temperature range of 4–37° C.

Cultures at pH 10

It is very important to study the flocculation effects of alginate and chitosan in microalgal cultures at high pH value, in many cases, microalgal cultures became highly alkaline and the pH value reaches 10 and sometimes 11.However, the trend of flocculation efficiency for microalgal culture under investigation at pH 10 was completely different from acidic and lightly alkaline cultures using dual system flocculants (Fig.4).

At this high pH, the flocculation efficiency of all alginate concentrations under study conditions tended to increase with increasing chitosan concentrations (Fig.4). The obtained data exhibited that the highest sedimentation percentage was achieved at 50 ppm chitosan regardless the applied alginate level. The flocculation efficacy ranged from 18.3% and 27.8% at 10 ppm chitosan to reach its peak (73.8% - 80.4%) at 50 ppm chitosan level. At pH 10, chitosan as single flocculation agent showed higher harvesting percentage, in comparison to dual flocculent system, which reached 93% at 50 ppm treatment (Fig.1 b).

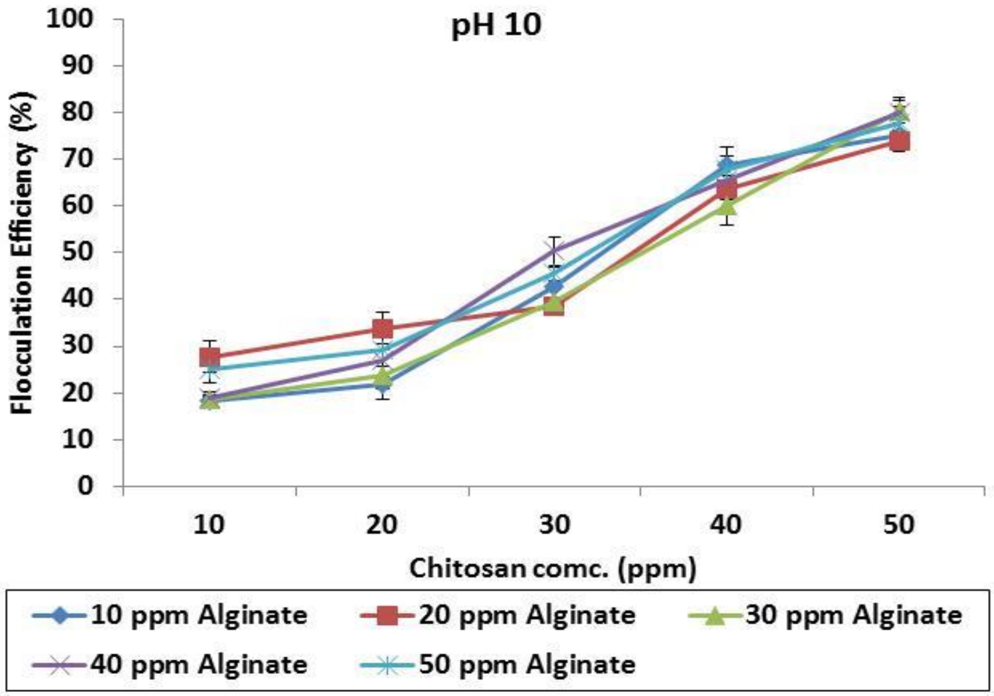

Fig. 4. Flocculation efficacy of *S. obliquus* using dual chitosan-alginate system at pH 10.

The obtained data could be explained as sodium alginate lost the ability to form polyelectrolyte complexes (PECs) with chitosan at high alkaline solutions. This deduction is compatible with the findings of Sibaja et al.,, (2015), who documented that the PECs keeps its stability at lower pH values. Furthermore, Zhu et al., (2014) study the flocculation effects of chitosan, sodium alginate and cationic starch, and stated that no flocculation effect occurred due to addition of sodium alginate or cationic starch at alkaline conditions (pH 8-10).

CONCLUSION

Auto-flocculation of *Scenedesmus obliquus* microalgae could be increased by increasing the cultural pH but the flocculation efficiency is not dependable in all cases. Despite sodium alginate could be used as flocculating agent in many cases; it did not achieve any flocculation effect on the microalgae under investigation conditions when it used individually. Chitosan as flocculating agent was a suitable means for microalgae harvesting and its flocculation efficiency could be enhanced by dual system of chitosan and sodium alginate. This research could be (according to our knowledge) the first investigation to utilize dual system chitosan-alginate to form polyelectrolyte complexes (PECs) which enhance microalgae flocculation in acidic and slightly alkaline cultures
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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all authors. All authors participated in planning, designing and doing the experiments. Additionally, all authors contributed in data analysis as well as writing and preparation of the manuscript.

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