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Chitosan as a Flocculant: An Approach to Improve its Solubility for Efficient Harvesting of Microalgae

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Abstract – Chitosan is a promising flocculant for microalgae harvesting, but its scale-up application is not economically supported yet. Low solubility of chitosan in microalgae suspension demands high dosage (as a flocculant) to destabilize the cells, and thus, increases the cost of microalgae harvesting. This study identifies efficient solvents for the chitosan, and optimizes the concentration of solvents and chitosan dose to improve the harvesting efficiency. Chitosan was dissolved in different acids, and subsequently used as a flocculant. The flocculant efficacy was measured in terms of harvesting efficiency and reduction in chemical oxygen demand (COD) of the microalgae suspension. It was found that chitosan dissolved in 0.05 M HCl showed the highest harvesting efficiency (89 ± 0.87%) at only 30 mg/L of dosage. In comparison, 270 mg/L of $FeCl_3 \cdot 6H_2O$ was required to attain 86 ± 0.083% of the harvesting efficiency. H_2SO_4 dissolved chitosan required high flocculant dose (150 mg/L) and resulted in relatively low harvesting efficiency (77±0.11%). It was concluded that the efficacy of chitosan is solvent dependent, and the selection of proper solvent can decrease the dosage requirement for microalgae harvesting.

Key words: Microalgae, Harvesting, Chitosan, Flocculation, Solubility

1. Introduction

Microalgae have received significant attention as an alternative feedstock for biofuel production [1,2]. Microalgae-based biofuel production involves many challenges from species selection to oil extraction [3]. Harvesting is one of the major challenges due to the dilute and stable nature of microalgae suspension. Typically, microalgae culture density is 2~4 g/L, and cells size (2~10 um). It needs to be concentrated 10~15 times to extract metabolites out of it [4-7]. A number of techniques including centrifugation, filtration, flotation, and sedimentation have been developed to harvest microalgae. Unfortunately, none of them has proved economical yet [8]. In this perspective, flocculation can be the promising approach to harvest microalgae [9]. In flocculation, microalgae suspension is destabilized by the aid of an oppositely charged flocculant. The particles interact with each other, their size and density increase over time, which forces them to settle via sedimentation [10]. Several inorganic flocculants like ferric chloride, ferric sulfate, and aluminum sulfate have been used for harvesting [11,12]. However, inorganic flocculants pose high cost, contaminate the microalgae biomass, cause excess concentration of ions in the supernatant, and produce large volume of sludge [12].

Alternatively, chitosan, a natural polymer, may be the promising choice. It offers unique advantages over other flocculants [13,14]. Chitosan is non-toxic, biodegradable, and does not interfere with downstream processing of biomass. Chitosan shows higher COD (chemical oxygen demand) removal efficiency (of microalgae supernatant) than the other flocculants. Chitosan causes no deleterious effect on fatty acids profile of microalgae. It can be recovered by growing microalgae in the spent media [7-8,19]. It has high charge density, long chain of polymers and excellent chelating properties. However, its chelating properties depend on its solubility. Chitosan is insoluble in water or organic acids. Acetic acid is the most commonly used solvent for the chitosan [6,12]. Huang *et al*. have shown that acetic acid increases the organic content of the suspension, which can affect its flocculation potential [8]. They also indentified that the solubility of chitosan depends on the type and concentration of the solvents [12,19,22]. High concentration of the solvent can reduce the solution viscosity, which negatively impacts its chelating ability. Moreover, the use of concentrated solvents is not affordable from an economical viewpoint. Our previous investigations have shown that the solubility of chitosan impacts the harvesting efficiency of microalgae [12,15]. In a poor solvent, chitosan does not mix properly, results in low harvesting efficiency, and demands high dose (as a flocculant). Ultimately, the overall cost of microalgae harvesting increases. Chitosan is a promising flocculant, but its scale-up application for microalgae harvesting is not economically supported (65 USD/Kg of chitosan) [7]. One

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way to reduce the cost is to improve the solubility of chitosan by selecting proper solvents, optimizing its concentration, and achieving harvesting at low dose. Thus, this study was carried out to experimentally verify the aforementioned concept. The chitosan was dissolved in different concentration of acids. The mixture was used as a flocculant and its impact of microalgae harvesting was investigated. The effect of chitosan dose (in the mixture) on the harvesting efficiency was also observed.

2. Materials and Methods

2-1. Sample collection

Freshwater mixed culture microalgae samples were collected in 5 L plastic bottles from a natural lake located in Havelian city of District Abbottabad, Pakistan. The samples were immediately stored at 4 °C. The samples were transferred to room temperature (25 °C) about 5~6 hours prior to use.

2-2. Preparation of chitosan solution

Chitosan was obtained from MP Biomedicals IllKirch, France. 250 mg dry weight of chitosan was mixed in 250 ml of 0.05 M, 0.1 M and 0.2 M of each HCl and H_2SO_4 solution with continuous stirring by magnetic stirrer on a hot plate. Stirring was done till chitosan was completely dissolved in the solution. 0.05 M, 0.1 M, and 0.2 M solutions of HCl and H_2SO_4 each, were prepared in 250 ml flasks; 0.003 M of $FeCl_3 \cdot 6H_2O$ solution was also prepared by dissolving its corresponding weight in DI water.

2-3. Microalgae harvesting

Harvesting experiments were carried out by taking 10 ml of the microalgae sample in 15 ml falcon tube. A sample of microalgae cells with no addition of chitosan was set as a control. The flocculant (chitosan/ferric chloride) with various concentrations (depending on experimental condition) was added in the culture, mixed at rotor (~150 rpm). The cells were allowed to settle for 25 minutes. 2 ml supernatant was carefully collected (without disturbing the cells settling) with the help of pipette from the mid-point of each falcon tube. The optical density of the collected sample was measured at 600 nm with a spectrophotometer (T80+ UV/VIS spectrometer PG instruments Ltd). Demineralized water was used as a reference for absorbance measurements. All the experiments were performed in triplicate.

The harvesting efficiency was calculated as follows:

$$\text{Harvesting effieicny (\%)} = \frac{OD_{to} - OD_t}{OD_{to}} \times 100$$

where OD_{to} and OD_t are the optical densities of the samples taken at time zero and a specific time t, respectively.

2-4. COD measurements

After harvesting, the COD of each supernatant was measured. The samples showing the highest harvesting efficiency were selected for COD measurements. 2.5 ml of each sample was added in 1.5 ml of $K_2Cr_2O_7$ and 3.5 ml of the H_2SO_4 for COD measurement. Vials were placed in a COD digester for 2 hours, and the measurements were taken with a COD meter by correlating it with absorbance.

3. Results and Discussion

3-1. HCl dissolved chitosan as a flocculant

Chitosan after dissolving in different concentrations of HCl was used as a flocculant. The effect of chitosan dose on the harvesting efficiency was investigated. The harvesting efficiency in control (without chitosan) was 18% only. The maximum harvesting efficiency (89 ± 0.87%) was achieved at 30 mg/L, while the minimum (63 ± 1.90%) at 150 mg/L for chitosan dissolved in 0.05 M HCl. For chitosan dissolved in 0.1 M HCl, the highest harvesting efficiency (89 ± 0.24%), was obtained at 30 mg/L, and the lowest (58 ± 4.7%) at 150 mg/L. In 0.2 M HCl, the highest harvesting efficiency (81 ± 1.95%) was obtained at 30 mg/L, and the lowest (20 ± 2.9%) at 150 mg/L as shown in Fig. 1.

"Control" (without flocculant) showed the least harvesting efficiency. Results showed that high dose of chitosan (>30 mg/L) is unfavorable for harvesting. At low flocculant dose (30 mg/L), the microalgae cells completely occupy the available binding sites of chitosan, forming strong and stable flocs, due to high electrostatic attraction and minimal repulsion [10,16-18]. At high flocculant dose, (up to150 mg/L), the excess cationic charge restabilizes the cells and increases the electrostatic repulsion, and reduces the harvesting efficiency [15-19]. At 0.05 and 0.1 M HCl, the maximum amino groups are protonated and chitosan becomes completely soluble. Completely soluble chitosan when used as a flocculant improves the harvesting efficiency [20-22]. This phenomenon was evidenced by our results, and hence, validates the pre-set hypothesis. With 0.2 M HCl, chitosan solubility was low. Wu *et al*. (2007) confirmed that hydrochloric acid, when used in excess, behaves like a neutral electrolyte (salt) and leads to a repulsive interaction between ionic groups of chitosan [29]. An increase in HCl concentration causes the contraction of chitosan chains from

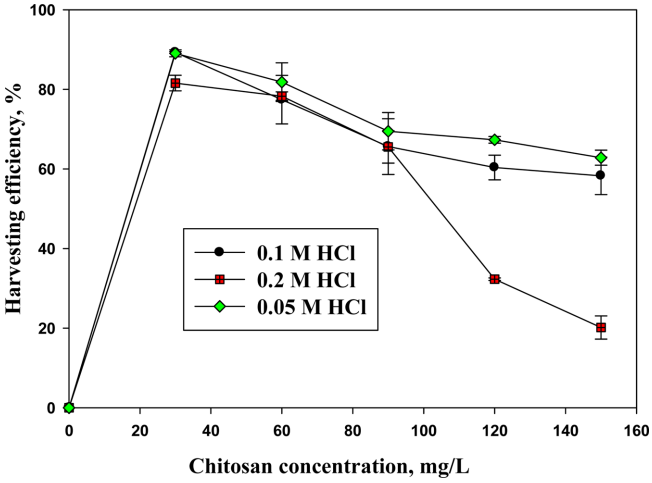

Fig. 1. The effect of chitosan dose on harvesting efficiency by using different concentration of HCl.

rod-like to coiled conformation; as a result, the size of macromolecules is reduced, which governs low harvesting efficiency when used as a flocculant.

The surface charge of microalgae changes with the pH, affecting microalgae harvesting efficiency. To determine the effect of pH on the harvesting efficiency, final pH of the samples was measured. pH after harvesting with chitosan (dissolved in 0.05 M HCl) changed to 7.0 for 30 mg/L, and 5.0 for 150 mg/L. (data not shown). With chitosan dissolved in 0.1 M HCl, pH after harvesting changed to 7.0 for 30 mg/L of chitosan and 4.7 for 150 mg/L. While, with chitosan dissolved in 0.2 M HCl, 30 mg/L of chitosan showed a change in pH of 6.8, and with 150 mg/L of chitosan, pH of the sample changed to 4.5. Heasman et al. (2000) reported that the medium pH affects the harvesting efficiency of microalgae [11]. This study supported such observations. The highest harvesting efficiency (89%) was found at pH closer to 7.0. A further decrease in pH, (< 5.0) reduced the harvesting efficiency. At low pH, excessive cations were released by the chitosan in the form of NH_2 [22]. When the concentration of cations is high in culture medium, they start to repel each other. As a result, the interaction between negatively charged microalgal cell and cations decreases, which inhibits floc formation [23-26,29-31].

3-2. H_2SO_4 dissolved chitosan as a flocculant

Chitosan was dissolved in H_2SO_4 (0.05, 0.1 and 0.2 M). The harvesting efficiency was measured at different chitosan concentrations (30, 60, 90, 120 and 150 mg/L). The harvesting efficiency in the control was 18% only. The highest harvesting efficiency (75 ± .41%) was obtained at 150 mg/L, and the lowest efficiency (57 ± 6.7%) at 30 mg/L of chitosan dissolved in 0.05 M H_2SO_4. For the chitosan dissolved in 0.1 M H_2SO_4, the highest harvesting efficiency (77 ± 1.1%) was at 150 mg/L and the lowest (57 ± 6.7%) %) was at 30 mg/L. Chitosan in 0.2 M H_2SO_4 also showed the same results. 150 mg/L of chitosan showed the highest harvesting efficiency (60±7.1%) %), and 30 mg/L showed the lowest harvesting efficiency (41±1.31%) %), as shown in Fig. 2. Results showed that in comparison to HCl, the harvesting efficiency of H_2SO_4 dissolved chitosan was low. The solubility of chitosan in different acids might have governed this change in harvesting efficiency. Studies revealed that chitosan is soluble in all monovalent acids of wide concentration, while partially soluble in divalent acids [9]. Chitosan dissolved in monovalent acid solutions like formic, acetic, propionic, butyric, isobutyric, lactic, nitric, hydrochloric, and chloroacetic acid is completely soluble [9,22], while least soluble in divalent solvents like H_2SO_4. Less solubility in H_2SO_4 is due to ionic cross-linking with sulfate ions [13,14,28]. This study supported such observations. Chitosan was not completely soluble in sulfuric acid. Partially soluble chitosan resulted low harvesting efficiency when used as a flocculant.

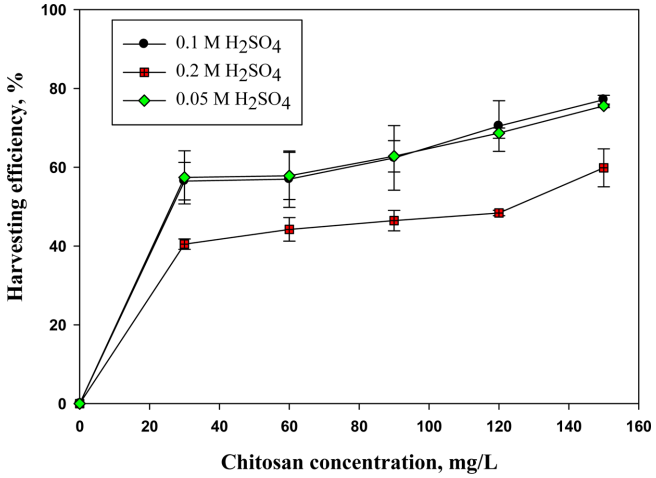

Fig. 2. The effect of chitosan dose on harvesting efficiency by using different concentration of H_2SO_4.

pH of the medium affects the harvesting efficiency of microalgae. Thus, the final pH was determined after harvesting of microalgae under different chitosan treatments. Initial sample pH was 7.35, which decreased after treating with chitosan (dissolved in 0.05 M, 0.1 M, and 0.2 M H_2SO_4). The samples pH after harvesting with chitosan dissolved in 0.05 M H_2SO_4 changed to 6.1 for 30 mg/L and 3.5 under 150 mg/L. For chitosan dissolved in 0.1 M H_2SO, the pH after harvesting changed to 5.3 with 30 mg/L, and 2.6 with 150 mg/L (data not shown). While, the chitosan dissolved in 0.2 M H_2SO_4 after harvesting changed the pH to 4.3 with 30 mg/L and 2.4 with 150 mg/L as shown in Table 1. 150 mg/L of chitosan in different H_2SO_4 concentrations (0.05, 0.1 and 0.2 M) decreased the sample pH, and increased the harvesting efficiency. The possible reason could be that the maximum solubility of the chitosan at low pH and high acid concentration.

3-3. Microalgae harvesting with $FeCl_3 \cdot 6H_2O$

$FeCl_3 \cdot 6H_2O$ was also used as a flocculant to compare its efficiency with the chitosan. Different concentrations of $FeCl_3 \cdot 6H_2O$ (30~300 mg/L) were employed. Results showed harvesting efficiencies of 86 ± 0.007%, 87 ± 0.005% and 86 ± 0.083% with 30, 240, and 270 mg/L of $FeCl_3 \cdot 6H_2O$, respectively. The harvesting efficiency of $FeCl_3 \cdot 6H_2O$ was less than HCl dissolved chitosan but higher than H_2SO_4 dissolved chitosan. This might be due to variation in chitosan solubility in different acids [9]. Furthermore, the results showed that 30 mg/L of $FeCl_3 \cdot 6H_2O$ showed an increase in the harvesting efficiency, and then declined till 210 mg/L. However, a further increase in dose (240, and 270 mg/L) again showed an increase in the harvesting efficiency as shown in Fig. 3. This is because a minimum concentration of $FeCl_3 \cdot 6H_2O$ (30 mg/L) is required to overcome electrostatic stabi-

Table 1. Change in pH and COD by using ferric chloride and chitosan as flocculants after dissolving in different concentrations of solvents

Flocculant dose	Solvent concentration	Final pH	Final COD, mg/L
Chitosan	0.05 M HCl	4.97	73
	0.1 M HCl	4.64	133
	0.2 M HCl	4.5	150
	0.05 M H_2SO_4	3.53	50
	0.1 M H_2SO_4	2.57	76
	0.2 M H_2SO_4	2.36	114
$FeCl_36H_2O$	DI Water	4.27	72

*Final pH and COD reflect the values obtained after harvesting

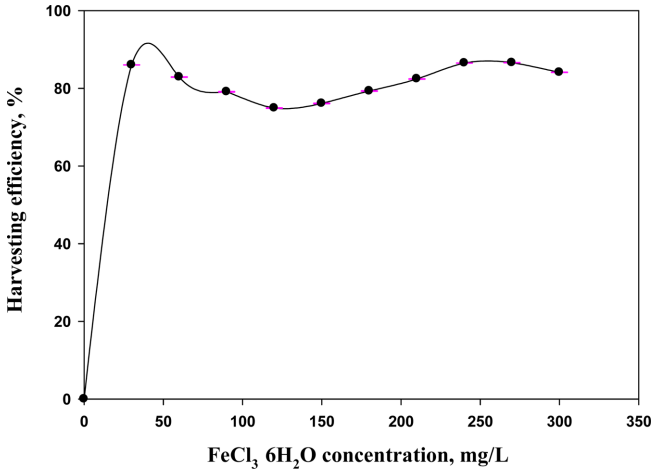

Fig. 3. Harvesting efficiency of microalgae under different concentrations of $FeCl_3 \cdot 6H_2O$.

lization of the cells, which then causes bridging of algae microalgae cells by precipitates, and thus, enhances flocculation. With a further increase in the flocculant dose, precipitates of ferric hydroxide are formed. These precipitates can physically sweep the colloidal particles from the suspension by a mechanism called sweep-flocculation [22-25].

The final pH was measured after harvesting with various concentrations of $FeCl_3 \cdot 6H_2O$. pH decreased from 7.6 to 7.3 with 30 mg/L, 4.3 with 240 mg/L, and 4.3 with 270 mg/L (Table 1). The highest harvesting efficiency was at 30 mg/L and the solution pH was almost neutral (7.0). Results showed that pH is an important parameter for flocculation process. At 30 mg/L, the harvesting efficiency was high, which corresponds to high final pH. It is because at high pH, precipitates of ferric hydroxide are formed, which promotes flocculation. While an increase in the concentration of $FeCl_3 \cdot 6H_2O$, pH decreased and the harvesting efficiency increased. It implies that when a high dose of ferric chloride is added, a series of soluble hydrolysis species are formed. They are positively charged at low pH (<6). The positively charged hydrolysis species can absorb onto the surface of colloidal particles and destabilize the stable colloidal particles. This mechanism is called *charge neutralization* [23].

3-4. COD reduction with chitosan

Chemical oxygen demand (COD) is an important parameter to assess the quality of wastewater and its impact on environmental health. It is a measure of oxygen uptake for the degradation of organic or inorganic pollutants. COD is important in microalgae harvesting perspective too. Reduction in COD of supernatant (obtained after harvesting) is considered to evaluate the efficacy of a flocculant [19,21]. The COD of supernatant should be reduced significantly for its safe disposal to water bodies or its reuse for other microalgae processes. [3] The use of chitosan to remove COD can be more promising than the chemical flocculants, as it is biodegradable and poses no threat to the environmental recipients. Thus, in this experiment, the COD removal was observed under different treatments while using chitosan as a flocculant.

In results, 30 mg/L of chitosan in (0.05M, 0.1M, and, 0.2 M) HCl, showed more COD reduction than 60 mg/L. COD reduction was the highest (71%) with 30 mg/L in 0.05 M HCl, while the lowest (30%) in 0.2 M HCl. With 60 mg/L, COD reduction was the highest 60% (in 0.05 M HCl), and the lowest 17% (in 0.2 M HCl) as mentioned in Table 1. Results showed that the harvesting efficiency was positively correlated with COD reduction. Chitosan dissolved in 0.05 M HCl, showed more COD reduction than 0.1 and 0.2 M HCl. Also, 30 mg/L concentration of chitosan showed more COD reduction as compared to 60 mg/L. At the lowest concentration of chitosan (30 mg/L), COD removal efficiency was 71%. With an increase in chitosan concentration, the harvesting efficiency decreased to 30%. It might be due to the high concentration of polyelectrolyte, which forms an envelope on the suspended particles and causes them to remain in suspension, thus reducing COD removal efficiency [21]. Similar results were obtained in this study, showing a decrease in COD with an increase in chitosan concentration.

COD reduction was investigated after harvesting microalgae with chitosan dissolved in H_2SO_4. 120 mg/L and 150 mg/L of chitosan showed more harvesting efficiency as compared to 30 and 60 mg/L. COD of the samples, treated with 120 and 150 mg/L of chitosan, was measured. We found that COD reduction by both 120 and 150 mg/L in 0.05 M H_2SO_4 was more as compared to 0.1 and 0.2 M H_2SO_4. COD removal efficiency was the highest (72%) with 150 mg/L in 0.05M H_2SO_4 while the lowest (37%) in 0.2 M H_2SO_4. At 120 mg/L of chitosan, the COD reduction efficiency was the highest (59 %) in 0.05 M and the lowest (29%) in 0.2 M H_2SO_4. Results showed that with an increase in chitosan dose, pH of the sample would decrease. Samples with low pH showed more COD reduction. The possible reason could be the maximum COD reduction in acidic pH. As H_2SO_4 is used in COD experiment for digestion, a sample containing H_2SO_4 lowers the pH, which enhances COD reduction.

COD was also measured after harvesting microalgae with $FeCl_3 \cdot 6H_2O$. The samples showing the highest harvesting efficiency were used for COD measurement. We found that 30 mg/L of ferric chloride showed 60% COD reduction. Increased concentration of 240 and 270 mg/L decreased COD almost 72%, and 73%, respectively. With further increase in concentration (300 mg/L), the COD reduction efficiency again started decreasing (59%).

Results showed that COD removal efficiency increased by increasing $FeCl_3 \cdot 6H_2O$ concentration. Birjandi *et al*. (2013) confirmed that at high coagulant doses, metal hydroxides are produced. Organic substances are removed by sorption onto hydroxide flocs [5]. Thus, an increase in ferric chloride concentration increased COD removal. But, when the dosage exceeded a threshold value, there were no additional improvements in COD reductions. At high coagulant dose, floc break up may occur due to charge reversal and dispersion.

4. Conclusion

This study has provided a baseline to reduce the cost of microalgae

harvesting. Economical microalgae harvesting is possible by increasing the solubility of chitosan and then using it as a flocculant. The results showed that by increasing chitosan solubility, the flocculant dose can be reduced significantly (up to 30 mg/L) only, to attain microalgae harvesting efficiency 90%. It was found that chitosan solubility depends on the solvent type and its molar concentration. Process optimization turned out that chitosan dissolved in 0.05 M HCl resulted in the maximum solubility, and thus, harvesting efficiency. The use of low concentration of chitosan also reduced the COD of microalgae culture, which confirmed the environmentally friendly nature of chitosan unlike other inorganic flocculants.

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