The Use of Chitosan as Non-toxic Flocculant for Harvesting Microalgae Spirulina sp

by Nur Rokhati

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Abstract. *Spirulina* is a type of microalgae widely consumed as a food supplement due to its high nutritious benefits. Furthermore, it is very small and lives floating in water, making it quite difficult to harvest. One effective method of harvesting *Spirulina* is by coagulation/flocculation. In order to successfully harvest *Spirulina*, choosing the right flocculant material is very important. Chitosan, a natural polymer that has a cationic amine group, is often used in the food industry. This is because colloidal particles (polymer) that exist in nature are mostly negatively charged (including microalgae) and electrostatic interactions between cationic polymers with anionic polymers lead to the formation of flocculants. Therefore, this study was carried out to measure the feasibility of chitosan as a flocculant in the flocculation process of harvesting microalgae. The experiments were carried out with the variation of the concentration of chitosan, pH and slow-stirring speed, using efficiency of flocculation and sedimentation speed as parameters. The result showed that optimum condition was achieved at a slow-stirring speed of 40 rpm, sedimentation time of 2 hours, chitosan concentration of 100 mg/L and pH of 7-8. In this condition, the flocculation efficiency was 99.57%.

Keywords: *Spirulina*, harvesting, flocculant, chitosan

1. Introduction

In the field of biotechnology, microscopic-sized algae (microalgae) have various applications [1]. One of the trends in that application is often associated with edible blue-green microalgae, including *Spirulina* which is small, lives floating in water and needs sufficient sunlight intensity for its growth. Furthermore, it has a mass concentration of less than 1g/l during harvest. As a tropical country, Indonesia has great potential for microalgae cultivation.

Spirulina is a type of microalgae widely employed as a food supplement due to its high nutritious benefits [2]. It contains high protein, vitamin A, B, and K, as well as minerals and fatty acids needed by the body. In addition to being consumed as a food product, *Spirulina* is known to have therapeutic implications, such as diabetes, arthritis, anemia, cardiovascular disease, and cancer. It is also famous for its phycocyanin which is used as an antioxidant. They exhibit antiviral activity by inhibiting the entry of hidden viruses such as herpes simplex, human cytomegalovirus and measles into cells [3].

The high protein content of *Spirulina* causes it to break down quickly and emits a fishy odor. This damage may occur 24 hours after harvesting. One effective method of harvesting is by coagulation/flocculation [4]. Coagulation and flocculation are successive processes, with coagulation

been related to the destabilization of colloidal suspensions by neutralizing electric forces among suspended particles and flocculation been the process of forming macromolecules to destabilize the colloid by adsorption and interparticle binding. After coagulation and flocculation, the bigger floccules appear and the precipitate is removed by filtration, centrifugation or flotation [5]. Colloidal particles that exist in nature are mostly negatively charged, including microalgae.

In the coagulation/flocculation process, choosing the coagulant and flocculant is very significant because it affects the efficiency of the process. Natural polymeric flocculants are preferable compared to synthetic [6]. Conversely, the use of chitosan and its derivatives as flocculants has received significant attention due to its numerous advantages, including adequate availability, environmentally friendly, non-toxic, biodegradable and biocompatible [7]. Chitosan has characteristics of both coagulant and flocculants for its high cationic charge density, long polymer chains, bridging of aggregates and precipitation in neutral and alkaline conditions [8,9]. The amine group in the cationic chitosan chain is capable of reacting with other anionic polymers. Flocculants are formed due to the electrostatic interactions between cationic polymers and anionic polymers [6]. In Indonesia, the number of study carried out in relation to chitosan as an assisting agent for harvesting *Spirulina* is still limited. Therefore, it is necessary to study the potential of chitosan and its performance as a flocculant in the process of harvest.

2. Materials and Methods

2.1. Materials

Spirulina was obtained from the Balai Besar Perikanan Budidaya Air Payau (Brackish Water Fisheries Cultivation Agency), Jepara, Indonesia. Furthermore, chitosan was obtained from PT. Biotech Surindo Indonesia with an 80.4% degree of deacetylation while, acetic acid and sodium hydroxide were obtained from Merck.

2.2. Culture of Microalgae

Spirulina obtained from the Brackish Water Fisheries Cultivation Agency was cultured in a batch process, placed into a 1 L container made of plastic and diluted to a volume 10 times bigger. The containers were equipped with aerator as a source of oxygen and LED lights. Cultivation was carried out at room temperature and pressure with continous aeration, maintaining a pH of ± 8. The nutrition for *Spirulina* was urea, which was given daily at a dose of 100 ppm. *Spirulina* was declared ready for harvest in the logarithmic phase (after 6 days of culture) after which, it was prepared for use in the experiment.

2.3. Coagulation-Flocculation Process

Chitosan was placed into the *Spirulina* culture solution and the pH was adjusted according to variables (pH 5-10). *Spirulina* culture solution was stirred using a jar test meter, with rapid stirring at 180 rpm for 1 minute using magnetic stirrer. This was carried out to disperse chitosan in the *Spirulina* solution to ensure that a good coagulation process is obtained. Furthermore, a slow-stirring process was carried out at a speed of 10-50 rpm for 2 minutes to form a large floc. After the flocculation process, turbidity of the solution and height of the floc at any given period were measured.

The flocculation efficiency was measured using the following equation:

$$\text{Flocculation efficiency } (\%) = \left[\frac{C_i - C_f}{C_i}\right] \text{x } 100 \qquad (1)$$

with

Ci = turbidity of the initial *Spirulina* solution

Cf = turbidity of the solution after the coagulation-flocculation process

3. Results and Discussion

3.1. Effect of Stirring Speed on Flocculation Efficiency

The stirring process aims to increase contact between chitosan and *Spirulina*. The performance of flocculation was investigated at a slow-stirring speed of 10, 20, 30, 40 and 50 rpm, respectively in the various dosage of chitosan.

The purpose of the stirring was to maximize the interaction between chitosan's active cationic group and *Spirulina*'s active anionic group. It was necessary to stir at high speed (fast stirring) at the beginning to ensure that the mixture of chitosan may be evenly distributed in all parts of the *Spirulina* solution [4] this was followed by slow stirring.

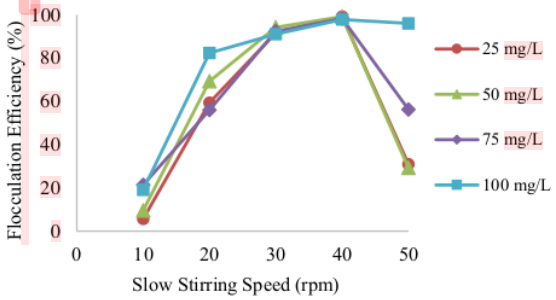

Figure 1. Effect of slow stirring speed on flocculation efficiency at various concentrations of chitosan

According to figure 1, the highest flocculation efficiency was obtained when the solution was stirred at 40 rpm. This is supported by previous study which achieved best result at 40 rpm of slow stirring [10]. In the speed above that, the efficiency declined in all chitosan concentration, due to the floc which was merged split again. Meanwhile, if the stirring was too slow, the energy given for floc formation would be less than optimal [11].

Slow stirring shortened the distance between particles and increased the incorporation of solid particles in solution to form aggregates, which grew into a large-sized floc. After the mass and floc size became maximal, a sedimentation process occurred from the floc to the bottom of the container, due to the gravitational force. Furthermore, two layers were formed in the container, namely, the clear water and floc sediment layers [8,12].

3.2. Effect of Chitosan Concentration on Flocculation Efficiency

The cationic properties of chitosan may be used for coagulation by charge neutralization, where the neutral colloidal particles will combine to form floc [13]. The effect of chitosan concentration on flocculation efficiency is shown in figure 1, which shows that the dosage of 100 mg/L acquired highest flocculation efficiency in most slow stirring speed. Generally, chitosan effectively flocculates algae species at 5 mg/L to 200 mg/L [14].

Colloidal particles with the same electric charge will repel each other. Consequently, it will be dispersed in the solution. The result of colloidal particles could not settle at the bottom even if left for a long time. However, if the solution was added to a differently charged material, the colloidal particles would be neutralized, and each colloidal particle will come closer to each other and combine to form a small floc [15].

The main contents of *Spirulina* are anionic carbohydrates and protein. Chitosan is a natural polymer containing a cationic amine group. When the chitosan was added to the *Spirulina* solution, its cationic properties weakened the repulsive forces between the *Spirulina* particles. Subsequently, each particle

came closer to each other and merged into a fine floc. The fine flocs further combined into large flocs and settled at the bottom of the container [12].
However, adding excessive flocculant (chitosan) re-stabilized the suspended *Spirulina* by repulsing the forces between particles therefore, reducing flocculation performance. Meanwhile, the addition of excessive flocculants did not usually result in a significant decrease in flocculation efficiency [16]. It is also known that excessive chitosan concentration is capable of saturating the polymer bridging or charge neutralization. This fact confirms the re-dispersion of the particles and increases residual turbidity [17].

3.3. Effect of pH on Flocculation Efficiency

Acidity (pH) affects the behavior of chitosan in solution, such as protonation of chitosan amine groups and changes in the conformation of molecular chains. Therefore, the flocculation process at pH 5-8 was carried out in order to observe the effect of acidity.

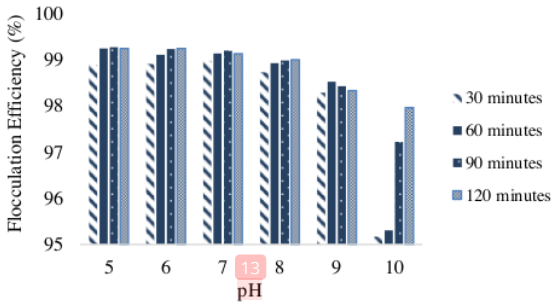

Figure 2. Effect of pH on the flocculation efficiency at chitosan concentration of 15 mg/L

It also affects the behavior of chitosan solutions, such as protonation of chitosan amine groups and changes in the conformation of molecular chains. The chitosan intrinsic pKa approahed 6.5, therefore most of the amine groups became protonated under the intrinsic pKa. Consequently, more than 90% of amine groups were protonated at pH 5 and most amine groups are deprotonated at high pH [13].
Based on figure 2, in the pH range of 5 to 8, the efficiencies of flocculation were mostly around 99%. This variation in pH did not provide a significant efficiency difference. These results were aligned with previous studies, which obtained better performance using chitosan as a flocculant agent with pH values from 4.5-6.5. At lower pH than that, chitosan's particle would be destabilized because of surface charge reversal and would finally become unstable at pH 2 [18,19]

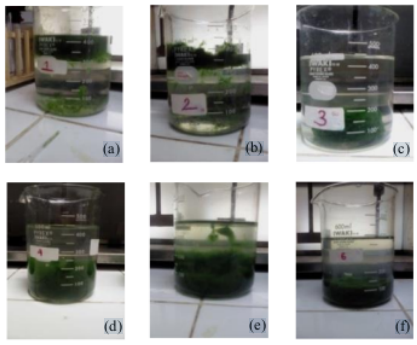

Figure 3. *Spirulina* floc formed at various pH ((a) pH 5, (b) pH 6, (c) pH 7, (d) pH 8, (e) pH 9, (f) pH 10)

The separation of floc formed in the solution also needs to be considered. Figure 3 shows that at pH 5-6, the floc formed tended to float above the solution. Meanwhile, for pH 7-10, the *Spirulina* floc formed settled under the solution (at the bottom of the glass beaker). To simplify the harvesting process, it is usually desirable that the *Spirulina* floc formed settles completely under the storage. Therefore, the flocculation process will not be optimal if the process is carried out in acidic pH of 5 and 6.
Figure 3 also shows that at pH 10, the blue color of *Spirulina* becomes more dominant. This blue color came from the phycocyanin pigment, which is very beneficial to humans mainly because of its designation as a food supplement.
Conversely, at pH above neutral, increasing the pH reduces the efficiency of flocculation. This is due to chitosan being deprotonated and losing its charge.

3.4. Effect of pH on Sedimentation Speed

One of the main process variables measured to justify the flocculation efficiency is the settling rate of flocs [20]. It is also actually the manifestations of the floc or aggregate size distribution and the shape and structure of flocs produced during the flocculation process. For good sedimentation, large, strong and solid flocs are preferred [6]. Sedimentation speed is determined by measuring the height of the sediment on the bottom of the beaker every 30 minutes for 2 hours.

Table 1. Effect of pH on sedimentation speed

pH	Sedimentation Speed [mm/sec]
5	Floc floating
6	Floc floating
7	0.85
8	0.8
9	0.7
10	0.8

As seen in table 1, the highest sedimentation speed was obtained at neutral to base pH medium due to the enlargement of floc size at neutral/base conditions therefore, making it easier for them to go down as affected by the force of gravity. Meanwhile, in acidic conditions, the floc formed was unable to settle at the bottom of the solution because the size of the floc was smaller, which made it difficult to undergo sedimentation.
There are two mechanisms of flocculation formation. Firstly, coagulation due to charge neutralization (protonated amine groups) and secondly, flocculation by trapped colloidal mineral in polymer tissue. At high pH, most amine groups are deprotonated and the coagulation process is inactive to neutralize colloidal particles due to the trapped colloidal particles of *Spirulina* in the polymer tissue. Furthermore, neutralization of the protonated amine group and the re-sedimentation of chitosan occur simultaneously. The sedimentation of this polymer contributed to the trap of colloidal particles and their deposition [21].

4. Conclusion

From this study, it was concluded that Chitosan is an effective type of flocculant. The results showed that at acidic pH (5-6), the floc formed was floating above the solution, at neutral to basic pH (7-10) and the formed floc settled at the bottom of the solution. Furthermore, at pH 10, the blue color of *Spirulina* became more dominant. In addition, the best-operating conditions for *Spirulina* flocculation were obtained at a slow stirring speed of 40 rpm, sedimentation time of 2 hours, chitosan concentration of 100 mg/L and pH of 7-8. In this condition, the efficiency of *Spirulina* flocculation was 99.57%.

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