The effect of chitosan concentration on flocculation efficiency microalgae *Porphyridium cruentum* (Rhodophyta)

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Abstract. *Porphyridium cruentum* is a species of red microalgae belongs to the family Porphyridiophyceae, divisi Rhodophyta. *P. cruentum* contains a lot of nutrients which are very useful as functional food. The purpose of this research is to determine the effect of chitosan concentration on the flocculation efficiency of *P. cruentum*. In this study, there were two treatments, namely the concentration of chitosan and the time of flocculation with 3 replications. Chitosan used were 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm. The flocculation efficiency were carried out at 10, 20, 30 and 40 minutes. The highest density was reached on the seventh day as amount 1720x10^4 cell/mL. The results showed that there was an effect of Chitosan concentration on flocculation efficiency (Anova two way; F_count = 4.109; df (9; 80); p=0.01). In addition, there was an effect of flocculation time on flocculation efficiency (Anova two way, F_count = 4.498; df (3;80)). Furthermore, there was an interaction effect between the chitosan concentration and the time of flocculation. (Anova two way; F_count = 26.635; df (2;80)). The greater the value of chitosan concentration given the greater the value of flocculation efficiency.

1. Introduction

The use of marine microalgae as natural food for aquaculture and as functional food has been carried out by many researchers [1]. *P. cruentum* is a unicellular red alga belonging to the Family Porphyridiaceae. Microalgae *P. cruentum* contain total lipid 43.6 %-dw at 24 hours light photoperiod culture [11], contain sulfated polysaccharides, [2] aspartic acid (11.21 ± 0.45 g/100 g), glutamic acid as amount 8.17 ± 0.29 g/100g [14] and astaxanthin, so that it is appropriate to be used as functional food, cosmetic, and pharmacy. The concentrations of soluble polysaccharides of Porphyridium from 0.1-0.7 g.L-1 [3]. However, the harvesting processing for species of *P. cruentum*, *Nannochloropsis* sp, *Nitzschia* sp, *Skeletonema* sp, and *Chaetoceros* sp still have some problems [4]. The technology of freeze-drying, and centrifuge which have been used for harvesting microalgae, still requires a large cost so that the price of dry microalgae is still expensive. Likewise, the use of some heavy metals for the flocculation process on a lot of microalgae has an effect that is not good for human health.

The success of the microalgae biomass production process is largely determined by the harvesting of microalgae. Some methods for harvesting microalgae such as (a) centrifugation technique, (b) filtration techniques, (c) sedimentation process, (d) microalgae flotation process, (e) flocculation process, (f) electro flocculation, and (g) magnetic separation of microalgae. Currently, microalgae industries use a centrifuge to harvest microalgae, so that can be collected and dried. The use of...
The use of flocculation technology is an alternative for solving these problems. Flocculation is one of some methods of microalgae harvesting which effectively, low cost, and low energy. Chitosan is one of the flocculants that are harmless and can be eaten with food-grade standards [5][6]. Chitosan is a linear polysaccharide derived from the deacetylation of the abundant natural polymer chitin, which is mainly composed of N-acetyl-D-glucosamine monomer units [7]. In this study, chitosan was used as a material for microalgae flocculation [8][9][10].

According to this problem, so this research has proposed to study was to determine the optimization of flocculation of microalgae P. cruentum in order to obtain optimum biomass production and to determine the value of flocculation efficiency.

2. Material and Method
Microalgae P. cruentum had been taken from pure culture, Laboratory of Natural Feed, Brackish Water Research Center, Jepara. The culture media used a 100-liter container and was treated with air filtered 0.2 µm at 30 W/m2 irradiances. Media has temperature with range 18 - 26°C, Water salinity has range air 29-32 ppt and value of pH has range 7.2 – 8.2. Walne fertilizer was used in this research with the concentration of 1 ml of fertilizer for 1 liter of culture media.

Harvesting was carried out on day 7 in the initial stationary phase, this is in accordance with the study P. cruentum [11] [12]. The volume of microalgae used for the flocculation test was 1000 ml. Flocculation treatment using chitosan 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/L. Before being put into the culture of P. cruentum, chitosan powder was dissolved with acetic acid. After that, the P. cruentum culture was added with NaOH 0.1 N till the culture had a pH value of 10. The culture P. cruentum stirred until homogeneous. The observations on optical density were carried out at stages 10, 20, 30, and 40 minutes. Every treatment was done in 3 replicates. Measuring OD was conducted at the beginning before flocculation and the last of flocculation. Measuring optical density used wavelength 440 nm [6][5]. Blanko solution used sterile seawater with a value of salinity 30 ppt. Flocculation efficiency was counted in Percentage (%). The higher the flocculation efficiency value, the more microalgae cells flocculated. [13][6]. Formulation to count Flocculation Efficiency [6] is

\[ Flocculation \, efficiency \, (FE, \, in\%) = \left(1 - \frac{OD \, Sample}{OD \, control}\right) \times 100 \]

Analysis of variance two way was applied to test hypotheses such as (a) there was an effect of Chitosan concentration on flocculation efficiency (b) there was an effect of flocculation time on flocculation efficiency and (c) there was an interaction effect between the chitosan concentration and the time of flocculation.

3. Result and Discussion
Based on the observation, the density increased drastically from the sixth (12.4 x 10^6 cell.mL\(^{-1}\)) to the seventh day (17.20 x 10^6 cell.mL\(^{-1}\)). In this research, the density of P. cruentum was higher than that research who were conducted [12] with a density range of 1.08 x 10^6 cell.mL\(^{-1}\) to 4.17 x 10^6 cells.mL\(^{-1}\).
Figure 1. Density of microalgae *P. cruentum* culture

Based on observations, the greater the concentration of chitosan given, the greater the value of flocculation efficiency (%) both at 10, 20, 30 and 40 minutes. The 40-minutes observation showed a higher flocculation efficient (%) value compared to the 30 minutes, 20 minute, and 10-minute observations (Table 1 and Figure 2).

The higher the flocculation efficiency value, the more *P. cruentum* cell flocculated. This fact is in accordance with which states that the higher the flocculation efficiency value, the more microalgae will flocculate [12].

Based on the observation of *P. cruentum*, the highest flocculation efficiency (%) was observed in the 40 minutes with a concentration of 100 ppm chitosan with a flocculation efficiency value of 99.76% (Table 1). Each species of microalgae has a different flocculation efficiency. This is also seen in the study that revealed that at a concentration of 22 ppm chitosan and a final pH of 10, the microalgae *N. oculata* could be flocculated well in just 20 minutes [6].

Table 1. Flocculation Efficiency (%) Microalgae *P. cruentum* in the Difference Concentration of Chitosan (ppm)

| Concentration of Chitosan (ppm) | After 10 minutes | After 20 minutes | After 30 minutes | After 40 minutes |
|---------------------------------|------------------|------------------|------------------|-----------------|
| 10                              | 10.08            | 13.15            | 36.22            | 41.1            |
| 20                              | 17.06            | 20.77            | 38.69            | 48.63           |
| 30                              | 27.14            | 33.38            | 41.22            | 53.84           |
| 40                              | 31.42            | 38.25            | 55.6             | 60.99           |
| 50                              | 35.54            | 44.93            | 62.78            | 65.63           |
| 60                              | 41.78            | 51.8             | 64.84            | 69.07           |
| 70                              | 47.77            | 61.51            | 71.33            | 83.53           |
| 80                              | 57.48            | 70.26            | 76.18            | 82.45           |
| 90                              | 62.62            | 74.4             | 84.19            | 97.49           |
| 100                             | 67.24            | 85.13            | 88.78            | 99.76           |
| Control                         | 64.88            | 66.34            | 48.02            | 60.77           |

The results showed that there was an effect of Chitosan concentration on flocculation efficiency (Anova two-way; F count = 4.109; df (9; 80); p=0.01). In addition, there was an effect of flocculation time on flocculation efficiency (Anova two-way, F count = 4.498; df (3;80)). Furthermore, there was an
interaction effect between the chitosan concentration and the time of flocculation. (Anova two way; F count = 26.635; df (27;80)). This indicates that the higher the concentration of chitosan, the higher the flocculation efficiency value. This is in accordance with the study of Morales and Picard (1985) that the highest flocculation for harvesting microalgae (Skeletonema costatum, Dunaliella tertiolecta, Thalassiosira nordenskoldii, Chlorella sp. and Thalassionema sp.) occurs at concentration of chitosan 40 ppm. Furthermore, in the harvesting of Nannochloropsis graditana, the use of chitosan concentration of 30 ppm resulted in a flocculation efficiency value of 85% and the use of chitosan concentration of 60 and 100 ppm in Nannochloropsis sp resulted in flocculation efficiency values of 97 and 90% [6]. This shows that the greater chitosan concentration is given, the greater the value of flocculation efficiency [6]

Based on the analysis, the results showed that there was a positive correlation between chitosan concentration and flocculation efficiency at 10 minutes, 20 minutes, 30 minutes, and 40 minutes. The correlation value between chitosan concentration and flocculation efficiency is 0.99 (Figure 2). This is also in accordance with the results of the determination regression value (r2) is 0.98 for correlation between chitosan concentration and flocculation efficiency [6]

![Figure 2](image-url)  
**Figure 2.** The Relationship between Concentration of Chitosan (ppm) and Flocculation Efficiency (%) on microalgae *P. cruentum*

4. Conclusion  
The chitosan concentration has an effect on the flocculation efficiency of the microalgae *P. cruentum* harvesting process. The longer the deposition time, the greater the flocculation efficiency value. There is an interaction effect between the concentration of chitosan and the time of deposition on the value of flocculation efficiency. The using chitosan concentration 100 ppm in the *P. cruentum* harvesting process gave a flocculation efficiency value of 99.79% at 40 minutes deposition.

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References

[1] Barkia I, Saari N and Manning S R 2019 Microalgae for high-value products towards human health and nutrition Mar. Drugs 17 304

[2] Bayona K C D, Navarro S M, Lara A D, Colorado J R, Atehortúa L G and Martínez M 2012 Activity of sulfated polysaccharides from microalgae Porphyridium cruentum over degenerative mechanisms of the skin Int. J. Sci. Adv. Technol. 2 85–92

[3] Balti R, Le Balc’h R, Brodu N, Gilbert M, Le Gouic B, Le Gall S, Sinquin C and Massé A 2018 Concentration and purification of Porphyridium cruentum exopolysaccharides by membrane filtration at various cross-flow velocities Process Biochem. 74 175–84

[4] Branyikova I, Prochazkova G, Potocar T, Jezkova Z and Branyik T 2018 Harvesting of microalgae by flocculation Fermentation 4 93

[5] Vandamme D, Foubert I and Muylaert K 2013 Flocculation as a low-cost method for harvesting microalgae for bulk biomass production Trends Biotechnol. 31 233–9

[6] Chua E T, Shekh A Y, Eltanahy E, Thomas-Hall S R and Schenk P M 2020 Effective harvesting of Nannochloropsis microalgae using mushroom chitosan: a pilot-scale study Front. Bioeng. Biotechnol. 8 771

[7] Dimzon I K D and Knepper T P 2015 Degree of deacetylation of chitosan by infrared spectroscopy and partial least squares Int. J. Biol. Macromol. 72 939–45

[8] Christenson L and Sims R 2011 Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts Biotechnol. Adv. 29 686–702

[9] Milleedge J J and Heaven S 2013 A review of the harvesting of micro-algae for biofuel production Rev. Environ. Sci. Bio/Technology 12 165–78

[10] Lee A K, Lewis D M and Ashman P J 2013 Harvesting of marine microalgae by electrofloculation: the energetics, plant design, and economics Appl. Energy 108 45–53

[11] Irwani I, Ridlo A and Widianingsih W 2013 Optimalisasi Total Lipid Mikroalga Porphyridium cruentum Melalui Pembatasan Nutrien dan Fotoperiod Bul. Oseanografi Mar. 2 16–23

[12] Putri A D A and Tjahjaningsih W 2018 Manajemen Pasca Panen Kultur Mikroalga Porphyridium cruentum Pada Skala Laboratorium Dan Skala Intermediet Di Balai Besar Perikanan Budidaya Air Payau, Jepara Jawa Tengah J. Aquac. Fish Heal. 7 111–7

[13] Praharyawan S and Putri S A 2017 Optimasi Efisiensi Flokulasi Pada Proses Panen Mikroalga Potensial Penghasil Biodiesel Dengan Flokulan Ion Magnesium-(Optimization of Flocculation Efficiency in the Harvesting Process of Potential Biodiesel Producing Microalgae by Using Magnesium Ions) Biopropal Ind. 8 89–98

[14] Safi, C., B. Zebib, O. Merah, P. Pontalier and C. Vaca-Garcia. 2014. Morphology, composisition, production, processing and applications of Chlorella vulgaris: A review. Renewable and Sustainable Energy Reviews, 35 : 265-278.