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Chitosan Powder vs. Chitosan Macrobead: Feasibility Test for Separation of Microalgae from Real Pond Water

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Microalgae are microscopic organisms that are commonly found in freshwater. Freshwater surfaces with excessive microalgae will result in unpleasant surface scum, severe oxygen depletion, and fish die-offs. One of the usually employed techniques to remove microalgae is through chitosan powder; in this approach, the cationic chitosan molecules are used to flocculate the anionic microalgae. However, this technique has the potential to generate sludge and requires considerable quantities of flocculant. To avoid such limitations, chitosan macrobeads were proposed in this study. This study aimed to compare the microalgae removal efficiency via flocculation (using chitosan powder) and adsorption (using chitosan macrobead). The removal experiment was conducted using microalgae from similar local pond water. Results showed that both methods are effective in removing wild microalgae. In particular, 60 %, 44 %, and 54 % of microalgae were removed by 2 g of chitosan powder at pH 2, pH 7, and pH 10. On the other hand, 2 g chitosan macrobeads removed 99 %, 91 %, and 95 % of microalgae at pH 2, pH 7, and pH 10. Such observation implies that the use of chitosan macrobeads for microalgae adsorption is less affected by pH. The isolation of spent chitosan macrobead amicrobial was done as there was no sludge formation. More importantly, the chitosan macrobead succusses removed almost all of the microalgae from the pond water within 24 h regardless of the medium pH, leaving a clear solution. Microscopic studies further verified the attachment of microalgae (in cluster form) on the chitosan macrobead. With the encouraging results, future study is suggested to further optimize the microalgae removal efficiency by modifying the surface pore structure of the chitosan macrobead.

1. Introduction

The existence of microalgae blooms in water resources has been a serious environmental issue worldwide. The microalgae bloom reduced the amount of oxygen dissolved in the water and prevented sunlight from accessing the water. However, these methods' slow response times, sensitivity, huge working areas, and high energy needs are significant limitations. On the other hand, flocculation has been a preferred method for microalgae removal (Burgstaller et al., 2018). During the flocculation step of water treatment, solids are gathered into larger flocs to be removed from the water (Cruz et al., 2020). As a superior substitute to synthetic flocculants, 312.5 mg biomass recovery, and 96.12 % flocculation efficiency were investigated together (Zhang et al., 2020). This procedure could take place naturally or with the aid of substances like chitosan. Chitosan has a higher charge density when compared to other coagulants such as cationic starch (Ahmad et al., 2006), and polymers produced by plants (polysaccharides and poly-phenolic substances) tannins (Ahmad et al., 2011). In another word, it can be described as a coagulant with a high charge density that requires less quantity to destabilize the targeted pollutant particles (Kurniawan et al., 2021).
This strategy, meanwhile, has significant operational limitations. Large volumes of coagulant and flocculant may be needed to achieve the proper level of flocculation. Hadiyanto et al. (2022) concluded that the flocculation process probably stabilized the microalgae cell solution since adding doses over the optimal concentration made the microalgae more stable and hindered separation (Loganathan et al., 2018). There is also a small amount of

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sludge produced, which is tedious to be handled particularly when wastewater is produced in big volume (Lydia et al., 2019), the expenses to treat the produced sludge may rise significantly (Sathya et al., 2022). Adsorption may be used instead of pollution removal because of its simplicity and high efficiency (Zhang et al., 2020). As a matrix for the creation of adsorbent materials, chitosan, a bio-polymer with a high density of hydroxyl groups (-OH) and primary amines (-NH_2) that serve as active adsorption sites is attracting a lot of attention (Daniele et al., 2021). Widely utilized as a biodegradable adsorbent for the elimination of color (Morais et al., 2020), heavy metal (Vigneshwaran et al., 2021), and organic contaminants (Sirajudheen et al., 2020), chitosan macrobead is created by cross-linking the chitosan molecules (Di Giacoma et al., 2022).

In this work, the viability of employing chitosan macrobead to remove microalgae from local pond water was evaluated. The removal efficiency was systematically done in three pH values and compared with the removal efficiency obtained through the flocculation method. In addition, the optical microscopic study was conducted to view the adsorbed microalgae. This microalgae removal study using chitosan macrobead was the first trial and to the best of the author's knowledge, a direct comparison of microalgae removal (from actual pond water) using flocculation and adsorption has been scarcely reported elsewhere.

2. Materials and methods

2.1 Chemicals

Acetic acid (AcOH)(Sigma Aldrich), sodium hydroxide (NaOH)(Chemiz), sulphuric acid (H_2SO_4)(Sigma Aldrich), and chitosan powder (molecular weight = 30,000 - 190,000 g/mol)(Sigma Aldrich) were obtained from the respective suppliers. All employed chemicals were of the analytical grade and were not further purified. Microalgae solution containing wild species of microalgae was collected from local pond water located at Bukit Pelanduk, Sepang. Note that analysis of the actual species of the microalgae was excluded at the current stage of the study.

2.2 Preparation of chitosan macrobead

First, 2 g of chitosan was dissolved in 100 mL of 1 % diluted AcOH solution to create a chitosan stock solution. To create the stock solution depicted in Figure 1 (a), the mixture was stirred with a magnetic stirrer at 400 rpm for 24 h. On the other hand, 40 g of NaOH pallets were mixed inside a beaker with 100 mL of water to dissolve the NaOH pallets as shown in Figure 1 (c). After that, the chitosan stock solution was then carefully dropped into the dissolved NaOH by using a dropper while keeping the NaOH solution homogeneously stirred at 125 rpm to avoid the sticking/stacking of the chitosan macrobead. The formed chitosan macrobeads then were kept in the same beaker for the next 24 h to give them a more rigid structure. The macrobeads (which have a size of around 0.3 cm) were then rinsed with distilled water in order to wash away the remaining NaOH from the bead's surface (Figure 1 (d-e)).

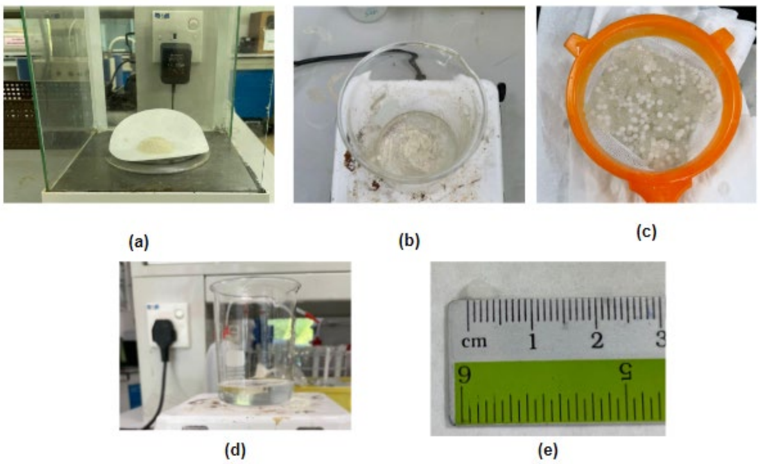

Figure 1: Photos showing the step-by-step formation of chitosan macrobead (a) chitosan powder was weighed, (b) dissolution of chitosan powder in 1 % AcOH, (c) preparation of NaOH solution, (d) chitosan macrobead formed upon dropping chitosan solution into NaOH solution, (e) the as-prepared chitosan macrobeads were ~ 0.3 cm in size

2.3 Removal of microalgae from pond water using flocculation

A batch test was conducted using chitosan to examine how medium pH affected the flocculation of microalgae. First, added 20 mL of microalgae solution was added into a 50 mL glass bottle and pH was adjusted to pH 2, pH 7, and pH 10 using 0.1M H_2SO_4 and 0.1M NaOH solutions. Then, 2 g of chitosan powder was added to the glass bottles. All three samples were then agitated at a rapid stirring speed of 200 rpm for 3 h in an orbital shaker. Then, after 3 h of stirring, the three samples were placed on a laboratory table (free-standing state) for another 21 h. On the next day, the sample bottles were turned over a few times before proceeding with UV-vis measurement, this action is to ensure the algae that settle to the bottom of the bottle was due to attachment onto the macrobead instead of self-sedimented. The final absorbance values of the microalgae solution were measured at wavelength 500 nm using a UV Spectrophotometer (S-2150) and compared with the initial absorbance value of the initial microalgae solution. The microalgae removal efficiency was calculated using Eq(1):

$$R(\%) = \frac{C_o - C_e}{C_o} \times 100\% \qquad (1)$$

where the initial and equilibrium concentrations of microalgae are Co (mg/L) and Ce (mg/L).

2.4 Removal of microalgae from pond water using adsorption

A similar procedure to section 2.3 was conducted by replacing the chitosan powder with 2 g of chitosan macrobeads. Similarly, the medium pH was adjusted to pH 2, pH 7, and pH 10 to study the effects of medium pH on microalgae adsorption. UV-vis measurement was conducted to determine the microalgae removal efficiency.

3. Results and discussion

3.1 Flocculation of microalgae from pond water using chitosan powder

In the first part of this study, microalgae were removed through the flocculation method using chitosan powder. As shown in Figures 2a-c, the microalgae removal efficiency through flocculation at pH 2, 7, and 10 was 60.82 %, 44.74 %, and 54.29 %. Results showed the best flocculation occurred in pH 2. The error bar in Figure 2c also indicated that the flocculation of microalgae at pH 2 gave more consistent data compared to pH 7 and 10, this is because the statistical analysis comparison between pH 2, 7, and 10 was significantly different (T-test analysis among all of the samples indicates that the *p*-value is lesser than 0.05), it is apparent that increasing the pH value has a detrimental effect on the removal efficiency. After 24 h, the solution, as depicted in Figure 2a-b, became less greenish but cloudy due to the sludge formation. Apparently, a massive sludge has been formed as shown in Figure 2b.

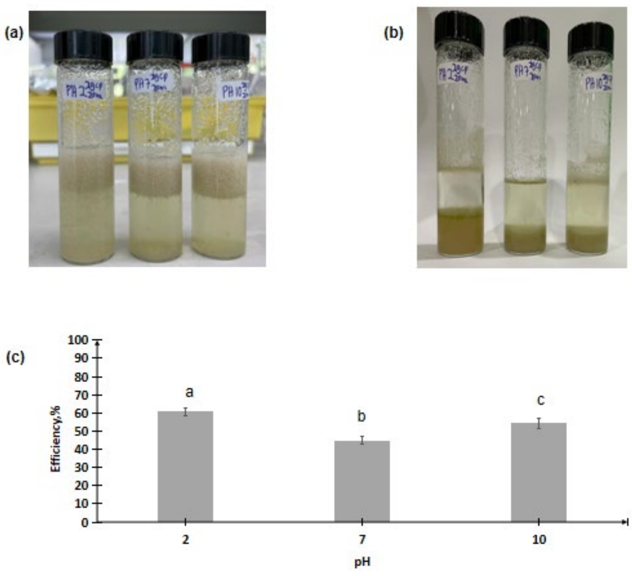

Figure 2: Images showing (a) microalgae flocculation with chitosan powder at 0 mins, (b) microalgae flocculation with chitosan powder after 24 h (Red circle indicates the formed sludge), (c) effects of medium pH on the microalgae removal efficiency through flocculation by chitosan powder.

Charge neutralization is most likely the mechanism that governs the flocculation process (Zhang et al., 2020). In contrast to microalgae cells, which have a net negative surface charge (Li et al., 2018), chitosan has a characteristically high positive charge density (Niemi et al., 2021). Chitosan molecules have a strong ability to neutralize charges due to the rapid adsorption caused by their large positive charge (Taghavijeloudar et al., 2022). In line with this observation, Mohd Yunos et al. (2017) observed that chitosan, which is known to have positive charges in acidic conditions, can be utilized to remove microalgae cells with negative charges with a removal efficiency of 80 %. According to Mahmoodi et al. (2011), the strong electrostatic interaction between the microalgae and the $-NH^{+}_{3}$ of chitosan led to high removal efficiency.

3.2 Adsorption of microalgae from pond water using chitosan macrobead

Figure 3c showed the adsorption results associated with the removal of microalgae using chitosan macrobead. The adsorption results at pH 2, 7, and 10 were 99.07 %, 91.81 %, and 95.49 %. Interestingly, the removal of microalgae through adsorption by chitosan macrobead significantly outperformed the one achieved through flocculation by chitosan powder by 1.62 times. Similarly, the highest adsorption took place at a pH of 2, indicating the role of electrostatic attraction between the negatively charged microalgae and positively charged chitosan macrobead. Chitosan is a polysaccharide with good adsorbing capability on a variety of negatively charged pollutants (Daniele et al., 2021) because it contains positively charged hydroxyl and amino groups (Alyasi et al.,2022). In fact, it was found that the error bar of removal efficiency at pH 2 is smaller than at pH 7 and pH 10 (see Figure 3 (c)), indicating that the removal efficiency achieved at pH 2 is more consistent and reproducible. The statistically significant analysis result was obtained as significantly different between the adsorption efficiency at pH 2 with that at pH 7 and pH 10 (i..e T-test analysis between the adsorption efficiency at pH 2 and pH 7, as well as between pH 2 and pH 10 showed *p*-value is lesser than 0.05). In addition, the T-test analysis indicates that the adsorption efficiency at pH 7 and 10 was not significantly different (i.e *p*-value is more than 0.05). As shown in Figure 3 (a-b), the solution turned less greenish after 24 h, indicating the successful separation of most of the microalgae. Interesting to note that the attachment of green microalgae on the surface of the macrobead can be clearly seen in the enlarged image of Figure 3b.

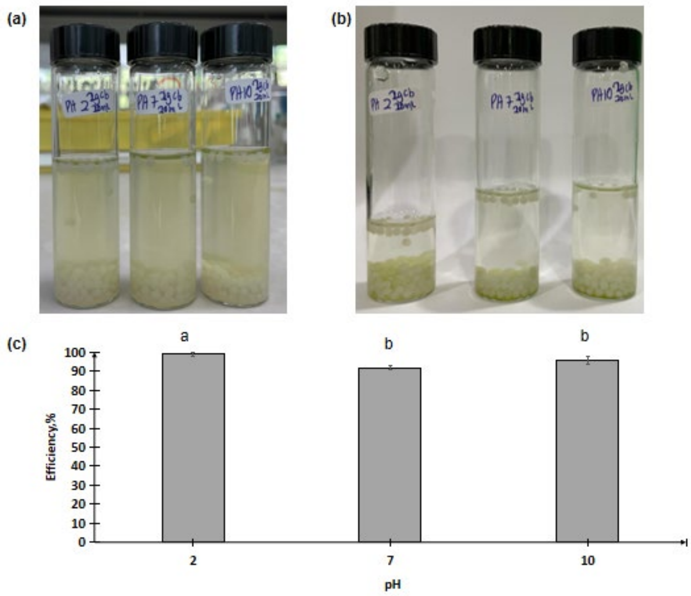

Figure 3: Images displayed (a) microalgae adsorption with chitosan macrobead at 0 mins, (b) microalgae adsorption with chitosan macrobead after 24 h (After adsorption, the macrobeads are indicated by a red circle), (c) effects of medium pH on the microalgae removal efficiency through adsorption by chitosan macrobead.

3.3 Optical microscopic analysis

First, optical microscopic analysis was used to observe the microalgae upon flocculation by chitosan powder (Figure 4) or adsorbed by chitosan macrobead (Figure 5). Before adding the chitosan powder, the microalgae cells were evenly dispersed throughout the liquid medium (Figure 4a); singly dispersed microalgae cells could be readily seen in the absence of a floc. Upon addition of chitosan powder (Figure 4b), it was found that the green microalgae were successfully flocculated (Figure 4c).

On the other hand, the attachment of green microalgae on the chitosan macrobead was verified in Figure 5. As shown in Figure 5, the microalgae were randomly distributed in the solution (Figure 5 (a)) before being subjected to chitosan macrobead. Upon completion of adsorption, the white surface of the chitosan macrobead (Figure 5 (b)) turned green, (Figure 5 (c)) indicating the successful attachment of microalgae on the chitosan macrobead. Worth noting that the microalgae were adsorbed in groups (cluster form) instead of individually adsorbed.

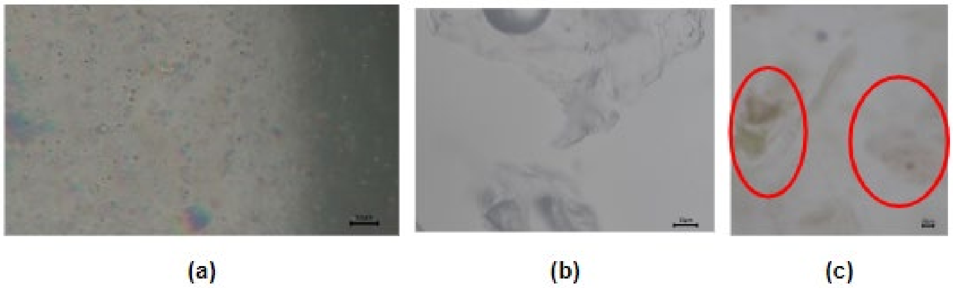

Figure 4: The microscopic image of (a) pure microalgae solution (scale bar = 50 μm), (b) chitosan powder and microalgae solution (scale bar = 50 μm), (c) chitosan powder after spending for the microalgae flocculation (scale bar = 50 μm). (Red circle indicates the flocculated microalgae)

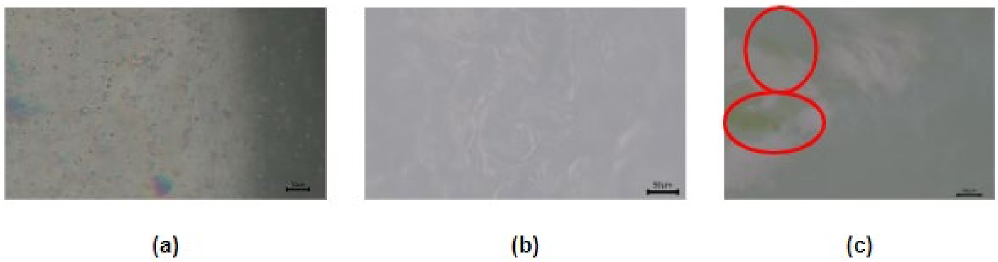

Figure 5: The microscopic image of (a) pure microalgae solution (scale bar = 50 μm), (b) chitosan macrobead before the adsorption process (scale bar = 50 μm), (c) chitosan macrobead after the adsorption process (scale bar = 50 μm). (Red circle indicates the adsorbed microalgae)

4. Conclusions

In this study, the removal of microalgae from local pond water using chitosan powder (flocculation) and chitosan macrobead (adsorption) was systematically compared. Results showed that the chitosan macrobead adsorption method outperformed the chitosan powder flocculation method in microalgae removal by 1.62 times. With the former method, more than 90 % of microalgaes were successfully removed regardless of the medium pH; while the latter method resulted in 44 – 61 % removal efficiency depending on the medium pH. More importantly, the formation of sludge was avoided using the chitosan macrobead adsorption method. The attachment of microalgae on the chitosan macrobead was successfully verified through optical microscopic analysis. With this encouraging result, it is suggested that future studies be done to further optimize the microalgae removal efficiency by modifying the surface pore structure of the chitosan macrobead to evaluate the role of the surface pore of the macrobead on the microalgae adsorption efficiency.

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