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THE INFLUENCE OF CHITOSAN FLOCCULANT ON THE FLOCCULATION OF MICROALGAE CHLORELLA sp.

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ABSTRACT

The use of the chemical flocculants can cause health and environmental problems. The alternative bio-flocculants is chitosan. In this study, the chitosan was prepared as flocculants for harvesting the microalgae chlorella sp. The aim of this research is to investigate the flocculation behavior and mechanism of a chitosan. Chitosan, nanopartikel chitosan and chitosan magnetite were prepared as a flocculant. Nanoparticle chitosan was prepared by crosslinking reaction with Sodium Tri Poly Phospat (STPP). The chitosan magnetite was prepared using iron (III) chloride and iron (II) chloride. Various concentrations (25, 50, 75 and 100 mg/L) of chitosan were tested. The Fourier Transform Infrared spectrometry (FTIR) showed that the FTIR spectra of nanoparticle chitosan the peak of 1655 cm^{-1} disappear and 2 new peaks at 1645 cm^{-1} and 1554 cm^{-1} appears. The transmission electron microscopy (TEM) identified the average diameter of particles was about 23.08 -61.54 nm. The magnetic property of the obtained magnetic composite nanoparticles was confirmed by X-ray diffraction (XRD) and FTIR. The saturated magnetisation reached 21.5 emu/g. The flocculation efficiencies of three flocculants were investigated. The effect of chitosan dose on harvesting efficiency of *Chlorella sp* was also investigated. Results showed that Chitosan nanomagntite was the best flocculants in their category in terms of flocculation efficiency at 98 % floc rate. The higher floc rate was conducted at 100 mg/l of chitosan nanomagnetite.

Keywords: flocculation behavior, chitosan, Microalga Chlorella sp.

INTRODUCTION

Flocculating agents are widely used in industrial processes such as, drinking water purification, food processing, harvesting marine microalgae and wastewater treatment. Flocculants are classified into inorganic flocculants such as polyaluminum chloride (PAC), synthetic organic flocculants such as polyacrylamide derivatives, natural occurring flocculants such as bio flocculants [1], nanoparticle such chitosan nanoparticle, nanomagnetite (Fe_3O_4) [2].

Chemical flocculants are commonly used in water and wastewater treatment industries because of their cost effectiveness and efficient flocculation activities [3]. However, the use of these chemical flocculants can cause health and environmental problems. For example, residual aluminium from PAC and acryl amide monomers from polyacrylamide is known to be neurotoxic and carcinogenic toward humans [4]. Therefore, nanoparticles, with their unique properties, could be considered as alternative flocculants. In hence, chitosan has attracted research and industry interests, as alternative flocculants, due to their high flocculation performance, ecofriendly, biodegradability and some of them could be produced from agro-industrial wastes [5].

The reactivity of chitosan for coagulation and flocculation is presumed due to the same characteristics that were described above for sorption reactions, i.e. cationic charge and ability to specifically bond to certain solid phases. In addition, chitosan is a polymer with moderate to high molecular weight (MW) so that bridging mechanisms are possible. Also, chitosan exhibits solubility changes as a result of changes in pH or salt concentration. The solubility of chitosan is increased in acid media due to protonation of the amine groups, except in sulfuric acid solutions, although dissolving properties are dependent on other characteristics of chitosan such as molecular weight, degree of deacetylation, and ionic composition of the solution.

Farid *et al*. [7] reported the repeated use of bare Fe_3O_4 particles for harvesting of oleaginous Chlorella sp. with high lipid content (0.35–0.41 g FAME or fatty acid methyl ester/g cell). Microalgae cells could be effectively harvested by Fe_3O_4 particles at low pH due to the electrostatic attraction between cells and Fe3O4 particles [6].

Farid *et al*. [7] developed chitosan nanopolymer by an ionic gelation method and tested it for harvesting of Nannochloropsis sp. They reported that the nano-chitosan, in comparison to bulk chitosan, resulted in an approximately 40% decrease of flocculant dosage and 9% enhanced biomass recovery [7].

Biocompatible chitosan has been successfully utilized as Fe_3O_4 nanoparticle surface-functionalization material for better than 95%-efficient harvesting of Chlorella spp. [8]. Lee *et al*. [6] reported increased harvesting efficiency with increasing concentration of composites as well as chitosan/magnetic nanoparticle ratio. They proved that the reuse of culture medium after microalgae harvesting did not show any adverse effect on cell growth, indicating the biocompatibility of chitosan-coated Fe_3O_4 and the possibility of the economic reuse of residual culture media [6]. Toh *et al*. [8] investigated the biodistribution of bare Fe_3O_4 and chitosan- Fe_3O_4 composite inside cells as a possible eventual influence on the quality of biodiesel produced [8]. The internalization

of both nanoparticles, according to transmission electron microscopy (TEM) and energy-dispersive

In this study, the efficiency of chitosan materials for the coagulation microalga Chlorella sp was investigated. In addition, the flocculation performance of chitosan flocculant as chitosan, nanoparticle chitosan and chitosan magnetite was investigated at different dose.
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MATERIALS AND METHOD

Chitosan were donated by Laboratory Material Science, BATAN, Jakarta, acetic acid, iron (III) chloride, iron (II) chloride, sodium hydroxide, sodium tripolyphosphate (STPP), glutaraldehyde (Merck).

Chitosan nanoparticle (Chitosan np)

Chitosan 500 mg was dissolved in 100 ml acetic acid 1% (v/v) to obtain chitosan solution, and raised to pH 4.6-4.8 with 10 N NaOH. STPP (1%) with pH 6.0 was slowly dropped into a chitosan solution. Under magnetic stirring at room temperature, 1 ml STPP 1% solution was added dropwise to 25 ml of chitosan solution. The mixture was stirred for a further 20 min followed by sonification. The resulting suspension was subsequently centrifuged at 12.000 rpm for 10 min. We added additional STPP into supernatant after centrifugation, and observed a milky emulsion, which implied that not at all the chitosan had been converted to nanoparticles. The mixture was stirred again for a further 20 min followed by sonification. The resulting suspension was subsequently centrifuged at 12.000 rpm for 10 min. The chitosan nanoparticles were obtained by freezing the emulsion at -4 ^{0}C. The frozen emulsion was then thawed in the atmosphere and the nanoparticles were precipitated and then dried at 100 ^{0}C for 96 h. The Chitosan nanoparticles was characterized by X-Ray Diffractometry, Fourier Transform Infrared spectrometry and TEM.

Chitosan nanomagnetite

The 7.4×10^{-4} moles of iron (III) chloride and 3.7×10^{-4} moles of iron (II) chloride were added to a 10 mL of chitosan 1% in acetic acid 1% at room temperature. After stirring at 2000 RPM for 5 min, a given 0.5 mL volume of glutaraldehyde 25% was added and stirred for 5 min, then, kept under rest for 5 h. After polymerization, the solid gel was gently smashed and then transferred to a 50 mL of a 20 w% NaOH solution. Immediately, the brownish gel changed to a blackcolor. The samples were washed with deionized water and centrifuged until a pH = 8, then, dried in the oven for 1 h at 80 ^{0}C and grounded to obtain a powder. The chitosan nanomagnetite was characterized by X-Ray Diffractometry and Fourier Transform Infrared spectrometry (FTIR).

Determination of flocculating rate

The flocculating rate of the chitosan materials was measured using a microalgae suspension. Different concentration of chitosan materials were added to 200 mL aliquots of microalgae suspension with cell concentration of 0.5 g/L dry cell and the pH value was adjusted to 7.0 using 1 M NaOH or HCl.in 500 mL beaker. The mixture was then vigorously stirred at 150 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and then allowed to stand for 5 min using a 6-breaker jar tester The optical density (OD) of the clarified solution was measured with a spectrophotometer (Shimadzu 160 A, Japan) at 550 nm. Finally, the flocculating rate was calculated according to the following equation:

$$\text{Flocculating rate } \% = (A - B)/A \times 100\%$$

Where, A and B are the OD550 of the control and sample supernatant, respectively.

RESULTS AND DISCUSSION

a) Characterization chitosan nanoparticle
The FTIR analysis

The FTIR spectra of nanoparticle chitosan is shown in Figure-1. A characteristic band at 3449 cm^{-1} is attributed to –NH$_2$ and OH groups stretching vibration and the band for amide I at 1655 cm^{-1} is seen in the infrared spectrum chitosan. Whereas in the FTIR spectra of nanoparticle chitosan the peak of 1655 cm^{-1} disappear and 2 new peaks at 1645 cm^{-1} and 1554 cm^{-1} appears. The disappearance of the band could be attributed to the linkage between the phosphoric and ammonium ions. The nanoparticle chitosan also showed a peak for P = O at 1155 cm^{-1}. Li Feng *et al* [8] and Devika and Varsha [10] observed similar results in their study of the formation nanoparticle chitosan and chitosan film treated with phosphate.
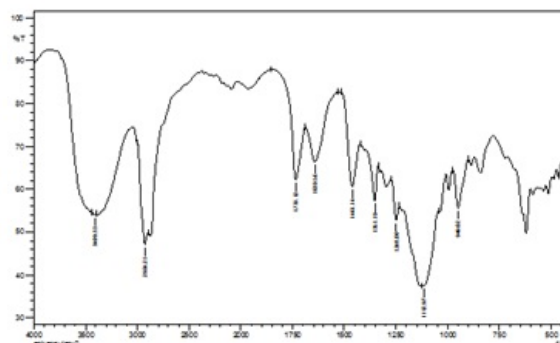

Figure-1. IR Spectra of chitosan nanoparticle.

The mechanism of cross linking of chitosan with STPP could be either by deprotonation or ionic interaction as shown in Figure-1. Chitosan with a pKa of 6.3 is polycationic when dissolved in acid and presents –NH$_3$$^+$ sites. STPP (Na$_5$P$_3$O$_{10}$) dissolved in water dissociates to give both hydroxyl and phosphoric ions. Since the cross linking of chitosan due to the availability of the cationic sites and the negatively charged species, it was expected that the pH of STPP would play a significant role in same. pH would bring about a change on the extent and type cross linking. Hence in the present study pH conditions pH 8.6 were used for reaction. At pH 8.6 both OH$^-$ and

phosphoric ions were present and may compete each other to interact with the $- NH_3^+$ of chitosan.

XRD analysis

X-ray diffraction pattern of chitosan nanoparticle are shown in Figure-2. However, no peak is found in the difractogram of chitosan nanoparticle. The XRD pf chitosan nanoparticle is characteristic of an amorphous polymer. The crystal structure of the amorphous chitosan nanoparticle has been destroyed after cross linked with sodium tripolyphosphate.
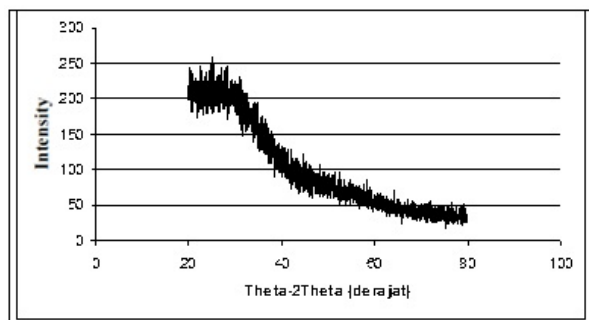

Figure-2. X-ray powder diffraction patterns of chitosan nanoparticles

b) Morphology of chitosan nanoparticles

Figure-3 depicts the TEM image of chitosan nanoparticle along with SAED particles shown. The average diameter of particles was about 23.08 -61.54 nm. Zhi *et al* [9] reported when NaOH was selected as the precipitator, the average diameter chitosan nanoparticle was 32-142 nm.
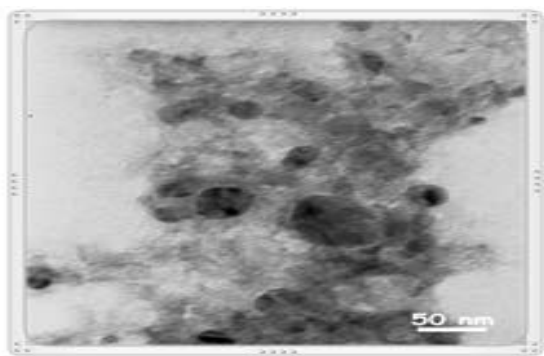

Figure-3. Images and size distribution of chitosan nanoparticle

c) Characterization chitosan nanomagnetite (CMNS)

FTIR spectral analysis CMNS

Figure-4 describes FTIR spectra of chitosan–magnetite nanocomposite (CMN), respectively. In the spectrum display bands at 3200–3400 cm^{-1} due to the stretching vibration mode of OH and NH_2 groups. The peaks seen at 2950–3000 and 2870 cm^{-1} are due to sp^3 and

sp^2 hybridization, respectively (corresponding to C–H group). The peak due to C–O stretching along with N–H deformation mode has been observed at 1615 cm^{-1}. The bands at 1550, 1430 and 1340 cm^{-1} are attributed to N–H deformation, symmetrical deformation of CH_3 and CH_2 group, COO− group in carboxylic acid salt. The FTIR peaks seen at 1150 and 925 cm^{-1} are assigned to β(1–4) glucosidic band in the polysaccharide unit, 1080cm^{-1} is attributed to Figure-2   describing FTIR spectra of chitosan–magnetite nanocomposite (CMNs), respectively. In the spectrum display bands at 3200–3400 cm^{-1}, it is due to the stretching vibration mode of OH and NH_2 groups. The peaks seen at 2950–3000 and 2870 cm^{-1} are due to sp^3 and sp^2 hybridization, respectively (corresponding to C–H group). The peak due to C–O stretching along with N–H deformation mode has been observed at 1615 cm^{-1}. The bands at 1550, 1430 and 1340 cm^{-1} are attributed to N–H deformation, symmetrical deformation of CH_3 and CH_2 group, COO− group in carboxylic acid salt.

The IR peaks seen at 1150 and 925 cm^{-1} are assigned to β(1–4) glucosidic band in the polysaccharide unit, 1080 cm^{-1} is attributed to stretching vibration mode of the hydroxyl Moreover, the characteristic peak, present at 3400.43, 2869.59, 1638.42, 1376.19, 1027.97 and 566.84 cm^{-1}, confirms the metal–oxygen stretching. The FTIR spectra CMNs exhibits characteristic IR bands of the functional group corresponding to pure CH and the Fe_3O_4 nanoparticles. The IR bands corresponding to the –NH and OH stretching modes are shifted to the lower wave number in the IR spectra of the -CH–Fe_3O_4 nanocomposite corresponding to pure CH. This indicates that amine group of CH is involved in the assembling of Fe_3O_4 nanoparticles. The presence of IR band at 566.84 cm^{-1} pertaining to the stretching vibration mode and the torsional vibration mode of Fe–O bonds in the tetrahedral sites and octahedral site reveals the formation of complex between surface charged Fe_3O_4 nanoparticles and cationic CH matrix, indicating the formation of -CH-Fe_3O_4 hybrid nanobiocomposite. Finally, it can be seen in that all these characteristic peaks are also present in spectrum of chitosan–magnetite nanocomposite.
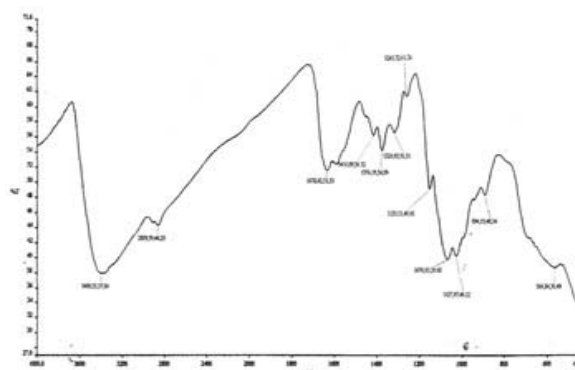

Figure-4. FTIR spectrum chitosan magnetite nanocomposite.

XRD analysis

Figure-5 describes XRD patterns of chitosan magnetite nanocomposite. The power XRD patterns of bare CMNs, all the diffraction peaks match the five diffraction peaks at 220, 311, 400, 422, 511, 440 and 553 by comparison with Joint Committee on Powder Diffraction Standards (JCPDS card no. 79-0418) , which are indexed to the cubic spinel phase of Fe_3O_4. It suggests that the synthesis process will not affect the peak positions of the particles.
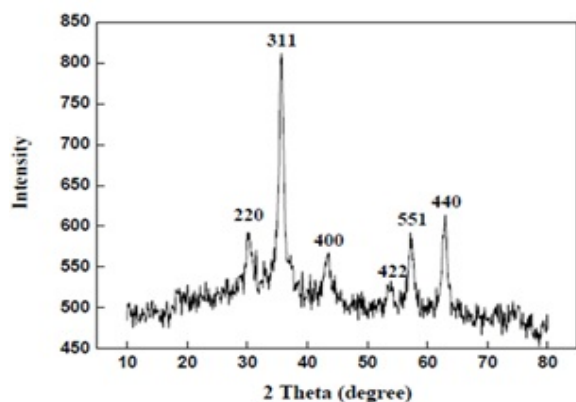

Figure-5. XRD patterns of Chitosan Magnetite nanocomposite.

Saturated magnetisation

The saturated magnetisation reached 21.5 emu/g. Figure-6 showed the characteristics of super paramagnetic which it indicated that the diameter of the particles was less than 30 nm. When CS was removed by calcinations, the saturated magnetisation of pure Fe_3O_4 particles without CS reached from 21.5-63.0 emu/g, and still showed the characteristics of super paramagnetic.
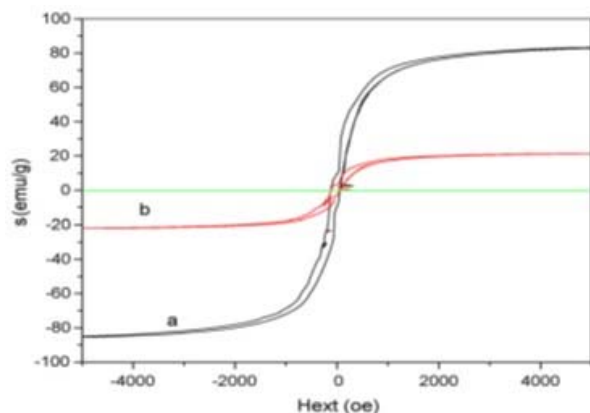

Figure-6. Hysteresis loop of magnetic nanoparticles after roasting (a) and before roasting (b).

d)   Flocculation efficiencies

Figure-7 shows the corresponding flocculation efficiencies (calculated using OD680) for the 5 tested flocculants.   In early stage of the study, different flocculants were tested individually. It was the goal of that stage to pick out the more efficient flocculants for further experimentation using flocculants. From the experiments, it was found that chitosan, chitosan nanoparticle and chitosan nanomagnetite flocculants used, the flocculation efficiency was close to the experiment control. Our study has shown that the third coagulant has similar efficiency to flocculation. Thus, sedimentation efficiency of about 90% was achieved in 60 min for third flocculants.   This phenomenon could be explained based on charge density. Chitosan has a high charge density compared to the other coagulants [10]. Moreover, polymer adsorption increased as the charge density of the polymer increased [11]. Therefore, this signified the rapid destabilization of the particles; and can also be concluded that the amount of the flocculant required to destabilize the particles was lower for a flocculant of higher charge density of chitosan. Moreover, the operating pH of all beer samples was between 4.5 and 4.7 which could influence the chitosan behavior. In acid solution, the amino groups of chitosan are protonated and under these conditions chitosan would be expected to exhibit behavior typical of a polyelectrolyte [12]. The protonation of amino groups of chitosan in solution makes chitosan positively charged (act as cationic polyelectrolytes) and since the particles in microalga suspension are negatively charged, chitosan is very attractive as a flocculant by allowing the molecule to bind to negatively charged surface via ionic or hydrogen bonding [13]. This will further reduce or neutralize the particles surface charge. Therefore, the particles destabilization by chitosan could be explained by charge neutralization mechanism. This is in agreement with the published information that non-ionic and anionic flocculants are not suitable to flocculate the microalgae C. vulgaris, Chlorella fusca, Scenedesmus subspicatus b Scenedesmus sp., which was obtained in [14]. Here, it is reported that chitosan materials were proved successfully in microalgae harvest.
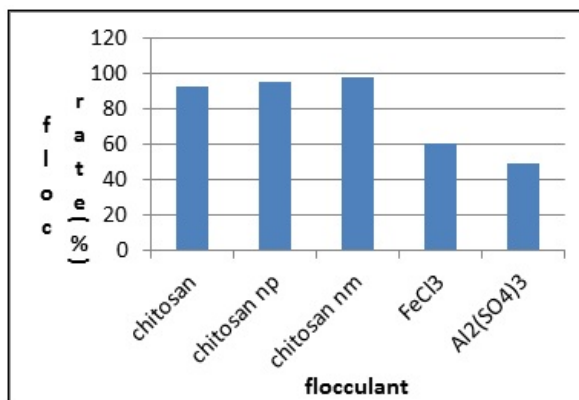

Figure-7. Flocculation of chitosan materials.

Three flocculants, chitosan, chitosan nanoparticle and chitosan magnetite were selected to further investigate their flocculation potential for microalgae harvesting. The

flocculants were chosen based on their diverse chemical properties and their potential applicability to different markets and products. Nanoparticle Chitosan and chitosan nanomagnetite were the best flocculants in their category in terms of flocculation efficiency and dose.

e) Effect of chitosan material dose

The effect of chitosan dose on harvesting efficiency of *Chlorella sp* was investigated. Various concentrations (25, 50, 75 and 100 mg/L) of chitosan was tested. The corresponding percentage ratios of chitosan dosages to microalgae biomass were 10%. A control experiment (without chitosan) was also performed as a reference. A dramatic decrease in optical density was found just after 3 min settling time. The optical density decreased with increase in chitosan dose. We have presented the decrease in optical density in terms of harvesting efficiency. The maximum decrease in optical density was found at 100 mg/L of chitosan and the lowest at 25 mg/L. The highest harvesting efficiency ($92 \pm 0.4\%$) was obtained at 100 mg/L (Figure-8). The efficiency decreased with the decrease of flocculant dose; the lowest efficiency ($32 \pm 0.5\%$) was found at 30 mg/L. The harvesting was almost complete within 3 min. This rapid harvest, even at low dose, is one distinct advantage of chitosan. The harvesting efficiency achieved in this experiment using chitosan was higher than the reported data. Figure-8 shows a comparison of harvesting efficiencies at various chitosan dosages.
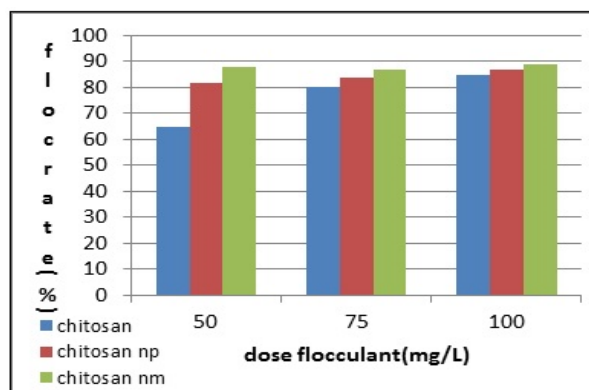

Figure-8. Effect dose flocculant (mg/L) on flocculation rate (%)

CONCLUSIONS

Chitosan particles such as chitosan, nanoparticle chitosan and chitosan magnetite used in this research holds tremendous potential for high flocculation from microalgae culture. Low dose requirement and short settling time are the distinct advantages of chitosan over commonly used flocculants. Dosage of chitosan materials consumption was decreased from 100 to 25 mg/l and flocculation rate increased from 60-85% by using chitosan materials. Chitosan nanomagnetite was the best flocculants in their category in terms of flocculation efficiency at 98%

floc rate. The higher floc rate was conducted at 100 mg/l of chitosan nanomagnetite.

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