The chitosan cembrane cffectiveness of *Anadara granosa* clam shells to remove total coliform in greywater

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ABSTRACT

Clam shell is a solid trash that has a rough texture, difficult to digest, and polluting the environment. Blood clam shells (*Anadara granosa*) contain chitin, which is converted into antimicrobial chitosan. The purpose of this study was to see the effectiveness of chitosan membrane from blood clam shells in reducing total coliform bacteria in household greywater. This study was carried out using experimental research method design. This research focused on household greywater waste. The chitosan concentrations on the membrane varied between 2.5% and 3.5% with filtration times of 20 and 60 minutes. The Anova Quadratic model test was further used to examine the data. Chitosan was separated from the shell of the blood clam at peak angles of 19.66° and 26.04° according to X-ray diffraction (XRD) characterization. It was obtained that chitosan membrane, with a concentration of 3.5% and a filtering duration of 60 minutes, provided the highest percentage of total coliform bacteria reduction of 99.9%. The conclusion of this study is that blood clam shells used as chitosan membranes can reduce total coliform bacteria in household greywater waste.

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1. INTRODUCTION

One of the marine resources found in several kinds of shellfish. Blood clam (*Anadara granosa*) belongs to the *Arcidae* family and the *Bivalvia* class. The red-brown meat of *Anadara granosa* gives it the name "blood clam." This clam, popularly known as blood clam, is a type of clam that possesses a red blood-producing pigment (hemoglobin) that binds oxygen to mussel meat, allowing it to exist in low-oxygen environments and survive even without water [1], [2].

One species of shellfish that is commonly consumed by the general public is blood clam shell (*Anadara granosa*) [3]. The more shellfish consumed; the more waste is produced. As reported in the Surabaya Story daily on March 4, 2019, shellfish trash that continues to build due to its difficulty in decomposition would progressively harm the surrounding ecosystem. Since the landfill cannot handle the big volume of blood clam shell debris, it is piled on Kenjeran beach [4].

The community's processing is still ineffective in reducing the accumulation of shellfish waste on Kenjeran Beach, so this study was conducted by converting blood clam shells (*Anadara granosa*) waste into chitin and chitosan, which were then modified into filtration membranes and made into useful products that have a great monetary worth [5]. Total coliform bacteria and heavy metals can be reduced using chitin and chitosan as filtration membranes [6], [7]. Chitin and chitosan are carbohydrate molecules that can be made from the waste of marine animals, particularly shrimp, crabs, and shellfish [8], [9].

The presence of amine functional groups and the capacity to absorb positively charged chitosan, while the microbial cell membrane is negatively charged, gives chitosan its antibacterial characteristics [10], [11]. These positive and negative charges interact electrostatically, causing a permeability pressure in the membrane, which generates an osmotic pressure imbalance in the cell, inhibiting microbial growth [12], [13]. As a result, chitosan can cause intracellular contents to leak out, causing germs to die, including complete coliform bacteria [14], [15]. The chitosan membrane produced was used as a filtration medium for household greywater waste in a housing complex in Candi Sidoarjo, which had total coliform bacteria exceeding a predetermined threshold.

Total coliform bacteria are an indicator of the presence of pathogenic bacteria, which are microorganisms that can be used to assess whether a water source has been polluted with pathogens or not [16], [17]. Organic and inorganic particles, pathogenic organisms, nutrients, and a variety of hazardous components may be found in high concentrations in a municipal wastewater or household sewage [18]. The existence of total coliform bacteria in the water body specifies that the water has been polluted with the fecal substance of warm-blooded animals [19]. The goal of reducing total coliform bacteria is to reuse household greywater waste as a new water source that does not contaminate the environment. Based on the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number 68 of 2016 concerning domestic wastewater quality standards, the threshold for total coliform bacteria content in domestic wastewater is 3,000 most probable number (MPN)/100 ml. Various membrane-based processes have been widely implemented to treat greywater for creating high quality water to meet greywater reuse standards [20]. This study aimed to analyze the effectiveness of blood clam shells (*Anadara granosa*) as raw material for making chitosan membranes in reducing the total coliform bacteria content in household greywater waste.

2. RESEARCH METHOD

This study was designed as a genuine experiment with a pretest-posttest control group. The study focused on household greywater waste, which contains high levels of total coliform bacteria. The membrane was made by combining chitosan powder (2.5 gr and 3.5 gr) with 100 ml of acetic acid (1%), 29 ml poly vinyl alcohol (PVA) (25%), and 14 ml poly ethylene glycol (PEG 400) [21].

Isolating and modifying chitin into chitosan are the two processes to generate chitosan from shells. Deproteination, demineralization, and depigmentation were used to produce chitin. Deproteination was achieved by mixing 50 gr shellfish powder with 500 ml of NaOH 3.5% and stirring for two hours at 65°C. Aquadest was used to filter the solution and neutralize it. The next step was demineralization by putting 30 grams of deproteinized shell powder into a beaker glass with 300 milliliters of 1N HCl. The mixture was then heated for 30 minutes at 40°C before being cooled down. After filtering and neutralizing the solids using aquadest, they were then dried for two hours at 80°C. Depigmentation was the final step in the chitin isolation process. Depigmentation was performed in a beaker glass, 20 grams of demineralized powder were mixed with 200 ml of 30% H_2O_2, heated for one hour at 50°C, and then cooled down. Aquadest was used to filter and neutralize the solids. The resulting solid was dried for two hours at 90°C.

The chitin obtained was then deacetylated to produce chitosan powder. Furthermore, 10 gr of chitin powder obtained from the depigmentation process was added to 100 ml of 50% NaOH in a beaker glass and then heated for two hours at 95°C before cooling down. The solids were then filtered and neutralized using aquadest until they reached neutral, and the chitosan was ready to be tested after three times heated in the oven at 90°C for eight hours. The chitosan powder (2.5 gr and 3.5 gr) was further used to make chitosan membranes by adding 1% acetic acid, PVA, and PEG into the chitosan powder. After the membrane had dried, it was soaked for 20 minutes in 1% NaOH to remove it from the petridish. Chitosan membrane concentration variations were 2.5% and 3.5%, respectively, with filtration times of 20 and 60 minutes. In this case, three replications were carried out. In addition, Anova was also employed to evaluate the data, which was displayed in tabular form of the Anova Quadratic model test.

3. RESULTS AND DISCUSSION

3.1. Characterization of chitosan powder

Figure 1 shows the characteristics of chitosan powder from blood clam shells (*Anadara granosa*) after being examined for its crystallinity with an angle of 2θ using the X-ray diffraction (XRD) test. Peaks at an angle of 2θ were found in the chitosan diffraction pattern, with the lowest being 19.66° and the highest being 26.04°. The XRD result shows that calcium carbonate (CaCO3) is the dominant mineral in chitosan powder from blood clam shells (*Anadara granosa*).

The peak represents the formation of chitosan from the shells of blood clams (*Anadara granosa*). The best characteristic peaks of chitosan, according to [22], [23], appear at 2θ 5-40°, reflecting the 020, 110, and 130 planes. Peaks around 2θ 35° reflect the 020 planes, peak 2θ 21.1° reflects the 110 planes, and peak 2θ 22.5° reflects the 130 planes, according to the XRD data. These results indicate that the chitosan powder generated has the best chitosan qualities.

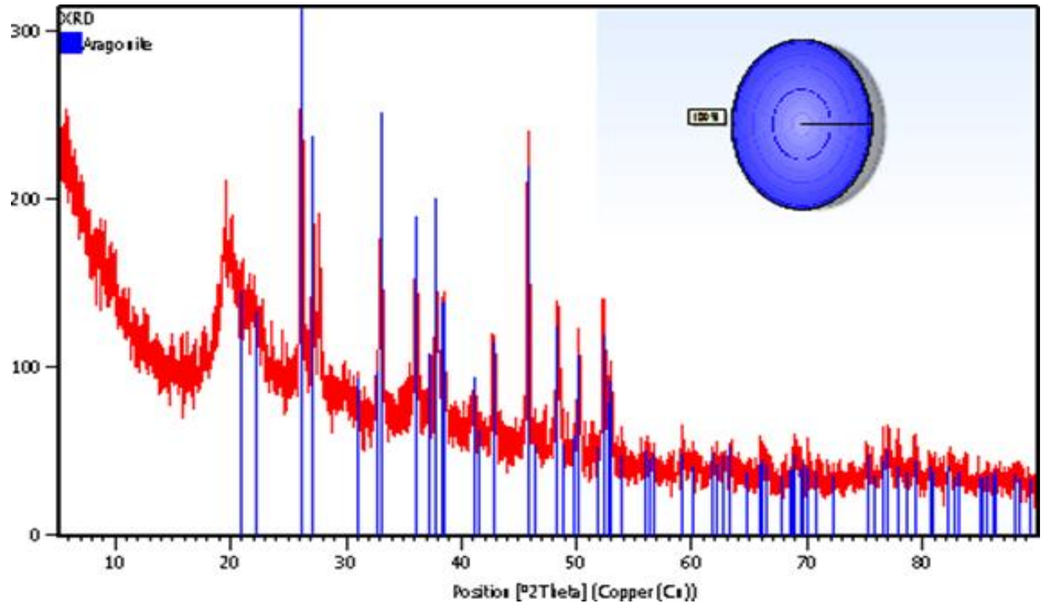

Figure 1. Chitosan powder diffractogram

3.2. Characterization of chitosan membrane

The morphology of the chitosan membrane from the shells of blood clams (*Anadara granosa*) was examined using the scanning electron microscope (SEM) test with magnifications of 1000x, 2500x, 5000x, and 10000x both surface and cross-sectional. Figures 2 and 3 show the results of the SEM on the membrane that were obtained.

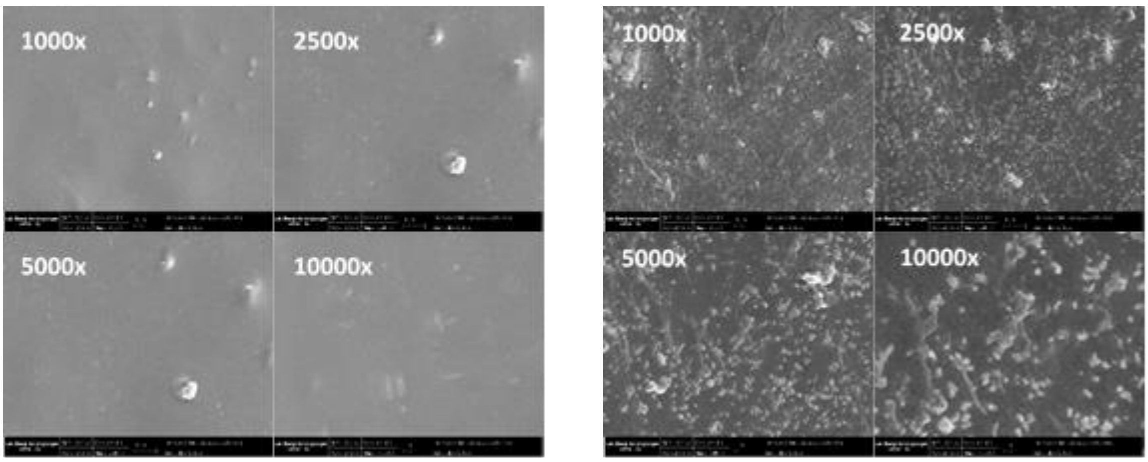

Figure 2. Scanning electron microscope (SEM) of chitosan membrane of blood clam shell (*Anadara granosa*) at 2.5% (left) and 3.5% (right) chitosan concentration on surface

The morphological structure of the chitosan membrane of blood clam shells (*Anadara granosa*) was examined with variations in the concentration of chitosan on the surface. It was observed that the chitosan membrane had a smooth textured surface structure with evenly distributed small lumps at a concentration of 3.5%. There was also saturation throughout the dissolution process on the chitosan membrane with a concentration of 3.5%, indicating that some chitosan powder had not been melted, whereas the chitosan membrane with a concentration of 2.5% had a smoother surface structure. Because the chitosan powder is blended a bit, no solids accumulate on the membrane's surface. This implies that the chitosan powder melted entirely.

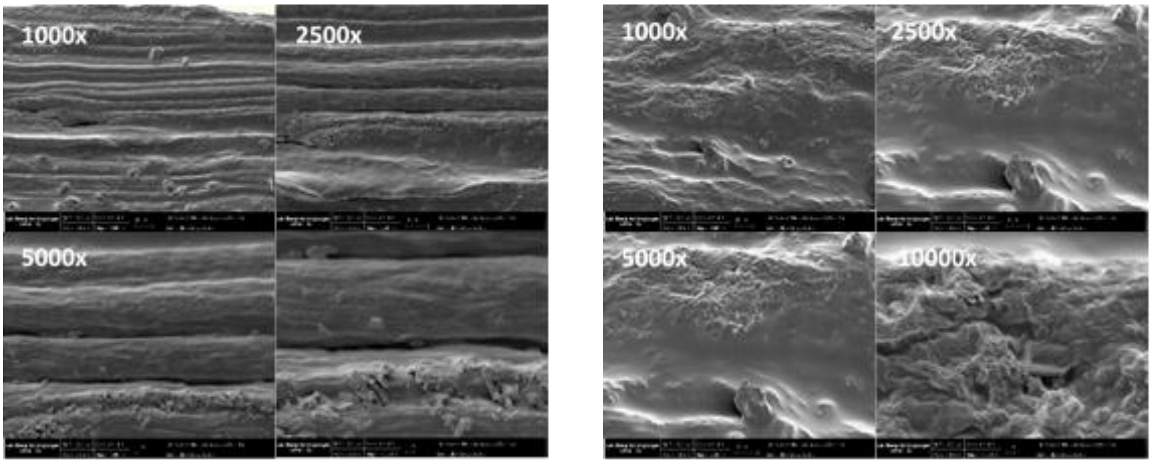

Figure 3. Scanning electron microscope (SEM) of chitosan membrane of blood clam shell (*Anadara granosa*) at 2.5% (left) and 3.5% (right) chitosan concentration on cross-section

According to cross-sectional examination, the chitosan membrane with a concentration of 2.5% appeared to have pores that were not excessively tight, whereas the chitosan membrane with a concentration of 3.5% appeared to have tight pores. From the results of morphological analysis, chitosan membrane at a concentration of 3.5% had a very good characteristic to be applied as a filter membrane in the filtration process of household greywater waste. The results of the morphological structural characterization of the membrane are consistent with the previous study [24], which indicated that the higher the chitosan concentration utilized, the denser the pores of the resulting membrane.

3.3. Total coliform bacteria in household greywater waste before and after the application of chitosan membranes

The result of total coliform bacteria in household greywater waste before and after the application of chitosan membrane from blood clam shells can be seen in Table 1. The average total coliform bacteria in household greywater waste is calculated in Table 1 for each application of chitosan membrane to reduce total coliform bacteria in household greywater waste. Table 1 shows the total coliform bacteria level in household greywater waste as a function of chitosan membrane concentration and filtering time. According to the table, the application of chitosan membranes with a 3.5% chitosan concentration and filtration duration of 60 minutes resulted in the greatest reduction of the total coliform bacteria content.

Table 1. Average of total coliform bacteria in household greywater waste before and after the application of chitosan membrane

Chitosan concentration	Filtration time (MPN/100ml)		Standard
	20 minute	60 minute	
Pretest	$2.6x10^5$		3,000 MPN/100ml
Control	$1.9x10^5$		
2.5%	$4.4x10^4$	$3.2x10^4$	
3.5%	$1.4x10^2$	$0.7x10^1$	

Variations in concentration and filtration time of chitosan membrane application affected the total coliform bacterial content in household greywater waste, resulting in differences in total coliform bacteria content in each sample that has been treated. This is consistent with the previous study [25], which found that the higher the chitosan concentration and the longer the filtering time, the more effective chitosan's antibacterial effect is. Chitosan possesses a positively charged amine functional group (-NH4) that can attach negatively charged molecules that form proteins in bacteria, making it an antibacterial. The ability of chitosan to bind to negatively charged molecules in microbes can bind to total coliform bacteria found in household greywater waste, resulting in a reduction in total coliform bacteria in water samples that have passed through the membrane [26]. Based on the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number 68 the year 2016 concerning domestic wastewater quality standards, the threshold for the content of total coliform in domestic wastewater is 3,000 MPN/100 ml.

3.4. Analysis of difference in average decreased total coliform bacteria in household greywater waste after application of chitosan membranes

The results of the analysis of the difference in average and percentage decrease in total coliform bacteria in household greywater waste after application using chitosan membranes from blood clam shells can be seen in Table 2.

Table 2. The percentage of total coliform bacteria decreased in household greywater waste after the application of chitosan membranes

Chitosan concentration	Filtration time	
	20 minutes	60 minutes
2.5%	215666.67 (83%)	228333.33 (88%)
3.5%	258566.67 (99.4%)	259999.27 (99.9%)

Table 2 shows that after using chitosan membranes, the percentage of total coliform bacteria in household greywater waste decreased. The average decrease in total coliform bacteria showed that the content of total coliform bacteria in each sample varied significantly depending on chitosan concentration and filtering time. The result of decreasing the total coliform bacteria content indicated that the process of separating and binding the negative charge of total coliform bacteria had been carried out by the chitosan membrane [27].

The results showed that the sample with a 3.5% membrane concentration and a filtration time of 60 minutes had the largest decrease in total coliform bacteria content by 99.9%, while the sample with a 2.5% membrane concentration and a filtration time of 20 minutes had the smallest decrease by 83%. The addition of variations in concentration and duration of filtration greatly affected the magnitude of the decrease in total coliform bacteria content in household greywater waste [28].

The chitosan membrane can reduce total coliform bacteria in household greywater, as seen by the decrease in percentage. At a concentration of 3.5%, the membrane reduced total coliform bacteria by 99.9%, and the membrane could not hold the remaining escaping bacteria. The amount of chitosan in the membrane determines the density of membrane pores. As the chitosan membrane's chitosan content increases, the pores become smaller [29]. The density of the resultant membrane pores can be modified by the concentration of the chitosan membrane. The tighter the pores of the chitosan membrane, the more increase the concentration of membrane chitosan, thus increasing the process of binding the negative charge of the microorganisms with the positive charge of the chitosan. The lower the overall total coliform bacteria content in the sample that has gone through the membrane, the more microorganisms that can bind to the positive charge of chitosan.

Filtration time has an impact on the reduction of total coliform bacteria in household greywater waste. The longer the filtration process takes, the longer the process of binding the negative charge of bacteria takes, resulting in a significant reduction in the total coliform bacteria content in household greywater waste that has passed through the membrane. This is consistent with the previous study [30], which found that the concentration of chitosan and the length of filtering time have a significant impact on the antibacterial activity of chitosan.

The difference in average total coliform bacteria content in household greywater waste shown in this study, where total coliform bacteria before treatment was higher than after treatment, showed that the chitosan membrane can reduce total coliform bacteria content in household greywater waste. Chitosan membranes from blood clam shells have several limitations. They can't be utilized for too long since they get saturated, making the process of lowering total coliform bacteria content ineffective.

4. CONCLUSION

Based on the research conducted, it can be concluded that the blood clam shells can be used as chitosan membranes in the process of reducing total coliform bacteria in household greywater waste with the addition of 1% acetic acid, PVA, and polyethylene glycol (PEG). The X-ray diffraction (XRD) test results for chitosan powder, which is isolated from blood calm shells, show a peak diffractogram at an angle of 2θ of 19.66° and 26.04° with the dominating mineral Calcium Carbonate in the chitosan powder characterization and reflected fields 020, 110, 101, 130. The chitosan membrane performed best in an SEM test at a concentration of 3.5% chitosan. The higher the amount of chitosan used in the membrane surface method, the denser the density, and the smaller the pore size of the membrane. The results show that a chitosan membrane made from blood calm shells (*Anadara granosa*) can reduce total coliform bacteria in household greywater, with the best percentage of total coliform bacteria reduction occurring at a 3.5% chitosan concentration membrane within 60 minutes of filtration, with the decrease in total coliform bacteria of 99.9%. It is suggested for other researchers to utilize this chitosan membrane as a reference in household greywater waste treatment. In addition, further research is needed on the effectiveness of chitosan membranes in reducing the other bacteria.

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