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# Effectiveness of eco-absorbent modified chitosan membrane from *Pila ampullacea* as urban water filter to provide healthy sanitary water in Kediri

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**Abstract.** Kediri is well known as a city of cigarette, sugar refinery, and tofu industries. This condition has an impact on high waste disposal to the environment such as rivers. One of the cases in Dermo village Kediri, where the river water turned black and well water turn yellow, high turbidity and smelly when the factory worked. The solution to provide a healthy sanitary water is the use of membrane for filtering the water. Chitosan from *Pila ampullacea* shell, PVA and PEG were mixed to make a membrane. The purpose of this study was to analyze the effectiveness of chitosan membranes as polluted well water filters. Twelve samples of water collected from the wells at a distance in range 10 meters from the river aseptically. Then the water was filtered using the membrane that varies 1, 2, 3, 4 mm thickness. This research compared the water quality before and after filtered by membrane. Temperature, Total Dissolved Solid (TDS), pH, Dissolved oxygen, Dissolved CO<sub>2</sub> and bacterial presence parameters were measured. The result show that chitosan membrane with 4 mm thickness had the most effective membrane to filter and provide the best water quality.

## 1. Introduction

Kediri is well known as a city of cigarette, sugar refinery, and tofu industries. This condition has an impact on high waste disposal to the environment such as rivers. Rivers in Kediri as one of fresh water source for domestic use. Recent condition of river will give the effect to quality of urban well which constructed less than 10 meters. Fidani [1], reported that the well water at Dermo village in Kediri got pollution from domestic and sugar factory. The condition of water from well are yellow in water color, and smell bad [2]. This content is not good for health if used daily. From the case above, we need to process river water into clean water efficiently and effectively. The treatment of waste polluted water using chitosan is effective for absorbing the color and odor of waste and killing pathogenic bacteria also as metal absorbent [3,4].

Chitosan is one of the most widely used materials as a membrane material because it has the ability to form films, easy processing and abundant availability[3,5]. Currently chitosan membranes have been used in desalination, pervaporation of organic dehydration, gas separation, and fuel cells[6,7,8,9]. However, the chitosan membrane has several deficiencies, which are easily brittle, and hydroscopic so it caused low mechanical stability in large scale use [5]. The addition of poly vinyl chloride (PVA) and polyethylene glycol (PEG) can improve the quality of chitosan in reducing COD



and BOD and absorb heavy metal ions so that modified chitosan membrane PVA and PEG can help overcome environmental pollution problems. PVA itself play a role as emulsifier which can minimize adhesion between proteins and cells while PEG has functions as an elasticity, hydrophilicity, and antifouling agent [10].

Generally, chitosan used as membrane filtration comes from crab or shrimp shell waste. However, chitosan used in this modified chitosan membrane products is made from rice field shells *Pila ampullacea* waste. This snail shell was obtained from the local food industry in Kediri. The industry uses rice snails as satay, or cooking, while the shell is removed. There have been no attempts to utilize the shell so we took the initiative to utilize and process the shell into chitosan. *Pila ampullacea* shells contain chitin (20%), oligosacarida, protein, minerals, and lime [11,12]. Chitin content in the shell is a basic ingredient that can be converted into chitosan. The objectives of this study wasto analyse the effectiveness of modified chitosan membrane as water filter to provide healthy water.

## 2. Materials and Methods

### 2.1. Chitosan preparation from *Pila ampullacea*

**2.1.1. Preparation of samples.** Shell of fresh water snail *Pila* washed and dried, then crushed until have a finely texture. This powder sifted on 100 mesh. Shell powder that passes the 100 mesh sieve will be isolated from the chitin content and then transformed into chitosan.

**2.1.2. Demineralization.** Shell powder from 100 mesh were reacted by HCl solution 1,5 M in ration 1:15 (%mass /volume). The reaction run with agitation 50 rpm at 60-70 °C for 4 hours. After that, the solids obtained are washed using distilled water until the pH is neutral. Solids are dried at 80 °C for 24 hours using an oven and weighing is done after drying. The results of the demineralization stage are referred to as crude chitin.

**2.1.3. Deproteinizing.** Crude chitin obtained from the results of demineralization is then reacted with 3.5% NaOH solution with a ratio of 1:10 (% b / v). The mixture is then heated at a temperature of 60-70 °C for 4 hours while stirring at a speed of 50 rpm. The solids obtained are then washed using distilled water until the pH is neutral. After that, the solid is dried in an oven with a temperature of 80 °C for 24 hours then weighed. The results of this deproteinizing stage are called chitin.

**2.1.4. Deacetylation.** At this stage, chitin is reacted with 50% NaOH solution with a ratio of 1:10 (% b / v). Then the mixture is heated at a temperature of 100-110°C for 4 hours while stirring at a speed of 50 rpm. The solids obtained were washed using distilled water until the pH was neutral, then dried in an oven at 80 °C for 24 hours. Weighing is done after chitosan is cooled in a desiccator. The chitosan obtained can be tested using ninhydrin solution or characterized using FTIR to determine the degree of deacetylation (DD).

### 2.2 Chitosan Characterisation

**2.2.1. Solubility Test.** Chitosan is dissolved in 1% acetic acid solution. The higher the solubility of chitosan, the better the quality or quality of chitosan.

**2.2.2. Ninhydrin Test.** Chitosan contains an amine group so it can be identified using ninhydrin solution. Chitosan which has been dissolved in 1% acetic acid is dripped with ninhydrin solution. A positive result for the ninhydrin test is the change in the color of the sample to purple.

**2.2.3. Degree of Deacetylation (DD).** The degree of deacetylation is used to determine the quality of chitosan, where chitosan which has DD is greater, the quality is better. DD measurement is based on

the results of chitosan FTIR analysis at wave number 1588 for identification of amide uptake and wave number 3410 for identification of hydroxyl uptake.

### 2.3. PVA-Chitosan membrane composite Preparation

Composite membrane was made by mixing 1% chitosan solution with polyvinyl alcohol (PVA) and 0.25 g PEG while stirring using a magnetic stirrer at a speed of 300 rpm until homogeneous. Comparison of the solution of chitosan and PVA used is 1: 1. After being homogeneous, the solution was printed using a petri dish with a thickness variation of 2.3, and 4 mm and then it was placed for 24 hours. The film formed was soaked in 1% NaOH solution and allowed to stand until the film detaches from the mold. Before use or for storage purposes, membrane films are washed or soaked in distilled water.

### 2.4. Effectiveness analysis of chitosan-PVA composite membrane

The composite membrane formed is then mounted on the Buchner funnel. Contaminated well water is filtered using a Buchner funnel that has been installed as a composite membrane. Furthermore, water before and after filtered (past the composite membrane) was analyzed for physical, chemical and biological parameters. The physical parameters measured were temperature and TDS test (Total Dissolved Solid), while the chemical properties seen included acidity (pH), measurement of DO and dissolved CO<sub>2</sub>. Biological parameters obtained from the bacterial presence test. Document standard for evaluating the quality of water as hygiene sanitation use Regulation of The Health Minister Republic of Indonesia Number 32 year 2017.

2.4.1. *Total Dissolved Solid (TDS) Test.* Total dissolved solid measured based on SNI 06-6989.27-2005 gravimetric method.

2.4.2. *Temperature and pH.* Measurement of water temperature use thermometer and pH done with digital pH meter. The measurement replicate three times then calculated the average.

2.4.3. *Dissolved Oxygen and CO<sub>2</sub>.* Calculation of DO and dissolved CO<sub>2</sub> levels to determine whether there is contamination in water samples. DO and CO<sub>2</sub> are measured using the Winkler titration method.

2.4.4. *Bacterial presence test.* Presence of bacteria in water counted by cultivation technique both MPN method and pour plate method. MPN is used to estimate the amount of *Escherichia coli* contamination while pour plate calculates the number of all types of bacteria in general [13].

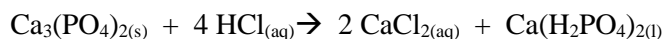
## 3. Results and Discussion

### 3.1. Characterization of chitosan

The Pila ampullacea shell must be washed with clean water to remove dirt and remaining meat that is still attached to the base of the shell. Then the shell is dried in the sun to eliminate water content and facilitate storage. The dried shell is then pounded and sieved using a 100 mesh size sieve to expand the surface area so that the sample interaction process with solvent when chitosan isolation is more effective.

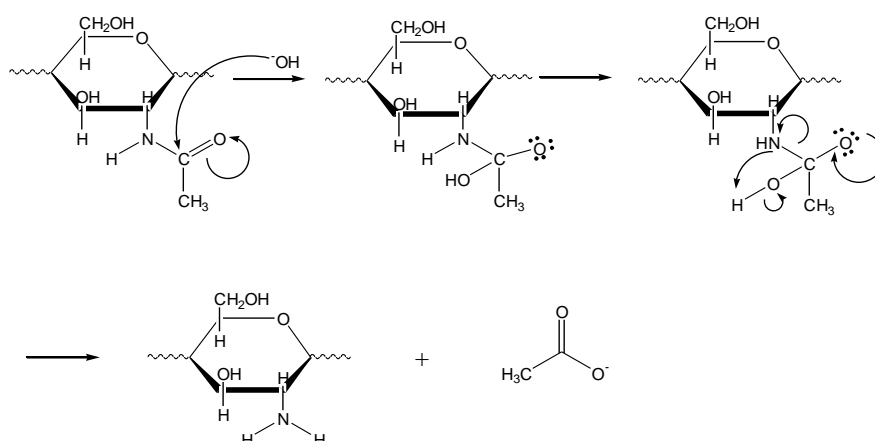
The objective of demineralization stage is to remove minerals or inorganic compounds contained in the conch shell. Generally minerals found in the conch shell are CaCO<sub>3</sub> (calcium carbonate) and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (calcium phosphate). The removal process is carried out by reacting shell powder with 1.5 M HCl solution at a temperature of 60-70 °C while being sterilized for 4 hours. The reactions that occur are as follows [14].





According to the reaction above, the  $\text{CO}_2$  gas formed can be seen in the presence of bubbles in the demineralization process. The results of this demineralization reaction form crude chitin which is brown or light yellow.

In the deproteinization phase, there will be a breaking of the bond between the protein and chitin so that the color changes to brownish yellow. The deacetylation stage uses 50% NaOH solution which functions to break carboxyl bonds with nitrogen atoms in chitin. The high NaOH concentration causes the amino function group ( $-\text{NH}_3^+$ ) which substitutes chitin acetyl groups to become more active so that the deacetylation process becomes better. The reaction mechanism that occurs is as follows:



**Figure 1.** The mechanism of reaction of chitin transformation to chitosan

The final of deacetylation process are white bone to white powder. Overall, the results of chitin and chitosan isolation are as follows:

**Table 1.** Result of chitosan synthesis.

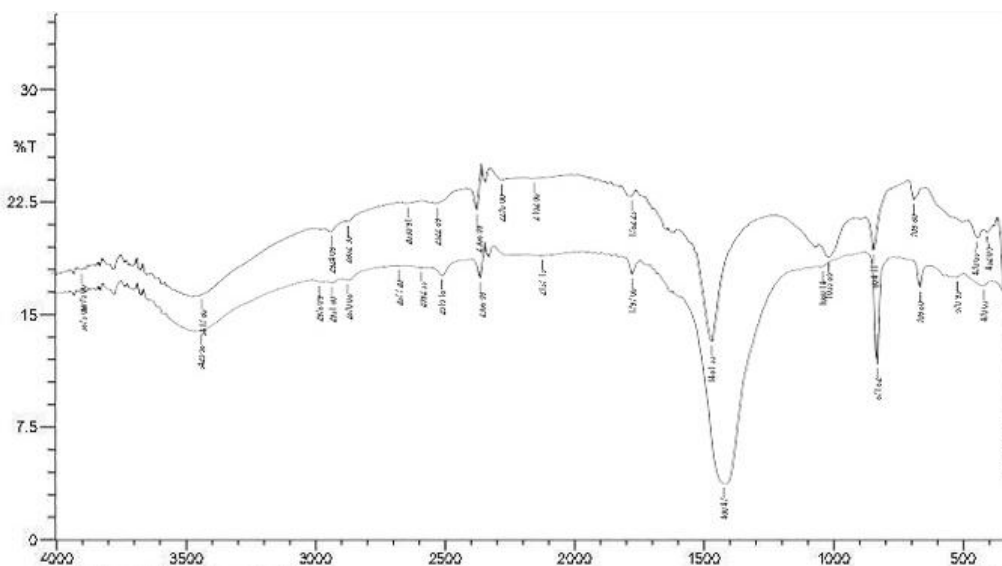
Shell mass (g)	Crude chitin (g)	Crude chitin (%)	Chitin (g)	Chitin (%)	Chitosan (g)	Chitosan (%)
75	37,71	50,28	34,81	46,41	29,87	39,83

The solubility test find out whether chitosan has actually formed or not because chitosan will dissolve in dilute acetic acid while chitin does not dissolve. Chitosan can dissolve in dilute acetic acid due to the formation of hydrogen bonds between carboxyl groups of acetic acid and amine groups of chitosan. Solubility of chitosan in acetic acid is influenced by the degree of deacetylation, where the higher the degree of deacetylation, the higher the solubility of chitosan. This is because the higher the deacetylation degree of chitosan, the lower the acetyl group and the higher the amine group in chitosan [5,6]. Thus, more hydrogen bonds can be formed between amine groups of chitosan and carboxyl groups from acetic acid.

Ninhydrin test is a qualitative test to determine the presence of amine groups in amino acids, proteins, or protein derivatives. The positive result of this ninhydrin test is the formation of purplish blue. The success of the deacetylation process is seen from the formation of amine groups ( $\text{NH}_2$ ) in chitosan, so that the ninhydrin test can be used to determine whether chitosan has been formed or not. The results of ninhydrin test on chitosan samples from conch shells showed the formation of blue cranium which indicated that chitosan was successfully isolated from the rice snail shell.

Another test to proofing the presence of chitosan can be analyses by FT-IR. IR spectroscopic tests were carried out to analyze functional groups and to determine the deacetylation degree of chitin and chitosan by IR spectroscopy. Chitin and chitosan were first prepared before being analyzed, which was

crushed with KBr with a ratio of 1:10 (w / w). The use of KBr because the cell where the sample must be made of materials that can penetrate infrared light, such as NaCl and KBr. Based on IR spectra it can be seen that at wave scale 3500-3300 cm<sup>-1</sup> there is an absorption band for (N-H), indicating the presence of an amide (acetamide) group.



**Figure 2.** IR chitin (above) and chitosan (bottom) spectra

### 3.2. Chitosan-PVA composite membrane analysis

Membranes that have been made are used to filter water samples which taken from well that build in 10 meters from river. A distance of 10 meters is a minimum distance requirement that is allowed to build buildings around the river. According to Waluyo [15] the distance is ideal for minimizing contamination. Well water source conditions are too close to river water bodies, allowing well water to get recharge from polluted rivers and reduce the quality of the water [16].

Measurements result of water quality parameters before filtered and after passing through the membrane are presented in Table 2. The results for each membrane thickness seem different. The difference in results supports the influence of thick membrane on water quality. The thicker the membrane, the better the water quality. Temperature parameters, TDS, dissolved CO<sub>2</sub>, TPC, and E. coli MPN are decreasing in value along with the increase in membrane thickness. DO and pH increase following thickness. Temperature does not change too far and thickness has no significant effect. For sanitary hygiene requirements with a range of 25-27 °C is normal.

Total dissolved solid illustrates the number of particles present in both organic and inorganic particles. The results show that even though it has been filtered but there are still particles contained in water which means that the particles can pass through the membrane pore. The selection ability of membran chitosan to particles dissolved in water is caused by the nature of chitosan as a positively charged biomultipolymer and contains an amine group so that it binds strongly to negatively charged molecules such as fat, protein, mineral ions and various metal ions [13]. The selective properties of chitosan are also strengthened by PVA composites. Based on statistical analysis a membrane thickness of 4 mm is most effective (p value < 0,05) in filtering out dissolved particles in water. The pH of clean water that is good for health is in the range of 6.5 to 8.5. This study obtained the pH value of the water before being filtered near the minimum threshold (6.8). The value of interpreting well water experiences pollution at a very low level. Low degree of acidity can increase pollutant toxicity [17]. In this study, chitosan membrane significantly affected pH changes. The thicker the membrane, the pH of the water increases closer to normal (7). pH properties showed the significant difference in 4 mm

thickness membrane. Increasing of water pH that has been filtered with chitosan membranes is also obtained by Pontius [18]. This result proves that the modification of chitosan with PVA and PEG can be effective as a filter of wastewater into clean water.

Dissolved oxygen is needed by aquatic organisms to carry out body metabolism. The normal DO water level of 2 ppm is not contaminated with pollutants [19]. DO well water tends to be low because the well is in a calm condition there is no water agitation. Increasing in DO levels in water filtered by chitosan membranes is determined by membrane thickness. Higher DO is obtained from the thickest membrane (4 mm). This is confirmed by the statement that the ability of chitosan membranes to absorb oxygen depends on its physical shape and is related to the surface area [23]. High levels of oxygen in water also come from the reduction in microorganisms present in the water itself because of the ability of chitosan as an antibacterial.

High CO<sub>2</sub> content in water before filtering is related to pH, low pH has high CO<sub>2</sub> [20,18]. Decreasing of CO<sub>2</sub> is not only influenced by the increase in pH that occurs but also influenced by chitosan activity. Carbon dioxide will be captured and act with the amine group contained in chitosan [24]. The well water before being filtered with membranes has several parameters that do not qualified as hygiene sanitation water requirements because the index of MPN E. coli showed 1600 CFU / 100ml. E. coli contamination in well water is caused by the proximity of wells with domestic waste disposal sources such as septic tanks, bathrooms, or garbage disposal [21].

**Tabel 2.** Membrane effectiveness measurement

Parameter of water quality	Membrane thickness (mm)					National Standarta
	0	1	2	3	4	
Temperature (oC)	27	25	25	25	25	Air temp±3 (32oC)
TDS (mg/L)	780±76,4	500±77,3	420± 48,4	400± 75,4	200±14,1*	1000
pH	6,8±0,2	7,3± 0,1	7,3± 0,2	7,4± 0,2	7,8± 0,1*	6,5 – 8,5
DO (ppm)	0,4±0,5	1,7±0,7	2,5±1,0	4,8±1,0*	6,4± 1,1*	-
Dissolved CO <sub>2</sub> (ppm)	1± 0,5	0,85± 0,4	0,72± 0,04	0,34± 0,04	0,25±0,03*	-
TPC (CFU/ml)	6,6 x 10 <sup>4</sup>	5,3 x 10 <sup>4</sup>	5,1 x 10 <sup>4</sup>	3,8 x 10 <sup>4</sup>	1,5 x 10 <sup>4</sup> *	-
MPN E.coli CFU/100ml	1600	350	110	4	0	0

aRegulation of The Health Minister Republic of Indonesia Number 32 year 2017.

\* Significant difference (p value< 0,05)

The use of chitosan membrane *Pila ampullacea* with a thickness of 4 mm is able to filter bacteria in well water until the water has a quality that qualify with Regulation of The Health Minister Republic of Indonesia Number 32 year 2017 [25]. Based on the ability to hold bacterial cells that are 2-6 µm long and 1-1.5 µm wide, the pore properties of the modified chitosan-PVA membrane are included in the membrane microfiltration [26]. In addition to pore properties, the reduction in the number of bacteria is an effect of the ability of chitosan as an antibacterial [27]. The ability of chitosan to inhibit bacterial growth is caused by an electrostatic reaction between the amine group on chitosan and phosphoryl from phospholipids on the bacterial cell wall [13,18,22].

#### 4. Conclusion

Chitosan modified membrane *Pila ampullacea* and PVA have the ability to filter water. The quality of water that has been in contact with the membrane has improved the quality and qualify to the requirements of Regulation of The Health Minister Republic of Indonesia No. 32 2017 in provide

sanitation hygiene .The thicker the more effective it is to reduce total bacteria, the amount of E.coli and dissolved CO<sub>2</sub> while DO increases.

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