



Optimization of the use of shellfish waste as a coagulant material for liquid waste from the paper industry in waste processing using the knorr method

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ABSTRACT

Background: The paper industry, in its process, utilizes a large amount of water, which in turn generates liquid waste containing chemicals with the potential to pollute the environment. The negative impact of this pollution not only impacts the balance of the environmental ecosystem, but also poses a risk to human health. In addition to the paper industry's wastewater problem, another problem is the accumulation of clamshell waste around the Kenjeran coastline. **Findings:** Poorly managed shell waste can cause air pollution due to unpleasant odors, damage the aesthetics of the beach environment, and become a hotbed of Coli bacteria that can cause diarrheal diseases in local residents. Thus, processing clamshell waste is an attractive solution to overcome the problem of paper industry liquid waste. This study aims to utilize shell waste from Kenjeran beach, Surabaya, to produce chitosan that can be used as a coagulant in the treatment of paper industry wastewater. **Methods:** The processing of green mussel shell waste (*Perna viridis*) into chitosan was carried out in several stages, namely: preparation stage, chitin extraction stage from green mussel shells (*Perna viridis*), chitosan synthesis stage using the Knorr method, and the last stage is the test stage of chitosan use in liquid waste. **Conclusion:** The shell waste treatment process involves chitin extraction by deproteination and demineralization, followed by chitosan synthesis using the Knorr method. The resulting chitosan, with a yield of 73.7%, proved effective as a coagulant in capturing colloidal particles in the effluent and forming floc precipitation. The use of chitosan at a dose of 600 ppm showed optimal results with a decrease in concentration and turbidity of the effluent reaching 59.35%, and lowering the pH by 0.3. **Novelty/Originality of this article:** the problem of liquid waste from the paper industry can be overcome with an environmentally friendly approach, while shell waste on Kenjeran Beach, Surabaya can be utilized economically by being converted into chitosan.

KEYWORDS: chitosan; coagulant; liquid waste; shell waste.

1. Introduction

Surabaya is one of the main contributors of Hazardous and Toxic Materials (B3) waste from the industrial sector in East Java. Paper or pulp industry waste is one type of liquid waste produced on an industrial scale. The pulp and paper production process requires a large amount of water, ranging from 20,000 to 60,000 gallons per ton of product, resulting in a significant volume of liquid waste (Ramos et al., 2009). Components such as phosphorus, heavy metals, and toxic organic substances that are difficult to settle are part of the content of liquid waste from the pulp and paper industry. The presence of these compounds poses a serious threat to aquatic ecosystems, especially rivers. Therefore, the

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development of waste processing technology is very important to overcome the problem of liquid waste produced by paper factories in the area.

In North Surabaya, especially in the Kenjeran area, in addition to industrial scale waste, household waste is also a major concern. This area is known as an area that is widely inhabited by fishermen who produce various processed seafood products, such as fish, shellfish, sea cucumbers, shrimp, and others. As a result of this activity, waste in the form of shells is one of the wastes that are often found around Kenjeran. Unfortunately, until now, the utilization of shells is still very limited, so that the shells are often ignored and become waste along the coast. As a result, not only is there an unpleasant odor around the coast, but environmental pollution also occurs along with the accumulation of this waste.

Considering the challenges of the two types of waste found in North Surabaya, namely liquid waste from the paper industry and solid waste in the form of shells in the Kenjeran area, a proposed solution is to utilize shell waste, especially the green mussel type (*Perna Viridis*), as a coagulant in the processing of liquid waste from the paper industry. Shells contain chitin polysaccharides with a β -bound N-acetyl-D-glucosamine structure. This chitin can be converted into chitosan by converting the acetamide group into an amine group, which is an important step in the processing of shell waste (Rafaat & Shal, 2009). Chitosan, with the structure β (1-4)-2-amino-2-deoxy-D-glucose, has the ability to act as a coagulant in binding several hazardous substances in liquid waste. The hydroxyl and amino groups in chitosan are effective in capturing heavy metal cations and organic compounds.

The process of isolating chitin to become chitosan involves a series of steps, including demineralization, deproteinization, and deacetylation (Salsabila et al., 2022). By proposing the use of mussel shell waste as a chitosan coagulant in paper mill liquid waste, it is hoped that the quality of paper industry liquid waste can be improved. This can be realized by minimizing the content of heavy metals and organic compounds in the waste using natural coagulants based on nature, namely chitosan obtained from green mussel shell waste (*Perna viridis*). In addition to the significant environmental benefits of using mussel shell waste, this approach is also expected to reduce the amount of mussel waste that accumulates on the Kenjeran coast. Thus, this solution will support and maintain the stability of the ecosystem around the coast. Testing the effectiveness of chitosan as a coagulant for liquid paper waste is also important to ensure an increase in the quality of liquid waste, which will be reflected in a decrease in pH value, turbidity, and concentration of organic compounds in it.

2. Methods

2.1 Research design and preparation stage

The processing of green mussel shell waste (*Perna viridis*) into chitosan is carried out in several stages, namely: (1) Preparation stage, (2) Chitin extraction stage from green mussel shells (*Perna viridis*), (3) Chitosan synthesis stage using the Knorr method, and the final stage is (4) Chitosan use test stage on liquid waste. The entire research flow diagram is shown in Figure 1. This stage is carried out to clean the waste of green mussel shells that have been collected until they are ready to be used in the next method, the chitin extraction stage. The mussel shells that have been collected from Kenjeran Beach are cleaned, rinsed with running water to ensure that the shells are clean from dirt. The mussel shells are then dried using an oven (70°C for 5 hours). The dried shells are ground and filtered with a 60 mesh test sieve.

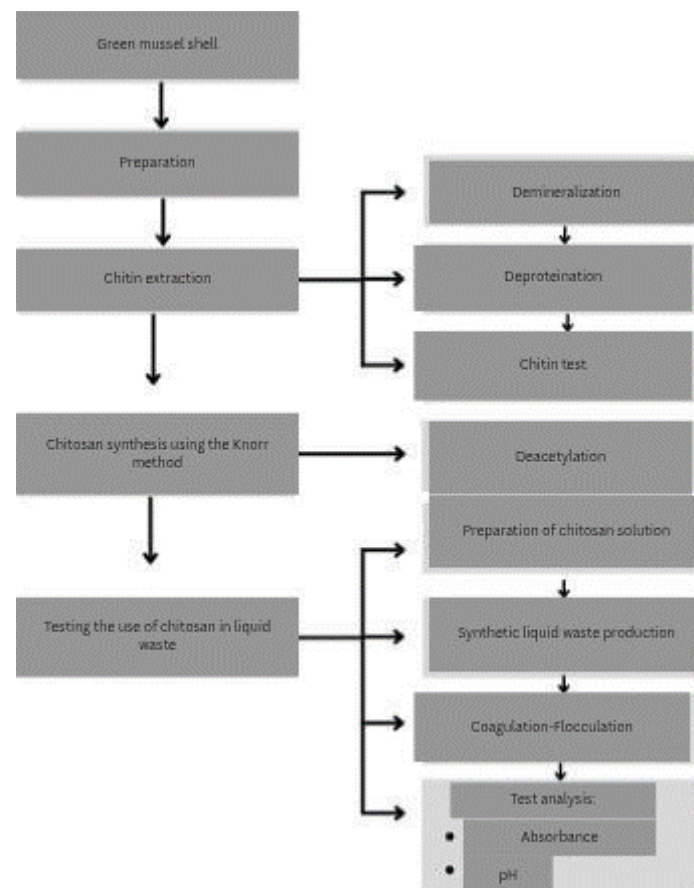


Fig 1. Chitosan synthesis process from green mussel shells

2.2 Chitin extraction stage from shellfish

This stage aims to extract chitin from green mussel shells through demineralization and deproteination stages. The mineral and protein content in green mussel shells must be removed so that pure chitin is successfully obtained and functions optimally. Qualitative testing of the chitin obtained was carried out by adding lugol iodine solution and H_2SO_4 . Demineralization stage: 120 g of mussel shell powder was put into a beaker glass and 1M H_2SO_4 was added with a ratio of 1:10 w/v. The mixture was stirred at 70 rpm at a temperature of 70°C for 1 hour. The mixture was filtered and washed with distilled water until the pH was neutral. The cake obtained was soaked with 4% ethanol (1:10 w/v for 10 minutes) and filtered again and dried in an oven (60°C for 5 hours). The dried mussel shells were then put into a desiccator.

Deproteination stage: demineralized powder (shells from stage 2.2.1) was added with 3.5% NaOH with a ratio of 1:10 w/v (Nitazul et al., 2018). The mixture was agitated at 70 rpm at 70°C for 2 hours. Furthermore, the solid was filtered and washed with distilled water until the pH was neutral. The filtered results were soaked with 4% ethanol with a ratio of 1:10 w/v for 10 minutes. Then dried in an oven at 60°C for 5 hours and put into a desiccator. Chitin test: The chitin obtained was identified qualitatively by reacting chitin with lugol iodine solution (I₂ in KI) and adding H_2SO_4 . The qualitative test is positive if the solution turns brown with the addition of lugol solution and then violet with the addition of H_2SO_4 solution.

2.3 Chitosan synthesis stage using the knorr method

This stage aims to synthesize chitin into chitosan by changing the acetyl group ($-\text{COCH}_3$) in chitin into an amine group ($-\text{NH}_2$). This synthesis is carried out using a high concentration of strong base. Deacetylation: Chitin powder is mixed with 50% NaOH with a

ratio of 1:20 w/v. Furthermore, the mixture is heated and stirred at a temperature of 100 C at a speed of 50 rpm. After that, the solid is filtered and washed using distilled water until the pH is neutral. Furthermore, the solid is dried in an oven at a temperature of 80 C and weighed.

2.4 Test stage of chitosan use in liquid waste

Chitosan obtained from the research results was tested on liquid waste as a coagulant. Before use, Chitosan was dissolved using organic acid, pH 4-6.5. The liquid waste used in this test was synthetic liquid waste whose content was made similar to paper liquid waste. Making chitosan solution: 0.3 g of chitosan was dissolved in 1 L of 1% acetic acid, so that a chitosan solution of 300 ppm was obtained. With the same ratio, chitosan solutions of 400 ppm and 600 ppm were also made. Making synthetic liquid waste: The process of making synthetic waste by delignifying rice husks. A total of 10 g of rice husks was put into a beaker glass then 180 mL of distilled water containing 1.5% H₂O₂ was added with a ratio of 9:1 (v/w). The mixture was added with 1 N NaOH and the mixture was heated at a temperature of 100°C for 2 hours. Finally, the solution was separated from the solids by filtering. Coagulation flocculation process of liquid waste: Liquid waste is prepared as much as 100 mL with the specifications of liquid waste that have been explained previously (sub-chapter 2.4.2). The coagulant dose is prepared in various variants, namely 100 ppm, 200 ppm, and 300 ppm.

The coagulation process is carried out by mixing synthetic liquid waste and coagulant solution from chitosan. The two mixtures are then stirred at a speed of 120 rpm for 1 minute and followed by a second stirring at a speed of 60 rpm for 15 minutes. Finally, the sedimentation process is carried out for 1 hour. During the sedimentation process, stirring is stopped. Absorbance test of liquid waste: The tool used is a UV-Vis spectrophotometer with a wavelength of 480 nm. First, the spectrophotometer must be calibrated with a blank, until %T shows 100 and absorbance is 0. Then the sample is inserted into the cuvette and the %T and absorbance are measured. Liquid waste pH test: Before testing the pH of the sample, the pH meter is first calibrated with a buffer solution. First, the glass electrode is washed with distilled water, dried and calibrated with a buffer solution of pH = 4, pH = 7, and pH = 10 with the same steps. After the pH meter calibration is complete, the pH is ready to be used to measure sample solutions, liquid waste.

3. Result and Discussion

3.1 Shellfish waste

Shellfish waste is one of the by-products of fisheries activities, especially in coastal areas. This waste generally consists of shellfish, most of which are biomaterials that do not decompose naturally quickly. The main composition of shellfish waste includes calcium carbonate (CaCO₃) of 85–90% and chitin of 20–30% in dry conditions (Yusan, 2023). The Kenjeran area, Surabaya, which is one of the main fisheries centers, produces large amounts of shellfish waste every day. Unfortunately, most of this waste ends up as garbage without adequate processing, potentially polluting the environment and causing aesthetic problems in coastal tourism areas (Kurniawati et al., 2020). Shellfish waste can be processed through three main stages: demineralization, deproteinization, and deacetylation. The demineralization process uses an acid solution such as HCl to remove calcium carbonate, while deproteinization uses a base solution such as NaOH to remove residual protein (Yusan, 2023).

The advantage of shellfish waste as a source of chitin compared to other sources, such as shrimp or crab shells, is the high mineral content that provides additional flexibility for other applications, such as fertilizer or carbon dioxide absorber material (Arvanitoyannis & Kassaveti, 2008). Processing shellfish waste into high-value products such as chitosan not only reduces the volume of organic waste that pollutes the environment, but also creates

new economic opportunities for local communities. In coastal areas such as Kenjeran, Surabaya, processing shellfish waste can be a solution to support a circular economy, where waste is processed into products that provide added value, such as heavy metal absorbers, fertilizers, or chitosan for coagulant applications (Wang et al., 2020). A study by Singh et al. (2018) showed that processing fishery waste can reduce up to 70% of waste that has the potential to pollute the environment. In addition, this processing also creates products that can compete in the global market, increasing the economic value of local waste.

3.2 Chitosan

Chitosan is a natural biopolymer produced from the deacetylation process of chitin, which is commonly found in the exoskeletons of crustaceans such as shrimp, crabs, and shellfish. Chitin itself is a polymer consisting of N-acetylglucosamine units, while chitosan has a higher proportion of glucosamine units, which gives it soluble properties at low pH. This property is due to the presence of positively charged amine groups in the chitosan molecule, which allows electrostatic interactions with negatively charged compounds. The molecular structure of chitosan, including the degree of deacetylation (DD), greatly affects its physicochemical properties, such as solubility, viscosity, and adsorption capacity. Generally, chitosan with a DD of more than 50% is considered quite soluble in weak acid solutions, such as acetic acid or citric acid (Rinaudo, 2006; No & Meyers, 1995). The appeal of chitosan as a biomaterial lies in its biocompatible, biodegradable, and non-toxic properties, making it a promising choice as a drug carrier that can be modified as needed (Darma, 2015). Chitosan is soluble in most organic acid solutions with a pH below 6.5, such as formic, acetic, tartaric, and citric acids, but insoluble in phosphoric and sulfuric acids.

In wastewater treatment, chitosan is used as an effective coagulant and flocculant agent to adsorb suspended particles, heavy metals, and organic compounds. Studies have shown that chitosan can remove up to 95% of pollutants in industrial wastewater, including paper and textile wastewater, making it an environmentally friendly alternative to metal-based synthetic coagulants such as aluminum sulfate (Singh et al., 2018). Despite its many advantages, challenges in the production and application of chitosan remain, including high production costs, the need for quality standardization, and the scalability of the technology for industrial use. However, with the development of more environmentally friendly technologies and approaches, such as the use of enzymes for the deacetylation process, the prospects for chitosan as a versatile material continue to grow, especially in supporting environmental and economic sustainability (Arvanitoyannis & Kassaveti, 2008; Safari, 2020).

3.3 Coagulant

Coagulants are substances used in the clean water treatment process to help remove unwanted small particles (Ampera, 2018). This process involves the clumping or flocculation of smaller particles so that they are easily precipitated. Basically, river water or polluted water has colloidal particles scattered in it. The coagulation process aims to disrupt the stability of these colloidal particles. Destabilization can be achieved by adding chemicals such as alum or through rapid stirring, plunging, or mechanical methods (Oktavianto, 2024). The benefits of using coagulants include their ability to facilitate the sedimentation process of dissolved particles in water, increase the clarity of the water produced, eliminate unwanted odors and colors, and ensure drinking water is free from bacteria and viruses that have the potential to endanger public health. In addition, the use of coagulants can also increase the efficiency of water treatment in industry.

3.4 Industrial liquid waste

Industrial liquid waste is waste generated from various industrial processes and is no longer used (Ratnani, 2024). This waste can come from various sectors such as industry,

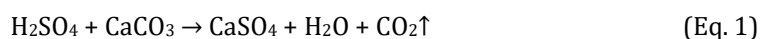
households, livestock, agriculture, and others. The main component in liquid waste is water, but other compositions vary depending on the source of the waste. In general, substances in liquid waste can be divided into organic and inorganic. One of the important characteristics and quality parameters in liquid waste is Biochemical Oxygen Demand (BOD). BOD is a measure of the organic content in liquid waste (Fakhrurozi, 2010). BOD measurements are carried out by measuring the amount of oxygen absorbed by a sample of liquid waste over a certain period of time, usually five days, at a certain temperature, generally 20 ° C. BOD is used as an indicator of waste strength and estimates its impact on oxygen in the receiving water body. In wastewater treatment, it is important to choose a method that can reduce the effluent BOD so as not to damage the quality of the receiving water.

3.5 Preparation stage

In the preparation stage, the dried green mussel shells were ground, and filtered using a 60 mesh test sieve, as much as 120 g. The process of refining and filtering the mussel shells was carried out to increase the reaction rate. The fine particle size produces a larger surface area, thus increasing the possibility of collisions between particles. Thus, the reaction rate becomes faster due to increased contact between the reacting materials.

3.6 Chitin extraction stage from shellfish

A total of 120 g of fine shells were demineralized using H₂SO₄ solution. This process aims to remove inorganic salts and mineral content contained in the shells. Minerals such as CaCO₃ and Ca₃(PO₄)₂ can be minimized by acid treatment (Sinardi et al., 2013). The demineralization process is characterized by the release of CO₂ during the mixing process. The decomposition of CaCO₃ into water-soluble calcium salts according to the following reaction equation:



Demineralization process at high temperature will accelerate the reaction (Truong et al., 2007). The reaction must be perfect to ensure total removal of all minerals. So the use of excess or high concentration mineral acid is required (Shahidi et al., 1991). Furthermore, the mixture of fine shells and H₂SO₄ solution is filtered using filter paper to obtain demineralized shell powder. Shell powder of 110 g is produced at the end of the demineralization stage.

The demineralized powder is continued to the deproteinization stage; aims to remove protein content in the shell. The protein contained in the shell is soluble in alkaline conditions so that the covalent protein will separate from the chitin functional group. The preferred base used is NaOH with a concentration range of 0.125-5 M at varying temperatures (Younes & Rinaudo, 2015). NaOH will break the intermolecular bonds between chitin and protein. Furthermore, the protein will bind to water-soluble Na⁺ proteinate. A ratio of 1:10 w/v of shell powder and NaOH solution was used, based on previous research with blood cockle samples (Windari et al., 2019). This deproteinization stage only produced 70 g of powder from 100 g of processed shell powder (70.00% yield). Chitin is the final powder from the demineralization and deproteinization process. Therefore, to ensure that chitin has been obtained in the powder, a qualitative chitin test was carried out. The chitin test was carried out using Lugol iodine and H₂SO₄. Lugol iodine and H₂SO₄ were used because chitin is a type of polysaccharide carbohydrate. The chitin test is positive when the color changes to brown when tested using Lugol Iodine and to violet when using H₂SO₄ (Silvia-Garcia et al., 2020).

3.7 The results of the synthesis stage of chitin into chitosan using the knorr method

The main process, chitosan synthesis, occurs in the deacetylation process. The acetyl group is removed ($-\text{COCH}_3$) to become an amine group ($-\text{NH}_2$) in this deacetylation process. However, in the deacetylation process, the depolymerization process still occurs. The synthesis of chitin into chitosan can be identified by the change in molecular weight (Figure 2).

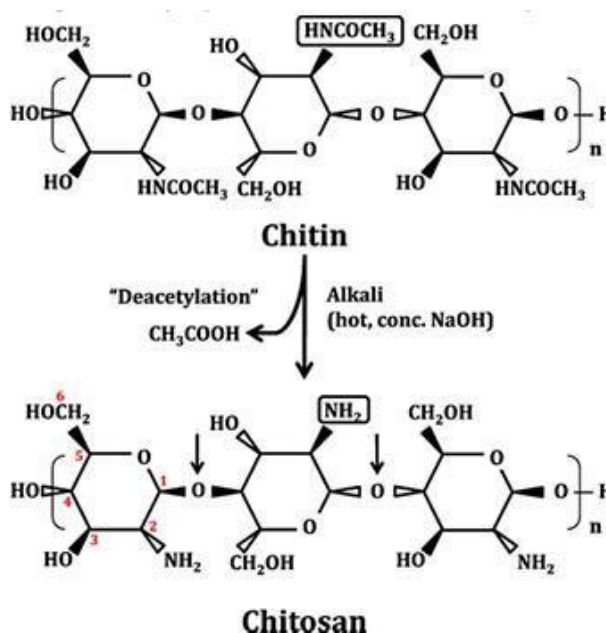


Fig 2. Structure of chitin and chitosan
(Rafaat & Shal, 2009)

The use of base in the deacetylation process produces better chitosan than using acid. The use of acid in the deacetylation process can cause chain destruction due to the susceptibility of glycosidic bonds to acid (Aranaz et. al., 2016). This study used 50% NaOH so that the degree of deacetylation can be increased. The higher the concentration and reaction temperature will increase the number of substances that react and collide (Toan, 2009). A total of 51.62 g of chitosan was obtained by processing 70 g of chitin (73.70% yield). The yield of each stage of the study is shown in Table 1.

Table 1. Yield obtained at each stage of chitosan isolation from green mussel shells

Process	Yield (%)	Color
Demineralization	83.3%	Gray
Deproteination	70.0%	Beige
Deacetylation	73.7%	White

The yield of chitin was 70% (Table 1), this result is higher than previous research of 46.5% (Arsyi et al., 2018). The yield of chitin from green mussels is also higher than the yield when chitin is made from shrimp shells, 36.76% (Agustina, 2015). Meanwhile, the yield of chitosan from green mussel shells is 73.7% and is also higher than the yield from shrimp shells, which is 67.08% (Agustina, 2015). Thus, green mussel shells have more potential to produce both chitin and chitosan than shrimp shells. Chitosan is catheterized to determine the suitability of the quality value of chitosan with the standard value. The results of chitosan catheterization from this study can be seen in Table 2.

Table 2. Characterization of chitosan

Parameter	Chitosan obtained	Standard Value (SNI No.7949.201)
Solubility in 2% acetic acid	Late	Late
Texture	Powder	Powder
Color	White	White to pale yellow

The solubility of chitosan in acetic acid is one of the standards for chitosan catheterization, the higher the solubility of chitosan in 1% acetic acid means the better the quality of chitosan produced. This happens because chitosan is very soluble in acidic solutions (especially pH <6) and becomes a weak base due to the presence of amine groups. When the pH is low, the amine group is positively charged because it is protonated so that chitosan can become a cationic polyelectrolyte that is soluble in water. However, when the pH increases (pH > 6), the amine group of the chitosan residue is protonated and the biopolymer loses its charge so that it becomes an insoluble polymer (Roy et al., 2017). The test results showed that chitosan was completely soluble in 1% acetic acid.

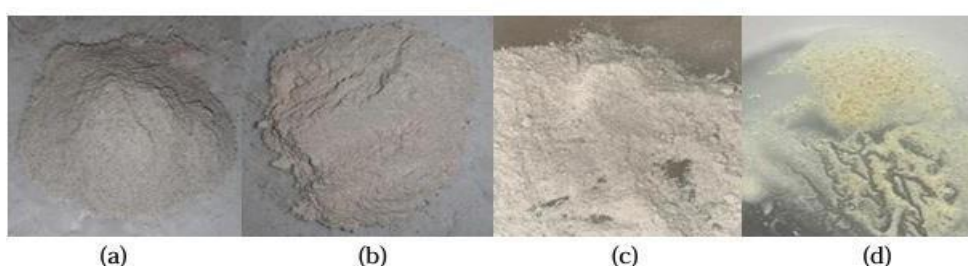


Fig 3. Green mussel shell powder from (a) demineralization; (b) chitin; (c) chitosan; and (d) chitosan test.

3.8 Test stage of chitosan use in liquid waste

Paper industry liquid waste is brown to blackish due to wood and pulp processing. Given its color, this liquid waste is called black liquor and is the main waste in the paper industry. This waste is considered a pollutant and the brown color of the waste is caused by complex compounds originating from polymerization between lignin and tannin degradation products during the pulping and bleaching process. Black liquor waste contains 50% lignin. Synthetic liquid waste was used in the study. Synthetic liquid waste is made from a substitute material similar to wood, namely rice husks. Rice husks contain cellulose of 34.34-43.80% and lignin 21.40-46.97% (Novia et al., 2017).

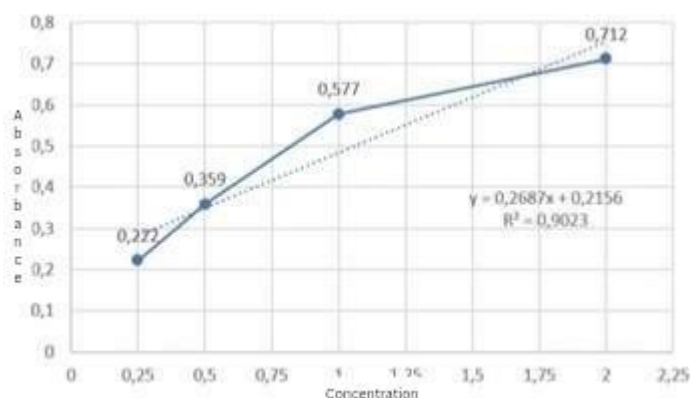


Fig 4. Calibration curve of standard solution

Chitosan test as a coagulant was applied to synthetic liquid waste. Previously, chitosan was dissolved first with 1% acetic acid, with a pH of around 4-6.5. Chitosan will not dissolve at a lower or higher pH. Thus, chitosan will coagulate organic compounds in waste. The amine group ($-NH_2$) in chitosan will bind H^+ from acetic acid to form NH_3^+ . This group will

be adsorbed and bonded to negatively charged colloidal particles, forming a polymer bridge that combines between colloidal particles to form flocs. The addition of chitosan as a coagulant will increase the sedimentation rate and increase the density of the sediment so that the TSS will decrease and the color of the liquid waste will decrease. First, a calibration curve of various concentrations of liquid waste was made (Figure 4). Then, each sample of liquid waste was tested for absorbance, both before and after the addition of chitosan (Table 3).

Table 3. Absorbance of each chitosan solution

Variable	ABS	Concentration	Turbidity (NTU)
Waste	0.575	2.12	4.9
Waste + 300 ppm chitosan	0.373	1.39	3.2
Waste + 400 ppm chitosan	0.381	1.42	3.27
Waste + 600 ppm chitosan	0.234	0.87	2.004

Chitosan coagulant can reduce the concentration of liquid waste and can also decolorize dyes from waste solutions as evidenced by a decrease in absorbance and turbidity values (Table 3). Of the various chitosan dose variables, the most effective dose was 600 ppm seen from the lowest concentration, absorbance and turbidity with a decrease of 59.35%. Furthermore, the pH of liquid waste was also measured (Table 4).

Table 4. pH of liquid waste

Variable	pH
Waste	12.96
Waste + 300 ppm	12.92
Waste + 400 ppm	12.9
Waste + 600 ppm	12.66

The greater the addition of chitosan, the greater the decrease in pH. This is because the chitosan solution has a pH of 4-6.5; slightly helps to a lower the pH of the waste. In addition to lowering the pH, the coagulation and flocculation process also reduces the levels of dissolved oxygen in the waste. This is because the chitosan solution coagulates the a organic solution from the waste.

3.9 Economic value of chitosan from green mussel shell waste

The process of making chitosan from green mussel shell waste requires several chemicals. The following is Table 5 which shows the economic value of chitosan from green mussel shell waste.

Table 5. Economic value of chitosan from green mussel shell waste

Stages	Material	Price/Gram of Shellfish Powder
Demineralization	H ₂ SO ₄ 1M	33,300 IDR & 1,200 IDR
TIDeproteinization	NaOH 3.5% Aquadest	25,300 IDR & 12,000 IDR
Demineralization	NaOH 50%	289,600 IDR & 2,400 IDR
Chitosan solution dose 600 ppm (1 liter)	CH ₃ COOH Aquadest	340,000 IDR & 120,000 IDR
Total		823,800 IDR

The production of chitosan from green mussel shell waste requires a fairly affordable cost, with an estimated cost of Rp. 3,638.00 for each gram of mussel powder. Furthermore, to prepare a chitosan solution with a dose of 600 ppm, a cost of Rp. 8,238.00 is required. The availability of affordable production costs, along with the availability of easily obtained raw materials, makes this method effective for large-scale development. Thus, this method promises significant potential for mass use in industry or other applications.

4. Conclusion

Based on the research, it was concluded that green mussel shell waste taken from Kenjeran Beach, Surabaya, was successfully converted into chitosan with a chitosan yield from chitin reaching 73.7%. Furthermore, it was found that the optimal dose for processing paper industry liquid waste is 600 ppm chitosan. The effectiveness of using chitosan coagulant in processing paper industry liquid waste is proven by a decrease in waste concentration and turbidity by 59.35%, a decrease in pH by 0.3, and a decrease in Total Suspended Solids (TSS). The method of processing liquid waste using chitosan coagulation has proven to be more cost-effective and more environmentally friendly. This is because chitosan is a non-toxic biopolymer compound, so it does not cause the formation of additional waste. Thus, the problem of paper industry liquid waste is overcome with an environmentally friendly approach, while mussel shell waste at Kenjeran Beach, Surabaya can be utilized economically by being converted into chitosan.

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Author Contribution

All authors contributed equally to the conceptualization, methodology, analysis, and writing of this review. They collaboratively reviewed and approved the final manuscript for submission.

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Not available.

Conflicts of Interest

The authors declare no conflict of interest.

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