ABSTRACT

Objective: Crustacean shell waste is not currently used to its full potential. Most waste from crustaceans used in food pollutes the environment. Widely found in crab shell waste and shrimp shell waste, chitosan is a modification of chitin compounds. This study aims to utilize crustacean shell waste (crab shell waste and shrimp shell waste) as a natural adsorbent against heavy metals and dyes in the form of chitosan.

Methods: This study includes the steps of extracting chitosan from crab shell waste and shrimp shell waste, followed by adsorption capacity tests against heavy metals (mercury and arsenic) and dyes (tartazine and amaranth).

Results: Chitosan sourced from both crab shell waste and shrimp shell waste met the physical and chemical characteristic requirements, and the yield was 28.19% and 18.33%, respectively. The adsorption capacity against heavy metals and dyes from crab shell waste chitosan ranged from 43.4% to 55.6% and the shrimp shell waste chitosan ranged from 50.8% to 60.2%.

Conclusion: Crustacean shell waste can be processed into chitosan, which is valuable and can be used as a natural adsorbent against heavy metals and dyes for wastewater treatment in several industrial sectors.

Keywords: Chitosan, Crab, Shrimp, Adsorbent, Heavy metals, Dyes

INTRODUCTION

Crustaceans are a subphylum that includes shrimp and crabs, which are harvested to be used in different various ways. In the crab and shrimp industry, usual processing only takes the crab and shrimp meat and throws away the excess, including the shells [1]. This waste is disposed of, which does not fully take advantage of this material and in some cases adds to pollution. The proper utilization of crab shell waste can reduce the potential for environmental contamination and can make the crab shell waste into a valuable material [2].

Chitin is produced from hard-shelled marine invertebrates, which are commonly known as crustaceans. The crab shell and shrimp shell contain chitin which has the potential to be developed and can be processed into chitosan through the deacetylation process using a strong base, which has unique properties because this polymer has a positively charged amine group [3]. Therefore, chitosan can be used as a source of natural materials because chitosan as a natural polymer has good characteristics, such as biodegradability, non-toxicity and adsorption [4].

Water pollution or water quality degradation is caused by a number of human activities, one of which comes from when an industry is not managed properly and discharges wastewater directly into waterways or the ground surface [5]. Industry waste that is directly discharged into rivers can cause pollution in many forms: changes in color, smell, and taste in water; inhibition and loss of aquatic biological activity; pollution of soil and groundwater; and physical changes in plants, animals and humans by chemical substances [6]. At a certain level, the pollution will be bound and neutralized by the soil layer, but if it exceeds the capacity of the soil, then the waste content will reach groundwater and pollute it [7].

Heavy metals such as mercury (Hg) and arsenic (As) are included in the group of metals that are toxic and harmful to living things. The high content of heavy metals in the water will cause polluted aquatic biota and accumulated in aquatic biota. If a person consumes the biota that live in the polluted water, it can be harmful to their health [8]. Dyes are one type of these many pollutants. Synthetic dyes are more stable than natural dyes and are one of the non-biodegradable organic pollutants in water. Almost all chemical dyes are toxic and if they enter the human body, they will stimulate the growth of cancer cells. It is necessary to find an effective alternative to remove the pollution of heavy metals and synthetic dyes from water [9].

Chitosan has specific properties that make it useful in different ways. It is bioactive, biocompatible, chelating, anti-bacterial, and biodegradable. It is most commonly used as a preservative for fishery products and a color stabilizer for food products, as a flocculant that assists the reverse osmosis process in water purification, and as an additive for agrochemical products and seed preservatives [10]. The aim of this study was to compare the adsorption effect of chitosan derived from fishery waste, namely crab shell and shrimp shell, against heavy metals (mercury and arsenic) and dyes (tartazine and amaranth).

MATERIALS AND METHODS

The technique used for chitosan extraction from crab shell waste and shrimp shell waste was based on the technique found in Tan et al., 2020 with slight modifications [11]. The technique used for the adsorption capacity test of crab shell waste chitosan and shrimp shell chitosan against heavy metals and dyes is based on the technique developed by Iukum et al., 2020 and Soewarna et al., 2021 with slight modifications [12, 13].

Materials

Crab shell waste and shrimp shell waste (Cinta Damai Sub District, Percut Sei Tuan District, Deli Serdang Regency, Sumatera Utara Province, 20371, Indonesia), Hydrochloric Acid (Smart Chemical), Sodium Hydroxide (Smart Chemical), Purified Water (Bratoco),
Chitosan (Smart Chemical), Mercury Chloride (Smart Chemical), Arsenic Chloride (Smart Chemical), Tartrazine (Smart Chemical), Amaranth (Smart Chemical).

**Tools**

Glassware (Borosil), Analytical Balance (Mettler Toledo), Water Purifier (Merck), Filter Paper (Whatman), Filter Cheesecloth (Ima), Oven (Pharma Technic), Blender (Sanken), Magnetic Stirrer (Ika), Thermometer (Thomas), Infrared Spectrophotometry (Agilent), Atomic Absorption Spectrophotometry (Agilent), Ultraviolet-Visible Spectrophotometry (Agilent).

**Chitosan extraction**

The crab shell and shrimp shell waste were obtained from a traditional market in Sumatera Utara. After being transported to the laboratory, the crab shell and shrimp shell waste was rinsed under running water, boiled for 10 min, rinsed once more under running water, and laid to dry under the sun for 3 d. The cleaned crab shell and shrimp shell waste were powdered using a blender and then sieved using a laboratory sieve at size 200 mesh. Then, 200 g of crab shell and shrimp shell waste was separated to begin the process. First, it underwent a demineralization process which used 2 L HCl solution 0.5 M for 1 hour at 80 °C. This demineralization was repeated 5 times. The resulting material was filtered and then washed with distilled water until the pH was neutral, then the material was dried in the oven for 1 hour at 100 °C. The material continued with a deproteinization process using 3 L of NaOH solution 0.3 M for 1 hour at 80 °C, which was repeated 3 times. It was then filtered and washed with distilled water until the pH was neutral and dried in the oven for 1 hour at 100 °C. Following this, the material underwent the deacetylation process using 4 L of NaOH solution 50% for 3 h at 120 °C. This was repeated 5 times. The material was filtered and washed with distilled water until the pH was neutral and then the material was dried in the oven for 1 hour at 100 °C.

**Adsorption capacity test**

In order to test the adsorption capacity of this chitosan derived from crab shell and shrimp shell waste, a controlled material containing heavy metals and dyes was produced. To simulate heavy metals contained in wastewater, mercury and arsenic were added at a concentration of 5 ppm. For the synthetic dyes found in wastewater, tartrazine and amaranth were added at a concentration of 50 ppm. The heavy metal solution at a concentration of 5 ppm and the dyes solution at a concentration of 50 ppm were tested separately with 100 ml of heavy metals solution of dyes solutions and 1 g of chitosan for 1 hour at room temperature (±25 °C). The mixture was filtered through filter paper and the solution of the heavy metal was measured by using Atomic Absorption Spectrophotometry, resulting in 235.7 nm for mercury and 193.7 nm for arsenic. The solution with synthetic dyes was measured using Ultraviolet-Visible Spectrophotometry and resulted in 569.0 nm for tartrazine and 516.0 nm for amaranth. The adsorption capacity of chitosan derived from crab shell and shrimp shell waste were presented as removal percentage of heavy metal (mercury and arsenic) and dye (tartrazine and amaranth) with 6 times replication.

**RESULTS**

This process of producing chitosan from dried crab shell waste proved to have a 28.19% yield. This yield was an odorless, light brown powder. The shrimp shell waste produced a 18.33% yield of chitosan and was an odorless off-white powder. Fig. 1 shows the physical appearance of chitosan sourced from crab shell waste, chitosan sourced from shrimp shell waste, and standard chitosan.

![Fig. 1: Physical appearance of chitosan sourced from crab shell waste (left), chitosan sourced from crab shell waste shrimp shell waste (middle), and standard chitosan (right)](image)

Fig. 2: Overlay infrared of spectrum chitosan sourced from crab shell waste (blue line), chitosan sourced from shrimp shell waste (green line), and standard chitosan (red line)
The chitosan derived from crab shell waste and shrimp shell waste were analyzed to find the active functional group contained in chitosan. Infrared spectrophotometry analysis was carried out on chitosan derived from crab shell waste, chitosan derived from shrimp shell waste, and standard chitosan. The standard chitosan was used as the reference or control chitosan. The chitosan sourced from crab shell waste and chitosan sourced from shrimp shell waste was designated as extracted chitosan (or test chitosan). Fig. 2 shown the overlay infrared spectrum of chitosan derived from crab shell waste, chitosan derived from shrimp shell waste, and standard chitosan. Table 1 shows the obtained wavenumber of the three types of chitosan.

The results of the analysis show that crab shell waste chitosan has a qualitative level of 96.92% against standard chitosan, while shrimp shell waste chitosan has a qualitative level of 99.90% against standard chitosan.

Table 1: Obtained wavenumber of chitosan sourced from crab shell waste, chitosan sourced from shrimp shell waste, and standard compared to chitosan references

| Functional group | Wavenumbers of chitosan |
|------------------|--------------------------|
|                  | References | Standard | Crab shell waste | Shrimp shell waste |
| N–H Stretching   | 3361 cm⁻¹  | 3362.1 cm⁻¹ | 3354.6 cm⁻¹ | 3362.1 cm⁻¹ |
| O–H Stretching   | 3291 cm⁻¹  | 3280.1 cm⁻¹ | 3287.5 cm⁻¹ | 3287.5 cm⁻¹ |
| C–H Stretching (Symmetric) | 2921 cm⁻¹  | 2929.7 cm⁻¹ | 2907.3 cm⁻¹ | 2914.8 cm⁻¹ |
| C–H Stretching (Asymmetric) | 2877 cm⁻¹  | 2877.5 cm⁻¹ | 2877.5 cm⁻¹ | 2877.5 cm⁻¹ |
| C–O Stretching (Amide) | 1645 cm⁻¹  | 1647.5 cm⁻¹ | 1647.5 cm⁻¹ | 1647.5 cm⁻¹ |
| N–H Bending (Amide) | 1589 cm⁻¹  | 1587.8 cm⁻¹ | 1580.4 cm⁻¹ | 1587.8 cm⁻¹ |
| C–H Bending      | 1423 cm⁻¹  | 1423.8 cm⁻¹ | 1416.4 cm⁻¹ | 1416.4 cm⁻¹ |
| C–H Bending      | 1375 cm⁻¹  | 1379.1 cm⁻¹ | 1379.1 cm⁻¹ | 1379.1 cm⁻¹ |
| C–N Stretching (Amide) | 1325 cm⁻¹  | 1319.5 cm⁻¹ | 1319.5 cm⁻¹ | 1319.5 cm⁻¹ |
| C–O–C Stretching (Asymmetric) | 1282 cm⁻¹  | 1252.4 cm⁻¹ | 1259.8 cm⁻¹ | 1259.8 cm⁻¹ |
| C–O Stretching   | 1066 cm⁻¹  | 1066.0 cm⁻¹ | 1058.6 cm⁻¹ | 1058.6 cm⁻¹ |
| C–O Stretching   | 1028 cm⁻¹  | 1021.3 cm⁻¹ | 1021.3 cm⁻¹ | 1021.3 cm⁻¹ |
| C–O Bending      | 896 cm⁻¹   | 879.7 cm⁻¹  | 879.7 cm⁻¹  | 879.7 cm⁻¹  |

The extracted chitosan (crab shell waste chitosan and shrimp shell waste chitosan) was tested for adsorption capacity against a solution containing heavy metals (mercury and arsenic) and a solution containing dyes (tartrazine and amaranth). The measurements were done by atomic absorption spectrophotometry for the heavy metals and ultraviolet, visible spectrophotometry for the dyes. The concentrations for heavy metals and dyes before and after filtration through the two types of test chitosan can be seen in table 2. Measurement results of adsorption capacity can be seen in fig. 3.

Table 2: Heavy metals and dyes concentration before and after filtration through chitosan derived from crab shell waste and shrimp shell waste

| Concentration                  | Treatment with chitosan | Crab shell waste | Shrimp shell waste |
|--------------------------------|-------------------------|------------------|-------------------|
| Mercury Concentration Before Treatment | 5.00 ppm               | 5.00 ppm         |
| Mercury Concentration After Treatment  | 2.72 ppm               | 2.46 ppm         |
| Arsenic Concentration Before Treatment    | 5.00 ppm               | 5.00 ppm         |
| Arsenic Concentration After Treatment     | 2.55 ppm               | 2.12 ppm         |
| Tartrazine Concentration Before Treatment | 50.00 ppm              | 50.00 ppm        |
| Tartrazine Concentration After Treatment  | 28.32 ppm              | 24.15 ppm        |
| Amananth Concentration Before Treatment  | 50.00 ppm              | 50.00 ppm        |
| Amananth Concentration After Treatment    | 22.18 ppm              | 19.88 ppm        |

Fig. 3: Adsorption capacity of chitosan sourced from crab shell waste and shrimp shell waste against heavy metals (mercury and arsenic) and dyes (tartrazine and amaranth)
In the preparation of chitosan, the washing step to remove the impurities and the drying step to remove the water moisture could cause a decrease in the final weight and yield [14]. In this study, the extraction process begins with demineralization and continues with deproteinization to increase the yield. The selection of the extraction process sequence is because the minerals form a hard shield on the shrimp shells, so the mineral removal process as the first step will facilitate the removal of the proteins in the next step, which has an impact on greater chitosan yield [15].

The demineralization stage aims to remove minerals (sodium, potassium, magnesium, calcium, iron, manganese, phosphorus and sulfur) by using dilute hydrochloric acid to dissolve minerals contained in crab shell and shrimp shell waste so that minerals will be released from the chemical matrix and leave a mineral-free residue [16]. The deproteinization stage aims to break the bond between protein and chitin by using dilute sodium hydroxide to dissolve protein contained in the shell waste so that the protein that is covalently bound to the chitin functional group will separate and leave a free protein residue [17]. The deacetylation stage aims to convert functional groups from amide to amine by using concentrated sodium hydroxide to hydrolysis the acetyl from an amide functional group to form an amine functional group. The higher the degree of deacetylation, the fewer acetyl groups, allowing interaction through ionic bonds and hydrogen bonds between chitosan and heavy metals and dyes [18].

The differences between the types of chitosan can be seen above. The chitosan derived from crab shell waste and shrimp shell waste are in powder form, while the standard chitosan is in particle form. The chitosan obtained from the crab shell waste was a light brown color and the chitosan obtained from shrimp shell waste was an off-white color, while the standard chitosan was white. All three kinds of chitosan were odorless. The different physical characteristics of chitosan are due to the different species of crustaceans as a source of the raw material to produce chitosan [19]. Overall, both chitosan derived from crab shell waste and shrimp shell waste meets the requirements of chitosan in terms of physical characteristics, particle size, and odor [20].

The results showed that crab shell waste chitosan and shrimp shell waste chitosan showed absorbance wavenumbers that were not significantly different from absorbance wavenumbers shown by standard chitosan. The two types of test chitosan have similar infrared spectrum shapes and absorbance wavenumbers to the reference chitosan [21]. The analysis of shell-derived chitosan was continued by analyzing the qualitative level between crab shell waste chitosan and shrimp shell waste chitosan with standard chitosan. The qualitative level showed that crab shell waste chitosan and shrimp shell waste chitosan meets the requirements. A substance is declared qualitatively similar if the substance has a qualitative level not less than 90% [22].

Chitosan has many benefits in various different industries. In the chemistry sector, it is used as a raw material for the manufacture of biomaterials. In the environmental sector, it can be used as an adsorbent for heavy metals and dyes. In the pharmaceutical sector, it is used as a moisturizer, stabilizer (suspending agent or emulsifying agent), and preservative [23].

The adsorption capacity results show that chitosan derived from crustacean waste removed from 45.6% to 57.6% of heavy metals and for dyes, the removal ranged from 43.4% to 60.2%. The crab shell waste chitosan showed a lower removal percentage than the shrimp shell waste. This phenomenon may be due to species differences, but the chitosan extraction process was carried out through the same procedure. Differences in species, varieties, nutrition, locations and conditions of growth can cause differences in the chemical makeup of the crab and shrimp shells, which require extra steps to optimize the extraction process (demineralization, deproteinization, and deacetylation) in order to produce similar chitosan quality [24].

The interaction between chitosan and heavy metals and dyes is an adsorption reaction in the surface of the chitosan. Active functional groups such as amines, hydroxyl, and carbonyl contained in the structure of the chitosan support the adsorption capacity and increase the removal percentage of chitosan against the pollutants [25]. The reaction between chitosan and heavy metals is due to the formation of complex compounds between chitosan and metal ions, where chitosan acts as a ligand and metal ions acts as the central ion. This happens because of the abundance of lone pairs of electrons on oxygen and nitrogen in the molecular structure of chitosan; chitosan acts as a donor of lone pairs of electrons (Lewis base) and metal ions as receptors of lone pairs of electrons (Lewis acid) [26].

Chitosan is an adsorbent that can be used in the decolorization process of industrial wastewater. Chitosan has a very high affinity for dyes. This is because chitosan has a unique structure with several active functional groups such as amines, hydroxyls, and carbonyls as active sites [27]. The amine group, under acidic conditions, will react with a proton (H+) from its environment so that the amine group is protonated to ammonium (NH4+) and can be used to adsorb anionic dyes. The adsorption of cationic dyes utilizes the presence of lone pairs of electrons on the amine (NH2), hydroxyl (OH), and carbonyl (C=O) groups, which act as ligands and can interact with cationic dyes through the mechanism of formation of coordination (complex) covalent bonds [28].

CONCLUSION

Based on the results of the research, it can be concluded that crab shell waste and shrimp shell waste can be converted into chitosan by simple extraction. This can be used as a natural adsorbent which is a valuable commodity. Both types of chitosan derived from crustacean waste have good adsorption capacity against heavy metals (mercury and arsenic) and dyes (tartrazine and amaranth).

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in the research and the manuscript.

REFERENCES

1. Azra MN, Okomoda VT, Tabatabaee M, Hassan M, Ikhwanuddin M. The contributions of shellfish aquaculture to global food security: assessing its characteristics from a future food perspective. Front Mar Sci. 2021;8(4). doi: 10.3389/fmars.2021.654897, PMID 654897.
2. Santos VP, Marques NSS, Maia PCSV, Lima MAB, Franco LO, Campos-Takáli GM. Seafood waste as an attractive source of chitin and chitosan production and their applications. Int J Mol Sci. 2020;21(12):4290. doi: 10.3390/ijms21124290, PMID 32560250.
3. Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs. 2015;13(3):1133-74. doi: 10.3390/md13031133, PMID 25738328.
4. Li B, Elango J, Wu W. Recent advancement of molecular structure and biomaterial function of chitosan from marine organisms for pharmaceutical and nutraceutical application. Appl Sci. 2020;10(14):4715. doi: 10.3390/app10144715.
5. Ferronato N, Torretta V. Waste mismanagement in developing countries: a review of global issues. Int J Environ Res Public Health. 2019;16(6):1060. doi: 10.3390/ijerph16061060, PMID 30909625.
6. Ayilara MS, Olanrewaju OS, Babalola OO, Odeyemi O. Waste management through composting: challenges and potentials. Sustainability. 2020;12(11):4456. doi: 10.3390/su12114456.
7. Manalisidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and health impacts of air pollution: a review.
13. Shrimp shell waste to mercury absorption efficiency. IOP Conf. Ser. Earth Environ Sci. 2020;589(1):012018. doi: 10.1088/1755-1315/589/1/012018.

14. Development of bio adsorbent chitosan from seafood waste using sugars derived from fruit waste-stream. AMB Expr. 2020;10(1):17. doi: 10.1186/s40668-020-0954-7.

15. Adsorption using chitosan and Nano zerovalent iron composite. Tan YN, Lee PP, Chen WN. Microbial extraction of chitin from water bacteria and viruses using electrosyn nanofibers. Sci Total Environ. 2021;751(1):141673. doi: 10.1016/j.scitotenv.2020.141673.

16. Biopolymer (chitin) from various marine seashell wastes: isolation and characterization. J Polym Environ. 2018;26(6):2207-18. doi: 10.1007/s10924-017-1118-y.

17. Zhao D, Huang WC, Guo N, Zhang S, Xue C, Mao X. Two-step separation of chitin from shrimp shells using citric acid and deep eutectic solvents with the assistance of microwave. Polymers. 2019;11(3):409. doi: 10.3390/polym11030409, PMID 30960393.