OPTIMIZATION OF CHITOSAN PREPARATION FROM CRAB SHELL WASTE

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Abstract

Crab shell waste from seafood restaurants is potential to be used as chitosan source. This material contain 20-30 % of chitin which could be converted into chitosan through deacetylation process. While, chitin could be isolated from crab shell by deproteination and demineralization. Chitosan is fine chemical used to adsorb fat from body, heavy metal absorbent, and medicine. This research looked into the prospect of crab shell as raw material to produce chitosan. In this case, the process variable of chitosan preparation was investigated involving operation time and NaOH concentration to determine optimum condition. Whereas, the other parameters including operation temperature, NaOH to chitin ratio is respectively fixed at 70-80°C and 5:1. As response, the yield of chitosan is calculated. In this case, the deacetylation time is varied from 1-4 hours with the time step 1 hours and the concentration of NaOH is changed from 20-50% with the step size of 10%. The results showed that the maximum yield of chitosan is 9.15%, which could be achieved at operation time of 3 hours and NaOH concentration of 20%.

Key words : crab shell, deproteination, demineralization, deacetylation, chitosan.

1. Introduction

A rapid of seafood restaurants growth still faces many problems. One of the problem is the acummulation of crab shell waste which can cause environmental problem. As consideration, the rate of crab shell waste from 100 seafood restaurants in Semarang could achieve 500 to 1000 kg per days. The crab shell is one of material that is very difficult to be degraded by microorganism because of high $CaCO_3$ content. The main compound of crab shell consist of protein 21%, chitin 24-29%, mineral ($CaCO_3$) 40-45%, and ash 5-10% (Taryoko and Irma, 2002).

According to first paragraph crab shell waste with 24-29% chitin content could be used as chitin or chitosan source. These fine chemical are very useful in chemical industries as a medicine and drugs supplement, fat coagulant, metal adsorption and cosmetic (Hanafi et al, 2000). Chitin is a polymer formed primarily of repeating units of beta(1-4) 2-acetamido-2-deoxy-D-glucose or N-acetyl-glucosamine. Its structure resembles that of cellulose, except that the hydroxyl groups in position 2 have been replaced by acetylamino groups. While the chitosan is a polymer derived from chitin through deacetylation process. The chitosan is formed by repeating units of beta (1-4) 2-amino-2-deoxy-D-glucose or D-glucosamine. Figure 1 presents the chitin and chitosan molecular structure (Suhardi, 1993, Hanafi et al, 2000).

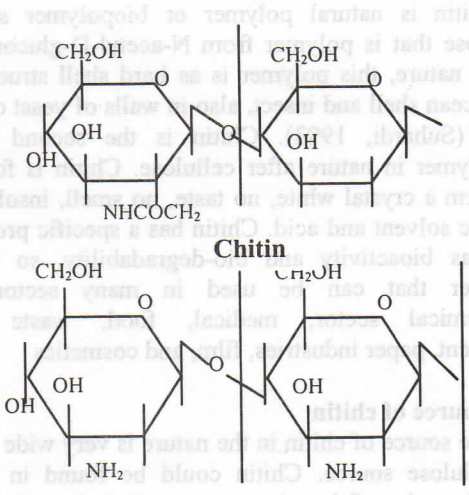

Figure 1: The Structure of Chitin and Chitosan

Chitin could be isolated from crab shell through two steps process namely deproteination and demineralization (Mekawati, et al 2000). The deproteination is a process to reduce protein content by extraction process using strong alkali solution in the concentration of 3 to 6 N (Hanafi, et al 2000). Occasionally, the alkali used for this process is sodium hydroxide, sodium carbonate, potassium hydroxide or potassium carbonate. However, the use

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of sodium hydroxide is prefer due to its lower cost (Taryoko and Irma, 2002).

After deproteination process, the solid product is washed by aquedest to reduce remaining alkali in solid. Product is then reacted with hydrochloric acid (HCl) 20% to remove $CaCO_3$ content. In this step, the $CaCO_3$ combined with HCl forming $CaCl_2$ which is soluble in water. The mixture was filtered to obtain solid chitin. This material was then washed using aquadest and dried to obtain solid chitin.

Chitin from the above process could be converted to chitosan compound through deacetylation process. The acetylation is a process to remove acetyl groups (CH_3CHO^-) bounded on amine groups in chitin compound by adding NaOH in the concentration of 20 to 50%. Here, the acetyl groups is reacted with NaOH producing sodium acetic. The main product, deacetylated chitin popular as chitosan is yielded in solid phase of mixture. The chitosan is the separated form the mixture by filtration process. The cake, wet chitosan is then dried to obtain dry chitosan with the purity of 70-80% (Hanafi et al, 2000). Chitosan is fine chemical that is used for medicine, fat coagulation, and heavy metal absorbent. The price of chitosan with the purity of 70% in world market could achieve US $ 750/kg (Djaeni et.al, 2002).

2. Chitin and its Properties

Chitin is natural polymer or biopolymer such as cellulose that is polymer from N-acetyl-D-glucosamine. In the nature, this polymer is as hard shell structure or crustacean shell and insect, also in walls of yeast cell and fungi (Suhardi, 1993). Chitin is the second largest biopolymer in nature after cellulose. Chitin is found in the form a crystal white, no taste, no smell, insoluble in organic solvent and acid. Chitin has a specific properties such as bioactivity and bio-degradability, so it is a polymer that can be used in many sectors like biochemical sector, medical, food, waste water treatment, paper industries, film, and cosmetics

2.1. Source of chitin

The source of chitin in the nature is very wide as well as cellulose source. Chitin could be found in marine animals such as fish and crustacean shell. It could be also obtained from protozoa cell and insect (Suhardi, 1993). In crustacean shell, the chitin content could achieve 20-80% in dry weight. While, in the insect and protozoa, the chitin content are in the range of 16-75% (Muzzareli, 1997).

Other sources of chitin are fungi and algae. In this organism, the chitin is bounded with poly-saccharide in fungi cell. The content of chitin in fungi could achieve 45%. Recently, one of a large potential as chitin source is *Aspergillus's niger* that contains high sufficient of chitin approximately 45% from organic materials (Suhardi, 1993).

2.2. Chitosan from chitin

Chitosan is modified natural carbohydrate polymer derived from chitin which occurs principally in animals of arthropods. The primary is 2-deoxy-2-(acetylamino) glucose (Suhardi, 1993). This units are combined by 1-4 glycoside linkage, forming a long chain linear polymer, as presented in Figure 1. Removal most of the acetyl groups of chitin by treatment with strong alkaly yielding chitosan (Padmono and Nasution, 1998).

At present, many potential products using chitosan have been developed, including flocculating agents for removal of traces of heavy metals from aqueous solutions, coating to improve dyeing characteristics of glass fibers, wet strength additives for paper, adhesive, photographic, and printing applications (Peniston and Johnson, 1980,; Campbell, 2000).

3. Methodology

The aim of this research is to determine optimal condition of chitosan production from crab shell. In this case, the crab shell was obtained from five seafood restaurants located in Semarang. The investigation was conducted in four step namely, size reduction, deproteination, demineralization, and deacetylation, as illustrated in Figure 2, as follows:

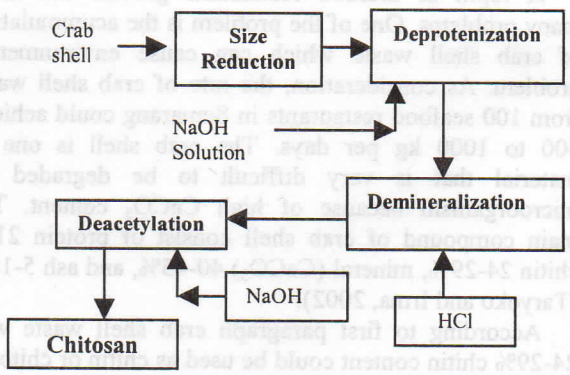

Figure 2 Schematic Diagram of Chitosan Production

3.1. Process description

The side reduction step was objected to reduce the size of crab shell up to 16 mesh. In this step, crab shell was milled using hammer mill. Exit from hammer-mill, crab shell powder is manually screened at 17 and 16 mesh. The product that was held on 16 mesh screen is taken 100 grams to be processed in the next step namely deproteination (Djaeni et al, 2002).

The crab shell powder was then added by 2 N of NaOH in the ratio of (1:6) to obtain chitin. The process is carried out in Stirred tank mixture at 70°C for 1 hours. After that, the mixture is then cooled and filtered. The deproteinated crab shell is washed by pure water and dried until 10% water content

(Djaeni et al, 2002; Hanafi et al, 2000; and Efrina and Rafiah, 1999). The deproteinated crab shell is reacted with hydrochloric acid to remove $CaCO_3$ content. The process is done in stirred-mixer for 2 hours at 70°C. Here, $CaCO_3$ was converted to $CaCl_2$ that is soluble in water. The mixture is then filtered by vacuum filter to separate solid and liquid phase. The solid phase was washed by pure water and then dried in 90 to 100°C for 2 hours to yield dry chitin. Meanwhile, the filtrate is neutralized and disposed.

The dry chitin is a raw material to produce chitosan through deacetylation process. The chitin powder is mixed with high concentration of NaOH to remove acetyl groups bounded in amine groups of chitin. Here, the acetyl reacted with NaOH forming sodium acetic (Mekawati, et al, 2000). The sodium acetate will dilute in solution, while deacetylated chitin namely chitosan could be obtained as solid product. The mixture was separated by vacuum filter to obtain chitosan as solid phase (cake). The cake was washed by pure water and then dried in electric oven for 2-4 at 105°C. The dried chitosan is weighed using electrical balance. The chitosan product is analyzed the water content and ash content to determine the purity of chitosan. In this investigation, the yield of chitosan was also calculated based on the weight of dried chitosan divided by the weight of crab shell fed, as expressed in the following equation.

$$Y = (Wc/Wsh)x100\% \quad (1)$$

Where:

Y is the yield of chitosan (%)

Wc is the weight of chitosan product (gram)

Wsh is the weight of crab shell (gram)

3.2. Determination of process variables

This investigation is focused to study the effect of sodium hydroxide concentration (%) and operation time on deacetylation process. The concentration of sodium hydroxide takes important role on deacetylation process since it reacts with acetyl groups forming sodium acetic. In this case, the concentration has to be optimized to avoid more sodium hydroxide loss after processing. While, the operation time is varied to get opimum of process length. The optimum time is useful to design process in larger scale, and it relates to production rate. The production rate will determine the dimension of equipment related with capacity.

On the other hand, the other process parameter including temperature and NaOH to chitin ration is respectively fixed at 75-80°C and 5:1. As an output indicator, the yield and purity of chitosan were measured based on the chitosan weight, water content, and ash content.

The experiment was conducted in two steps. The first step is, the variation of operation time at constant NaOH concentration. In this step, deacetylation time is varied at 1.0, 1.5, 2, 2.5 and 3 hours, while the NaOH is set at 30%. This step resulted optimum deacetylation time which will be used at second step.

The second step involved the variation of NaOH concentration at 20, 30, 40, and 50 %. Meanwhile the operation time is set at optimum condition obtained from first step. The optimal NaOH concentration was determined based on maximum yield.

4. Result And Discussion

4.1. The influence of operation time on chitosan yield

The effect of operation time on chitosan yield is depicted in Table 1. The result showed that the yield of chitosan increase as well as time operation prolonged especially in the range of 1 to 3 hours. This indicated that the greater amount of chitosan product could be obtained. In this range, the chitosan yield increases from 8.28% to 9.07%. This phenomena is caused by the contact time between chitin and NaOH being longer in which makes the conversion of acetyl groups in chitin to sodium acetic become greater. However, at operational time longer than 3 hours, the process operation could not enhance the chitosan yield, even it reduces the product from 9.07 to 8.26%. This because, the content of acetyl groups in chitin becomes low even approximately to be the zero. Hence, NaOH with the high concentration will introduce the chitin compound, which makes the chitosan product declining.

Meanwhile, the others parameters related with product quality were also measured including water and ash content. The results showed that different value of these indicators in the range of 2.00 to 2.02% for water content, and 5.12 to 5.30 to ash content are not significant with the change of time operation. Thus, it could be diagnosed that the optimum operation time is 3 hours.

Table 1: The effect of operation time on chitosan yield

Time (hour)	Water content (%)	Ash Content (%)	Yield Dry bases (%)
1	2.02	5.02	8.28
2	2.01	5.30	8.90
3	2.02	5.25	9.07
4	2.00	5.22	8.26

4.2. The influence of NaOH Concentration on chitosan yield

This step was conducted at operation time of 3 hours with various of NaOH concentration, as mentioned in Section 3.2. The results indicated that increasing concentration of NaOH makes the chitosan product decreasing as depicted in Table 2. This phenomena is due to the excessiveness of NaOH concentration for removing acetyl groups from chitin. Hence, the remaining OH^- will attack the chitin ring that causes the destruction of chitin

compound. However, when the concentration of NaOH is diminished fewer than 20%, the reaction between NaOH with acetyl groups could not occur since the concentration of OH^- is too low.

In the other hand, the process parameters including water and ash content were also analyzed. In this case, the change of NaOH will not affect those parameters significantly. As presented in Table 2, the water and ash content in various NaOH concentrations is in the range of 2.00 to 2.01, and 4.85 to 5.03 respectively. Therefore, it could be concluded that the optimum of NaOH concentration is 30%.

Table 2: The effect of NaOH concentration on chitosan yield

NaOH Concentration (%)	Water content (%	Ash Content (%)	Yield Dry bases (%)
20%	2.01	4.97	9.15
30%	2.00	5.03	8.92
40%	2.00	4.85	8.26
50%	2.01	4.95	8.15

5. Conclussion

The investigation to determine the optimum process condition on deacetylation process yielding chitosan from chitin has been implemented. The results showed that the maximum chitosan product could achieve 9.15% dry bases. This condition is reached at operation temperature of 75-80°C, NaOH to chitin ratio of 5:1, operation time of 3 hours, and NaOH concentration of 20%. However, the results still need extent analysis namely the degree of acetylating for chitosan. This parameter is important since it determines the quality and use of chitosan product.

6. Acknowledgement

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