Isolation of β-Chitosan from Squid Bone as Raw Material to Synthesize of Hybrid Photocatalysts TiO₂-Chitosan

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Abstract. Synthesis of hybrid photocatalyst of TiO₂-chitosan has been done. Chitosan as a raw material was chitosan-β obtained from chitosan isolation from squid bone. Chitosan from squid bone has a soft structure and very little to contain minerals and other impurities so easily obtained. Hybrid photocatalyst TiO₂-chitosan was synthesized by a sol-gel method in which the incorporation of chitosan as a supporting material and TiO₂ having a function as a photocatalytic agent was dispersed on the β-chitosan surface. The hybrid of TiO₂-chitosan photocatalysts was characterized by FTIR and SEM/EDX shows the functional groups and surface morphologies of hybrid photocatalyst TiO₂-chitosan covered by uniformly distributed TiO₂ nanoparticles. The FTIR spectrum showed the O-Ti-O absorption bands at the wavenumber of 678.98 cm⁻¹ and the typical absorption of chitosan the -OH at the wavenumbers of 3425.58 cm⁻¹, 3834.49 cm⁻¹, and 3873.06 cm⁻¹, respectively.

Keywords: chitosan-β, photocatalyst, TiO₂, sol-gel method, nanoparticles

1. Introduction
Chitosan is a natural polymer that has a structure similar to cellulose and can be formed into thin films. Chitosan that has the properties of nontoxic, biodegradable, and biocompatible resulting from the deacetylation of chitin by using high temperature and high concentrated alkali solvents [1,2]. Chitosan has the structure of poly β-(1,4)-2-acetamide-2'-deoxy-D-glucose. Chitosan is specific because it has a reactive amine group on C-2 and primary and secondary hydroxyl groups in C-3 and C-6 which cause chitosan to have high chemical reactivity [3,4,5]. The structure of chitin and chitosan have the difference lies in the comparison of the amine group (-NH₂) with an acetyl group (-CH₃ CO) called the degree of deacetylation [6]. The degrees of deacetylation depending on the raw material and the method used in the preparation of chitosan. The degree of deacetylation is a quality parameter of chitosan which shows the percentage of acetyl groups which can be removed from the chitin rendemen. The release of acetyl groups on chitin causes the positively charged chitosan, to able to bind negatively charged compounds or...
molecule. Methods for determining the acetyl groups released from chitin include potentiometric titration, ninhydrin test, NMR, titration of hydrogen bromide, IR, and UV-Vis spectrophotometry [7,8]. XRD analysis shows that chitosan has 3 structures, that is structure α, β, and γ. Molecule α-Chitosan has a very dense antiparallel bond because it is stabilized by its intermolecular hydrogen bond. In contrast to β and γ chitosan, where the hydrogen bond in the water increases. The high level of hydration and decreased attachment causes the β and γ chitosan structure to be more flexible and reactive. Chitosan with α structure is mostly found in animal shells of crustaceans, while β and γ chitosan can be found in squid bones and fungi, respectively.

Chitosan is insoluble in water but is soluble in most organic solutions, such as acetic acid, formic acid, and lactic acid, and has a finite solubility in phosphoric acid and is insoluble in sulfuric acid. The commonly used chitosan solvent is an acetic acid with a concentration of 1-2% [9]. Photocatalyst technology used for processing and degraded of wastewater contaminant, efficiently and can be done by using titanium dioxide (TiO₂) through the process photo-oxide the dye into pollutants compounds in the water. The Titanium dioxide (TiO₂) is a semiconductor material that has a relatively high photocatalytic activity. In this study, TiO₂ will be dispersed into chitosan which acts as a supporting material, wherein the chitosan-TiO₂ formed to combine the functions of chitosan as an adsorbent and TiO₂ that has high photocatalytic activity [10,11].

2. Experiment
Materials used in this research were squid bone, hydrochloric acid, sodium hydroxide, nitrogen gas, and distilled water, while instrumentation applied for analysis were spectrophotometer FTIR Shimadzu Prestige-21, and SEM-EDX JEOL JED-2300.

2.1. Preparation of Chitosan from Squid Bone
Fresh squid bone washed and dried in the sunlight for about four days. Once the squid bone dry milled using a mortal and sieved to pass the 80 mesh size. Amount of 50 g of 80 mesh squid bone put into a 500 mL beaker glass, then adding 1 M HCl solution with a ratio of 1:10 (w/v) for demineralization process. The mixture is stirred with a magnetic stirrer at room temperature for 3 hours and then filtered through filter paper while continuously washed with distilled water until no residual chloride ion remains in the samples. The washing process is stopped if no turbid solution formed when the filtrate drops with an AgNO₃ solution. Residue from demineralization put into a 500 mL beaker glass and added with 1 M NaOH solution at a ratio of 1:10 (w/v). The mixture stirred and heated at 60 °C on a hotplate stirrer for 1 hour and then filtered with Whatman 41μm filter paper. The residue was found on the filter paper was washed with distilled water until the residue namely chitosan in neutral pH. Chitosan was dried in an oven at a temperature of 70 °C until dry to constant weight. Chitosan has been obtained then characterized by FT-IR spectrophotometer to identify the functional groups of chitosan, and determine the degree of deacetylation product by base line method.

In this FT-IR spectrum analysis for chitosan does area of functional groups and the fingerprint region with a frequency of 4000 cm⁻¹ - 400 cm⁻¹. Deacetylation degree of chitosan determined by base line by FT-IR spectra, with the formula:

\[ DD = 100 - \left( \frac{A_{1655}}{A_{3450}} \right) \times \frac{100}{1.33} \] (1)

Where, A₁₆₅₅ showed absorption at amide band, A₃₄₅₀ showed absorption at hydroxyl and a factor of 1.33 shows the value of the ratio A₁₆₅₅/A₃₄₅₀ to completely deacetylation degrees of chitosan.

2.2. Synthesis of Hybrid Photocatalysts TiO₂-Chitosan from Squid Bone
Amount of 10 g of chitosan from squid bone added into 100 mL of 0.1 M HCl solution in 250 mL beaker glass, while continuously stirring until it dissolved evenly and the TiO₂ particle was added with a ratio of 1:1 (w/w). The solution stirred to homogenize for 20 minutes then filtered through Whatman 41μm filter
paper, then the precipitate obtained is dried in an oven at a temperature of 50°C about 4 h in the form of TiO$_2$-chitosan gel. The product of TiO$_2$-chitosan gel washed with distilled water until the pH of water residue is neutral, then dried in the oven at a temperature of 70 °C for 12 h to obtain a hybrid of TiO$_2$-chitosan. The hybrid TiO$_2$-Chitosan characterized by a FT-IR spectrophotometer to see functional groups and the elemental contained by SEM-EDX analysis.

3. Results and Discussion
Squid bone powder (80 mesh) that has been prepared was added 1 M HCl to remove mineral content, and continue to the second stage is deproteinize, which in this process aims to break the protein bonds and other crosslinked intramolecular in squid bone chitin using 1M NaOH. The yield produced at this stage is about 65.6% which estimated that the protein content in squid bone around 30-40% [12],[13]. The deacetylation process of chitin to chitosan by removed of the acetyl group (CH$_3$CO-) by using a concentrated solution of NaOH 50% (v/v). The process of deacetylation of chitin takes place under alkaline conditions because the N-acetyl group is not removed with an acid reagent without polysaccharides [14]. Thus, in this process the yield obtained from squid bone about 80.15% (w/w) with the texture of chitosan in shaped hydrogel [15].

Based on the FT-IR spectra on Figure 1. (A) and the calculation using equation (1) the degree of deacetylation by methods base line on a FT-IR spectrum obtained the degree of deacetylation of chitosan from squid bone ranged around ± 93.6%. The degree of deacetylation levels off at relatively high values while normal commercial chitosan has of ± 80% deacetylation. Figure 1. was confirmed the characterizations of chitosan (A), and a hybrid of TiO$_2$-chitosan (B), the presence of carboxyl and
The peak at (3380 to 3480) cm$^{-1}$ is $\nu$(O-H) and $\nu$(NH$_2$); the peak at (2920 to 2940) cm$^{-1}$ is from $\nu$(CH$_3$, CH$_2$, CH, and OH); the peak at (1630 to 1650) cm$^{-1}$ is from $\nu$(C=O) in low absorbances indicate that the C=O group of chitin have had reduced in the form of chitosan [17]. The adsorption band of O-Ti-O for hybrid of TiO$_2$-chitosan from squid bone appear at (524 to 680) cm$^{-1}$ showed in Figure 1. (B) with presence of $\nu$(O-H) and $\nu$(NH$_2$) stretching vibration overlap at 3425 cm$^{-1}$ indicate that that functional group is still active as adsorbent in the form of hybrid of TiO$_2$-chitosan.

The hybrid of TiO$_2$-chitosan surface morphology characterized by SEM and the elemental analyses performed by EDX. According to the SEM photograph in Figure 2, it is seen that the TiO$_2$ particles have had evenly distributed over the surface of chitosan in the hybrid of TiO$_2$-chitosan with particle size almost in nano size. The surface of hybrid of TiO$_2$-chitosan mass percentage had dominated by TiO$_2$ particle while the N atom from the aminne group (NH$_2$) of chitosan confirmed as the active site of the hybrid agrees to the FTIR spectra in Figure 1. and the detail of elementals percentage on the hybrid of TiO$_2$-chitosan given in Table 1.

![Figure 2. Scanning Electron Microscope (SEM) photograph of hybrid of TiO$_2$-chitosan from squid bone with (A) 10.000x and (B) spectrum of Energy Dispersive X-ray (EDX)](image)

Table 1. The percentage of elements and oxides on hybrid of TiO$_2$-chitosan from squid bone

| Elements | % Mass | % Atom | Oxides |
|----------|-------|-------|--------|
| O        | 44.20 | 62.10 | TiO$_2$ |
| Ti       | 43.66 | 20.49 | NO     |
| N        | 9.36  | 15.03 |        |

The TiO$_2$ on a hybrid of TiO$_2$-chitosan from squid bone has 62.10% in oxide form, while the N atom contributes 15.03%, and 20.49% of NO, respectively. That mean 15.03% of nitrogen atom from chitosan exist as aminne group (NH$_2$) and 20.49% also in the form of oxide. The in oxide form (NO) of chitosan has been by oxidation process when amine direct contacted to the TiO$_2$ particle on chitosan surface to form of hybrid of TiO$_2$-chitosan.

4.Conclusions

The product of β chitosan isolated from squid bone gain about 80.15% (w/w) yield. The degree of deacetylation of the β chitosan determines by base line methods on a chitosan FT-IR spectra results about ± 93.6% degree of deacetylation. TiO$_2$-chitosan from squid bone was used as raw material to synthesis of
hybrid TiO$_2$-chitosan. Base on FT-IR spectra and SEM-EDX analysis the absorbances appear at (524 to 680) cm$^{-1}$ and $\nu$(O-H) and $\nu$(NH$_2$) stretching vibration overlap at 3425 cm$^{-1}$ indicate that those functional group of NH$_2$ from $\beta$ chitosan is still active as adsorbent in the form of hybrid of TiO$_2$-chitosan even though TiO$_2$ particle were distribute evenly on the surface of chitosan.

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6. References

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