Preparation of Calcium Carbonate (from Shellfish)/Magnesium Oxide Composites as an Antibacterial Agent

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Abstract. We have performed research on antibacterial substance from a natural substance, one of them is calcite from shellfish (Anadara granosa) in Kenjeran Beach Surabaya. This calcite is composed of magnesium oxide using PEG 4000 (Polyethylene glycol) as a solvent and then heated at 800 °C for 30 minutes. Weight variety of calcite used was 80% wt, 85% wt, and 90 wt%. Subsequently, that composites characterized using XRD, antibacterial activity test (Escherichia coli and Staphylococcus aureus), and SEM. The result of antibacterial assay shows that composite of CaCO₃/MgO with 80% wt composition have the best activity inhibitory of 31.96 mm for Escherichia coli bacteria and 32.26 mm for Staphylococcus aureus bacteria.

Keyword: calcium carbonate, shellfish, magnesium oxide, composite, antibacterial.

1. Introduction

Food entered into the human body through the mouth and teeth. Both of these organs have a very vital role which is vulnerable to the entry of pathogenic bacteria and viruses which may cause various types of diseases. There are more than 500 species of bacteria living in the oral cavity of humans [1], [2]. The bacteria live in the oral cavity, which can form plaque on the teeth, and cause inflammation of the oral cavity [3]. One type of bacteria in dental plaque is Staphylococcus aureus [4]. And other bacteria in dental plaque is Escherichia coli [5].

The antibacterial agent is required which can inhibit bacterial activity in the oral cavity to reduce the disease in the oral cavity and tooth caused by bacterial buildup. Generally, to control the activity of bacteria in the oral cavity used abrasive substances such as calcite and hydroxyapatite contained in toothpaste to control bacterial activity in the oral cavity. The abrasive material can be obtained from calcium carbonate contained in the shellfish which content of 98% [6]. Calcium carbonate is known to use as a material to clean up the remains of food, stains on the teeth and kill bacteria [7]. However,
abrasive is not enough to remove bacteria because it has no antibacterial activity [8]. Therefore, it needs materials that have two functions such as an abrasive as well as shows an antibacterial properties.

Antibacterial compounds can be made from organic and inorganic materials. Inorganic materials are more in demand because they have good stability compared to organic one [9], [10]. Magnesium oxide is one of the inorganic materials that have good antibacterial activity [11] and as safe for humans [12], [13]. Thus the composite of CaCO₃/MgO fit to two functions as abrasive and antibacterial.

Yamamoto et al.[14] reported his study of antibacterial characteristics of carbon-coated CaCO₃/MgO powder which led to the pyrolysis of poly(vinyl alcohol)-dolomite mixture showed strong antibacterial properties in Staphylococcus aureus and Escherichia coli. The antibacterial action on Staphylococcus aureus was greater than towards Escherichia coli [8]. They used the in-situ method to obtain the CaCO₃/MgO composite. So more expensive tools and materials are needed for high sample preparation and heating with long holding time. The further reported from XRD analysis that impurities such as Al, Si, and Fe [8] existed. The CaCO₃/MgO composite can be prepared by an ex-situ method that combines pure CaCO₃ and MgO materials.

In this work, we report the study to determine the inhibitory power of composite material made by CaCO₃/MgO to the activity of Escherichia coli and Staphylococcus aureus bacteria. We also report the preparation of CaCO₃/MgO composite material by the optimum clear zone and able to utilize shell waste shells to be extracted into calcium carbonate and composed with magnesium oxide as one of the antibacterial ingredients of natural materials.

2. Materials and methods

2.1 Preparation of CaCO₃ Extract
The stage of extracting calcium carbonate from shellfish (Anadara granosa) begins by cleansing the fur shells from feathers using 2 M HCl. Then dried under the sun for 24 hours and calcined at 900 °C for 5 hours. Furthermore, the shellfish was smoothed and flowed CO₂ gas with a speed of 2.8 L/min and then precipitated for 24 hours. The precipitate was filtered and heated at 90 °C for 24 hours to obtain calcite phase on calcium carbonate.

2.2 Preparation of CaCO₃/ MgO Composites
This composite was prepared by using the ex-situ method of combining pure materials. The calcium carbonate and magnesium oxide powder were dissolved in PEG 4000 solution then stirred using magnetic stirrer at 80 °C for 30 minutes at 6 rpm. The solution was then filtered and calcined at 800 °C for 30 minutes. The calcined product pounded to get CaCO₃/MgO composites powder. The calcite mass was varied as 80% wt, 85% wt, and 90 wt%.

2.3 Antibacterial assay
Referring to [14] the research using Escherichia coli (hereafter, E. coli) bacteria as gram-negative bacteria and Staphylococcus aureus (hereafter, S. aureus) as gram-positive bacteria. Tests were performed by isolation of CaCO₃/MgO composite and bacterial isolation. The bacteria then incubated the bacteria at a temperature of 28-30 °C for 24 hours to observe the bacterial reactivity of the CaCO₃/MgO alloy material. A clear zone of inhibiting zone indicated the antibacterial activity test. The diameter expresses this activity. Based on the dimension of the inhibition zone, one can obtain bacterial activity against CaCO₃/MgO composite material. The inhibitory power was calculated by measuring the diameter of the clear visible region. The width of the clear zone was then reduced by 6 mm from the diameter of the well [15]. The measurement results are presented as an average for each treatment.
3. Results and discussions

3.1 XRD Characterization
The X-ray data collection specification uses Philips X'Pert MPD (Multi Purpose Diffractometer) type system, with anodic radiation source Cu, 40 kV, 30 mA, CuKα 1.54056 wavelength and Bragg-Brentano optical light. XRD patterns of the CaCO3 powder sample from shellfish analyzed quantitatively with Match Software. The technique used searches and match database on application to identify the phase formed. This technique also applies to the analysis of XRD composite CaCO3/MgO. Based on Fig. 1, it shows that the extraction results in this study were 100% CaCO3, with miller index [012], [104], [110], [113], [202], [018], [116], [1010].

![Figure 1. XRD patterns of the calcium carbonate extract in the analysis using Match! software.](image1)

![Figure 2. XRD patterns of CaCO3/MgO composite in the analysis using Match! software.](image2)
Figure 3. SEM-EDX image of CaCO$_3$/MgO composite (80% wt CaCO$_3$ and 20% wt MgO) magnification x15k (a) SEM, (b) SEM-EDX CaCO$_3$/MgO.

The results of the composite CaCO$_3$/MgO XRD patterns are shown in Fig. 2 which shows the presence of peak at 2θ CaCO$_3$ or MgO diffraction angles corresponding to the available databases in the Match, e.g. at 42.8°, and 62.17° for MgO. The results of the Match analysis indicates that the phase formed in Fig. 2 is composed by 64.2% calcite CaCO$_3$ and 35.8% MgO.

3.2 SEM-EDX Analysis

Based on Fig.3 SEM-EDX results for composites having the greatest inhibitory power in composites 80% wt CaCO$_3$ and 20% wt MgO formed composite particle bonds. Fig.3 (a) Composite morphology is shaped like a solid cube which indicating the calcite phase CaCO$_3$ [16] is supported with Fig.3 (b) light blue. Calcite is the most stable phase when compared with other phases such as vaterite and aragonite.

The composite is composed of C, O, Mg and Ca atoms with weight percentage and atoms symbolized by different colors (Figure 3). In the picture there is a dark and bright side, the dark side shows the location of Mg atoms while the light-colored side shows the location of Ca atoms. The red spots on the image indicate the presence of C atoms, which are not very dominant. This sample is dominated by O atoms that can be seen from the point of green color that is quite large and evenly distributed. It is very reasonable because the O atoms in this composite have four molar fraction of 3 atoms in CaCO$_3$ and one atom from MgO.

3.3 Clear Zone of Antibacterial Activity

The results of the antibacterial assay against $E. \text{coli}$ and $S. \text{aureus}$ bacteria showed that the highest clear zone in the composite was 80% wt CaCO$_3$, 20% wt MgO for each bacteria (Table 2). CaCO$_3$/MgO composites obtained at 800°C have clear zone diameter greater than MgO such as research conducted by the data shown in Table 3 [13].

| Table 1. Percentage by weight of composite atoms of CaCO$_3$/MgO |
|-----------------|---------|---------|-------|
| **Element**     | % wt    | % Atoms | Color |
| O               | 60.35   | 67.64   | Green |
| Mg              | 16.92   | 12.48   | Dark blue |
| Ca              | 13.45   | 6.02    | Light blue |
| C               | 9.29    | 13.86   | Red   |
The existence of differences in structure and chemical composition in both types of bacteria cause antibacterial inhibition in *S. aureus* bacteria greater than *E. coli*. The structure and chemical composition of the *S. aureus* cell surface consist only of the peptidoglycan layer, whereas the surface of *E. coli* cells includes a thin layer of lipid, lipopolysaccharide, and peptidoglycan [8]. This research relevant to previous studies suggesting that the antibacterial inhibition of *S. aureus* bacteria is more significant than that of *E. coli* [8], [11], [14].

The composite with concentration 80% wt CaCO₃, 20% wt MgO is able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. CaCO₃/MgO is the inorganic material of metal oxide. The presence of metal elements causes the increase of pH [14] in bacteria when the membrane cell contact with the material so that the metabolism of bacteria is disrupted. MgO has metal elements less than CaCO₃/MgO composite. It is more effectively used as an antibacterial agent.

Antibacterial results of the study can be classified as antibacterial in the medium category. This category is based on observations of the bright zone formed by each composite. The results showed that each concentration has different inhibitory power based on the clear zone formed. Clear zones visible around the well characterized antibacterial activity. The mechanism of action of microbial control with antimicrobial compounds is to cause barriers to the formation of cell walls. Failure of this cell wall synthesis process will cause the cell to die due to lysis. The inhibition of cell wall synthesis and protein is due to these antimicrobial compounds by joining the 50S ribosomal subgroup so that all processes are inhibited [7].

4. **Conclusion**

The CaCO₃/MgO composite prepared has a very variable inhibitory of bacterial activity (*E. coli* and *S. aureus*). The best inhibitory effect on bacterial activity in this study was 80% wt CaCO₃ and 20% wt MgO composite, ie 31.96 mm for *E. coli* bacteria and 32.26 mm for *S. aureus* bacteria. This study is stating antibacterial composite CaCO₃/MgO activity on *S. aureus* is stronger than on *E. coli*.

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