Utilization of rice husk (*Oryza sativa*) for amorphous biosilica (SiO$_2$) production as a bacterial attachment

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**Abstract.** Rice husk as a by-product of the rice milling process has not been utilized optimally. The yield of rice husk waste continues to increase from year to year, considering that Indonesia is the third largest rice producer in the world. Rice husk has a fairly large silica content; therefore, rice husk is widely used as an alternative source of silica. The purpose of this study is to synthesize silica from rice husk and analyse its characteristics. The research method used in silica isolation was carried out by non-thermal and thermal methods. Non-thermal method for removal of inorganic compounds and hydrolysis of organic compounds by acidic solutions (HCl), while the thermal method is carried out by burning rice husks at a temperature of 500 °C to produce rice husk ash. The resulting rice husk ash was processed by the sol-gel method. The resulting silica was determined of its morphology by Scanning Electron Microscopy (SEM), composition, and structure with Fourier Transform Infrared Spectroscopy (FT-IR). The results obtained in this study were white silica with a yield of 84.782%. Biosilica produced is then mixed with certain bacteria, either *Serratia sp* or *Bacillus pumilus*. Then, the attachment of bacteria was evaluated by using SEM (Scanning Electron Microscopy).

**Keywords:** Rice husk; Amorphous biosilica; Bacterial attachment; Rice milling process

1. Introduction

Rice (*Oryza sativa*) is widely available in Indonesia as an agricultural country. Rice is raw material for many foodstuffs in Indonesia. Rice plants have many benefits for Indonesian people; ranging from stems, leaves, and grain in the paddy milled can be used for various purposes. In Indonesia, rice husk is a by-product of the paddy milled industry which contains of 65 % rice, 20 % rice husk, and the rest is in other forms such as bran or rice bran which is commonly used for animal feed and others [1]. Rice husk is considered less useful and has low nutritional value, so it is used as stoves fuel in small industries or as a soil mixture for planting media. If a number of rice husks produced from the rice milling industry are not managed and utilized properly, it will lead to an environmental pollution. Therefore, it is necessary to look for the effective utilization. One of the possibilities is the isolation of silica (biosilica) from the rice husk which is reported to contain 17.90 % of silica [2–4]. Rice husk ash is one of the largest silica-producing sources after a complete combustion. Combustion of rice husk ash in controlled temperatures (500-600 °C) will produce silica that can be used for various chemical processes. It was reported [5–7] that rice husk ash contains silica as much as 86 % - 97 %. Besides being abundant, rice husk silica can be obtained very easily and at a relatively low cost by using alkaline extraction [8]. The extraction method is based on the high solubility of amorphous silica in
alkaline solutions, such as KOH, Na₂CO₃, or NaOH, and followed by precipitation of dissolved silica using acids, such as hydrochloric acid, citric acid, acetic acid, and oxalic acid.

Silica gel is a form of silica that is produced by clumping sodium silicate sol (Na₂SiO₃). Sol is similar to agar [9], so that it can be dehydrated into solids or transparent beads that are not elastic. These properties make silica gel useful as an adsorbent, dryer, and support catalyst. Silica gel is a product that is safe to use to maintain food moisture, medicines, sensitive materials, electronics, adsorbents of bacteria and films. This product absorbs moisture without changing the condition of the substance. Even if held, these silica gel beads remain dry.

From the description above, a study was conducted on the synthesis of silica from rice husk ash as a safe product for various purposes, in this case, directed to bacterial adhesion or adsorbent. Adsorbents are solid substances that can adsorb fluid particles in an absorption process. Adsorbents are specific and made from porous materials, either polar materials (activated charcoal, silica gel, and zeolite) or non-polar compounds such as polymers. This research was carried out by processing rice husk ash into sodium silicate, which was then continued with the sol-gel process using strong acids and weak acids.

2. Materials and Methods
The materials used in this study were rice husk, distilled water, HCl, H₂SO₄, and NaOH solution. Chemicals were purchased from E.Merck (Darmstadt, Germany), and the main tools used were Hot Plate Magnetic Stirrer, thermometers, scales and furnace.

One hundred grams of rice husk was cleaned from the dirt such as rice leaves, sand, and gravel. Then, the rice husk was washed by immersing it in tap water for 90 minutes. After washing, the rice husk was rinsed with distilled water and then soaked in HCl 1N 1.5 L for 4 hours. Then, the husk was put in the oven at 60 °C until it dries. This husk then put in a porcelain cup for further heating in a furnace for 6 hours with a temperature of 500 °C to become white ash, hereinafter referred to biosilica.

A 500 mL of 0.5 M NaOH solution was made, then 5 grams of husk ash was dissolved into the NaOH solution. Then, it was incubated at 100 °C with stirring for 4 hours, chilled down to room temperature, and followed by filtering using Whatman filter paper No. 41. The filtrate is known as sodium silicate solution and stored at room temperature.

A 10% H₂SO₄ solution is slowly added to the extracted silicate solution and adjusted until pH 7 is reached. Then, the mixture is stirred continuously using a magnetic stirrer for 1×24 hours, then stored 2×24 hours to form a white gel-shaped precipitate. After that, the precipitate is washed with aquadest, so a clear white gel is obtained.

3. Results and Discussion
From rice husk, silica is found in higher amounts compared to inorganic compounds, such as potassium, sodium, and phosphorus. Organic and inorganic impurities must be removed to obtain pure silica so that the rice husk is pre-treated with soaking using hydrochloric acid (HCl). Inorganic impurities in rice husks could be removed, and most of the organic compounds such as hemicellulose and cellulose will be reduced. Inorganic impurities in rice husk will accelerate carbon fixation in rice husk ash. Therefore, inorganic impurity ions will cause melting on the surface. It can accelerate crystallization from amorphous to cristobalite silica. By doing pre-treatment with hydrochloric acid, the presence of inorganic impurity ions can be reduced so that carbon will be more easily oxidized to form whiter ash. Whiter ash indicates higher silica content. Also, HCl is proven to be the most effective in removing impurities in rice husks, especially metals, compared to other acids (H₂SO₄, HNO₃). The total amount of metal contained in the acid solution produced by hydrolysis with H₂SO₄ is lower. This result is due to the metal sulphate formed, which is not easily dissolved in water. Whereas the treatment with HNO₃ - only tends to remove iron (Fe) and cannot remove all metals contained in rice husks, while organic compounds such as cellulose, hemicellulose, and lignin would be hydrolysed [10].
Then the combustion is carried out using a furnace at a temperature of 500 °C for 6 hours, combustion at high temperatures serves to remove the organic fraction of rice husk so that only the inorganic fraction is left. Combustion is carried out at lower than 800 °C to prevent the transformation of amorphous silica structure into crystalline. The temperature used is not too low (350 °C) in order not to produce black husk ash due to the decomposition of organic materials, such as cellulose and lignin to form carbon, which is marked by the appearance of black colour on the rice husk ash. Rice husks that are completely burned will be grey to white, while rice husk ash that is not completely burned will be blackish. The white ash obtained from burning rice husks at 500 °C for 6 hours has an average yield circa 17.12 %.

3.1. Making sodium silicate solution from rice husk ash

Sodium silicate is obtained by reacting rice husk ash with an alkaline solution, sodium hydroxide. The reaction that occurs between silica contained in rice husk ash and NaOH, which forms sodium silicate [11] is as follows:

\[
\text{SiO}_2 + 2 \text{NaOH} \rightarrow \text{Na}_2\text{SiO}_3 + \text{H}_2\text{O} \tag{1}
\]

High electronegativity of the oxygen atom in SiO\textsubscript{2} causes Si to be more electropositive, and form the unstable intermediates (SiO\textsubscript{2}OH). After that, dehydrogenation will occur. The second -OH ion will bind to hydrogen to form a water molecule, and two Na\textsuperscript{+} ions will balance the negative charge of the SiO\textsubscript{3}\textsuperscript{2-} ion to form sodium silicate. The resulting sodium silicate solution has a yellowish-white color (Figure 1)

![Figure 1. From left to right is synthesis of biosilica from rice husk.](image)

3.2. Silica gel synthesis

Silica gel is one of the synthetic silica compounds which has an amorphous structure and could be synthesized through a sol-gel process, in the form of solids and has the potential to be used as an adsorbent. The nature of an absorber is also called adsorptive due to the presence of active sites on the surface [12]. The formation of silica gel is done by adding of an acid to the sodium silicate solution. Sulphuric acid solution 10 % v/v (168 mL) was used. However, before adding the acid, the pH of sodium silicate solution is measured first (as the initial pH = 14) then the acid solution is added up to pH 7. The sulphuric acid solution functioned as a precipitating agent. However, it is known that silica compounds dissolve easily in an alkaline atmosphere, and will settle in an acidic atmosphere. Based on these characteristics, to have silica gel compounds from rice husks, a basic solvent is used. In this experiment NaOH solution and 10% v / v H\textsubscript{2}SO\textsubscript{4} acid solution is added to recover the silica due to the following equation [9]:

\[
\text{Na}_2\text{SiO}_3 + \text{H}_2\text{SO}_4 \rightarrow \text{SiO}_2 + \text{Na}_2\text{SO}_4 + \text{H}_2\text{O} \tag{2}
\]

It was reported that at pH 7 the silica gel has the highest yield and surface area [13]. The process of gel formation occurs through the formation of siloxane bonds - Si-O-Si- from silicates as follows [14,15]:

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SiO$_3$ + xH$_2$O + 2H$^+$ SiO$_2$(x+1)H$_2$O + 2Na$^+$

(3)

After acid addition, a clear white hydrogel is formed. The formed gel is then allowed to stand for 2×24 hours for gel maturation, then washed with aquadest three times. The obtained white gel later then stored in a refrigerator to remove H$_2$O content in silica, which requires a long time to dry. Dry silica gel is known as xerogel [16][17]. The obtained silica is white which then this dry silica is mashed so that it would be easy for determination using Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red (FTIR). A total of 26.488 gr rice husk with a yield of ash 84.7815 %. Morphological observation using SEM shows that there is no real crystals formed (Figure 2). Also, it can be seen that the silica morphology (SiO$_2$) has an in homogeneous form (tetrahedral and tetraglomeration), the resulting is amorphous silica. Furthermore, a qualitative analysis using the FTIR shows the character of extracted silica (Figure 3).

![Figure 2. SEM analysis of silica results with magnification of (a) 1,000; (b) 5,000; (c) 10,000; (d) 30,000 times](image-url)
Figure 3. FTIR spectra of biosilica from rice husk

Figure 4. SEM of biosilica from rice husk attaching of (a) *S. marcescens*, (b) *B. pumilus* at magnification of 7500 x.

From (Figure 3) can be seen the vibration signals around 1066.13 cm\(^{-1}\), 962.03 cm\(^{-1}\), and 785.31 cm\(^{-1}\) are typical of Si–O–Si bands attributed to the asymmetric stretching, symmetric stretching and bending, respectively\cite{18-21}. These three peaks are the main indices of the silica materials, which represent the successful production of silica nano-particles. The ability of isolated bio-silica from rice husk as an adsorbent later on observed by mixing the biosilica with bacterial strain *Serratia mercerescens* and *Bacillus pumilus*. A portion of biosilica was added to the bacterial suspension and centrifuged for water separation. Later then fixed using glutaraldehyde and washed with ethanol series solutions. Scanning Electron Microphotograph was determined after coating with gold shown in (Figure 4). From (Figure 4), it can be seen that the bacteria are very numerous and thick, possibly due to imperfect fixation processes, therefore are needed other chemicals that can help strengthen the attachment of bacteria to silica particles so that the shape of the bacteria can be seen clearly.

4. Conclusion
Silica produced from rice husk has advantages because it has fine grains, is more reactive, and can be obtained easily with relatively low cost since it is supported by the availability of abundant and renewable raw materials. With these advantages, it shows that the potency of rice husk silica is large enough to be used as a source of biosilica. Biosilica considered as a material that has a reasonably
wide application, one of them is as a bacterial attachment. Other applications of biosilica should be investigated due to the characteristics of the material.

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