

PRESERVATION OF *RHIZOPUS* IN BENTONITE CLAY AS BIO-STARTER

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ABSTRACT

Effective and efficient preservation process is necessary in terms of increasing the fungal usage for industrial scale as biostarter. The objective of this study was to identify bentonite characteristic to be carrier to preserve of *Rhizopus* spore and to determine its viability after preservation process. The clay of bentonite characteristics were identified by BET (*Brunauer Emmett Teller*) and SEM-EDS (*Scanning Electron Microscopy-Energy Dispersive Spectroscopy*) for determining surface properties and elements within the minerals, XRD (*X-Ray Diffraction*) for identifying the mineral, and AAS (*Atomic Absorption Spectroscopy*) for determining chemical composition. The growth of microbial preserved in bentonite tablet after stored for 20, 40, and 60 days was identified by TPC (*Total Plate Count*). Bentonite has a main component as silica-SiO₂ dan montmorillonit with some elements existence of magnesium (Mg), iron (Fe), aluminum (Al), and silica (Si), and Sodium (Na). The spores after preserved need two days longer to grow back into the mycelium. Viability the spore after storage for 60 days could be revived 3.0×10^{10} CFU/g. The results suggest that bentonite could be used as carrier for the spore of *Rhizopus*.

Keywords: Bentonite; Carrier; Preservation; *Rhizopus*; Spore

1. INTRODUCTION

Rhizopus was a superior indigenous microbe role in ramie fiber degumming that hydrolyzes pectin (gum) by *pectinase* activity (Wulandari et al., 2011). Application of biodegumming in large-scale production requires starters in sufficient quantities. Starters defined as amount of microbe added in bioprocess. It is required proper media and preservation methods for storage and maintenance. Preservation was the most important stages for the survival viability of the industry because of influence the ability to produce a metabolite or biomass (Badjoeri, 2010; Coinceicago et al., 2005; Susilawati et al., 2016; Yulneriwarni, 2008). Dry preservation method is the most effective method for the culture that produces spores or other dormant structures (Nakasone et al., 2005).

Bentonite is clay that contains more than 85% montmorillonite has active surface area to allow for intercalation of molecules or such as cell of bacteria (Sembiring & Sumarnadi, 2011). Mineral absorbent has potentially for microorganism preservation because it has pores cavity 12-20Å, not contains heavy metals, and has big pore cavity. In order to be used as preservation carrier for spore effectively and efficiently, activation necessary for a modification of physical or chemical properties without to damage the crystal structure (Drastinawati et al., 2010).

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This research was important to identify bentonite characteristic to be carrier to preserve of *Rhizopus* spore and to determine its viability after preservation process.

2. METHODOLOGY

2.1. Bentonite Characterization and Activation

Material used in this study was Ca-bentonite from Desa Sarimanggu, Karangnunggal, Tasikmalaya, West Java. Bentonite characteristics were identified by BET (*Brunauer Emmett Teller*) and SEM-EDS (*Scanning Electron Microscopy-Energy Dispersive Spectroscopy*) for determining surface properties and elements within the minerals, XRD (*X-Ray Diffraction*) for identifying the mineral, and AAS (*Atomic Absorption Spectroscopy*) for determining chemical composition analyzed with SNI 13-3608-1994 Standard Method. Bentonite was activated with H₂SO₄ 5 % for 2 h, soaked 150 rpm, and dried at 100°C then ground and sieved with size (-60 + 80) mesh and sterilized before used as carrier.

2.2. Microorganism Preparation

Isolate fungi of *Rhizopus* was a collection of Microbiology Laboratory, Department Biology, University of Padjadjaran. Culture of *Rhizopus* was grown in *Potato Dextrose Agar* (PDA) slant and incubated 8 days. Spore harvesting were done by dredged parts mycelium and the spore suspension was transferred into 90 mL physiological saline with 0.1% (v/v) Tween. Spore concentrations were calculated using a haemocytometer.

2.3. Bentonite Activation

Bentonite was activated with modification method from Sembiring (2010), 40% solid bentonite was soaked with sulfuric acid is 0.6 N for 2 h. The bentonite was dried at 100°C then crushed and checked the porosity using SEM.

2.4. Immobilization Bentonite with Spore Suspension

Fifty grams of bentonite added to 100 mL of sterile distilled water. Adsorption spores on bentonite has been carried out with agitation for 30 minutes at 320 rpm, and then dried at 42°C. Spore-contained bentonite packaged in tablet and stored in dark bottles covered and analyzed with SEM to determine the nature of the surface.

2.5. Viability Test of Bentonite Tablet Contains Preserved-spore

A spore-preserved tablet that has been stored for 20, 40, and 60 days homogenized in 9 ml physiological saline diluted to 10⁻⁶ poured in to PDA medium and incubated at 27°C. Viability of fungal cells was observed from growth of mycelium on PDA medium.

3. RESULTS

3.1. General Characteristics of Bentonite

Characterization of bentonite was conducted to determine the general chemical or physical properties of its components. Physically, these clay minerals were reddish, creamy white, hard in the dry state and swelling in water. XRD phase analysis of the composite is shown in Figure 1. The peaks have been identified as belonging to the phases of monmorilonit (sodium calcium alumunium silicate hydroxide) and tridimit (silicon oxid) as main minerals. Figure 2 shows the EDX analysis of the bentonite to confirm the major elements in bentonite existence of magnesium (Mg), iron (Fe), aluminum (Al), and silica (Si), and Sodium (Na) in different quantity, with the most dominate was silica as 27.94%.

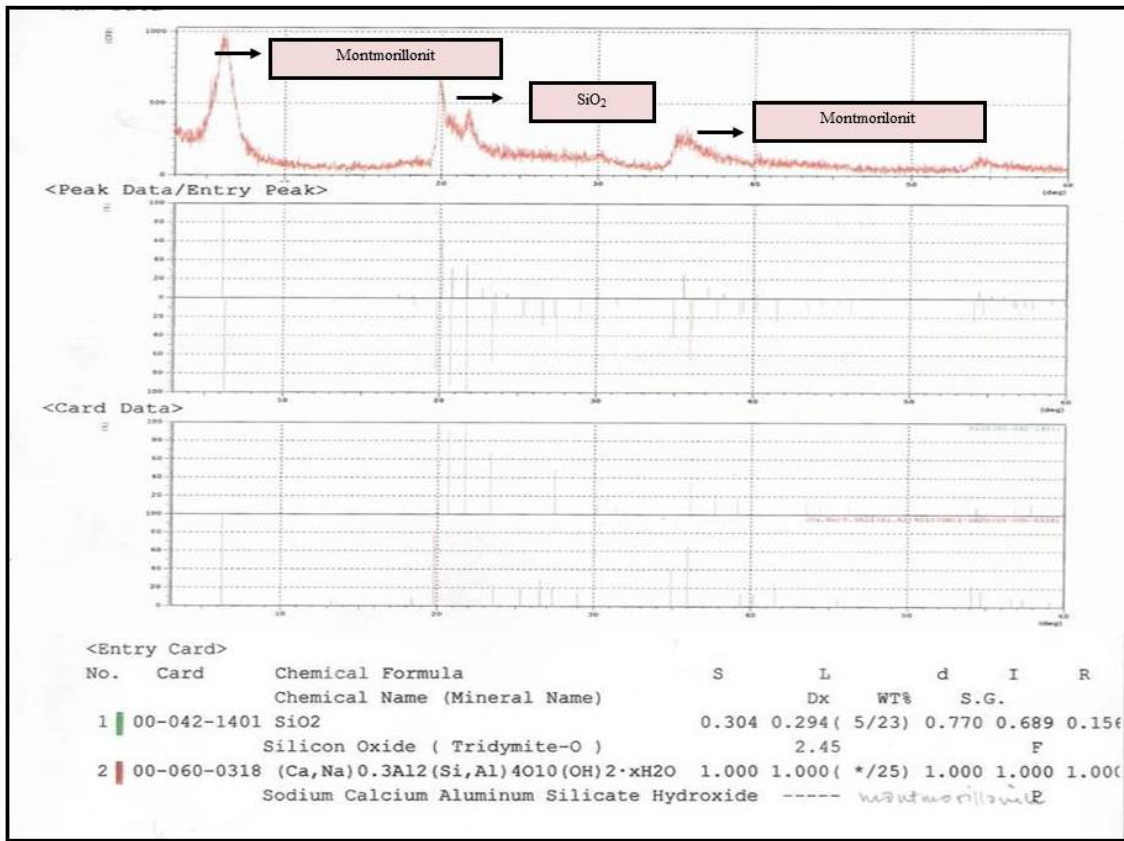


Figure 1 XRD diffractogram showing the main mineral compound in bentonite

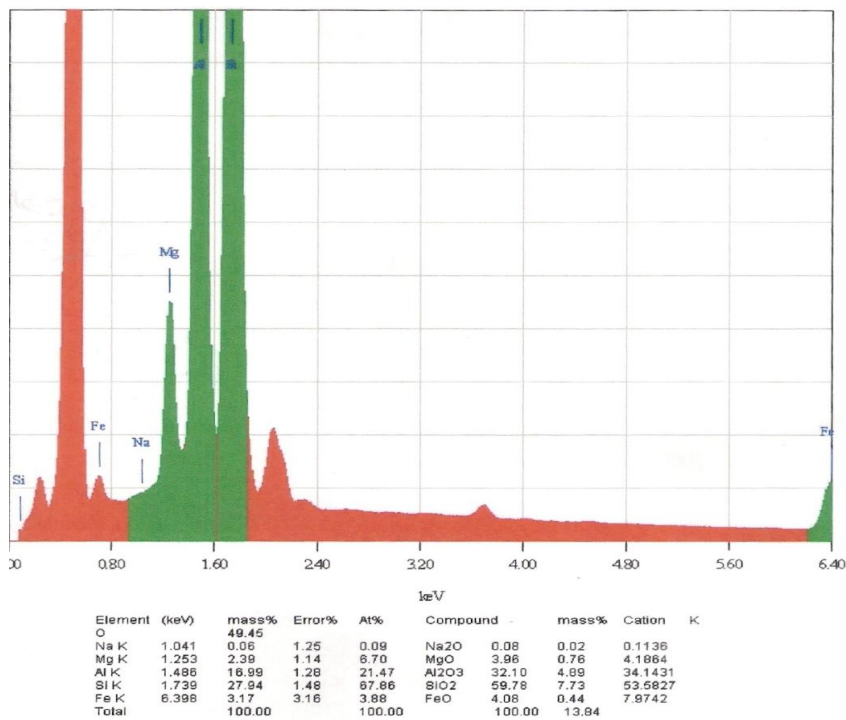


Figure 2 SEM /EDS diffractogram of the bentonite to detect the presence of major elements

The results of the physical analysis further confirmed by the chemical composition of bentonite results are shown in Table 1. Bentonite has a main component constituent indicated as silica-

SiO₂ (66.9%), Al₂O₃ (16.07%), and other elements. Both of the chemical composition seems as a major component forming of structure and properties of Sarimanggu-bentonite. The results of chemical analysis showed the composition of bentonite as shown Table 1.

Tabel 1 Chemical composition of bentonite Sarimanggu

Chemical composition	Levels (%)
SiO ₂	66.9
Al ₂ O ₃	16.07
Fe ₂ O ₃	2.56
K ₂ O	0.17
Na ₂ O	0.082
CaO	0.39
MgO	4.15
LOI	9.36

Figure 3 shows the SEM photomicrographs of the bentonite at different magnification. Purity of the particle bentonite has pores (Figures 3a and 3b), with more detailed observations may know microporous structure (Figure 3c) allowing adsorption of *Rhizopus* spore into pore of bentonite contacted by adhesion bond. Further analysis using *Surface Area Analyzer*, the bentonite surface had 85.94 m²/g, volume micro pore 0.016–0.020 cc/g and micro pore area 32.66–39.71 m²/g.

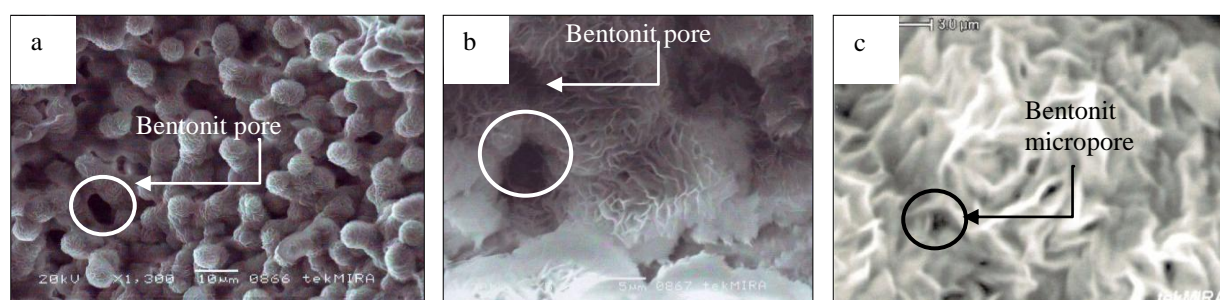


Figure 3 SEM micrographs of particles of bentonite: (a) 1,300×; (b) 5,000×; (c) 10,000× magnification

Based on observations (SEM), *Rhizopus* spores existed on the tablet surface and cavity. The existence the spores from the bentonite tablet was also seen after it was suspended with physiological saline and viewed under a fluorescence microscope shown in (Figure 4).

3.2. Viability *Rhizopus* Spore

Rhizopus viability before and after preservation for 20, 40, and 60 days in bentonite showed high purity for all treatment, however there is a difference growth of fungal cells without preserved as a control, that only need two days for growing mycelium in the plate. After preservation treatment of the tablet, mycelium growth to be 4 days, need a longer time for the spores after a dormant phase (Figure 5). For each 1 g tablet bentonite containing 1.3×10^{15} spores, however after storage for two months, the spores could be revived 3.0×10^{10} CFU/g of bentonite.

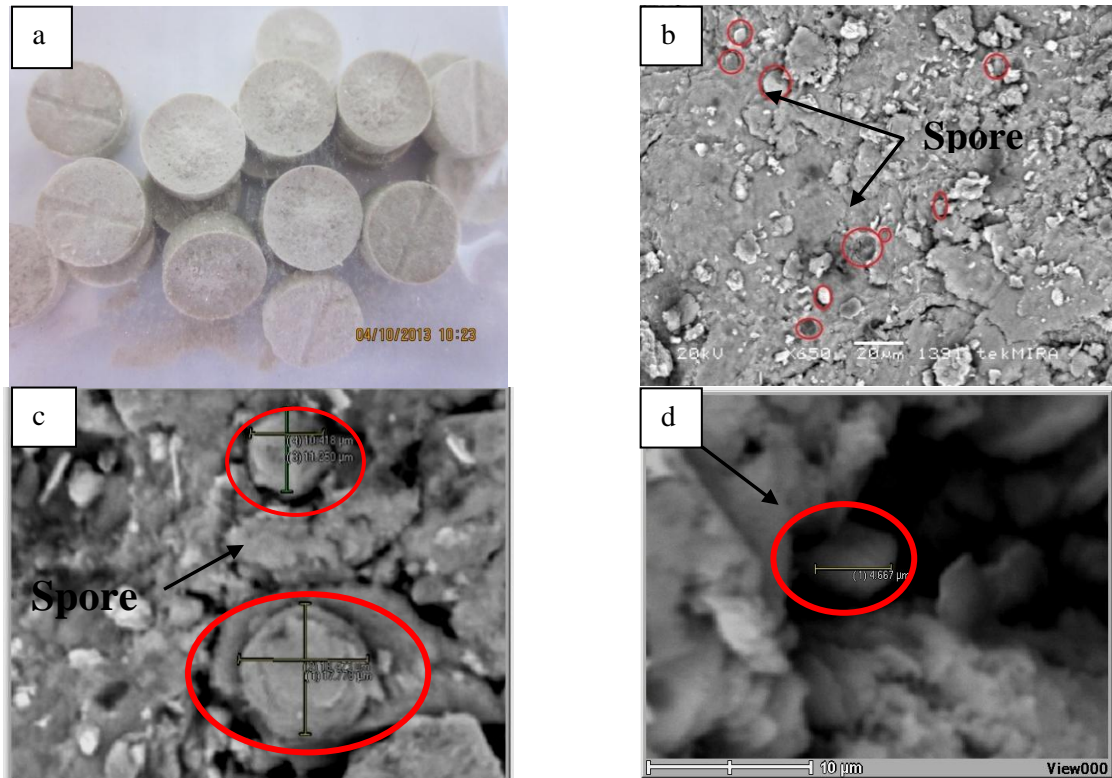


Figure 4 Results of analysis (SEM): (a) tablet containing spores *Rhizopus.sp*; (b) the surface of the tablet (650 \times); (c) spores of *Rhizopus* with a diameter of 17.78 μm on the surface of the tablet (4500 \times); (d) spores of *Rhizopus* with a diameter of 4.66 μm in the cavity bentonit tablet (4,500 \times)

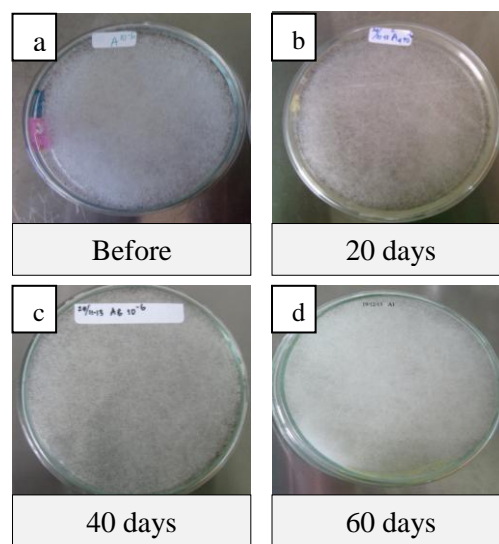


Figure 5 Growth of *Rhizopus.sp*: (a) before preservation in bentonite tablets; and after storage: (b) 20 days; (c) 40 days; (d) 60 days

4. DISCUSSION

The results in this study indicate that the local bentonite from Desa Sarimanggu, Indonesia showed potential to become a carrier of the spore from fungus *Rhizopus sp*. Bentonite used as preservation media of *Rhizopus.sp* was sodium-bentonite (NA-bentonite) types. Sembiring and Sumarnadi (2011) had used Na-bentonite as preservation of bacterial cells for applications *Bacillus.sp*, , in this study using bentonite for preservation of spore of fungi was a new one.

Spore survival after preserved to grow back to the mycelium takes 4 days, while untreated preservation of the fungus can grow only 2 days. It is strongly associated with release time for the spores to begin a process of germination. The biochemical properties related to the chemical reaction and the distinctive characteristics of metabolism occurs in the spore (Cánovas & Iborra, 2003). Without preservation, in the a suitable environment, the spores grow to stage the size of the enlargement (swelling) and tube bulge as the final stage of germination. This process involves a biochemical reaction in the cell spores that follow morphological changes in cells fungus.

Preparation of dried-biostarter with carrier materials such as bentonite depends on activation and adsorption processes. The purpose of activation of the carrier using 6 N sulfuric acid for 2 hours as the optimum conditions for the removal of impurities minerals such as ion Al_3^+ , Fe_3^+ , Mg_2^+ , and other materials to increase the porosity and surface area, so as to increase the adsorption capacity. Activated-bentonite used in this study was able to absorb 1.3×10^{15} spores / g, however only revived 3.0×10^{10} spores/g tablet. These results show the use of bentonite for the preservation of the spores did not differ with the zeolite as a medium for bacterial cells of 1.0×10^{10} cells/g tablets (Sembiring et al., 1998).

5. CONCLUSION

In conclusion, the bentonite from Simanggu have the main components as silica-SiO₂ and other elements Mg, Fe, Al, and Si and Na in different quantities. 1 g tablet of bentonite contain 1.3×10^{15} spores and the spores can be revived 3.0×10^{10} CFU/g after storage for 60 days, with mycelium grows for 4 days.

6. ACKNOWLEDGEMENT

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