Fabrication and Characterization of Immobilized Biosurfactant Produced by *Pseudomonas aeruginosa* Grown on Cassava Industrial Wastewater into Activated Allophane as an Adsorbent

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Abstract. The immobilization of biosurfactant into activated allophane has been conducted with mass ratio of biosurfactant:allophane of 1:5; 1:7 and 1:10 and contact time of 24 and 48 h. The optimum condition for immobilization was reached when the mass ratio of biosurfactant: allophane of 1:10 with the contact time of 24 h was applied. The result yielded the immobilization product having the specific surface area of 82.42 m^2/g and the surface acidity of 9.12 mmol/g. A better adsorbent has been produced. In respect to the activated allophane, there was a decreasing of specific surface area about 20% and increasing of surface acidity value about 120%.

1. Introduction

Allophane is an amorphous clay mineral which is naturally available in volcanic soils. It is found in the mountain areas with an altitude of 0-3000 m above sea level [1-2]. Allophane is found in many Indonesian volcanic mountains. Natural allophane has been reported as an adsorbent for heavy metal ions, such as Cr, Fe, Cd, Cu, Pb and Mn [3]. Natural allophane which has been activated by H_2SO_4 and NaOH giving higher specific surface area and surface acidity value [4].

On the other hand, biosurfactants have also been applied for remediation of both organic and inorganic pollutants [5-6]. The application of biosurfactants as adsorbents for heavy metals has been reported [7-8]. Biosurfactants are surface active compounds which are produced by a variety of bacteria, fungi and yeast which have various structures having many functional groups for ion binding. They possess better environmental compatibility than that of the chemical surfactants, e.g biodegradable and lower toxicity [9-11]. Therefore, research in developing method of biosurfactants application as adsorbents is still need to be conducted.

The immobilization methods have been reported to increase the properties of adsorbents [12]. Our previous work demonstrated the production of biosurfactant by *Pseudomonas aeruginosa* using cassava industrial wastewater (*manipuera*) as media [13]. The abilities of allophane and biosurfactants as adsorbents may be increased by surface modification. The purpose of this research was to develop a better adsorbent by immobilization of biosurfactant into activated allophane.

2. Experimental

2.1. Materials and Instruments

Chemicals and solvents were used are analytical grade from e-Merck. The *manipuera* used was obtained a local cassava industry. The strain *P. aeruginosa* FNCC 0063 was purchased from IUC Food and Nutrition, Universitas Gadjah Mada, Indonesia. Allophane was collected from Lawu Mount, Central Java, Indonesia. The instruments were used are Shimadzhu FTIR-8201 PC Spectrometer and X-Ray Diffractometer Shimadzu 6000.

2.2 Media Used and Growth Condition

Cultures of bacteria were maintained on nutrient agar. Biosurfactant was produced using media composed of nutrient broth (8 g/l), NaCl (5 g/l) and *manipuera*. The media were sterilized prior used. Fermentation was carried out for 4 days at room temperature on a rotary shaker (100 rpm).

2.3. Biosurfactant recovery

Culture liquid of *P. aeruginosa* was centrifuged at 12,500 *g* for 15 mins. The supernatant was acidified to pH 2.0 with HCl 6N and leaved overnight at 4°C. The supernatant then was extracted using chloroform:methanol = 2:1. The organic layer was evaporated to give the biosurfactant.

2.4. Preparation of activated allophane

The natural allophane was washed, dried and ground. The powder obtained was sieved on sieves of 150 mesh. Activation of allophane was conducted by addition of 1 L H_2SO_4 5 M to the 200 g of allophane and then stirred for 3 h. The allophane was then washed for several times until the pH was neutral. Finally, the activated allophane was then dried in oven at 100°C for 1 h.

2.5. Immobilization of biosurfactant into allophane

Mass ratio of biosurfactant : allophane used in this immobilization process were 1:5; 1:7 and 1:10 with contact time of 24 and 48 h. Aquadest (5 mL) was added to the biosurfactant (0.4 g) and stirred for 5 mins. The activated allophane was added as much as 2, 2.8 and 4 g. The water (25 mL) was then added and the mixtures were stirred for 24 or 48 h at room temperature. The solid was filtered using Whatman filtration paper No. 42 and washed with aquadest. The resulting solid was dried in oven at 40°C.

2.6. Characterization of biosurfactant, allophane and immobilization products (IP)

Characterization of biosurfactant, allophane and immobilization products (IP) were performed for functional groups determination using FT-IR spectrophotometer, specific surface area using methylene blue absorption test and surface acidity value using ammonia adsorption method [14-15]. Additional characterizations of allophane and immobilization products were conducted by sodium-flouride (NaF) test and X-Ray diffraction [16].

3. Results and Discussion

3.1. Biosurfactants characterization

The obtained FT-IR spectra (Figure 1a) revealed a strong and broad band at 3402 cm^{-1} for O-H stretching vibration. The band at 2927and 2854 cm^{-1} was related to the –CH stretch. The asymmetric stretching vibration of C=O carboxylate was identified by the band at 1654 and 1253 cm^{-1} and the peak at 1056 cm^{-1} was confirmed the presence of ester. The weak band at 1458 and 1377 cm^{-1} were obtained for -CH_3- and CH_2-, respectively. The resulted peaks of biosurfactants confirmed that rhamnolipids biosurfactants were produced by *P. aeruginosa* [17]. The specific surface area and surface acidity value of biosurfactant were 55 m^2/g and 88.24 mmol/g, respectively.

3.2. Allophane characterizations

The presence of allophane in the soil samples can be validated by NaF test which pH sample is greater than 9.4 [3]. The pH sample was obtained for 9.5 confirming the presence of allophane in the soil samples. The resulted FT-IR spectrum also verified the presence of allophane in the sample because it showed similar characteristic peaks with the standard allophane, such as 3447 cm^{-1} for -OH (Al-OH/Si-OH); 1035 cm^{-1} for Si-O/Al-O and 550, 432 and 471 cm^{-1} for O-Al-O/O-Si-O [3].

X-Ray powder diffraction of soil sample was recorded (Figure 1) and its *d-spacings* were confirmed with JCPDS for allophane (JCPDS 38-0449) as shown in Table 1. The XRD analysis revealed the peaks at 2θ = 27.854; 27.300 and 66.470 with intensity of 100, 7 and 9, respectively. The mineral compositions of sampel soil were allophan (A) and Klo-Ver-Mont (K-V-M) with the precentage of 34.81 and 9.07%, respectively. The specific surface area and surface acidity value of allophane were 104 m^2/g dan 4.12 mmol/g, respectively.

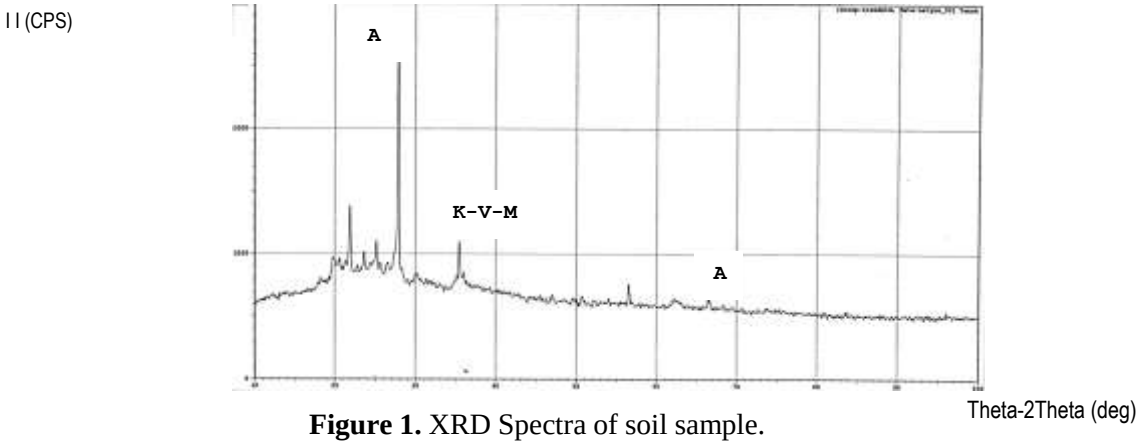

Figure 1. XRD Spectra of soil sample.

Table 1. Typical *d-spacings* data of the soil sample compared to the JCPDS 38-0449.

	***d-spacings* (Å)**				
Standar (JCPDS 38-0449)	3.30	2.25	1.86	1.40	1.23
Sample soil	3.17	2.23	1.78	1.41	1.23

3.3. Immobilization Products (IP) characterization

Figure 2a and 2b shows the FT-IR spectra of the biosurfactant and immobilization product of sample IP-110-24, respectively. Table 2 presents the comparison peaks obtained for biosurfactant, activated allophane and immobilization products. Some peaks of activated allophane were found to be shifted in the immobilization product. However, the peaks for all immobilization product were similar to the peaks of activated allophane possibly due to the mass ratio of activated allophane was higher than that of biosurfactants.

Table 3 presents the specific surface area and surface acidity value of biosurfactant, activated allophane and immobilization products. The specific surface are of all immobilization products were smaller than that of activated allophane. These results were unexpected. Possibly, the porous of activated allophane were occupied by biosurfactants leading to the decreased of the specific surface area. The specific surface area of immobilization product with 48 h immobilization process were lower than that of with 24 h immobilization process. These findings indicated that the longer immobilization process, the more surface area were occupied by biosurfactant leading to the decreased of the specific surface area. The immobilization product with the mass ratio of 1:10 and the

immobilization process of 24 h (IP-110-24) had the highest of specific surface area. In respect to the specific surface area of activated allophane, the specific surface area of IP-110-24 decreased 20.4 %.

The surface acidity value of all the immobilization products were higher than that of the activated allophane (Table 3). The surface acidity value reflects the number of ionic active sites of the samples. The higher of surface acidity value, the better adsorbent was produced. The porous of activated allophane were occupied by biosurfactant in the immobilization products resulting the higher surface acidity values were obtained These results possibly due to the additional functional groups from biosurfactants The surface acidity value of immobilization product with 24 h immobilization process were higher than that of with 48 h of immobilization process. These results suggested that the longer immobilization process, the lower number of active sites of the immobilization product was obtained resulting to the decrease of the surface acidity value. In respect to the surface acidity value of activated allophane, the highest of surface acidity value was obtained for IP-110-24 with 121.5 % increased.

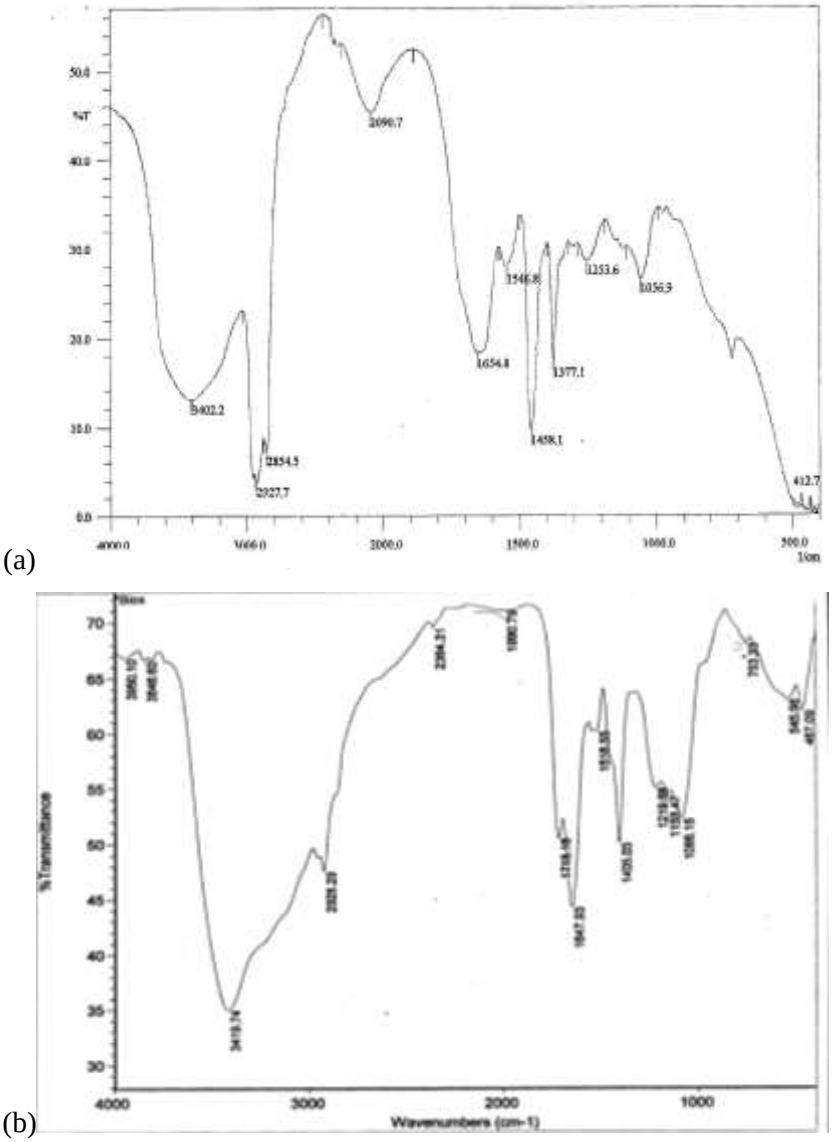

Figure 2. FT-IR spectra of: (a) biosurfactant and (b) immobilization product of sample IP-110-24.

Table 2. The peaks obtained from FT-IR spectra analysis for biosurfactant, activated allophane and immobilization products.

Samples	Wavenumber (cm^{-1}) for Fuctional Groups			
	-OH	C-O	Si-O/ Al-O	O-Al-O/ O-Si-O
Biosurfactants	3419	1086	-	-
Activated allophane	3449	1039	1039	542;471
IP-15-24	3449	1039	1039	542;471
IP-15-48	3449	1039	1039	543;471
IP-17-24	3451	1039	1039	542;471
IP-17-48	3453	1040	1040	542;471
IP-110-24	3449	1037	1037	540;471
IP-110-48	3451	1040	1040	542;471

Table 3. The specific surface area and surface acidity value of biosurfactant, activated allophane and immobilization products.

Samples	Specific Surface Area (m^2/g)	Surface Acidity Value (mmol/g)
Biosurfactants	54.86	88.24
Activated allophane	103.56	4.12
IP-15-24	73.85	9.12
IP-15-48	73.07	7.94
IP-17-24	78.44	9.12
IP-17-48	73.24	7.35
IP-110-24	82.44	9.12
IP-110-48	72.56	7.06

Based on the specific surface area and surface acidity value data, the optimum condition of immobilization process was obtained for sample IP-110-24 which had mass ratio of biosurfactant and allophane of 1:10 with 24 h of immobilization process. Although the specific surface area decreased in this immobilization products in respect to the activated allophane, its surface acidity value increased significantly by 121.5% indicating the higher number of active sites were obtained.

4. Conclusion

The novel adsorbent could be obtained by immobilization of biosurfactant produced by *P. aeruginosa* grown on *manipuera* into allophane. The optimum condition of immobilization process was achieved for sample IP-110-24 which had mass ratio of biosurfactant and allophane of 1:10 with 24 h immobilization process. The specific surface area and surface acidity value of this sample were 82.44 m^2/g and 9.12 mmol/g, respectively. In respect to activated allophane, there was a decreasing of specific surface area about 20% and increasing of surface acidity value about 120%. The significantly increasing of surface acidity value indicated that the immobilization product was better adsorbent than that of activated allophane. This adsorbent has potential applications in environmental remediation.

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