

Removal of Heavy Metals Pb, Zn and Cu from Sludge Waste of Paper Industries Using Biosurfactant

Nurul Hidayati*, Tini Surtiningsih and Ni'matuzahroh

Biology Department, Faculty of Science and Technology, Airlangga University, Indonesia

Abstract

Increasing public awareness of environmental pollution influences the search and development of technologies that help in cleanup of organic and inorganic contaminants such as metals. Sludge waste of paper industries as toxic and hazardous material from specific source containing Pb, Zn, and Cu metal from waste soluble ink. An alternative and eco-friendly method of remediation technology is the use of biosurfactants and biosurfactant-producing microorganisms. Soil washing is among the methods available to remove heavy metal from sediments. The purpose of this research is to study effectiveness of biosurfactant with concentration=CMC for the removal of heavy metals, lead, zinc and copper in batch washing test under four different biosurfactant production by microbial origin; *Pseudomonas putida* T1(8), *Bacillus subtilis* 3K, *Acinetobacter* sp, and *Actinobacillus* sp was grown on mineral salt medium that had been already added with 2% concentration of molasses that it is a low cost application. The samples were kept in a shaker 120 rpm at room temperature for 3 days. Supernatants and sediments of sludge were separated by using a centrifuge and samples from supernatants were measured by Atomic Absorption Spectrophotometer. The highest removal of Pb was up to 14.04% by *Acinetobacter* sp. Biosurfactant of *Pseudomonas putida* T1(8) have the highest removal for Zn and Cu was up to 6.5% and 2.01% respectively. Biosurfactant have a role for removal process of the metals, including wetting, contact of biosurfactant to the surface of the sediments and detachment of the metals from the sediment. Biosurfactant has proven its ability as a washing agent in heavy metals removal from sediments, but more research is needed to optimize the process of removal heavy metals.

Keywords: Biosurfactant; Molasses; Removal heavy metal; Sludge waste paper industries

Introduction

Among the different contaminants, heavy metals have received special attention due to their strength and persistence in accumulating in ecosystems, where they cause damage by moving up the food chain to finally accrue in human beings, who are at the top of the chain food. The toxic potential of heavy metals with regards to the human body is diverse and, because of their toxicity and persistence in nature, the levels of heavy metals in the environment need to be controlled by mandating waste treatment at the sources of pollution. The development of new treatment technologies is required at these sources; however, even though there is awareness of this problem, sustainable solutions are not easily accessible. In general, the conventional treatment methods used to remove metals from wastewater are inefficient and cost-prohibitive [1]. Sludge waste of paper industries as toxic and hazardous material from specific source containing Pb, Zn, and Cu metal from waste soluble ink. According to regulation of Ministry of Environmental no.33/2009 that all the industries are mandatory to clean up contaminated soil from hazardous waste [2].

The use of microbial metabolic ability or their enzymes for degradation/removal of environmental pollutants provides an economic and safe alternative compared to other physicochemical methodologies. Biosurfactants are a structurally diverse group of surface-active substances produced by microorganisms. All biosurfactants are amphiphiles, they consist of two parts-a polar (hydrophilic) moiety and non polar (hydrophobic) group. A hydrophilic group consists of mono-, oligo- or polysaccharides, peptides or proteins and a hydrophobic moiety usually contains saturated, unsaturated and hydroxylated fatty acids or fatty alcohols [3].

Biosurfactant activities depend on the concentration of the surface-active compounds until the critical micelle concentration (CMC) is obtained. At concentrations above the CMC, biosurfactant molecules associate to form micelles, bilayers and vesicles [4].

Biosurfactants produced by microorganisms show promise for enhancing organic compound biodegradation in the presence of metals. Application of biosurfactants or microorganism produced biosurfactants in in situ co-contaminated sites bioremediation seems to be more environmentally compatible and more economical than using modified clay complexes or metal chelators [5].

Material and Methods

Bacterial isolates

A Biosurfactant-producing bacterium used in this study was isolated from oil contaminated soil in PERTAMINA storage-Tanjung Perak Surabaya. The isolated bacteria were stored in Microbiologi Laboratory, Biology Departemen Airlangga University Surabaya. The bacterium was maintained on nutrient agar (NA) and was incubated at 28°C for 24 hours.

Bacterial culture

Synthetic mineral salt medium with adding molasses 2% (v/v) was used as growth substrate. Mineral salts medium developed by Pruthi and Comeotra [6] used in this study contained per liter distilled water, KH_2PO_4 , 5.0g; K_2HPO_4 , 2.0g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0006g; $(\text{NH}_4)_2\text{SO}_4$, 3.0g; NaCl, 10g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2g; CaCl_2 , 0.01g; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$,

*Corresponding author: Nurul Hidayati, Biology Department, Faculty of Science and Technology, Airlangga University, Indonesia, Tel: +62 31 5914042; E-mail: ukhti.abel@gmail.com

Received August 25, 2014; Accepted October 17, 2014; Published October 20, 2014

Citation: Hidayati N, Surtiningsih T, Ni'matuzahroh (2014) Removal of Heavy Metals Pb, Zn and Cu from Sludge Waste of Paper Industries Using Biosurfactant. J Bioremed Biodeg 5: 255. doi:10.4172/2155-6199.1000255

Copyright: © 2014 Hidayati N, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

0.001g; H₃BO₃, 0.001g, ZnSO₄ · 7H₂O, 0.001g; CuSO₄ · 5H₂O, 0.001g; CoCl₂·6H₂O, 0.005g; and Na₂M₁₀O₄ · 2H₂O, 0.001 g, pH was adjusted to 7.0. In each flask containing various microbial producing biosurfactant; *Pseudomonas putida* T1(8), *Bacillus subtilis* 3K, *Acinetobacter sp.*, and *Actinobacillus sp.* were inoculated at 4% (v/v), OD=0.5, λ=650 nm. Cultivations were performed in 250 mL flasks containing 50 mL broth medium. The mixture was placed on a reciprocal shaker at 120rpm, at 28°C, for 4 days incubation to produce a well-dispersed suspension and to isolate biosurfactant producing bacteria.

Test of biosurfactant production

Bacterial culture centrifuged at 9000 rpm for 15 min for separating cells from supernatant. Biosurfactant production from obtained supernatant was determined by measuring emulsification activity, surface tension reduction and crude biosurfactant yield.

Measurement of emulsification test (E24)

E24 of samples were determined by adding 1 mL of kerosene to the same amount of cell free cultures, mixing with a vortex for 2 minutes, and leaving to stand for 24 hours. The E24 index is given as percentage of height of emulsified layer (cm) divided by total height of the liquid column (cm) [7].

Measurement of surface tension value

The sample is inserted into the petri dish, which is previously washed with 5% HCl solution, rinsed with distilled water, and dried. Surface tension measurement of the liquid sample was conducted using tensiometer Du Nouy. The value of surface tension is the value seen on the tensiometer when the tensiometer ring was untied from the sample surface. The value of surface tension was expressed in mN / m and reported as a result by using the formula [8].

$$r = r_0 \times \theta / \theta_0$$

r= surface tension of liquid samples

r₀= surface tension of water at t°C

θ= surface tension of the sample.

θ₀= surface tension of water

Measurement of crude biosurfactant yield

Biosurfactant was extracted from supernatant by 20% saturation (NH₄)₂SO₄ precipitation. The remained product was lyophilized using freeze dryer to obtain crude biosurfactant, and then weighed. The value was assessed as crude biosurfactant yield.

Batch Washing Test

The biosurfactant solution was diluted with buffer phosphate pH 7: KH₂PO₄ (mr 136.08) 7.11 g/L and K₂HPO₄ (mr 174.1) 11.59 g/L. Measuring pH of buffer using pH meter. NaOH and HCl used for adjusted pH until get pH 7 [9]. A quantity of 12 g from sludge was placed in each 500 mL vial. 120 mL of biosurfactant solution was added to each vial. The samples were kept at room temperature on a shaker 120 rpm for 3 days. Supernatants and sediment particles were separated by using a centrifuge (3000 rpm, 30 min) and samples from supernatants were taken. All test were performed in triplicates. The sample were digested in order to release the heavy metals trapped in the biosurfactant micelles and then the concentrations of copper and other metals in each sample were measured by the Anatomic Absorption Spectrophotometer [10].

Results and Discussion

Biosurfactant production

Molasses used as substrate is important in biosurfactant production. It is a viscous by-product of the processing of sugar cane, grapes or sugar beets into sugar and usually constitutes an industrial waste. Molasses are an important carbon source for microbial growth. Dessai and Banat [11] used molasses and cornsteep liquor as the primary carbon and nitrogen source to produce biosurfactant.

Evaluation of biosurfactant activity was done by surface tension determination and emulsification activity. This process also to detect biosurfactant existence.

Surface tension determination

Surface tension determination is an important point in biosurfactant production by *Acinetobacter sp.* A best level of biosurfactant production is characterized by its capacity to reduce surface tension [12].

From the Table 1, it can be explained that *Pseudomonas putida* T1(8) can reduce the surface tension from 61.67 dyne/cm to 32.14 dyne/cm (29.53 dyne/cm of decrease). A potential bacterium in biosurfactant production can reduce the surface tension ≥ 10 dyne/cm. It means that in this research, *Pseudomonas putida* T1(8) biosurfactant had the highest potentiality to produce biosurfactant than 3 others.

Emulsification activity test of supernatant

In this research, kerosene was used to determine the emulsification activity of supernatant. The result is shown on the Table 2. The stability value (%) indicate the power of the biosurfactant produced by microbe on hydrocarbon emulsification test [9].

The data in Table 2 explain that the highest emulsification activity is *Pseudomonas putida* T1(8) supernatant that can emulsify kerosene at a range of 35.18% (1 hour). After 24 hours the emulsification percentages drop to 31.69% which can explain emulsification instability. *Pseudomonas putida* T1(8) has a high potentiality in emulsification activity, comparing with control (water: 0%). Being highly potential in kerosene emulsification, *Pseudomonas putida* T1(8) has a high capacity in the production of bioemulsifier substance.

Critical Micelle Concentration (CMC) Value

The CMC is commonly used to measure the efficiency of surfactant.

Treatments	Surface Tension (dyne/cm)
Molasses medium	61.67 ± 0.99
Molasses + <i>Pseudomonas putida</i> T1(8)	32.14 ± 0.99
Molasses + <i>Bacillus subtilis</i>	39.04 ± 0.51
Molasses + <i>Acinetobacter sp.</i>	36.16 ± 1.86
Molasses + <i>Actinobacillus sp.</i>	35.80 ± 2.09

Table 1: Surface tension value (supernatant) by four days of incubation.

Treatments	Emulsification activity (%)	
	1 jam	24 jam
Water (Control)	0 ± 0	0 ± 0
<i>Bacillus subtilis</i>	34.9 ± 0.39	19.6 ± 0.39
<i>Acinetobacter sp.</i>	13.15 ± 0.04	10.88 ± 0.18
<i>Pseudomonas putida</i> T1(8)	35.18 ± 0.54	31.69 ± 0.29
<i>Actinobacillus</i>	15.73 ± 0.15	9.86 ± 0.11

Table 2: Emulsification activity value culture supernatant (1 hour and 24 hours).

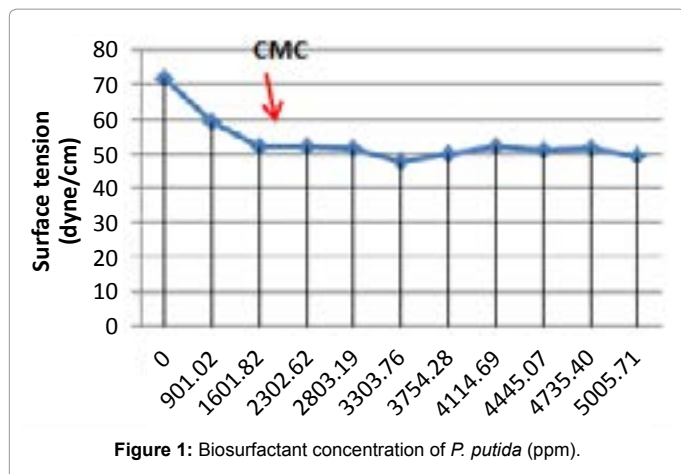


Figure 1: Biosurfactant concentration of *P. putida* (ppm).

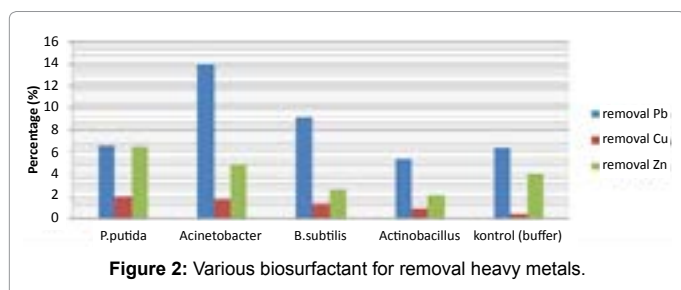


Figure 2: Various biosurfactant for removal heavy metals.

Efficient biosurfactants have a low CMC, which means that less biosurfactant is required to decrease the surface tension [11].

The highest CMC value is from *Actinobacillus sp*, it have CMC value 3303.76 mg/L or 3.30376 g/L (dry weight) with surface tension value 57.17 dyne/cm. Then the lowest CMC value in this research is from *Pseudomonas putida* T1(8). CMC value of *Pseudomonas putida* T1(8) is 1601.82 mg/L or 1.60182 g/L (dry weight) with surface tension value 51.91 dyne/cm. It showed on the Figure 1.

Surface tensions, CMC, emulsification stability value constitute the biosurfactant characteristics which depend on substrate type [10]. A biosurfactant is efficient if the CMC is about 1- 2000 mg/L [13].

Removal heavy metal Pb, Zn, and Cu using biosurfactant

The diagram showed that the highest removal of Pb was up to 14.04% by *Acinetobacter sp*. Biosurfactant of *Pseudomonas putida* T1(8) have the highest removal for Zn and Cu was up to 6.5% and 2.01% respectively. Compare with Dahrazma [10], removal Cu and Zn using 5% Rhamnolipid are 12.9% and 10.7%. It caused by different kind of biosurfactant and substrate (Figure 2).

The value removal heavy metal using buffer phosphate as control have a high enough, it maybe because buffer phosphate support absorption process for removal heavy metal. According to Roane and Pepper [14], they explain that most heavy metals are cations and this determines their sorption to negatively charged functional groups that are present in: cell surfaces, which are generally anionic at a pH of between 4 and 8; surfaces with residual hydroxides (OH⁻) or thiol (SH⁻) and anionic salts, such as PO₄⁻ and SO₄⁻, humic acid, and clay minerals.

The usefulness of biosurfactants for bioremediation of heavy metal contaminated soil is mainly based on their ability to form complexes

with metals. The anionic biosurfactants create complexes with metals in a nonionic form by ionic bonds. These bonds are stronger than the metal's bonds with the soil and metal-biosurfactant complexes are desorbed from the soil matrix to the soil solution due to the lowering of the interfacial tension. The cationic biosurfactants can replace the same charged metal ions by competition for some but not all negatively charged surfaces (ion exchange). Metal ions can be removed from soil surfaces also by the biosurfactant micelles. The polar head groups of micelles can bind metals which mobilize the metals in water [15].

Conclusion

In conclusion, the highest removal of Pb was up to 14.04% by *Acinetobacter sp*. Then biosurfactant of *Pseudomonas putida* T1(8) have the highest removal for Zn and Cu was up to 6.5% and 2.01% respectively. These molasses could be of great alternative substrate for biosurfactant production for using continuously. Biosurfactant has proven its ability as a washing agent in heavy metals removal from sediments, but more research is needed to optimize the process of removal heavy metals.

Acknowledgement

This work was supported by Biology Department, Faculty of Science and Technology, Airlangga University. The authors are very grateful to microbiology laboratory staffs and other students for their help and support.

References

- Volesky B (2001) Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy 59: 203-216.
- Hardiyani H (2011) Bioremediation of soil contaminated heavy metal Pb from deienking process sludge waste in the paper industries. 31-41.
- Lang S (2002) Biological amphiphiles (microbial biosurfactants) Curr. Opin. Colloid Inter. Sci 7: 12-20.
- Whang LM, Liu PW, Ma CC, Cheng SS (2008) Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. J Hazard Mater 151: 155-163.
- Sandrin TR, Maier RM (2003) Impact of metals on the biodegradation of organic pollutants. Environ Health Perspect 111: 1093-1101.
- Pruthi V, Comeotra S (1997) Rapid Identification of Biosurfactant Producing Bacterial Strain Using a Cell Surface Hydrophobicity Technique, Biotechnology Technique 11: 671-674.
- Tabatabaee A, Mazaheri Assadi M, Noohi AA, Sajadian VA (2005) Isolation of Biosurfactant Producing Bacteria from Oil Reservoirs. Iranian J Env Health Sci Eng 2: 6-12.
- Pornsunthomtawee O, Wongpanit P, Chavadej S, Abe M, Rujiravanit R (2008) Structural and Physicochemical Characterization of Crude Biosurfactant Produced by *Pseudomonas aeruginosa* SP4 Isolated from Petroleum-Contaminated Soil. Bioresource Technology 99: 1589-1595.
- Ulum B (2004) The effect of Molasses Concentration and Natrium Nitrate for Biosurfactant Production of *Bacillus subtilis* 3KP. Thesis. Airlangga University. Surabaya.
- Dahrazma B, Mulligan CN (2007) Investigation of the removal of heavy metals from sediments using rhamnolipid in a continuous flow configuration. Chemosphere 69: 705-711.
- Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 61: 47-64.
- Willumsem PA (1997) Karlson U (1997) Screening of bacteria isolated from PAH-contaminated soils for production of biosurfactants and bioemulsifiers. Biodegradation 7: 415-423.
- Mulligan CN, Gibbs BF (1989) Correlation of nitrogen metabolism with biosurfactant production by *Pseudomonas aeruginosa*. Appl Environ Microbiol 55: 3016-3019.
- Roane TM, Pepper IL (1999) Microbial Responses to Environmentally Toxic Cadmium. Microb Ecol 38: 358-364.
- Mulligan CN, Gibbs BF (2004) Types, production and applications of biosurfactants. Proc. Indian Nat. Sci. Acad 1: 31-55.