Preliminary Study of Biosurfactant Consortium Producing Bacteria from Palm Oil Mill Effluent (POME) in East Kalimantan

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Abstract-Biosurfactants are surface active agents from living organism, especially from bacteria and fungi. Many types of biosurfactants are synthesized by a number of microbes during their growth on non-polar substrates and the majority of biosurfactants are produced by bacteria. Emulsification activity toward n-hexane, drop collapsing test as well as oil displacement test were used to determine biosurfactant producing bacteria consortium activity from palm oil mill effluent (POME). Samples were collected from waste water treatment in PTPN XIII Palm Oil Mill Factory, Paser Regency, Provincial of East Kalimantan, Indonesia. Total of six samples were screened in order to find out which consortium was producing biosurfactant. All of the samples were showing activity in the drop collapsing method, but only sample #5 and #6 were showing Emulsification activity of 50 and 14%, respectively. Moreover, sample #5 and #6 were showing oil displacement activity which have 0.37 cm and 0.26 cm in diameter, respectively.

Keywords-Palm oil mill effluent, biosurfactant, screening, bacteria consortium

I. INTRODUCTION

Biosurfactants are surface-active substances synthesized by living cells. They are generally lipid compounds whose features are related to two ends present in the molecule, one end is hydrocarbon part which is less soluble in water (hydrophobic end).The hydrophobic part of the molecules is a long- chain of fatty-acids, hydroxyl fatty acid or α-alkyl hydroxy-fatty acids. The other end is hydrophilic, more soluble in water and consists of carbohydrate, amino acid, cyclic peptide, phosphate and carboxylic acid or alcohol [1].

Almost all surfactants currently in use are chemically derived from petroleum. However, in recent years, the interest in chemical surfactants has been substituted by the increase of microbial surfactants. The used of synthetic surfactant to enhanced the contaminant solubility are often toxic and representing an additional source of contamination, but microbially produced surface-active compounds have similar properties but provide less toxic, biodegradable and can be produced *in situ*. In order to spread the application of biosurfactants, methods of possible cost reductions have been sought.

Palm oil mill effluent (POME) is a liquid waste from palm oil mill which contains very high amount of organic materials. POME until now is still problem regarding its proportion, odour and other environment problem. Thus, POME could be utilized as source of biosurfactant production bacteria and also carbon source for its growth. Nowadays, cost of biosurfactant production is relatively higher than chemical based surfactant. Several attempt was conducted in order to decreace of the cost including utilization of renewable resources as carbon source i.e. olive oil mill effluent (OOME), sludge palm oil (SPO), animal fat, frying oil, soap stock, molases, whey and starch-rich waste [2,3,4].

The aim of this research was to search biosurfactant producing consortium in POME, screening and analyzing its properties by oil displacement test, drop collapse test and emulsification activity.

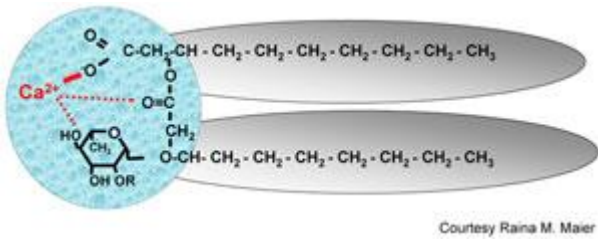

Figure 1. An example of biosurfactant, Rhamnolipid. Note the head (polar part) is dissolved in water meanwhile the tail (non-polar part) is dissolved in lipids (http://superfund.pharmacy.arizona.edu/content/sbrp-research-brief-119-novel-applications-microbially-produced-surfactants)

II. METHODS

Materials

POME samples were collected from waste water treatments ponds in PTPN XIII Palm Oil Mill Factory, Paser Regency, East Kalimantan, Indonesia. Used lubricating oil was provided by the motor vehicle maintenance garage located at Samarinda, East Kalimantan, Indonesia. 10W-30 motor oil was provided by P.T. Pertamina (Indonesia). All other chemicals used were of analytical grade.

Substrate preparation

The mineral salts medium (MSM) used was composed of (gram/liter) K_2HPO_4, 0.8; KH_2PO_4, 0.2; $CaCl_2$, 0.05; $MgCl_2$, 0.5; $FeCl_2$, 0.01; $(NH_4)_2SO_4$, 1.0; and NaCl, 5.0. The pH was adjusted to 7.0 with either hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions.

Condition of cultivation

Enrichment and isolation of biosurfactant producing bacteria was carried out using waste lubricating oil as a sole carbon and energy source. Initially, a bacterial consortium was enriched by 1 mL of POME to a 100 mL flask containing 25 mL MSM-oil medium at 30^{o}C in a shaking incubator at 100 rpm for 120 h or until oil emulsion is observed. Then 1 mL aliquot of the culture broth was transferred to fresh 25 mL MSM in 100 mL flask and incubated with the same condition as described above. This procedure was repeated 5 times. The biosurfactant producing bacteria consortia was stored at -20°C in MSM medium containing 25% glycerol.

Emulsification activity (%EA)

The 1 mL of culture supernatant and 1 mL of *n*-hexane was added and vortexed at high speed for 2 min. The mixture was allowed to stand for 10 min prior to measurement. The emulsification activity was defined as the height of the emulsion layer divided by the total height and expressed as percentage.

Oil displacement area (ODA)

Ten-µL micro litter of weathered crude oil was placed on the surface of distilled water (40 mL) in a petri dish (250mm in diameter). Then, 10 µL of the culture supernatant was gently put on the centre of the oil film. The diameter of clear halo area visualized under visible light was measured and calculated.

Drop-collapse test

Drop-collapse tests were performed in th polystryrene lid of a 96 microwell plate. A tin coat of 10W-30 oil was applied to each well. The coated wells were equilibrated for 24 h and then a 20 µL aliquot of supernatant was delivered into the center of each well. If the drop remained beaded, the result was scored as negative. If the drop spread and collapsed, the result was scored as positive for presence of biosurfactant. The mineral salt medium alone had a negative drop-collapse test [5].

III. RESULTS

POME samples were collected from waste water treatments ponds in TPN XIII Palm Oil Mill Factory, Paser Regency, East Kalimantan, Indonesia. Total of six samples were screened in order to find out which consortium was producing biosurfactant. Then these samples tested qualitatively for biosurfactant-production with the drop collapse test, emulsification index (EI_{24}) test and drop collapse test. The results are shown in Table1. The waste water treatment facility was shown in Figure 2.

Figure 2. The waste water treatment facility in PTPN XIII

TABLE 1. Oil displacement test, emulsification index (EI_{24}) and drop collapse test of consortium supernatant culture

Sample #	Testing method		
	Oil displacement test (cm)	EI_{24} (%)	Drop collapsing method
1	< 0.1	0	+
2	< 0.1	0	+
3	< 0.1	0	+
4	< 0.1	0	+
5	0.37	50	++
6	0.26	14	+
7 (MSM media)	< 0.1	0	--

From the result above, sample #5 exhibited the highest activity for oil displacement test toward crude oil (0.37 cm) and emulsification activity and was a good biosurfactant consortium producer. It was writted that the sample with higher emulsifying activity tendence to showed greater oil displacement activity. Figure 3 showed the oil displacement test from sample #5. The clear hollow in the middle of used lubricating oil indicating that the consortium could produce biosurfactant. While on the other samples and MSM solution only forming buble (figure not shown).

Figure 3. Oil Displacement activity from sample #5

In the other hand, drop collapsing methods also a rapid method for pre-eliminary screening of biosurfactant. If the supernatant culture contained biosurfactant, the droplets of the supernatant in the oil-coated wells collapsed. If not, there was no change in the shape of droplets. All of the ulture supernatant droplets collapsed, meanwhile the MSM media was not collapsed (Figure 4). Tugrul and Cansunar used this method for detecting surfactant-producing microorganism from *Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans* and *Phanerochaete chrysosporium* [6].

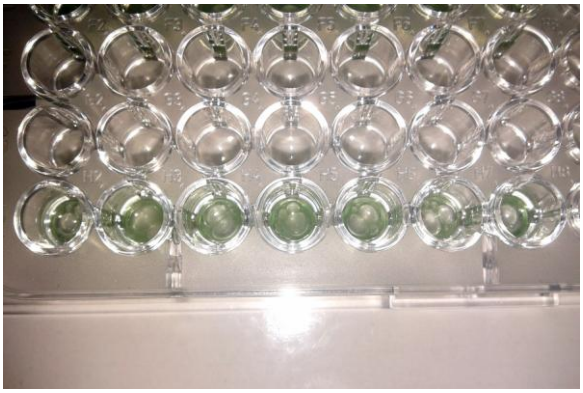

Figure 4. Drop collapse test from culture supernatant

From above data, the sample #5 and #6 are the promising consortium that produce biosurfactant. This is a pre-eliminary research that should be continued with the experiment to know the application of these findings.

ACKNOWLEDGEMENT

This research was funding by Directorate General of Higher Education, Republic of Indonesia by Hibah Bersaing Research Grant 2012.

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