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Comparative study: Different recovery techniques of rhamnolipid produced by Pseudomonas aeruginosa USMAR-2

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Abstract. Rhamnolipid, a glycolipid-type biosurfactant, exhibits its potential in many industrial applications. The cost of downstream separation and purification, however, remains significant, limiting the widespread use of this biosurfactant. Consequently, it is prudent to seek a practical and economical recovery technique to minimize production cost. This paper gives an overview of three recovery techniques that has been used to recover the rhamnolipid produced by *Pseudomonas aeruginosa* USMAR-2 from the fermentation process. These are acid precipitation, ammonium sulfate precipitation and organic solvent extraction. Organic solvent extraction using methanol, 1-butanol and chloroform was found as the best technique in this study, giving the highest recovery at 89.70 % (w/v). The resulting powder rhamnolipid presents a purified compound and consequently offers further cost reduction in transportation and packaging. Besides giving a high product recovery, the crude rhamnolipid also showed a stable activity at high temperature, 121°C.

Keywords: *Pseudomonas aeruginosa* USMAR-2, biosurfactant, rhamnolipid, recovery.

1. Introduction

Biosurfactants are surface active molecules produced by variety of microorganisms and have several advantages over the synthetic surfactants. They are structurally diverse group of surface active molecules which contained hydrophobic and hydrophilic moieties in the same molecules. Due to this nature, they can reduce the surface tension and interfacial tension between individual molecules at the surface and interface, respectively [1]. In addition, They are of commercial interests because of their unique characteristics such lower toxicity, higher biodegradability, higher surface activity, can be produced from renewable and cheaper substrates and have stable activities at extreme pH, salinity and temperatures.

Rhamnolipid, a glycolipid-type biosurfactants have been widely utilized in industries like agriculture, enhanced oil recovery and bioremediation of oil-contaminated sites [2]. Despite improving fermentation strategy, an efficient and economical product recovery technique is needed for maximum product recovery. It is essential to recover and purify the biosurfactants in a cost-effective manner to lower down the whole cost of production as the industrial demand for biosurfactants is constantly growing. Therefore, the present study compares various recovery techniques for rhamnolipid produced by *Pseudomonas aeruginosa* USMAR-2.

2. Materials and Methods

2.1. Solvent extraction

Modified method from Lee *et al*., [3] was applied. Cells were removed from the culture broth by centrifugation at 10 000 x *g*, 4°C for 10 min. The supernatants were lyophilized and extracted with a mixture of the extraction solvents with the following ratio; (methanol/chloroform/1-butanol, 1:1:1 by volume). The mixture was continuously shaken at 200 rpm (Certomat R&H, B. Braun, Germany) and 30ºC for 5 hours. After 5 hours, 2 layers of precipitation were obtained. The upper layer was discarded and the lower layer was

poured onto a clean glass Petri dish. The Petri dish was put inside the fume hood until dry, brown-colored powder was obtained.

2.2. Ammonium sulfate precipitation

Cells were removed from culture broth by centrifugation at 10 000 x *g*, 4°C for 10 min. The supernatant containing biosurfactant was precipitated with 40% (w/v) ammonium sulfate and incubated overnight at 4°C. The precipitate was then collected by centrifugation at 10,000 x *g* for 10 min at 4°C and extracted with acetone. The volume of acetone used was as same as the volume of the supernatant. After the extraction process, the precipitate was dried inside fume hood until dry, white-colored powder was obtained.

2.3. Acid precipitation method

Cells were removed from culture broth by centrifugation at 10 000 x g, 4°C for 10 min. The supernatant containing biosurfactant was acidified with 2N HCl until pH 2.0 was obtained and the mixture was incubated overnight at 4°C. The precipitate was collected by centrifugation at 10 000 x g, 4 °C for 30 min and washed with acetone. The precipitate was then dried inside the fume hood. Black colored paste was obtained after 24 hours of drying process.

The formula used to calculate the percentage of rhamnolipid recovery for the 3 methods as listed above is as follows:

Rhamnolipid recovery, (%) =

$$\frac{\text{rhamnolipid concentration after recovery process}}{\text{rhamnolipid concentration before recovery process}} \times 100$$

3. Results and discussion

From the three different techniques studied, the solvent extraction using the mixture of chloroform, 1-butanol and methanol gave the highest recovery percent, approximately 90% of recovery as indicated in Figure 1. The final product was brown in colored powder. This result can be explained as biosurfactant is a lipid containing amphiphatic molecules [2]. Due to the presence of hydrophobic end, the biosurfactants were soluble in the organic solvent and were extracted out. On the other hand, the ammonium sulfate precipitation method was successfully produced white particles. However, the recovery percent was lower compared to the solvent extraction method as this method was specifically used to salting out protein rich biosurfactant. The acid precipitation method gave a sticky dark brown-colored paste and the lowest recovery percent. Moreover, the sticky paste was difficult to handle and further purification should be done to increase the recovery percent. Therefore, the solvent extraction method was chosen as the best purification method for rhamnolipid produced by *Pseudomonas aeruginosa* USM AR-2.

Stability study of rhamnolipid was carried out to evaluate the performance of the purified rhamnolipid at a high temperature. As illustrates in Figure 2, the purified rhamnolipid was able to maintain its activity stability at various temperature including the highest temperature, 121°C. The purified rhamnolipid has a maintained activity, as stable as the rhamnolipid contained in the culture and supernatant. This finding is very crucial as it will help the researcher to determine the suitable application for the rhamnolipid produced. For an example, as the purified rhamnolipid can restrain at a very high temperature, it is applicable in various industry such as bioremediation and the microbial enhanced oil recovery (MEOR) area [4].

4. Acknowledgment

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5. References

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Tables and figures

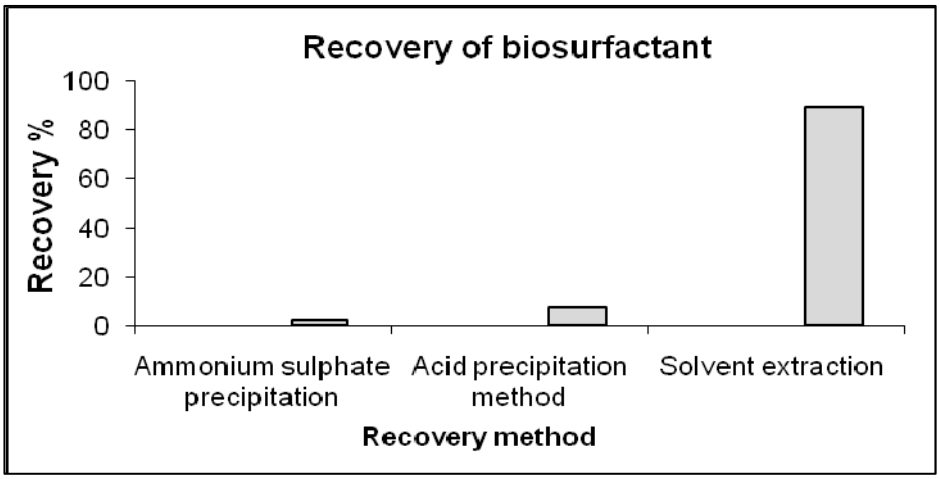
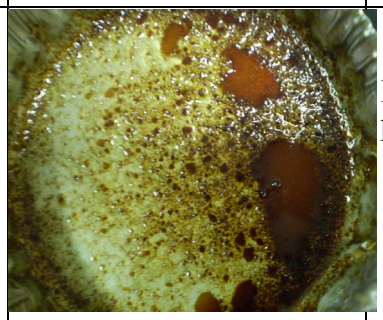


Figure 1: The recovery percent of rhamnolipid with different methods

Table 1: Comparison of purified rhamnolipid obtained from different technique.

Method	Final product	Appearance
Acid precipitation method		Brownish-black colored and oily paste
Ammonium sulphate precipitation		Coarse white colored powder

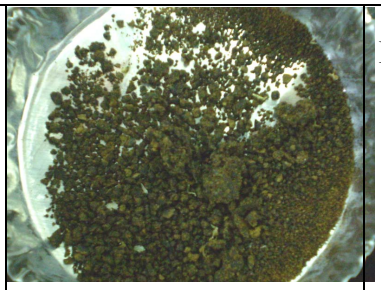
Solvent extraction		Fine brownish colored powder

Figure 2: Stability of rhamnolipid at different temperatures