



Investigation of Bio clean-up of Crude oil-contaminated Soil using Rhamnolipid Biosurfactant

Shokoufeh Malek Mahmoodi¹, Hossein Amani^{1*}, Seyed Morteza Hosseini¹ and Hasan Kariminezhad²

1. Department of Biotechnology, Faculty of Chemical Engineering, Babol Noshirvani University of Technology, Iran

2. Department of physics, Faculty of Basic Sciences, Babol Noshirvani University of Technology, Iran

hamani@nit.ac.ir

DOI: 10.22078/pr.2018.3356.2541

Received: May/10/2018

Accepted: November/25/2018

INTRODUCTION

Today, the production and consumption of biological materials has increased considerably due to biocompatibility [1]. These materials are widely used in the agricultural, cosmetic, food, pharmaceutical, oil and environmental industries [2]. The most important characteristic of biosurfactants is low toxicity, biodegradability, high surface activity, better performance in hard conditions (high pH, saline, and high temperatures), low concentrations of CMC, availability of raw materials Production and control of environmental pollution [3]. Rhamnolipid biosurfactant is produced from *Pseudomonas aeruginosa* bacteria and used to remove oil contamination soil [4]. The purpose of this paper is to investigate the purification of soil contaminated with crude oil by using the rhamnolipid biosurfactant from *P. aeruginosa* PTCC 1340.

MATERIALS AND METHODS

BACTERIA CULTURING

P.aeruginosa PTCC 1340 was prepared from the Persian type culture collection. LB¹ medium was used as a pre-culture for the production of rhamnolipid. In addition, 100 ml of LB was autoclaved for 20 minutes at 121 °C. After inoculation into the pre-culture medium, the solution was placed in a shaker incubator for 24 hours at 37 °C and 150 rpm. In this study, solutions 1, 2, and 3 are separately autoclaved according to Table 1. The solution 4 is sterilized due to the sensitivity to heat by 0.22-micron syringe filter [5].

Solutions 1 and 3 are mixed equally, and then sunflower oil are added to them at sterile condition. Finally, 1 ml of solution 4 is added to flask.

1. Lysogeny Broth

EXTRACTION OF RHAMNOLIPID BIOSURFACTANT

Equal volume of Normal hexane and culture medium was combined to be centrifuged at a rate of 4600 rpm for 20 minutes.

The aqueous phase is separated, and H₃PO₄ is added to reach the pH values which are equal to 2 to 3. Then, it is combined in a ratio of 1.25 with ethyl acetate and centrifuged for 20 minutes at 4600 rpm. Finally, the organic phase is separated and evaporated in an evaporator at a temperature of 50 and rate of 2000 rpm, ethyl acetate evaporated, and the yellow liquid of the rhamnolipid remained.

METHOD OF THIN LAYER CHROMATOGRAPHY ANALYSIS

Thin layer chromatography (TLC) was used to prove the rhamnolipid production. The sample of biosurfactant was spotted on TLC. Finally, the TLC entered the moving phase including chloroform: methanol: acetic acid, with a volume ratio (2:15:65). When the moving phase reaches the end of the TLC paper, it is removed from the moving phase and placed in the detector solution containing sulfuric acid: acetic acid, with a volume ratio (50: 1). After 10 minutes, it was removed and dried at 150 °C. Finally, the yellow rhamnolipid appeared on the paper. Equation 1 is used to calculate the delay factor (Rf) ¹.

$$Rf = \frac{\text{distance of desired item}}{\text{distance of solvent}} \tag{1}$$

Table 1: Culture media for rhamnolipid production.

Solution 4		Solution 3		Solution 2		Solution 1	
Concentration(g/l)	compound	Concentration(g/l)	com- pound	Concentration(g/l)	com- pound	Concentration(g/l)	compound
2	C ₇ H ₅ NaO ₇ .2H ₂ O	23	NaH ₂ PO ₄ .2H ₂ O	120	Sunflower oil	0.05	MgSO ₄ .7H ₂ O
0.28	FeCl ₃ .6H ₂ O	11	Na ₂ HPO ₄ .2H ₂ O	-	-	0.1	KCl
1.4	ZnSO ₄ .6H ₂ O	-	-	-	-	1.5	NaNO ₃
1.2	CoCl ₂ .5H ₂ O	-	-	-	-	-	-
1.2	CuSO ₄ .5H ₂ O	-	-	-	-	-	-
0.8	MnSO ₄ .H ₂ O	-	-	-	-	-	-

CRUDE OIL REMOVAL FROM THE SOIL BY PRODUCED RHAMNOLIPID

Oil removal was recorded by spectroscopy. The concentration of oil in the samples calculated with the calibration curve and Equation 2 was used to obtain the percentage of removal of crude oil.

$$\text{oil removal percentage} = \frac{\text{initial oil} - \text{amount of oil remaining}}{\text{initial oil}} * 100 \tag{2}$$

1. Retention Factor

RESULTS AND DISCUSSION

The highest amount of rhamnolipid was obtained 11.49 g/l after 144 h. The result of the TLC is shown in Fig. 1. According to Figure 1, *Paeruginosa* has the ability to produce rhamnolipid biosurfactant types 1 and 3. The result showed that the Maximum purification of crude oil contaminated soil was achieved by 75.17% Rhamnolipid solution.

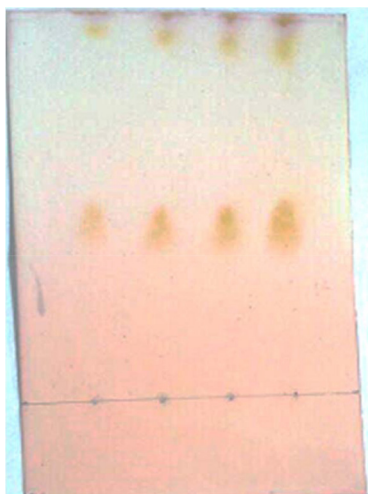


Figure 1: TLC analysis.

In addition, the results showed that Rhamnolipid supernatant eliminated 75.17% of the crude oil in the soil, while the highest removal efficiency of the crude oil from the soil was 76.92% by SDS surfactant. According to the successful results, it can be suggested that the produced rhamnolipid biosurfactant can be used as an alternative to chemical surfactants in various industries.

CONCLUSIONS

In this study, by considering the importance of producing biosurfactants and their high utilization in different industries, the production of rhamnolipid as one of the most important biosurfactants was studied. The production of rhamnolipid biosurfactant was confirmed by TLC tests. According to our results, rhamnolipid eliminated 75.17% of the crude oil in the soil. Therefore, we can propose that the produced rhamnolipid can be used as an alternative to chemical surfactants in various industries. Finally, the results of this research can be considered by researchers and industry in the field of crude oil removal from contaminated soil.

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