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Research Article

Biodegradation of Phenol by Local Strain of Bacteria Isolated from Crude Oil Contaminated Soil

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Abstract

Phenol is one of the major organic pollutant, which present in numerous industrial wastewater products, including oil refineries and petrochemical. Phenol can be removed by biological treatment using microorganisms which may solve this problem completely. In this study we sought to use some local bacterial strains to investigate their ability to treat industrial wastewater and remove phenolic compounds from it. This study was carried out using nine local bacterial strains isolated from crude oil contaminated soil from Alzawia oil refining company in Libya. The strains were cultivated in minimal salt medium with 200 mg/l phenol concentration as the sole carbon source. Bacterial growth was measured by the optical density at (OD600 nm) and phenol percentage. Among all strains used *Pseudomonas aeruginosa* was found the best strain has phenol removal 88% within 24h at37 C in the initial concentration of phenol (200 mg/l) and 97% within 48h. The local isolated strain can lead to a good application for bioremediation of pollutants phenolic compounds.

Keywords: Biodegradation; Phenol; Industrial Wastewater; *Pseudomonas aeruginosa*

Introduction

Phenol compounds are considered as environmental pollutant, generally found in the wastewater and various industrial processes such as petrochemical, oil refining manufacturing and pharmaceutical industries H. Movahedyan., *et al*. [1]. Additionally, can be found in the ground water. Phenol and its derivatives have been categorized as toxic compounds to human and environment Bhavan., *et al*. [2]. The removal of such organic compounds from polluted environment can be achieved by different ways physically, chemically, and biologically. Bioremediation techniques economically and environmentally are the best to get rid of phenol. There are many studies used microorganisms for phenol removing and have been proved to eliminate phenol pollution: S. Chakraborty., *et al*. [3]. In the present study we investigate indigenous phenol biodegradation by local strains of bacteria to determine their ability and potential levels.

Materials and Methods

Sample collection

The contaminated soil samples with crude oil were collected from three different places in Alzawia oil refining company, industry area, which located 45 km west of Tripoli, the samples were collected in sterile bags and kept at 4 C, then transferred to the laboratory.

Isolation and identification

Isolation of bacteria was done by using serial dilution method, the serial dilution was made to reduce the cells in the samples. 10 gm of contaminated soil was added to 90 ml of nutrient broth and then incubated at 37C with shaking (170 rpm for 24h) Bhavna., *et al*. [2]. o.1 ml was plated onto mineral salt agar medium complemented with 200 mg/l phenol concentration as sole of carbon source, the

Plates were incubated aerobically at 37C and pH 7.2 until colonies have appeared. The isolates were screened macroscopically to check the morphology and size of each colony. biochemically we used standard biochemical assays according to the scheme Berge's manual and all results confirmed by Analytical Profile Index 20(A PI 20) system technique.

Preparation of inoculums

By loop pickup selected bacteria isolates and inoculated separately in individual Erlenmeyer flask containing nutrient broth medium and incubated for 24h at 37C, in the rotary shaker at 170 rpm. All isolates were centrifuged at 6000 rpm for 10 min. Followed by discarding supernatant and cells were re-suspended in normal saline. Microbial growth for each strain measured by using spectrophotometer at 600 nm (OD600): R. Shawabeh., *et al*. [4]. Each strain (0.5 mcf or $1.5x10^8$ cfu) Mc Farland.

Screening of phenol utilizing bacteria

10 ml of each strain (0.5 mcf) was inoculated in 500 ml flask containing 240 ml (MSM), 2.25 g KH_2PO_4, 0.1g NaCl, 2.75g K_2HPO_4, 10 g $(NH_4)2SO_4$, 0.2 g $MgCl_2.6H_2O$, 0.02 g $FeCl_3.6H_2O$ and 0.01 g $CaCl_2$ and supplemented with 200 mg/l phenol as carbon source: H. Movahedyan., *et al*. (2009). All flasks were incubated in rotary shaker at 170 rpm for 5 days. Samples were collected every 24h to monitor the bacterial growth by measuring the optical density at 600 nm, whereas biodegradation rate of phenol concentration was measured by spectrophotometer at 470 nm (palintest 8000), phenol reacts with 4-amino-antipyrine in the presence of ferricynide ion to form a red color.

Result and Discussion

In this study we used nine different local bacterial strains named S0 - S8 to determine their ability of phenol degradation at optimum pH 7.2 and incubation temperature 37C with fixed phenol concentration 200 ppm. The phenol performance of all strains were evaluated by measuring the degradation rate and optical density every 24h. The isolated strains showed that the fastest growing strain during 24h was S0 which identified and found *pseudomonas aeruginosa* bacteria as showing in table (1), this strain gives highly phenol removal ability, about 88% within 24h at 37 ◦ C in the initial phenol concentration 200 mg/l, and 97% within 48h comparing to other strains used in this study, and phenol degradation resulted in increasing of cell biomass as showing in figure 1. The remaining five strains *Bacillus* spp., *Breviobacillus* spp., *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. exhibited good degradation rate after 48h meaning that more time is required for phenol degradation as showing in figure 2. However, other strains (S3, S4, S6) found have no phenol degradation ability even after 5 days incubation. Our findings are in the same concept with Mitra Shourian [5]. Mitra and his colleagues suggested that all members of the genus tested in their study have the ability for good phenol degradation [6].

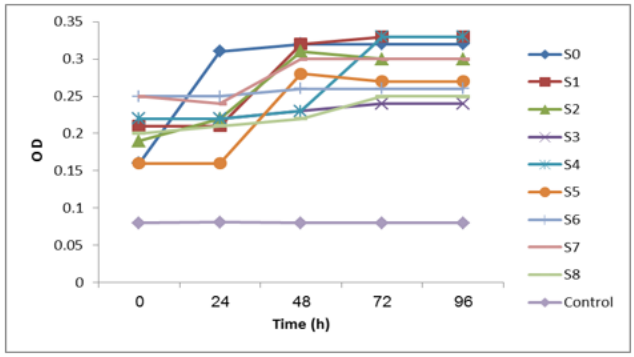

Figure 1: Growth pattern of bacteria in (MSM).

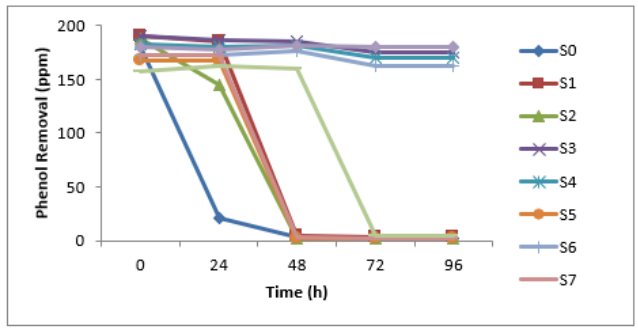

Figure 2: Potential of phenol biodegradation by bacterial strain at 200 (ppm).

Ps. Aeruginosa	NO_3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLUa	ARAa
	+	-	-	+	-	-	+	-	+	-
	MNEa	MANa	NAGa	MALa	GNTa	CAPa	ADIa	MLTa	CITa	PACa
	-	+	+	-	+	+	+	+	+	-

Table 1: Biochemical test of identified *pseudomonas aeruginosa* (API20NE).

Conclusion

Our findings suggest that phenolic contaminated wastewater can be treated by using the local strain, *pseudomonas aeruginosa*, to remove phenol compounds. Moreover, some other parameters such as PH, temperature, and concentration of phenol should be taken in consideration when biological treatment used.

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