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Biodegradation of Crude Oil by *Kocuria* sp.

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Abstract

Oil fields are not uniformly dispensed on the world's map, but are limited in several areas such as Persian Gulf region. Frequent international transport by tankers and shipping accidents makes the oil and its products a most important pollutant of ecosystems. After these accidents, different methods have been developed for clean-up of the polluted areas, out of which bioremediation has attracted highest attention. The present paper describes the crude oil degradation by bacteria *Kocuria sp*. 27/1, isolated from contaminated wastewater by oil hydrocarbons. This bacteria was used for inoculation of mineral salt medium containing 500; 1000; 2000; and 4000 ppm of crude oil (v/v). Incubation was performed in orbital shaker (250 rpm) at 30°C for 7 days. The optical density of the liquid culture and final oil concentration were measured. Results showed that *Kocuria sp*. 27/1 was capable of crude oil degradation. Throughout the experiment, the optical density was rapidly increased comparing to the control. The results obtained by this research showed that *Kocuria sp*. 27/1 can potentially be used for application in crude oil biodegradation of natural environments.

Key words: biodegradation, bioremediation, crude oil, microorganisms, oil hydrocarbons-contaminated waters

1. Introduction

Crude oil is a liquid mixture of thousands hydrocarbons and nonhydrocarbon compounds. For industries and daily life, crude oil products are important source of energy [1]. However, the development of oil industry usually leads to the environment pollution [2] during the production, transport and storage of final crude oil [1].

Because of harmful biological effects of crude oil pollutants on marine and soil ecosystems, mechanical, physical or chemical methods are usually proposed for removal of these pollutants. Unfortunately, these technologies are costly and can lead to incomplete degradation of pollutants [3].

Bioremediation as a technique which uses microorganisms for pollutants removal from the environment [4], is very important in treating crude oil pollutants. In comparison to the other techniques, bioremediation has shown to be beneficial in many ways especially regarding its high efficiency and low cost [5, 6]. The results of experiments in laboratory conditions and field researches, demonstrated the practicability of bioremediation technologies in cleaning of oil-polluted environments [2].

Several studies conducted in recent years have investigated bacterial potential to use oil hydrocarbons as a unique carbon and energy source [7, 8, 9]. These bacteria, such as species from genera *Sphingomonas*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Rhodococcus* etc. tolerate different oil hydrocarbons concentrations and are capable of their degradation [10].

Because of enormous quantity of crude oil that is being introduced into the different ecosystems and its relatively slow natural attenuation [11], development of bacterial strains with novel biodegradation capabilities may be suitable for microbiological control of crude oil-polluted environments [12]. The aim of this paper is to show the potential of bacterial strain *Kocuria* sp. isolated from oil hydrocarbons-contaminated wastewaters in degrading crude oil in laboratory conditions.

2. Material and Methods

Three bacterial strains *Kocuria* sp. 27/1, used in this research, were isolated from oil hydrocarbons-contaminated wastewater of "API separator" in Oil Refinery (Pancevo, Serbia) and previously described as MTBE-degrading strains [13].

The culturing of bacterial strains was performed in flasks containing liquid mineral medium [14] supplemented with 0.5; 1.0; 2.0; and 4.0 % (v/v) of light crude oil. Flasks were incubated on rotary shaker at 120 rpm and 28°C in dark. Flasks with same crude oil-containing medium without bacterial cultures were used as a control. All experiments were conducted in three replication.

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Optical density (OD_{550}) of the liquid cultures was determined at time zero, and also after 1; 3; 5; and 7 days of incubation, using T70 UV/VIS spectrometer (PG Instruments) and 0.5-cm cuvettes. The residual crude oil was extracted after 7 days of incubation by *n*-hexane following the previously described method [15].

3. Results and Discussions

Biodegradation is one of the most frequently applied methods for oil hydrocarbons removal from the environment [16]. In this removal process different organisms, as well as consortia, are involved [17]. The role of microorganisms in degradation of oil hydrocarbons is well described [18, 19]. Degradation ability of *Kocuria* species was previously described during the biodegradation of crude oil [20], diesel oil [21] and some BTEX compounds [22].

Pure culture of *Kocuria* sp. 27/1 was capable to grow in light crude oil-containing liquid medium (table 2). Growth rate of bacteria was controlled by time of sampling and initial crude oil concentration (Table 1).

Table 1. Optical density (OD_{550}) of liquid cultures

BS*	COC**	Sampling dynamics				
		0h	1d	3d	5d	7d
Kocuria sp. 27/1	500	1.365	1.313	1.319	1.467	1.504
	1000	1.403	1.409	1.459	1.651	1.872
	2000	1.457	1.394	1.269	1.306	1.521
	4000	1.537	1.549	1.410	1.637	1.594
Control	500	0.026	0.020	0.011	0.018	0.017
	1000	0.044	0.029	0.012	0.017	0.016
	2000	0.126	0.041	0.030	0.041	0.039
	4000	0.059	0.046	0.042	0.041	0.038

* Bacterial Strain (BS)
** Crude Oil Concentration (COC) in ppm

The presented results (Table 1) show that the growth of selected strain was low. Slow growth of bacteria during the crude oil degradation was also observed in previous investigations [23]. During the degradation of crude oil by *Kocuria* sp. 27/1, rapid changes of optical density in initial degradation phases (day 0 to day 1) were not observed. Rapid increase of optical density was observed after 3 days in all treatments. At the end of incubation period, highest values of optical density in all treatments were observed (except in treatment with highest initial crude oil concentration). The similar increase of optical density during oil degradation was noticed previously [24, 25].

This research has shown that pure cultures of *Kocuria* sp. 27/1 were capable of light crude oil biodegradation. Degradation rate of light crude oil depend on the initial oil concentration. The presented results (Table 2) illustrate changes in crude oil concentrations after 7 days in darkness.

Table 2. Degradation of light crude oil by bacterial strains

Bacterial strain	Crude oil concentration (ppm)		Degradation rate (%)
	Time zero	After 7 days	
Kocuria sp. 27/1	500	402	19.6
	1000	760	24.0
	2000	543	72.8
	4000	1413	64.7
Control	500	478	4.4
	1000	944	5.6
	2000	1921	3.9
	4000	3784	5.4

The obtained results suggest that *Kocuria* sp. shows different efficiency during degradation of low initial crude oil concentrations. Upgrade of oil concentration stimulated the biodegradation efficiency, which may be linked with Michaels Menten theory of enzyme kinetics, where upgrade in substrate concentration can stimulate enzyme activity, i.e. biodegradation capability [26]. Comparing to all inoculated treatments, crude oil depletion in treatment representing abiotic control was insignificant (Table 2).

4. Conclusion

The results of this study showed that the initial amount of crude oil influences the bacterial growth and biodegradation ability. Strain *Kocuria* sp. 27/1 was capable of using crude oil as a sole carbon and energy source. Based on the biodegradation data, this bacteria has the potential to be used in the partial bioremediation of crude oil-contaminated environments. However, more studies need to be conducted for further improvements of biodegradation process.

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