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Research note

Effect of Electrokinetic on Biodegradation of Fluorene and Phenanthrene in Soil

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ABSTRACT

Bioremediation of soil contaminated by polycyclic aromatic hydrocarbons (PAHs) was studied using Bacillus subtilis DSMZ 3256 (B.subtilis) strains. The effect of electrokinetics on biodegradation of PAH was investigated. Fluorene and phenanthrene as selected PAHs were mixed with soil. The bioremediation experiments were initially performed at 30°C and different humidities. The biodegradation percentages of fluorene and phenanthrene after 7 days at 40% relative humidity were 12.2 and 11.9%, respectively. The effects of electrokinetics on this process were studied at different current densities. It was found that the electrokinetic can accelerate the biodesulfurization rate. According to the results, the removal percentages of fluorene and phenanthrene after 4 days under current density of 1.82 mA/cm^2 were 39.4 and 37.2, respectively.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a major group of environmental contaminants. These organic compounds consist of two or more aromatic rings in various structural configurations [1]. The removal of PAHs is of great importance due to their toxic, mutagenic, and carcinogenic properties [2]. PAHs are hydrophobic compounds and generally exist as colorless solids with a, pale yellow or white color [3]. The hydrophobicity of a PAH is increased by increasing the aromatic part in its structures that reduces the solubility in water [4].

PAHs, which are found in crude oil, creosote, and coal tar, can enter into the soil, water, and air as a result of industrial activities such as oil and gas processing. Removal of PAHs from soil is difficult as these chemicals are persistent in the soil. Bioremediation is a suitable method for complete removal or destruction of contaminants in the soil. This method uses living organisms, mainly microorganisms, to degrade the environmental contaminants into less toxic forms [5]. The technology is cost effective and environmentally

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friendly. As PAHs are biodegradable [6,7], bioremediation has been studied extensively for removal of these chemicals from polluted soils. The literature review reveals that a number of microorganisms (bacteria, fungi, and algae) are capable of degrading PAHs [8]. The bioremediation process can take place under both aerobic and anaerobic conditions. As Lu *et al*. [9] have mentioned, microorganisms play an important role in bioremediation of PAH-contaminated soils. Various bacteria have been recognized for biodegradation of PAHs with common names of Pseudomonas [10], Rhodococcus [11], Sphingomonas [12], Polaromonas [13], Janibacter [14], some thermophilic bacteria of Nocardia [15], Bacillus [16], etc. According to literature, the bacillus and rhodococcus are the only strains which can remove these compounds from the soils and sediments [7]. A brief discussion on bioremediation of PAH and the factors affecting the biodegradation rate have been reviewed by Lu *et al*. [9]. There are several influencing factors on the process efficiency such as pH, temperature, and nutrition while adding surfactants and biostimulation enhance the degradation rate of PAHs [17]. On the other hand, the electrokinetic (EK) phenomena stimulate the degradation rate of contaminated soils by the microorganisms. In this technology, a direct electrical current is introduced across polluted soil through inert electrodes [18]. EK and bioremediation supply nutrients, electron donors/acceptors, and bacteria to the soil uniformly and rapidly [18,19]. Heavy metals and organic chemicals may be removed by this method [20,21]. The EK-bioremediation may be performed in situ and is particularly effective for soils with low permeabilities [22].

The electrical current leads to migration of contaminants via electro osmosis, electro migration, and electrophoresis [23]. The electrical currents enhance the microbial activity. This technique has been extended for removal of organic compounds from contaminated soil in recent years [23-27]. Wick *et al*. [24] reviewed the fundamental interactions of electro-bioremediation of hydrophobic organic soil-contaminants. They studied the influence of direct current on microbial physiology and the physico-chemistry of organism–soil and organism–compound interactions. The removal of pentadecane from kaolinite and diesel from contaminated soil using a bacterial consortium consisting of several Pseudomonas have been studied by Kim *et al* [18,22]. As applying EK-bioremediation process for removal of PAHs from the soil is new, there are only a few researches in literature that mention this subject. Meanwhile experimental data are needed to apply this technology for biodegradation of PAH in the soil.

Shi *et al* [25-27] determined the EK-bioremediation of PAH in bench scale aquifers. The effects of different parameter on the degradation of fluorine were investigated [27]. Xu *et al*. [28] studied the bioremediation of phenanthrene in soil with EK. They found that 8% of phenanthrene in soil is degraded after 20 days and in order to remove more phenanthrene EK should be added.

As the biodegradation of PAH in the soil is a slow process, applying EK to accelerate the process is of great importance.

This research is the continuation of our previous research to investigate the effect of EK on biodegradation of organic compounds [23,29]. The effect of EK on biodegradation of PAH (fluorene and phenanthrene) in contaminated soil are studied. The applied microorganisms are the strain of bacillus subtilis DSMZ 3256. This strain produces a biosurfactant, namely surfactin.

2. Materials & methods

2.1. Chemicals

Peptone, yeast extracts, agar, nutrient broth, acetonitrile, methanol, phenanthrene, and fluorene were purchased from Merck Company. Bacillus subtilis 3256 was prepared from DSMZ in Germany. The double distilled deionized water was used in the experiments.

2.2. Microorganism & culture conditions

Bacillus subtilis 3256 was cultivated in nutrient agar and transferred into the LB media. The LB media contained 10 g/l peptone, 5 g/l yeast extract, and 10 g/l NaCl in water. According to the experimental results, the Bacillus subtilis attains the maximum growth after 24h. The media was then transferred into a 250-mL flask and inserted into a shaker incubator (Kuehner Lab-Therm) at 30°C and 200 rpm for 24 h.

The growth of microorganism was determined by optical density measurements at 600 nm using a UV spectrophotometer (JENWAY 6715, Germany).

2.3. Analytical procedure

Phenanthrene and fluorene concentrations were analyzed by HPLC (Shimadzu LC-8A model) with C_{18} column and a UV detector. The mobile phase was the solution of acetonitrile and deionized water (3:2, v/v). The phenanthrene and fluorene concentrations were determined by comparison with the standard solution prepared by dissolving the known amount of phenanthrene and fluorene in acetone. The standard solution was then mixed with the dry soil and water. No microorganism existed in the sample while the other samples consisted of the culture media and microorganism. Phenanthrene and fluorene were extracted from each sample and their concentrations were determined. In order to extract the phenanthrene and fluorene from the soil, a known concentration of methanol was added to each sample and mixed for 15 min. The soil mixtures were then inserted in an ultrasonic bath (Bandelin electoconic, sonorex, RK103H) for 30 min [30]. Subsequently, a float suspend solution was taken and centrifuged (SIGMA, model 3K30) at a rate of 6000 rpm for 10 min to eliminate the soil particles in the solution.
The population of bacteria in the soil and bioreactor was measured according to the Varon & Peterson method [31].

2.4. Experimental procedure

The experimental setup applied in the present research is similar to that in our previous research with a brief modification as shown in Fig. 1 [23]. It consisted of a horizontal rectangular Plexiglas container (30×11×7cm) for the contaminated soil. The soil was autoclaved and mixed up with known concentrations of phenanthrene and fluorene. Two stainless steel electrode chambers (3×5×5cm) were inserted in both sides of the soil container 2 cm apart from the container walls. The

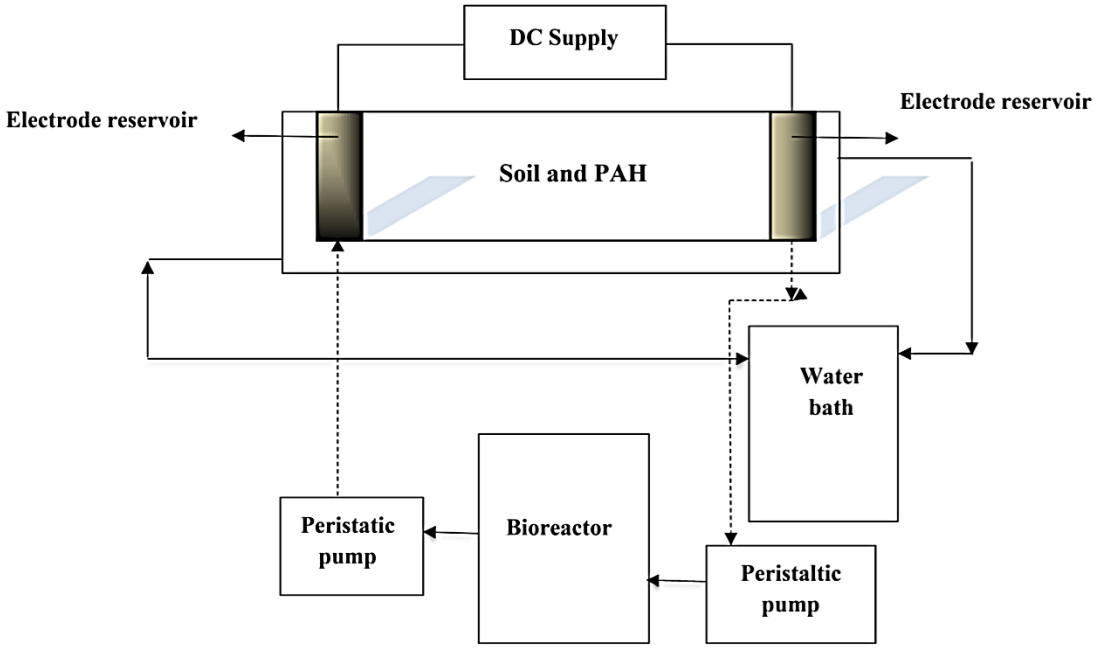

Figure 1. Experimental set up for EK- bioremediation of fluorene and phenanthrene.

soil container was separated from the electrode chambers by a porous Plexiglas sheet covered by a paper filter.

The cultivation media were charged/ discharged from the anode and cathode respectively using two peristaltic pumps (Heidolph, PD5006, Germany). The injection of cultivation media regulated the pH of soil and prepared suitable conditions for microorganism growth. The temperature of the setup was set to 30˚C using a circulator (Julabo, FP50, Germany). The pH of the soil was determined with a pH meter (Metrohm, model 627). As the generation of oxygen in the anode significantly reduces the pH of the soil, the pH was controlled by injection of a buffer solution. The duration time of experiments for EK-bioremediation was 2-7 days. In order to measure the phenanthrene and fluorene concentrations, the EK cell was divided into four separated sections. The concentrations of phenanthrene and fluorene in each section were measured and the average concentrations of four sections were reported.

The phenanthrene and fluorene bioremediation experiments were performed in 60 mL vial glasses. Before each experiment, 5 mg phenanthrene and 5 mg fluorene were dissolved in acetone and mixed with 20 g soil. The mixture was autoclaved in an incubator at 121˚C for 15 min where the acetone was vaporized. The humidity of the soil was controlled by addition of deionized water to the mixture. This mixture was named as the blank sample. In a second vial, the media culture of the studied microorganism was added to the mixture. Both vials were inserted in an incubator for different experimental times (2-6 days) and the concentrations of phenanthrene and fluorene were determined.

3. Results & discussion

The removal percentage of fluorene and phenanthrene in biodegradation experiments by *Bacillus subtilis* at different humidity at 30˚C after seven days

is reported in Table 1. The initial concentrations of fluorene and phenanthrene were 0.5 mg/g soil. Each experiment was repeated three times and the average values were reported.

As shown in Table 1, the best humidity range for biodegradation of fluorene and phenanthrene are 40 and 45, respectively. The results of biodegradation process in different days, performed at 40 and 45% relative humidity, are reported in Table 2.

Table 2 shows that the maximum removals of fluorene and phenanthrene are observed after 5 days and the optimum humidity is 40%. This behavior was also observed in other humidity. According to the experimental results the removal

Table 1
Removal percentage of fluorene and phenanthrene at different humidity after seven days using Bacillus subtilis strain.

Humidity	Fluorene removal %	Phenanthrene removal %
30	11.4	11
35	11.7	11.4
40	12.2	11.9
45	12.7	11.5
50	12	11.2
55	11.5	10.6
60	11	10.2

Table 2
Removal percents in biodegradation of fluorene and phenanthrene in different days using Bacillus subtilis strain.

day	Humidity= 40%		Humidity= 45%	
	Fluorene removal %	Phenanthrene removal %	Fluorene removal %	Phenanthrene removal %
1	0	0	0	0
2	12	11.3	11.6	11
3	12.8	12.2	12.3	11.8
4	13.4	13	13	12.6
5	14.7	13.5	14.1	13
6	13.5	12.4	13.1	12
7	12.2	11.9	12.7	11.5

percentage for fluorene and phenanthrene is very low and biodegradation of these materials needs a great deal of time. However, this amount is more than the 8 removal percentage for phenanthrene, reported by Xu *et al*. [28]

The effect of EK on bioremediation of fluorene and phenanthrene in soil was studied by applying different current densities. The experimental results are given in Tables 3-4.

The data in Table 3 show that the removal percentage of fluorene and phenanthrene are increased up to 4 days and removal percentages are then decreased.

According to Table 4, the optimum current density range for removal of fluorene and phenanthrene is within 1.82-2.42 mA/cm^2. Applying an optimum current density increases the growth rate and facilitates the transfer of Microorganisms [24] while a high current density reduces the microorganism's activities [27]. The experimental data indicated the EK- bioremediation significantly reduce the removal time for fluorene and phenanthrene. Similar

Table 3
Removal percentage of fluorene and phenanthrene in different days for EK-bioremediation using bacillus subtilis strain at current density of 0.6 mA/cm^2 and temperature 30˚C.

Day	Fluorene removal%	Phenanthrene removal %
2	16.7	14.2
3	20.7	18.8
4	35.7	33.4
5	32.8	30.6
6	25.2	23.4
7	23.6	22.8

Table 4
Effect of current density on removal percentage of fluorene and phenanthrene in EK-bioremediation after 4 days using Bacillus subtilis strain at 30˚C.

Current density (mA/cm^2)	Fluorene removal %	Phenanthrene removal %
0.6	33.7	32.4
1.21	35.6	33.3
1.82	39.4	37.2
2.42	37.2	36.2
2.85	36	35
3.42	35.2	33.4

behavior is observed for biodegradation of other types of organic contaminants [22,23,29]. The electric current stimulates the microorganism to degrade the organic contaminants [27] and this phenomenon reduces the time for biodegradation of fluorene and phenanthrene.

The gradient of fluorene and phenanthrene removal from the anode to the cathode is shown in Fig. 2 versus the normalized distance. The figure reveals that the anode region has the highest removal percentage of fluorene and phenanthrene, and the removal percentage decreases from the anode to the cathode. This may be due to the electrical affinity between the anode and the microorganism. Due to electrolysis of water in the anode, the generated oxygen promotes the microorganism activities. This behavior was also observed by Kim *et al*. [22].

The populations of the microorganism after 7 days EK-bioremediation experiment were measured in three points between the anode and the cathode (at the anode point, a point between anode and cathode, and at the cathode) where the current density was set to 1.82 $mA.cm^{-2}$ as represented in Fig. 3. The figure shows that the microorganism populations are

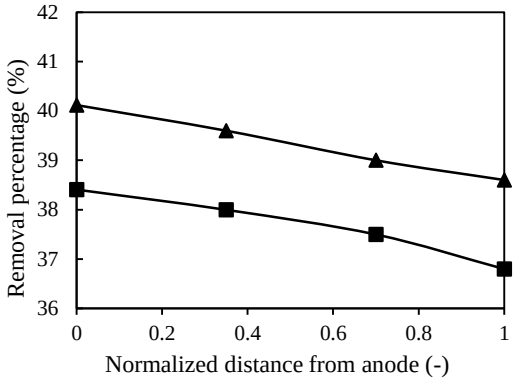

Figure 2. Gradient of fluorene and phenanthrene removal percentages from anode to cathode at current density of 1.82 mA/cm^2 and 30˚C; ▲, fluorene; ■, phenanthrene.

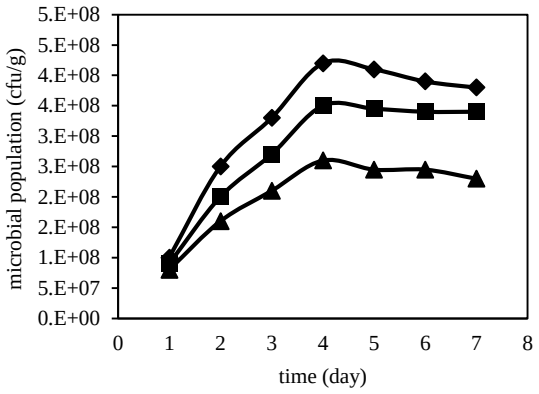

Figure 3. Population of *Bacillus subtilis* microorganism for removal of fluorene and phenanthrene in current density of 1.82 mA/cm^2 and 30°C; ♦, anode; ■, between anode and cathode; ▲, cathode.

increased up to 4 days and then decreased. The highest population of microorganisms is in the anode point. This is in good agreement with the results of Kim *et al*. [18].

4. Conclusions

Biodegradation of PAH is limited by slow rate of bioremediation. The combination of electrokinetic and bioremediation accelerates the biodegradation process. In this research, the EK– bioremediation technique was proposed for biodegradation of fluorene and phenanthrene in the soil

for the first time. The experimental results revealed that the removal percentages for bioremediation of fluorene and phenanthrene are 12.2 and 11.9 at 40% relative humidity after 7 days, respectively. However, the combination of EK–bioremediation in 4 days with the current density of 1.82 mA/cm^2 enhanced the removal of fluorene and phenanthrene up to 39.4% and 37.2%, respectively. Comparison of those processes showed that the electrokinetics significantly reduces the time for biodegradation of fluorene and phenanthrene. It was found that the electrokinetic stimulates the microorganisms' activities and increases the biodegradation of PAHs. Due to the generation of oxygen, the population and activity of the microorganisms are increased in the electrokinetic process. EK–bioremediation can be applied as a promising hybrid technology for degradation of fluorene and phenanthrene.

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