Effect of Electrokinetic on Bioremediation of Disulfide Oil Contaminated Soil

Mohammad Asgari[2], Babak Mokhtarani[1,*], Ahmad Ataei[2], Kurosh Tabar Heidar[1]

1. Chemistry and Chemical Engineering Research Center of Iran, P.O. Box 14335-186, Tehran, Iran
2. Department of Chemical Engineering, University of ShahidBahonar Kerman, P.O. Box 76169113, Kerman, Iran

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* Corresponding author:
Email: *mokhtaranib@ccerci.ac.ir*
Tel.: *+98 21 44580751*
Fax: *+98 21 44580781*

ABSTRACT

In this study, the removal of disulfide oil (DSO) from contaminated soil was studied by the bioremediation method and the influence of electrokinetic on the bioremediation process was investigated. The *bacillus subtilis* strain was used in the bioremediation process. The effects of humidity, time and DSO concentration in soil was studied. The experimental results for the bioremediation of DSO showed that the removal percent of DSO reached to 67% at 30^{o}C and 26% humidity after six days. For the electrokinetic bioremediation (EK – bioremediation) experiments, the optimal current density was determined and several experiments at different times were performed. The concentration of DSO and the humidity was 20 μL/g. soil and 26% respectively. The DSO removal percent reached to 61% after two days. The maximum DSO concentration in soil was 50 μL/ g. soil. The comparison of the EK – bioremediation with the bioremediation method shows that the EK – bioremediation significantly reduces the biodegradation time for DSO.

1. Introduction

The removal of organic sulfur compounds from fossil fuels is a major problem in oil and gas industries. These compounds produce so much contamination for the environment. A major group of the organic sulfur compounds is mercaptan. The removal of mercaptans from gas or petroleum hydrocarbon streams is known as sweetening process. The Merox unit removes the mercaptans compounds from oil or gas streams. The Merox process is an efficient and economical catalytic process developed for the chemical treatment of petroleum distillates for the removal of sulfur. The following main reactions relevant to this treatment for the removal of mercaptans occur in this unit:

$$NaOH + RSH \rightarrow NaSR + H_2S \quad (1)$$

$$4NaSR + O_2 + 2H_2O \rightarrow 2RSSR + 4NaOH \quad (2)$$

The organic dialkyl disulfides (RSSR) are the byproduct of this process. The mixtures of the dialkyl disulfides are called disulfide oil (DSO), a waste whose disposal from demercaptanization units is still an unresolved problem [1]. It is not practical to dispose DSO and its storage creates a safety hazard. The production of DSO all over the world continuously grows. DSO is a mixture of dimethyl disulfide (DMDS), diethyl disulfide (DEDS), methyl ethyl disulfide (MEDS). It is an extremely flammable substance with a relatively high vapor pressure and low water solubility. At room temperature, the material exists as a yellow liquid with an extremely foul and obnoxious odor.

The leakage of DSO from the refineries to the soil and water generates a lot of environmental problems. Bioremediation is a suitable method for the contaminated soils. This technology is cost effective and environmentally friendly. The biodegradation of DSO in soil by microorganism has not been widely investigated. The literature surveys reveal that a few researches have been done in this area. Kanagawa and Mikami have studied the removal of methanethiol, dimethyl sulfide (DMS), DMDS and hydrogen sulfide from contaminated air by *ThiobacillusThioparus TK-m* [2]. The biodegradation of hydrogen sulfide, methanethiol, DMS and DMDS with a fungus have been studied by Phae and Shoda [3].

Lee et al. [4] have investigated the ultraviolet photo dissociation of DMDS and dimethyl sulfide (DMS). Reichert et al. [5] isolated *Pseudnocardia*which use DMDS as the source of carbon.

Ito et al. [6] studied the degradation of DMDS by *Pseodomonasfluorescens* strain 76. They have shown that the *strain76* has the highest growth rate on glucose/DMDS medium. EsmaeiliTaheri et al. [7] studied the bioremediation of DSO in soil. They have examined the impact of seven strains on the removal of DSO from contaminated soil and found that, the strains of *Rhodococcus* and *Paenibacillus* have a high potential for biodegradation of DSO. The DSO removal efficiency was 19.3% and 24.3% after 48 h for *Rhodococcus* and *Paenibacillus* respectively.

According to the literature, bioremediation is a suitable technology for the removal of pollutant from the contaminated soil; however, the removal efficiency for DSO is low. In order to increase the removal efficiency, the selection of a

suitable microorganism has a great importance. Moreover, the combination of the bioremediation technique with other technologies may improve the removal of the DSO from soil. The combination of the bioremediation with electrokinetic may increase the biodegradation of DSO. EK is an emerging technology that relies on the application of a low-intensity, direct current through the soil to separate and extract organic contaminants from unsaturated soil, sludge, and sediment [8-9]. The combination of EK and bioremediation, namely EK- bioremediation, can uniformly and rapidly supply nutrients, electron donors/acceptors, and bacteria to soil [10-11]. This process was applied for a wide range of pollutants in soil such as heavy metals and organic chemicals [12-13]. The main advantages of EK-bioremediation are that this process can be performed in situ. It is also particularly effective for soils with low permeability [14]. In EK, the electrical current is introduced into the soil. The introduced electric current leads to the migration of contaminants via electro osmosis, electro migration, and electrophoresis [14]. The electrical currents enhance the microbial activity. The application of this technique for the removal of organic chemicals from contaminated soil has been investigated in recent years. Kim et al. [10] studied the removal of pentadecane from kaolinite using a bacterial consortium consisting of several *Pseudomonas*.

The removal of diesel from contaminated soil with this method was also investigated by Kim et al. [14]. They found that the soil pH and direct electric current have significant effects on microbial activity.

Wick et al. [15] reviewed the fundamental interaction on Electro-bioremediation of hydrophobic organic soil-contaminants. They studied the influence of direct current on the microbial physiology and the physico-chemistry of organism–soil and organism–compound interactions. Shi et al. [16-18] determined the EK- bioremediation of poly aromatic hydrocarbon (PAH) in bench scale aquifers. They found that electrokinetic is a valuable mechanism for PAH degrading bacteria in soil and sediments. In this research, the removals of DSO from contaminated soil were studied by the bioremediation method and the effect of EK was investigated on the bioremediation process for the first time. The microorganism was the strain of *bacillus subtilis*. According to the literature, the *bacillus* and *rhodococcus* are the only strains which can remove these compounds from soil and sediments [7]. *Bacillus subtilis* produce surfactin. Surfactin is one of the most effective biosurfactants with the ability to reduce the surface tension of water to 27 mN/m at a trace concentration [19-20].

2. Research Method

2.1 Chemicals

Peptone, Yeast extracts, agar, Nutrient broth and Isooctane were purchased from Merck Company and Dodecane was purchased from Fluka. *Bacillus subtilis* 3256 was prepared from DSMZ in Germany. DSO was taken from South Pars Gas Refinery, located in Bushehr province in south of Iran. The double distilled deionized water was used in the experiments.

2.2 Microorganism & culture condition

Bacillus subtilis 3256 was cultivated into nutrient agar in 25 ml LB media. The LB media contains 10 g peptone, 5 g yeast extract and 10 g NaCl in 1 lit water. The LB media was transferred into a 250 ml flask. The flask was inserted

into a shaker incubator (Kuehner Lab-Therm) at 30ºC and 200 rpm for 24 hours. The growth of *Bacillus subtilis* was determined by measuring the optical density at 600nm by using a UV spectrophotometer (JENWAY 6715, Germany). According to the experimental results, after 24 hours, the *Bacillus subtilis* attains to the maximum growth.

For the EK- bioremediation experiments, one liter of the LB media was used as the electrolyte in order to supply the conductivity of the soil.

2.3 Analytical procedure

DSO concentration was analyzed using a Varian CP-3800 gas chromatograph equipped with a capillary column (chrompack CP sil 5CB 30m, inner diameter 0.25mm, film thickness 0.25µm) and a flame ionization detector with Nitrogen as the carrier gas. The temperatures of injector and detector were 250ºC. Temperature programming was 40 ˚C for 4 min and 280˚C for10 min.

The DSO concentration was determined through addition of 1 ml of the standard solution to the two different samples of DSO contaminated soils. The standard solution was prepared by adding 400µl dodecane in 40 ml isooctane. In the first sample, a known concentration of DSO was mixed with dry soil and water. There were no microorganisms in the first sample. Instead of water, the culture media and microorganism were added to the second sample. The extraction of DSO from each of the samples was carried out by the addition of known concentration of the isooctane to the samples. The soil mixtures were inserted in an ultrasonic bath (Bandelinelectoconic, sonorex, RK103H) for 30 min [6]. After that, a float suspend solution was taken and centrifuged (SIGMA, model 3K30) at a rate of 6000 rpm for 10 min to eliminate the soil particle in solution. Finally, 1 ml of the extracted solution of each of the samples was added to a 1 ml of the standard solution and was injected to the gas chromatography. The DSO removal percent was calculated from the difference in concentration of DSO in the first and second samples. The first sample without microorganism shows the actual DSO concentration in soil. The area ratio of DSO's peaks to dodecane's peak was compared for each sample and the removal percent of DSO was calculated. This method prevents any error due to the DSO vaporization during the experiment.

The population of bacteria in soil and bioreactor was measured according to the Varon& Peterson method [21].

2.4 Experimental procedure

The experiments were done in two stages. In the first stage, the bioremediation of DSO in contaminated soil was performed. The experiments for the bioremediation of DSO were studied in the 60 ml vial glass. Before each experiment, the vial glass and the soil were autoclaved at 121˚C for 15 min. A known volume of DSO was added to 20 g of dry soil. The humidity of the soil was adjusted by the addition of deionized water into the mixture. This mixture was the blank sample. In the second vial, the media culture of the *bacillus subtilis* was added to the mixture of the dry soil and DSO. Both vials were inserted in an incubator for different days (2-6 days) and the concentration of DSO was determined.

In the second stage, the EK – bioremediation experiments were performed. The diagram of the experimental set up is shown in Figure 1. After autoclaving of 200 g soil, a known amount of DSO was mixed with the soil. The

soil moisture was increased to 30% by the addition of deionized water. The soil mixture was loaded into the EK test cell. The EK test cell was constructed from pelexi glass (25×5×5 cm) and two porous stainless steel electrodes in electrode reservoir (3×5×5 cm). The soil was separated from electrode reservoir by a porous pelexi glass sheet (0.5×5×5 cm). A paper filter (125 nm) coated the sheet. The cultivation media were circulated using two peristatic pumps. The first pump charged the cultivation media from bioreactor to anode chamber at a definite rate and the second pump discharged it from cathode chamber to bioreactor. This action prevented the sudden change of pH and prepared a suitable condition for microorganism growth. The bioreactor was constructed from double layer glass (height: 34cm, inner diameter: 12cm, outer diameter: 14cm). A directly driven stirrer (SDSS-20 D, Korea) mixed the cultivation media. The temperature of the EK test cell and bioreactor was regulated at 30˚C by a circulator Julabo model (FP50, Germany). The pH of the soil was determined with a pH meter Metrohm model 627. The time length of the EK – bioremediation experiments was 2-7 days. In order to measure the DSO concentration, the EK cell was divided into four 50 g samples. The concentrations of DSO in each sample were measured and the average of the DSO concentration in four compartments was reported.

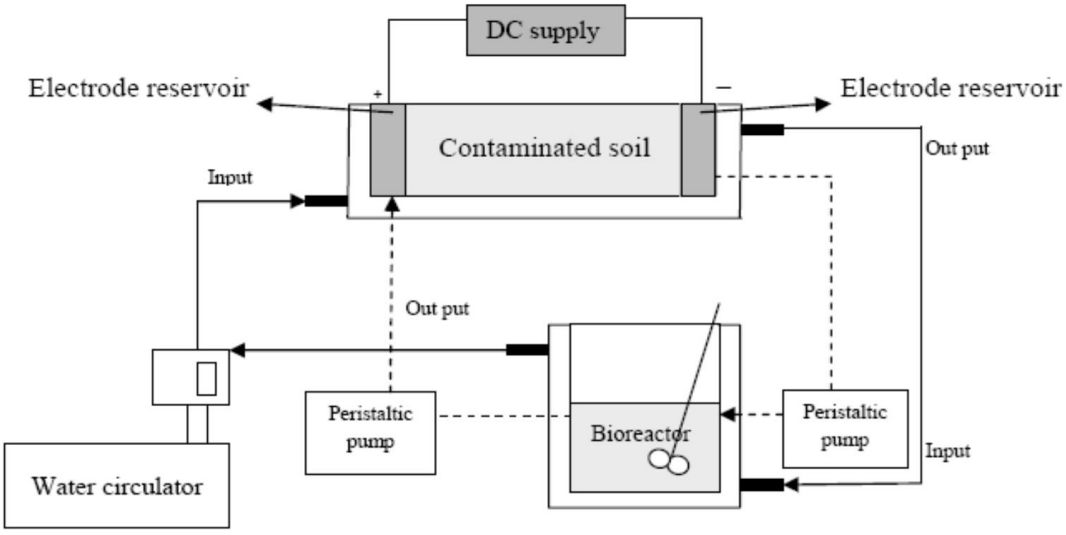

Figure 1. Schematic diagram of EK – bioremediation cell

3. Results and Analysis

The bioremediation of DSO contaminated soil by *bacillus subtilis* was studied in different humidity at 30°C for six days. The concentration of DSO was 20 μL/g. soil. Each experiment was repeated three times and the averages of the humidity were reported. The experimental results are reported in Table 1. As Table 1 shows, the optimum humidity is around between 26-30%.

Table 1. The removal percent of DSO at different humidity

Humidity	DSO removal %
20	58.3
26	67.0
30	66.0
35	61.4

The effect of EK on bioremediation of DSO in soil was studied and the optimum current density was determined. The experiments were performed in the EK – bioremediation cell (Figure 1), the DSO concentration was 20 μL/g. soil and the removal percentages of DSO were measured after two days. As the results reported in Table 2 show, the optimum current density for the removal of DSO is between 1.82 to 2.42 $mA.cm^{-2}$.

Table 2. The effect of current density on the removal of DSO in soil at 30°C

Current density ($mA.cm^{-2}$)	DSO removal %
0.6	48.9
1.21	50.2
1.82	61.8
2.42	61.4
2.85	53.1
3.42	52.0

The DSO removal percent gradient from anode to cathode is shown in Figure 2. In this Figure, the distance between anode and cathode was normal to unity, the pH measurement points were calculated based on this normalization. Based on the Figure, the anode region has the highest removal percent of DSO, and the removal percent decreases from anode to the cathode. This may be due to the electrical attraction between anode and microorganism. The removal percent of DSO increases by raising the current density.

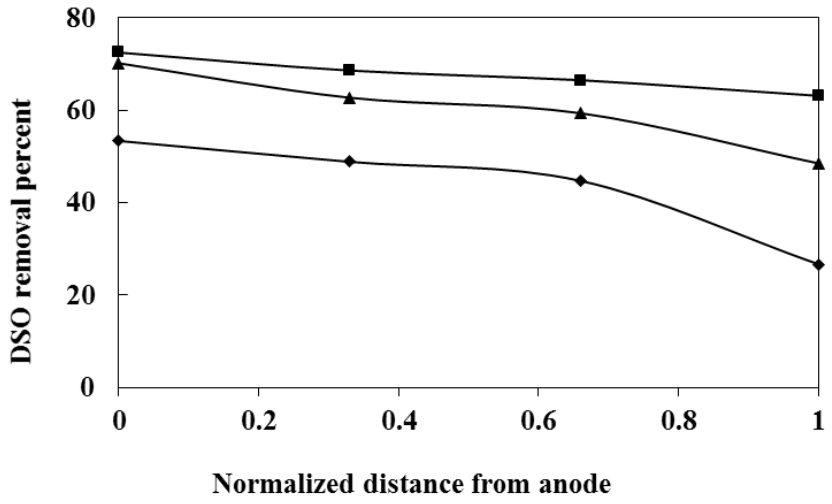

Figure 2. DSO removal percent gradient from anode to cathode in different current densities, ♦, 0.6 $mA.cm^{-2}$; ▲, 2.85 $mA.cm^{-2}$; ■, 2.42 $mA.cm^{-2}$.

Table 3 shows the variation of DSO biodegradation in soil with times for bioremediation and EK – bioremediation at 30°C. The concentration of DSO and the humidity was 20 μL/g. soil and 26% respectively. The current density for the EK – bioremediation process was 2.42mA.cm^{-2}. As Table 3 shows, the EK – bioremediation enhances the biodegradation of DSO. The maximum removal percent of DSO for the bioremediation process was observed after six days, while for the EK – bioremediation method it was reduced to two days. The comparison of the results of this research for the bioremediation method with the results of EsmaeiliTaheri et al. [7] shows that the *bacillus subtilis* strain improves the removal percent of DSO in soil. The maximum removal percent of DSO was reported to be 24.3% after 2 days by EsmaeiliTaheri et al. [7] while in this study a measure of 38.2% was observed.

Table 3. The comparison of DSO biodegradation in soil with times for the methods of bioremediation and EK – bioremediation

Times (day)	DSO removal %	
	Bioremediation	EK - Bioremediation
1	14.3	30.0
2	38.2	61.4
3	40.0	62.0
4	59.7	67.4
5	64.0	62.4
6	67.0	63.9
7	56.6	62.8

In order to determine the maximum concentration of DSO that can be biodegraded, different concentrations of DSO were injected in soil and the removal percent of DSO was determined for the both methods. The removal percent of DSO for bioremediation method and EK- bioremediation were determined after six and two days, respectively. The temperature of experiments was at 30°C and the current density was 2.42 mA.cm^{-2}. The results are reported in Table 4. The removal percent of DSO for both methods were reduced by increasing DSO concentration in soil. The comparison of both methods shows that the EK – bioremediation method enhances the DSO biodegradation. The maximum concentration of DSO in soil was 50 μL/g. soil because for more than this amount, the soil was saturated from DSO and a layer of DSO appeared on the surface of the soil. The removal percent of DSO for the EK – bioremediation method for DSO concentration of 50 μL/g. soil, was 55% after six days.

Table 4. The comparison of DSO removal percent in soil with concentration for the methods of bioremediation and EK – bioremediation

DSO concentration μL/g. soil	DSO removal %	
	Bioremediation (6 day)	EK – Bioremediation (2 day)
20	67.0	61.4
30	58.0	58.0
40	44.0	45.6
50	33.2	36.2 (55)*

* The removal percent for 6 day

The variations of the bacterial population from anode to cathode were measured in four points of the experimental cell with the current densities of 2.85 $mA.cm^{-2}$ during EK – bioremediation. The results are presented in Figure 3. As Figure 3 shows, the anode region has the highest microbial population because of the electrical attraction between anode and bacteria. This result has a good agreement with the results of Kim et al. [10]. By increasing the microbial population, the removal percent of DSO increased up to the fourth days of the experiment (Table 3). After the fourth day, the microbial population decreased and the DSO removal percent reduced.

Control of pH in the EK – bioremediation influences the biodegradation process. The electrical current passes through the soil and the full redox reactions take place. The electrolysis reactions generate an acidic medium at anode and an alkaline medium at cathode. The pH gradients of soil from anode to cathode in EK – bioremediation were measured at different electrical currents after two days in Figure 4. Based on Figure 4, the pH in anode is lower than cathode. The soil remains in neutral pH and complete oxidation-reduction processes take place.

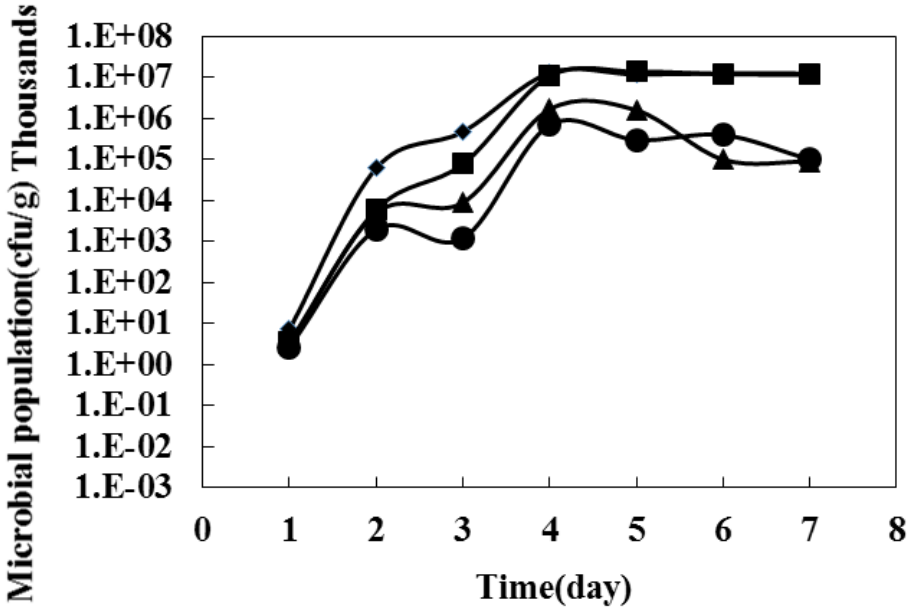

Figure 3. The variation of microbial population in anode (♦), after anode (■), before cathode (▲) and cathode (●) regions of soil during EK – bioremediation with the current densities of 2.85 $mA.cm^{-2}$.

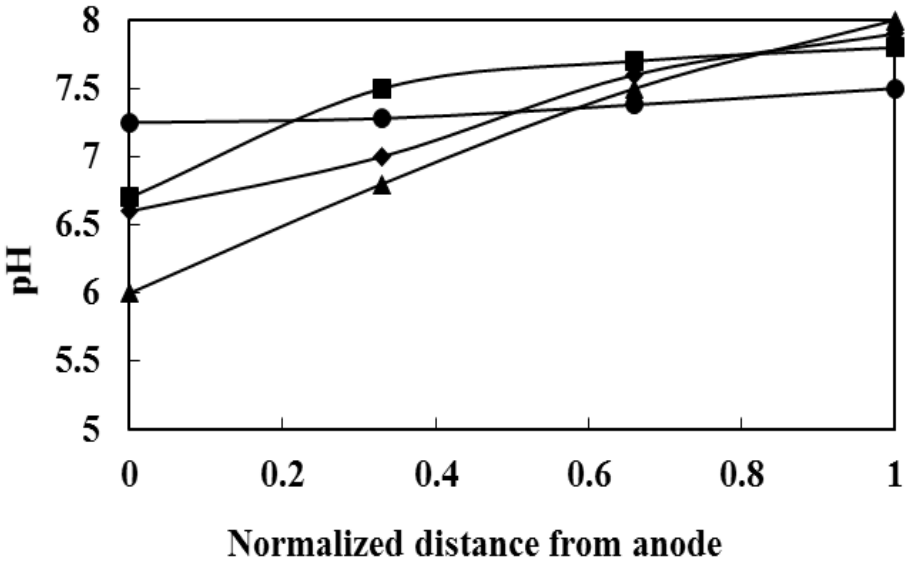

Figure 4. The pH gradient from anode to cathode in different current densities after 2 days during EK – bioremediation process, ♦, 0.6 mA.cm^{-2}; ■, 1.21 mA.cm^{-2}; ▲, 2.42 mA.cm^{-2}; ●, 2.85 mA.cm^{-2}.

4. Conclusion

Biodegradation of DSO contaminated soil was studied by *bacillus subtilis* microorganism. The removal percent of DSO contaminated soil was about 67% at 30^{o}C after six days with DSO initial concentration of 20 μL/g. soil. The effect of the EK – bioremediation on biodegradation of DSO contaminated soil was investigated for the first time. The optimum current density for the removal of DSO in EK – bioremediation process was in the range of 1.82 to 2.42 mA.cm^{-2}. The comparison of bioremediation process and EK – bioremediation for the removal of DSO shows that the EK – bioremediation significantly reduces the time for the biodegradation of DSO. The removal percent of DSO in bioremediation method was about 38.2% while for the EK – bioremediation method it was 61.4% after two days. The maximum DSO concentration in soil was 50 μL/g. soil and the removal percent for bioremediation and EK – bioremediation after six days was 33.2% and 55%, respectively.

Finally, EK – bioremediation could be a promising hybrid technology for the treatment of organic and inorganic contaminant in soil. Furthermore, this method is superior to the conventional bioremediation.

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تأثیر الکتروکینتیک بر زیست پالایی خاک‌های آلوده به دی سولفید اویل

محمد عسگری[۲]، بابک مختارانی[۱،*]، احمد عطایی[۲]، کوروش تبار حیدر[۱]

۱. پژوهشگاه شیمی و مهندسی شیمی ایران، صندوق پستی ۱۸۶-۱۴۳۳۵، تهران، ایران
۲. بخش مهندسی شیمی، دانشگاه شهید باهنر کرمان، صندوق پستی ۷۶۱۶۹۱۱۳، کرمان، ایران

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باسیلوس سابتیلیس

* عهده دار مکاتبات:
رایانامه: *mokhtaranib@ccerci.ac.ir*
تلفن: ۰۲۱ ۴۴۵۸۰۷۵۱ ۹۸+
دورنما: ۰۲۱ ۴۴۵۸۰۷۸۱ ۹۸+

چکیده

در این تحقیق حذف آلودگی دی سولفید اویل از خاک آلوده به آن با استفاده از روش زیست پالایی مورد بررسی قرار گرفت و تاثیر فرآیند الکتروکینتیک بر زیست پالایی به عنوان یک روش جدید پیشنهاد گردید. از گونه *باسیلوس سابتیلیس* به عنوان میکروارگانیسم استفاده شد. و تاثیر پارامترهای رطوبت، زمان و غلظت دی سولفید اویل در خاک در روش زیست پالایی مطالعه گردید. نتایج آزمایشگاهی برای زیست پالایی دی سولفید اویل نشان داد، درصد حذف در دمای ۳۰ درجه سانتیگراد و رطوبت ۲۶٪، بعد از گذشت ۶ روز، به ۶۷٪ خواهد رسید. با استفاده از فرایند الکتروکینتیک - زیست پالایی و دانسیته جریان بهینه آزمایش‌هایی در زمان‌های مختلف و دانسیته جریان بهینه صورت پذیرفت. میزان رطوبت وغلظت دی سولفید اویل به ترتیب، ۲۶٪ و ۲۰ µl /g dry soil بود که در این شرایط درصد حذف دی سولفید اویل طی ۲ روز به ۶۱٪ رسید.مقایسه دو روش زیست پالایی و الکتروکینتیک - زیست پالایی نشان داد، تلفیق دو روش با هم مدت زمان حذف دی سولفید اویل را بطور قابل توجهی افزایش می‌دهد.