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Coupling electrokinetics with microbial biodegradation enhances the removal of cycloparaffinic hydrocarbons in soils

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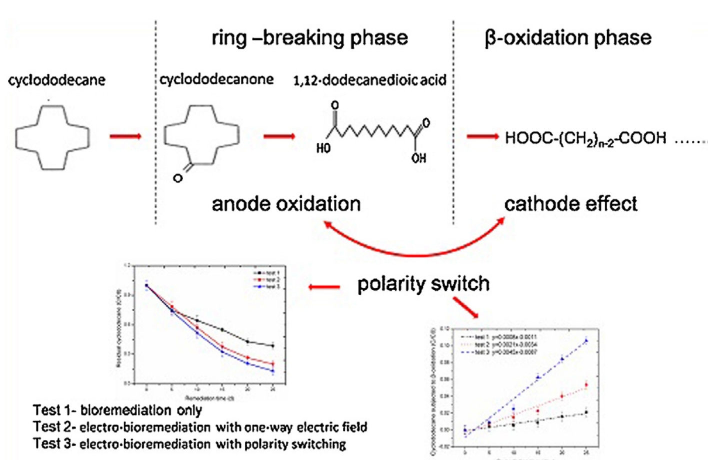
HIGHLIGHTS

- Electro-biodegradation of cycloparaffinic hydrocarbons in soil is established.
- Electric field remarkably increase ring-breaking extent of cyclododecane.
- β-oxidation of intermediate products can be accelerated by electric field.
- Periodically switching polarity may be a preferable technology.
- *Bacillus* sp. and *Arthrobacter* sp. can be domesticated by electric field.

GRAPHICAL ABSTRACT



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ABSTRACT

An innovative approach that couples electrokinetics with microbial degradation to breakdown cycloparaffinic hydrocarbons in soils is described. Soils were spiked with cyclododecane, used as a model pollutant, at approximately 1000 mg kg^{-1}. A mixture of petroleum-utilizing bacteria was added to achieve about 10^6–10^7 CFU g^{-1}. Then, three treatments were applied for 25 days: (1) no electric field, control; (2) a constant voltage gradient of 1.3 V cm^{-1} in one direction; and (3) the same electric field, but with periodical switching of polarity. The degradation pathway of cyclododecane was not changed by the electric field, but the dynamic processes were remarkably enhanced, especially when the electric field was periodically switched. After 25 days, 79.9% and 87.0% of the cyclododecane was degraded in tests 2 and 3, respectively; both much higher than the 61.5% degraded in test 1. Analysis of the intermediate products strongly indicated that the competitive advantage of the electric field was the increase in ring-breaking of cyclododecane, resulting in greater concentrations of linear substances that were more susceptible to microbial attack, that is, β-oxidation. The conditions near the cathode were more favorable for the growth and metabolism of microorganisms, which also enhanced β-oxidation of the linear alkanoic acids. Therefore, when the electric field polarity

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was periodically switched, the functions of both the anode and cathode electrodes were applied across the whole soil cell, further increasing the degradation efficiency.

1. Introduction

Total petroleum hydrocarbon (TPH) contamination has been a worldwide environmental problem for decades. The high-volume alkanes, including cycloalkanes, are particularly important [1]. Of these, cycloparaffinic hydrocarbons have been identified as the most persistent and hazardous soil pollutants. This is because of their higher resistibility to soil microorganisms and adhesion to the surface of soil particles [2,3].

There has been a large amount of research into specialized enzymes and metabolic pathways for the degradation of linear alkanes, and numerous novel strains of bacteria, capable of degrading linear alkanes, have been described [4]. However, the microbial degradation of cycloalkanes has received little attention.

Cyclohexane is the most prominent model cycloalkane molecule used and can be degraded by many microorganisms, such as *Nocardia, Pseudomonas* and *Rhodococcus* [5–7]. The pathways described for cyclohexane degradation by these microorganisms contain common key steps. Initially, the ring is hydroxylated by a monooxygenase, yielding cyclohexanol. Then, the corresponding cyclohexanone is generated, and subsequently an oxygen atom is inserted into the ring to generate a lactone. The resulting lactone is further cleaved to produce a linear alkanoic acid, which will be subjected to β-oxidation. The ubiquitous monooxygenases or hydroxylases generated by the microorganisms that metabolize the linear alkanes can transform cycloalkanes into the corresponding cycloalkanones [8]. Few microorganisms can secrete oxygenases or hydroxylases for cycloalkanes alone. Therefore, the complete mineralization of cycloalkanes is generally achieved through co-metabolism or commensalism [3]. Consequently, accelerating lactone cleavage is essential for the fast and effective degradation of cycloalkanes.

There has been much research into enhancing the microbial degradation of total petroleum hydrocarbons using electrokinetics. Fan et al. [33] reported an increase in the microbial degradation of total petroleum hydrocarbons in soil as a result of electrokinetics; they achieved degradation efficiency of up to 45.5% in their electro-biodegradation treatments, while the natural degradation efficiency was only 7.9%, after 100 days. Ramírez et al. [34] employed an electro-biodegradation method using polarity reversal, which achieved higher diesel removal efficiencies than treatments with only biodegradation or electro-biodegradation using an electric field in only one direction, over 14 days. Li et al. [35] reported that polarity-reversal enhanced microbial counts and maintained neutral pH conditions, resulting in an improvement in pyrene degradation.

Although cycloalkanes can constitute 15% of standard gasoline and 40% of crude oil[12], they have not yet been focused on in studies of electrokinetics remediation. Cyclododecane has been proposed for inclusion in the initial 'Substances of very high concern' (SVHC) List, being compiled by the European Chemicals Agency (ECHA), due to its relatively high persistence, bioaccumulation potential and toxicity. Therefore, cyclododecane was employed as the model compound to represent cycloalkanes in crude oil. The present study was driven by the following two questions: (i) can microbial degradation of cyclododecane be enhanced by electrokinetics? and (ii) if so, what is the microbial degradation pathway and enhancement mechanism?

Table 1
Original properties of the test soil (after being air-dried and sieved).

Soil properties	Value
pH	6.68
Density (g cm^{-3})	2.65
Porosity (%)	51.9
CEC (cmol kg^{-1})	7.44
Organic C (g kg^{-1})	10.65
Total phosphorus (g kg^{-1})	0.11
Total nitrogen (g kg^{-1})	0.82
Fe_2O_3 (g kg^{-1})	5.06
MnO_2 (g kg^{-1})	0.08
Texture (mm, %)	
<0.002	18.6
0.002–0.02	29.8
0.02–2.00	51.6

2. Materials and methods

2.1. Soil and chemicals

Pristine soil was sampled from the National Field Research Station of Shenyang Agro-ecosystems (Shenyang, China), and was classified as a sandy loam. The samples were air-dried and sieved through a 2-mm mesh to create a homogeneous mixture (Table 1). The soils were also sterilized to remove any indigenous microorganisms.

Cyclododecane (>98%) and cyclododecanone (>99%) were purchased from Tokyo Chemical Industry (Japan). Boron trifluoride-methanol (14%) and authentic standards of dimethyl-esters, including dimethyl dodecanedioate (>99%), dimethyl decanedioate (>99%) and dimethyl octanedioate (>99%) were supplied by Sigma-Aldrich Chemicals (USA). All other chemicals used were purchased from the Sinopharm Chemical Reagent Company (China), and were analytical reagent or GC grade.

2.2. Bacteria

A mixed culture of petroleum-utilizing bacteria was used. The bacteria were isolated from a petroleum-contaminated soil adjacent to Daqing Oil Field (Heilongjiang province, Northeast China) using a mineral salt medium, with components as described by Yin et al. [13]. The microbial cells were cultivated in a basic culture medium (1% peptone, 0.5% beef extract, 0.5% NaCl, 0.07% K_2HPO_4, 0.03% KH_2PO_4, and 0.3% NH_4NO_3; pH 7.2) on a constant-temperature shaker (30°C, 160 rpm). They were harvested in their exponential growth phase by centrifugation (8000 rpm at 5°C for 5 min). The cells were resuspended in sterilized deionized water and evenly added into the sterilized test soils to obtain an initial concentration of 10^6–10^7 CFU g^{-1} of dry soil.

2.3. Experimental design and set-up

Three treatments were performed: in Test 1, bioremediation was performed without the application of an electric field; in Test 2, electro-bioremediation was performed with a constant voltage gradient of 1.3 V cm^{-1} for the duration of the experimental period; and in Test 3, electro-bioremediation was performed with the same voltage and the electrode polarity was switched periodically (every 2 h). The treatments were applied to the contaminated soils for