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Interactions between electrokinetics and rhizoremediation on the remediation of crude oil-contaminated soil

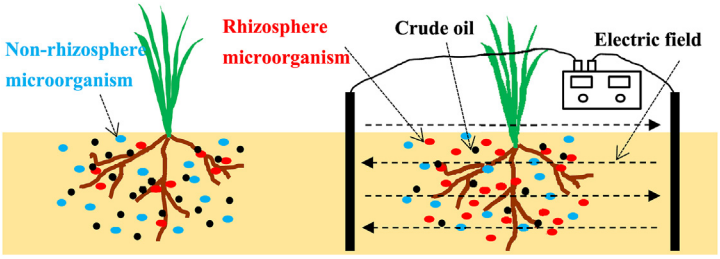
Hua Huang [a, b], Jingchun Tang [a, *], Zhirui Niu [b], John P. Giesy [c, d, e, f, g]

[a] Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin Engineering Center of Environmental Diagnosis and Contamination Remediation, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China
[b] School of Petroleum and Environmental Engineering, Yan'an University, Yan'an, 716000, Shaanxi, China
[c] Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[d] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[e] School of Biological Sciences, University of Hong Kong, Hong Kong, China
[f] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, China
[g] Department of Biology, Hong Kong Baptist University, Hong Kong, China

HIGHLIGHTS

- An electrokinetics-enhanced phytoremediation (EK-R) was constructed to remediate crude oil-contaminated soil.
- Removal of TPH and microbial activity were increased by use of EK-R.
- Absolute and relative numbers of bacteria in bulk soil of EK-R were similar to that in rhizosphere soil.

GRAPHICAL ABSTRACT



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ABSTRACT

An electrokinetics (EK)-enhanced phytoremediation system with ryegrass was constructed to remediate crude oil-polluted soil. The four treatments employed in this study included (1) without EK or ryegrass (CK-NR), (2) EK only (EK-NR), (3) ryegrass only (CK-R), and (4) EK and ryegrass (EK-R). After 30d of ryegrass growth, EK at 1.0 $V \cdot cm^{-1}$ with polarity reversal (PR-EK) was supplied for another 30 d. The electric current was recorded during remediation. The pH, electrical conductivity, total petroleum hydrocarbon content (TPH), 16S rDNA, functional genes of *AlkB*, *Nah*, and *Phe*, DGGE, and dehydrogenase activity in soil were measured. The physical-chemical indexes of the plant included the length, dry mass, and chlorophyll contents of the ryegrass. Results showed that EK-R removed 18.53 ± 0.53% of TPH, which was higher than that of other treatments (13.34–14.31%). Meanwhile, the values of 16S rDNA, *AlkB*, *Nah*, *Phe*, and dehydrogenase activity in the bulk soil of EK-R all increased. Further clustering analysis with numbers of genes and DGGE demonstrated that EK-R was similar to the ryegrass rhizosphere soils in both EK-R and CK-R, while the EK treatment of EK-NR was similar to that of CK-NR without EK and ryegrass. These results indicate that the PR-EK treatment used in this experiment successfully enlarged the existing scale of the rhizosphere microorganisms, improved microbial activity and enhanced degradation of TPH.

* Corresponding author. 94 Weijin Road, Tianjin 300071, China.
E-mail address: tangjch@nankai.edu.cn (J. Tang).

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1. Introduction

As petroleum hydrocarbons and their derivatives are important in the modern world, crude oil extraction, transport, refinement, and utilization are increasing. Concomitantly, the occurrence of accidental spillages has also increased, such that water, soil, and sediments have become contaminated, which can adversely affect both the environment and human health (Varjani and Upasani, 2017). Various techniques have been applied to remediate soils contaminated with oil, including the extraction of oil with solvents, chemical oxidation, thermal technologies, ultrasonication, and flotation with density gradients. As they are both cost-effective and "environmentally friendly", less active methods of natural attenuation, including phytoremediation, have been applied to pollution hotspots (Feng et al., 2017).

Phytoremediation is defined as a "green technology" that uses plants and the associated microorganisms in their rhizosphere (roots) to degrade organic pollutants in soil (Feng et al., 2017). In this system, various exudates released by plants, such as carbohydrates, amino acids, organic acid anions, and secondary metabolites, provide substrates for bacteria that can then either internally or externally degrade petroleum hydrocarbons by co-metabolism (Martin et al., 2014; Sasse et al., 2018). These exudates can serve as carbon or energy sources, or provide micronutrients that support and/or enhance the growth and activity of microorganisms in the rhizosphere, and also activate functional genes that result in the co-metabolism of organic pollutants, including petroleum hydrocarbons (Martin et al., 2014; Sasse et al., 2018). Specific microbial communities associated with the rhizospheres of plants that can degrade chemicals that are potentially toxic to plants or animals have evolved, and can be used in phytoremediation to reduce the concentrations of pollutants (Alagić et al., 2015). The results of several studies have confirmed that phytoremediation could enhance the degradation of organic pollutants (Acosta-Santoyo et al., 2017; Ancona et al., 2017). However, in soils, the scale of this is limited and restricted to areas near the rhizospheres of plants. This, combined with the movement of exudates by passive diffusion, which is slow, and the immobility of microbes in soils, has restricted the effectiveness of phytoremediation for practical application to large-scale contamination (Corgie et al., 2004; Tu et al., 2017). To address these limitations, plants have been seeded to increase the density of the rhizosphere in a certain area, or nutrients have been added to soils to provide nitrogen, which often limits the growth of microbes (Jagtap et al., 2014; Liu et al., 2014). Furthermore, in some cases, specific microbes that can degrade oil have been added to soils, or soils have been tilled back into local soils to move microbes or provide aerobic environments (Tang et al., 2010). These amendments have increased the rates of degradation, but each has limitations. The addition of a nitrogen fertilizer can result in the formation of ammonia, which can be toxic to invertebrates in soils and sediments. Further, the benefits of adding specific microbes normally are typically only short-term due to their inability to compete with natural soil microflora or their loss of genetically modified capabilities through hybridization. Finally, these active augmentations of bioremediation by bacteria are time-consuming and costly. An alternative, less active, and less costly method of facilitating the diffusion of rhizosphere microorganisms from the rhizosphere zone into bulk soil to overcome the slow nature of this process is required.

By imposing weak electric fields on contaminated soils through electro-kinetic phenomena, such as electro-osmosis, electromigration, and/or electrophoresis, electro-kinetic (EK) technologies can facilitate the movement of water, metals, microorganisms, terminal electron acceptors, and nutrients, as well as organic pollutants, through soils to promote degradation (Lima et al., 2017). This movement of materials is especially attractive when combined with other methods to maximize the effectiveness of those for degradation and/or the removal of contaminants, such as total petroleum hydrocarbon (TPH), as such a combination can overcome the inherent limitations of the formation of depleted zones due to slow diffusion (Lima et al., 2017). The results of previous studies that have used EK in combination with other bioremediation methods, including bio-stimulation and bio-augmentation, have improved the effectiveness of remediation (Wang et al., 2013; Hassan et al., 2017; Zhang et al., 2017). In those studies, EK mainly increased the bioavailability of organic pollutants by facilitating contact between microbes and nutrients/pollutants, however, the weak electric current may have also directly stimulated microbial activity (Kim et al., 2010; Velasco-Alvarez et al., 2011) or degraded some of the pollutants through an electrolytic reaction (Acimovic et al., 2017). EK was also successfully combined with phytoremediation to remove metals from soils (Jamari et al., 2014; Acosta-Santoyo et al., 2017; Luo et al., 2018a). In these studies, EK not only moved metals to plants, but may have also stimulated their growth and respiration.

Phytoremediation for treating organic polluted soils has been studied previously and combined with EK for the removal of metals (Cameselle et al., 2013; Putra et al., 2013; Jamari et al., 2014; Acosta-Santoyo et al., 2017). However, to our knowledge, there has been no previous research into joint effects of EK and phytoremediation for treatment of soils contaminated with organic pollutants. Based on previous studies, this combination is hypothesized to not only increase the mobility of contaminants and nutrients, but also enlarge the effective biodegradation zone by diffusing rhizosphere microorganisms into bulk soil to overcome the inherent limitations of phytoremediation. In this way, EK could improve both the spatial scale and speed of remediation by plants.

Thus, an EK-enhanced phytoremediation system was constructed for remediating crude oil-polluted soils. The effects of EK on remediation, as well as the growth of plants and responses of the microbial communities in bulk soil, were studied after 30 d of incubation.

2. Materials and methods

2.1. Pristine soil, crude oil, and the EK system

Soil that had not been previously contaminated by petroleum hydrocarbon spills was collected from the campus of Nankai University at Tianjin, China, from a depth of 0–20 cm. After air-drying, small rocks, plant residues, and other non-soil components were removed and the soil was passed through a 2-mm sieve, before being stored at a cool temperature and ventilated prior to use. Some major soil properties are listed in Table 1. Before use, approximate 30% of river sand and 12.5 $g \cdot kg^{-1}$ of crude oil were mixed into the soil. The crude oil was obtained from the Dagang Oil Field near Tianjin, China. Ryegrass was selected for use in this study owing to its strong vitality and good performance in petroleum remediation (Tang et al., 2010; Han et al., 2016). Dry seeds were purchased from Suqian, Jiangsu, China.

The EK system was similar to that used in a previous study, with some modification (Fig. 1). The setup consisted of twelve Perspex® soil chambers (21 × 8 × 8 cm), twenty-four plated graphite electrodes (8 × 8 × 1 cm) that supplied uniform electric field (Fig. S1), a direct current power supply that could periodically reverse the polarity, 6 A meters, and one voltmeter. Though the circuit was parallel, the same electric intensity could be supplied to each replicate. All soil chambers were covered with black plastic bags to protect the plant roots from light, and were separated into five sites with a 0.35-μm nylon net as previously described (Cang et al., 2011).

Table 1
Properties of the pristine soil.

Characteristics		Characteristics	
pH	8.11	Sand (%)	21
Electrical conductivity ($\mu S \cdot cm^{-1}$)	194	Slit (%)	46
Organic matter (%)	3.42	Clay (%)	33
Ca ($mg \cdot g^{-1}$)	19.55	Texture	Clay loam
Fe ($mg \cdot g^{-1}$)	22.68	16S rDNA copies	7.6×10^7
Mn ($mg \cdot g^{-1}$)	0.71	Dehydrogenase activity ($\mu g\ TPF \cdot g^{-1} \cdot h^{-1}$)	13.43
Mg ($mg \cdot g^{-1}$)	2.83	Catalase (mg $H_2O_2 \cdot g^{-1}$ soil 20 min)	2.56
P ($mg \cdot g^{-1}$)	1.03	CO_2 release (mg $CO_2 \cdot g^{-1} \cdot d^{-1}$)	1.03

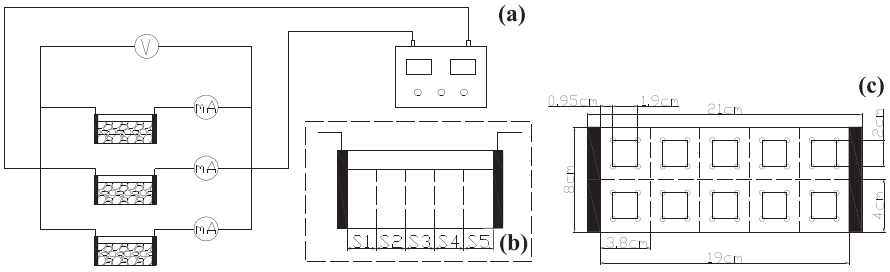

Fig. 1. Schematic diagram of the testing system. Note: (a) A power supply was used to supply a constant direct current to the electrokinetic reactors at 1 $V \cdot cm^{-1}$, and the polarity was reversed hourly through the parallel circuit. Only three reactors are shown in this figure. (b) Sections were divided by nylon meshes. (c) Ryegrass planting positions.

2.2. Experimental setup and process

Four treatments were used, with each tested in triplicate: a control without an electric field or ryegrass (CK-NR), a treatment with an electric field but without ryegrass (EK-NR), a treatment with ryegrass and without an electric field (CK-R), and a treatment with both an electric field and ryegrass (EK-R).

Approximately 1.25 kg of dry soil contaminated with petroleum hydrocarbons was carefully packed layer by layer into each chamber, and the thickness of the soil layer was approximately 7 cm. Soils were watered to a water content of 25% following the gravimetric method, and incubated under the room's conditions for 10 d to ensure good contact between the soil and the graphite electrodes. The surfaces of the seeds were sterilized in a 20% (v/v) NaOCl solution for 10 min, washed five times with sterile water, and soaked for 24 h before planting. Floating seeds were removed, and full seeds were planted in the designed sites of the chambers (Fig. 1). Two seeds were sown into one site, and only one ryegrass plant that had germinated was left at the site after one week. After 30 d of ryegrass growth, EK with periodic polarity reversal (PR-EK) was supplied continuously for 30 d. The electric intensity was 1 $V \cdot cm^{-1}$ and the polarities of the electrodes changed hourly. The water content was maintained by daily watering and adjusting, while the gravimetric method was applied every three days. When the remediation process was stopped after 60 d, soil and plant samples were harvested.

Forty ryegrasses plants were obtained for each treatment – they were washed with tap water five times, rinsed twice with deionized water, and blotted dry before being weighed. As the biomass of the roots was relatively small, all 120 roots from the triplicates of each treatment were pooled to measure their wet masses. The lengths of the roots and shoots were measured using a ruler. Shoots were randomly divided into aliquots, and one aliquot was dried in an oven at 40 °C for 24 h to measure its dry mass. The other part was used for chlorophyll quantification.

The soil that was strongly adhered to the ryegrass roots was collected as part of the rhizosphere. These samples were labeled with a subscript "r" to identify them as the rhizosphere soil of CK-R and EK-R, i.e., they were denoted as CK-R-r and EK-R-r, respectively. Other soil samples were separately collected from different sites and designated as bulk soil. The bulk soil samples were separated into three aliquots: the first was immediately used to measure the pH and electrical conductivity, the second was air-dried to measure the TPH, and the third aliquot was stored at −20 °C before the expressions of genes and enzyme activities were quantified.

2.3. Analytical methods

For ryegrass, the contents of chlorophyll were quantified following previously published spectrophotometric methods (Han et al., 2016). The electrical conductivity and pH of soils extracted at 1:5 (w/v) with deionized water were measured using a pH (Sartorius, Germany) or conductivity meter (Mettler Toledo, Swiss), respectively. TPH was determined gravimetrically through Soxhlet-extraction with dichloromethane after the extracted mass was determined by previously reported methods (EPA, 1998). Dehydrogenase activity was detected following the triphenyltetrazolium chloride method provided in ISO 23753-1:2005(E) (2005). DNA was extracted from soils using ZR Soil Microbe DNA MiniPrep (Zymo Research, CA, USA). The extracted DNA was then used for the genomic analysis of the microbial community structure by using DGGE according to a previous method (Muyzer et al., 1993) and to quantify the 16S rDNA from the bacterial V3 region for the total microbial numbers and three metabolic genes (*AlkB*, *Nah*, and *Phe*) involved in the biodegradation of petroleum by qPCR. Detailed procedures for all methods for determining the chlorophyll contents, TPH, dehydrogenase activity, and DGGE are given in the Supporting Information. Details of the primers and qPCR conditions have been published previously (Liu et al., 2015, 2017).

2.4. Statistical analysis

SPSS 18.0 (SPSS Software, USA) was used for clustering analysis. A Student's T-test or one-way ANOVA with a post-hoc Tukey test was conducted to evaluate the data and ensure that they met the assumptions of independence, normality, and homogeneity of

variance. OriginPro 8.5.0 SR1 (OriginLab, USA) was used for linear regression analysis. Quantity One v4.62 (Bio-Rad, USA) was used to analyze the DGGE bands and construct the phylogenetic tree.

3. Results and discussion

3.1. pH, electrical conductivity, and electric current

EK with a direct electric current can alter the pH of soils through an electrolytic process that releases H^+ near the anode and OH^- near the cathode (Mena et al., 2016b). Thus, the pH levels of the soils were measured when remediation was completed, which were 7.72–8.03 for treatments, and evenly distributed in EK-NR and EK-R (Fig. 2). This suggested that protons and hydroxyl ions were neutralized under the PR operation mode, and that PR-EK had little influence on the pH of soils, which is consistent with the results of previous studies (Barba et al., 2017; Li et al., 2018a). The electrical conductivity of treatments CK-R and EK-R were 8.2% and 9.3% less than that of CK-NR and EK-NR respectively, while no clear differences were found between CK-NR vs. EK-NR, and CK-R vs. EK-R (Fig. 2). Spatially, the electrical conductivity of EK-NR on one side (S_1) was notably greater than that on the other (S_5) (Fig. S2), which was consistent with the results of previous studies (Mena et al., 2016a; Barba et al., 2017; Li et al., 2018a). In contrast, the electrical conductivity was evenly distributed in the EK-R (Fig. S2), suggesting that planting ryegrass could inhibit the free movement of ions and improve the evenness of their distribution in soil.

The electric currents varied between treatments (Fig. 3). For EK-NR, the electric current was approximately 13.3 mA with small fluctuations – it increased slightly during the first 10 d and then decreased during the following 20 d, which was consistent with results of previous PR-EK studies (Li et al., 2018a; Zhou et al., 2018). In contrast to EK-NR, the electric current of EK-R decreased notably from 13.9 mA on the first day of the study to 9.8 mA after 30 d. As the nature of the electric current in EK is a function of the flow of charged ions under electric fields, planting ryegrass decreased the electric current in PR-EK as it limited the movement of ions and water in soil. This was supported by the strongly positive correlation between the electrical conductivity and average electric current ($R^2 = 0.84$, Fig. S3) in this experiment, and by a similar observation in a previous study (Cang et al., 2012).

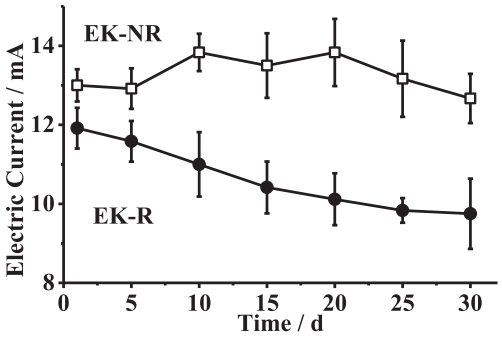

Fig. 3. Changes in the electric current during the experiment.

3.2. Growth of plants

Plants grew well in all treatments (Fig. S4). The shoot length, dry mass, and chlorophyll contents of plants were determined after harvest to determine their biomass. Results showed that there were no significant differences in CK-R and EK-R ($p = 0.56–0.83 > 0.05$) (Table 2). Previous studies found that a weak electric current can improve the growth of plants by enhancing the uptake of nutrients as the electromigration of ions and electroosmosis of water can improve the availability of nutrients, and electrical stimulation can promote the permeability of cell membranes and metabolic activity of plants (Bi et al., 2011; Chirakkara et al., 2015; Acosta-Santoyo

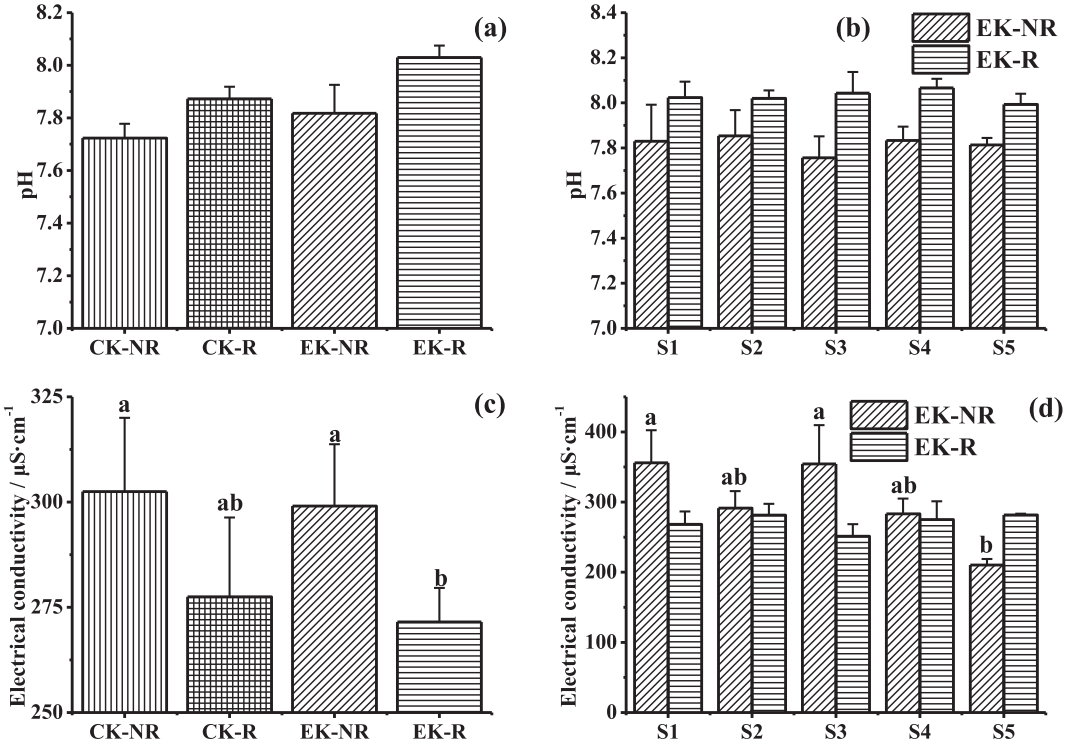

Fig. 2. Changes in pH (a, b) and electrical conductivity (c, d). **Note**: the distribution of pH and electrical conductivity are displayed in b and d, respectively.

Table 2
Growth of ryegrass with and without PR-EK.

		EK-R	CK-R
Length (cm)	Root	5.7 ± 0.7	5.4 ± 0.4
	Shoot	18.4 ± 1.0	18.0 ± 0.8
Dry weight (g)	Root[a]	0.16	0.14
	Shoot	0.29 ± 0.03	0.27 ± 0.03
Chlorophyll ($mg \cdot g^{-1}$ dw)	Chl[a]	1.26 ± 0.29	1.15 ± 0.30
	Chl[b]	0.29 ± 0.11	0.27 ± 0.05
	Car	0.35 ± 0.04	0.33 ± 0.05

Note :
[a] All 120 plants from the triplicates were included for root biomass; there was no standard error.
[b] t-tests (n = 3) were conducted and no significant difference was observed.

et al., 2016, 2017). However, it would also improve the uptake of pollutants, such as heavy metals, that may damage the health of plants (Aboughalma et al., 2008; Acosta-Santoyo et al., 2017). Some other indirect effects of EK include the development of an extreme soil pH, toxic metals, peroxides, release of nitrite nitrogen by the corrosion of electrodes, and electrochemical reactions (Bi et al., 2011; Cang et al., 2012; Acosta-Santoyo et al., 2017). Therefore, the final effect of EK on plants is a balance between the above-mentioned positive and negative effects, which depend on several factors such as the type and intensity of the electric field, electrode materials, plant types, soil properties, and pollutant types (Bi et al., 2011; Cang et al., 2012; Acosta-Santoyo et al., 2017; Luo et al., 2018b).

3.3. TPH removal

After remediation for 60 d, the TPH contents were reduced by 13.34%–18.53% in different treatments (Fig. 4), and were in even distribution in EK-NR and EK-R. Increases in the removal of only 0.39% or 0.97% from that of CK-NR were achieved by EK-NR or CK-R alone, and the differences were not statistically significant. This result suggests that solely supplying PR-EK or planting ryegrass had little influence on the removal of TPH. However, when combining PR-EK and ryegrass together as EK-R, the removal of TPH increased by 38.9%. This indicates that combining PR-EK with planting ryegrass could strongly enhance the removal of TPH.

3.4. Changes in dehydrogenase activities

When the dehydrogenase activities of samples were used as a general measure of microbial activity, the activity of EK-R was significantly greater than that of the other treatments, and there was no significant difference between CK-NR, EK-NR, and CK-R (Fig. S5). Therefore, it can be concluded that the interactions of the main effects of combining PR-EK with planting ryegrass resulted in the significantly greater microbial activities observed during this study. Previous studies showed that dehydrogenase activity was positively influenced by microbial numbers (Lu et al., 2014), residual TPH (i.e., metabolic substrates) (Wang et al., 2016), and electric stimulation near electrodes (Wang et al., 2013; Zhang et al., 2017). In this experiment, the effect of electric simulation on dehydrogenase activity was negligible, which was confirmed by the similar dehydrogenase activities, 16S rDNA lg copies, and residual TPH of CK-NR and EK-NR. Thus, the higher microbial number of EK-R was responsible for the higher dehydrogenase activity (See below).

3.5. Changes in degrading genes and microbial community

The 16S rDNA was used to estimate the total mass of bacteria, *AlkB* was used to estimate the numbers of bacteria capable of degrading n-alkanes, and *Nah* and *Phe* were used as indicators of the potential for degrading PAHs. These were measured using qPCR in bulk soils and the rhizosphere, and there were significant differences in the microbial communities between treatments (Fig. 5) (Liu et al., 2015, 2017; Gurav et al., 2017). For treatments without PR-EK, the numbers of copies in the 16S rDNA of CK-R and CK-R-r (rhizosphere soil of CK-R) were 0.33 and 0.68 lg higher than that of CK-NR, respectively, suggesting that planting ryegrass enhanced the total numbers of bacteria in soils. The enhancement was even greater for soils in the rhizosphere than that for bulk soils (Han et al., 2016). With the supply of PR-EK, the 16S rDNA of EK-NR was similar to that of CK-NR, suggesting that PR-EK alone had little effect on the total numbers of bacteria, which was consistent with the results of previous studies (Mena et al., 2016a; Li et al., 2018a, 2018b). However, the total mass of bacteria in EK-R was significantly greater than that in CK-R, but similar to that in CK-R-r and EK-R-r (rhizosphere soil of EK-R). This indicates that the

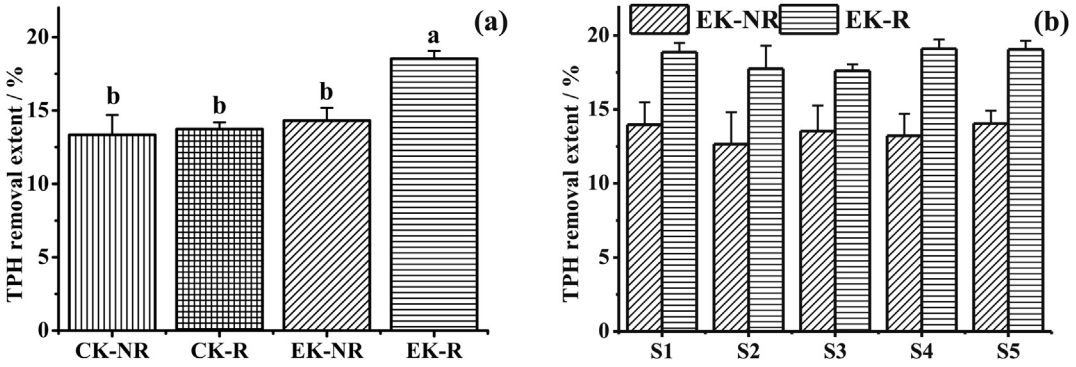

Fig. 4. Removal of TPH in each treatment (a) and space (b).

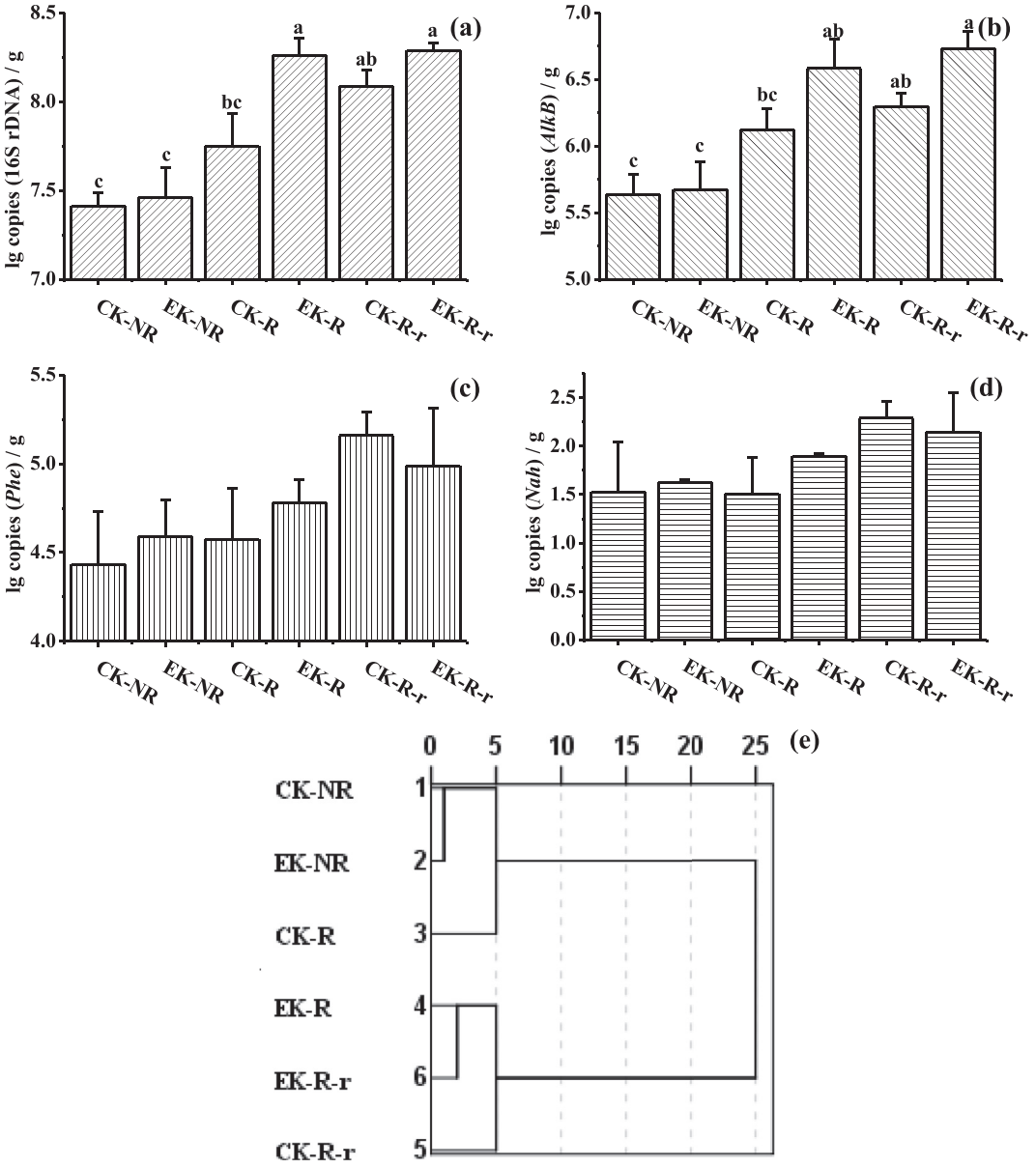

Fig. 5. lg copies of 16S rDNA (a), *AlkB* (b), *Phe* (c) and *Nah* (d) for each treatment and the results of clustering analysis (e). **Note**: The one-way ANOVA were significant for 16S rDNA and *AlkB* (both $p = 0.001 < 0.01$) but were not significant for *Nah* and *Phe* ($p = 0.124$ and $0.084 > 0.05$).

synergistic effect of PR-EK and planting ryegrass could significantly increase the total bacterial numbers in (bulk) soil.

The magnitudes of genes responsible for the degradation of petroleum hydrocarbons, including *AlkB* (10^5 - 10^6 copies), *Nah* (10^2 - 10^5 copies), and *Phe* (10^5 - 10^6 copies), were less than that of 16S rDNA (10^7 - 10^8 copies) by several orders of magnitude. The expressions of *AlkB*, between treatments were similar to the results of 16S rDNA: EK-R > CK-R > EK-NR ≈ CK-NR; the expressions of *AlkB* in EK-R were similar to those in CK-R-r and EK-R-r. Thus, the conclusions based on the total *AlkB* were similar to those based on 16S rDNA. The expressions of *Nah* and *Phe* in EK-R were greater than that in CK-NR, EK-NR, or CK-R, but lower than that in CK-R-r and EK-R-r. Due to the relatively large standard deviation (SD), this difference was not significantly different ($p = 0.124$ and $0.085 > 0.05$ for *Nah* and *Phe*, respectively).

When clustering analysis were used to explore the effects of planting ryegrass and PR-EK on microbial communities (Fig. 5e), CK-R, CK-NR, and EK-NR were clustered in the first group, while EK-R, EK-R-r, and CK-R-r were clustered in the second group, which exhibited greater amounts of 16S rDNA, *AlkB*, *Nah*, and *Phe*. To ensure that this result was not an artifact caused by chance, 16S rDNA-DGGE technology was also used to develop clusters based on the entire microbial communities (Fig. S6b). The same clustering results were obtained by both technologies, which provides confidence in the conclusions reached. In addition, the microbial diversities, as measured by the Shannon index, of both EK-R and EK-R-r were lower, while those of CK-R and CK-R-r were higher when data from DGGE were used (Fig. S6c). The structure of microbial communities typically changes to adapt to the biodegradation of TPH (Guo et al., 2014; Zhang et al., 2017).

Previous studies showed that the microbial communities in oil-polluted soil had little been influenced by PR-EK alone (Guo et al., 2014; Wang et al., 2016; Yuan et al., 2016) or the plant rhizosphere when it was at a great distance (Corgie et al., 2004; Tu et al.,

2017), which explains the similarity of CK-NR vs. EK-NR and CK-NR vs. CK-R. On the other hand, EK can move almost everything, including charged ions, microbes, organic or inorganic compounds, or nano-materials, by electro-migration, electro-osmosis, and electrophoresis (Hassan et al., 2017; Sun et al., 2017). When EK was combined with phytoremediation of ryegrass in EK-R, it may have moved root exudates or rhizosphere microorganisms, or both, from the rhizosphere to the bulk soil. Finally, the interactions between EK and planting ryegrass resulted in the similar microbial community of EK-R to that of the ryegrass rhizosphere's soil, which exhibited higher microbial numbers (16S rDNA), functions (*AlkB*, *Nah* and *Phe*), structures (DGGE bands), and activities (dehydrogenase activity). Thus, the change in microbial community was responsible for the higher TPH removal rate. However, it was unclear whether the movement of rhizosphere microorganisms or root exudates that activated functional microbes was the trigger of variations of microbial communities, and further study is required.

4. Conclusion

In a PR-EK enhanced phytoremediation system with ryegrass, plants decreased electric current and inhibited ions movement. PR-EK had little influence on growth of plants but changed microbial community in soil. The combination of electrokinetics with ryegrass resulted in higher microbial total mass, activity and function for higher removal rate of TPH. This critical change might come from the movement of rhizosphere microorganisms from rhizosphere soils to the bulk soils, or migration of root exudates which could activate functional microbes. Further study is still required to investigate whether EK-enhanced movement of rhizosphere microorganisms or root exudates was the trigger of variations of microbial communities in the soil.

Author disclosure statement

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.04.150.

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Supplementary materials

Interactions Between Electrokinetics and Rhizoremediation on the Remediation of Crude Oil-Contaminated Soil

Hua Huang[1,2], Jingchun Tang[1*], Zhirui Niu[2], John P Giesy[3,4,5,6,7]

[1]Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), Tianjin Engineering Center of Environmental Diagnosis and Contamination Remediation, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

[2]School of Petroleum Engineering and Environmental Engineering, Yan'an University, Yan'an 716000, Shaanxi, China.

[3]Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

[4]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

[5]School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China.

[6]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China.

[7]Department of Biology, Hong Kong Baptist University, Hong Kong, SAR, China.

∗ Corresponding author at: 94 Weijin Road, Tianjin 300071, China.

Tel.:+86 2283614117; fax: +86 2283614117.

E-mail address: tangjch@nankai.edu.cn (J. Tang).

The following is included as additional **Supplementary materials** for this paper.

Details of methods used in this study

Figure S1. A schematic diagram of electrodes configuration and electric field lines.

Figure S2. Electrical conductivity distribution in EK-NR (a) and EK-R (b).

Figure S3. Relationship between the electric current and electrical conductivity values.

Figure S4. Appearance of ryegrass with and without PR-EK.

Figure S5. Dehydrogenase activity in each treatment.

Figure S6. DGGE photograph (a), clustering analysis (b) and Shannon index (c).

Details of methods used in this study

Chlorophyll contents: 0.10 g fresh shoots were soaked in 10 mL mixture of absolute ethyl alcohol, acetone and water (V/V/V=45:45:10) for 1 week at 4 °C without light. After centrifugation, absorbance of supernatant was measured at 663, 645 and 470 nm by spectrophotometer and calculated.

TPH: 10 g air-dried sample was Soxhlet-extracted with dichloromethane for 24 h. The extract was condensed to almost dry in a rotary evaporator, then evaporated for half an hour in fume cupboard. Before weighing, residual TPH was evaporated under 35 ℃ for 2 h in a drying oven and determined gravimetrically.

Dehydrogenase activity: Air-dried soil (2 g) was added with 2 mL 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution which was dissolved in 0.1 $mol \cdot L^{-1}$ tris–HCl buffer solution (pH=7.4). The mixture was shaken for 2 min before incubated at 37 ℃ for 24 h. Control without soils was also conducted. After incubation, 10 mL toluene was added and centrifuged at 2500 rpm. Supernatant was filtered into volumetric flask. The process was repeated 3 times and then diluted with toluene to 100 mL. The absorbance of extract was determined at 485nm wave length. DHA was expressed as the amount of triphenylformazan (TPF) formed.

PCR and DGGE: universal bacterial primers 338F-GC (5'-CGGTGAATACGT TCYCGG-3') / 518-R (5'-GGWTACCTTGTTACGACTT-3') was used to amplify 16S rDNA genes with PCR. In each 50 μL PCR reaction system, it contained 3μL soil DNA template, 1 μL of each primer, 25μL GoTaq Colorless Master Mix (Promega, USA) and 20 μL ddH_2O. The thermal cycles for PCR were as follows: initial denaturation at 94 ℃

for 5min and 35 cycles of 94 ℃ for 1 min, annealing at 57 for 1 min, extension at 72 ℃ for 1 min, followed by final extension at 72 ℃ for 10 min.

Before DGGE analysis, products of PCR were detected by electrophoresis with 1% agarose gel and EB staining. DGGE were done as follows: 8% polyacrylamide with 25-55% denaturing gradient was used; electrophoresis was conducted at temperature of 60 ℃ and a constant voltage of 80 V for 30 min initially and 100 V for 5 h, using Bio-Rad D-Code Universal Detection Mutation system (BioRad, Hercules, CA). Subsequently, gel was stained with EB for 45min.

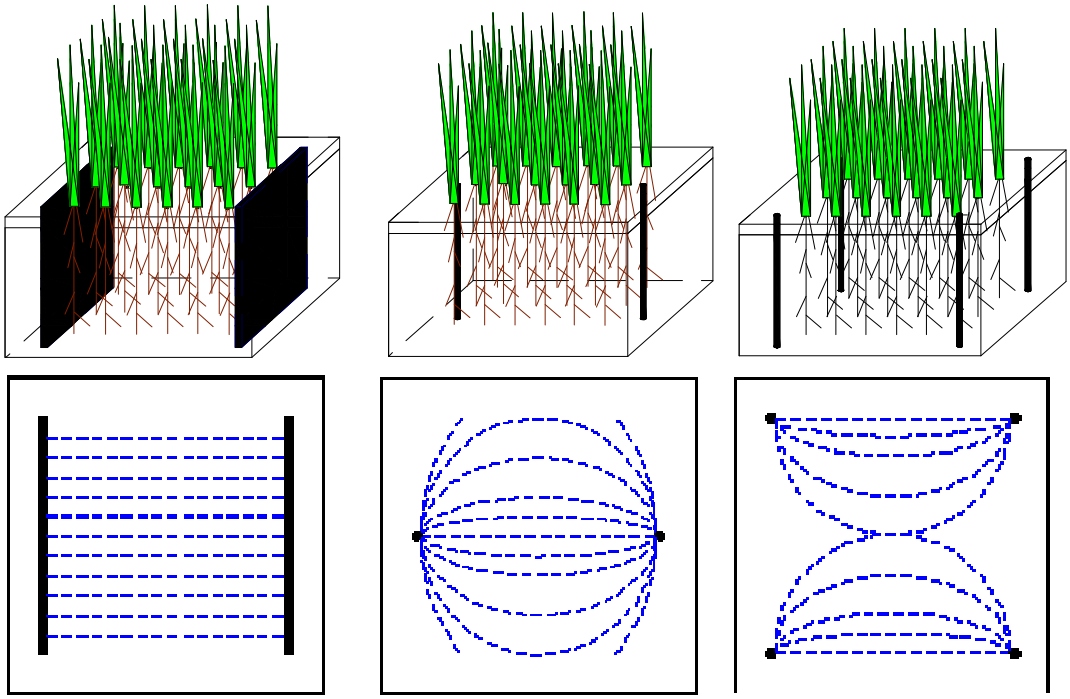

Figure S1. A schematic diagram of electrodes configuration and electric field lines.

Note: The thicker electric field lines was corresponding to the higher electric intensity.

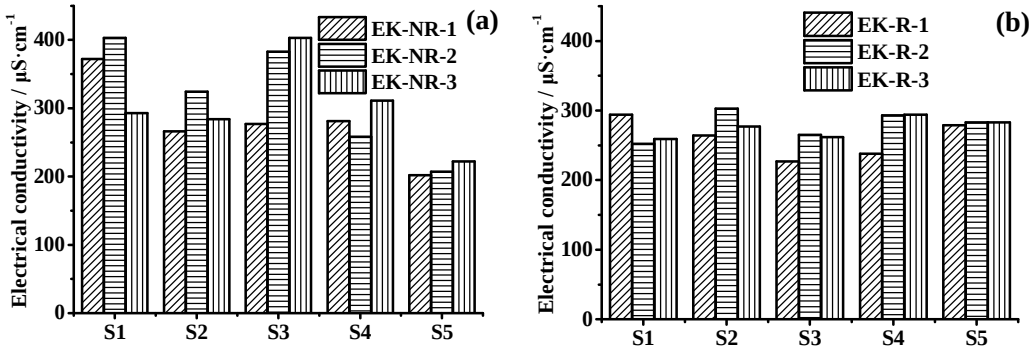

Figure S2. Electrical conductivity distribution in EK-NR (a) and EK-R (b)

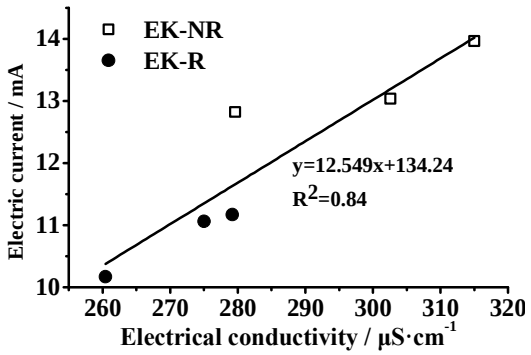

Figure S3. Relationship between the electric current and electrical conductivity values.

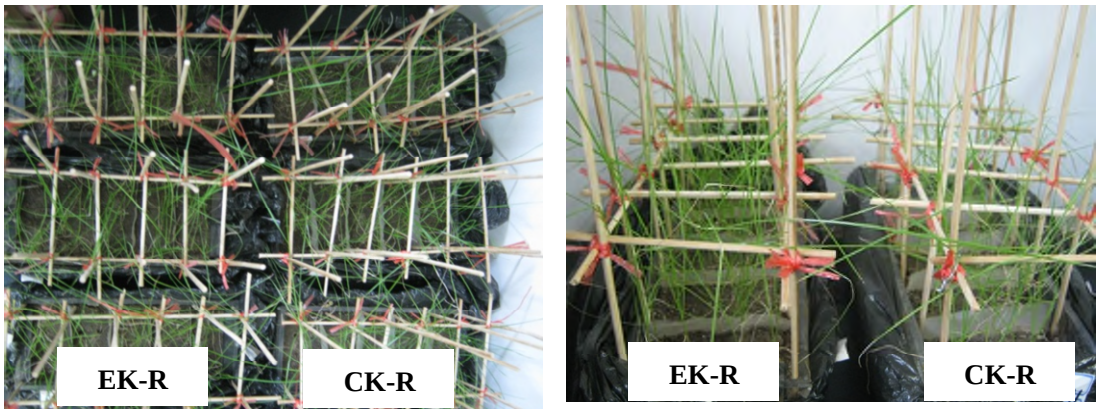

Figure S4. Appearance of ryegrass with and without PR-EK.

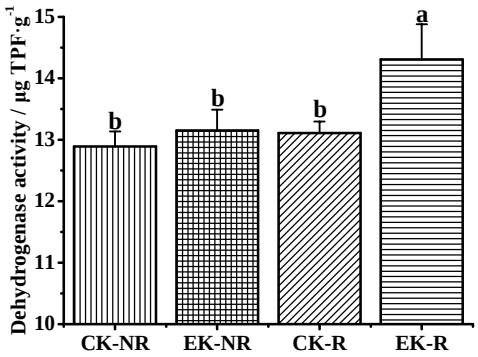

Figure S5. Dehydrogenase activity in each treatment.

Note: Dehydrogenase activity was expressed as the amount of triphenylformazan (TPF) formed.

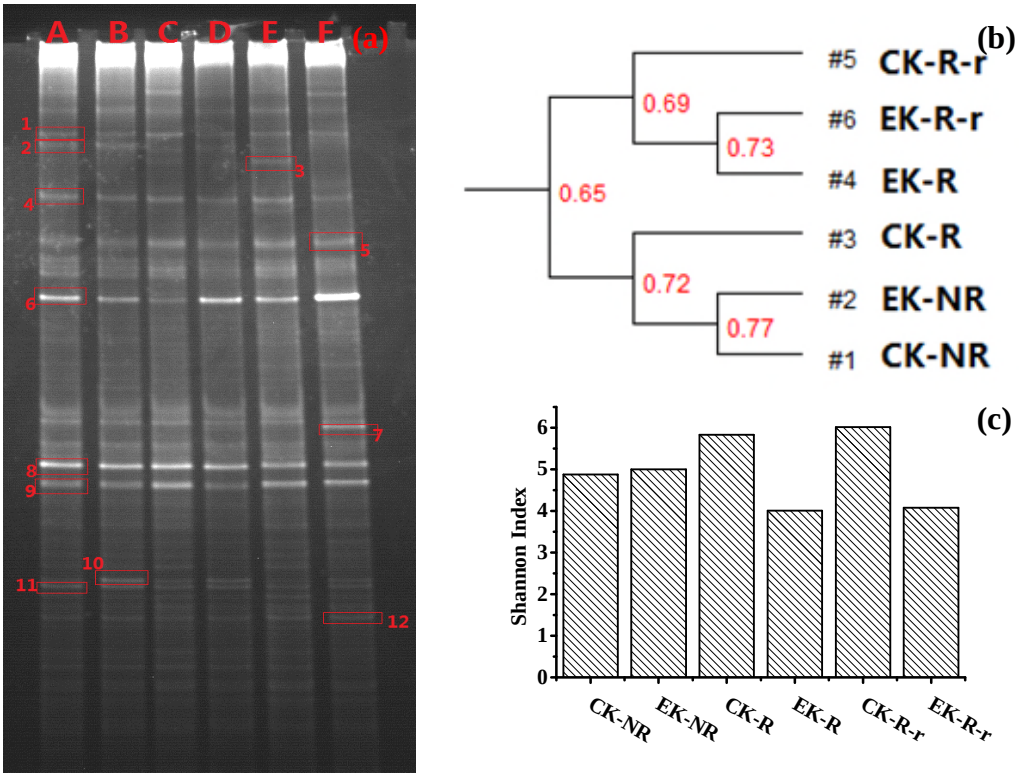

Figure S6. DGGE photograph (a), clustering analysis (b) and Shannon index (c).

Note: CK-NR, EK-NR, CK-R, EK-R, CK-R-r and EK-R-r were labelled as A~F in DGGE photograph, respectively.