Ecotoxicology and Environmental Safety 160 (2018) 207–215

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Factors influencing the removal of antibiotic-resistant bacteria and antibiotic resistance genes by the electrokinetic treatment

Hongna Li[a,*,1], Binxu Li[a,1], Zhiguo Zhang[a,b], Yunlong Tian[a], Jing Ye[a], Xiwu Lv[b], Changxiong Zhu[a]

[a] Agricultural Clean Watershed Research Group, Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China

[b] School of Energy and Environment, Southeast University, Nanjing 210096, PR China

ARTICLE INFO

Keywords:
Antibiotic-resistant bacteria
Antibiotic resistance genes
Electrokinetic
Correlation analysis

ABSTRACT

The performance of the electrokinetic remediation process on the removal of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) was evaluated with different influencing factors. With chlortetracycline (CTC), oxytetracycline (OTC), and tetracycline (TC) as template chemicals, the removal of both ARB and ARGs was enhanced with the increase of voltage gradient (0.4–1.2 V cm^{-1}) and prolonged reaction time (3–14 d). The greatest removal (26.01–31.48% for ARB, 37.93–83.10% for ARGs) was obtained applying a voltage of 1.2 V cm^{-1}, leading to the highest electrical consumption. The effect of polarity reversal intervals on the inactivation ratio of ARB followed the order of 0 h (66.06–80.00%) > 12 h (17.07–24.75%) > 24 h (10.44–13.93%). Lower pH, higher current density, and more evenly-distributed voltage drop was observed with a polarity reversal interval of 12 h compared with that of 24 h, leading to more efficient electrochemical reactions in soil. Compared with *sul* genes, *tet* genes were more vulnerable to be attacked in an electric field. It was mainly attributed to the lower abundance of *tet* genes (except *tet*M) and the varied effects of electrokinetic remediation process on different ARGs. Moreover, a relatively less removal ratio of *tet*C and *tet*G was obtained mainly due to the mechanism of the efflux pump upregulation. Both *tet* and *sul* genes were positively correlated with TC-resistant bacteria. The efflux pump genes like *tet*G and the cellular protection genes like *tet*M showed different correlations with ARB. This study enhances the current understanding on the removal strategies of ARB and ARGs, and it provides important parameters for their destruction by the electrokinetic treatment.

1. Introduction

Antibiotics are commonly used to protect human health, decrease diseases, and promote animal growth worldwide for several decades (Kumar et al., 2012). A major concern from antibiotic contamination of the environment is the rapid and increasing number of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) under selection pressure from antibiotics (Ji et al., 2012). Once acquired, antibiotic resistance may be rather stable in the environment (Novo et al., 2013). As a result, there has been increasing attention in the impacts of antibiotics perturbation. The environmental effects caused by antibiotics are identified on microbial biomass, activity, and community structures (Ma et al., 2014). The abundance of ARB is found to be 10^5–10^6 CFU g^{-1} soil for anti-tetracycline (TC) bacteria in soil with no detection of tetracyclines (TCs) (Li et al., 2018a). It is also pointed out that elevated diversity of ARGs is obtained in the receiving environment, including natural water and field soils, compared with animal feedlots (He et al., 2016; Zhou et al., 2017). Considering the serious situation of ARB and ARGs, effective controlling measures should be carried out immediately.

Several techniques had already been reported in the removal of ARB and ARGs in view of the serious pollution situation. For example, Munir et al. (2011) found that *tet*W was not significantly removed after chlorination and UV radiation, while a significant decrease was observed with UV/H_2O_2 (Ferro et al., 2016). However, few studies focused on the reduction of antibiotics and ARGs in the polluted soil. As a novel clean-up technology, electrokinetic remediation is promising in *in situ* soil remediation and has received increasing attention due to its unique applicability even for the low-permeable soils (Ma et al., 2010). Recently, it has already been successfully applied in soil to enhance the remediation effects of petroleum oils and polycyclic-aromatic hydrocarbons (Gomes et al., 2012; Mena et al., 2016a). For the optimal effects, there are several key points to be addressed, including the electric field (the voltage gradient and the polarity reversal strategies), the

* Corresponding author.
E-mail address: lihongna828@163.com (H. Li).
[1] Hongna Li and Binxu Li, these authors contributed equally to this work.

https://doi.org/10.1016/j.ecoenv.2018.05.028
Received 25 February 2018; Received in revised form 10 May 2018; Accepted 11 May 2018

reaction time, and initial pollutant concentration. Mena et al. (2016a,2016b) studied the effect of the electric field (within the range 0.0–1.5 V cm^{-1}) on the performance of electrobioremediation with diesel spiked kaolinite. The highest removal ratio of diesel was achieved by using the highest electric field. The periodic changes in the polarity of the electric field resulted in a more efficient treatment, and it does not require the addition of a buffer to keep the pH within a suitable range (Gill et al., 2014). Ribeiro et al. (2011) investigated the effects of the initial organic pollutant contents in soils, electrolyte solutions, and reaction time on the electrokinetic efficiency, and demonstrated its possibility to handle the herbicides in soils.

Our previous study shows that electrokinetic treatment is a promising technology for the removal of ARB and ARGs in soil, with chlortetracycline (CTC), oxytetracycline (OTC), and TC as template antibiotics (Li et al., 2018a). However, information about the removal efficiency with different operational parameters is limited. Furthermore, the interactions between ARB and ARGs in the process remains unclear, which is important to understand the removal mechanisms of persistent antibiotic resistance. Therefore, the main objective of the current work is the evaluation of the influencing factors (voltage gradient, reaction time, initial antibiotic concentration, and polarity reversal interval) on the removal of ARB and ARGs by the electrokinetic remediation. More importantly, the correlation analysis of ARB and ARGs is carried out. This is vitally important for providing references in the condition establishment of various factors during the electrokinetic treatment of ARB and ARGs.

2. Materials and methods

2.1. Materials

CTC, OTC, and TC were from Sigma-Aldrich (St. Louis, MO). Methanol of HPLC grade was from Fisher Scientific (Houston, TX). Other chemicals of at least analytical grade were from Beijing Chemical Reagents Co. (Beijing, China).

Soil used in this work was from the surface field of the Experimental Base in Shunyi District, Beijing, China. The antibiotic-polluted soil, with an initial concentration of 10 mg kg^{-1} for the three TCs at the beginning of experiments, was prepared according to Li et al. (2018a). The concentration of TCs at 0, 5, or 20 mg kg^{-1} was also applied to study the effect of initial TCs concentration on the efficacy of electrokinetic treatment.

2.2. Electrokinetic setup

The experimental setup (30.0 cm × 10.0 cm × 10.0 cm) used in the work was described elsewhere (Li et al., 2018b). In order to observe the voltage drop at different positions during electrokinetic remediation, four stainless steel nails were fixed equidistantly on the central axis of the bottom of the soil tank (0–4, 4–8, 8–12, 12–16, and 16–20 cm). The graphite anode and the cathode (10.0 cm × 10.0 cm × 0.5 cm) were connected to a power supply (DH1716, Dahua, Beijing). The electrolyte was composed of 80.75 mg L^{-1} Na_2SO_4, 70.0 mg L^{-1} $NaHCO_3$, and 30.36 mg L^{-1} $NaNO_3$. The antibiotic-polluted soil, with a moisture content of ~30%, was compacted to the highest degree.

A voltage gradient of 0.8 V cm^{-1} was applied during the 7-day experiment, and 0.0, 0.4, and 1.2 V cm^{-1} were also introduced to assess the influence of voltage gradient. The electric field was reversed every 12 h, except for the polarity reversal interval contrast experiments, in which it was adjusted to 24 h or 0 h (no polarity reversal). ARB and ARGs were also analyzed on Day 3 and 14 so as to investigate the influence of reaction time. The parametric design of the treatments in this work was listed in Table 1. All treatments were replicated at least three times.

Table 1
The parametric design of the treatments in the work.

Treatment	Voltage gradient (V cm^{-1})	Reaction time (d)	Initial antibiotic concentration (mg kg^{-1})	Polarity reversal interval (h)
1	0.0	7	10	12
2	0.4	7	10	12
3	0.8	7	10	12
4	1.2	7	10	12
5	0.8	0	10	12
6	0.8	3	10	12
7	0.8	14	10	12
8	0.8	7	0	12
9	0.8	7	5	12
10	0.8	7	20	12
11	0.8	7	10	0
12	0.8	7	10	24

2.3. Sample analysis

The electrical current and the voltage drop of the setup, as well as the pH and conductivity of the electrolytes were monitored every 12 h.

Considering the different reactions occurred near the anode/cathode zones, soil samples collected at 0–2, 8–12, and 18–20 cm were used in the analysis of the total bacteria, ARB, and ARGs after the remediation. The sampling procedure for the soil was illustrated in previous work (Li et al., 2018b).

The total bacterial abundance was measured with beef extract medium (pH 7.2). An extra final concentration of 10 mg L^{-1} CTC, OTC, TC or the three antibiotics combined (MIX) in beef extract medium was used for the enumeration of anti-CTC, anti-OTC, anti-TC, and anti-MIX bacteria. The antibiotic concentrations were based on previous studies (Novo et al., 2013). These plates were cultured for 48 h at 37 °C and the resulting colonies were then counted as colony-forming units (CFU g^{-1} soil).

Real-time qPCR was applied to quantify the presence of *tet*C, *tet*G, *tet*W, *tet*M, *sul*I, *sul*II, and class 1 integron (*intI*1). The qPCR mixtures (total 15.0 μL) consisted of 7.5 μL of SYBR Premix Ex Taq (TaKaRa), 0.3 μL of ROX reference dye, 0.2 μM concentration of each primer (Cheng et al., 2016), 2.0 μL of template DNA, and 4.6 μL of ddH_2O. Positive controls were used to construct the standards by transforming the gene bearing plasmids into *Escherichia coli* using TOPO Cloning kit (Invitrogen™) (Munir et al., 2011). Sterile water was used as the negative control in every run. Product specificity was confirmed by melting curve analysis and visualization in agarose gels. The external reference method was used to calculate the copy number of ARGs, with r^2 of the standard curve higher than 0.99 and the PCR efficiency of 95–110%. The PCR protocol was: 5 min at 95 °C, followed by 45 cycles of 15 s at 95 °C, 30 s at 60 °C, and a final step for the melting curve.

All analyses were performed in triplicate, and the result was calculated as the average. The values of ARB and ARGs for soils sampled at 0–2, 8–12, and 18–20 cm were then calculated as an average value representing the corresponding treatment.

The electrical consumption was calculated according to the following equation (Yang et al., 2001):

$$EC = \frac{1}{m}\int_0^t UIdt \quad (1)$$

where EC is the electrical consumption (Wh kg^{-1}), m is the soil weight (kg), U is the electric potential difference across the electrodes (V), I is the electric current (A) and t is the treatment time (h).

2.4. Statistical analysis

All data was analyzed using SPSS Version 16.0 and Microsoft Excel. P values < 0.05 were considered to be statistically significant.