Augmentation of chloramphenicol degradation by *Geobacter*-based biocatalysis and electric field

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ABSTRACT

Electroactive microorganisms and electrochemical technologies have been separately used for environmental remediation such as antibiotics removal, yet the efficiency of coupling these two methods for chlorinated antibiotics removal is poorly known. Here we tested the synergy of *Geobacter sulfurreducens* PCA, an electroactive bacteria, and an electrical field, on chloramphenicol removal. Removal is increased two-fold by increasing the temperature from 30°C to 37°C. The cyclic voltammograms and chronoamperometry tests demonstrated that *G. sulfurreducens* PCA catalyzed chloramphenicol chemical reduction with electrode as exclusive electron donor. A critical voltage, -0.6 to -0.5 V vs. Ag/AgCl, was discovered for chloramphenicol degradation with an increase of removal rate about 2.62-folds, from 31.06% to 81.41%. Combined removal with both *G. sulfurreducens* PCA and an electrical field increased the apparent rate constant and reached 82.77% removal at -0.5 V. Specially, the combined removal at -0.5 V even presented more robust removal efficiency compared to -0.6 V (78.64%) without *G. sulfurreducens* PCA. Mass spectrometry of degradation products indicates the reduction of nitro into amine groups, and dechlorination into less toxic compounds. Overall, combined biocatalysis and an electrical field is a promising method to remove antibiotics from polluted environments.

1. Introduction

Antibiotics have been frequently detected in soil, surface water, groundwater and anaerobic engineering systems (Huang et al., 2019; Pan and Chu, 2016; Xiao et al., 2021; Yin et al., 2018). The extensive use of antibiotics poses a threat to the environment and human health by inducing the emergence of antibiotic resistant bacteria and genes (Aydin et al., 2015; Biancullo et al., 2019; Li et al., 2020). Chloramphenicol is a broad spectrum antibiotic intensively used in clinical practice, it is as an effective bacteriostatic antimicrobial for diverse bacteria (Liang et al., 2013; Zhang et al., 2020). The intensive use of chloramphenicol is of increasing concern due to its mutagenic, carcinogenic and toxic effects, notably for hematopoietic and digestive system in humans and animals

(Deng et al., 2017; Yang et al., 2020a). Nonetheless, chloramphenicol is still widely used in many developed countries due to its low manufacturing cost and extensive availability (Boeckx and Brett, 2019).

Chloramphenicol and some other antibiotic removal have been tested using physicochemical approaches such as adsorption (Zhao et al., 2016), advanced oxidation (Karaolia et al., 2018; Wang and Zhuan, 2020), nanoparticles treatment (Guo et al., 2019; Xu et al., 2020), UV/chlorine treatment (Dong et al., 2017), photocatalysis (Dou et al., 2020; Wei et al., 2020) and electrochemical oxidation/reduction (Garcia-Segura et al., 2014). Yet, these technologies are limited by high energy needs, chemical cost or secondary pollution. Alternatively, microbial degradation of chlorinated compound appears as a more sustainable method for chloramphenicol removal because some

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microorganisms tolerate chlorinated contaminants and perform dechlorination, thus decreasing toxicity (Boyd et al., 1983; Zhao et al., 2015).

Electroactive bacteria (EAB) have been recently used for bioremediation (Aulenta et al., 2010; Chen et al., 2018; Palma et al., 2018). For instance, *Geobacter* spp. are electroactive bacteria that are able to degrade monoaromatic hydrocarbons and tetracycline hydrochloride (Kunapuli et al., 2010; Liu et al., 2017a; Zhang et al., 2013). Further revealed that *Geobacter* spp. are involved in the dehalogenation of polychlorinated biphenyls in anaerobic sediments. Biochar-assisted dechlorination of pentachlorophenol by *Geobacter sulfurreducens* (Yu et al., 2015), has suggested the benefit of adding a conductive material because biochar conductivity has been later shown to improve performances (Xiao et al., 2019a, 2020a). This is explained by the fact that *Geobacter* spp. grow well on electrodes by harvesting electricity from wastewater or sediments, due to their excellent ability of extracellular electron transfer (Wang et al., 2018; Yang et al., 2017). Therefore, both electromicrobiology and electrochemical technology appear promising for pollutant removal.

So far, either electroactive microorganisms or electric fields have been used separately for the removal of pollutants such as phenanthrene tetracycline hydrochloride (Peng et al., 2020; Sharma et al., 2020). Yet the feasibility, efficiency and mechanism of combining these two methods is poorly known, notably for chlorinated antibiotics. Here we studied: (1) the capability of *Geobacter* on chloramphenicol removal at diverse chloramphenicol concentrations and temperatures; (2) the reinforcing role of exogenous electric field for chloramphenicol removal; (3) a co-augmentation strategy, the combination of microbes and electric field, for chloramphenicol removal; and (4) the metabolic pathway of chloramphenicol at above co-augmentation strategy. This study proposes a promising strategy for removing antibiotics from wastewater.

2. Materials and methods

2.1. Bacteria strain and growth conditions

G. sulfurreducens PCA (ATCC 51573) was donated by Professor Lovley (Coppi et al., 2001), and was cultured with NBF medium containing 10 mM acetate and 40 mM fumarate as the electron donor and electron acceptor. Basic medium composition is (g/L): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.4, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1, NaHCO_3 1.8, $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$ 0.5, Na_2SeO_4 0.0002, K_2HPO_4 0.22, $\text{KH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.42, NH_4Cl 0.2, NaCl 0.36, KCl 0.38. The mineral and vitamin medium elements are: Free Acid Non-Trisodium Salt (NTA) 21.4, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 3, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 1.7, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 2, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 0.3, $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ 0.05, H_3BO_3 0.05, $\text{Na}_2\text{MnO}_4 \cdot 2\text{H}_2\text{O}$ 0.9, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ 1.1, $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ 0.2, biotin 0.03, pantothenic acid 0.07, B-12 0.002, p-aminobenzoic acid 0.07, thioctic acid 0.07, nicotinic Acid 0.07, thiamine 0.07, riboflavin 0.07, pyridoxine HCl 0.15, folic acid 0.03. All incubations and experiments were performed at 30 °C in the dark under strict anaerobic conditions unless indicated otherwise.

2.2. Removal of chloramphenicol by *G. sulfurreducens* PCA without electrical field

Chloramphenicol removal was studied using the following procedure at two temperature: 25 °C (experiment 1) and 37 °C (experiment 2). Incubations were carried out in 100 mL serum bottles with 40 mL NBF medium using chloramphenicol (>98% purity) purchased from Aladdin (Shanghai, China). Bottles were sealed with thick butyl rubber and aluminium caps. About 5% (v/v) of *G. sulfurreducens* PCA at stationary phase was added into serum bottles under the condition of nitrogen-saturated atmosphere. Chloramphenicol was tested at 5, 10, 20, 30 and 50 mg/L. Liquid samples were collected after 0, 24, 48, 72 and 96 h with a syringe in an anaerobic glovebox (Coy Laboratory Products),

similarly as previous investigations (Li et al., 2018; Xiao et al., 2019b; Xiao et al., 2019c). All the incubation experiments were carried out in triplicate. After sampling, the samples were filtered through 0.22 µm filters. Chloramphenicol concentrations were monitored using a 1260 Infinity high-performance liquid chromatography (HPLC) for Agilent Technologies, USA, equipped with a photodiode array detector. Analysis was performed using a 5 µm; 5 × 250 mm C18 column from Agilent, eluting methanol/water 55/45, v/v 1 mL/min with ultraviolet detection at 275 nm. Cell density was measured using a TU-1810 ultraviolet spectrophotometer at wavelength of 600 nm.

2.3. Analysis of chloramphenicol removal by electrochemistry

Experiment 3: chloramphenicol removal were done by cyclic voltammetry and chronoamperometry in a three-electrode system using CHI660 electrochemical workstation form ChenHua, China, as Xiao et al. (2020b). A 3 mm-diameter glassy carbon electrode, and a 1.5 cm × 1.5 cm platinum sheet electrode served respectively as working electrode and auxiliary electrode. Reference electrode was Ag/AgCl electrode. The working electrode was polished using successively 0.3 µm and 0.05 µm alumina slurries for 5 min, rinsed with distilled water and ethanol 3 times, then dried in clean bench. The working electrode was then activated by cyclic voltammetry in a 0.1 M H_2SO_4 and examined in 10 mM potassium ferricyanide until the peak potential difference was below 80 mV; sweep between −1 V and 1 V at scan rate of 25 mV/s.

G. sulfurreducens PCA solution of 0.5 OD₆₀₀ was harvested, 8000 rpm centrifuged at 4 °C then and resuspended in 10 mM phosphate buffer solution (PBS, pH = 7) for further analysis by cyclic voltammetry and chronoamperometry. Cyclic voltammetry was done in 30 mL oxygen-free PBS with centrifuged *G. sulfurreducens* PCA and 5 mg/L chloramphenicol. The voltage were between −1 V and 1 V with a scanning rate of 10 mV/s. PCA suspension was replaced with PBS solution in the control group. Chronoamperometry test was performed at a constant potential of −0.6 V with and without *G. sulfurreducens* PCA of 0.5 OD₆₀₀. About 20 µL chloramphenicol was added into the electrolyte every 100 s after the baseline reached steady state. All the experiments were conducted under strict anaerobic environment at 30 °C.

2.4. Effect of electric field on chloramphenicol removal alone

Experiment 4: The effect of electric field on chloramphenicol removal was carried out in a 100 mL single compartment electrolytic cell made of organic glass under galvanostatic conditions, after addition of 40 mL oxygen-free NBF medium containing 30 mg/L chloramphenicol. The electrolyte was acetate-free. Carbon cloths (20 × 20 × 1 mm) served as electrodes and were twisted by titanium wire of 0.8 mm diameter. Reference electrode was Ag/AgCl. Cathode potentials of −0.4, −0.5, −0.6 and −1.0 V vs. Ag/AgCl were applied using a CHI660 electrochemical workstation. An open circuit experiment was used as the control. Solution was sampled at different time intervals then 0.22 µm-filtered. All operations were conducted under strict anaerobic condition at 30 °C.

2.5. Synergistic effect of electric field and *G. sulfurreducens* PCA on chloramphenicol removal

Experiment 5: The synergy of an electric field and *G. sulfurreducens* PCA for chloramphenicol removal was tested. That is, chloramphenicol removal may be promoted from the cooperation by biocatalysis from *G. sulfurreducens* PCA and physicochemical action from the electrode. In previous studies, a synergistic effect for antibiotic degradation occurred by coupling electrolysis with persulfate oxidation (Liu et al., 2018). Moreover, Liu et al. (2017b) proposed an effective tetracycline degradation attributed to the synergistic effects of direct and indirect electrochemical oxidation. Here, the synergistic action biocatalysis and electrochemistry was tested. 40 mL *G. sulfurreducens* PCA in the

exponential phase was centrifuged at 8000 rpm then washed with oxygen-free 10 mM PBS at pH 7.0 three times. The collected cells were dissolved in 1 mL PBS and transferred into the electrolytic cell. The electrolyte was 40 mL oxygen-free sterile NBF medium with or without 10 mM sodium acetate. The final concentration of chloramphenicol was 30 mg/L in the electrolyte. The applied cathode potential was -0.5 and -0.6 V vs. Ag/AgCl, and other operations refer to experiment 4. Experiments were conducted in the dark under strict anaerobic conditions at 30°C .

2.6. Metabolic products analysis

Experiment 6: chloramphenicol metabolites were studied by HPLC-MS/MS (TSQ Quantum Access MAX, Thermo Fisher, USA) according to Schymanski et al. (2014). Three types of samples were tested in the end of experiment: 1. A sample from experiment 4 at -0.6 V. 2. A sample from experiment 5 at -0.6 V with *G. sulfurreducens* PCA excluding sodium acetate. 3. A sample from experiment 5 at -0.6 V with *G. sulfurreducens* PCA and sodium acetate. HPLC-MS/MS was set to positive ion mode. After cultivation, the samples were $0.45\ \mu\text{m}$ filtered then the supernatants were placed into a separatory funnel with equal volume of ethyl acetate with a 15-minute shock. The ethyl acetate layer was transferred to a rotary evaporation flask, evaporated to near dryness in a 45°C water bath, dilute with 1 mL methanol and $0.22\ \mu\text{m}$ filtered prior measurement. The HPLC-MS/MS was equipped with an electrospray ionization source and operated in the positive/negative polarity mode. The column and detection conditions were consistent with the above HPLC detection method.

2.7. Statistical analysis

Data are presented as means $\pm$ standard deviation of triplicate cultures. All statistical analyses were performed with the Origin 8.5 software from Origin Lab Corporation, USA. T-test was used to analyze the significance level, and a p value below 0.05 was considered statistically significant.

3. Results and discussion

3.1. Chloramphenicol removal dynamics by electroactive bacteria

We tested the performance of *G. sulfurreducens* PCA for chloramphenicol removal at concentrations of 5, 10, 20, 30, 50 mg/L in experiment 1 at 30°C (Fig. 1, Table 1). Results show that chloramphenicol removal within 96 h is 100% at 5 mg/L then decrease to 20% at 50 mg/L. This removal can be attributed to microbial processes because our previous study showed that physico-chemical adsorption of chloramphenicol by *Geobacter* is negligible (Xu et al., 2019). Removal can be modelled by first order kinetics (Fig. 1b), as follows:

$$\ln\left(\frac{C}{C_0}\right) = -k_{\text{app}}t \quad (1)$$

where C and C_0 are chloramphenicol current and the initial concentrations, k_{app} is the apparent rate constant, and t denotes time. k_{app} at different chloramphenicol concentration was achieved through fitting curves (Table 1). Chloramphenicol removal dynamics provides comprehensive information such as the apparent rate constant (k_{app}), which measures the removal efficiency affected by single biological or electrochemical actions. Furthermore, k_{app} is also a very important parameter to characterize the synergistic operation of biocatalysis and the electric field. Results show that k_{app} values decrease from $0.0397\ \text{h}^{-1}$ at 5 mg/L to $0.0043\ \text{h}^{-1}$ at 50 mg/L, in agreement with removal rates. This could be explained by chloramphenicol toxicity on *G. sulfurreducens* PCA, as suggested by previous reports showing the antibacterial effect on rate constants (Mao et al., 2018a; Pan and Chu, 2016). Therefore, we hypothesized that *G. sulfurreducens* PCA biomass decreased with increasing chloramphenicol concentration. This antibacterial effect is confirmed by biomass monitoring that shows that the optical density is sharply decreasing with chloramphenicol concentration, and that optical density is correlated with the removal rate (Fig. 1c, d). This is also supported by the fact that bioaugmentation should be done to improve bioremediation of antibiotics-contaminated soil by bioaugmentation (Cycon et al., 2019; Hong et al., 2020).

Fig. 1. Time course of the removal of chloramphenicol by *G. sulfurreducens* PCA under different initial concentrations (a) and kinetic fitting (b) at 30°C . The OD_{600} values of *G. sulfurreducens* PCA at five initial chloramphenicol concentrations (c) and the relevance to $\ln(C/C_0)$ (d). Values are the mean of three biological replicates. Error bars represent the SD.

Table 1Removal rate (%), optical density OD₆₀₀ and removal rate constant (k_{app}) at different initial concentrations of chloramphenicol.

Concentration (mg/L)	30 °C			37 °C		
	Removal rate (%)	OD ₆₀₀	k_{app}	Removal rate (%)	OD ₆₀₀	k_{app}
5	100	0.249 ± 0.029	0.0397	100	0.401 ± 0.009	0.0658
10	76.51 ± 0.234	0.169 ± 0.014	0.0305	100	0.362 ± 0.010	0.0498
20	43.02 ± 0.169	0.059 ± 0.007	0.0144	85.28 ± 4.28	0.196 ± 0.006	0.0433
30	28.01 ± 0.289	0.04 ± 0.006	0.0054	74.06 ± 2.62	0.121 ± 0.001	0.0136
50	20.10 ± 0.24	0.03 ± 0.006	0.0043	59.93 ± 0.22	0.119 ± 0.005	0.0096

Overall, results of chloramphenicol removal with *G. sulfurreducens* PCA alone at 30 °C show that the removal rate decreases with increasing chloramphenicol levels, and that removal is likely due to microbial processes, versus physicochemical adsorption. In the next section we tested the effect of temperature to try to improve the removal.

3.2. Effect of temperature on chloramphenicol removal by electroactive bacteria

The biodegradation rate of antibiotics should improve with temperature (Wen et al., 2010). Therefore we tested the effect of elevation from 30 °C to 37 °C on chloramphenicol removal. Results show that

bacterial biomass measured by optical density is much higher at 37 °C than at 30 °C (Figs. 1c and 2a, Table 1). Chloramphenicol was completely removed after a cultivation of 72 h when chloramphenicol concentration was less than 20 mg/L (Fig. 2b). Compared with 30 °C, the removal rates of 30 mg/L chloramphenicol (74.06%) and 50 mg/L chloramphenicol (59.93%) presented a twofold increase (Table 1). The values of k_{app} of 5 and 10 mg/L chloramphenicol were 0.0658 and 0.0498 h⁻¹, which were about 1.66 and 1.63 times compared to 30 °C. It indicated that low concentration of chloramphenicol (<10 mg/L) could be eliminated completely by a small amount of *G. sulfurreducens* PCA. While, more biomass was necessary for efficient removal along with the increasing of chloramphenicol contents. In the same vein, the removal rate is much higher at 37 °C, yielding for example a threefold increase at 50 mg/L, from about 20% removal at 30 °C to 60% removal at 37 °C. Beside biomass, other biological factors, such as specific enzyme activities, might also be impacted with the increase of temperature. Hence, the promotion of chloramphenicol degradation could be the consequence of coaction of biomass and biological factors with a higher temperature, 37 °C.

3.3. Electrical responses during chloramphenicol removal

In experiment 3 we used cyclic voltammetry and chronoamperometry to characterize the catalytic ability of *G. sulfurreducens* PCA to degrade chloramphenicol without exogenous organic carbon. This experiment was aimed at optimizing conditions of removal. Results show a weak reduction peak, at 10.1 nA/cm², in the control group at -0.6 V (Fig. 3a), which is the chloramphenicol reduction potential as previous reported (Alizadeh et al., 2012; Mao et al., 2018b). Therefore chloramphenicol probably undergoes weak reductive degradation with the electric field. In the presence of *G. sulfurreducens* PCA, the peak current density of 27.5 nA/cm² was 2.3-fold higher compared to the control group, which implies that *G. sulfurreducens* PCA directly induced chloramphenicol reduction using the electrode as electron donor. This enhancement of peak current by electroactive microorganisms and degradation rate is in line with previous reports (Liang et al., 2013; Xu et al., 2019). The catalytic activity of *G. sulfurreducens* PCA was further investigated by chronoamperometry at a fixed potential of -0.6 V (Fig. 3b). With each chloramphenicol addition, some small reduction peaks appeared, suggesting that weak abiotic degradation of chloramphenicol existed. Results show almost no variation without bacteria, and the very small peaks are probably due to abiotic degradation, as suggested also by a weaker redox peak in cyclic voltammetry. By contrast, the current density increased sharply with bacteria with the involvement of *G. sulfurreducens* PCA, reaching 12.38 nA/cm², which is three times that of the control, of 4.03 nA/cm². Comprehensive results verified that chloramphenicol degradation occurred directly in the electric field and was further catalyzed by *G. sulfurreducens* PCA.

Detoxification of chloramphenicol using a biocathode with an external power source has been shown in various media (Cotillas et al., 2018; Guo et al., 2018; Liang et al., 2013). Although it was demonstrated that electrochemical reduction decreases the antibacterial activity of chloramphenicol (Kong et al., 2015), the critical voltage for chloramphenicol degradation is unknown. Therefore we tested cathode potentials of -0.4, -0.5, -0.6 and -0.1 V to provide electrons in experiment

Fig. 2. Time course of the removal of chloramphenicol by *G. sulfurreducens* PCA at 37 °C with increasing biomass. The OD₆₀₀ values of *G. sulfurreducens* PCA at five initial concentrations (a) and the degradation kinetics (b) and kinetic fitting (c). Values are the mean of three biological replicates. Error bars represent the SD.

Fig. 3. The electrical signal during chloramphenicol degradation. Cyclic voltammograms (a), chronoamperometry (b) and external electric field (c) were used. The red arrows in (b) represented the intermittent addition of chloramphenicol. Values are the mean of three biological replicates. Error bars represent the SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Results show that removal increases from 11.11% at -0.4 V to 81.41% at -1.0 V (Fig. 3c). In details, about 11.11% of chloramphenicol was reduced at -0.4 V. In contrast, there was no decrease of chloramphenicol content under the condition of open circuit. As the potential was lowered to -0.5 V, the removal rate of chloramphenicol was increased to about 31.06%. The decrease of the potential from -0.5 V to -0.6 V significantly accelerated the removal rate of chloramphenicol, from 31.06% to 81.41%, an increase of about 2.62 times without bacteria. Robust decrease of the potential to -1.0 V further increased the degradation rate of chloramphenicol, but the benefit was slight. Therefore, the optimum potential for chloramphenicol degradation should be between -0.5 V and -0.6 V.

Overall, our results show the effective catalysis of chloramphenicol degradation by *G. sulfurreducens* PCA in an electric field, and that degradation should be critical between -0.5 V and -0.6 V. The next section explores the synergy of electroactive bacteria and an electrical field.

3.4. Synergistic operation of biocatalysis and electric field for chloramphenicol removal

Since we found a sharp drop of removal efficiency from -0.6 V to -0.5 V in the electric field alone, in experiment 5 we added *G. sulfurreducens* PCA into the system to test a possible improvement of degradation efficiency (Fig. 4). At -0.6 V, the addition of *G. sulfurreducens* PCA slightly improved the degradation ability (Fig. 4a). In sharp contrast, the degradation is highly enhanced at -0.5 V ($p < 0.05$). The corresponding k_{app} is 0.0157 h^{-1} , which is close to the efficiency of the cathode potential at -0.6 V, 0.0184 h^{-1} , and more than four-fold that of the control group, of 0.0038 h^{-1} . These results demonstrate the synergy of electroactive bacteria and the electrical field for chloramphenicol degradation. Previous work showed that 17α -ethinylestradiol removal was improved by coupling electrochemical methods with anaerobic bacteria (He et al., 2017), yet critical potentials are still unknown which is important for energy costs. *G. sulfurreducens* PCA could make up for the required potential difference, namely it was feasible to achieve the same degradation efficiency even at a higher potential (from -0.6 to -0.5 V). Our results thus imply on the applied side that combining electroactive bacteria and an electric field requires higher voltage, and thus less energy.

Acetate is an important substrate and electron donor for *Geobacter* spp (Caccavo et al., 1994; Xiao et al., 2018, 2019a). Therefore, we investigated the effect of adding sodium acetate to enhance the exoelectrogenic degradation of chloramphenicol. Results show that adding sodium acetate stimulated the capability of *G. sulfurreducens* PCA with a high removal rate at both -0.5 and -0.6 V (Fig. 4b, Table 2). A such improvement is in agreement with the fact that *Geobacter*-affiliated phylotypes accounted to more than 40% of total bacteria following acetate feeding in microbial fuel cells (Zhang et al., 2014). Our data show that chloramphenicol degradation follows a first-order reaction kinetics

Fig. 4. Time course of removal of chloramphenicol under different cathode potentials with and without *G. sulfurreducens* PCA. Chloramphenicol degradation under potentials of -0.5 and -0.6 V with and without *G. sulfurreducens* PCA (a). The strengthening of chloramphenicol degradation by *G. sulfurreducens* PCA with extra acetate addition. Values are the mean of three biological replicates (b). Error bars represent the SD.

Table 2

Removal rate and removal rate constant (k_{app}) under electric field. (chloramphenicol concentration was 30 mg/L).

Cathode alone	Removal rate (100%)	k_{app}	Cathode-G.s. PCA	Removal rate (100%)	k_{app}
-0.4 V	10.62 ± 0.12	0.0012	-0.5 V	82.77 ± 0.75	0.0157
-0.5 V	40.46 ± 1.32	0.0038	-0.6 V	87.44 ± 2.88	0.0285
-0.6 V	78.64 ± 2.13	0.0184	-0.5 V-NaAC	94.40 ± 1.57	0.0201
-1.0 V	97.11 ± 1.94	0.0373	-0.6 V-NaAC	98.95 ± 1.49	0.0417

with a removal efficiency reaching 98.95% at -0.6 V by combining biocatalysis and electric field, which is 11.51% higher than that of the negative group without sodium acetate ($p < 0.05$). Moreover, the presence of electroactive bacteria raised k_{app} 1.34-fold versus the control without bacteria. Noteworthy, the degradation rate at -0.5 V was even higher with bacteria than that of the electric field alone at -0.6 V, with rate constants of 0.0201 h^{-1} versus 0.0184 h^{-1} , and removal of 94.40% versus 81.81%, respectively ($p < 0.05$). Overall our findings demonstrate the synergy of electroactive bacteria and an electrical field to remove chloramphenicol, and that addition of sodium acetate further increases the degradation efficiency.

3.5. Analysis of degradation products

Chloramphenicol metabolites were analysed after degradation using both *G. sulfurreducens* PCA and an electric field in experiment 6 (Fig. 5). Chloramphenicol, which show typical m/z ratios at 321, 323 and 325 by HPLC-MS/MS (Xu et al., 2019), was not detected at the end of our experiments, suggesting complete removal. We detected small amounts of an aromatic amine (AMCl_2) at m/z 295 (Fig. 5a), suggesting that nitro groups of chloramphenicol was reduced to amino substituents. This is in line with nitro reduction and dechlorination observed previously for chloramphenicol (Kong et al., 2015; Lin et al., 2019; Yang et al., 2020b). Noteworthy, it is reported that AMCl_2 induces 1/500 of the toxicity of the corresponding nitroaromatic precursor (Donlon et al., 1995; Xu et al., 2019). In our experiment, AMCl_2 was probably further dechlorinated to produce inactive antibacterial products AMCl at the m/z of 279 [$\text{M} + \text{Na}^+$] (Fig. 5a). This suggests that combining electroactive bacteria and an electric field has the potential to markedly reduce the biotoxicity of some antibiotics in the environments.

In brief, the degradation process of chloramphenicol by synergistic operation of biocatalysis and electric field is proposed (Fig. 5b). Chloramphenicol was firstly converted to AMCl_2 , i.e. the nitro group of chloramphenicol was replaced by amino group. Then, AMCl_2 was translated to AMCl by dechlorination. These findings showed that cathode served an electron donor to promote effective chloramphenicol reduction. Under abiotic cathode condition, electrons directly reduce chloramphenicol to finish the nitro reduction or dechlorination process. By comparing the degradation progress of chloramphenicol under electric field alone at -0.6 V (Experiment 4, Fig. S1), degradation products were similar with treatments in the presence of *G. sulfurreducens* PCA but lacking sodium acetate (Fig. 5a). After augmentation of *G. sulfurreducens* PCA by providing extra sodium acetate, the degradation products did not show significant difference (Fig. 5a and Fig. S2), suggesting that biocatalysis and abiotic reduction may follow the similar route for chloramphenicol degradation in this study (Fig. 5b). There are mainly two strategies for the degradation process: (1) *G. sulfurreducens* PCA could act as biocatalyst to realize nitro reduction or dechlorination reaction; (2) *G. sulfurreducens* PCA, as typical electroactive microorganism, could facilitate electron flow from cathode to chloramphenicol.

Fig. 5. The potential reduction products of chloramphenicol (a) and degradation pathway (b).

4. Conclusion

Chloramphenicol, a recalcitrant endocrine-disrupting contaminant, was efficiently removed by coupling *G. sulfurreducens* PCA with electric field. An obvious inhibitory effect on the dechlorination activity and growth of *G. sulfurreducens* PCA appeared when the dosages exceeded 20 mg/L. Individual *G. sulfurreducens* PCA demonstrated relatively poor removal capability at 30 °C, such as 20% at 50 mg/l. More effective removal was achieved with the increase of temperature to 37 °C with the value of about 60% at 50 mg/l. This study showed that single electrochemical method was with the capability of catalyzing chloramphenicol reduction, which could be impacted greatly by cathode potential. Notably, synergistic operation of biocatalysis by *G. sulfurreducens* PCA and electric field maximized chloramphenicol removal efficiency. The apparent rate constant at -0.5 V was more than fourfold improvement with the addition of *G. sulfurreducens* PCA. Very interestingly, biocatalysis compensated or even stimulated the more robust chloramphenicol removal at a higher potential (from -0.6 to -0.5 V). The work proposes a feasible strategy for pollutant poisoning treatment by the synergistic operation of electroactive microorganisms and electric field.

CRedit authorship contribution statement

F.L., L.X. and J.L. designed the research. L.X., J.L., Z.L. and Q.W. performed the experiments. L.X., J.L. E. L. and F.L. analyzed the data. L. X., J.L., E. L. and F.L. wrote and revised the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124977.

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