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Biodegradation of perchloroethylene and chlorophenol co-contamination and toxic effect on activated sludge performance

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HIGHLIGHTS

- Dehydrogenase more sensitive than urease and phosphatase in PCE and 2-CP.
- PCE and 2-CP co-contamination much more toxic than single pollutant.
- Short-term exposure of PCE and 2-CP not significantly effect on activated sludge.
- Long-term exposure significantly changed microbial community diversity.

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ABSTRACT

This study investigated the effects of PCE and 2-CP co-contamination on growth of microbial community in terms of enzymatic activity and microbial diversity in activated sludge. Results showed that the activities of three key enzymes (dehydrogenase, phosphatase and urease) decreased significantly when PCE (in the range of 5–150 mg/L) was mixed with 2-CP (in the range of 25–150 mg/L). Especially, activity of dehydrogenase decreased by more than 93% as the concentration of PCE and 2-CP both reached 150 mg/L. PCR–DGGE revealed that short-term exposure with PCE and 2-CP did not lead to shift in the microbial community structure, while clone library demonstrated a significant change in the microbial diversity after long-term exposure. As the population of *Alphaproteobacteria* and *Gamaproteobacteria* decreased, with *Actinobacteria* eventually disappeared, species including *Firmicutes*, *Bacteroidetes* and *Synergistetes* became dominating groups. This study demonstrated that co-contamination with PCE and 2-CP affected the performance of activated sludge in a significant way.

1. Introduction

Perchloroethylene (C_2Cl_4) is a widespread organic contaminant in groundwater due to its wide usage as a soil and grain fumigant, an industrial solvent and a dry-cleaning or degreasing fluid because of its high recalcitrance and volatility (Doherty, 2000). The presence of PCE in the environment has brought up numerous contamination problems and health concerns, it has been proven to be carcinogenic, mutagenic and teratogenic to human and animal health (US-EPA, 2009). PCE was listed as a priority pollutant by US EPA, and numerous physiological toxicity studies have been performed (Green et al., 2001). However, the influence of PCE on the growth of micro-organisms in the environment was not well understood, and most studies have been conducted with PCE as an individual contaminant. In reality, PCE often co-exists with other contaminants including alkanes, chlorinated solvents and polycyclic aromatic hydrocarbons commonly found in the wastewaters (Lim et al., 2008; Qiu et al., 2004). Especially, 2-chlorophenol was the most frequently detected pollutant to couple with PCE in industrial wastewater (Qiu et al., 2004). Recently, anaerobic biological technique was developed to degrade such co-contaminated compounds and the anoxic active sludge became a widely-accepted process for biological wastewater treatment for abatement of chlorinated solvent-based pollution (Lin et al., 2011; Xu et al., 2011).

It was well known that the performance of bio-treatment was affected by many factors including various operation parameters, and the toxicity was regarded as critical factors (Kim et al.,

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2005). Many researchers have studied the effects of toxic organic compounds on the microbial community of activated sludge (Prado et al., 2009; Samaras et al., 2009). Tetrahydrofuran (THF) were demonstrated to have a significantly negative impact on the performance of the activated sludge system (Yao et al., 2010). Trichloroethylene (TCE) was able to noticeably inhibit the activity of anaerobic bacterial community ability, and TCE degradation rate was evidently reduced when influent containing TCE 60 mg/L was released into the anaerobic bioreactor (Siggins et al., 2011). PCE and 2-CP were both important and popular industrial chemical intermediates, especially for the syntheses of pesticides, and showed acute toxicity to microbes of anaerobic systems. However, so far little is known about the impact of the co-contamination by PCE and 2-CP on environment and wastewater treatment systems and in most cases there is no knowledge of how existing populations in activated sludge respond to their mixed contaminants (Thavamani et al., 2012).

Microbial community diversity and enzyme activity were two sensitive indices for activated sludge performance in bio-treatment process (Vivas et al., 2009). Microbial activity is normally expressed by the key enzymes' activity, such as dehydrogenase, phosphatase, and urease (Molina-Muñoz et al., 2010) were easily to be inhibited by toxic organic compounds (Li et al., 2012; Yao et al., 2010). Microbial community structure often substantial shifted in activated sludge when it was exposed to the toxic substances (Kabelitz et al., 2009) and the PCR–DGGE profile was successfully applied to assess the effects of pollutants on microbial community diversity, stability and growth dynamics (Li et al., 2012; Yao et al., 2012).

The objective of this work is to evaluate the cross-effect of PCE and 2-CP towards the performance of activated sludge in the anaerobic bioreactor. The activated sludge was maintained at PCE and 2-CP exposure from 0 to 150 mg/L. Three key enzymes' activities were determined during the short- and long-term exposure. And the toxic effect of PCE and 2-CP on the microbial community structure was examined by PCR–DGGE and clone library methods. The results could provide reliable and accurate information about the effects of PCE and 2-CP co-contamination on the activated sludge performance.

2. Methods

2.1. Activated sludge culture

Activated sludge was obtained from the anaerobic tank of Shanghai Changqiao sewage treatment plant. The basic characteristics of the activated sludge were as following: pH 6.89, dehydrogenase 40.15 ($\mu g(g\,MLSS)^{-1}\,h^{-1}$), urease 18.70 ($mg(g\,MLSS)^{-1}\,h^{-1}$) and phosphatase 4.87 ($mg\,(g\,MLSS)^{-1}\,h^{-1}$). The activated sludge was acclimated 2 weeks with artificial wastewater (NaCl 1.0 g, $MgCl_2{\cdot}6H_2O$ 0.40 g, $CaCl_2{\cdot}2H_2O$ 0.075 g, NH_4Cl 0.25 g, KH_2PO_4 0.2 g, KCl 0.50 g, glucose 3.0 g, yeast extract 0.06 g). Lab-scale anaerobic sequencing batch cultures of activated sludge were then conducted. Each culture enrichments contained 20 mL activated sludge in a 100 mL serum bottle with the mixed liquor suspended solids (MLSS) of 9500 mg/L. A set of concentration of PCE and 2-CP were added into the serum bottles (Table 1). The short-term effect on activities of three kinds of enzymes and the composition of the microbial community were determined after the 24 h and 3 day incubation at 30 °C, 140 rpm. The long-term effects on the microbial diversity in activated sludge were determined after 60 day incubation at 30 °C, 140 rpm. All treatments were replicated three times.

2.2. PCE and 2-CP degradation assay

The degradation of amended PCE and 2-CP was monitored using a gas chromatograph (GC). Media samples (10 μL anaerobic medium in 10 mL double distilled water) were obtained from the serum bottles every day. PCE, 2-CP and byproducts were analyzed by GC (Agilent 6890) equipped with a flame ionization detector (FID) and a DB-VRX capillary column, 60 m × 0.25 mm × 1.4 μm. N_2 (ultra pure) served as both carrier and make up gas at flow rates of 20 and 25 mL/min, respectively. The flow rates of H_2 and air were at 40 and 400 mL/min, respectively. The GC was operated using the following conditions: set at 45 °C for 0 min, then to 190 °C at a rate of 12 °C/min, and held at 190 °C for 2 min. Nitrogen was used as the carrier gas. Inlet temperature: 240 °C, samples were injected using a split rate, 10:1 and detector temperature, 300 °C.

2.3. Enzyme activity assay

Five kinds of enzymes were chosen for the activity analysis based on the methods described by Lv et al. (2008); some modifications were made as described below.

2.3.1. Dehydrogenase activity assay

Dehydrogenase activity is often used as the activity index of activated sludge. It was tested by the reduction amount of 2,3,5-triphenyltetrazolium chloride (TTC). Two milliliters of activated sludge was shaken with 1.5 mL of Tris–HCl buffer solution (0.2 M, pH 7.3), 0.5 mL of TTC (4 g/L), 0.5 mL Na_2SO_3 (35%) and 0.5 mL of glucose solution (0.1 M) at 140 rpm for 20 min and then incubated at 37 °C for 12 h. The deoxidization reaction was stopped by the addition of 100 μL of concentrated sulfuric acid. The following steps were performed as previously described by Lv et al. (2008). The absorbance of the supernatant was checked at 492 nm with an UV spectrometer. The enzyme activity was expressed as the TTC amount reduced per mg activated sludge per hour. The dehydrogenase activity was calculated using the equation previously described by Yao et al. (2010): $E_{dehydrogenase} = 0.08M/(Vs \times MLSS)$, where $E_{dehydrogenase}$ is the dehydrogenase activity ($\mu g(g\,MLSS)^{-1}\,h^{-1}$), M is the calculated TTC quantity (μg) and Vs is the volume of activated sludge sample (L).

2.3.2. Phosphatase activity assay

Phosphatase activity was determined as previously described by Lv et al. (2008), except that 5 mL of activated sludge was used. The phosphatase activity was defined as the production of phenol from disodium phenyl phosphate catalyzed by phosphatase per g MLSS per hour. Phosphatase activity was calculated according to the equation (Yao et al., 2010) $E_{phosphatase} = 4.17M/(Vs \times MLSS)$,

Table 1
PCE and 2-CP concentration levels in different treatment groups (mg/L).

Factor	Level																								
	CK	C1	C2	C3	C4	P1	P2	P3	P4	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
PCE	0	0	0	0	0	5	20	60	150	5	5	5	5	20	20	20	20	60	60	60	60	150	150	150	150
2-CP	0	25	75	125	150	0	0	0	0	25	75	125	150	25	75	125	150	25	75	125	150	25	75	125	150

CK represented the control sample. C1 to S16 represented the different samples.