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Toxicity in different molecular-weight fractions of sludge treating synthetic wastewater containing 4-chlorophenol

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ABSTRACT

This study investigated sludge toxicity in removal of 4-chlorophenol (4-CP) in reactor system with addition of methanol. The toxicity was evaluated in two sequential batch reactors (SBRs), namely acclimated sludge reactor with addition of 10 mg l^{-1} 4-CP and control sludge reactor without 4-CP addition. Results showed that even though 4-CP both in aqueous and sludge phases was undetectable, the toxicity of the acclimated sludge was still higher than the control sludge, which was assessed with *Photobacterium phosphoreum* (*P. phosphoreum*). Adsorption and biodegradation assays indicated that secondary metabolites from microorganism also contributed to sludge toxicity in addition to 4-CP itself. The different molecular-weight (MW) distribution of organic substances in sludge analysis validated that the mass fractions of TOC at >10 K and 10 K-500 were significantly related to the sludge toxicity.

1. Introduction

4-chlorophenol (4-CP) is widely used in oil refining, pulp and paper manufacturing, insecticide and fungicide production, wood preservation, *etc.* (Monsalvo et al., 2009; Sahoo et al., 2010; Lim et al., 2013). As a result, it is extensively distributed in soil, water bodies, and sediment (Diez et al., 2012; Basak et al., 2013; Lim et al., 2013). Owing to its high toxicity and suspected carcinogenic and mutagenic characteristics, 4-CP has been regulated as a priority pollutant by the United Stated Environmental Protection Agency (Wen et al., 2006; Nalbur and Alkan, 2007; Haddadi and Shavandi, 2013; Lim et al., 2013). Therefore, the removal of 4-CP from industrial wastewater has been an important environmental issue (Gomez et al., 2009). At present, some physical, chemical and biological treatment methods are widely applied to investigate 4-CP removal efficiency (Lima et al., 2004; Bian et al., 2011; Oh et al., 2011; Diez et al., 2012; Elghniji et al., 2012). However, if taking economical efficiency and natural nutrient circulation into consideration, biological treatment methods for 4-CP removal are the most promising. Many studies show that 4-CP could be effectively biodegraded with sequencing batch reactors (SBRs) (Monsalvo et al., 2009; Carucci et al., 2010). However, a great deal of excess sludge is produced in the biological treatment process: how to treat this excess sludge has become a significant challenge in wastewater treatment plants (WWTPs) (Zhao et al., 2014).

Some research results show that agricultural land application is the most promising and economical way of disposing excess sludge (Zhao et al., 2014). Because excess sludge contains abundant nutrients, such as N and P, which are therefore able to improve soil characteristics and increase agricultural yields (Ferreiro-Domínguez et al., 2012). According to the EC (2008), 44% of excess sludge will be recycled to the land in 2020. Nevertheless, in addition to nutrients, excess sludge maybe contains harmful ingredients. Activated sludge which once treated toxic and refractory organics, can damage human health through accumulation via the food chain (*e.g.* residual organic pollutants, toxic intermediate products and microorganism secretions) (Banerjee et al., 2010; Wang and Zhang, 2010; Rodríguez-Rodríguez et al., 2012). Therefore, sludge organic toxicity has to be considered when excess sludge is recycled to agricultural land. If not, the ecological risks maybe underestimated.

In fact, the removal of organic pollutants using biological treatment methods mainly focuses on the aqueous phase (Dong et al., 2015). With regard to 4-CP biodegradation, previous studies

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concentrated on the following several aspects: the degradation capacity and removal efficiency of 4-CP in aqueous solution; the identification and analysis of dominant microorganism species for 4-CP biodegradation; the biodegradability and degradation kinetics of pure 4-CP degrading microorganisms *etc.* (Lima et al., 2004; Kargi and Eker, 2005; Wen et al., 2006; Uysal and Turkman, 2007; Li et al., 2011; Diez et al., 2012; Lim et al., 2013). However, after 4-CP was removed by activated sludge, the ecological risk from excess sludge was seldom concerned when it was considered for application to agricultural land. Therefore, to judge the ecological risk from excess sludge, 4-CP was chosen as a target pollutant to investigate sludge organic toxicity and *Photobacterium phosphoreum* (*P. phosphoreum*) was employed to determine sludge organic toxicity for its sensitivity and accessibility (Sponza and Kuscu, 2011; Qu et al., 2013). The objectives of this study are: firstly, to investigate the variation of sludge organic toxicity over the whole treatment process of 4-CP; secondly, to assess the influence on adsorption and biodegradation of 4-CP on sludge toxicity; thirdly, to analyse the correlation between sludge toxicity and different molecular-weight (MW) fractions of sludge in the stable operation stage of acclimated and control SBRs. The aim is to provide a reference for land application of excess sludge, which once had to be used to biodegrade recalcitrant pollutants.

2. Materials and methods

2.1. Chemicals

The 4-CP was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) at a purity of at least 98.0%. HPLC-grade methanol at 99.9% purity was provided by J & K Scientific Ltd. (Beijing, China) and ultrapure water was prepared in the laboratory using an ELGA ultrapure water machine (including water column) (ELGA, UK). An ultrafiltration (UF) membrane (diameter 80 mm) and MSC 300 ultrafiltration cup (effective volume 300 ml and maximum critical pressure 0.22–0.25 MPa) were supplied by Shanghai Mosu Science Equipment Co., Ltd. (Shanghai, China). All other chemicals applied were of analytical reagent grade and supplied by Shanghai Ling-Feng and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Seed activated sludge and composition of synthetic wastewater

Two 5-L beakers were used as laboratory-scale SBRs to culture the 4-CP degradation biomass. One SBR was an acclimated SBR formed by injecting 10 mg l^{-1} 4-CP, the other one was a control SBR without 4-CP. Seed activated sludge was obtained from a local municipal sewage treatment plant. Before injecting the SBR, the activated sludge was washed three times by using tap water and aerated for 24 h to eliminate organics therein. The influent was pumped to the SBR using a peristaltic pump. The mechanical mixers were operated for 2 h and then stopped for 2 h throughout its continuous operation cycle. The hydraulic retention time (HRT) and sludge retention time (SRT) were 12 h and 20 d, respectively. The operating temperature was maintained at a constant temperature (25 ± 1 °C) by placing the SBR on a heater and the influent mixed liquor suspended solids (MLSS) was controlled to 2500 ± 200 mg l^{-1}. The ratio of aeration/non-aeration in the SBR was maintained at 1:1. The aerobic process was aerated by an air-pump and diffusion aerator to maintain the dissolved oxygen (DO) concentrations at 1.5 ± 0.5 mg l^{-1}. The whole HRT was 0.25 h for influent, 11 h for operation, 0.50 h for settling and 0.25 h for discharge.

The influent COD concentration varied within 300 ± 20 mg l^{-1} with methanol. The composition of synthetic wastewater was prepared in tap water by dissolving metallic elements and N, and P to satisfy the nutrient requirements of activated sludge (Table 1). The formula was revised according to Seyhi et al. (2013). The appropriate pH (7.4 ± 0.2) was obtained by the addition of 180 mg l^{-1} $NaHCO_3$ to the influent.

2.3. Sample preparation and analysis

2.3.1. COD and MLSS analysis

After the SBR had settled, the supernatant was centrifuged at 4000 r min^{-1} for 5 min and the COD was measured in parallel according to APHA, 2002. MLSS was measured using a gravimetric method, i.e., 100 ml activated sludge mixture, which was sufficiently mixed, was acquired from the SBR, and then filtered through a filter paper which was oven-dried to constant mass at 105 °C.

2.3.2. 4-CP analysis

The 4-CP contents both in aqueous and sludge phases were measured in parallel by high-performance liquid chromatography (HPLC, LC-10ATVP, Kyoto, Japan) using a reversed-phase C-18 column (250 nm × 4.6 nm, 5 μm) as the stationary phase and a mixture of methanol (80%) and H_2O (20%, containing 1% acetic acid) as the mobile phase. The flow rate was maintained at 1 ml min^{-1} and a wavelength of 280 nm was used. The sample injection volume was 10 μl. The samples were pre-treated before performing the HPLC. Aqueous phase 4-CP content was directly measured through 0.45 μm filtration; sludge phase 4-CP content was measured through ultrasonic extraction.

2.3.3. Sludge toxicity bioassay

A luminescent bacteria bioassay was used to determine the sludge acute toxicity according to the standard methods of China (GB/T 15441-1995, China). The freeze-dried powder of *P. phosphoreum* was purchased from the Institute of Soil Science, Chinese Academy of Sciences (Nanjing, China). Before this bioassay, the dried powder was resuscitated using a sterilization medium which included: 0.5 g yeast extract, 0.5 g peptone, 3 g NaCl, 0.5 g Na_2HPO_4, 0.1 g KH_2PO_4, 0.3 g glycerol and 100 ml deionized water. The pH of the medium was adjusted to 7 ± 0.5 and sterilized for 20 min at 121 °C. The *P. phosphoreum* was used to measure sludge toxicity during the logarithmic phase.

Before sludge toxicity bioassay, the sludge flocs need to be crushed and undergo cell lysis because the sludge toxicity is likely to be reduced if its flocs are not collapsed (Ren, 2004). The pre-treatment method of sludge lysis proposed by Zhao et al. (2014) was used. Sludge toxicity was characterised by the percentage of inhibiting luminosity (Eq. (1-1)), and the variations of the visible light intensity were detected using a Toxicity Determinator (DXY-2, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China). Meanwhile, the sludge toxicity was measured over three replicates for each sludge sample so as to ensure accuracy.

Table 1
Composition of synthetic wastewater.

Compound	Concentration (mg l^{-1})
$MgSO_4 \cdot 7H_2O$	23.9
$CaCl_2 \cdot H_2O$	7.6
$FeCl_3 \cdot 6H_2O$	7.0
$CuSO_4 \cdot 5H_2O$	0.047
$MnSO_4 \cdot H_2O$	0.06
$ZnCl_2$	0.09
$CoSO_4 \cdot 7H_2O$	0.20
$Na_2MoO_4 \cdot 2H_2O$	0.05
$CO(NH_2)_2$	32
KH_2PO_4	13