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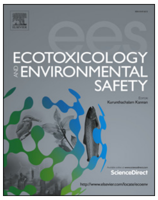

Impact of sludge conditioning treatment on the bioavailability of pyrene in sewage sludge

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ABSTRACT

Conditioning is an indispensable step to improve mechanical dewatering of municipal sewage sludge. However, it is still unclear how sludge conditioning treatments impact the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge that potentially influences the biodegradation of PAHs during the composting of dewatered sludge cake. In the present study, five sludge conditioning treatments, including chemical acidification, bioleaching driven by *Acidithiobacillus ferrooxidans*, chemical conditioning with Fe[III] and CaO, and chemical conditioning with either aluminum polychloride (PACl) or polyacrylamide (PAM), were investigated to reveal their respective impacts on the bioavailability of pyrene in sewage sludge. The bioavailability of pyrene in conditioned sludge was evaluated by using the *n*-butanol extraction method. The results showed that the bioavailable fraction of pyrene increased from 59.1% in raw sludge to 68.7% in chemically acidified sludge and 79.3% in bioleached sludge, while the other three conditioning approaches did not significantly change the bioavailability of pyrene. During chemical acidification or bioleaching of sludge, cellular membrane damage of sludge microbial cells induced changes in sludge chemical and physical properties. Ridge regression analysis revealed that during these two conditioning processes the contribution rates of the changes in sludge chemical properties and physical properties on the enhancement of pyrene bioavailability were 33.0% and 67.1%, respectively. Therefore, chemical acidification and bioleaching conditioning treatments can enhance the bioavailability of pyrene in sewage sludge, mainly through changing the relative hydrophobicity and particle size of sludge flocs.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are mainly discharged in wastewater during chemical industry processes. During wastewater treatment, only a small fraction of PAHs is degraded by microorganisms, while a large fraction is adsorbed onto sludge flocs or particles (Mailler et al., 2017). Consequently, PAHs are frequently detected in sewage sludge. For example, it was reported that the content of PAHs in sewage sludge can reach 1.4–33 mg/kg dried sludge (DS) in China (Cai et al., 2007) and 0.31–50 mg/kg DS in Europe (Suciu et al., 2015). Such high contents of PAHs usually restrict the use of sludge for agricultural land application (Oleszczuk et al., 2014), because of the ecotoxicity and environmental risk of PAHs to the environment. Thus, the Europe Union (EU) proposed that the content of nine PAHs should not exceed 6 mg/kg DS in sewage sludge for agricultural use (Suciu et al., 2015). In accordance with the directive draft of National Standards of the People's Republic of China, the total content of PAHs in sewage sludge used for agricultural reuse must be less than 5 mg/kg DS for Class A sludge and 6 mg/kg DS for Class B sludge (Cai et al., 2007).

Anaerobic digestion and composting are widely used to treat sewage sludge and convert it into biosolid or compost (Mowla et al., 2013). Unfortunately, many studies revealed that anaerobic digestion and composting cannot completely eliminate or degrade PAHs in sewage sludge because of the extremely low bioavailability of most PAHs. For instance, Ozaki et al. (2017) found that the contents of PAHs were only decomposed by 65% during the composting of sewage sludge. In addition, Aemig et al. (2016) reported that only phenanthrene was dissipated during the anaerobic digestion of sewage sludge, while the other PAHs, including fluoranthene, pyrene and benzo[b]fluoranthene, were not degraded because of their low bioavailability. The term bioavailability refers to the most available fraction for bioaccumulation, biosorption and/or uptake by organisms, which can be solubilized

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and/or easily extracted by mild solvent (Cachada et al., 2014). The bioavailability of PAHs is dependent on physicochemical properties (i.e., particle size and organic matter) of the matrix and their interactions with organisms. One way of effectively improving the degradability of PAHs is to enhance their bioavailability (Barret et al., 2010a; Bezza and Chirwa, 2017). In fact, Haudin et al. (2013) found that the fate and behavior of fluoranthene depend not only on its intrinsic properties but also on its distribution between the available and non-available fractions in composts. Moreover, Oleszczuk (2009) reported that the amount of PAHs disappearance during the composting of sewage sludge was linearly correlated with the bioavailable PAHs determined by using the *n*-butanol extraction method.

Sewage sludge usually contains very high content of moisture (around 98%), and thus, mechanical dewatering is necessary before the disposal of sewage sludge to remove moisture in sludge and reduce the volume of sludge (Lu et al., 2017, 2018). Due to the very low dewaterability of sewage sludge, sludge conditioning is indispensable prior to mechanical dewatering to drastically enhance the dewaterability of sewage sludge (Wang et al., 2015; Lu et al., 2018). To date, many approaches have been successfully applied to condition sewage sludge, such as chemical conditioning with Fe[III] and CaO (Deneux-Mustin et al., 2001), chemical conditioning with either aluminum polychloride (PACl) (Niu et al., 2013) or polyacrylamide (PAM) (Fu et al., 2010), chemical acidification (Chen et al., 2004; Lu et al., 2017), and bioleaching conditioning driven by *Acidithiobacillus ferrooxidans* (Zheng et al., 2016; Lu et al., 2018). Previous studies found that some sludge conditioning approaches, including chemical acidification or bioleaching conditioning, led to serious lysis of microbial cells in sludge and the content increases of sludge extracellular polymeric substances (Lu et al., 2017, 2018). Consequently, physical and chemical properties of sewage sludge, including the dissolved organic matter (DOM) content in the liquid phase of sewage sludge, total organic matter (TOM) content in the solid phase of sewage sludge, relative hydrophobicity and particle size of sludge flocs, may be greatly changed during chemical acidification or bioleaching treatment of sewage sludge. These changes have been proved to relate with the transformation, sorption and desorption of organic contaminants in soils, sediments and sludge (Ling et al., 2009; Barret et al., 2010a, 2011). As a result, it is speculated that some conditioning processes may enhance the bioavailability of PAHs in sewage sludge, facilitating the degradation of PAHs during composting treatment of dewatered sludge cake. However, the detailed impact of sludge conditioning treatment on the bioavailability of PAHs in sewage sludge has not been investigated previously, and it remains unclear how the changes in sludge properties induced by conditioning treatments influence the bioavailability of PAHs in sewage sludge.

Pyrene is extensively distributed in municipal sewage sludge collected from different countries (Suciu et al., 2015; Cai et al., 2007), and Oleszczuk (2009) reported that pyrene accounted for 21.8–48.6% of the total content of ten PAHs in four municipal sewage sludge samples collected throughout Poland. Thus, the present study aims to (1) study the impacts of various sludge conditioning treatments on the bioavailability of pyrene in sewage sludge; (2) investigate the changes in sludge properties during chemical acidification and bioleaching conditionings of sewage sludge; and (3) use ridge regression analysis to find the dominant factors impacting the bioavailability of pyrene during sludge conditioning treatments. The findings of the present study contribute to increasing the bioavailability of pyrene in sludge and, thus, potentially enhancing the biodegradation efficiency of PAHs during the composting of dewatered sludge cakes.

2. Materials and methods

2.1. Reagents

Pyrene (purity ≥ 98%) was purchased from Sigma-Aldrich Chemical Co., Ltd. Dichloromethane (DCM), acetone, *n*-butanol, acetonitrile and *n*-hexadecane were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) at analytical grade.

2.2. Preparation of pyrene-contaminated sewage sludge

The municipal sewage sludge used in this study was collected from a sludge thickening-pond of the Taihu New City Wastewater Treatment Plant in Wuxi City, Jiangsu Province, China. The pH (7.54), solid content (3.61%), and organic matter content (52.1%) of the sludge were determined immediately upon collection, following to the standard methods (APHA, 2005). Sewage sludge was freeze-dried and passed through a 0.2 mm sieve in order to improve its homogeneity. Pyrene was dissolved in acetone and spiked into the sludge to obtain sewage sludge contaminated with 18.91 mg/kg of pyrene, which is within the contamination range of pyrene in municipal sewage sludge collected from Jiangsu Province, China (Zhang et al., 2008). To ensure the even distribution of pyrene in the contaminated sludge, this artificially contaminated sludge was stirred vigorously using a glass rod for 30 min. The acetone in the mixture was allowed to evaporate for one week at 30 °C in a fume hood, and the contaminated sludge was aged for 30 days at room temperature. Before conditioning treatments, supernatant of fresh sewage sludge was mixed with the contaminated sludge to obtain pyrene-contaminated sewage sludge with pH 7.50 and solid content 3.03%.

2.3. Conditioning treatment of pyrene-contaminated sewage sludge

Acidophilic chemoautotrophic bacteria *A. ferrooxidans* LX5 (CGMCC No. 0727) were cultivated in modified 9 K medium and then spiked with 44.2 g/L of 0.22 µm membrane-filtered $FeSO_4·7H_2O$ as the energy source (Zheng et al., 2009). The inoculum was cultured at 180 rpm and 28 °C until the density of *A. ferrooxidans* LX5 reached approximately 10^8 cells/mL (approximately 48 h).

The bioleaching experiments were conducted in 1000 mL Erlenmeyer flasks containing 450 mL pyrene-contaminated sewage sludge and 50 mL *A. ferrooxidans* LX5 culture as the inoculum. Each flask was added with 10 g/L $FeSO_4·7H_2O$ as the energy source. All flasks were shaken in a gyratory shaker at 180 rpm and 28 °C. The change of sludge pH during bioleaching treatment is shown in Table S1. Sludge samples were collected at 0.5, 4, 24, and 48 h, and the pH of each sludge sample was 5.53, 4.51, 3.54, and 2.52, respectively. The bioleaching conditioning of pyrene-contaminated sewage sludge was accomplished until the sludge pH dropped to around 2.50 (Zheng et al., 2016).

The chemical acidification conditioning was conducted in 500 mL Erlenmeyer flasks containing 250 mL pyrene-contaminated sewage sludge. The sludge was acidified to pH 5.50, 4.50, 3.50 and 2.50 with sulfuric acid (9.20 M, AR). The flasks were shaken in a gyratory shaker at 180 rpm and 28 °C for 30 min. Owing to the buffering capacity of sludge, the above procedure was repeated until the respective sludge pH value was stable. Our previous study revealed that chemical acidification to pH 2.50 is optimum for achieving the best dewatering performance of sewage sludge (Lu et al., 2017), and thus the chemical acidification treatment was completed when the sludge pH dropped to around 2.50.

The method for chemical conditioning with Fe[III] and CaO was adopted from Deneux-Mustin et al. (2001). At a dosage of 0.3 g CaO/g DS, 2.27 g CaO was added to 500-mL Erlenmeyer flasks, each containing 250 mL of pyrene-contaminated sewage sludge. All flasks were shaken at 28 °C and 180 rpm for 30 min, in a gyratory shaker, to ensure the full reaction. Subsequently, 1.75 g $FeCl_3·6H_2O$ was added to the above sludge at a dosage of 0.08 g Fe[III]/g DS, and then flasks were shaken for 30 min at 28 °C and 180 rpm, in a gyratory shaker, to accomplish the whole conditioning.

Chemical conditioning with PAM was conducted according to the method recommended by Fu et al. (2010). PAM was prepared as an