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Estrogenic activity and identification of potential xenoestrogens in a coking wastewater treatment plant

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ABSTRACT

In this study, the estrogenic activities in influent and effluents of coking wastewater from different treatment stages were studied using Yeast Estrogen Screen (YES) bioassays. Raw extracts were further fractioned to identify the potential xenoestrogens combined with YES bioassays and gas chromatography–mass spectrometry analysis. Influent, primary effluent, and anaerobic effluent showed high estrogenic activities, with potencies of 1136 ± 269, 1417 ± 320, and 959 ± 69 ng/L of 17β-estradiol (E2) equivalent (EEQ), respectively. The potency of estrogenic activity was gradually removed through the treatment processes. In the final effluent, the estrogenic activity was reduced to 0.87 ng EEQ/L with a total removal efficiency of more than 99%, suggesting that the estrogenic activity was almost completely removed in the coking wastewater. For the fractions of raw extracts, bioassay results showed that the estrogenic activities were mostly present in the polar fractions. Correlation analysis between estrogenic activities and responses of identified chemicals indicated that potential xenoestrogens were the derivatives of indenol, naphthalenol, indol, acridinone, fluorenone, and carbazole. The estrogenic activity in the final effluent was higher than the predicted no effect concentration (PNEC) for E2, implying that the discharged effluent would probably exert estrogenic activity risk to the aquatic ecosystem in "the worst-case scenario."

1. Introduction

Industrial wastewaters are the main pollution sources for the presence of toxicants in the receiving environment. Coking wastewater is one major type of industrial wastewater in China. The discharge amounts of chemical oxygen demand (COD), ammoniacal nitrogen (NH_3–N), and oil from coking wastewater effluents in China are 125 kt, 19 kt, and 2 kt every year, respectively, which accounts for 2.5%, 4.6%, and 8.5% of the corresponding total national industrial discharges (Wei et al., 2011; Ren et al., 2007). Coking wastewater has a very complex chemical composition. Various pollutants, including metals, phenols, cyanides, polycyclic aromatic compounds, and nitrogen-, oxygen-, and sulfur-containing heterocyclic compounds, have been found in coking wastewater (Ren et al., 2007; Kim et al., 2008; Marañón et al., 2008; Zhang et al., 2013; Wei et al., 2011). Thus, coking wastewater leads to toxic effects on organisms in the environment due to the presence of mixed pollutants. Crude coking wastewater showed inhibition effect on the growth and embryo development of maize (*Zea mays* L.) seed (Han et al., 2011; Wei et al., 2012). Effluents of coking wastewater from different treatment stages have also displayed toxic effects on Japanese medaka (*Oryzias latipes*) (Zhu et al., 2013).

In recent years, hormone activities caused by xenobiotics from industrial wastewaters have attracted great concern due to their adverse effects on aquatic organisms even at low activity units (Hewitt et al., 2006; Wartman et al., 2009). Estrogenic activity is the commonly screened hormone activity in industrial wastewaters (Schilirò et al., 2012; Bazin et al., 2012;). Textile industrial wastewaters have reported weak estrogenic activity, because textile dyes have displayed estrogenic toxicity at the potency of several ng/L of 17β-estradiol (E2) equivalent (EEQ) quantity (Schilirò et al., 2012; Bazin et al., 2012). Industrial effluents of textile and dyeing plants, pulp and paper mills, fine chemical factories, and municipal wastewater treatment plants (WWTPs) in the Pearl

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River Delta Region were also screened estrogenic activity up to several tens of ng EEQ/L (Fang et al., 2012a). However, the knowledge of estrogenic activity in coking wastewaters is still lacking. In light of the large discharge volume of coking wastewater in China (Wei et al., 2011; Ren et al., 2007), it is necessary to evaluate the estrogenic activity of coking wastewater for assessing the removal efficiency of estrogenic activity and the potential risk to the environment before the final effluent is discharged (USEPA, 1991).

Toxicity identification and evaluation (TIE) (USEPA, 1992; Petrović et al., 2004; Fang et al., 2012b) and effective directed analysis (EDA) (Brack et al., 2003) have been the commonly used methods to identify the potential toxic substances in complex matrices. The EDA method usually focuses on the identification of organic extracts combined with fast in vitro bioassay and chemicals analysis (Brack et al., 2007), which has been successfully applied to identify the potential toxic substances in environmental samples after the fractioning of extracts (Burnison et al., 2003; Nakada et al., 2004; Oh et al., 2009; Luo et al., 2011). The identification of estrogenic substances in municipal effluents has been well demonstrated, and the potencies are usually contributed by some known estrogens, such as natural estrone and estradiol, and synthetic ethinylestradiol (Metcalfe et al., 2001). While the potential estrogenic substances in industrial wastewater were mostly the xenoestrogens, such as nonylphenol and its ethoxylates in textile wastewater (Sumpter et al., 2006) and bisphenol A in effluent of pulp and paper mills (Fernandez et al., 2007). So far, the potential xenoestrogens in coking wastewater are still not well studied. The derivatives of polycyclic aromatic hydrocarbons and carbazole showed estrogenic activity (Von Angerer and Prekajac, 1986; Liu et al., 1994; Machala et al., 2001), which were also regularly detected in coking wastewater. Hence, these pollutants may be the potential xenoestrogens in coking wastewater.

The aim of the present study is to evaluate the estrogenic activity of coking wastewaters from different treatment stages using the in vitro recombinant yeast estrogen screen (YES) method. Removal efficiency of estrogenic activity in the wastewater treatment plant was evaluated. Raw extracts of coking wastewaters were further fractioned by high performance liquid chromatography (HPLC), according to the EDA method. Scanning of raw extracts and fractions for the potential toxic substances was conducted using gas chromatography–mass spectrometry (GC–MS). The potential xenoestrogens were identified by correlation analysis of the estrogenic potency and the response of pollutants in different fractions. The results are contributed to understanding the potential estrogenic substances in the coking wastewater and potential risk to the environment.

2. Materials and methods

2.1. Coking wastewater treatment plant and samples

The coking wastewaters tested in this study were sampled from a WWTP at the Songshan coking plant in Shaoguan city in the Guangdong Province of South China. The WWTP receives effluents from ammonia stilling and cleaning plants, and has a treatment capacity of 2000 m^3/d. The treatment processes used in the WWTP include primary treatment, biological treatment, and coagulation treatment. In primary treatment, a flotation-degreasing tank coupled with an equalization basin is used to separate particles and tar from the raw influent. Then, the primary effluent is subjected to biological treatment, which is composed of an anoxic–oxic–hydrolytic–oxic system coupled with a biological fluidized-bed. In coagulation treatment, the biological effluent is mixed with a polyacrylamide (PAM) and polyferric sulfate (PFS) solution for 4 min. Then, the mixed effluent enters into the secondary clarifier with a hydraulic retention time of 4 h. After these treatments, the final effluent is discharged.

The schematic diagram of the coking WWTP and sampling sites are shown in Fig. 1. Sampling was carried out in June 2012. Twenty-four hour composite water samples of raw influent, primary effluent, biological effluent (anaerobic effluent, the first aerobic effluent, hydrolytic effluent, and the second aerobic effluent), and final effluent were collected using flow proportional samplers (cooled at 4 °C) with a sampling interval of 2 h. Water samples were transported to the laboratory in ice coolers and stored at 4 °C until treatment.

2.2. Sample extraction and fraction

One litre of each water sample was filtered by GF/F glass fiber filters (0.7 μm pore size, Whatman). A solid phase extraction (SPE) method was used to enrich the organic pollutants using Waters Oasis HLB cartridges (6cc, 500 mg sorbents, Waters), because the HLB cartridge could simultaneously enrich a large range of substances (from nonpolarity to high polarity) in industrial and municipal wastewaters (Fang et al., 2012a; Fernandez et al., 2007). The HLB cartridge was first conditioned with 10 mL of methanol and 10 mL of Milli-Q water in sequence. Then, 1 L of wastewater was loaded on the HLB cartridge at a speed of 3–5 mL/min. After the sample was loaded, 10 mL of Milli-Q water was used to rinse the cartridge, and the cartridge was dried under a vacuum for 2 h and eluted by 10 mL of dichloromethane and 10 mL of methanol in sequence. The dichloromethane and methanol elutes were combined and dried under a gentle nitrogen stream. The extract was reconstituted in 1 mL of methanol for fractioning and bioassay.

Extracts of coking wastewater were further fractioned using HPLC, which was conducted using an Agilent 1200 HPLC system

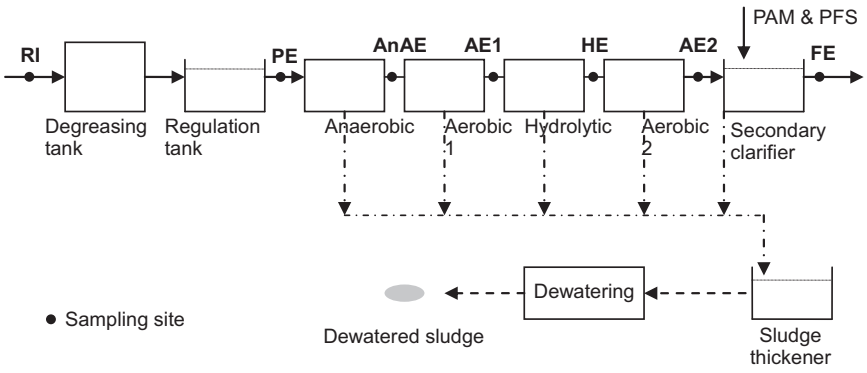

Fig. 1. Flowchart of coking wastewater treatment plant (WWTP) and sampling sites. RI: raw influent; PE: primary effluent; AnAE: anaerobic effluent; AE1: first aerobic effluent; HE: hydrolytic effluent; AE2: second aerobic effluent; FE: final effluent. PAM & PFS: polyacrylamide (PAM) and polyferric sulfate (PFS) solution.