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Biological removal of antiandrogenic activity in gray wastewater and coking wastewater by membrane reactor process

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ABSTRACT

A recombinant human androgen receptor yeast assay was applied to investigate the occurrence of antiandrogens as well as the mechanism for their removal during gray wastewater and coking wastewater treatment. The membrane reactor (MBR) system for gray wastewater treatment could remove 88.0% of antiandrogenic activity exerted by weakly polar extracts and 97.3% of that by moderately strong polar extracts, but only 32.5% of that contributed by strong polar extracts. Biodegradation by microorganisms in the MBR contributed to 95.9% of the total removal. After the treatment, the concentration of antiandrogenic activity in the effluent was still 1.05 μg flutamide equivalence (FEQ)/L, 36.2% of which was due to strong polar extracts. In the anaerobic reactor, anoxic reactor, and membrane reactor system for coking wastewater treatment, the antiandrogenic activity of raw coking wastewater was 78.6 mg FEQ/L, and the effluent of the treatment system had only 0.34 mg FEQ/L. The antiandrogenic activity mainly existed in the medium strong polar and strong polar extracts. Biodegradation by microorganisms contributed to at least 89.2% of the total antiandrogenic activity removal in the system. Biodegradation was the main removal mechanism of antiandrogenic activity in both the wastewater treatment systems.

Introduction

Recently, numerous examples of adverse effects of endocrine disrupting chemicals (EDCs) in invertebrates, fish, wildlife, domestic animals, and humans have aroused great interest and concern in scientists (Hotchkiss et al., 2008). Besides the well-documented environmental estrogens, some researchers have focused on additional mechanisms of endocrine toxicity, *e.g.*, antiandrogens. Since vinclozon was found to be an antiandrogen in 1994 (Gray et al., 1994; Kelce et al., 1994), a series of compounds had been proven to produce antiandrogenic effects *in vitro* and *vivo*, *e.g.*, phthalate esters, phenols, parabens, pesticides, and so on. Environmental antiandrogens could adversely affect the development of androgen-dependent tissues (Monosson et al., 1999), and cause malformations of male reproductive organs (Foster, 2006), and depression of androgen production (Blystone et al., 2007), as well as other demasculinization effects.

Antiandrogens have been found to be prevalent in various environments. Antiandrogenic activity has been detected in many kinds of wastewater and solid samples, *e.g.*, effluents from domestic wastewater treatment plants (Rostkowski et al., 2011), oil production platforms (Tollefsen et al., 2007; Thomas et al., 2009), chemical industry wastewater (Shi et al.,

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2009), and river water as well as river sediment (Urbatzka et al., 2007). Moreover, many antiandrogens were found in different kinds of industrial wastewater, although their antiandrogenic activities have not been reported. Pothitou and Voutsa (2008) found that an important antiandrogen, triclosan, was present in textile wastewater and tannery wastewater. Some of the estrogenic chemicals can exert androgen receptor antagonistic activity, *e.g.*, bisphenol A, as well as butyl benzyl phthalate (Sohoni and Sumpter, 1998; Kolle et al., 2010). These estrogenic chemicals are also widely present in wastewater. Butyl benzyl phthalate was found in 13 of a total of 18 investigated sewage treatment plant effluents, with concentrations between 80 and 450 ng/L (Spengler et al., 2001). Bisphenol A was found in textile wastewater (Pothitou and Voutsa, 2008), paper mill wastewater (Balabanic et al., 2012), livestock effluent (Ahn et al., 2012), and mixed industrial wastewater (Sánchez-Avila et al., 2009).

Graywater is wastewater from households, hotels, and schools, as well as some types of industries, where no contributions from toilets or heavily polluted process water are included (Eriksson et al., 2002). Graywater has been considered to have high reuse potential since graywater was found to contribute only 9%–14%, 20%–32%, 18%–22%, and 29%–62% of nitrogen, phosphorus, potassium, and organic matter in domestic wastewater, respectively (Ghunmi et al., 2011). The main xenobiotic source in gray wastewater is the chemicals used in households, *e.g.*, laundry detergents, dish-washing liquids, cleaning detergents, shampoos, soaps and toothpastes (Eriksson et al., 2003). Some antiandrogens, *e.g.*, triclosan, 4-nonylphenol, bisphenol A, octocrylene, butylparaben, propylparaben, benzophenone-3, and 3,4-dichlorophenol, were found in gray wastewater (Leal et al., 2010). We had previously reported the occurrence and removal of antiandrogenic activity of gray wastewater in a MBR treatment system (Ma et al., 2013). Nevertheless, further research is still needed on the specific properties of antiandrogens in gray wastewater and their removal in the treatment system.

Coking wastewater is produced during the coal coking, coal gas purification, and by-product recovery processes of coke factories (Zhang et al., 1998). High concentrations of ammonia and organic compounds, especially refractory and inhibitory organics, are two main problems in coking wastewater treatment. However, it should be noted that there might also be some endocrine-disrupting chemicals at high concentrations in this wastewater. In coking wastewater, about 80% of the total COD derives from phenolic organic pollutants. Many phenols have been proven to exert androgen antagonistic activity, *e.g.*, 2-naphthol (Rostkowski et al., 2011), 2-tert-butylphenol (Li et al., 2010), and 4-n-dodecylphenol (Satoh et al., 2005) were shown to be antagonists for androgen receptors. In addition, some antiandrogenic phenols, *e.g.*, 2,4-dichlorophenol and 2-naphthol, have been detected in coking wastewater as well as its treatment system. Thus, it is necessary to investigate the occurrence of antiandrogens in coking wastewater and their removal in the treatment system. To date, studies on the antiandrogens of coking wastewater and their removal during the treatment systems are relatively few. Thus, further investigations are required.

In the current study, the occurrence of antiandrogenic activity during gray wastewater treatment system and coking wastewater treatment system was investigated. In order to investigate the removal of antiandrogens during wastewater treatment processes, the recombinant human androgen receptor (AR) yeast assay was applied to study the antiandrogenic activity in a graywater treatment system and a coking wastewater treatment system. The recombinant human androgen receptor (AR) yeast assay has been proven to be a rapid, efficient, and economical tool to assess the antiandrogenic activity of chemicals and environmental samples (Sohoni and Sumpter, 1998; Rostkowski et al., 2011). Both the gray wastewater and coking wastewater treatment systems employed the MBR as the main process. The objectives of this study were (1) to enrich the knowledge of antiandrogens in coking wastewater, (2) to compare the transport of different polar antiandrogens during the treatment, and (3) to assess the effects of biodegradation on the removal of antiandrogenic activity in the two treatment systems. Our results provide new data for understanding the existence of antiandrogens in the wastewater and the removal during the treatment.

1. Method and material

1.1. Chemicals

Flutamide (DHT, purity ≥ 99%), tert-butyl methyl ether (MTBE, purity ≥ 99.8%), and dimethyl sulfoxide (DMSO, purity ≥ 99.8%) were obtained from Sigma Chemical Co., St Louis, MO, USA. The androgen receptor ligand dihydrotestosterone (DHT, purity ≥ 94.5%) was purchased from Dr. Ehrenstorfer, Augsburg, Germany. HPLC grade solvents, including hexane, dichloromethane, and methanol, were purchased from Fisher Scientific Worldwide Company Limited, Shanghai, China.

1.2. Wastewater treatment systems and sample collection

Gray wastewater was obtained from dormitory buildings on the test campus in Beijing, China. The capacity of the pilot gray wastewater treatment system is 1200 m^3/day. The treatment employed a membrane bioreactor (MBR) as the main process, with a sludge age of 20 days and effective MBR volume of 250 m^3. Raw coking wastewater was collected from the regulation tank of the coking wastewater treatment plant of an iron and steel corporation in China, which had been pretreated. The supernatant of the raw coking wastewater was introduced to the treatment system. The lab-scale coking wastewater treatment system consists of an anaerobic reactor (A1), an anoxic reactor (A2), and an aerobic MBR, as shown in Fig. 1. The reactors were operated at a hydraulic retention time of 73 hr and no sludge was discharged from the system. Table 1 shows the basic parameters of the two treatment systems. Both of the treatment systems had been run steadily for at least 3 years. Mixed water samples were taken from the regulation tank (0.5 L, G_RW), the aerobic tank (2 L, G_SW), and the reclaimed water tank (2 L, G_EF) of the gray wastewater treatment system. A suspended solid sample (108 mg, G_SS) from the regulation tank and sludge sample (4 g dry weight (dw), G_SL) from the aerobic tank were also collected. The effluents from each of the three reactors of the coking wastewater treatment system were collected. Samples are referred to as C_RW (100 mL, raw coking wastewater), C_A1 (100 mL, effluent from anaerobic reactor), C_A2 (100 mL,

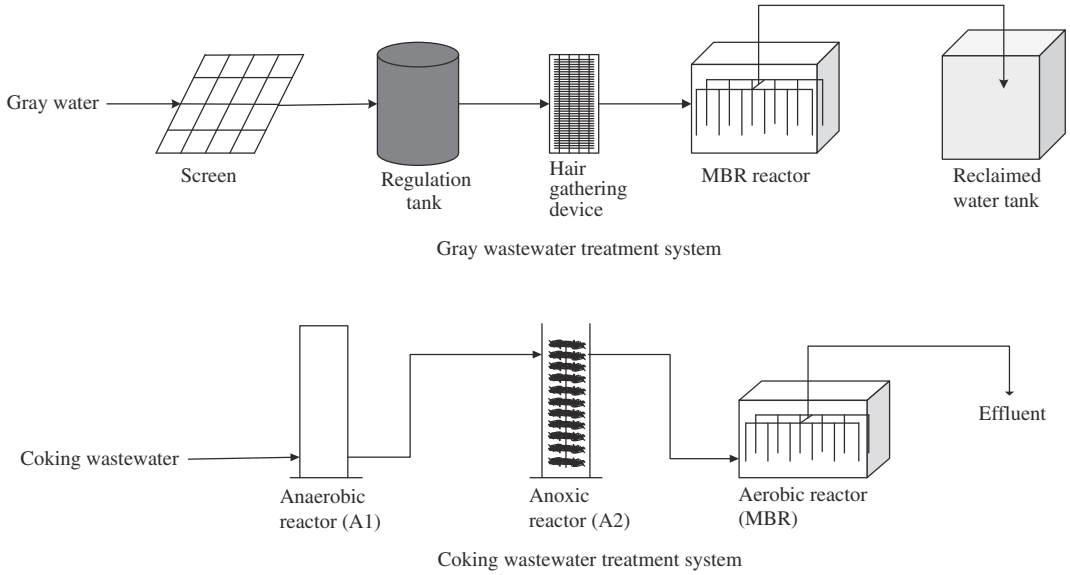

Fig. 1 – Schemes of the two investigated treatment systems.

effluent from anoxic reactor) and C_MBR (500 mL, effluent from aerobic MBR). Samples were taken in solvent-rinsed glass containers, which were soaked overnight in 10% nitric acid and soaked in chromic acid solution for 30 min, then washed three times with double-distilled water and methanol. Before the collection, the containers were also washed three times with the sample water.

1.3. *Samples pretreatment*

Pretreatment was conducted according to the previous study with proper modification (Ma et al., 2013). In order to suppress possible biotic activity, an appropriate amount of methanol (2 mL/L) was added to each sample right after the sampling. Samples were processed immediately upon arrival in the laboratory. During treatment, all the samples and procedure blank were first filtered with GF/F glass fiber filters (Whatman GF/F, GE Healthcare Life Sciences, Maidstone, UK). The solid samples (G_SS and G_SL) were lyophilized in a freeze drier (FD-1, Boyikang Laboratory Instrument Company, Beijing, China) and extracted by methanol (15 mL/g) in an ultrasonic instrument. Afterwards, the supernatants from the same sample were combined and redissolved in 2 L of ultrapure water, obtained from an ultrapure water system (Milli-Q Gradient, Millipore Corporation, Billerica, MA 01821, USA). The reconstituted solid samples and the water samples were introduced to Oasis HLB cartridges (6 mL/500 mg, Waters Corporation, USA) on a vacuum extraction manifold to extract the analytes at a flow rate of approximately 6 mL/min. The HLB cartridges were successively conditioned with 5 mL of dichloromethane, 5 mL of methanol, and 5 mL of deionized water twice before the solid phase extraction (SPE). After the samples were introduced to the cartridges, they were then kept under vacuum aspiration for 20 min to dry any residual water. The cartridges of samples from gray wastewater treatment were serially washed with 5 mL of hexane/dichloromethane (7/3, V/V) twice, 5 mL of tert-butyl methyl ether (MTBE) twice, 5 mL of dichloromethane/methanol (9/1, V/V) twice, and 5 mL of methanol. The cartridges of samples from the coking wastewater treatment were serially washed with 5 mL of hexane twice, 5 mL of dichloromethane twice, and 5 mL of methanol twice. The wash was conducted at a flow rate of 1 mL/min. Then the extracts of some samples were combined. The combined samples and separated fractions were dried by anhydrous sodium sulfate to remove water, and then evaporated to 1 mL in a rotary evaporator (RE-2000B, Yarong Company, Shanghai, China). The dehydrated extracts were blown to dryness under a gentle nitrogen flow. The residues were redissolved in 0.2 mL of DMSO and stored at −20°C for the yeast bioassay. Ultrapure water was treated by the same procedure as samples as the procedure blank.

1.4. *Yeast bioassay procedure*

The antiandrogenic activity of the samples was quantified using a recombinant human androgen receptor (AR) yeast

Table 1 – Basic parameters of the two investigated wastewater treatment systems.

Parameters	Gray water treatment	Coking wastewater treatment
Capacity (m^3/day)	1200	0.006
COD of influents (mg/L)	106–229	1490–2100
Total nitrogen of influents (mg/L)	12.3–38.7	170–270
COD of effluents (mg/L)	7–14	197–217
Total nitrogen of effluents (mg/L)	9–27	30–140

assay (YAS). This assay has been proven to be a rapid, efficient, and economical tool to assess the antiandrogenic activity of chemicals and environmental samples (Sohoni and Sumpter, 1998; Rostkowski et al., 2011; Li et al., 2008). The yeast assay was carried out as described by Routledge and Sumpter (1996) and Ma et al. (2013) with some modifications. The extracts were assayed in a 96-well plate including a positive control (10^{-8} mol/L DHT) and a negative control (DMSO). The androgen receptor antagonistic activities were detected by co-incubating with the natural androgen DHT (10^{-8} mol/L). The enzyme reaction was started by adding o-nitrophenyl-â-D-galactopyranoside (4 mg/mL) and terminated by adding Na_2CO_3 (1 mol/L). Some toxic chemicals in the extracts could inhibit the growth of yeast cells, resulting in the â-galactosidase activity inhibition. Thus, the cell density (OD_{600}) in the assay medium was determined as a measure of the change in cell viability. In order to evaluate the androgen receptor antagonistic effects of the samples, the flutamide equivalence (FEQ) was calculated as described by Wu et al. (2002). FEQ was only calculated when the samples showed no toxicity toward the yeast.

2. Results

2.1. Occurrence of antiandrogenic activity in gray wastewater treatment system

Androgen receptor antagonistic activity was detected in most of the fractions of water and solid samples in the gray wastewater treatment system (Fig. 2). For the raw gray wastewater (G_RW), the antiandrogens were concentrated in the extracts eluted by the mixture of hexane and dichloromethane (7/3, V/V) and the mixture of dichloromethane and methanol (9/1, V/V). The antiandrogenic activity of the two extracts constituted 83.6% of the overall 0.67 mg FEQ/L. The antiandrogenic activity of the extract eluted by the mixture of dichloromethane and methanol (9/1, V/V) was highest, about 10 times higher than the lowest extract, eluted by MTBE. However, for the supernatant in the aerobic tank (G_SW), and the effluent (G_EF), the extracts eluted by the mixture of hexane and dichloromethane (7/3, V/V) exerted the highest antiandrogenic activity of the four fractions. The antiandrogenic activities of the MTBE extracts in all of the three water samples were the lowest. As for the two solid samples, the antiandrogenic activities of extracts eluted by the mixture of hexane and dichloromethane (7/3, V/V) were the highest, followed by the extracts obtained with the mixture of dichloromethane and methanol (9/1, V/V). The overall antiandrogenic activity of the suspended solid sample (G_SS) was 2.0 mg FEQ/g, which is about 7 times higher than that of the sludge sample (G_SL).

2.2. Occurrence of antiandrogenic activity in coking wastewater treatment system

Androgen receptor antagonistic activity was detected in all four of the water samples and most of the fractions in the coking wastewater treatment system (Fig. 3). The A1–A2-MBR treatment system could remove the antiandrogenic activity of the coking wastewater along the process, as shown in Fig. 3. The antiandrogenic activity of raw coking wastewater was 78.6 mg FEQ/L, and the effluent of the treatment system had only 0.34 mg FEQ/L, which was only 0.4% of the activity of the influent. For all four samples, extracts eluted by methanol exerted the highest antiandrogenic activities, which were slightly higher than extracts eluted by dichloromethane. The antiandrogenic activities of extracts by hexane were much lower than the other two fractions. In addition, the sum of the antiandrogenic activities of the three fractions was higher than that of the samples. Overall, the anaerobic reactor and anoxic reactor respectively removed 43.4% and 45.8% of the antiandrogenic activity inflow, and the MBR reactor only accounted for 10.4% of the removal of the antiandrogenic activity. In other words, 99.2% of the total antiandrogenic activity was removed by the whole treatment system.

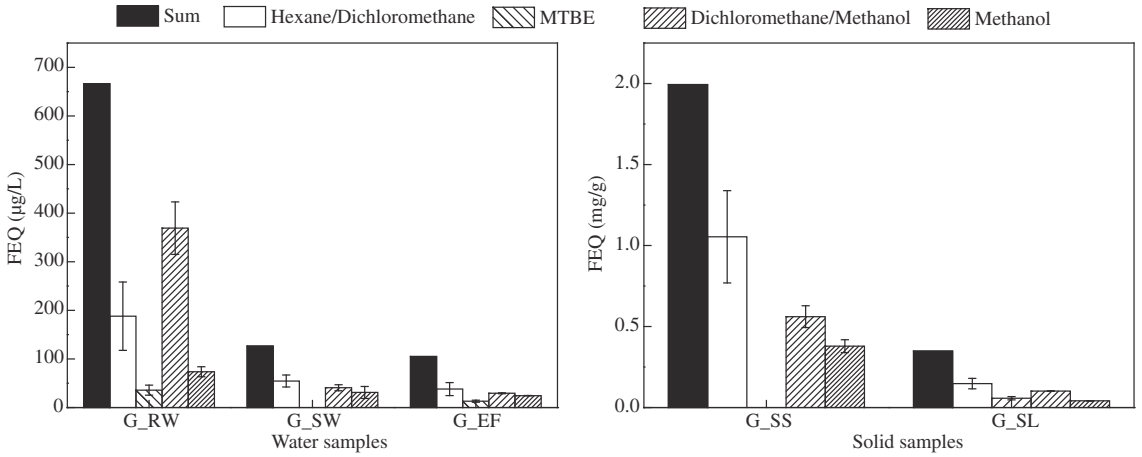

Fig. 2 – Antiandrogenic activity of different fractions in gray wastewater treatment system. FEQ: flutamide equivalence; MTBE: tert-butyl methyl ether; G_RW, G_SW and G_EF refer to mixed water samples taken from the regulation tank, the aerobic tank and the reclaimed water tank, respectively; G_SS: suspended solid sample from the regulation tank; G_SL: sludge sample from the aerobic tank.

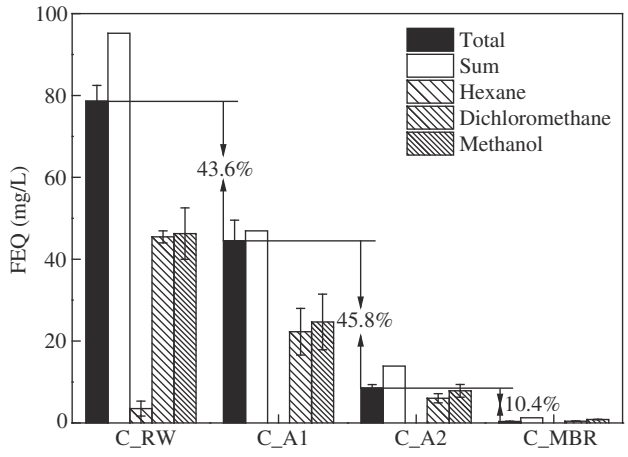

Fig. 3 – Antiandrogenic activity of samples in coking wastewater treatment. C_RW, C_A1, C_A2 and C_MBR refer to samples from raw coking wastewater, effluent from anaerobic reactor, effluent from anoxic reactor and effluent from aerobic membrane reactor (MBR), respectively.

3. Discussion

3.1. Removal of antiandrogenic activity in gray wastewater and coking wastewater treatment systems

In this study, antiandrogenic activity was detected in all of the samples from gray wastewater and coking wastewater treatment systems. We reported the occurrence and removal of the antiandrogenic activity of gray wastewater by the same treatment system previously (Ma et al., 2013). The antiandrogenic activity of gray wastewater was 1.1 mg FEQ/L, and the sum of the four fractions of the graywater in this study was slightly less, at 0.66 mg FEQ/L. The antiandrogenic activity sum of the four fractions of effluent in this study was 1.05 μg FEQ/L, which was slightly higher than the antiandrogenic activity of the effluent (0.54 μg FEQ/L) reported before. As for the solid samples, the antiandrogenic activity sum of the four fractions of suspended solids was a little less than the total sample antiandrogenic activity we reported before (Ma et al., 2013). However, the sludge sample was contrary to the suspended sample, that is, the antiandrogenic activity sum of the four fractions of sludge was higher than the total sample antiandrogenic activity. Thus, the sum of the antiandrogenic activities of the fractions was not always consistent with that of the total sample, which also occurred in HPLC fractions (Rostkowski et al., 2011). Differences between the sum of the fractions and the total samples could be due to a number of factors; that either there were combined effects between different antiandrogens in different fractions or that androgens in different fractions together could mask the detection of antiandrogenic activity. The combined effects of mixed antiandrogens are complicated, including synergistic effects, antagonistic effects, and interaction (Ma et al., 2014). The sums of activities of different fractions were slightly higher than that of the total samples, as shown in Fig. 3. It was speculated that an antagonistic effect existed in the samples. As a matter of fact, many antiandrogens were found in the wastewater with different concentrations (Leal et al., 2010), and other kinds of combined effects might exist.

The polarity of the eluents for samples of the gray wastewater treatment system and the compounds in the extracts increased. The extracts eluted by the mixture of hexane and dichloromethane and MTBE contained mainly weakly polar compounds. The moderately strong polar compounds were eluted by the mixture of dichloromethane and methanol, and the strong polar compounds were eluted by methanol. It could be inferred from Fig. 2 that the antiandrogenic activity of the samples in the gray wastewater treatment system, including the water samples and the solid samples, was concentrated in the weakly and moderately strong polar extracts. The results were coincident with a previous study, in which most of the potential antiandrogens in graywater had octanol–water partition coefficients over 3.0 (Ma et al., 2013).

This is the first time that the antiandrogenic activity of coking wastewater has been reported. As mentioned before, some antiandrogenic phenols were detected in coking wastewater as well as its treatment system. 2,4-Dichlorophenol might contribute antiandrogenic activity of 5.0–39.5 ng FEQ/L in coking wastewater (Tamura et al., 2006; Czaplicka, 2003). 2-Naphthol was also found in a coking wastewater treatment system, with a concentration of 2.937 mg/L in the raw water and 5.948 μg/L in the discharged water (Zhang et al., 2010). It might contribute antiandrogenic activity of 0.94 mg FEQ/L in the raw coking wastewater and 1.9 μg FEQ/L in the discharged water (Rostkowski et al., 2011). Thus, 2,4-dichlorophenol and 2-naphthol could account for about 4.9% of the total antiandrogenic activity in the coking wastewater and 2.3% of that of the effluent of the biological wastewater treatment system. Other suspected antiandrogens in the coking wastewater treatment system might be alkylphenols. Some previous studies reported the existence of alkylphenols in coking wastewater, but they were mainly the short chain alkyl phenols (Zhang et al., 2013). As mentioned before, some long chain alkyl phenols have been proven to be androgen receptor antagonists. Thus, more work should be done on the long chain alkylphenols and other suspected antiandrogens in coking wastewater.

The eluents for samples from the coking wastewater treatment system were different from those of the gray wastewater, but their octanol–water partition coefficients were similar. The weakly polar, moderately strong and strong polar compounds were eluted by hexane, dichloromethane and methanol, respectively. As shown in Fig. 3, the moderately strong and strong polar fractions accounted for at least 96.3% of the total antiandrogenic activity of water samples in the coking wastewater treatment system. Thus, the antiandrogens in coking wastewater were mainly moderately strong and strong polar compounds, which was very different from the gray wastewater. In addition, it was also found for the coking wastewater treatment system that the sum of the antiandrogenic activities of the fractions was not the same as the total sample antiandrogenic activity, as shown in Fig. 3.

Table 2 – Comparison of the antiandrogenic activities in the environment.

	Environment	Bioassay	Concentration	Reference
Water samples (μg FEQ/L)	Effluents from oil production platforms	Recombinant hAR yeast bioassay	20–8000	(Tollefsen et al. (2007); Thomas et al. (2009))
	Effluents from chemical industry wastewater treatment systems	Recombinant hAR CV-1 cell bioassay	12.6–25.3	Shi et al. (2009).
	Domestic wastewater	Recombinant hAR yeast bioassay	34–1100	(Li et al. (2010); Ma et al. (2013))
	Effluents from domestic wastewater treatment system	Recombinant hAR yeast bioassay	21.3–1231	Johnson et al. (2007)
	Gray wastewater	Recombinant hAR yeast bioassay	1100	Ma et al. (2013)
			670	This study
	Effluents from gray wastewater treatment system	Recombinant hAR yeast bioassay	0.54	Ma et al. (2013)
			1.05	This study
	River	Recombinant hAR yeast bioassay	1.34–17.1	Kloas et al. (2007)
	Bile of fish	Recombinant hAR yeast bioassay	1840000–9860000	(Hill et al. (2010); Rostkowski et al. (2011))
	Coking wastewater	Recombinant hAR yeast bioassay	78600	This study
	Effluent from coking wastewater treatment system	Recombinant hAR yeast bioassay	340	This study
Solid (mg FEQ/g)	River sediment	Recombinant hAR yeast bioassay	Not detectable-0.154	Zhao et al. (2011)
	Suspended solids in wastewater	Recombinant hAR yeast bioassay	6.9–10	Ma et al. (2013)
	Sludge	Recombinant hAR yeast bioassay	0.00069–0.0036	Ma et al. (2013)

hAR: human androgen receptor; FEQ: flutamide equivalence.

3.2. Comparisons of antiandrogenic activity in different environmental samples

Tollefsen et al. (2007) reported for the first time that AR antagonists were detected in both the dissolved and oil-associated phase at concentrations of between 0.02 and 8 mg FEQ/L. Then, a series of environmental samples were reported to exert antiandrogenic activity, *e.g.*, domestic wastewater, industrial wastewater, sludge and suspended solids as well, as shown in Table 2. Compared with the antiandrogenic activities in other environmental samples, the estimated antiandrogenic activity of gray wastewater was similar to that of domestic wastewater, and much higher than in the natural environment, *e.g.*, rivers (Urbatzka et al., 2007), and much less than in organisms, *e.g.*, bile of fish (Hill et al., 2010; Rostkowski et al., 2011). The estimated antiandrogenic activity of effluent from a gray wastewater treatment system was still about ten times higher than that in the river Lambro (Urbatzka et al., 2007). A rise of antiandrogenic activity could occur in the river if the effluent from the gray wastewater treatment system were discharged to the river directly. The estimated antiandrogenic activity of suspended solids in wastewater was higher than that of river sediment, and the estimated antiandrogenic activity of the sludge was similar to that of river sediment. Coking wastewater exerted much higher antiandrogenic activity than domestic wastewater and other industrial wastewater. What's more, the antiandrogenic activity of the effluent from the A1–A2-MBR treatment system was still more than ten times higher than that of the river. Thus, biologically treated coking wastewater should be given advanced treatment for the safety of the environment.

3.3. Removal effects in gray wastewater and coking wastewater treatment systems

Graywater is considered to have high reuse potential, yet the endocrine-disrupting chemicals in graywater have recently raised concerns due to their adverse effects (Hernández-Leal et al., 2011). Use of a biological treatment system was recommended due to its advantages of being efficient, simple and affordable (Ghunmi et al., 2011) with high removal efficiency for micropollutants, including antiandrogens (Leal et al., 2010).

As shown in Fig. 4, in the studied gray wastewater treatment system, there were mainly two antiandrogenic activity sources, *i.e.*, dissolved antiandrogens in the raw gray water and antiandrogens adsorbed on the suspended solids in the raw water, and three major kinds of removal mechanisms, *i.e.*, extractable sorption to the biomass; a series of effects, *e.g.*, biodegradation, irreversible adsorption, and so on; and membrane filtration, *e.g.*, adsorption and size exclusion as well as charge repulsion, as reported before (Ma et al., 2013). By mass balance analysis, about 1316.5 mg FEQ of antiandrogens flowed into the gray wastewater treatment system every day, 41.1% of which was contributed by weakly polar extracts. The moderately strong polar extracts accounted for 44.7% of the total antiandrogenic activity, and the strong polar compounds only contributed 14.2% of the total. After the treatment, there were still about 126.0 mg FEQ of antiandrogens flowing out of the gray wastewater treatment system every day, 51.4% of which was contributed by weakly polar extracts. There was still 36.2% of the total antiandrogenic activity exerted by strong polar compounds. The gray wastewater treatment system could remove 88.0% of weakly polar extracts and 97.3% of

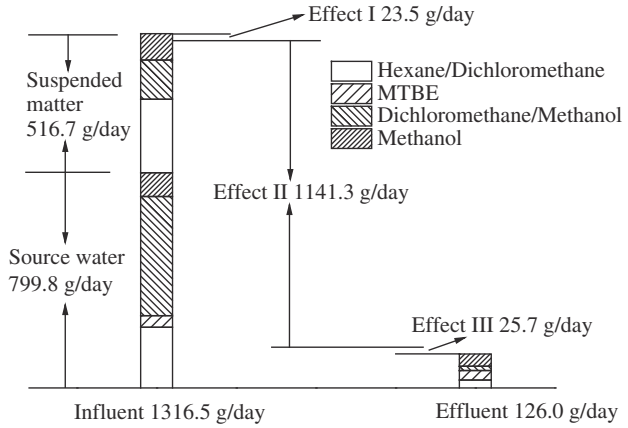

Fig. 4 – Removal of antiandrogenic activity by different effects in gray water treatment system. Effect I: extractable sorption to biomass; Effect II: a series of effects, *e.g.*, biodegradation, irreversible adsorption, and so on; Effect III: membrane filtration, *e.g.*, adsorption and size exclusion as well as charge repulsion.

moderately strong polar extracts but only 32.5% of strong polar extracts. In all, 90.4% of the antiandrogenic activity inflow was removed every day. The extractable sorption to sludge in the MBR only accounted for 2.0% of the total removal and the ultra filtration (UF) membrane in the aerobic zone retained only 2.2% of the total removed antiandrogenic activity. Most of the antiandrogenic activity, namely 95.9%, was removed by the effect of either degradation by microorganisms or was unavailable to the extraction procedure, *etc.* It could be surmised that the biodegradation played an important role in antiandrogens removal in the gray wastewater treatment system since many of the detected antiandrogens were easily biodegradable (Leal et al., 2010).

Great attention has been paid to coking wastewater because of its high concentrations of inorganic and organic pollutants (Kim et al., 2008). However, this is the first time that the removal of antiandrogens in coking wastewater has been reported. The supernatant of the pretreated coking wastewater after sedimentation for several months was introduced to the studied coking water treatment system. Thus, the inflow antiandrogenic activity could be estimated as 471.4 mg FEQ/day. Since there was no sludge discharged in the system, there were only two main removal effects after long-term operation, *i.e.*, biodegradation and membrane filtration, *e.g.*, adsorption and size exclusion as well as charge repulsion. Everyday, 469.4 mg FEQ of antiandrogenic activity was removed by the studied coking wastewater treatment system, only 10.4% of which was contributed by aerobic biodegradation and membrane filtration in the MBR. Biodegradation by microorganisms in the anaerobic reactor accounted for 43.6% of the total antiandrogenic activity removal, which was slightly less than that in the anoxic reactor. Thus, at least 89.6% of the total antiandrogenic activity removal was contributed by biodegradation by microorganisms in the A1–A2-MBR coking wastewater treatment system.

4. Conclusions

Biodegradation by microorganisms contributed most of the antiandrogenic activity removal in the gray wastewater and coking wastewater treatment systems. A total of 95.9% of the total antiandrogenic activity removal in the gray wastewater treatment system was contributed by biodegradation. After the treatment, there was still about 126.0 mg FEQ of antiandrogens flowing out of the system everyday, 36.2% of which was from strong polar extracts. In the A1–A2-MBR coking wastewater treatment system, the antiandrogenic activity of raw coking wastewater was 78.6 mg FEQ/L, and the effluent of the treatment system was only 0.34 mg FEQ/L, with removal efficiency of 99.6%. The biodegradation by microorganisms contributed at least 89.2% of the total antiandrogenic activity removal in the system.

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