Article

Determination of 4-nonylphenol and 4-*tert*-octylphenol compounds in various types of wastewater and their removal rates in different treatment processes in nine wastewater treatment plants of Iran

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ABSTRACT

Alkylphenols (APs), considered as xenoestrogenic compounds, mainly exist as 4-nonylphenol (4-NP) and 4-*tert*-octylphenol (4-*t*-OP) in environments. The high stability and accumulation of APs in aquatic systems have caused endocrine disruption. In this study we measured APs in the wastewater influent and effluent samples, from the urban, rural, livestock, commercial and hospital wastewater treatment plants (WWTPs) in Iran. Dispersive liquid–liquid microextraction (DLLME) combined with gas chromatography–mass spectrometry (GC–MS) was used for the extraction and determination of 4-NP and 4-*t*-OP. In these treatment plants, various processes such as activated sludge, aerated lagoon, moving bed biofilm reactor and activated sludge along with wetland were applied. The highest concentration of 4-NP and 4-*t*-OP was observed in commercial and livestock sewages. The activated sludge along with wetland and then the MBBR process showed the highest removal rates of pollutants. The rates of biodegradability and accumulation in sludge were determined and also the specific adsorption coefficient K_d and the organic carbon–water partition coefficient k_{OC} of the sludge for APs were calculated.

1. Introduction

In recent years, the presence of emerging pollutants, especially in the aquatic environments, has drawn the attention of environmental experts [1]. These chemicals are widely used in various industries and include many substances such as endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs) and persistent organic pollutants (POPs) [2]. The sewage and sludge of municipal and industrial wastewater treatment plants are one of the entry routes of emerging contaminants to water resources [3]. Many of priority pollutants are from EDCs [2]. Alkylphenols (APs) fall in the EDCs group and have estrogenic effects. These materials are used in the production of lubricants, phenolic resins, rubber and plastic, polymers, antioxidants, heat stabilizers and other materials [4]. The long chain APEs degrade quickly to the corresponding APs, which leads to permanent entry of these compounds into the environment [5]. APs which are the metabolites of APEs, are more stable, toxic, lipophilic compounds and with stronger estrogenic properties than their precursors. 4-NP and 4-*t*-OP are the most important and widely used Alkylphenolic compounds [2]. According to the research conducted on the APs, they are highly toxic to many aquatic organisms, humans, animals and the environment. These effects may include cancerous tumors, reproductive disruptor, immune system disorders and obesity [6]. As mentioned above, one of the most important ways to transfer 4-NP and 4-*t*-OP to the environment is through wastewater [7]. Thus, in recent years, many studies have reported monitoring of APs in wastewaters and water resources and the fate of ones in wastewater treatment plants [2,4,8–15]. However, most of these studies have been focused on surface water resources. In addition, these researches have been done on wastewater treatment plants without considering the factors like the type of sewage such as urban wastewater treatment plants (U-WWTPs), rural wastewater treatment plants (R-WWTPs), livestock wastewater treatment plants (L-WWTPs), commercial wastewater treatment plants (C-WWTPs) and hospital wastewater treatment plants (H-WWTPs) [16].

4-NP and 4-*t*-OP due to high K_{ow}[1] (4-NP lg K_{ow} = 4.48, 4-*t*-OP lg K_{ow} = 5.3), have a high tendency to be adsorbed to surfaces such as sludge, sediments and soils. Also persistence of APEs and APs in wastewater sludge is an important problem because among other sludge handling techniques, land application is the most desirable due to its low cost and beneficial recycle of nutrients. But wastewater sludge does not only contain nutrients, it also have contaminants such as APs

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[1] K_{ow}: The *n*-octanol–water partition coefficient.

compounds; they should monitored appropriately to judge about their capability in land application. It allows avoiding these compounds entering the food chain and accumulate in the fatty tissues of living organisms. Concerns about the presence of these contaminants in the wastewater, sewage and sludge have encouraged the researchers to monitor APs Specially 4-NP and 4-*t*-OP in the water and wastewater resources [8].

Despite the development of several methods for measuring organic compounds with small amounts, the study on methods for determining the values of 4-NP and 4-*t*-OP in a variety of samples is still ongoing.

These compounds are determined mostly by techniques such as gas chromatography (GC) coupled with mass spectrometric detection (MS), liquid chromatography (LC) coupled with MS and high-performance liquid chromatography (HPLC). The GC–MS method achieves significantly higher separation, sharper peak shapes and better resolution. However, the derivatization phase prior to injection to GC must be done for polar compounds that have low volatility. Chemical derivatization of the analytes is often used to improve volatility, increase thermal stability or to enhance the detection sensitivity in GC. Methods such as solid phase micro extraction (SPME) and liquid phase micro extraction (LPME) have been applied in many studies. The SPME method is time-consuming and expensive. The LPME method has also some Difficulties such as operational problems, instability of the micro-drop and bubble formation formed by the gas produced during the reaction [17]. Such disadvantages have led to new sample preparation techniques, aiming for faster and simpler methods that consume less solvent with higher recovery and compatibility with GC techniques. Therefore, in 2006, the dispersive liquid–liquid microextraction (DLLME) technique was introduced. In this method, a combination of extraction and dispersive solvent is injected quickly to the liquid sample, which will produce a cloudy solution. This solution is centrifuged, and finally, the contaminant is dissolved in the extraction solvent and will accumulate at the bottom of the centrifuge tube as a tiny drop [18]. The DLLME method has been used in recent years to extract various pollutants, such as steroid hormones [19], Bisphenol A [20], a variety of pharmaceuticals [21], NP and OP [17].

In this paper, a comprehensive study was conducted of the occurrence and distribution of 4-NP and 4-*t*-OP discharged from important sources such as in urban, rural, commercial, livestock and hospital wastewater. Therefore, we investigated APs in raw sewage, effluent and sludge to study their occurrence and fate in various WWTPs. A total of 4 U-WWTPs, 2 R-WWTPs, a L-WWTP, a H-WWTP and a C-WWTP were selected for observations.

The treatment processes in the studied WWTPs include activated sludge (AS), moving bed biofilm reactor (MBBR), aerated lagoon (AL) and activated sludge along with wetland (AS + WL). Accordingly, the ability of various processes for the removal of 4-NP and 4-*t*-OP and the adsorption rates of pollutants into the sludge was evaluated.

2. Materials and Methods

2.1. Standards and reagents

Standard solutions of 4-NP (CAS: 84852-15-3), 4-*t*-OP (97%, CAS: 140-66-9), internal standard of *n*-OP (99%, CAS: 1806-26-4, liner compound) and the derivatization agent (BSTFA + TMCS, 99: 1, CAS: 25561-30-2) were purchased from Sigma-Aldrich Company. GC grade methanol, chloroform and *n*-hexane were also obtained from Merck Company. Standard and internal standard Solutions of 4-NP, 4-*t*-OP and *n*-OP at concentrations of 1000 mg·L^{-1} were prepared in methanol and kept in the dark at −18 °C. Working solution was prepared freshly by suiable dilution of the mixed stock solution with distilled water.

2.2. Sampling

The influent and effluent samples were collected by 24 h composite samples approach from 4 U-WWTPs (Activated sludge and aerated lagoon), 2 R-WWTPs (Aerated lagoon), a L-WWTP (Activated sludge with wetland), a C-WWTP (MBBR) and a H-WWTP (Activated sludge). Samples collection is done in triplicate in each WWTP and its average reported. The information of the studied treatment plants are given in Table 1. The samples were kept in the 500 ml dark glass bottles at 4 °C. All the samples were analyzed within one week.

In the activated sludge process, sludge samples were collected and analyzed from the thickener tank. Thickened Sludge is mixture of primary and secondary Sludge. In the MBBR process, due to lack of primary sedimentation tank, the samples were taken from the waste sludge of the sedimentation tank. By determining the flow and the percentage of solids in the sludge, the daily sludge solids were calculated. Then, the amounts of pollutants adsorbed into the sludge were obtained.

The sludge samples were transported to the laboratory as soon as possible and were kept in the dark at a temperature of −18 °C until analysis. Prior to performing analysis the samples were dried in lab at room temperature during about 3 days. The dried sludge was crushed and passed through a 1 mm sieve.

2.3. Extraction

In the extraction procedure, 5 ml of sample was poured into a 10 ml glass conical tube. 5 μl of the internal standard (*n*-OP) was added to the sample. Then, 500 μl methanol as dispersive solvent were mixed with 100 μl chloroform as extraction solvent, and the 600 μl resulting mixture was injected rapidly into the sample by using a 1000 μl syringe. The resulting cloudy solution was centrifuged for 5 min at 5000 r·min^{-1}. The fine droplets of extraction solvent that contain analytes were sedimented at the bottom of the centrifuge tube. The sedimented phase [(30 ± 2) μl] was withdrawn and calculated by a 50 μl syringe. The extracts from DLLME were evaporated to dryness in a gentle passage of nitrogen gas at room temperature.

Table 1
Information of WWTPs surveyed in this study

Type	Name	Treatment process	Capacity Q/m^3·d^{-1}	Thickened sludge Q_s/kg·d^{-1}	Source
Urban	U1	AS	120000	32520	Man-made and residential wastewater
	U2	AS	42000	11176	
	U3	AS	85000	24220	
	U4	AL	60000	–	
Rural	R1	AL	12000	–	Man-made, residential and livestock wastewater
	R2	AL	8000	–	
Livestock	L1	AS + WL	115	–	livestock wastewater
			115	–	
Hospital	H1	AS	125	38.5	Hospital wastewater (500 beds)
Commercial	C1	MBBR	2500	485	Commercial, man-made and residential wastewater

Subsequently the sonication-assisted extraction method for sludge was selected, 1 g of dried sludge samples were placed in 15 ml glass centrifuge tube. Then 10 ml methanol were added in to tube and the mixture was placed in an ultrasonic bath and sonicated for 10 min. The vials were then centrifuged at 5000 r·min^{-1} for 5 min. The sludge was then separated from methanol by a 0.45 μm filter. With nitrogen gas passage, the resulting methanol was dried at laboratory temperature and analyzed.

2.4. Derivatization

Due to the polar and semi-volatile nature of Alkylphenolic compounds, the derivatization process seems to be essential. Derivatization turns these compounds into derivatives with more volatility and higher thermal stability. In these circumstances, these derivatives would gain more favorable conditions for analysis in the GC–MS device. By the experiments conducted in this study, the *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) (1:99) was selected as the derivatization agent.

The derivatization reagent 10.0 μl and n-hexane 10 μl were added to the test vial containing extracts from DLLME which generates sharper peaks and higher sensitivity. The sample was vortexed for 1 min. Finally, 3 μl sample was injected to the GC–MS for analysis.

2.5. Instruments

The analyses were done using GC–MS instrument (Agilent Technologies, USA), which includes gas chromatography (7890A series) and mass spectrometry (5975C series). The column used in this device was HP-5MS column (30 m × 0.25 mm, 0.25 μm), and the carrier gas was helium at a constant rate of 1 ml·min^{-1}. Then, 3 μl of the derived sample were injected into the GC–MS system with a 1:10 split at 280 °C. The final program used in this study for analysis in the GC–MS system was as follows:

The initially oven temperature was 60 °C and held on it for 1 min; then temperature reached to 170 °C with temperature ramp of 10 °C·min^{-1}; continue to reach 300 °C with a temperature ramp of 15 °C·min^{-1} and kept on this temperature for about 2 min.

For all compounds the selective ion monitoring (SIM) mode was used. The mass-to-charge (*m/z*) ratio values for derivatized 4-NP, 4-*t*-OP and *n*-OP were (207, 179 and 193), (207) and (179 and 278), respectively. The 4-NP is indeed a combination of different isomers that are generated due to diversity in the branches of the nonyl chain section. These isomers are seen as a cluster of peaks in the chromatogram. Therefore, the total area of these peaks is used to determine the amount of 4-NP. Due to the unique structure, *n*-OP and 4-*t*-OP appear as single peaks. For each compound, the target ions and their exit times during analysis in the GC–MS device were specified. Fig. 1, shows the final chromatogram obtained with the selected program of Alkylphenolic compounds.

2.6. Determining the limit of detection (LOD) and the limit of quantitation (LOQ)

The calibration curves were drawn for each of the 4-NP and 4-*t*-OP compounds in the intervals of 1 ng·L^{-1} − 1 mg·L^{-1} using 10 standard solutions. The correlation coefficient (R^2) was obtained more than 99% for the calibration curves.

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated using the response standard deviation and calibration curve slope, which are given in the following equations [23]. The values of LOD, LOQ and recoveries percentage in the analysis can be observed in Table 2.

$$LOD = 3.3 \times (s/S) \tag{1}$$

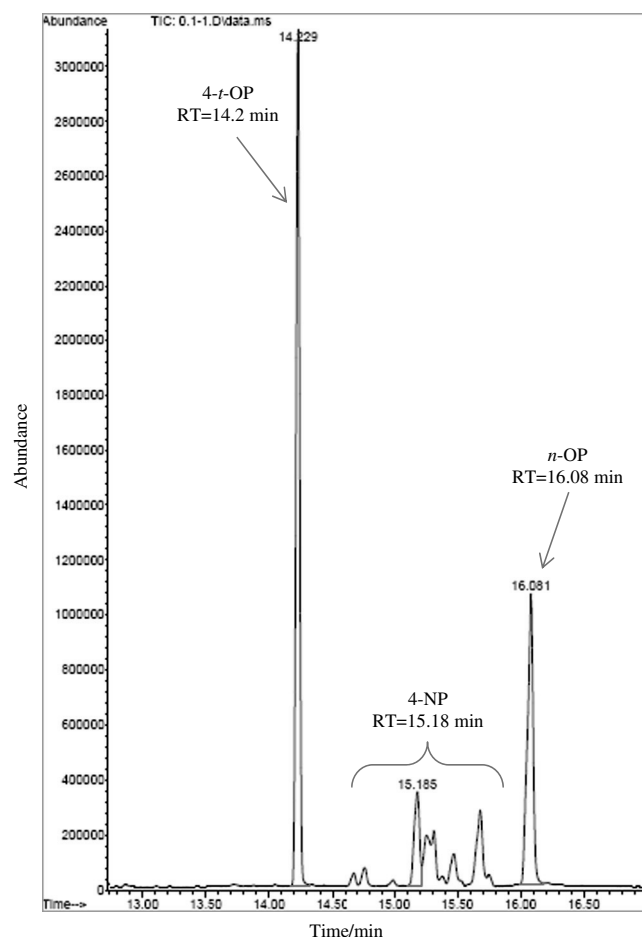**Fig. 1.** The chromatogram of 4-NP and 4-*t*-OP compounds and internal standard of *n*-OP after extraction by DLLME method and derivatization.

$$LOQ = 10 \times (s/S) \tag{2}$$

s- response standard deviation
S- calibration curve slope

2.7. Determining the adsorption coefficients of APs by sludge

Adsorption to biomass is a key mechanism which results in the removal of APs from wastewater. One of the most important indicators used to evaluate the adsorption behaviors of different compounds is the distribution coefficient or specific adsorption coefficient (k_d, kg^{-1}), which is calculated by Eq. (3) [22].

$$k_d = \frac{C_s}{C_w} \tag{3}$$

This parameter can be normalized with organic matter of the adsorbent by Eq. (4). The resulting value is the organic carbon–water partition coefficient (k_{OC}, kg^{-1}), which reflects the fact that the organic carbon content is the main adsorption area for neutral organic materials [22,23].

$$k_{OC} = \frac{k_d}{OC} \tag{4}$$

Table 2
LOD, LOQ and recoveries in 4-NP and 4-*t*-OP analysis

Compounds	Aqueous sample			Sludge sample		
	Recovery/%	LOD/ng·L^{-1}	LOQ/ng·L^{-1}	Recovery/%	LOD/ng·g^{-1}	LOQ/ng·g^{-1}
4-*t*-OP	103.76	0.282	0.974	75.61	1.044	3.165
4-NP	96.39	0.76	2.364	64.3	1.459	4.751

C_s: the amount of pollutants in sludge µg·kg^{-1}, C_w: the amount of pollutants in liquid, µg·L^{-1}, OC: organic carbon content in sludge, %.

One of the objectives of this study was to investigate the adsorption coefficients of 4-NP and 4-*t*-OP by sludge in various WWTPs.

3. Results and Discussion

3.1. Concentrations of 4-NP and 4-t-OP in the influent wastewater

The concentrations of 4-NP and 4-*t*-OP in the raw sewage are summarized in Table 3. The target analytes were detected in the all wastewater samples. In the influent wastewater of the WWTPs, the concentration of 4-NP in livestock wastewater (17.02 µg·L^{-1}) and commercial wastewater (8.23 µg·L^{-1}) showed the highest values compared to urban wastewater (3.78–5.57 µg·L^{-1}), rural wastewater (1.25–2.56 µg·L^{-1}) and hospital wastewater (4.02 µg·L^{-1}).

In case of 4-*t*-OP, in which the endocrine disrupting effect is almost 25% more than of the 4-NP [24], the commercial wastewater (718.12 ng·L^{-1}) and livestock wastewater (686.41 ng·L^{-1}) showed higher concentration compared to urban wastewater (86.1–183.34 µg·L^{-1}), rural wastewater (51.45–35.0 µg·L^{-1}), and hospital wastewater (139.35 µg·L^{-1}).

The main use of APEs, which are the parents of APs, is in the production of surfactants and detergents which included about 90% of the total use of these materials in various industries [25]. In the commercial centers, the sewage source is sanitary type and APs are used in large quantities as detergents and surfactants; thus, high values of APs are observed in commercial wastewater [26]. Non-ionic surfactants have positive effects on degrading enzymes, increasing food consumption efficiency, and mass gain and milk production by livestock. Therefore, the non-ionic surfactants are used as permitted food additives in livestocks. In addition, in livestock farms where milking is done, the need to use detergents rises sharply. As a result, these can be the reasons for high concentration of APs in livestock wastewater [27,28].

The concentrations of 4-NP and 4-*t*-OP compounds in urban and hospital wastewaters have been higher than rural sewage. This indicates that detergents and surfactants containing AlkylPhenols have been used more in urban and hospital areas, which has caused the higher concentration of APs in these wastewaters.

The concentrations of NP and OP in urban wastewaters were measured in two treatment plants in Tokyo, Japan, by Isobe and Takada.

The concentrations of NP and OP were determined about 1 µg·L^{-1} and 40–190 ng·L^{-1}, respectively [12]. Gao *et al.* determined the amounts of NP, NP1E and NP2EO in sewage samples. The scope of changes of the mentioned compounds were obtained respectively as 3.9–7.0 µg·L^{-1}, 4.0–4.8 µg·L^{-1} and 5.2–7.2 µg·L^{-1} [13]. Lian *et al.* determined the concentration of NP (max: 2 µg·L^{-1}, min: 0.91 µg·L^{-1}) in four domestic wastewater treatment plants in Beijing [15]. Bergé *et al.* reviewed the literatures about monitoring NP in different wastewaters up to 2012. The values of NP in USA were reported in hospital wastewater (<LOQ-2.50 µg·L^{-1}), man-made wastewater (0.25–193 µg·L^{-1}, Median: 16.7 µg·L^{-1}), domestic wastewater (0.24–170 µg·L^{-1}, Median: 5.09 µg·L^{-1}) and industrial wastewater (0.25–400 µg·L^{-1}, Median: 10 µg·L^{-1}) [14]. In studies by Petrovic *et al.* the range of NP and OP in four wastewater treatment plants in Spain were respectively determined as 1–80 µg·L^{-1} and 0.01–5 µg·L^{-1} [11].

Several studies have been done on monitoring of AlkylPhenols in surface waters, sediments and sewages [4,8,13,24,29–31]. But there is not enough research on wastewaters with different sources that have been investigated in this study. For this reason, the amounts of 4-NP and 4-*t*-OP compounds were not mentioned for livestock, commercial and rural wastewaters in literature review.

3.2. Concentrations of 4-NP and 4-t-OP in effluent wastewater

The 4-NP and 4-*t*-OP concentrations in the effluent wastewater from U-WWTPs (0.605–2.12 µg·L^{-1}, 12.47–54.81 ng·L^{-1}), R-WWTPs (0.42–0.932 µg·L^{-1}, 11.24–14.63 ng·L^{-1}), L-WWTP (1.163 µg·L^{-1}, 5.35 ng·L^{-1}), H-WWTP (0.807 µg·L^{-1}, 22.21 ng·L^{-1}) and C-WWTP (0.671 µg·L^{-1}, 40.24 ng·L^{-1}) can be seen in Table 3. Very small concentrations of alkylphenol compounds have been observed in effluent wastewater of treatment plants. Given the wide range of concentrations of these pollutants in influent wastewater, the process of sewage treatment has had a significant impact on removal rate of APs from sewages, which will be examined below.

The concentrations of NP and OP in effluent wastewater from two domestic treatment plants in Tokyo, Japan, were measured Isobe and Takada. The concentrations of NP and OP were determined about 0.1 µg·L^{-1} and 10 ng·L^{-1}, respectively [12]. Gao *et al.* measured the NP concentration in the effluent wastewater of treatment plants in the range of 0.39–2.8 µg·L^{-1} [13]. Lian *et al.* determined the amount of NP in effluent wastewater of four domestic wastewater treatment plants

Table 3
Results from analyses conducted on 4-NP and 4-t-OP compounds

Type	Name	Treatment process	Wastewater						Sludge, C_s/µg·kg^{-1}		Adsorption coefficients			
			4-NP			4-*t*-OP			4-NP	4-*t*-OP	4-NP		4-*t*-OP	
			Influent /µg·L^{-1}	Effluent /µg·L^{-1}	Removal /%	Influent /ng·L^{-1}	Effluent /ng·L^{-1}	Removal /%			lg k_d	lg k_{OC}	lg k_d	lg k_{oc}
Urban	U1	AS	4.1	0.768	81.27	131.9	16.9	87.19	4760	15	3.79	4.39	2.95	3.69
	U2	AS	3.78	0.605	83.99	86.1	12.83	85.10	3243	6	3.73	4.33	2.67	3.72
	U3	AS	4.72	0.924	80.42	145.37	12.47	91.42	4381	9	3.68	4.28	2.86	3.96
	U4	AL	5.57	2.12	61.94	183.34	54.81	70.10	–	–	–	–	–	–
Rural	R1	AL	2.56	0.932	63.59	51.45	14.63	71.56	–	–	–	–	–	–
	R2	AL	1.25	0.42	66.40	35	11.24	67.89	–	–	–	–	–	–
Livestock	L1	AS + WL	17.02	1.163	93.17	686.41	5.35	99.22	–	–	–	–	–	–
Hospital	H1	AS	4.02	0.807	79.93	139.35	22.21	84.06	2852	15	3.55	4.15	2.83	3.75
Commercial	C1	MBBR	8.23	0.671	91.85	718.72	40.24	94.40	7136	32	4.03	4.63	2.90	4.07

in Beijing as about 0.09–0.5 $\mu g \cdot L^{-1}$ [15]. In studies by Petrovic *et al.*, the concentrations of NP and OP effluent wastewater of four wastewater treatment plants in Spain were respectively obtained as 1 $\mu g \cdot L^{-1}$ and 10 $ng \cdot L^{-1}$ [11]. Höhne *et al.* measured 4-NP and 4-*t*-OP compounds in the effluent wastewater samples at two treatment plants in Germany, respectively, less than 14.4 $\mu g \cdot L^{-1}$ and 392 $ng \cdot L^{-1}$ [9]. Ros *et al.* measured 4-NP and 4-*t*-OP compounds in the effluent wastewater of treatment plant less than 81 $ng \cdot L^{-1}$ and <LOD [31].

3.3. 4-NP and 4-t-OP removal efficiency in different treatment processes

The processes of target WWTPs are activated sludge (AS), aerated lagoon (AL), MBBR and activated sludge along with wetland (AS + WL). The influent and effluent concentrations, treatment processes and removal rates of 4-NP are shown in Table 3 and Fig. 2. Accordingly, for 4-NP the AS + WL process showed maximum removal rate (93.17%), followed by the MBBR (91.85%), AS (79.93%–83.99%) and AL (61.94%–66.40%).

The influent and effluent concentrations, treatment processes and removal rates of 4-*t*-OP are presented in Table 3 and Fig. 3. Generally, the removal rate of 4-*t*-OP has been higher than 4-NP in all treatment plants. As in the wastewater samples, the removal rates of 4-*t*-OP in the AS + WL (99.22%) were higher than those in the MBBR (94.40%), AS (84.06%–91.42%) and AL (67.89%–71.56%).

USEPA reported that 4-NP and 4-*t*-OP removal rates in 17 conventional AS WWTPs were about 76% and 79%, respectively. Also, their average removal rates were mentioned in processes with nitrification (78% and 87%) and denitrification (82% and 91%) [32]. According to the USEPA report, with increasing nitrification and denitrification rate, the APs removal efficiency has increased. In the MBBR method, nitrification occurs partly due to high sludge retention time (SRT) in the attached biofilm. Anoxic conditions have prevailed in the lower layers of the biofilm, and thus, denitrification would occur. Therefore, the MBBR method is expected to show a higher removal rate in compared with activated sludge process, which is consistent with the experiment results.

When AS and AS + WL removal rates are taken into consideration, it could be understood that WL process is the most effective process that would be able to increase the removal rates of 4-NP and 4-*t*-OP by about 15%. Alkylphenol pollutants such as 4-NP and 4-*t*-OP have a high k_{ow}, and thus, have a great tendency to be adsorbed to surfaces such as sludge, sediments and soil [8]. Therefore, it seems that by passing through the roots of plants in the wetland method; these compounds have been adsorbed by the plants roots. Hence, the factor increasing the AS + WL efficiency is the high tendency to adsorption of these compounds.

In AL process, due to low MLSS in the aeration tank, the rates of 4-NP and 4-*t*-OP absorbed into the sludge have been also lower. In addition, lower sludge retention time (SRT, day) and non-occurrence of nitrification and denitrification have caused the process to show lower efficiency than other processes for the removal of APs.

3.4. Absorption and biodegradation rates of 4-NP and 4-t-OP in activated sludge and MBBR processes

The units in activated sludge process and moving bed biofilm reactor are depicted in Fig. 4. The mass balance of these systems includes the influent wastewater, effluent wastewater and thickening sludge. Mass balance equations are given in Eqs. (5) and (6).

$$(1)Q_{in} \times C_{in} = (2)\dot{m}_{biodegradation} + (3)\dot{m}_{Thikened\ Sludge} + (4)Q_{out} \times C_{out} \quad (5)$$

$$\dot{m}_{Thikened\ Sludge} = C_s \times Q_s \quad (6)$$

Q_{in} = influent wastewater, $m^3 \cdot d^{-1}$, C_{in} = influent concentration, $\mu g \cdot L^{-1}$, Q_{out} = effluent wastewater, $m^3 \cdot d^{-1}$, C_{out} = effluent concentration, $\mu g \cdot L^{-1}$, $\dot{m}_{ThikenedSludge}$ = Mass flow of absorbed pollutants in the thickened sludge, $mg \cdot d^{-1}$, $\dot{m}_{biodegradation}$ = Mass flow of biodegraded pollutants, $mg \cdot d^{-1}$, C_s = absorbed pollutants in the thickened sludge, $mg \cdot kg^{-1}$, Q_s = Mass flow of thickened sludge, $kg \cdot d^{-1}$.

The Eq. (5) concept is that the influent pollutants (1) will have 3 different fates. The first one is biodegradation (2) and mineralization by bacterial activities. The next one is adsorption (3) to the sludge that eventually will be disposed by gravity thickener sludge. The last one is the unchanged pollutants that observed in the effluent wastewater (4). Given that the concentration of pollutants has been measured in the influent and effluent wastewater, the 1st and 4th parts of the balancing are known. The rate of pollutants adsorption into the sludge is also measured (C_s), which can be seen in Table 3. The mass flow rate of sludge (Q_s) in the thickeners is given in Table 1. Therefore, the third part of the equation is also known. Hence, a part of the pollutant biodegraded can easily be calculated. The results of these calculations can be seen in the diagrams of Fig. 5.

According to Fig. 5, biodegradation has been considered to be the principle route involved in the removal of APs during all technologies. The biodegradation rates of 4-NP and 4-*t*-OP in the activated sludge process in the studied treatment plants were respectively obtained more than 50% and 80%. In addition, the adsorption rates into the sludge in the activated sludge process for 4-NP and 4-*t*-OP were calculated as 22%–32% and 1.8%–3.3%, respectively.
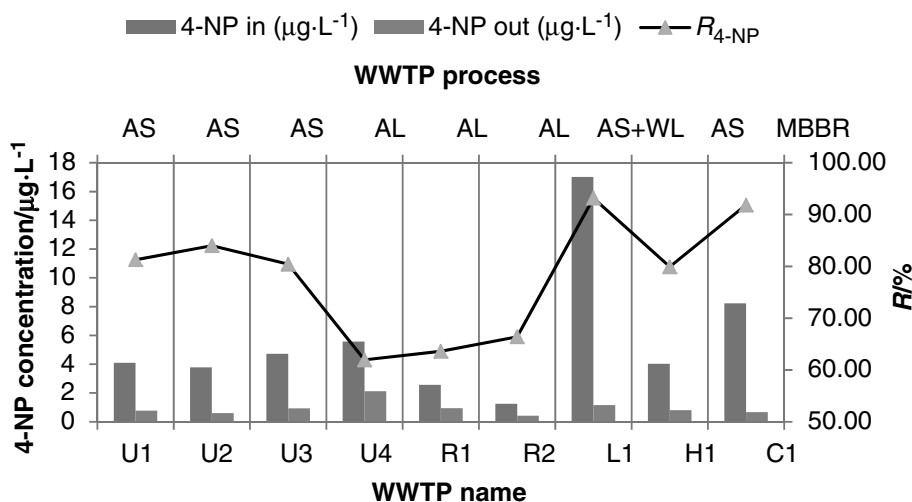

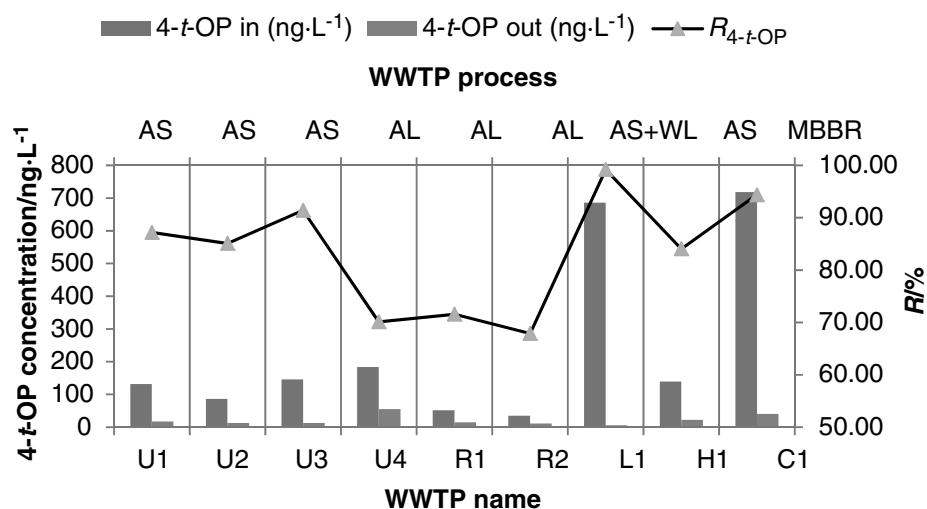**Fig. 2.** The removal rate of 4-NP in different processes.

Fig. 3. The removal rate of 4-*t*-OP in different processes.

In the MBBR process, due to high sludge retention time (SRT), there has been more time for biodegradation of the adsorbed pollutants, so its adsorption rate has reduced and its biodegradation rate has increased. The biodegradation rates of 4-NP and 4-*t*-OP were determined as 75% and 93.5%, respectively. Also, the rates of adsorption into the sludge in this process for 4-NP and 4-*t*-OP were calculated as 16.8% and 0.9%, respectively.

Bolz *et al.* examined the phenolic estrogen-like pollutants. In this study, the adsorption rates of 4-NP and 4-*t*-OP into the sludge were calculated as about 35% and 14%, respectively [33]. Isobe *et al.* measured
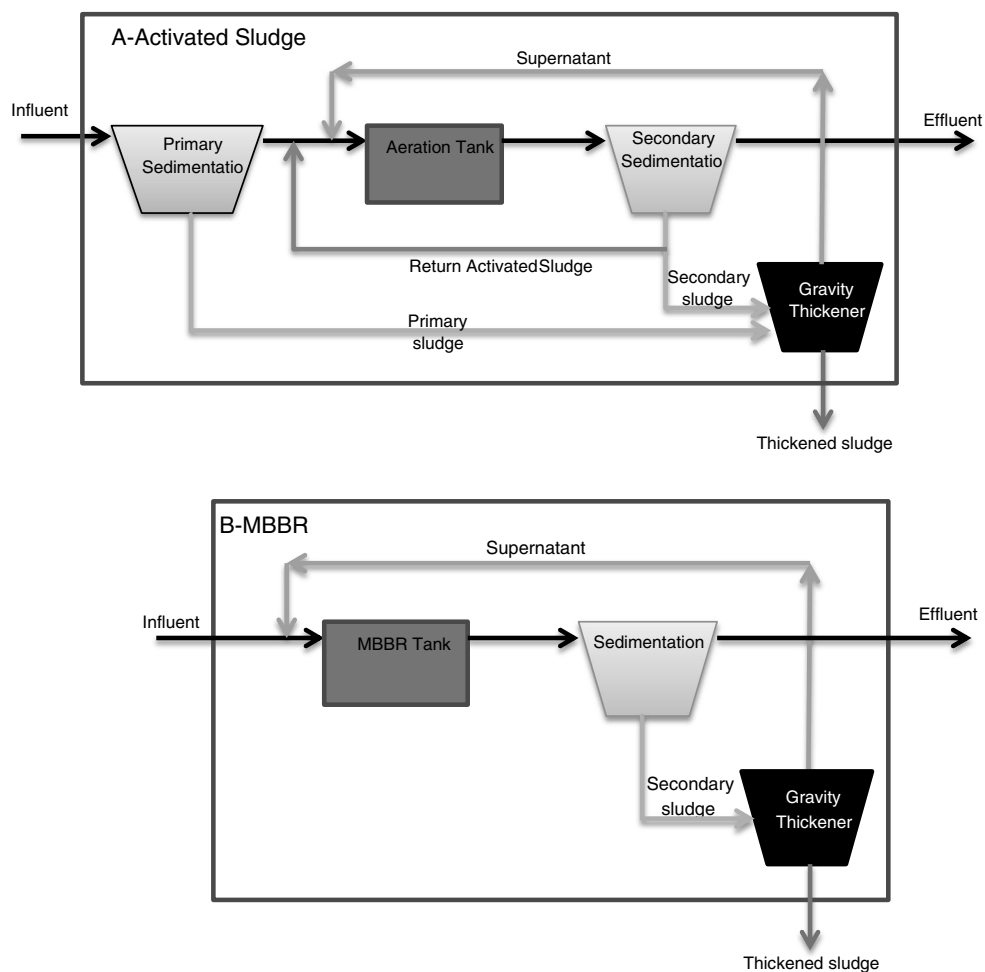

Fig. 4. Flow diagram of A-activated sludge and B-MBBR system for mass balance.
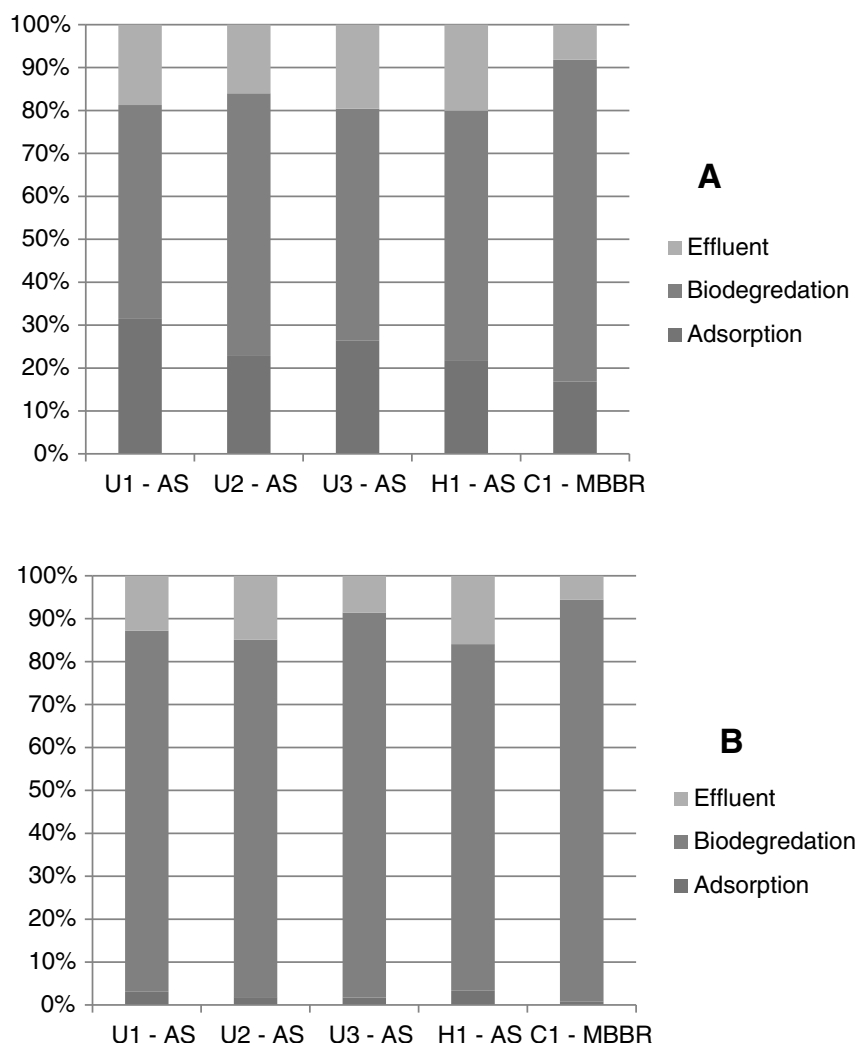
Fig. 5. The percentage of adsorption and biodegradation for (A) 4-NP and (B) 4-*t*-OP.

the NP adsorption rate into the sludge at about 20% [34]. Lian *et al.* measured the NP adsorption rate into the sludge in four treatment plants in Beijing as about 5% to over 70% [15].

3.5. Calculating the adsorption coefficients

As mentioned above, k_d and k_{OC} coefficients were calculated by Eqs. (3) and (4) in WWTPs with AS and MBBR processes, which can be observed in Table 3. The lg k_d for 4-NP and 4-*t*-OP was calculated respectively in the range 3.55–4.03 and 2.67–2.95. The lg k_{OC} was also determined for 4-NP and 4-*t*-OP in the range 4.15–4.63 and 3.69–4.07, respectively. Bergé *et al.* calculated the value of lg k_d coefficient for NP ranging from 2.70 to 3.82, and the lg k_{OC} coefficient as 5.39 [14]. In a review of studies conducted until then, Ying *et al.* reported the rate of lg k_{OC} for NP and OP, respectively, as about 4.7 to 5.6 and 3.54 to 4.25. They also announced the value of lg k_d for OP in the range from 0.77 to 2.85 [35].

4. Conclusions

In this study, two xenoestrogens compounds 4-NP and 4-*t*-OP were evaluated in the urban, rural, livestock, commercial and hospital WWTPs. L-WWTP and C-WWTP had the highest concentrations of 4-NP and 4-*t*-OP in influents, respectively. The processes of the examined WWTPs in this study include activated sludge, aerated lagoons,

MBBR and activated sludge along with wetland. The AS + WL process had the highest removal rate, while the MBBR process has been the most efficient after it. The biodegradation and adsorption rates of 4-NP and 4-*t*-OP were calculated. APs were mostly removed through biodegradation and adsorption to sludge is another effective process for APs removal. The lg k_d and lg k_{OC} coefficients of the sludge for 4-NP and 4-*t*-OP were also determined.

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