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Effects of sludge retention time (SRTs) on the removals of polycyclic aromatic hydrocarbons (PAHs), chemical oxygen demand (COD), and toxicity in a petrochemical industry wastewater

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ABSTRACT

In this study, it was aimed to investigate the effects of increasing sludge retention times (SRTs, 5, 10, 25, and 40 d) on dissolved chemical oxygen demand (COD_{dis}), polycyclic aromatic hydrocarbons (PAHs) and acute toxicity removals in an aerobic completely stirred tank (CSTR) reactor system treating raw petrochemical industry wastewater. The reactors were operated with and without rahmnolipid biosurfactant. In both conditions the effluent quality of the CSTR reactor improved with increasing SRT. Addition of 15 mg/l rhamnolipid increased significantly both COD_{dis} and PAH yields. For maximum COD and PAH removals (86 and 86%) the optimum sludge age was found to be 25 d. The effective PAHs concentration affecting half of the *Daphnia magna* organisms (EC_{50} value) was reduced from 65.61 to 1.86 ng/ml at the end of the aerobic treatment at a SRT of 25 d. The EC_{50} value for COD was reduced from 240 to 33 mg/l after aerobic treatment. Toxicity removals which originated from PAHs and COD were 97 and 86%, respectively.

Keywords: Aerobic; *Daphnia magna*; Petrochemical wastewater; Polycyclic aromatic hydrocarbons (PAHs); Sludge retention time; Toxicity

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread ubiquitous contaminants in the different areas of the environment [1]. They originate from anthropogenic inputs like incomplete combustion, oil spills, and urban runoff, domestic and industrial wastewater discharges [2]. Due to their toxic, mutagenic, and carcinogenic characteristics, PAHs are considered to be hazardous to the biota and the environment [3,4].

No further studies have been performed investigating the effects of sludge retention time (SRT) on the treatment of petrochemical wastewaters in aerobic activated sludge reactor systems. Manoli and Samara found that the total PAH removal efficiencies varied between 1 and 11% in the activated sludge systems at a SRT of 20 d [5]. Some investigators have considered the yields of PAHs through the biological reaction stage in an aerobic reactor at SRTs of 7 and 12 d. They found 49 and 53% total removals for the PAHs with high and low molecular weights [6,7].

Potential advantages of biosurfactants include their unusual structural diversity that may lead to unique properties, the possibility of cost-effective production, and their biodegradability [8]. These properties make biosurfactants a promising choice for applications in enhancing PAHs degradation. Mulligan [9] and Banat et al. [10] found that the biosurfactants increase the

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solubility of petroleum components including oil, aromatic, aliphatic hydrocarbons and polychlorinated biphenyls (PCBs). Yin et al. also found that biosurfactants play an important role in the degradation of polycyclic aromatic hydrocarbons (PAHs) from the petrochemical industry wastewaters [11]. Abalos et al. studied the effect of the rhamnolipid produced by *P. aeruginosa* AT 10 on crude oil degradation [12]. They determined that the biodegradation level of PAHs could be increased from 32 to 61% at a rhamnolipid concentration of 8 mg/l under anaerobic conditions at a SRT and HRT of 20 d and 8 l/d, respectively. Zhou and Zhu, 2007 [13] and Yu et al. 2007 [14] showed that some surfactants enhance both biodegradation and reaction rates of petrochemical wastes. They reported that biosurfactants like rhamnolipid, emulsan, surfactin and glycolipid are surface-active molecules that have both hydrophobic and hydrophilic domains and are capable of lowering the surface tension of PAHs with 5 and 6 benzene rings.

In Izmir, Turkey, wastewaters from the petrochemical industry are treated with conventional activated sludge systems. Since such systems are unable to completely remove the main PAHs present (ca. 15) these are released into receiving bodies. Sludge retention time (SRT) is commonly used in activated sludge systems to control the efficiency of biological wastewater treatment as well as the physical and biological characteristics of the sludge. Although the biomass production rate and biomass viability generally increased with decreasing SRTs, the SRT should be long enough to provide sufficient retention times for contact of the biomass with toxic organics like PAHs in activated sludge reactor systems treating refractory and inhibitory substances. Since the PAHs removal efficiencies are low in the petrochemical industry wastewater treatment plants for activated sludge processes in Izmir, in this study, it was aimed to detect the optimum SRT for maximum COD_{dis}, PAHs and toxicity removals in the aforementioned real petrochemical industry wastewater with and without biosurfactant administration. Therefore, in this study the effects of increasing SRTs (5–40 d) were investigated on the performance of the aerobic completely stirred tank reactor in rhamnolipid (biosurfactant) added and non added conditions. Furthermore, the effects of increasing SRTs on the acute toxicity removals were investigated using the *D. magna* test.

2. Materials and methods

2.1. Experimental set-up

A continuous aerobic stirred tank reactor (CSTR) made up of stainless steel was used in the experimental study.

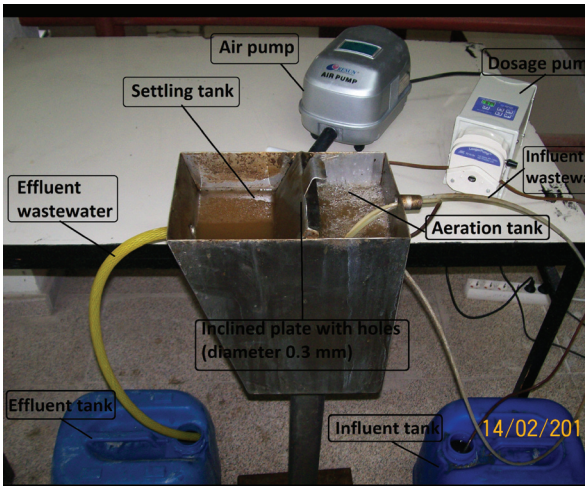

Fig. 1. Configuration of the aerobic CSTR system used in the treatment of petrochemical industry wastewater.

The configuration of the CSTR reactor is illustrated in Fig. 1. It consists of an aerobic (effective volume = 9 l) and a settling compartment (effective volume = 1.32 l). The CSTR reactor was continuously fed from the bottom by a feeding pump with the raw wastewater taken from the influent of the aeration tank of the wastewater of the petrochemical industry. The aerobic reactor was aerated by an air pump and porous diffusers to maintain the DO concentrations between 4 and 6 mg/l. The effluent wastewater from the aeration tank to the sedimentation tank passed through the holes in a plate inclined at 45° to the horizontal axis. Effluent leaving the sedimentation tank was collected in an effluent tank. The sludge retention times were adjusted to 5, 15, 25 and 40 d by discarding an appropriate volume of activated sludge daily from the aeration tank of the aerobic reactor.

2.2. Chemicals

Polycyclic aromatic hydrocarbons, solvents used in GC-MS and rhamnolipid were purchased from Aldrich chemical company and had purities of 98% or greater. A mixture of R1 and R2 rhamnolipid biosurfactants (commercially known as JBR natural biosurfactant) was used (R1, $C_{26}H_{48}O_9$ and R2, $C_{32}H_{58}O_{13}$) in this study.

2.3. Operational conditions

In this study a real wastewater was taken from the influent of the aerobic tank of a petrochemical industry wastewater treatment plant (Izmir, Turkey). The seed used in this study was taken from the recycle line of the

final settling unit of the aeration tank of the wastewater treatment plant. The flow rate and HRT were constant as 2 l/d, and 5 d, respectively. The SRT was increased from 5 to 10, 25 and 40 d. The F/M ratio and OLR in the aerobic CSTR reactor were measured as 0.13 g COD/gVSS·d and 0.33 g COD/l·d, respectively. The MLSS and MLVSS concentrations in the CSTR reactor were 2950 and 2356 mg/l, respectively.

2.4. Analytical procedures

2.4.1. Measurement of conventional parameters

Chemical oxygen demand (COD) measurements were carried out according to Standard Methods [15] in an AquaMED spectrophotometer at a wavelength of 600 nm. Oil-grease was measured using the gravimetric method following the Standard Methods 5520-B [15]. BOD_5 was measured using the WTW Oxi Top IS 6 system. Ammonium (NH_4-N kit number: 14752), Total nitrogen (TN kit number:14537), Nitrate (NO_3-N kit number:14773), Nitrite (NO_2-N kit number:14776) and Total phosphate (TP: kit number:14729) were quantified using Merck-Spectroquant, kits.

2.4.2. PAH analysis and extraction of samples

Wastewater samples were filtered through a glass fiber filter (47 mm-diameter) to collect particle–phase in series with a resin column (~10 g XAD-2) to collect dissolved-phase PBDEs (polybrominated diphenyl ethers). The volume of extracts was reduced and was transferred into hexane using a rotary evaporator and a high-purity N_2 stream. Afterwards, the samples were cleaned up on an alumina–silicilic acid column. All extracts were analyzed for 15 PAHs including acenaphthene (ACT), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), carbazole (CRB), fluoranthene (FL), pyrene (PY), benz[a] anthracene (BaA), chrysene (CHR), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benz[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA), and benzo[g,h,i]perylene (BghiP) with a gas chromatograph (GC) (Agilent 7890) equipped with a mass selective detector (Agilent 5975 inert MSD). A capillary column (HP 5-MS, 30 m, 0.25 mm, 0.25 μm) was used. High purity helium was used as the carrier gas at constant flow mode (1.5 ml/min, 45 cm/s linear velocity). The MSD was run in selected ion-monitoring mode. The signal/noise (S/N) values were taken into consideration for every PAHs compound at their lowest concentrations. The measured S/N ratios varied between 64 and 559. The limit of detection (LOD) and limit of quantification (LOQ) data varied at between 0.909 and 1.908 and at between 1.175 and 5.201, respectively.

2.4.3. D. magna *acute toxicity test*

Acute toxicity was tested using 24 h born *D. magna* as described in Standard Methods (2005) [15]. The cultivation of *D. magna* was performed in the Environmental Microbiology Laboratory of the Environmental Engineering Department at Dokuz Eylul University (DEU) in a temperature and moisture controlled room at 21°C. Experiments were carried out using 10 *daphnids* introduced into the test beakers with 100 ml effective volume at 7–8 pH, providing a minimum dissolved oxygen concentration of 6 mg/l at an ambient temperature of 20–22 °C. The samples were diluted at ratios varying between 1/2 and 1/100 to monitor the response of *daphnids* to the treated and untreated petrochemical industry wastewaters at increasing STRs. The results were expressed as the morality percentage of the *daphnids* after 24 h. The immobile animals which were not able to move were determined as dead *daphnids*. All the data given in Tables and Figures are the mean of the triplicate samplings or mean with SD.

2.4.4. Calculation of SRT in a CSTR with a recycle ratio of 100%

SRT is the total quantity of active biomass in the reactor divided by the total quantity of active biomass withdrawn daily. In activated sludge systems for a given SRT the excess activated sludge produced each day must be wasted. In this approach the sludge wasting in the CSTR occurred in the aeration tank and the solids in the effluent (X_e) were taken into consideration. The CSTR used in this study is a recycled reactor. In the other words, the sludge was recycled 100% from the settling tank to the aeration tank. If the concentration of microorganism in the effluent of the settling tank is low, X_e is negligible [16]. In this study, since the activated sludge was withdrawn from the inside of the aeration stage (Q_w), the microorganism concentration in the reactor (X) was equal to the wasted microorganism concentration (X_w). Therefore, in this study the SRT in CSTR reactor with a volume V_r was calculated using Eq. 1 [16].

$$\mathrm{SRT} = \frac{V_r}{Q_w} \tag{1}$$

SRTs in the aerobic CSTR reactor were adjusted to 5, 10, 25 and 40 d by wasting of 1.8, 0.9, 0.36, 0.23 l/d ($Q_w = V_r/\mathrm{SRT}$) mixed biomass (MLVSS) from the aeration basin of the reactor, respectively.

2.4.5. Models indicating the relationships between SRT, biomass and substrate

As given in Eq. 2 the reactor biomass concentration is a function of the system SRT, the aerobic tank (τ), the

synthesis yield coefficient (*Y, unitless*), the amount of substrate removed (S_0–S, mg/l) and endogenous decay coefficient (k/d) [16]. The same equations can be applied to describe an activated sludge process with no clarifier and thus no return sludge flow. For the case with no return sludge, all the solids produced are present in the effluent from aeration tank and the SRT equals the (τ) [16].

$$X = \left(\frac{\text{SRT}}{\tau}\right)\left[\frac{Y(S_0 - S)}{1 + (k_d)\text{SRT}}\right] \quad (2)$$

The importance of the system SRT in determining the aeration tank biomass concentration is clear from an examination of Eq. 1. As indicated by the waste sludge flow term (Q_w) in Eq. 1 a selected SRT value can be maintained by the amount of solids wasted per day to control the process performance. In other words, SRT can be controlled by wasting a given percentage of sludge (MLVSS) from the aeration tank.

3. Results and discussions

3.1. Characterization of the raw petrochemical industry wastewater used in this study

The characterization of raw wastewater taken from the influent of the aeration unit of the petrochemical industry wastewater treatment plant in Izmir are shown in Table 1. The raw wastewater was taken from the influent of the activated sludge process after mechanical treatment. The COD, total PAH, BOD and the concentrations for other parameters given in this table are characteristic for petrochemical industry wastewater and exhibited similarities with the petrochemical industry wastewaters used by Manoli and Samara [17] and Busetti et al. [18] in their studies.

3.2. Start-up period of the aerobic CSTR reactor for acclimation of bacteria to the petrochemical industry wastewater

The adaptation period is very important since the bacterial population used as seed is going to be exposed to the petrochemical industry wastewater in the aerobic CSTR reactor. To acclimate the aerobic biomass to the petrochemical wastewater, four lab-scale CSTR reactors were operated through 15 d to reach steady-state conditions at STRs varying between 5 and 40 d [19]. The steady-state conditions were defined with COD and total PAH removal efficiencies higher than 75 and 60%, respectively, for consecutive 5–7 d. During the start–up period the dissolved oxygen concentration and the redox potential were around 3 mg/l and + 90 mV [19]. The CSTR reactors reached steady-state conditions after an operation period of 10–15 d depending to the SRTs depending on the SRTs studied. After this operation time, the PAHs and the total COD removal efficiencies remained constant at approximately at 60–69% and between 70–79%, respectively, through continuous operation in CSTR reactors without rhamnolipid [19]. The aforementioned parameters were at between 77–86% and at between 83–86% in the CSTR reactor cotaining rhamnolipid. SRT is defined as the average length of time a unit of biomass remains in the treatment system.

Table 1
Characterizations of petrochemical industry wastewater

pH	6.3–7.8	Oil-grease (mg/l)	212.5
COD_{total} (mg/l)	1575	TSS (mg/l)	152
$COD_{dissolved}$ (mg/l)	1150	BOD_5 (mg/l)	584
NO_3–N (mg/l)	1.8	Total-N (mg/l)	15.4
NO_2–N (mg/l)	0.046	Total-P (mg/l)	10.6
NH_4–N (mg/l)	2.20	PO_4-P (mg/l)	6.8
		Total PAH (ng/ml)	66.27

3.3. Effect of SRT on the COD and total PAH degradation in aerobic CSTR system

The results of this study showed that as the SRTs were increased from 5 to 25 d both dissolved COD and total PAH removals increased from 70 to 79% and from 60 to 69% in the CSTR reactor containing no-rhamnolipid (Table 2). The dissolved COD and total PAH yields decreased from 79 to 77% and from 69 to 63% as the HRT were increased from 25 d up to 40 d. Although a part of dissolved COD oxidation occurred even at a SRT of 5 d, average dissolved COD removal efficiency increased with increasing SRT. This trend was to be expected based on Eq. 2, which shows the relationship between SRT and effluent substrate concentration, and is in agreement with the results of other studies reported in the literature [16]. This clearly shows the relationship between SRT and treatment performance of the CSTR reactor. On the other hand the bacteria concentration through a microbial growth into a mass balance equation is significant to detect the relationship between biomass and SRT through treatment of PAHs and dissolved COD in a CSTR reactor (Eq. 2) [16].

The SRT is used as a fundamental variable because it is functionally related to the steady-state specific growth rate of the biomass in a complete-mix reactor. Since at steady state, the mass of organisms wasted must equal the net mass formed; that is, the new growth minus the loss due to lysis, death, and decay, it follows that the SRT is inversely proportional to the net average specific growth rate of the entire system [16]. Consequently, the SRT controls the concentration of the growth-limiting

Table 2
Concentrations of COD_{dis} and PAHs and removal efficiencies of influent/effluent wastewater in CSTR system at increasing SRTs (without rhamnolipid)

	SRT 5 d		SRT 10 d		SRT 25 d		SRT 40 d	
	COD_{dis} (mg/l)	PAHs (ng/ml)	COD_{dis} (mg/l)	PAHs (ng/ml)	COD_{dis} (mg/l)	PAHs (ng/ml)	COD_{dis} (mg/l)	PAHs (ng/ml)
Influent	1200	66.27	1200	66.27	1200	66.27	1200	66.27
Effluent	360	26.51	324	24.52	252	20.54	276	24.52
Removal (%)	70	60	73	63	79	69	77	63

substrate in the reactor and determines important system characteristics, such as the electron acceptor requirement and the excess biomass production rate.

Heterotrophic bacteria in activated sludge are characterized by relatively large maximum specific growth rates, resulting in low values of minimum SRT. The range of SRTs necessary for efficient removal of soluble organic matter from municipal wastewater is generally between 1.5–4 d [16]. In practice, a safety factor is employed to guard against upsets in treatment performance and account for uncertainty in the kinetic parameters and influent characteristics, as well as natural. In general, a higher SRT results in a greater amount of biomass in the system and therefore a higher MLSS concentration. Several studies have demonstrated that for a given waste stream and reactor configuration, MLSS concentration increases with SRT.

Although the enzymatic activity of the biomass was highest at low SRTs in the activated sludge systems treating COD originating from the slowly degradable organics, long SRTs are necessary to treat the wastewaters containing CODs originating from the slowly degradable and inhibitory substances. In this study high SRTs such as 20–25 d provided enough contact time between microorganisms and PAHs to degrade these organics containing high and low benzene rings. The reason for the decrease in COD and PAH yields at HRTs > 25 d could be attributed to the inhibitory effect of the long contact time on the bacteria through degradation of PAHs with high benzene rings and to the bacteria aged which resulted a loss of enzymatic activities in the CSTR reactor. Table 2 shows the PAHs and dissolved COD removals at increasing SRTs from 5 up to 40 d in the CSTR reactors with 15 mg/l rhamnolipid biosurfactant. It was found that the optimum SRT was 25 d for maximum total PAH and dissolved COD removals. Addition of rhamnolipid biosurfactant significantly increased the PAH and COD removals. The COD and total PAH removals increased from 70 to 83% and from 79 to 86%, respectively, at a SRT of 25 d with a rhamnolipid dose of 15 mg/l (see Tables 1 and 2). These results agree with the studies performed by Shokrollahzadeh et al. [20], while disagree with the data obtained by Zhao et al. [21] since the latter researchers found that the aerobic reactor performance treating industrial wastewater containing PAHs and oils is independent from the SRT and CMC biosurfactant [21]. In the studies performed by Wei et al. [22] and Yin et al. [11] rhamnolipid was used to promote the biodegradation of PAHs from a petrochemical wastewater by reducing the interfacial tension of the wastewater. This contributed to PAH removals resulting in a 23% increase in PAH yield compared to rhamnolipid free conditions. Jeong et al. also found high removals (74–89%) in ACT, ANT, FL, PY, BaA, CHR, BbF and BkF PAHs through treatment in an aerobic basin at a SRT of 20 d [23]. In this study, the data obtained showed that the CSTR reactor without rhamnolipid exhibited low removal efficiencies compared to the CSTR reactor with rhamnolipid added at a SRT of 25 d. The concentration and the removal efficiencies of fifteen PAHs are shown in Table 3. The removal efficiencies

Table 3
COD_{dis} and PAHs concentrations and removal efficiencies of influent/effluent wastewater in CSTR at increasing SRTs (with 15 mg/l rhamnolipid)

	SRT 5 d		SRT 10 d		SRT 25 d		SRT 40 d	
	COD_{dis} (mg/l)	PAHs (ng/ ml)	COD_{dis} (mg/l)	PAHs (ng/ ml)	COD_{dis} (mg/l)	PAHs (ng/ ml)	COD_{dis} (mg/l)	PAHs (ng/ ml)
Influent	1200	66.27	1200	66.27	1200	66.27	1200	66.27
Effluent	205	15.28	195	12.94	165	9.15	145	10.88
Removal (%)	83	77	84	80	86	86	88	83

of three ring PAHs namely ACT-FLN-PHE-ANT-CRB increased from 85, 75, 69, 90%, and 62 to 87, 80, 74, 92, and 80% as the SRT was increased from 5 to 25 d. The removal efficiencies of four ring PAHs namely FL-PY-BaA-CHR increased from 68, 43, 32 and 62% to 70, 60, 37, and 79% as the SRT was increased from 5 to 25 d. Similarly as the SRT was increased from 5 to 25 d the removal efficiencies of the PAHs with five and six benzene rings (BbF, BkF, BaP, IcdP, DahA, and BghiP) increased from 59, 37, 67, 79, 77, and 72 to 74%, 54, 73, 95, 95, and 95% in the aerobic CSTR reactor. Increasing the SRT to 40 d did not contribute to all PAH removals. It can be concluded that for the maximum PAH removal efficiency the optimum SRT was 25 d. The removal efficiencies of PAHs with three, four, five and six benzene ring PAHs were higher than the PAHs removals obtained by Jing et al. who found 56, 67, 60, 45 and 40% degradation yields, respectively [24].

Treatment with rhamnolipid (15 mg/l) caused a significant increase in 5 and 6-ring PAHs degradation. However, no significant effect was detected in the case of some 3-ring PAHs (BaA, E = 31–33%) and one 4-ring PAH (BkF, E = 51%) removals (Table 4). In this study it was found that PAH treatment with rhamnolipid was beneficial for the degradation of all ring PAHs with a total PAH removal yield of 86 % in comparison to 79% in the rhamnolipid-free case. The efficiency in the removal of PAHs is not in relation to the number of aromatic rings. Aerobic degradation in the CSTR process was very efficient for all ring PAHs removal. The results of this study show that 15 mg/l rhamnolipid increased the removal efficiency of both lower (2, 3 and 4 ring PAHs) and higher (5 and 6 ring PAHs) molecular weight PAHs compounds. These results could be attributed to the combined effects of activated sludge which is resistant to PAHs, to the type of biosurfactant and the dose. The results found in this study are in contrast to the findings of Whang et al. [25] and Pathak et al. [26] which reported that PAHs with low carbon number are degraded more rapidly.

3.4. *Effect of increasing SRT on the* D. magna *acute toxicity*

In order to determine the acute toxicity of the raw wastewater, dilutions varying between 0, 1/10, 3/10, 1/2 and 4/5 were performed in the influent and effluents of the CSTR reactor. Table 5 shows the alive numbers and the percentage inhibitions of *D. magna* in rhamnolipid added influent samples. The inhibition percentage was calculated by the ratio of the number of dead *D. magna* to the total number of live *daphnids*. It was found that the percentage of dead *D. magna* was higher at high wastewater ratios. In other words, the inhibitions percentage increased at low dilutions in the influent samples (Table 5). For example, the inhibitions (the percentage of dead *daphnids*) decreased from 100 to 70% and 60% as the dilution ratios increased from zero to 1/2 and 4/5, respectively.

Table 4
PAHs concentrations and percent removal efficiencies in CSTR reactors at increasing SRTs (mean ±SD)

PAHs	PAH conc. influent CSTR (ng/ml)	PAH concentrations in the effluent CSTR (ng/ ml)							
		SRT 5 d[a]	% [b]	SRT 10 d[a]	%[b]	SRT 25 d[a]	%[b]	SRT 40 d[a]	%[b]
ACT	29.438±0.011	4.415±0.05	85	3.912±0.02	87	0.386±0.01	99	0.445±0.02	98
FLN	9.380±0.009	2.355±0.06	75	1.876±0.06	80	1.763±0.03	81	2.053±0.05	78
PHE	15.010±0.018	4.602±0.03	69	3.879±0.01	74	4.117±0.02	73	4.892±0.01	67
ANT	3.612±0.021	0.356±0.04	90	0.279±0.03	92	0.293±0.01	92	0.254±0.02	93
CRB	0.901±0.012	0.354±0.01	62	0.254±0.04	72	0.176±0.02	80	0.149±0.02	83
FL	2.986±0.008	0.983±0.06	68	0.913±0.06	69	0.886±0.01	70	0.974±0.07	67
PY	2.199±0.015	1.253±0.07	43	1.068±0.03	51	0.890±0.04	60	1.269±0.03	42
BaA	0.364±0.014	0.247±0.01	32	0.243±0.01	33	0.228±0.05	37	0.252±0.03	31
CHR	0.726±0.021	0.259±0.02	64	0.256±0.01	65	0.152±0.01	79	0.281±0.08	61
BbF	0.080±0.013	0.032±0.05	59	0.023±0.03	71	0.021±0.01	74	0.025±0.07	69
BkF	0.091±0.007	0.057±0.02	37	0.050±0.01	45	0.041±0.03	54	0.052±0.03	43
BaP	0.072±0.024	0.024±0.05	67	0.024±0.01	67	0.019±0.01	73	0.025±0.02	65
IcdP	0.325±0.035	0.067±0.03	79	0.034±0.06	89	0.017±0.02	95	0.034±0.01	89
DahA	0.742±0.028	0.172±0.01	77	0.119±0.05	84	0.038±0.02	95	0.125±0.01	83
BghiP	0.338±0.011	0.094±0.01	72	0.023±0.02	93	0.015±0.09	95	0.042±0.01	87
Total PAH	66.27	15.28	77	12.94	80	9.15	86	10.88	83

[a]PAH concentrations in the effluent of the aerobic CSTR reactor.
[b]PAH removal efficiencies.

Table 5
Acute toxicity tests of *D. magna* for influent wastewater with biosurfactant

Dilution ratios	Number of *Daphnids*	Influent	
	*a	*b	*c
0 (0 ml S+100 ml W)	10	0	100
1/10 (10 ml S+90 ml W)	10	1	90
3/10 (30 ml S+70 ml W)	10	2	80
1/2 (50 ml S+50 ml W)	10	3	70
4/5 (80 ml S+20 ml W)	10	4	60

*a: alive *D. magna* number at t = 0 h;
*b: alive *D. magna* number at t = 24 h;
*c: percentage of dead *D. magna* (%), S: distilled water, W: wastewater.

Table 6 shows the numbers of *D. magna* and inhibition percentages in the aerobic CSTR effluent samples containing 15 mg/l rhamnolipid at increasing SRTs from 5 d up to 40 d.

As the SRT increased from 5 to 25 d the *D. magna* inhibitions decreased from 50% (30 S+70 ml W) and 20% (50 S + 50 W) to 6 and 10%, respectively, in the effluent of the CSTR reactor containing 15 mg/l rhamnolipid. The inhibitions decreased to zero at dilution ratios of 1/2 and 4/5 at a SRT of 25 d. The acute toxicity test results showed that low toxicity removals were obtained at low and high SRTs such as 5, 10 and 40 d. The petrochemical wastewater was not treated effectively at low SRTs since short SRTs did not provide enough time to activated sludge bacteria to contact and metabolize the PAHs in the petrochemical industry wastewater. Therefore, low inhibitions were observed at low SRTs. The inhibitions were high at SRTs >25 d since the PAHs in petrochemical wastewater caused toxicity in the activated sludge bacteria at high contact times. The continuous accumulation of PAHs could not be metabolized by the bacteria in the activated sludge resulting in high inhibitions and lower PAH and COD yields compared to 25 d SRT. Therefore a high inhibition was observed at long SRTs. In this study it was found that the optimum SRT was 25 d for the lowest death rate of the *daphnids* at dilution ratios of 1/2 and 4/5. This could be explained by the uptake of the PAHs by the activated bacteria cells together with hydrolyzed dissolved COD through fast PAH diffusion with rhamnolipid. Dissolved COD is taken up by the activated sludge bacteria degrading PAHs in a matter of minutes and metabolized, giving rise to a high unit rate of oxygen demand for synthesis resulting in low inhibitions to *D. magna*. Table 7 depicts the dilution ratios (GL) for PAHs and COD_{dis}, and the EC_{50} values in the influent and effluent samples at increasing SRTs.

The dilution ratios (D_L) were measured as 9.7/10 both for PAHs and dissolved COD in the influent samples while these ratios decreased. The dilution ratios decreased from 3/10 to 2/10 as the SRT decreased from 5 to 25 d indicating that high SRTs up to 40 d decreased the acute toxicity to *D. magna*. Therefore low dilution ratios was obtained at 10, 25, and 40 d of SRTs. Table 8 depicted the EC_{50} values in the influent and effluent of the CSTR reactor at increasing SRTs based on PAH and COD. By using the D_L ratios the 50% inhibitions on the number of *D. magna* corresponds to an EC_{50} = 1200 mg COD_{dis}/l × 0.97 = 1164 mg COD_{dis}/l and EC_{50} = 66.27 ng PAHs/ml × 0.97 = 64.28 ng/ml in the influent samples. The 50% inhibitions of the *D. magna* (EC_{50} values) decreased from initial 1164 mg/l to EC_{50} = 61.5 mg/l, to EC_{50} = 39 mg/l to EC_{50} = 33 mg/l and to 29 mgl at SRTS 2, 10, 25 and 40 d, respectively, when the COD parameter was taken into consideration. The EC_{50} values decreased from an initial 64.28 ng/ml to EC_{50} = 4.58 ng/ml, to EC_{50} = 2.59 ng/ml to EC_{50} = 1.86 ng/ml and to 2.18 ng/ml at SRTS 2, 10, 25 and 40 d, respectively when PAH was taken into consideration. The initial EC_{50} values decreased significantly to $EC_{6.87}$ and $EC_{7.02}$ at a SRT of 25 d

Table 6
The number of alive *D. magna* and inhibition percentages in the aerobic CSTR system effluent at increasing SRTs after 24 h (with15 mg/l rhamnolipid)

Dilutions	SRT 5 d			SRT 10 d		SRT 25 d		SRT 40 d	
	*a	*b	*c	*b	*c	*b	*c	*b	*c
0 (0 ml S+100 mlW)	10	0	100	0	100	0	100	0	100
1/10 (10 ml l S+90 ml W)	10	3	70	3	70	4	60	3	70
3/10 (30 ml S+70 ml W)	10	5	50	6	40	6	40	6	40
1/2 (50 ml S+50 ml W)	10	8	20	9	10	10	0	9	10
4/5 (80 ml S+20 ml W)	10	10	0	10	0	10	0	10	0

*a: alive *D. magna* number at t = 0 h;
*b: alive *D. magna* number at t = 24 h;
*c: percentage of dead *D. magna*, S: distilled water, W: wastewater.

Table 7
Dilution ratios (D_L) for PAHs and COD_{dis} in the influent and effluent samples at increasing SRTs

	SRT (d)	Influent COD_{dis} (mg/l)	Effluent COD_{dis} (mg/l)	Influent PAHtotal (ng/ml)	Effluent PAHtotal (ng/ml)	Dilution ratios (D_L)			
						Influent COD_{dis}	Influent PAH	Effluent COD_{dis}	Effluent PAH
Raw ww* with rhamnolipid	–	1200	–	66.27	–	9.7/10	9.7/10	–	–
Effluent ww* in CSTR reactors	5	1200	205	–	15.28	–	–	3/10	3/10
	10	1200	195	–	12.94	–	–	2/10	2/10
	25	1200	165	–	9.15	–	–	2/10	2/10
	40	1200	145	–	10.88	–	–	2/10	2/10

*ww: wastewater.

Table 8
EC_{50} values measured in the influent and effluent samples of the CSTR system at increasing SRTs

		Influent/Effluent EC_{50} concentrations (ng/ml)				Effluent EC values	
		Influent COD_{dis} EC_{50} (mg/l)	Influent PAH EC_{50} (ng/ml)	Effluent COD_{dis} EC_{50} (mg/l)	Effluent PAH EC_{50} (ng/ml)	Effluent COD_{dis} conc EC value (mg/l)	Effluent PAH conc EC value (ng/ml)
Raw wastewater with rhamnolipid		1164	64.28	–	–	–	–
CSTR (15 mg/l biosürfactant)	SRT=5 d	1164	64.28	61.5	4.58	8.54	11.52
	SRT=10 d	1164	64.28	39	2.59	8.12	9.77
	SRT=25 d	1164	64.28	33	1.86	6.87	7.02
	SRT=40 d	1164	64.28	29	2.18	6.04	8.23

based on the COD and PAH. As shown the maximum reduction in EC values was observed at a SRT of 25 d.

4. Conclusions

The results of this study showed that 25 d SRT provided effective contact between the petrochemical wastewater and the biomass in an aerobic CSTR reactor to biodegrade the COD and the fifteen PAHs with maximum yields. In other words, the highest PAHs (E = 86%) and COD_{dis} (E = 86%) removal efficiencies were obtained at a SRT of 25 d among the other SRTs used. The EC_{50} value originating from the PAHs was 64.28 ng/ml in the influent of CSTR while this value decreased to 1.86 ng/ml at a SRT of 25 d in the effluent of this reactor. The maximum acute toxicity removals originated from the PAHs and dissolved COD were 97 and 86%, respectively at a SRT of 25 d.

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