Journal of Hazardous Materials 197 (2011) 404–416

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effects of sludge retention time (SRT) and biosurfactant on the removal of polyaromatic compounds and toxicity

Delia Teresa Sponza*, Oguzhan Gok

Dokuz Eylul University, Engineering Faculty, Environmental Engineering Department, Buca Kaynaklar Campus, 35160 Izmir, Turkey

ARTICLE INFO

Article history:
Received 9 March 2011
Received in revised form 5 August 2011
Accepted 30 September 2011
Available online 10 October 2011

Keywords:
Biosurfactant
Petrochemical industry
Polyaromatic hydrocarbons
Sludge retention time
Acute toxicity

ABSTRACT

A laboratory-scale aerobic activated sludge reactor (AASR) system was employed to investigate the effects of SRT on the removal of three less hydrophobic and six more hydrophobic PAHs in the presence of rhamnolipid (RD), emulsan (EM) and surfactine (SR) biosurfactants. Among the biosurfactants it was found that RD exhibits a better performance than the others in the removal of PAHs. At a RD of 15 mg l^{-1} aerobic treatment for 25 days SRT was enough to remove over 90% of the total PAHs, 88% of the COD originating from the inert organics (COD_{inert}) and 93% of the COD originating from the inert soluble microbial products (COD_{imp}). At this SRT and RD concentration, about 96–98% of the RD was biodegraded by the AASR system, 1.2–1.4% was accumulated in the system, 1.1–1.3% was released in the effluent, and 1.2–1.4% remained in the waste sludge. The addition of electron acceptors (NO_3^{-1}, SO_4^{-2}) and increasing of temperature up to 45 °C enhanced the PAH yields. The most effective PAH degradation occurred in high-oxygenated and neutral pH conditions. The PAH concentration affecting half of the *Daphnia magna* organism (EC_{50} value) was reduced from EC_{50} = 45.02 ng ml^{-1} to the PAH concentration affecting only 6% of the live *Daphnia magna* (EC_6 = 5.30 ng ml^{-1}) at the end of the aerobic treatment at a SRT of 25 days. Toxicity removals originating from the PAHs were 96%.

1. Introduction

Many PAHs are priority pollutants listed by the US Environmental Protection Agency and they cannot be removed effectively in activated sludge systems. Not many studies have been performed investigating the effects of sludge retention time (SRT) on the treatment of petrochemical wastewaters in aerobic activated sludge reactor systems. Namkung and Ritmann found that the total PAH removal efficiencies varied between 1% and 61% in the activated sludge systems at a SRT of 20 days [1]. Some investigators have considered the yields of PAHs through the biological reaction stage in an aerobic reactor at different SRTs [2,3]. Potential advantages of biosurfactants include their unusual structural diversity that may lead to unique properties, the possibility of cost-effective production, and their biodegradability [4]. These properties make biosurfactants a promising choice for applications in enhancing PAHs degradation.

In Izmir, Turkey, wastewaters from the petrochemical industry are treated with conventional activated sludge systems. Petrochemical industry wastewater treatment plants may have acted as a source of PAH pollution in the environment because high concentrations of PAHs are usually detected in petrochemical industry wastewater treatment plant influent, effluent and dewatered sludge [5,6].The studies performed by our team showed that the PAHs are usually removed by processes such as biodegradation, sorption, and volatilization, etc. in aerobic treatment plants [7]. We reported that volatilization and abiotic hydrolysis of PAHs can be ignored [7]. Therefore, biodegradation in activated sludge systems could be the major mechanism for PAH removal in petrochemical industry wastewater. Since such systems are unable to completely remove the three less hydrophobic PAHs with three benzene rings [(acenaphthene (ACT), flourene (FLN) and phenanthrene (PHE)] and six more hydrophobic PAHs with five and six benzene rings [(benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (IcdP), dibenz(a,h)anthracene (DahA) and benzo(g,h,i) perylene (BghiP)] these are released into receiving bodies. In activated sludge systems the SRT should be long enough to provide sufficient retention time for contact of biomass with toxic organics like PAHs and inhibitory substances. The SRT values typically used in full-scale aerobic wastewater treatment plants are in the range of 4–10 days for carbon oxidation [8]. For the treatment of wastewaters containing inert and toxic compounds the activated sludge systems should be operated as long as necessary in order to maintain enough contact time between the microorganism and organic substrates in question.

The studies performed until now have been concerned with the aerobic degradability of low molecular weight hyrophilic PAHs [9].

* Corresponding author. Tel.: +90 232 412 71 19; fax: +90 232 453 11 53.
E-mail address: delya.sponza@deu.edu.tr (D.T. Sponza).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.09.101

The fates of more hydrophobic PAHs with high molecular weights have not been investigated in detail for petrochemical wastewaters. On the other hand, high molecular weight compounds may be components of bacterial cells that lysed during the endogenous stage or some extracellular polymeric substances derived from suspended bacteria within the aerobic sludge reactors [10]. A large proportion of PAHs remained in the treatment system until discharged. In the presence of surfactants the solubility and the biodegradability of PAHs were stimulated so that they could be taken up by the bacterial cells, could be used as carbon and energy source or could be used throughout co-metabolism [8–10]. Fast PAH and inert organic substrate diffusion from aqueous media to bacteria cells occurred via increasing mass transfer rates in the presence of biosurfactants. This caused a decrease in the inert fraction of the COD and PAH in the effluent [8,10]. The dissolved COD (COD_{dis}) parameter used for substrate utilization cannot give enough information about the degradation of organic matter [11]. The inert soluble microbial products generated from the biomass decay, from the endogenous respiration and from the hydrolysis of slowly degradable organics in the AASR. The determination of soluble inert COD (COD_{inert}) of influent wastewater and COD_{imp} generated in the biological treatment gains importance for meeting the stringent discharge limits and aquatic toxicity. These fractions become the major constituents of the effluent COD and they by-passed the AASR system without being affected by the biochemical reactions between substrate and bacteria. This is important for refractory wastewater treatment, such as in the petrochemical industry [11].

PAH removal efficiencies are low in the petrochemical industry wastewater treatment plants for activated sludge processes in Izmir. For this reason, in this study, it was aimed to determine the best biosurfactant (among RD, EM and SR) to improve bacterial activity when treating the petrochemical wastewaters. The effects of increasing SRT and RD biosurfactant concentrations on the removals of three less hydrophobic (ACT, FLN and PHE PAHs with three benzene rings) and six more hydrophobic PAHs (BbF, BkF, BaP, IcdP, DahA, BghiP PAHs with five and six benzene rings) (Table 1) and on the removals of COD_{dis}, COD_{imp}, COD_{inert} removals in a real petrochemical industry wastewater were investigated in an AASR system. The fates of RD and PAHs were investigated in the AASR system. The effects of some environmental conditions (dissolved oxygen, temperature, electron donors and pH) on the PAH yields were investigated. Furthermore, the effect of increasing SRTs on acute toxicity removals was investigated using the *Daphnia magna* test.

2. Materials and methods

2.1. Experimental set-up

An aerobic activated sludge (AASR) system made of stainless steel was used in the experimental study. The configuration of the AASR reactor is illustrated in Fig. 1. It consists of an aerobic (effective volume = 9.0 l) and a settling compartment (effective volume = 1.3 l). The AASR was continuously fed from the bottom by a feeding pump with raw wastewater taken from the influent of the aeration tank of wastewater from the petrochemical industry. The AASR was aerated by an air pump and porous diffusers to maintain the DO concentrations between 4 and 6 mg l^{-1}. The effluent wastewater from the aeration tank to the sedimentation tank passed through holes in a plate inclined at 45° to the horizontal axis. Effluent leaving the sedimentation tank was collected in an effluent tank.

2.2. Chemicals

PAHs and solvents used in GC–MS, RD, EM and SRr were purchased from Aldrich Chemical Company and have purities of 99% or greater. A mixture of R1 and R2 RD biosurfactants (commercially known as JBR natural biosurfactant) was used in this study. The second biosurfactant EM is a complex extracellular acylated polysaccharide produced by the Gram-negative bacterium *Acinetobacter calcoaceticus*. SR is a very powerful surfactant commonly used as an antibiotic. It is a bacterial cyclic lipopeptide, mainly known for its exceptional surfactant power. Its amphiphilic properties help this substance to survive in both hydrophilic and hydrophobic environments.

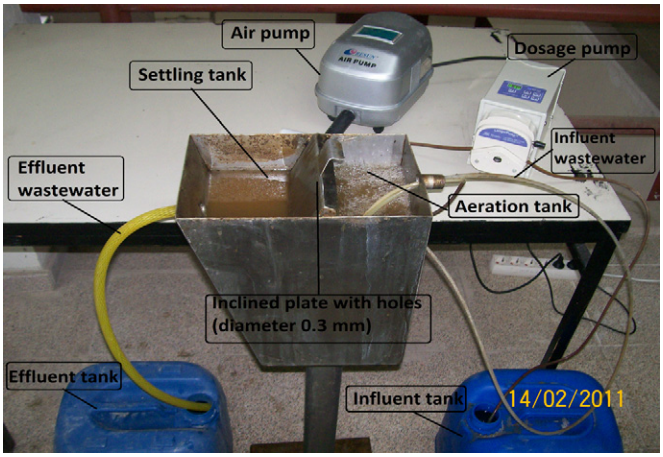

Fig. 1. Configuration of aerobic activated sludge reactor (AASR) system.

2.3. Operational conditions

In this study real wastewater was taken from the influent of the aerobic tank of the petrochemical industry wastewater treatment plant in Izmir, Turkey. The activated sludge was taken from the recycle line of the final settling unit of the aeration tank. The flow rate and hydraulic retention time (HRT) were constant as 2 l d^{-1}, and 5 days, respectively. The dissolved oxygen (DO) in AASR was adjusted to 4–6 mg l^{-1} with an air pump at flow rates of 2.5–4.5 l day^{-1} The SRTs were adjusted to 5, 15, 25 and 40 days by discarding an appropriate volume of activated sludge daily from the aeration tank of the AASR system. With each SRT change the AASR reactor was operated 20–25 days to reach steady-state conditions. Steady-state conditions were defined by stable COD and PAH removals higher than 65–70% for seven consecutive days. The results given in tables and figures are data representing the steady-state conditions. The food to mass (F/M) ratio and organic loading rate (OLR) in the AASR system were measured as 0.14 g COD g^{-1} VSS d^{-1} and 0.34 g COD l^{-1} d^{-1}, respectively. The mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentrations in the AASR were 2950 and 2356 mg l^{-1}, respectively.

The OU, OUR tests were performed in 500 ml glass flasks containing mixed samples of wastewater-biomass taken from the AASR. They were capped with silicone suba-seal septa (Sigma–Aldrich) and wrapped with aluminium foil to prevent photolysis.

The flasks were divided into five groups, high-oxygen and low-oxygen, anaerobic and anoxic conditions. For the high-oxygen condition the flask was aerated with an air pump every day to reach dissolved oxygen concentrations of 4–6 mg l^{-1}. For the low-oxygen condition, the flask was treated as that of non-oxygen condition but about 2 ml N_2 gas in the flask was then replaced with purified oxygen gas (>99.9%), and the 2 mg l^{-1} content was obtained by replacing N_2. During these tests the dissolved oxygen was measured frequently. During the biodegradation experiment, the

Table 1
Physical and chemical properties of the PAHs studied in this study.

PAHs	CAS-No	Molecular formula	M^w (g mol^{-1})	T^m (°C)	T^b (°C)	S^w (25 °C) (mg l^{-1})	V^p (25°C) (mmHg)	H (25 °C) (atm m^3 mol^{-1})	log K_{OA} (25 °C)	log K_{OW}
ACT	83-32-9	$C_{12}H_{10}$	154	93	279	390E−02	2.15E−03	1.84E−04	6.52	3.92
FLN	86-73-7	$C_{13}H_{10}$	166	115	295	169E−02	6.00E−04	9.62E−05	6.9	4.18
PHE	85-01-8	$C_{14}H_{10}$	178	99	340	115E−02	1.21E−04	3.35E−05	7.68	4.46
BbF	205-99-2	$C_{20}H_{12}$	252	168	–	1.50E−03	5.00E−07	6.57E−07	11.34	5.78
BkF	207-08-9	$C_{20}H_{12}$	252	217	480	8.00E−04	9.70E−10	5.84E−07	11.37	6.11
BaP	50-32-8	$C_{20}H_{12}$	252	177	495	1.62E−03	5.49E−09	4.57E−07	11.56	6.13
IcdP	193-39-5	$C_{22}H_{12}$	276	164	536	1.90E−04	1.25E−10	3.48E−07	12.43	6.70
DahA	53-70-3	$C_{22}H_{14}$	278	270	524	2.49E−03	1.00E−10	1.23E−07	12.59	6.75
BghiP	191-24-2	$C_{22}H_{12}$	276	278	>500	2.60E−04	1.00E−10	3.31E−07	12.55	6.63

Acenaphthene (ACT), flourene (FLN), phenanthrene (PHE), benz[*b*]fluoranthene (BbF), benz[*k*]fluoranthene (BkF), benz[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IcdP), dibenzo[*a*,*h*]anthracene (DahA), benzo(*ghi*)perylene (BghiP), M^w: Molecular weight, T^m: Melting point, T^b: Boiling point, S^w: Solubility in water, V^p: Vapor pressure, H: Henry's law constant, log K_{OW}: Octanol–water coefficient, log K_{OA}: Octanol–air coefficient.

low- oxygen condition was maintained by refilling oxygen gas every 2 days to compensate for the oxygen utilized by the microorganisms in the flask. The anaerobic condition was obtained by vacuuming the headspace in the conical flask for 30 s, then refilling it with about 90 ml purified N_2 gas (>99.9) with a syringe. This procedure was repeated three times to ensure that all of the oxygen inside the flask was replaced by N_2 gas. The anoxic condition was performed under zero dissolved oxygen and by adding 80–125 mg l^{-1} $NaNO_3$ to the glass flasks. The effects of Fe^{+2}, SO_4^{-2} on PAH yields were investigated by adding 40–60 mg l^{-1} $FeSO_4^{-2}$ and 55–125 mg l^{-1} $CaSO_4^{-2}$ to the glass flasks. The oxygen content in each flask was monitored with an oxygen-meter. The pH of the batch tests were adjusted by 0.5 N H_2SO_4 and 0.2 N NaOH (Table 1).

2.4. Analytical procedures

2.4.1. Measurement of conventional parameters

Dissolved chemical oxygen demand (COD_{dis}), Oil-grease, BOD_5, total suspended solid (TSS), mixed liquor volatile suspended solid (MLVSS) and mixed liquor suspended solid (MLSS) measurements were carried out according to Standard Methods [12]. The total nitrogen, total phosphate, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen were measured spectrophotometrically using Merck kits numbered 14537, 14729, 14752, 14547, and 14773, respectively. Dissolved oxygen (DO) was measured using an oxygen-meter (WTW Oxi330). The oxidation–reduction potential (ORP) and the pH were measured with ORP-meter (WTW DO-SET) and pH-meter (WTW pH 33i). The oxygen utilization rate (OUR) was measured by measuring the DO versus time in closed glass bottles containing mixed wastewater and biomass taken from the AASR. Heavy metals were measured in an ICP-OES following Standard Methods [12]. The COD_{inert} was measured using the glucose comparison method. This method involves running three batch reactors, two with the wastewater to be studied and the third with glucose. One of the wastewater reactors has the total COD, and the second has the total soluble COD, whereas the initial COD in the glucose reactor is adjusted to equal COD value. The experimental studies are performed until all the biodegradable COD is depleted, where the COD profiles reach a plateau and stay unchanged. The difference between glucose COD and wastewater COD gives the COD_{inert} [11]. The inert soluble COD generated as residual microbial products by means of growth or decay associated processes (COD_{imp}) was calculated from the [(final soluble COD (remaining COD) − (COD_{inert})] [11]. RD, EM and SR measurements were performed in an AquaMED spectrophotometer at wavelengths of 221, 298 and 302 nm. After centrifugation of the samples, 0.1 ml sample was added to 0.9 ml of a solution containing 0.19% sorcinol and 53% H_2SO_4 for RD measurement. After heating for 20 min. at 100 °C the samples were cooled at room temperature and the absorbances were measured in a spectrophotometer versus standard RD concentrations [13]. For quantification of EM, hexadecane and two-methylnaphthalene, in a 1:1 volumetric ratio, in a 20 mM tris–HC1 buffer solution containing 10 mM $MgSO_4$ was added, keeping a volumetric ratio of samples to water of 1:75. The assay mixtures were shaken at 30 °C in baffled flasks for 1 h. The resulting turbidity of the EM formed was measured in the spectrophotometer. The data were correlated with the standard curve for EM [14]. For SR measurement the samples were washed four times by filtration. Then methanol was added to the retentate until the concentration of methanol reached about 70% (v/v), and the solution was filtered through a 0.2 μm membrane. The final filtrate was concentrated by evaporation using a Heidolph VV 2000 Rotavaporator [15]. All the microbiological analyses were performed in media given in Standard Methods [12]. For *Pseudomonas aeruginosa* enumeration M-PA agar was used after 48 h incubation at 35 °C following the detection and the identification steps of this bacteria with growth in selective Bushneel Haas and Thioglycollate Broths, respectively, at 35 °C three days. The biochemical tests namely Voges Proskaver, Indol, Oxidase, Methyl Red and Catalase tests were performed. Gram staining was performed to the isolated bacteria. The floc bacteria based on *Zoogloea ramigera* were enumerated using the M-ZR agar with glucose and yeast extract after 150 h incubation at 30 °C. Some biochemical tests (flagella, pigment formations and hydrolysis of gelatin tests) applied to the colonies in M-ZR agar to identify the bacteria.

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). All extracts were analysed for nine PAHs with a gas chromatography (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^{-1}, 45 cm s^{-1} linear velocity). Signal to noise (S/N) was taken into consideration for every PAH compound at their lowest concentrations. The measured signal-to-noise (S/N) ratios varied between 64 and 559. The limit of detection (LOD) and limit of quantification (LOQ) data varied between 0.909 and 1.908 and between 1.175 and 5.201, respectively.

2.4.3. Daphnia magna acute toxicity test

Acute toxicity was tested using 24 h born *Daphnia magna* as described in Standard Methods [12]. Experiments were carried out using 10 *Daphnia magna* introduced into the test beakers with 100 ml effective volume at 7–8 pH, providing a minimum dissolved oxygen concentration of 6 mg l^{-1} at an ambient temperature of 20–22 °C. The results were expressed as the mortality percentage

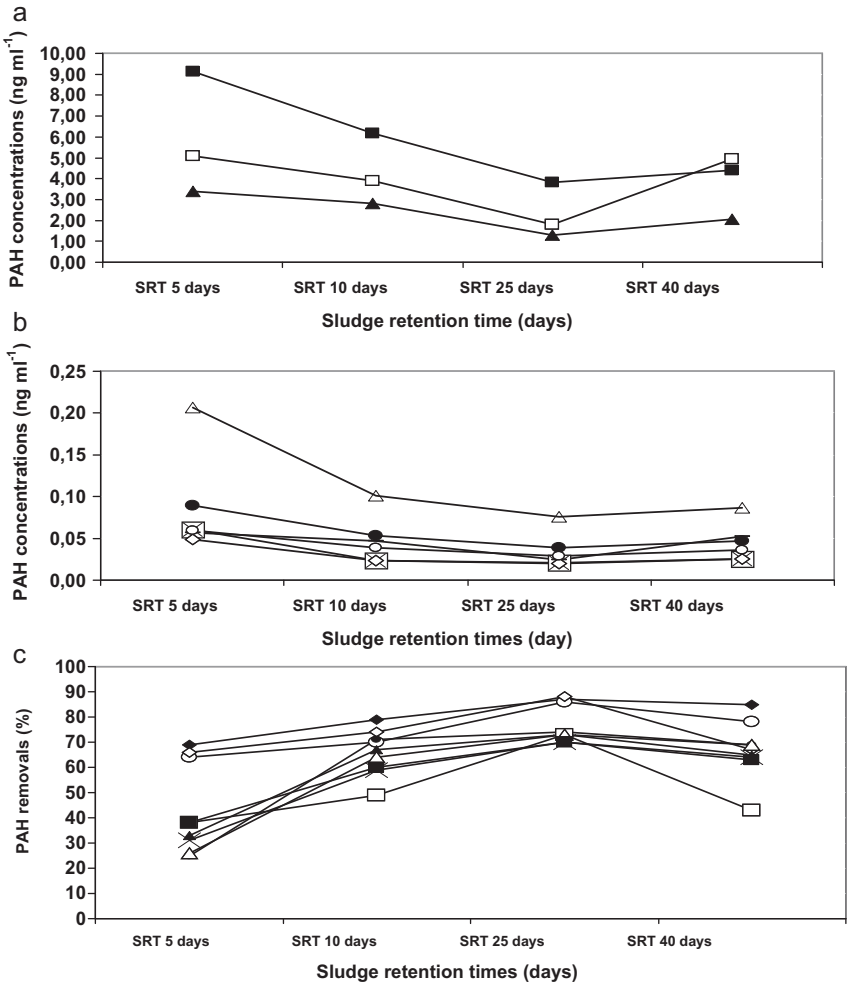

Fig. 2. Variations of PAH concentrations (a and b) and PAH yields (c) versus sludge retention times in AASR ■ ACT effl., ▲ FLN effl; □ PHE effl. (a); X BbF effl., – BkF effl., ◊ BaP effl., ♦ IcdP effl., Δ DahA effl., ○ BghiP effl. (b); ♦ ACT (%), ○ FLN(%), ◊ PHE (%), ● BbF (%), □ BkF(%), ▲ BaP (%), X IcdP (%), Δ DahA (%), ■ BghiP (%) (c) (mean values) (without RD).

of the *Daphnia magna* after 24 h. Animals which were not able to move were determined as dead *Daphnia magna*.

2.5. Statistical analysis

The regression analysis between *y* (dependent) and *x* (independent) variables was carried out using Windows Excel data analysis. An ANOVA test was performed in order to determine the statistical significance between *x* and *y* variables.

3. Results and discussion

3.1. Start-up period of the AASR system 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 AASR system. In order to ensure acclimation of the aerobic biomass to the petrochemical wastewater, four lab-scale AASR systems were operated through 15–20 days to reach steady-state conditions at each SRT from 5 up to 40 days [7]. Steady-state conditions were defined as COD and total PAH removal efficiencies higher than 75% and 65%, respectively, for 5–7 consecutive days. The AASR systems reached steady-state conditions after an operation period of 15–20 days, depending on the SRTs studied. After this operation time, the PAHs and the total COD removal efficiencies remained constant at approximately 69% and between 70 and 79%, respectively, through continuous operation in AASR systems without rhamnolipid [7].

3.2. Effects of increasing SRT on PAH, COD_{dis} and COD_{inert}, COD_{imp} removals in AASR system treating petrochemical wastewater

The results of this study showed that as the SRTs were increased from 5 to 25 days the removals of less hydrophobic PAHs with three benzene rings (ACT, FLN, and PHE) and more hydrophobic PAHs with five and six benzene rings (BbF, BkF, BaP, IcdP, DahA, and BghiP) increased from 25 to 74% and from 69 to 87% in the AASR system, respectively (Fig. 2). The removal yields of the PAHs in question decreased from 70% to 63% and from 88% to 67% as the SRTs were increased from 25 days up to 40 days. Although the enzymatic activity of the biomass was highest at low SRTs in the aerobic activated sludge reactor systems treating COD originating from the slowly degradable organics, long SRTs are necessary to treat the wastewaters containing hydrophobic PAHs, the COD_{inert} and the inhibitory substances. In this study high SRTs such as 25 days provided enough contact time between microorganisms and less and more hydrophobic PAHs with low solubilities (1.90E-04; 3.90E-02 mg l^{-1}), Henry's law constants (1.23E-07;1.84E-04 atm m^3 mol^{-1}) and low vapor pressures

(1.00E-10; 2.15E-03 mmHg) (Table 1). The reason for the decrease in PAH yields at SRTs > 25 days could be attributed to the low enzymatic activities of the aged biomass and to the biomass decay in the AASR system. Aged bacteria did not effectively degrade the hydrophobic PAHs. As a result the PAHs accumulated in the AASR at SRTs > 25 days. The SRT increase induced more release of the inert microbial products due to decay mechanisms, endogenous respiration and also metabolic products associated with bacteria. On the other hand, the increment of biomass in AASR was another factor because high biomass or low *F*/*M* condition could cause a decrease in the numbers of viable cells and consequently, an increase in specific decay rate of biomass. This caused more soluble inert isismetabolised cellular material concentration in the AASR system. This soluble inert microbial products COD is usually the major component of the soluble organic matter, e.g., soluble COD in effluents from biological wastewater treatment plants [11].

There are approximately three to six orders of magnitude difference between ACT and BghiP PAHs in aqueous solubility, a tendency to partition from hydrophobic organic matter into water, vapor pressure at room temperature, and a tendency to partition between water and air Henry's law constant. The octanol water partition coefficient increased from 3.92 up to 6.75 indicating that aqueous solubility decreased from PAHs with three benzene rings up to PAHs with six benzene rings (Table 1). In general, PAHs become increasingly less soluble in water with an increasing number of benzenoid or other rings, and increasing molecular weight. ACT, a three-ring PAH, is soluble, with an estimated aqueous solubility of around 390E-02 mg l^{-1} at 25 °C. BghiP a six-ring PAH, has a much more limited aqueous solubility at a room temperature of approximately 2.60E-04 mg l^{-1}. Lower molecular weight PAHs with three rings also tend to be more volatile (i.e., have a higher vapor pressure) and more readily partition into air from pure water (i.e., have a higher Henry's law constant). In this study, no significant difference in PAHs yields was observed between some PAHs with three (ACT, FLN, and PHE), five (BbF, BkF, and BaP) and six rings (DahA, BghiP and BghiP) although the PAHs with high benzene rings became increasingly less soluble in water with an increasing number of benzenoid ring and molecular weight, and lower Henrry's low constant and less volatility which were biodegraded effectively in the AASR system (Table 1).

Zheng et al. found that the lower weight PAHs with two, three, and four rings were biodegraded more rapidly than the higher weight (≥5 ring) PAH compounds [16]. The transfer of PAHs from the bulk liquid to the biomass is essential for aerobic removal of hydrophobic PAHs. Since the hydrophobic PAHs are not soluble and the low Henry's law constants characterize the relative amount of a substrate that will enter the biomass; the removal of these PAHs causes serious problems in petrochemical wastewaters. Haritash and Kaushik showed that the Henry's law constant is strongly associated with the transfer rate of PAHs to the biomass since the low Henry's law constants or low vapor pressures of the hydrophobic PAHs decrease their aerobic biodegradations [17]. On the other hand the studies performed by Feldmannova et al. showed a linear correlation between PAH yields and physichochemical properties of PAHs [18]. They reported that the PAH removals decreased in hydrophobic PAHs as the Henry's law constant and vapor pressure decreased. In contrast to these studies, in the present research a significant linear correlation was not found between hydrophobic PAHs and their physicochemical properties ($R^2 = 0.83$, $F = 2.45$, $p = 0.01$). The aerobic degradation of hydrophobic PAHs is not dependent on the physichocemical properties of PAHs. In this study PAHs with three benzene rings (ACT, FLN, and PHE) were rapidly removed with more than 87% reduction at a SRT of 25 days (Fig. 2). The removal of other PAHs with five (BbF, BkF, and BaP) and six (IcdP, DahA, and BghiP) benzene rings varied between 73 and 74% for the same sludge age indicating that the more hydrophobic PAHs were degraded with high removals although these were not as high as the PAHs with lower weights.

The recalcitrance of PAHs may play an important role in decreasing the COD removal efficiency in affected treatment systems. Substantial parts of the COD and PAHs may be biodegradable while other parts of the COD may be inert in petrochemical wastewaters. The COD parameter alone used for substrate utilization cannot give enough information about the degradation of the organic matter. Therefore, biological degradation parts and inert fraction of COD must be determined since all design calculations need to deal with biodegradable COD [11]. This is important for refractory and toxic wastewaters such as the petrochemical industry.

The amount of organic carbon is only meaningful when it is expressed in terms of various fractions with different mechanisms and rates of biodegradation in petrochemical industry wastewaters. In this respect, COD fractionation has been introduced as a very useful tool for the evaluation of the aerobic activated sludge process treating petrochemical industry wastewater. COD fractionation involves identification of COD_{dis} together with soluble COD_{imp} and COD_{inert} fractions since they do not give any reaction in the activated sludge system and are released together with wastewater discharge [7,11]. High PAH concentrations, CODs originating from the soluble inert compounds, from the bacterial metabolites and from the slowly degradable compounds such as in the petrochemical industry wastewater may reduce the performance of treatment plants [7]. The COD_{dis} is hypothesized to consist of simple soluble molecules that can be absorbed by the organisms. In the aerobic hydrolysis, the COD_{dis} is transformed into readily degradable COD and a small fraction of inert COD. The soluble COD_{imp} generated by the hydrolysis of slowly degradable organics to readily degradable organics and by the decay of biomass through endogenous phase is directly converted into stored material in bacterial cells [7,11]. These stored compounds are subsequently used as a carbon and energy source for growth purposes.

The COD_{inert} concentration in the influent wastewater was 260 mg l^{-1} while the COD_{imp} increased from 0 to 98 mg l^{-1} in the effluent samples (Table 2). The influent COD_{inert} was not consumed by the microorganisms throughout PAH degradation in the AASR system. The effluent organic matter of the AASR system treating petrochemical wastewater is composed of refractory compounds, residual degradable substrates, intermediates, end products, complex organic compounds, and soluble microbial products. The characteristics of the effluent organic compounds from different wastewater treatment plants vary due to differences in treatment processes and their operational conditions. Formation of inert microbial products is found to increase during stress conditions, e.g., hydraulic shock loads, low pH, nutrient deficiency, and in the presence of toxic non-biodegradable compounds such as hydrophobic PAHs. The results of this study showed that the COD_{inert} and COD_{imp} concentrations increased mainly at a SRT of 40 days in the effluent samples. This could be attributed to the death of aged bacteria through cell lysis. Furthermore the accumulation of hydrophobic PAHs and long contact times between bacteria and PAHs and their metabolites caused toxicity to the bacteria in the AASR system. The metabolites produced from some PAHs in the presence of RD and the toxicity of PAHs were given in next sections (Tables 5 and 9).

3.3. Selection of biosurfactant

To determine the influence of some biosurfactants on the growth and viability of the cells of floc bacteria and *Pseudomonas* sp. the petrochemical industry wastewaters were treated with three different biosurfactants, namely rhamnolipid (RD), emulsan (EM) and surfactin (SR) and tested at a constant SRT of 25 days, since the optimum sludge age for maximum total PAH and COD_{dis}

Table 2
Concentrations of COD_{dis}, COD_{inert}, COD_{imp}, total PAH and removal efficiencies in influent/effluent wastewater of AASR system at increasing SRTs ($n = 3$, mean values) (without RD).

	SRT 5 days				SRT 10 days				SRT 25 days				SRT 40 days			
	COD^{dis}	COD_{inert}	COD_{imp}	PAH	COD_{dis}	COD_{inert}	COD_{imp}	PAH	COD_{dis}	COD_{inert}	COD_{imp}	PAH	COD_{dis}	COD_{inert}	COD_{imp}	PAH
w^i	1345	260	0	54.57	1345	260	0	54.57	1345	260	0	54.57	1345	260	0	54.57
w^e	538	264	45	21.28	363	271	65	20.19	296	268	78	16.92	390	279	98	20.19
w^r	60	0	–	61	73	–	–	63	78	–	–	69	71	–	–	63

w^i: Influent wastewater; w^e: Effluent wastewater; w^r: Removal efficiency in wastewater(%); COD_{dis}: dissolved COD concentration ($mg\,l^{-1}$); COD_{inert}: COD concentration originating from the inert compounds ($mg\,l^{-1}$); COD_{imp}: COD concentration originating from the inert microbial products ($mg\,l^{-1}$); PAH: total PAH concentration ($ng\,ml^{-1}$).

removals was found to be 25 days (Table 2). These two organisms were selected since the predominant bacterial population in AASR was comprised of 93% Gram-negative bacteria principally *Pseudomonas aeruginosa* (53%), *Zoogloea ramigera* (floc bacteria, 30%), *Flavobacterium* (7%) and *Comamonas* (3%) (data not shown). Furthermore, the preliminary studies performed by our team showed that *Pseudomonas aeruginosa* and *Zoogloea ramigera* are able to isismetabolise the PAHs effectively in the presence of some biosurfactants at optimum doses [7]. According to the biochemical tests results it was found that the Voges Proskaver, the Indol, the Oxidase and Methyl Red tests were negative while the catalase test was positive. Gram staining showed that the isolated bacteria were Gram negative and rod shaped *Pseudomonas* bacteria. Among the biochemical tests applied to the colonies in M-ZR agar the flagella, pigment formations and hydrolysis of gelatin tests were positive while the acid production from glucose and hydrolysis of starch tests were negative. Gram staining result showed that the Gram-negative, rod-shaped bacterium that formed characteristic cell aggregates surrounded by gelatinous matrices, was *Zoogloea ramigera.*

Tables 3a and 3b show the effects of different concentrations of RD, EM and SR on the growth of Zoogloea *ramigera* and *Pseudomonas aeruginosa* bacteria in the AASR system. The biosurfactant concentrations were chosen according to the recent literature and our preliminary studies [5,7,12]. The presence of RD supported and stimulated the cell growth with respect to the control whereas EM and SR biosurfactants did not encourage the growth of floc bacteria and *Pseudomonas aeruginosa* significantly. The viability of bacteria cells decreased in the control AASR system, which contained only raw petrochemical wastewater without biosurfactant. The addition of $10\,mg\,l^{-1}$ RD to the AASR system resulted in an enhancement of cell growth compared to the control (Tables 3a and 3b). However, in the presence of $15\,mg\,l^{-1}$ RD the cell growth reached the highest values. Comparison of the cell growth and viability with different concentrations of RD also revealed that such surfactant is utilized by the floc and *Pseudomonas aeuruginosa* bacteria probably as an additional carbon source in the AASR system [19]. Biosurfactants can improve the PAH bioconversion process by increasing the mass transfer rates to cells [20]. In this study the contribution of the biosurfactant might be (i) to increase the mass transfer of PAH and the all COD fraction from the activated sludge liquid to bacteria cell and (ii) to change the hydrophobicity of the cell, and the changed hydrophobicity might enhance the direct cell attachment to PAHs and COD components.

As shown in Table 3a, the growth of floc (*Zoogloea ramigera*) and *Pseudomonas aeruginosa* bacteria increased, as the RD, EM and SR bisurfactant concentrations were increased from 10 to 15, 25 and $40\,mg\,l^{-1}$, respectively. These levels of biosurfactants were beneficial to both the floc and *Pseudomonas* sp. Bacteria growth. The maximum bacterial growth was between 10^7 and 10^8 colony in $100\,ml^{-1}$ wastewater sample at $15\,mg\,l^{-1}$ RD concentration. The number of bacteria were 10^5 colony and 10^4 colony in $100\,ml^{-1}$ wastewater samples for 25 and $40\,mg\,l^{-1}$ EM and SR biosurfactants, respectively. However, the growth of *Zoogloea ramigera* and *Pseudomonas aeruginosa* bacteria were significantly delayed or inhibited by the added surfactants at high levels, such as RD ($>15\,mg\,l^{-1}$), EM ($>25\,mg\,l^{-1}$),and SR ($>40\,mg\,l^{-1}$).When the concentrations of RD, EM and SR surfactants were $<15\,mg\,l^{-1}$, <25 and $<40\,mg\,l^{-1}$, respectively, no obvious effects on the growth of bacteria were noticed, indicating that these surfactants did not affect the uptake of the hydrophobic PAHs by the floc and *Pseudomonas* bacteria.

Table 3b shows the COD sub-categorisation in the presence of $15\,mg\,l^{-1}$ RD. The COD_{inert} concentrations decreased to $30\,mg\,l^{-1}$ in the effluent of AASR resulting in a inert COD removal efficiency of 88% at a SRT = 25 days. This could be explained by the uptake of inert COD to the cell together with hydrolyzed slowly degradable

Table 3a
Effects of biosurfactants on the bacterial cell number in AASR system treating PAHs (n = 3, mean values).

a^{bn}	b^{c}	c^{RD}			d^{EM}			e^{SR}		
		10	15	25	10	25	75	10	40	50
Z. ramigera [f]	2×10^2	4×10^3	8×10^7	9×10^5	3×10^2	2×10^5	2×10^4	4×10^2	5×10^4	6×10^2
P. aeruginosa[g]	6×10^2	4×10^3	6×10^8	9×10^4	3×10^2	2×10^5	2×10^4	2×10^2	5×10^4	6×10^3

a^{bn}: Bacteria number (colony 100 ml^{-1}); b^{c}: Control; c^{RD}: Rhamnolipid concentration (mg l^{-1}); d^{EM}: Emulsan concentration (mg l^{-1}); e^{SR}: surfactine concentration (mg l^{-1}); f: floc bacteria, *Zoogloea ramigera*; g: *Pseudomonas aeruginosa*.

organics through fast PAH diffusion with rhamnolipid. As shown in Table 3b, the COD_{imp} yield was 93% at a SRT = 25 days. In the presence of RD the bacteria released low extracellular materials since the PAH could be used effectively by the PAH degrading microorganisms compared to no RD amended AASR as shown in Table 2.

The existence of surfactants could increase the solubility of PAHs with three, five and six benzene rings and they were probably used with PAHs as the carbon and energy source [20]. Normally, bacteria only utilize solubilized PAHs. Thus, the presence of RD, EM and SR biosurfactants at optimum concentrations may stimulate the biodegradation of hydrophobic PAHs to be taken up by the bacteria. In the present study, the growth of both bacteria was slightly increased with low biosurfactant concentration (10 mg l^{-1}). It was observed that *Microbacterium* sp. did not grow when the RD level exceeded 40 mg l^{-1} [19]. Yin et al. showed that the addition of 200 mg l^{-1}, RD increased the PHE, ANT PAH removals in an oil industry wastewater [21]. Zhu and Zhang reported that a RD concentration of 50 mg l^{-1} increased the PHE and CHR PAH yields [22]. This optimum RD concentration is significantly higher than that the RD dose used in this study. Yin et al. found that the biodegradation of PAHs could be promoted by reducing the interfacial tension of wastewater [21]. An exponential increase in bacterial cell number even during hydrophobic PAHs degradation was observed with RD (Tables 3a and 4) and this can be attributed to the growth of biomass on PAHs and their catabolic intermediates [20]. The studies performed by Lei et al. showed that little or no PAH degradation was observed at high biosurfactant concentrations [23]. The negative observations regarding the biosurfactants can be explained by one or more of the following effects: (a) toxicity of surfactants due to surfactant-induced permeabilization or lysis of the bacterial cell membrane, (b) toxicity of biosurfactant enhanced aqueous PAH concentrations, (c) prevention of bacterial adhesion to the hydrophobic PAHs. Decreases in bacteria concentrations at high biosurfactant concentrations could be attributed to the reduced bioavailability of the biosurfactant as a barrier to hinder direct contact of bacterial cells to micellar-phase PAHs [22].

3.4. Effect of SRT and RD concentrations on the removal of PAHs

In order to determine the optimum RD dose for the maximum removals of PAHs with three, five and six benzene rings; 10 mg l^{-1} (RD 10), 15 mg l^{-1} (RD 15), 30 mg l^{-1} (RD 30), and 50 mg l^{-1} (RD 50) RD were administered to the feed of the AASR system at four increasing SRTs. As the SRT and the RD concentrations were increased from 5 to 25 days and from 10 to 15 mg l^{-1} the individual hydrophobic PAH removals increased from 30 to 70% and 37–85% to 70–90% and 94–97%, respectively (Table 4). In the presence of 15 mg l^{-1} RD a longer SRT remarkably increased the hydrophobic PAH removals in the AASR system. The yields of PAH increased as the SRT was increased from 5 to 25 days, but decreased as the SRT was further increased to 40 days. The presence of 15 mg l^{-1} RD increased the more hydrophobic PAH removals with five (BbF, BkF and BaP) and six benzene rings (IcdP, DahA and BghiP) from 70 to 74% up to 94–98%, remarkably, at a SRT of 25 days compared to control without RD. The high PAH removals with 15 mg l^{-1} RD could be attributed to the increase in the surface area of the PAHs, which subsequently increased the substrate, flux from aqueous medium to bacterial cells [23].

At low and high RD concentrations (5, 10, 50 mg l^{-1}) the PAH removals were low in the AASR systems for all studied SRTs compared to the 15 mg l^{-1} RD (Table 4). Addition of RD to the AASR reactors improved both less and more hydrophobic PAH removals (E > 95). Similarly, although BbF and BkF PAHs have similar (same number of benzene rings, low Henry's law constant, high octanol–air and octanol–water coefficients) physichochemical properties BkF PAH was removed with low yields compared to its counterpart BbF. During PAH degradation in the presence of RD, a significant linear correlation was observed between increasing SRT and PAH removals up to a SRT and a RD concentration of 40 days and 25 mg l^{-1}, respectively (R^2 = 0.84, F = 2.56, p = 0.05). The further addition of an external biosurfactant could have deleterious effects on bacteria in the AASR system. This may be due to the toxic effect of the biosurfactant on the biomass or it may be utilized partially as carbon and energy sources due to the restricted rate of mass transport between surfactant micelles and aqueous phase [24,25].

In the presence of 15 mg l^{-1} RD the metabolites produced from ACT, PHE, FLN and DahA PAHs were analysed in GC-MS for the samples taken from the effluent of the AASR at a SRT of 25 days. NAP and 5,8-dihyrox-1,4-naphthoquinone are the metabolites detected from the aerobic bio-degradation of ACT in the AASR (Table 5). From 29.43 ng ml^{-1} ACT, 17.27 ng ml^{-1} NAP and 3.89 ng ml^{-1} 5,8-dihyrox-1,4-naphthoquinone were produced. Although the ACT PAH yield was 96% in the AASR, the effluent pollutant concentration (0.10 mg ml^{-1}) consisted only of its aerobic metabolites (0.04 ng ml^{-1} NAP and

Table 3b
Variation in COD subcategories in the presence of 15 mg l^{-1} RD (n = 3, mean values).

SRT[a]	SRT 10 days			SRT 25 days		
	COD_{dis}	COD_{inert}	COD_{imp}	COD_{dis}	COD_{inert}	COD_{imp}
w^{i}	1345	260	0	1345	260	0
w^{e}	121	83	29(65[b])	78	30	5 (78[c])
w^{r}	86	68	55	95	88	93

w^{i}: Influent wastewater; w^{e}: Effluent wastewater; w^{r}: Removal efficiency in wastewater(%); COD_{dis}: dissolved COD concentration (mg l^{-1}); COD_{inert}: COD concentration originating from the inert compounds (mg l^{-1}); COD_{imp}: COD concentration originating from the inert microbial products (mg l^{-1}).

[a] SRT, Sludge retention time.

[b] The COD_{imp} yield was calculated according to COD_{imp} concentration obtained in RD free AASR at a SRT 10 days (Table 3a).

[c] The COD_{imp} yield was calculated according to COD_{imp} concentration obtained in RD free AASR at a SRT 25 days(Table 3a).

Table 4
Effects of SRT and RD concentrations on the removals of nine PAHs ($n = 3$, mean values).

PAHs	a^m	PAH removal efficiencies (%) (SRT 5 days)				PAH removal efficiencies (%) (SRT 10 days)				PAH removal efficiencies (%) (SRT 25 days)				PAH removal efficiencies (%) (SRT 40 days)			
		b^c	c^{10}	c^{15}	c^{30}	c^{50}	b^c	c^{10}	c^{15}	c^{30}	c^{50}	b^c	c^{10}	c^{15}	c^{30}	c^{50}	b^c	c^{10}	c^{15}	c^{30}	c^{50}
ACT	3	60	70	85	62	55	64	70	87	74	35	87	90	96	76	70	68	83	93	70	50
FLN	3	60	65	75	70	40	64	70	80	70	45	86	89	97	83	72	79	70	78	74	58
PHE	3	55	60	69	69	50	63	70	74	65	50	88	90	96	65	62	79	60	67	70	60
BbF	5	38	50	59	60	30	45	69	71	65	50	74	79	95	53	47	68	60	69	65	59
BkF	5	30	30	37	50	29	40	59	45	40	30	73	78	96	48	44	59	32	43	59	60
BaP	5	45	55	67	30	40	46	59	67	38	34	73	73	95	54	47	62	60	65	60	52
IcdP	6	50	58	63	30	40	53	62	89	46	40	70	70	95	67	68	62	74	89	60	50
DahA	6	43	50	56	50	40	47	61	84	34	29	73	80	94	61	31	64	78	83	62	54
BghiP	6	40	64	75	43	40	43	59	81	35	29	70	78	95	43	49	61	74	87	64	60

a^m: Ring number; b^c: PAH removal efficiency (%) in control; c^{10}: PAH removal efficiency (%) in reactor containing 10 mg l^{-1} RD; c^{15}: PAH removal efficiency (%) in reactor containing 15 mg l^{-1} RD; c^{30}: PAH removal efficiency (%) in reactor containing 30 mg l^{-1} RD; c^{50}: PAH removal efficiency (%) in reactor containing 50 mg l^{-1} RD. SRT: sludge retention time.

0.06 ng ml^{-1} 5,8-dihyrox-1,4-naphthoquinone). The metabolites of PHE and FLN are 9-phenanthrol and 9-hydroxyflourene (Table 5). 15.01 ng ml^{-1} PHE and 9.38 ng ml^{-1} FLN converted to 13.57 ng ml^{-1} 9-phenanthrol and 7.27 ng ml^{-1} 9-hydroxyflourene, respectively, throughout aerobic treatment in the AASR. Although these metabolites were removed with a yield of 97%, the effluent PAH concentration consisted only of PHE (0.45–0.25 = 0.15 ng ml^{-1})and FLN(0.39–0.20 = 0.19 ng ml^{-1}) not from metabolites (Table 5). The metabolites of 0.27 ng ml^{-1} DahA consisted of 0.004 ng ml^{-1} Benz(a)anthracene, 0.002 ng ml^{-1} Benz(a)anthracene-7,12dione and of 0.001 ng ml^{-1} 1,2-benzenedicarboxaldehyde (phthaldialdehyde). These metabolites were not removed in the AASR. The effluent of the AASR contained only DahA metabolites since 0.007 ng ml^{-1} is the sum of Benz(a)anthracene, Benz(a)anthracene-7,12 dione and phthaldialdehyde.

3.5. Effect of SRT on the fates of PAH and RD surfactant

The degradations and accumulations of PAHs and RD were examined in the AASR system with the presence of varying rhamnolipids additions and SRTs. Usually PAH and surfactant accumulation are not desirable in the AASR system due to environmental concerns and these are regarded as important factors in various applications. When the SRT was increased from 10 to 25 days, the overall removal efficiency of more and less hydrophobic PAHs by the AASR system increased from 88% to 97% ($R^2 = 0.87$, $F = 2.02$, $p = 0.01$). However, there was no significant change when the SRT was increased from 25 to 40 days (Table 6). The possible fate of PAHs in AASR system most likely includes being discharged with the final effluent and waste sludge, accumulating in the system, and being degraded by microorganisms in activated sludge. The fate of PAHs was calculated based on the following mass balance equations:

$$\text{PAH-}M_{\text{inlet}} - \text{PAH-}M_{\text{outlet}} = \text{PAH-}M_{\text{sludge}} + \text{PAH-}M_{\text{biodegradation}} + \text{PAH-}M_{\text{accumulation}} \quad (1)$$

$$\text{RD-}M_{\text{inlet}} - \text{RD-}M_{\text{outlet}} = \text{RD-}M_{\text{sludge}} + \text{RD-}M_{\text{biodegradation}} + \text{RD-}M_{\text{accumulation}} \quad (2)$$

The unit in Eq. (1) was ng day^{-1} while the unit in Eq. (2) was mg day^{-1}. Here M_{inlet}, M_{outlet}, and M_{sludge} are PAH masses in the inlet, effluent, and waste sludge, respectively. They were calculated based on the relevant PAH concentrations. $M_{\text{accumulation}}$ is PAH mass accumulated in the AASR system. $M_{\text{biodegradation}}$ is PAH mass degraded by the activated sludge in AASR which was obtained from Eq. (1). With a similar equation, the mass balance of RD was investigated in the AASR system. The PAH concentrations measured in different parts of the AASR were shown in Table 6. It was found that the PAHs were mainly biodegraded in the AASR. 94–97% of PAHs was biodegraded, 1.1–1.5% and 0.7–1.2% of PAHs were accumulated in the sludge and in the aerobic reactor, respectively, while 0.9–1.3% of the PAHs were in the effluent of the AASR (Table 6). The concentrations of RD in the aerobic activated sludge and in the effluent were in the range of 0.25–0.40 mg l^{-1} and 0.20–0.38 mg l^{-1}, respectively, at initial RD concentrations of 15.01–15.09 mg l^{-1} (Table 6), suggesting that 14.31–14.61 mg l^{-1} RD was biodegraded throughout 94–97% PAH treatment in the AASR at a SRT of 25 days. It was shown that the removal of rhamnolipids from the aqueous phase of activated sludge caused the increase in surface tension in the activated sludge system. This decrease in rhamnolipid concentration in the aqueous phase could be due to the biological utilization as carbon sources by the microorganism as reported by [23]. The quick adsorption of rhamnolipids on biomass could largely cause the initially rapid decrease in surface tension in the beginning upon

Table 5
Metabolites of ACT, PHE, FLN and DahA PAHs at a SRT of 25 days and at a RD concentration of 15 mg l^{-1} in AASR (n = 3, mean values).

PAH name	ACT	PHE	FLN	DahA
$İ^a$	29.43	15.01	9.38	0.27
E^f	0.10	0,45	0,39	0.007
PAH^c	96	96	97	94
Metabolite name	NAP and 5,8-dihyrox-1,4-naphthoquinone	9-phenanthrol	9-hydroxyflourene	Benz(a)anthracene; Benz(a)anthracene-7,12dione and 1,2-benzenedicarboxaldehyde (phthaldialdehyde)
M^d	17.27 and 3.89	13.57	7.27	0.004; 0.002 and 0.001
M^e	0.04; 0.05	0,25	0,20	0.004; 0.002 and 0.001
M^y	98	98	97	0

$İ^a$: PAH concentration in the influent of AASR (ng ml^{-1}); E^f: PAH concentration in the effluent of AASR (ng ml^{-1}); PAH^c: PAH removal efficiency (%); M^d: Metabolite concentration in the AASR (ng ml^{-1}); M^e: Metabolite concentration in the effluent of the AASR (ng ml^{-1}); M^y: Metabolite removal efficiency (%).

Table 6
Fates of PAHs and 15 mg l^{-1} RD in the AASR system at a SRT of 25 days and a HRT of 80 h (n = 3, mean values).

PAHs	$a^{r\ n}$	PAH^b	PAH^c	PAH^d	PAH^e	PAH^f	RD^g	RD^b	RD^i	RD^k	RD^l
ACT	3	29.4386	0.9606	1.1775	0.1092	96					
FLN	3	9.3800	0.3538	0.2814	0.0984	97					
PHE	3	15.0104	1.6347	0.6004	0.4502	96					
BbF	5	0.8030	0.0041	0.0028	0.0021	95					
BkF	5	0.4912	0.0920	0.0019	0.0025	96	15.01–15.09	0.20–0.38	0.25–0.40	0.23–0.41	14.31–14.61
BaP	5	0.0723	0.0028	0.0011	0.0018	95					
IcdP	6	0.1292	0.0788	0.0025	0.0035	95					
DahA	6	0.2797	0.0023	0.0068	0.0074	94					
BghiP	6	0.0966	0.0092	0.0020	0.0027	95					
Total		54.5783	2.8405	1.0986	0.0797	96					

$a^{r\ n}$: Ring number in PAHs; PAH^b: (PAH $_{inlet)}$, influent PAH concentration (ng ml^{-1}); PAH^c: (PAH $_{acc.)}$, concentration of PAH accumulated in sludge (ng g^{-1} dw^{-1}.); PAH^d: (PAH $_{outlet.)}$, PAH concentration in effluent (ng ml^{-1}); PAH^e: (PAH $_{AASR)}$, PAH concentration in aeration tank (ng ml^{-1}); PAH^f: PAH removal efficiency (%);RD^g: (RD $_{inlet)}$, RD concentration in inlet (mg l^{-1}); RD^h: (RD$_{outlet}$), RD concentration in outlet (mg l^{-1}); RD^i: (RD$_{sludge}$), RD concentration in sludge (mg l^{-1}); RD^k: (RD$_{AASR}$), RD concentration in aeration tank (ng ml^{-1}); RD^l: (RD$_{biodeg)}$, RD concentration biodegraded in aeration tank (mg l^{-1}).

aeration while the microbial biodegradation of rhamnolipids dominated the decrease in surface tensions at a late stage when the PAHs were consumed.

Among the removed RD in the AASR 96–98% was biodegraded by the activated sludge process, 1.1–1.3% was discharged with the effluent, 1.2–1.4% accumulated in the aerobic reactor and only 1.3–1.5% remained in the waste sludge at a SRT of 25 days (Table 6). Preliminary studies showed that the main removal mechanism of PAHs was biodegradation (87%) while the contributions of adsorption and volatilization were only 7% and 4% [7]. As the SRT was decreased from 25 to 10 and 5 days, the proportion of 15 mg l^{-1} RD removed decreased from 96 to 98% to 68 and 34%, respectively, (data not shown). In contrast, the proportion remaining in the sludge increased from 1.2 to 1.4% to 45 and 62%, respectively (data not shown). This showed that an optimum SRT is necessary for PAH-degrading bacteria in order to metabolise RD together PAHs in the AASR system.

If we assume that the accumulated PAHs and RD in the AASR system are not degraded and are eventually discharged with the sludge, we can also obtain a similar percentage in the sludge. Based on the present data, we observed that PAHs can be efficiently degraded in the AASR along with a high RD removal efficiency. The optimal SRT for both RD and PAH removal must be 25 days. The decrease in RD concentration in the aqueous phase could be due to uptake by the bacteria following the adsorption of activated sludge as well as waste PAHs for only a few minutes or the biological utilization as carbon sources by the microorganism [26]. If optimum surfactant concentrations are applied, the hydrophobic contaminants such as PAHs can be solubilized by incorporation into surfactant, i.e., aggregates of biosurfactant molecules where the hydrophobic moieties form a core which is insulated from the aqueous environment by the outward-oriented hydrophilic moieties.

The variations in DO, COD_{total}, BOD_5/COD ratios were investigated in the AASR reactors with and without 15 mg l^{-1} RD biosurfactant at the optimum SRT of 25 days throughout 35 days of continuous operation in the AASRs. In the RD amended AASR the BOD_5/COD ratio increased from 0.23 to 0.37 indicating the biodegradability of the effluent wastewater compared to AASR without RD (Table 7). The biodegradability of an industrial wastewater is dependent upon BOD_5/COD ratio. It has generally been accepted that when a biodegradability ratio is greater than 0.3, this represents a readily biodegradable effluent [27]. An increase in the BOD_5/COD ratio indicates an improvement in the biodegradability of the petrochemical wastewater containing PAHs due to formation of inter-metabolite-products more biologically degradable

Table 7
Variation of BOD_5, BOD_5/COD ratios, DO utilization and OUR (n = 3, mean values).

AASR	COD^a		BOD$_5$^{b}		BOD$_5$/COD^c		PAH^d		DO^e	OU^f	OUR^g
	$İ^h$	E^i	$İ^h$	E^i	$İ^k$	E^l	$İ^h$	E^i			
15 RD^m	2650	328	650	127	0.23	0.37	54.57	3.11	4–6	489	198
O RD^n	2650	1278	650	340	0.23	0.26	54.57	20.19	3–4	287	74

COD^a: Total COD concentration (mg l^{-1}); BOD$_5$^{b}: BOD_5 concentration (mg l^{-1}); BOD$_5$/COD^c: BOD_5 to COD ratio; PAH^d: PAH concentration (ng ml^{-1}); DO^e: Dissolved oxygen utilization (mg l^{-1}); OU^f: Final cumulative oxygen utilization (mg l^{-1}); OUR^g:Oxygene utilization rate (mg l^{-1} h^{-1}); $İ^h$: Influent concentration; E^i: Effluent concentration; $İ^k$: Ratio in influent; E^l: Ratio in effluent; m: AASR reactor containing 15 mg l^{-1} RD; n: AASR reactor containing no RD.

[27].The aerobic transformation of PAHs into more biodegradable inter-metabolites in the AASR increased the biodegradability ratio of the petrochemical wastewater (Table 5). Meanwhile, the oxygen utilization rate (OUR) levels increased from 70 to 198 mg l^{-1} h^{-1}, compared to the AASR containing no RD. The oxygen utilization (OU) values showed a short lag phase of 4 h which is reflective of the acclimatisation time of PAH degraders in the AASR containing RD (data not shown). The final measured cumulative OU value was 489 mg l^{-1} (Table 7). The OU values exhibited a long lag phase of 25 h (data not shown) indicating that the PAH degrading biomass takes a long time to acclimatise and the maximum cumulative final OU was recorded as 287 mg l^{-1} in the AASR without RD (Table 7). The COD_{total} and PAH yields were 89% and 95% in the AASR containing 15 mg l^{-1} RD while the BOD concentration decreased from 650 to 127 mg l^{-1} yielding a BOD_5 removal efficiency of 80% (Table 7).BOD_5 removal efficiency remained as 47% in the AASR containing no RD. In the AASR with 15 mg l^{-1} RD, the OUR increased since the PAHs in wastewater could be effectively uptaken by the biomass together with RD. As a result, the MLVSS concentrations in the AASR containing RD were higher than in the reactor without RD (data not shown). Therefore, the DO utilization levels and OUR of microorganisms increased in the AASR with RD. It was reported that the OUR provides a suitable measure of cell metabolic activity and information about the effects of different conditions on cell viability [28]. Haritash and Kaushik and Mohan et al. mentioned similar data with the DO levels in biological reactor containing different biosurfactants at optimum dose throughout aerobic PAH degradation [17,29].

3.6. Effects of environmental conditions on PAH yields

3.6.1. Temperature

Increasing the temperature from 21 to 25 to 45 °C showed that total PAH yields increased from 95% up to 99% in the presence of 15 mg l^{-1} RD in AASR as reported by Nie et al. [30] (Table 8). Higher temperatures increase the solubility and mass transfer rates of PAHs. It was reported that the diffusion coefficient and the solubility of PHE in water increase by factors of 1.5 and 2.5 when the temperature is raised from 20 to 45 °C. Similar enhancement of degradation rates at high temperatures has been previously observed in tests with PY and ANT (Feitkenhauer and Marki, [31]). Increasing of temperature to 60 °C reduced the total PAH yields significantly since the microorganisms present in activated sludge were mesophilic. Possibly these microorganisms could not acclimate to the high temperatures. A second factor that could limit the PAH yields at high temperatures is the low aqueous solubility of oxygen at high temperatures. Another factor that could lower PAH yields could be the lowering of octanol–water partition coefficients at higher temperatures [31].

3.6.2. Dissolved oxygen

The impact of oxic, anaerobic and anoxic conditions on the fate of PAHs was investigated. Five incubation conditions were chosen: two bioreactors were subjected to alternations in aeration (oscillating conditions). One of these reactors was aerated with 2–3 mg l^{-1} DO while the second reactor was aerated with 4–5 mg l^{-1} DO. These reactors were operated continuously for 35 days. Another reactor was operated without DO under anaerobic conditions while the last reactor was also operated under anoxic conditions (DO = 0 mg l^{-1}) by adding 25 mg l^{-1} NO_3-N to permanent anoxia. Permanent oxic (4–5 mg l^{-1} DO)and sequential oxic/anoxic oscillation conditions showed total PAHs removal of about 95% and 78%, respectively, after 35 days of operation, at a SRT of 25 days at 15 mg l^{-1} RD concentration (Table 8). The reactor containing 2–3 mg l^{-1} DO exhibited low PAH yield compared to oxygenated samples with 4–5 mg l^{-1} DO. The PAH yields decreased to 61%

Table 8
Effects of some environmental conditions (Temperature, DO, electron acceptors and pH) on mean total PAH yields (n = 3, mean values).

P^{a}	T^{a}			DO^{b}, ORP^{c}					EA^{d}						pH^{e}		
	21–25	45	60	2 – 3^{b} + 45^{c}	4 – 5^{b} + 145^{c}	0^{b} – 340^{c}	AN^{f} + 8^{c}	S A/AN^{g} + 145^{c}/+8^{c}	Fe^{+2}		NO_3^{-1}		SO_4^{-2}		4	7	10
									DO^{b}: 4–5	DO^{b}: 0	DO^{b}: 4–5	DO^{b}: 0	DO^{b}: 4–5	DO^{b}: 0			
M PY^{h}	95	99	65		95	61	83	78	95	98	95	99	95	99	56	95	53

P^{a}: parameter; T^{a}: Temperature (°C); DO^{b}:dissolved oxygene concentration (mg l^{-1}); ORP^{c}: oxidation reduction potential in low, high-oxygenated, anaerobic and anoxic conditions (mV); EA^{d}: Electron acceptors(Fe^{+2}, NO_3^{-1},SO_4^{2-}, mg l^{-1}; pH^{e}: −log[H]; AN^{f}: Anoxic conditions (DO = 0 mg l^{-1}); SA/AN^{g}: sequential aerobic/anoxic conditions; M PY^{h}: mean total PAH yields (%).

Table 9
Toxicity values in the influent, effluent of AASR system with *Daphnia magna* ($n = 3$, mean values).

a^{dl} ratio (%)	$e^{inf.ww}$			$f^{eff.\ SRT\ 5}$		$g^{eff.\ SRT\ 10}$		$h^{eff.\ SRT\ 25}$		$i^{eff.\ SRT\ 40}$	
	$b^{d.m.*}$	$c^{d.\ m.}$	$(I\%)^c$	$c^{d.\ m.}$	$(I\%)^d$	$c^{d.\ m.*}$	$(I\%)^b$	$c^{d.\ m.*}$	$(I\%)^d$	$c^{d.\ m*}$	$(I\%)^d$
0	10	0	100	0	100	0	100	0	100	0	100
10	10	1	90	3	70	3	70	4	60	6	40
30	10	2	80	5	50	6	40	6	40	7	30
50	10	3	70	8	20	9	10	10	0	9	10
80	10	5	50	10	0	10	0	10	0	10	0

a^{dl}: dilution ratio (%); $b^{d.m.*}$: number of living *Daphnia magna* at first start ($t = 0$); $c^{d.\ m.}$: number of living *Daphnia magna* after 24 h; $(I\%)^c$: percentage of dead *Daphnia magna* in raw wastewater ($t = 24$ h); $(I\%)^d$: percentage of dead *Daphnia magna* in effluent wastewater at different SRTs ($t = 24$ h); $e^{inf.ww}$: influent raw wastewater in AASR; $f^{eff.\ SRT\ 5}$, $g^{eff.\ SRT\ 10}$, $h^{eff.\ SRT\ 25}$, $i^{eff.\ SRT\ 40}$: treated effluent wastewater in AASR system at SRT 5, 10, 25 and 45 days, respectively.

under anaerobic conditions (Table 8). The addition of anaerobic sludge to the AASR increased the PAH yields to 89%. The inoculum effect was more significant under the non-oxygen condition [32]. The efficiency of the enriched consortia indicated that bioaugmentation with the enriched consortia from sediments was useful in bioremediation of PAHs in anaerobic contaminated sediment [32]. In the case of oscillating conditions, two days of aeration following the anoxic period was sufficient to reach the same percentage as under oxic conditions. The availability of oxygen exerts a strong influence on PAH yields. Very low PAH yields without oxygen have been reported [33]. In very low oxygen environments, there may be microbial degradation of PAHs with low molecular weights via non-oxygen dependent mechanisms [33,34]. Under the low-oxygen condition with the inoculation of the enriched consortium the anaerobic biodegradation of some three-rings (FL, PHE) and four-rings (FLN, PRY) PAHs were detected [33]. The oxidation reduction potentials (ORP) were measured as +145, +45, −340, and +8 mV in high, low oxygenated, anaerobic and anoxic reactors, respectively (Table 8). ORP value is considered an important factor affecting the growth of anaerobic bacteria, especially for those which required a lower ORP range and were sensitive to higher ORP values, such as methanogens [32].

3.6.3. Effects of electron acceptors

The effects of three electron acceptors (Fe^{+2}; NO_3^{-1} and SO_4^{-2}) on the PAH yields were investigated in AASR through high-oxygenated (4-6 mg l^{-1} DO) and un-oxygenated anaerobic conditions (O mg l^{-1} DO). 25–45 mg l^{-1} Fe^{+2}, 56–98 mg l^{-1} NO_3^{-1} and 30–65 mg l^{-1} SO_4^{-2} were added to the reactors. The effect of Fe^{+2} on the PAH yields were found to be insignificant under oxidative aerobic conditions (4–6 mg l^{-1} DO). Good PAH consumption was obtained in presence of NO_3^{-1} and SO_4^{-2} under anaerobic conditions while the PAH yields were not changed under oxic conditions (Table 8). The reason for this is the destruction of PAHs by the utilization of nitrate and sulphate as electron acceptors under reductive anaerobic conditions resulting in high PAH yields, in the presence of anaerobic specific bacteria. These data agree with the results obtained by Chang et al. under anaerobic PAH degradation [32].

3.6.4. Effect of pH

The pH was monitored through the experiment. No significant pH reductions were observed in aerated AASRs while a significant reduction in pH was observed due to acidification and volatile fatty acid (VFA) productions in anaerobic AASRs (Table 8). The maximum PAH yields were obtained at pH 7 and 8 for aerobic and anaerobic conditions, respectively. The accumulation of H^+ or other acidic metabolites could reduce the pH (Table 8). If the activity of methanogenic bacteria is slowed down by unfavorable environmental conditions they will not utilize the VFAs at approximately the same rate as the VFAs produced by the acid formers. The pH change may signify metabolic activity leading to production of acidic or alkaline metabolites during breakdown of PAHs. Kim et al. observed that acidic pH conditions promote uptake of PAHs for degradation [35]. Therefore, monitoring the pH of the media may be used to check the progress of PAH degradation.PAH degradation depends on the environmental conditions, number and type of the microorganisms and the nature and chemical structure of the chemical compound being degraded. They are biodegraded/biotransformed into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) and the rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium.

3.7. Effect of increasing SRT on the Daphnia magna acute toxicity

Daphnia magna test is accepted as an acute toxicity test to determine the toxicity of refractory organics [16,36,37]. Table 9 shows the *Daphnia magna* toxicity test results at an influent PAH concentration of 54.57 ng ml^{-1} in the AASR at SRTs of between 5 and 40 days. In order to determine the acute toxicity of the raw wastewater, dilutions varying between 0%, 10%, 30%, 50% and 80% were performed in the influent and effluents of the AASR system (Table 9). The inhibition percentage was calculated by the ratio of the number of dead *Daphnia magna* to the total number of living *daphnids*. The inhibition percentage increased at low dilutions in the influent samples. For example, the inhibitions (the percentage of dead *Daphnids*) decreased from 100% to 70% and 50% as the dilution ratios increased from 0 to 50% and 80%, respectively (Table 9). Table 10 shows the EC_{50} values (the concentration affected 50% of *Daphnia magna* number) in the influent and effluent of the AASR at increasing SRTs based on PAH. The 50% inhibitions of the *Daphnia magna* (EC_{50} values) decreased from an initial 45.02 ng ml^{-1} to $EC_9 = 8.38$, $EC_8 = 7.47$, $EC_6 = 5.30$ and $EC_{12} = 6.69$ ng ml^{-1} at SRTs 5, 10, 25 and 40 days, respectively, when PAHs were taken into consideration (Table 10 and Fig. 3a–d). As shown, the maximum reduction in EC_{50} values was observed at a SRT of 25 days. The petrochemical wastewater was not treated effectively at short SRTs since short SRTs did not provide enough time for sludge bacteria in the AASR to make contact with and isismetabolise the PAHs in the petrochemical industry wastewater. Therefore, low toxicity was observed at a SRT as high as 25 days. However, the toxicity was high at SRTs > 25 days since the PAHs in petrochemical wastewater caused toxicity to the activated sludge bacteria at high contact times. The continuous accumulation of PAHs could not be isismetabolised by the bacteria in the activated sludge resulting in high toxicity and lower PAH yields compared to a 25 day SRT. In this study it was found that the optimum SRT was 25 days for the lowest EC_{50} value at a dilution ratio of 75% (Table 10). This could be explained by the uptake of the PAHs by the activated bacteria cells through fast PAH diffusion with rhamnolipid.

Table 10
The effect of raw and treated petrochemical industry wastewater on *Daphnia magna* at increasing SRTs ($n = 3$, mean values).

	Influent			Effluent			*b*
a	*c*	Inf.$^{ww\ dl}$	PAH$^{inf.\ EC50}$	PAH^{eff}	Eff.$^{ww\ dl}$	PAH$^{eff\ .EC}$	
5	54.57	80	43.66	11.68	65	$EC_{15} = 7.59$	83
10	54.57	80	43.66	9.92	70	$EC_{10} = 6.94$	84
25	54.57	80	43.66	6.51	75	$EC_2 = 4.88$	89
40	54.57	80	43.66	7.69	70	$EC_{12} = 6.69$	88

c: (PAH^{inf}.): influent PAHs concentration in raw wastewater (ng ml^{-1}); Inf.$^{ww\ dl}$.: dilution ratio of influent wastewater (%); Eff.$^{ww\ dl}$: dilution ratio of effluent wastewater (%); PAH^{eff}: effluent PAHs concentration in treated wastewater (ng ml^{-1}); *a*: (SRT), sludge retention time (day); PAH- EC_{50}: PAH concentration inhibiting the half of the *Daphnia magna* number(ng ml^{-1}); PAH$^{inf.\ EC50}$: PAH concentration inhibiting the half of the *Daphnia magna* number (EC_{50} values) in the influent (ng ml^{-1}); PAH$^{eff.EC}$: EC values in enfluent (ng ml^{-1}); *b*: (ATR), acute toxicity removal (%).

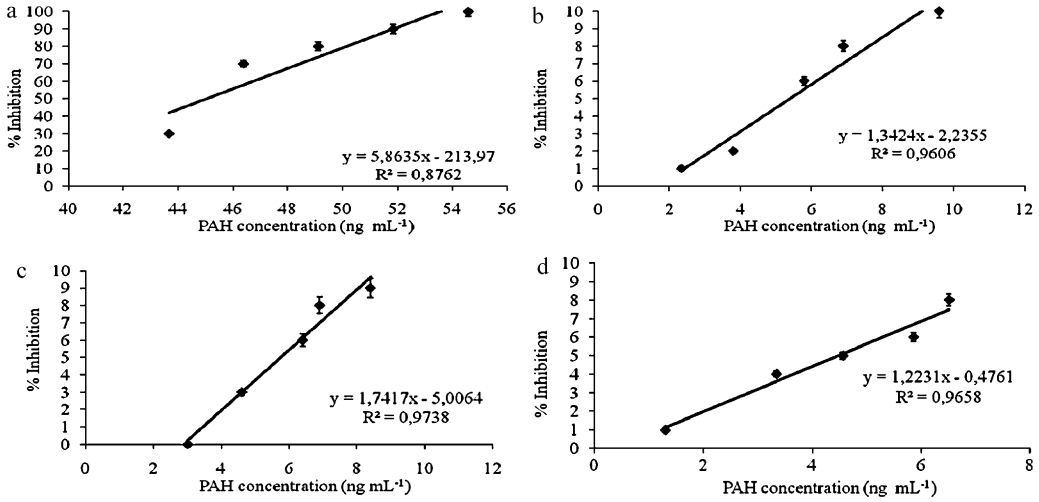

Fig. 3. Variation of percent inhibition in (a): influent wastewater ($EC_{50} = 45.02$ ng ml^{-1}); (b) effluent wastewater in AASR at SRT = 5 days ($EC_9 = 8.38$ ng ml^{-1}); (c) effluent wastewater in AASR at SRT = 10 days ($EC_8 = 7.47$ ng ml^{-1}); (d) effluent wastewater in AASRs at SRT = 25 days ($EC_6 = 5.30$ ng ml^{-1}).

Acute toxicity of aromatic hydrocarbon compounds (naphthalene, benzene, toluene and xylene) and toxic effects after an activated sludge continuous-flow completely mixed reactor were studied with the *Daphnia magna* test by Gomez et al. [36]. Although the removals of the organics in question varied between 77% and 93% the acute toxicity removals were low (34–56%) in untreated and treated effluents. The EC_{50} values were observed to be between 18.44 and 30.81 at 24 h (v/v). Eom et al. evaluated the toxicity of 16 PAHs (total concentration 17.7 ng ml^{-1}) in contaminated soil samples using *Daphnia magna* bioassay [37]. The ecotoxicity results of this study showed that the EC_{50} values were very toxic to *Daphnia magna*. In our study the EC_{50} values of PAH in the effluent of AASR system exhibited higher toxicity removals than those of Gomez et al. and Eom et al. [36,37]. Sepic et al. investigated the toxicity of 20 mg l^{-1}, fluoranthene and its biodegradation metabolites to pure bacterial strain *Pasteurella sp.* and *Daphnia magna* [38]. 34% and 56% acute toxicity removals were obtained for the organism in question, respectively, a result which is comparably lower than the toxicity yields obtained in our study.

4. Conclusions

A higher SRT is preferable to establish activated sludge in AASR system adapted to degrade the hydrophobic PAHs and tends to lower the effluent concentrations of PAHs. The results of this study showed that the optimum SRT and RD concentrations for total maximum PAH, COD_{inert}, COD_{imp} removals (96%, 88% and 93%, respectively) were 25 days and 15 mg l^{-1}, respectively. RD concentrations >15 mg l^{-1} decreased the number of bacteria present in the AASR system. The addition of RD to AASR system significantly improved the more hydrophobic PAH degradations. The RD was biodegraded by the sludge microbial communities and was thus environmentally friendly. Among the RD removed in the AASR, 96–98% was biodegraded by the activated sludge process, 1.1–1.3% was discharged with the effluent and only 1.3–1.5% remained in the waste sludge while a maximum PAH biodegradation efficiency of 97% occurred. The temperature increase up to 45 °C and both additions of NO_3^{-1} and SO_4^{-2} enhanced the PAH yields in the presence of RD. The BOD_5/COD ratio increased from 0.23 to 0.37 with simultaneous PAH biotreatment. The results of the toxicity tests performed with *Daphnia magna* showed that EC_{50} value decreased from $EC_{50} = 45.02$ ng ml^{-1} to $EC_6 = 5.30$ ng ml^{-1} with removal efficiency of 96% at the end of aerobic treatment at SRT of 25 days and 15 mg l^{-1} RD concentration.

Acknowledgments

This study was supported by the Scientific Research Foundation of Dokuz Eylul University (Project No. 2007. KB.FEN.057). The authors would like to thank Prof. Mustafa Odabaşı and Research Assistant Yetkin Dumanoğlu for the method used in the measurement of the PAHs and for their valuable advice during the sample preparation and PAHs analysis steps.

References

[1] E. Namkung, B.E. Rittmann, Estimating volatile organic compound emissions from publicly owned treatment works, Journal Water Pollution Control Federation 59 (1987) 670–678.
[2] B. Clark, B.C.J. Henry, D. Mackay, Fugacity analysis and model of organic chemical fate in a sewage treatment plant, Environmental Science and Technology 29 (1995) 1488–1494.

[3] E. Manoli, C. Samara, The removal of polycyclic aromatic hydrocarbons in the wastewater treatment process, experimental calculations and model predictions, Environmental Pollution 151 (2008) 477–485.
[4] C.N Mulligan, R.N. Yong, B.F. Gibbs, Remediation technologies for metal-contaminated soils and groundwater: an evaluation, Engineering Geology 60 (2001) 371–380.
[5] S.M. Randhır, H.J. Rockne, Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons, Environmental Toxicology and Chemistry 22 (2003) 2280–2292.
[6] A. Ramdani, P. Dold, S. Deleris, D. Lamarre, A. Gadbois, Y. Comeau, Biodegradation of the endogenous residue of activated sludge, Water Research 34 (2010) 2179–2188.
[7] O. Gok, D.T. Sponza, 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, Desalination Waste Water 26 (2011) 57–65.
[8] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering, Treatment and Reuse, fourth ed., McGraw-Hill, New York, 2003.
[9] I.F. Bautista, R. Sanza, M.C. Molina, N. Gonzalez, D. Sanchez, Effect of different non-ionic surfactants on the biodegradation of PAHs by diverse aerobic bacteria, International Biodeterioration and Biodegradation 63 (2009) 913–922.
[10] C. Park, J.T. Novak, Characterization of activated sludge exocellular polymers using several cation-associated extraction methods, Water Research 41 (2007) 1679–1688.
[11] G.A. Ekama, P.L. Dold, G.R. Marais, Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems, Water Science and Technology 18 (1986) 91–114.
[12] H.S. Jeong, D.J. Lim, S.H. Wang, S.D. Ha, J.Y. Kong, Rhamnolipid production by *Pseudomonas aeruginosa* immobilised in polyvinyl alcohol beads, Biotechnology Letters 26 (2004) 35–39.
[13] A.D. Eaton, L.S. Clesceri, E.W. Rice, A.E. Greenberg, Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF), 21st ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA, 2005.
[14] Y. Shabtai, D.C. Wang, Production of emulsan in a fermentation process using soybean Oil (SBO) in a carbon-nitrogen coordinated feed, Biotechnology and Bioengineering 35 (2009) 53–765.
[15] F. Coutte, D. Lecouturier, S.A. Yahia, V. Leclère, M. Béchet, P. Jennese, P. Dhulster, Production of surfactin and fengycin by *Bacillus subtilis* in a bubbleless membrane bioreactor, Applied Microbiology and Biotechnology 87 (2010) 499–507.
[16] X.J. Zheng, J.F. Blais, G. Mercier, M. Bergeron, P. Drogui, PAH removal from spiked municipal wastewater sewage sludge using biological, chemical and electrochemical treatments, Chemosphere 68 (2007) 1143–1152.
[17] A.K Haritash, C.P. Kaushik, Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review, Journal Hazardous Materials 169 (2009) 1–15.
[18] M. Feldmannova, K. Hilscherova, B. Mars, L. Blaha, Effects of n-heterocyclic polyaromatic hydrocarbons on survival, reproduction, and biochemical parameters in *Daphnia magna*, Environmental Toxicology 21 (2006) 425–431.
[19] J. Al-Halbouni, S. Traber, T. Lyko, T. Wintgens, D. Melin, A. Tacke, W. Janot, J. Dott, Hollender, Correlation of EPS content in activated sludge at different sludge retention times with membrane fouling phenomena, Water Research 42 (2008) 1475–1488.
[20] D.J. Barker, S.L. Salvi, A.M. Langenhoff, D.C. Stuckey, Soluble microbial products in ABR treating low-strength wastewater, Journal Environmental Engineering 126 (2006) 239–249.
[21] H. Yin, J. Qiang, Y. Jia, J. Ye, H. Peng, H. Qin, N. Zhang, B. He, Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater, Process Biochemistry 44 (2009) 302–308.
[22] L. Zhu, M. Zhang, Effect of rhamnolipid on the uptake of PAHs by ryegrass, Environmental Pollution 156 (2008) 46–52.
[23] L. Lei, A.P. Khodadoust, M.T. Suidan, H.H. Tabak, Biodegradation of sediment-bound PAHs in field contaminated sediment, Water Research 39 (2005) 349–361.
[24] H. Yu, L. Zhu, W. Zhou, Enhanced desorption and biodegradation of phenanthrene in soil-water systems with the presence of anionic-nonionic mixed surfactants, Journal Hazardous Materials 142 (2007) 354–361.
[25] C. Sartoros, L. Yerushalmi, P. Beron, S.R. Guoit, Effects of surfactant and temperature on biotransformation kinetics of anthracene and pyrene, Chemosphere 61 (2005) 1042–1050.
[26] Q. Yuan, R. Sparling, J.A. Oleszkiewicz, Waste activated sludge fermentation: effect of solids retention time and biomass concentration, Water Research 43 (2009) 5180–5186.
[27] G. Tchobanoglous, F.L. Burton, H.D. Stensel (Eds.), Stensel, Wastewater Engineering, Treatment, Disposal and Reuse, McGraw-Hill, New York, USA, 1991.
[28] K. Lee, J.-W. Park, I.-S. Ahn, Effect of additional carbon source on naphthalene biodegradation by *Pseudomonas putida* G7, Journal of Hazardous Materials 105 (2003) 157–167.
[29] P.K. Mohan, G. Nakhla, E.K. Yanful, Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions, Water Research 40 (3) (2006) 533–540.
[30] M. Nie, X. Yin, C. Ren, Y. Wang, F. Xu, Q. Shen, Novel rhamnolipid biosurfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium Pseudomonas aeruginosa strain NY3, Biotechnology Advance 28 (2010) 635–643.
[31] H. Feitkenhauer, H. Marki, Biodegradation of aliphatic and aromatic hydrocarbons at high temperatures, Water Science Technology 47 (2003) 123–130.
[32] B.V. Chang, S.W. Chang, S.Y. Yuan, Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge, Advances in Environmental Research 7 (2003) 623–628.
[33] C. Cravo-Laureau, G. Hernandez-Raquet, I. Vitte, R. Jezequel, V. Bellet, J-J Godon, P. Caumette, P. Balaguer, R. Duran, Role of environmental fluctuations and microbial diversity in degradation of hydrocarbons in contaminated sludge, Research in Microbiology (in press), doi:10.1016/j.resmic.2011.04.011.
[34] A.I. Janbandhu, M.H. Fule, Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment, Journal Hazardous Materials 187 (2011) 333–340.
[35] G.B. Kim, K.A. Maruya, R.F. Lee, J.-H. Lee, C.-H. Koh, S. Tanabe, Distribution and sources of polycyclic aromatic hydrocarbons in sediments from Kyeonggi Bay, Korea, Marine Pollution Bulletin 38 (1999) 7–15.
[36] C.E. Gomez, L. Contento, A.E. Carsen, Toxicity tests to assess pollutants removal during wastewater treatment and the quality of receiving waters in Argentina, Environmental Toxicology 16 (2001) 217–224.
[37] C. Eom, C. Rast, A.M. Veber, P. Vasseur, Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH) contaminated soil, Ecotoxicology and Environmental Safety 67 (2007) 190–205.
[38] E. Sepic, M. Bricelj, H. Leskovsek, Toxicity of fluoranthene and its biodegradation metabolites to aquatic organisms, Chemosphere 52 (2003) 1125–1133.