Asian Journal of Water, Environment and Pollution, Vol. 9, No. 2 (2012), pp. 11–23.

A Sonication Extraction Method to Determine PAHs in Activated Sludge (Supernatant and Solid) for the Monitoring of Aerobic Biodegradation

Yamen AlSalka*, Kai Lehnberg[1], Djamila Al-Halbouni[2], François Karabet, Mohammad Shahir Hashem and Rasha Al Misrabi

Department of Chemistry, Faculty of Sciences, University of Damascus, Damascus, Syria
[1]Institute for Hygiene and Environmental Medicine, RWTH Aachen University, Aachen, Germany
[2]Institute for Biology 1, Unit of Soil Ecology, RWTH Aachen University, Aachen, Germany
✉ yamensalka@yahoo.com

Received June 30, 2010; revised and accepted March 19, 2012

Abstract: A simple analytical method was developed and optimised for the determination of polycyclic aromatic hydrocarbons in activated sludge consisting of supernatant and solid, which collected from the aerobic reactor (aerated zone) of the biological treatment step in a wastewater treatment plant. The procedure accomplishes in feasible time of extraction and reasonable solvent volume without the need of any special technique to perform the extraction step. The method involves extraction of entire sample by sonication and mechanical shaking using *n*-hexane, preconcentration by solvent evaporation, and determination by high-performance liquid chromatography with diode array and fluorescence detectors. The developed method was optimised to give precise results in both low and high spiked samples: relative standard deviation lower than 10%, limits of detection lied between 0.2 and 5 μg kg^{-1}, and recoveries were between 66% and 109%. This method allows the monitoring of the bioremediation efficiency of PAHs by the activated sludge, since it permits the extraction of both PAHs; those that adsorbed to the solid phase, and the other that presented in supernatant phase. The activated sludge could be valuable in the bioremediation of lighter PAH compounds mainly those which consist of two and three fused aromatic rings.

Key words: PAHs, HPLC, extraction, activated sludge, wastewater, aerobic biodegradation.

Introduction

Wastewater treatment plants (WWTPs) receive complex mixtures containing a wide range of organic pollutants involving the polycyclic aromatic hydrocarbons. PAHs are listed as US-EPA and EU priority pollutants, and their concentrations therefore need to be controlled in treated wastewater effluents (Busetti et al., 2006; Blanchard et al., 2004) as well as generated sludges, since they are ubiquitous environmental pollutants with carcinogenic and mutagenic properties (Santos et al., 2007; Trably et al., 2004). PAHs constitute a large family of organic compounds widely distributed in the environment, and more than 74 PAHs have been identified, but only 16 are monitored by US-EPA and the environmental commission of European community.

Because of the physical-chemical processes involved in wastewater treatment plants, the sludge tends to concentrate a lot of organic contaminants with low water solubility and high adsorption capacity (Santos et al., 2007; Gibson et al., 2005). As a consequence of their strongly hydrophobic properties and their resistance to

*Corresponding Author

biodegradation, PAHs are almost removed from wastewaters by activated sludge treatments relocating them into sludges (Busetti et al., 2006; Rogers, 1996).

Many analytical methods for extraction and determination of PAHs in wastewater and sludge are available from literature. These analytes are commonly analysed by GC-MS or by HPLC coupled with fluorescence FLD and ultraviolet diode array detectors UV-DAD (Busetti et al., 2006; Blanchard et al., 2004; Marttinen et al., 2003). Both techniques possess high sensitivity and selectivity, but MS provides greater specificity than FLD or UV detectors (Busetti et al., 2006). However, LC chromatographic systems permit injection of much larger sample volumes (up to 200 μL) than GC-based techniques (Busetti et al., 2006; Miège et al., 2003; Marcé et al., 2000).

Several methods for the extraction of PAHs from sludges have been reported: traditional methods which consume much solvent and time such as Soxhlet (Santos et al., 2007; Lara et al., 2006; Helaleh et al., 2005; Morales et al., 2004; Vinther et al., 2003), reflux (Santos et al., 2007), and shaking (Santos et al., 2007; Vinther et al., 2003) extraction; and more recent extraction methods which require complex and expensive instrumentation: microwaves (Santos et al., 2007; Villar et al., 2007), pressurised-liquid extraction (Santos et al., 2007; Eichhorn et al., 2005) and supercritical fluid extraction (Santos et al., 2007; Priego et al., 2004). Extraction assisted by sonication has demonstrated to be an advantageous alternative for both conventional and novel extraction techniques (Busetti et al., 2006; Nimer et al., 2007; Miège et al., 2003). Common analytical extraction methods to extract PAHs from wastewater involve liquid-liquid extraction using non-polar solvents or SPE using reverse phase or polymeric sorbing materials (Busetti et al., 2006; Blanchard et al., 2004; Marttinen, 2003).

Bioremediation utilises the metabolic versatility of microorganisms to degrade hazardous pollutants. A goal of bioremediation is to transform organic pollutants into harmless metabolites or mineralise the pollutants into carbon dioxide and water (Seo et al., 2009). A promising approach to reduce PAHs pollution is the utilisation of the natural potential of microorganisms to utilise hydrocarbons since the bioremediation techniques are cheaper than the other alternatives used for cleaning up (Larsen et al., 2009). Bioaugmentation have been previously used as a tool for bioremediation of wastewaters especially in relation to activated sludge (Larsen et al., 2009; Dionisi et al., 2006; Boon et al., 2003). The aerobic biotransformation of PAHs in sludge has been observed in both pure and mixed cultures (Chang et al., 2003). Anaerobic PAHs biodegradation in synthetic medium was also studied with a mixed microbial consortium (Larsen et al., 2009) and with a pure culture (Larsen et al., 2009; Fuchedzieva et al., 2006) isolated from a wastewater treatment plant.

The aim of this work was to evaluate and validate a simple method for the determination of 13 PAH compounds in activated sludge samples which have been collected from a wastewater treatment plant and consisted of supernatant in addition to the solid sludge. The method was based on the extraction of PAHs from the entire activated sludge by sonication and mechanical shaking using *n*-hexane as extraction solvent, then the concentration of the extract prior to the analysis by HPLC-FLD/DAD. The developed method was then applied to monitor aerobic biodegradation of PAHs by activated sludge, in order to evaluate the bioremediation processes as a solution for the detoxification.

Experimental

Materials and Chemicals

Acetonitrile was of HPLC grade, purchased from Merck (Darmstadt, Germany), *n*-hexane was of HPLC grade, purchased from Biosolve (Valkenwaard, Netherlands), sodium azide was purchased from Merck (Darmstadt, Germany), and water of HPLC grade was produced using "Synthesis A10" device purchased from Millipore (Molsheim, Germany). A certified standard mixture solution (PAH-Mix 13), used for calibration, containing 100 mg l^{-1} of each substance: acenaphthene, acenaphthylene and naphthalene, and 10 mg l^{-1} of each other PAH compound was purchased from Dr. Ehrenstrofer reference materials (Augsburg, Germany).

A certified standard mixture solution (EPA 610 PAH Mix), using spiking, was purchased from Supelco Analytical (Bellefonte, USA) and containing 2000 mg l^{-1} of acenaphthylene, 1000 mg l^{-1} of each of acenaphthene and naphthalene, 200 mg l^{-1} of each of benzo(b) fluorenthene, benzo(g,h,i) perylene, dibenzo(a,h)anthracene, fluorene, fluorenthene, and 100 mgl^{-1} of each of anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthene, chrysene, indeno(1,2,3-cd)pyrene, phenanthrene, and pyrene.

Instruments

HPLC analysis was done using an 1100/1200 HPLC system produced by HP (USA) consisting of degasser, gradient pump, autosampler, column oven, photo diode array detector and fluorescence detector. Chromatographic separation was performed on

250 × 3.0 mm, 5 µm, MZ-PBM C18 column (MZ-Analysentechnik, Mainz, Germany). A PC interfaced to the HPLC using Agilent ChemStation software (Agilent Technologies) was used for data acquisition and processing.

Extraction steps were done as the following: sonication using ultrasonic bath model 5210 (Branson, Danbury, USA), mechanical shaking by horizontal shaker model HS500 (Ika Werke GmbH & Co. KG, Staufen, Germany), centrifugation in a centrifuge model Avanti J20XP (Beckman Coulter, Brea, USA) equipped with an aluminium fixed-angel rotor 6 × 500 ml and aluminium beakers with screw caps. Extract was concentrated using custom build nitrogen evaporator device with recirculation water bath utilise heater model DC10 (Thermo Scientific, Osterode, Germany).

Weighing tasks were done using analytical balance model 2004 MP (Sartorius, Göettingen, Germany). Incubation of sludge samples to monitor the bio-degradation was performed in incubator shaker model Multitron (Infors, Bottmingen, Switzerland).

Extraction and Concentration Method

Definite equivalent weights (150 g) of entire activated sludge samples (solid and supernatant) were placed in 500 ml aluminium beakers (which could be used in the centrifuge).

A volume of 20 ml of *n*-hexane was added to each sample, and the beakers were well stoppered and sonicated by the ultrasonic bath for 10 min, followed by shaking in the horizontal shaker for 15 min prior the centrifugation for 5 min at 20 ºC with 6000 rpm. The upper organic phase was collected and transferred to a stoppered glass bottle. The extraction steps were repeated for two more times, and the extracts were mixed together in the same stoppered glass bottle.

In order to concentrate the collected extract, *n*-hexane was evaporated by blowing a stream of nitrogen over it at 30 ºC to reach about 0.5 ml volume. Then, the solvent was changed by adding 1 ml of acetonitrile, and the remaining volume of *n*-hexane (the upper phase) was evaporated to reach a final volume of 1 ml which was then transferred to an HPLC vial in order to perform the HPLC separation procedure.

Analysis Procedure

An aliquot of 5 µL of the sample being extracted was injected into the HPLC system using a flow rate of 0.6 ml min^{-1}, and a gradient elution of water and acetonitrile as mobile phases. The separation was accomplished using the mentioned column at 35 °C for 55 min. Table 1 shows the program of gradient elution which has been used. The detection was carried out using photo diode array and fluorescence detectors. Table 2 shows the parameters which have been used in each detector to detect the analytes.

Collection of Activated Sludge Samples

Activated sludge samples were collected as grab samples from the aerobic reactor (aerated zone), which is used for nitrification processes in the biological treatment step. Samples were collected from wastewater treatment plant in Aachen Soers (50º48′24″ N, 6º06′01″ E), in 1000 ml containers which were completely filled and stoppered. All the samples were maintained below 4 ºC.

Table 1: Time table of the gradient elution used for HPLC separation

Time (min)	*Solvent A % (water)*	*Solvent B % (acetonitrile)*	*Flow rate (ml min^{-1})*
0.00	40.00	60.00	0.6
35.00	0.00	100.00	0.6
45.00	0.00	100.00	0.6
47.00	40.00	60.00	0.6
55.00	40.00	60.00	0.6

Table 2: HPLC detection parameters

Photo diode array detector			*Fluorescence detector*		
Signal	*Wavelength (nm)*	*Bandwidth (nm)*	*Time (min)*	*Excitation (nm)*	*Emission (nm)*
A	210	8	0.00	275	350
B	230	8	10.00	260	420
C	245	16	11.00	270	440
			15.00	260	420
Spectrum scan (nm)			21.50	290	430
From 190 to 600			29.50	250	500

Calibration Curves

Calibration standards were prepared in the concentration ranges from 1.25 to 200 µg l^{-1} of each PAH compound by diluting the certified standard mixture solution (PAH-Mix 13) in acetonitrile. Calibration curves were generated by linear regression of peak areas against their respective amount injected (in ng).

Method Performance

Eight glass containers with a volume of 500 ml equipped with stoppers were prepared. A 150 g of entire activated sludge (solid and supernatant) was weighed in each glass container. Four of these containers were spiked with a volume of 30 µl, and the other four containers were spiked with a volume of 0.3 µl of PAHs standard certified solution (EPA 610 PAH Mix). Recoveries and relative standard deviations were calculated. A comparison between signals obtained from the analysis of spiked samples, non-spiked samples, and supernatant phase only was done. All samples were extracted and analysed according to two preceding sub-sections—'Extraction and Concentration Method' and 'Analysis Procedure' respectively.

Limits of detection (LOD) and limits of quantitation (LOQ) were calculated as the concentrations corresponding to a signal-to-noise ratio of 3 and 10 respectively.

Aerobic Biodegradation Test

Twelve glass containers with a volume of 500 ml equipped with stoppers were prepared. A 150 g of entire activated sludge (solid and supernatant) was weighed in each glass container and spiked with a volume of 30 µl of PAHs standard certified solution (EPA 610 PAH Mix). Sodium azide was added to four of these containers (0.5 % each). The containers were then completely stoppered and incubated in the incubator shaker at 20 ºC and 150 rpm.

Results and Discussion

Chromatographic Separation

Under the described HPLC conditions, RP-HPLC procedure was performed to analyse a diluted standard solution (1:2000) in acetonitrile from the certified standard mixture solution (PAH-Mix 13). The chromatogram obtained of fluorescence detector in Figure 1 shows good resolution between the 13 separated peaks. Acenaphthylene is not fluorescent, so it has to be determined by photodiode array detector at 230 nm.

Calibration Curves

Mathematical verification of linearity was performed in term of correlation coefficient (R^2). Also, ANOVA lack of fit using Fisher's t-test was applied. Table 3

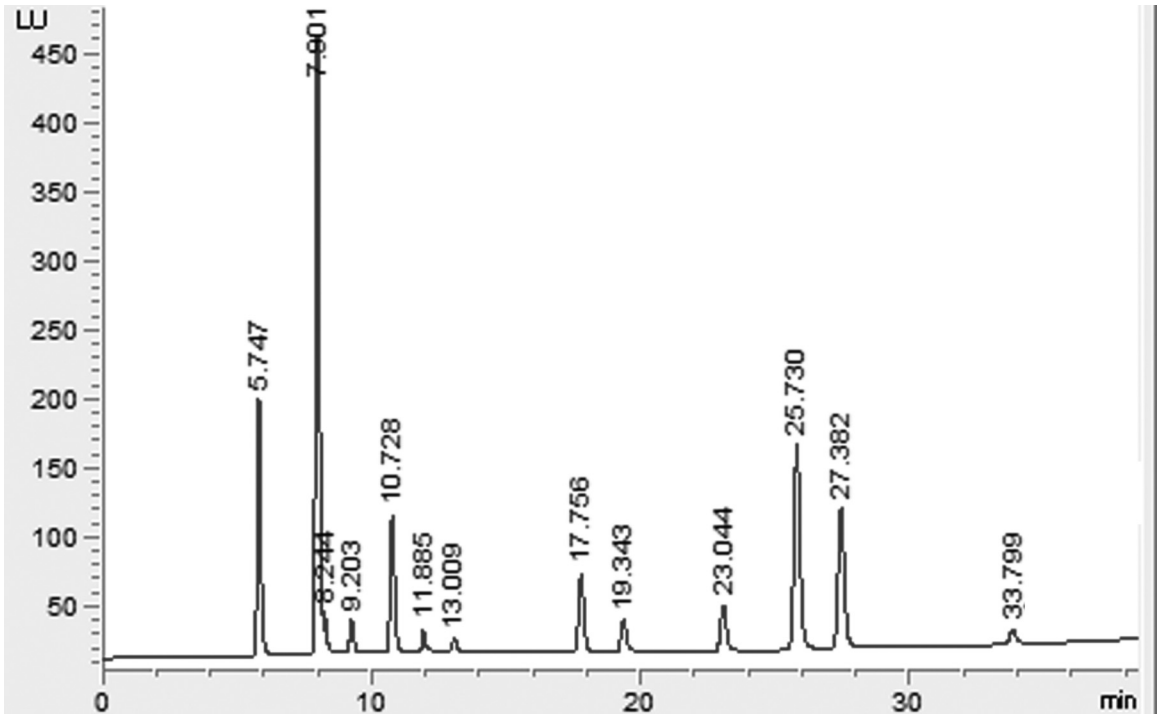

Figure 1: HPLC-FLD chromatogram of PAH-Mix 13 1:2000 in acetonitrile: Naph, Ace, Fluo, Phen, Ant, Flt, Pyr, BaA, Chry, BbF, BkF, BaP and IcdP.

Table 3: Linear range, coefficients and lack of fit test results

Compound	*Slope mean (b)*	*Intercept mean (a)*	R^2 *mean*	*LOF* F_{calc}
Naphthalene	5331.886	157.333	0.9987	3.01
Acenaphthylene [a]	26.779	2.648	0.9937	4.60
Acenaphthene	7861.023	95.458	0.9918	5.36
Fluorene	12270.414	2.356	0.9999	0.25
Phenanthrene	9066.136	9.440	0.9997	1.42
Anthracene	39803.131	14.430	0.9997	1.44
Fluorenthene	7365.076	-6.414	0.9983	3.75
Pyrene	3686.266	4.820	0.9996	1.53
Benzo(a)anthracene	27915.192	10.444	0.9998	1.36
Chrysene	11757.478	3.696	0.9997	1.59
Benzo(b)fluorenthene	17907.509	6.058	0.9998	1.14
Benzo(k)fluorenthene	80054.200	104.947	0.9998	0.99
Benzo(a)pyrene	59829.418	23.925	0.9998	1.27
Indeno(1,2,3-cd)pyrene	6573.990	5.305	0.9992	2.45

[a] PDA detection. Calibration fitting: $y = a + bx$, $F_{crit,\ 95\%} = 6.57$, $n = 3$

summarises the curve equation, and lack of fit test results. As shown, all correlation coefficients were above 0.99, and F_{calc} value of each linear range was less than F_{crit} (ANOVA with 95% confidence level), ensuring linearity and goodness of fit.

Development and Optimisation of the Extraction Method

Spiked activated sludge samples with 30 µl of PAHs standard certified solution (EPA 610 PAH Mix) were treated in different ways, in order to find the optimal extraction recovery: extraction of entire sludge sample using sonication and shaking repeated for two and for three times, extraction of entire sludge sample using shaking step only repeated for three times, and extraction of supernatant only repeated for three times. Figure 2 summarises the results of method development. The higher recoveries were achieved when a combination between sonication and mechanical shaking repeated for three times was used, except for naphthalene, acenaphthene and anthracene. These recoveries were higher than 75 % confirming the comparability of the proposed method with those mentioned in literature. Low recoveries resulted by the extraction of supernatant only indicate that the entire sludge sample, including solid, should be extracted to ensure transferring of the total PAHs content to the solvent extract during the extraction procedure.

According to the above mentioned results, we chose the combination of sonication and mechanical shaking repeated for three times with a centrifugation and concentration steps as optimum conditions to reach to the maximal recovery.

Activated Sludge Characteristics

In order to identify the content of PAHs presented in the collected activated sludge sample, the optimum extraction conditions were applied before the HPLC analysis. The analysis results of non-spiked activated sludge sample taken from the aerobic reactor (aerated zone) of the biological treatment step are shown in Table 4.

Method Performance

Figure 3 shows an HPLC-FLD chromatogram of PAH analytes resulted from the extraction and analysis of activated sludge spiked at low concentration. Recoveries with the relative standard deviations obtained from the analysis of entire activated sludge and the analysis of supernatant samples spiked at two concentrations are shown in Table 5, which indicates that the mean recoveries were in the range of 66% to 109% which were comparable to those reported by other authors, while the RSD values were from 0.1% to 9%. The analysis of supernatant only showed that it is not sufficient to determine the total concentration of PAHs in the entire activated sludge by analysing the supernatant phase only since the solid sludge tends to concentrate these contaminants because of its high adsorption capacity and the low water solubility of PAH compounds.

LODs and LOQs were determined in the extracts related to the low-spiked activated sludge samples, and were defined as the concentration of the analyte that produce a signal-to-noise ratio of 3 and 10 times the baseline noise respectively. LODs were in the range of 0.2 to 5 µg kg^{-1}, and LOQs were in the range of 1 to 16.5

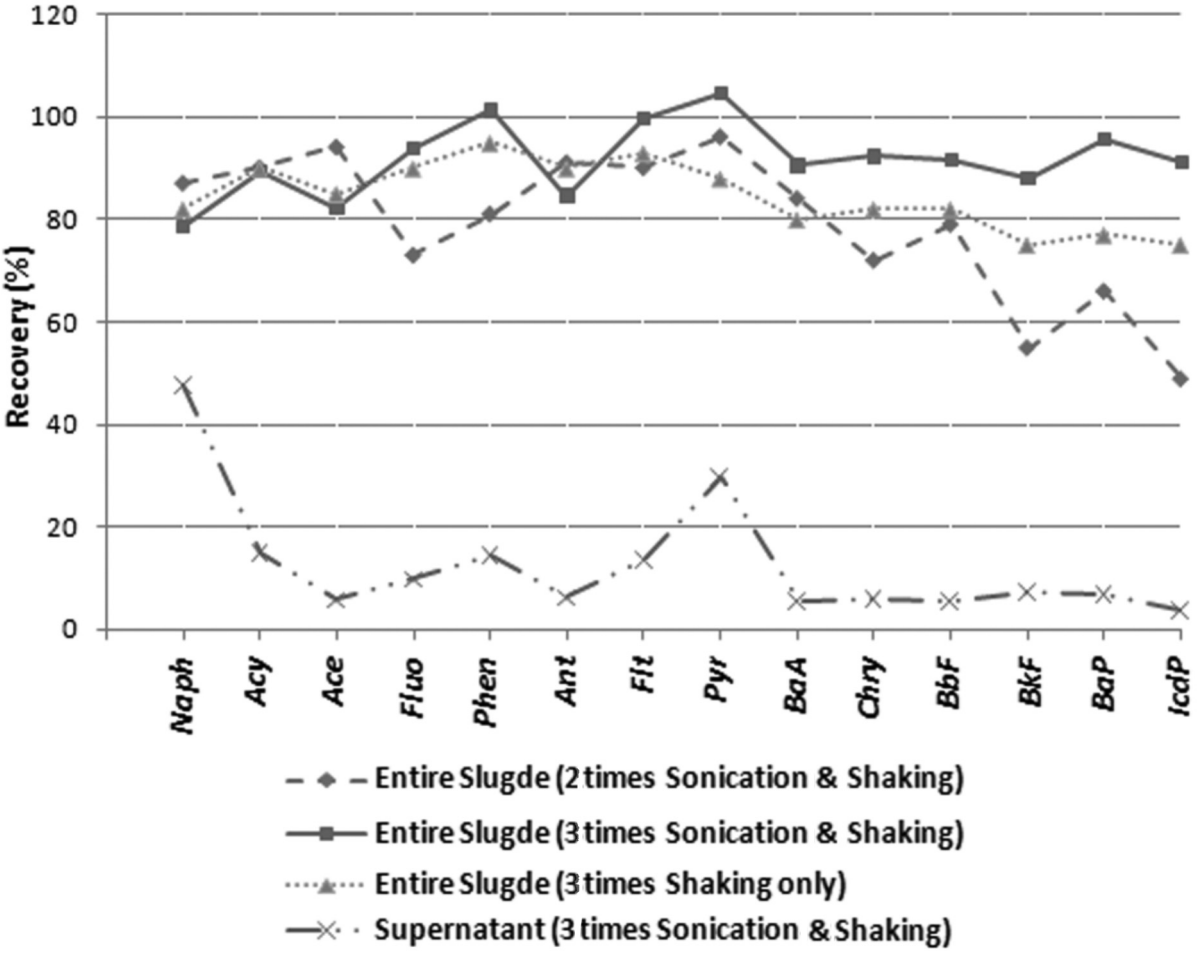

Figure 2: Influence of the sonication, shaking and their repetitions in the extraction of PAHs from activated sludge samples.

Table 4: Results of the analysis of PAHs in activated sludge sample

Compound	*Non-spiked activated sludge sample (µg kg^{-1})*	*RSD (%)*
Naphthalene	ND	-
Acenaphthylene [a]	ND	-
Acenaphthene	NQ	-
Fluorene	3.998	5.43
Phenanthrene	6.667	3.88
Anthracene	1.048	5.11
Fluorenthene	5.105	6.52
Pyrene	NQ	-
Benzo(a)anthracene	NQ	-
Chrysene	ND	-
Benzo(b)fluorenthene	ND	-
Benzo(k)fluorenthene	ND	-
Benzo(a)pyrene	ND	-
Indeno(1,2,3-cd)pyrene	ND	-

[a] PDA detection, ND: Not detected, NQ: Not quantified

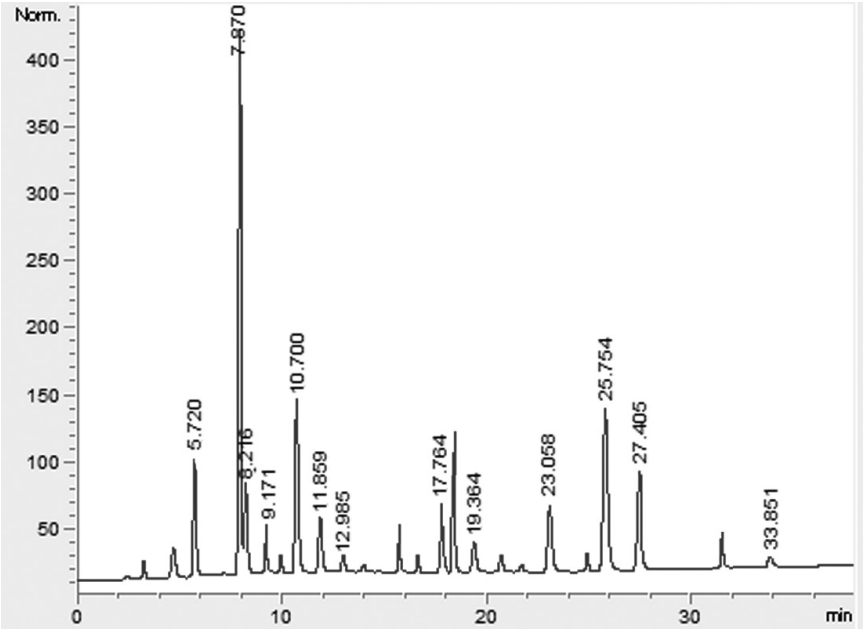

Figure 3: HPLC-FLD chromatogram of activated sludge spiked at low concentration.

Table 5: Results of method performance

Comp.	*Low-spiked supernatant*		*High-spiked supernatant*		*Low-spiked entire activated sludge*		*High-spiked entire activated sludge*	
	Recovery %	*RSD %*	*Recovery %*	*RSD %*	*Recovery %*	*RSD %*	*Recovery %*	*RSD %*
Naph	33.16	4.65	47.60	3.86	66.78	3.78	78.74	5.90
Acy[a]	12.37	3.93	20.02	3.15	77.91	3.32	89.38	3.92
Ace	6.38	2.56	12.65	3.41	92.87	4.88	82.30	3.96
Fluo	10.00	3.72	9.69	3.32	97.03	6.56	93.98	5.39
Phen	14.74	4.24	14.45	5.05	100.18	8.72	101.21	6.19
Ant	7.11	5.49	6.09	5.11	98.94	9.41	84.71	6.59
Flt	14.03	2.04	13.65	2.35	102.45	0.12	99.66	1.10
Pyr	24.77	3.96	29.68	4.87	109.89	8.76	104.54	6.68
BaA	3.13	3.31	6.32	2.92	85.18	4.15	90.59	3.78
Chry	4.68	3.66	6.02	3.12	71.83	1.00	92.39	2.09
BbF	3.76	4.01	5.44	4.17	69.37	3.89	91.58	3.50
BkF	5.15	4.82	7.12	5.22	68.92	4.99	88.07	4.60
BaP	6.67	5.03	6.83	5.06	88.56	3.24	95.75	2.92
IcdP	6.30	4.25	3.61	3.85	90.59	3.34	91.09	2.96

[a] PDA detection.

$\mu g\ kg^{-1}$ depending on the compound in concern as can be seen in Table 6.

Table 7 demonstrates a comparison between the developed method and some other methods described in literatures to determine PAHs in sewage sludge. The proposed method allows the extraction of PAHs from entire activated sludge (solid and supernatant), in feasible time of extraction and solvent volume with a comparable performance in terms of recovery and RSDs.

Monitoring of Aerobic Biodegradation of PAHs by Activated Sludge

For the monitoring of aerobic biodegradation of PAHs by activated sludge, every week, two incubated samples were monitored. The remaining amounts of PAHs were analysed in each sample (Table 8), and the obtained results were compared to those found in the samples containing sodium azide, which has been used to screen the activity of microorganisms preventing the biodegradation processes.

Table 6: Limits of detection / quantitation of PAHs in low spiked sludge

Compound	*LOD (µg kg^{-1})*	*LOQ (µg kg^{-1})*
Naphthalene	1.401	5.077
Acenaphthylene [a]	5.095	16.434
Acenaphthene	3.415	10.659
Fluorene	0.917	3.220
Phenanthrene	1.148	4.032
Anthracene	0.269	0.960
Fluorenthene	1.698	4.966
Pyrene	3.240	9.367
Benzo(a)anthracene	0.476	1.687
Chrysene	1.176	4.134
Benzo(b)fluorenthene	0.821	2.890
Benzo(k)fluorenthene	0.177	0.634
Benzo(a)pyrene	0.261	0.928
Indeno(1,2,3-cd)pyrene	2.591	8.086

[a] PDA detection.

Table 7: Comparison between the proposed method and some other methods

Extraction method	*Extraction solvent*	*Solvent volume (ml)*	*Extraction time*	*Recovery (%)*	*Determination*	*Ref.*
Proposed method	*n*-Hexane	60	2 h	66 - 109	LC-DAD/FLD	
Microwave-assisted	MeOH:Acetone (1:1)	20	20 min	52 - 110	LC-DAD/FLD	(García et al., 2006)
Soxhlet	CH_2Cl_2:Hexane (1:1)	150	18 h	65 - 90	GC-MS/MS	(Helaleh et al., 2005)
Soxhlet	CH_2Cl_2:MeOH (2:1)	150	48 h	No data	GC-MS	(Dionisi et al., 2006)
Soxhlet	Toluene	80	8 h	61 – 94	LC-DAD/FLD	(Miège et al., 2003)
Sonication	Toluene	80	30 min	56 - 81	LC-DAD/FLD	(Miège et al., 2003)
Sonication-assisted	MeOH	50	25 min	48 - 99	LC-DAD/FLD	(Santos et al., 2007)

Table 8: Aerobic biodegradation of PAHs by activated sludge

Compound	*1st week*	*1st week*	*2nd week*	*2nd week*	*3rd week*	*3rd week*
	With sodium azide	*Removal (%)*	*With sodium azide*	*Removal (%)*	*With sodium azide*	*Removal (%)*
Naph	ND	22.03	ND	59.89	ND	88.15
Acy [a]	ND	15.10	ND	33.97	ND	49.66
Ace	88.53	9.54	ND	18.19	ND	26.18
Fluo	66.98	4.12	82.72	7.11	98.02	11.96
Phen	54.25	2.07	68.10	5.02	86.37	9.02
Ant	36.80	0.98	45.16	2.12	52.78	4.02
Flt	43.45	1.11	52.14	2.09	59.16	4.21
Pyr	45.48	0.28	52.58	0.59	63.36	4.63
BaA	11.63	0.15	15.95	0.11	17.05	2.05
Chry	28.90	0.55	33.68	0.42	43.84	3.55
BbF	1.89	0.28	2.07	0.77	2.67	1.12
BkF	1.65	0.02	1.88	0.09	2.38	0.97
BaP	0.61	0.02	0.71	0.20	0.90	1.01
IcdP	16.28	2.03	19.13	3.14	22.27	3.84

[a] PDA detection, ND: Not detected

As can be seen from the results, during the three weeks, aerobic biodegradation was a good solution for the removal of most of PAH compounds. The degradation was varied according to the structure of PAHs. Under aerobic conditions, microorganisms which are present in activated sludge were capable of degrading lighter PAHs with two fused aromatic rings such as naphthalene, acenaphthene, acenaphthylene and fluorene, while most of the higher PAHs were more recalcitrant and took more time to degrade. Biodegradation rates were inversely correlated with the increasing molecular weight, and seemed limited by the low bioavailability of the heaviest PAHs (Trably et al., 2006).

The results obtained from the analysis of samples containing sodium azide showed there was a decrease in some PAH analytes even though the biological activity was inhibited. We could conclude that the removal of these compounds did not only occur through the aerobic biodegradation. Therefore, in addition to the biodegradation, the removal of lower PAHs could be a result of losses of volatile PAHs and/or of photo oxidation processes. Since lower PAH compounds have higher solubility in liquid phase, their distribution in supernatant phase is more than the higher PAHs which tend to adsorb into solid sludge; so the lower PAHs were more likely to evaporate and oxidise by light.

The monitoring of pH and nitrate ions in incubated sludge, Figure 4 and Figure 5 respectively, showed a decrease in pH values and increase in the concentrations of nitrate during time, while no changes happened in these parameters in samples containing sodium azide, ensuring the fatality of microorganisms. The principal genera in the activated sludge process, *Nitrosomonas* and *Nitrobacter*, are responsible for the oxidation of ammonium to nitrite (nitritification) and of nitrite to nitrate (nitratification), respectively (Wiesmann et al., 2007).

The stoichiometry for catabolism of NH_4^+ and NO_2^- oxidation are presented in Equations 1 and 2 respectively. The overall oxidation of ammonium to nitrate by both groups needs a large amount of oxygen, and the pH decreases in water with low buffer capacity if no pH control is performed (Wiesmann et al., 2007).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

In the aerobic catabolic funnel (Figure 6), most peripheral pathways involve oxygenation reactions carried out by monooxygenases and/or hydroxylating dioxygenases that generate dihydroxy aromatic compounds (catechol, protocatechuate, gentisate, homoprotocatechuate, homogentisate, hydroquinone, and hydroxyquinol). These intermediate compounds are the substrates of ring-cleavage enzymes that use molecular oxygen to open the aromatic ring between the two hydroxyl groups "ortho cleavage, catalysed by intradiol dioxygenases" or proximal to one of the two hydroxyl groups "meta cleavage, catalysed by extradiol dioxygenases" (Díaz, 2007).

Central pathways involve a series of reactions leading to the formation of Krebs cycle intermediates "central metabolism" (Díaz, 2007).

Naphthalene has often been used as a model compound to investigate the ability of bacteria to degrade PAHs because it is the simplest and the most soluble PAH (Seo et al., 2009). As Figure 7 indicates, degradation of naphthalene starts through the attacking of naphthalene

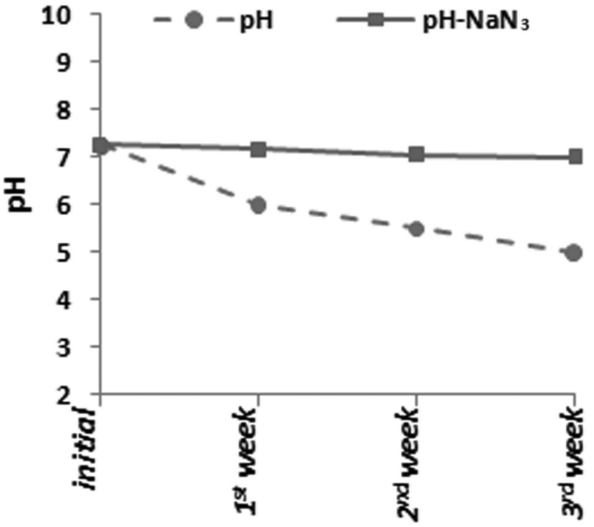

Figure 4: Changes in pH values during biodegradation.

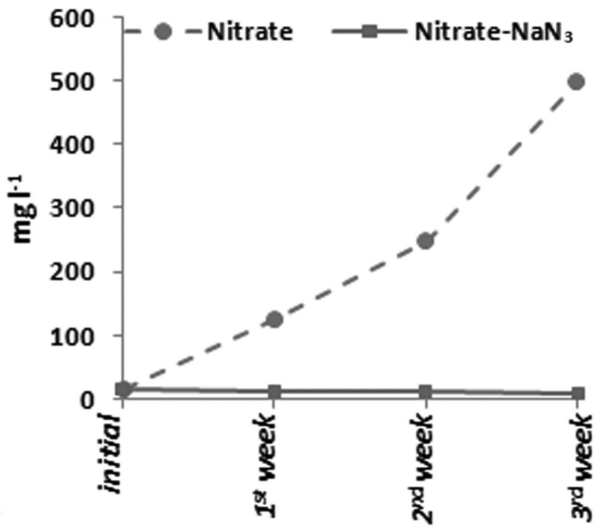

Figure 5: Changes in nitrate concentrations during biodegradation.

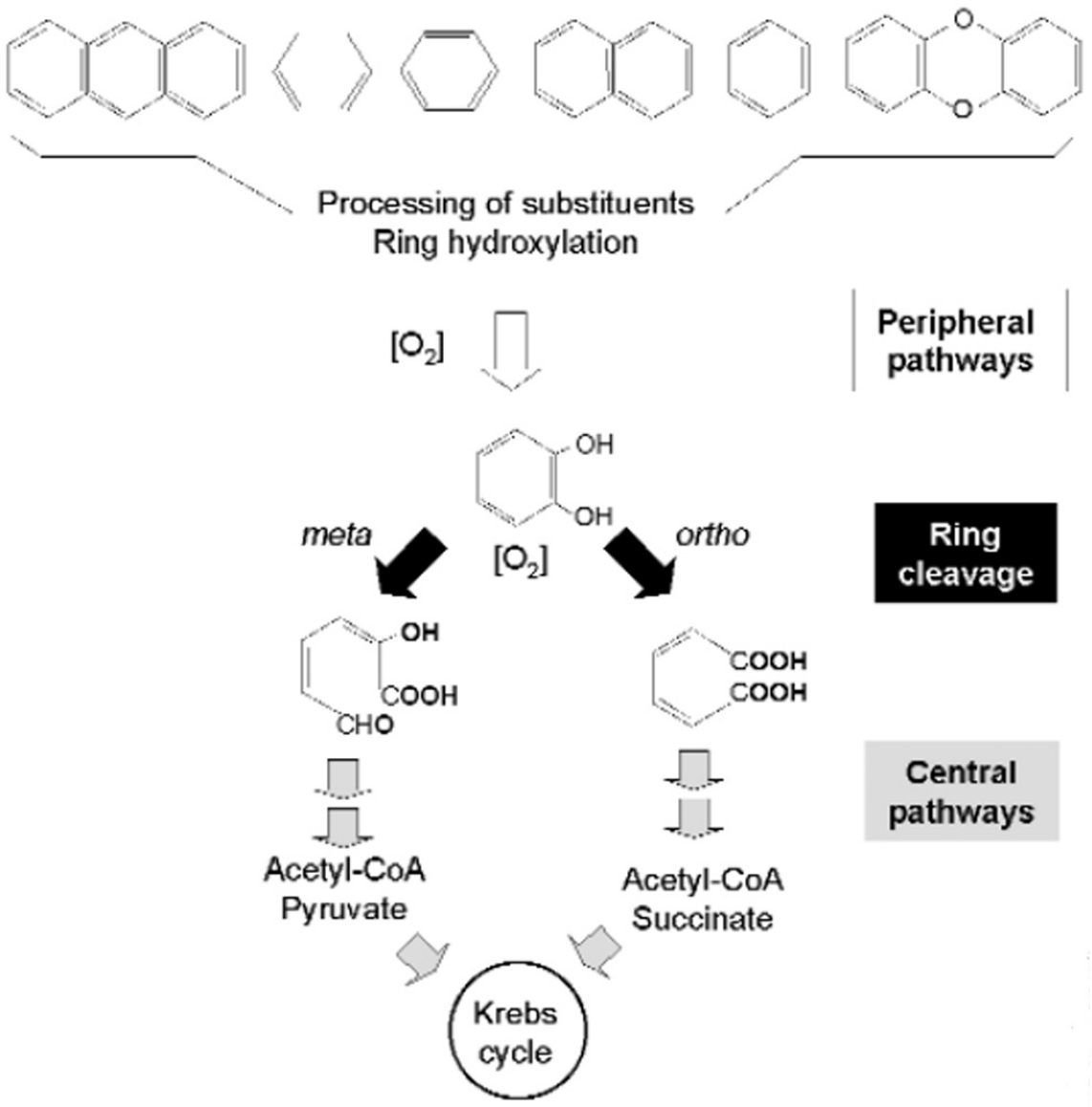

Figure 6: The catabolic funnel for the aerobic degradation of aromatics. White arrows: peripheral pathways, black arrows: cleavage of the ring; gray arrows: central pathways (Diáz, 2004).

dioxygenase enzyme, on the aromatic ring to form *cis* naphthalene dihydrodiol which is subsequently dehydrogenated to 1,2-dihydroxynaphthalene by a *cis*-dihydrodiol dehydrogenase. Subsequently, 1,2-dihydroxynaphthalene is metabolised to salicylate. Furthermore, 1,2-dihydroxynaphthalene is non-enzymatically oxidised to 1,2-naphthaquinone. Salicylate is typically decarboxylated to catechol, which is further metabolised by ring fission in *meta*- and *ortho*-pathways (Seo et al., 2009). Salicylate could be converted to gentisate by salicylate-5-hydroxylase (Fuenmayor et al., 1998).

Conclusion

A sonication and shaking extraction method has been proposed to extract PAH compounds and analyse them in activated sludge collected from WWTP. The analysis has been done of the entire activated sludge which includes supernatant and solid using HPLC equipped with diode array and fluorescence detectors. Recoveries, relative standard deviations, limits of detection and quantitation were consistent with those reported using similar methods. Considering feasible time consuming sample preparation, reasonable solvent volume, and the

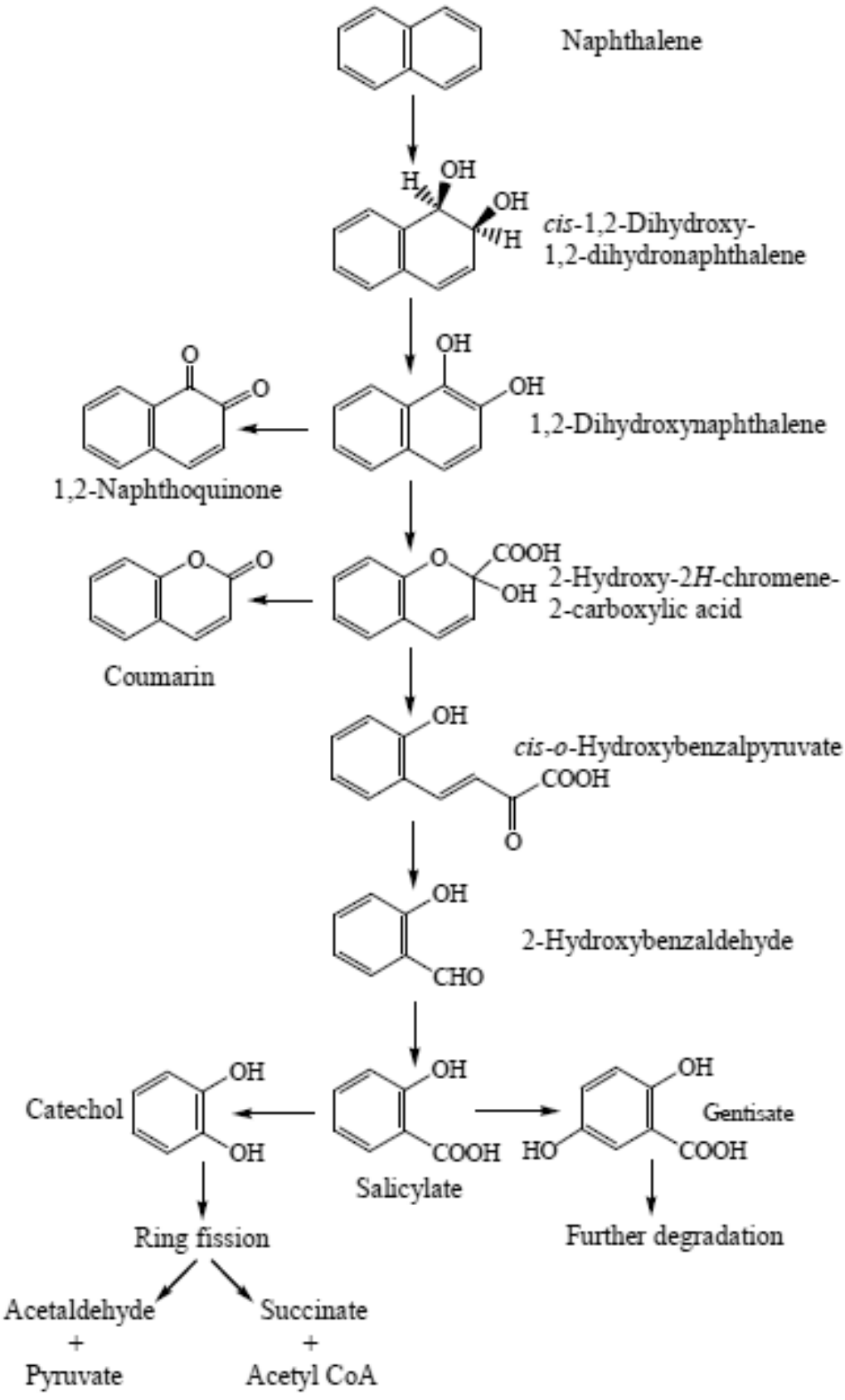

Figure 7: Proposed catabolic pathways of naphthalene by bacteria (Seo et al., 2009).

less expensive equipment used, it might be considered as a good method to perform the determination of PAHs in activated sludge. The applicability of the proposed method in the monitoring of aerobic biodegradation of PAHs was achieved. The results of biodegradation showed that during three weeks, the activated sludge was capable to degrade most of PAH pollutants, especially lighter compounds, confirming that as the number of benzene ring in PAH compounds increases the rate of biodegradation decreases. Furthermore, the results demonstrated the contribution of other factors like losses by evaporation and/or photo oxidation in the removal of lighter PAHs. In this short period of study, the activated sludge could be valuable in the bioremediation of PAHs—mainly lighter compounds which consist of two and three fused aromatic rings.

Acknowledgements

This work has been done as a result of a programme of students exchange between Germany and Syria for one month, which has been supported financially by German Academic Exchange Service (DAAD). All analysis has been done in the laboratories of the Institute for Hygiene and Environmental Medicine, RWTH Aachen University, Aachen, Germany. We wish to thank Professor Wolfgang Dott, Professor Ursula Priefer and the group of Institute for Hygiene and Environmental Medicine and Institute for Biology 1, Unit of Soil Ecology in RWTH Aachen University for the scientific supports for the realisation of this study.

References

Abad, E., Martínez, K., Planas, C., Palacios, O., Caixach, J. and J. Rivera (2005). Priority organic pollutant assessment of sludges for agricultural purposes. *Chemosphere*, **61(9):** 1358-1369.

Blanchard, M., Teil, M.J., Ollivon, D., Legenti, L. and M. Chevreuil (2007). Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewaters and sewage sludges from the Paris area (France). *Environmental Research*, **95(2):** 184-197.

Boon, N., Siciliano, E.M. and W. Verstraete (2003). Bioaugmentation as tool to protect the stucture and function of an activated-sludge microbial community against a 3-Chloraniline Shok load. *Applied and Environmental Microbiology*, **69:** 1511-1520.

Busetti, F., Heitz, A., Cuomo, M., Badoer, S. and P. Traverso (2006). Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant. *Journal of Chromatography A*, **1102(1-2):** 104-115.

Chang, B.V., Chang, S.W. and S.Y. Yuan (2003). Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. *Advances in Environmental Research*, **7(3):** 623-628.

Díaz, E. (2004). Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. *International Microbiology*, **7:** 173-180.

Dionisi, D., Bertin, L., Bornoroni, L., Capodicassa, S., Papini, M.P. and F. Fava (2006). Removal of organic xenobiotics in activated sludges under aerobic conditions and anaerobic digestion of the adsorbed species. *Journal of Chemical Technology and Biotechnology*, **81(9):** 1496-1505.

Eichhorn, P., López, O. and D. Barceló (2005). Application of liquid chromatography–electrospray-tandem mass spectrometry for the identification and characterisation of linear alkylbenzene sulfonates and sulfophenyl carboxylates in sludge-amended soils. *Journal of Chromatography A*. **1067(1-2):** 171-179.

Fuchedzieva, N., Karakashev, D. and I. Angelidaki (2008). Anaerobic biodegradation of fluoranthene under methanogenic conditions in presence of surface-active compounds. *Journal of Hazardous Materials*, **153(1-2):** 123-127.

Fuenmayor, S.L., Wild, M., Boyes, A.L. and P.A. Williams (1998). A Gene Cluster Encoding Steps in Conversion of Naphthalene to Gentisate in *Pseudomonas* sp. Strain U2. *Journal of Bacteriology*, **180(9):** 2522-2530.

García, M.T., Campos, Sánchez-Leal, E. and J.I. Ribosa (2006). Effect of linear alkylbenzene sulphonates (LAS) on the anaerobic digestion of sewage sludge. *Water Research*, **40(15):** 2958-2964.

Gibson, R.W., Wang, J., Padgett, E. and A.J. Beck (2005). Analysis of 4-nonylphenols, phtalates, and polychlorinated biphenyls in soils and biosolids. *Chemosphere*, **61(9):** 1336-1344.

Helaleh, M.I.H., Al-Omair, A., Nisar, A. and B. Gevao (2005). Validation of various extraction techniques for the quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludges using gas chromatography-ion trap mass spectrometry. *Journal of Chromatography A*, **1083(1-2):** 153-160.

Lara-Mártin, P.A., Gómez-Parra, A. and E. González-Mazo (2006). Simultaneous extraction and determination of anionic surfactants in waters and sediments. *Journal of Chromatography A*, **1114(2):** 205-210.

Larsen, S.B., Karakashev, D., Angelidaki, I. and J.E. Scmidt (2009). Ex-situ bioremediation of polycyclic aromatic hydrocarbons in sewage sludge. *Journal of Hazardous Materials*, **164(2-3):** 1568-1572.

Marcé, R.M. and F. Borrull (2000). Solid-phase extraction of polycyclic aromatic compounds. *Journal of Chromatography A*, **885(1-2):** 273-290.

Marttinen, S.K., Kettunen, R.H. and J.A. Rintala (2003). Occurrence and removal of organic pollutants in sewages

and landfill leachates. *Science of the Total Environment*, **301(1-3):** 1-12.

Miège, C., Dugay, J. and M.C. Hennion (2003). Optimisation, validation and comparison of various extraction techniques for the trace determination of polycyclic aromatic hydrocarbons in sewage sludges by liquid chromatography coupled to diode-array and fluorescence detection. *Journal of Chromatography A*, **995(1-2):** 87-97.

Morales-Muñoz, S., Luque-García, J.L. and M.D. Luque de Castro (2004). Screening method for linear alkylbenzene sulfonates in sediments based on water Soxhlet extraction assisted by focused microwaves with on-line pre-concentration/derivatisation/detection. *Journal of Chromatography A*, **1026(1-2):** 41-46.

Moreda, J.M., Arranz, A., de Betoño, S.F., Cid, A. and J.F. Arranz (1998). Chromatographic determination of aliphatic hydrocarbons and polyaromatic hydrocarbons (PAHs) in a sewage sludge. *Science of the Total Environment*, **220(1):** 33-43.

Nimer, M., Ballesteros, O., Navalón, A., Crovetto, G., Verge, C., López, I., Berna, J.L. and J.L. Vílchez (2007). New sample treatment for determination of linear alkylbenzene sulfonate (LAS) in agricultural soils by liquid chromatography with fluorescence detection. *Analytical and Bioanalytical Chemistry*, **387(6):** 2175-2184.

Priego-López, E. and M.D. Luque de Castro (2004). Superheated water extraction of linear alquilbenzene sulfonates from sediments with on-line preconcentration/derivatisation/detection. *Analytica Chimica Acta*., **511(2):** 249-254.

Rogers, H.R. (1996). Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. *Science of the Total Environment*. **185(1-3):** 3-26.

Santos, J.L., Aparicio, I. and E. Alonso (2007). A new method for the routine analysis of LAS and PAH in sewage sludge by simultaneous sonication-assisted extraction prior to liquid chromatographic determination. *Analytica Chimica Acta*., **605(1):** 102-109.

Seo, J., Keum, Y. and Q.X. Li (2009). Bacterial degradation of aromatic compounds. *International Journal of Environmental Research and Public Health*, **6:** 278-309.

Trably, E., Delgènes, N., Patureau, D. and J.P. Delgènes (2004). Statistical tools for the optimisation of a highly reproducible method for the analysis of polycyclic aromatic hydrocarbons in sludge samples. *International Journal of Environmental Analytical Chemisrty*. **84(13):** 995-1008.

Trably, E. and D. Patureau (2006). Successful Treatment of Low PAH-Contaminated Sewage Sludge in Aerobic Bioreactors. *Environmental Science and Pollution Research*, **13(3):** 170-176.

Villar, M., Callejón, M., Jiménez, J.C. and E. Alonso (2007). Optimisation and validation of a new method for analysis of linear alkylbenzene sulfonates in sewage sludge by liquid chromatography after microwave-assisted extraction. *Analytica Chimica Acta*., **599(1):** 92-97.

Vinther, F.P., Mortensen, G.K. and L. Elsgaard (2003). Effects of linear alkylbenzene sulfonates on functional diversity of microbial communities in soil. *Environmental Toxicology and Chemistry*, **22(1):** 35-39.

Wiesmann, U., Choi, I.S. and E.M. Dombrowski (2007). Fundamentals of Biological Wastewater Treatment. Weinheim, Wiley-VCH Verlag GmbH & Co. KGaA, Germany.

Asian Journal of Water, Environment and Pollution

Volume 8 Number 2 **April – June, 2011**

Contents