ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

RESPIROMETRIC ANALYSIS OF BENZO[A]ANTHRACENE INDUCED INHIBITION ON PEPTONE BIODEGRADATION

Ph.D. Thesis by
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Department : Environmental Engineering

Programme : Environmental Biotechnology

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PEPTONUN BİYOLOJİK OLARAK AYRIŞTIRILMASI SIRASINDA BENZO[A]ANTHRACENE'NİN SEBEP OLDUĞU İNHİBİSYONUN RESPİROMETRİK OLARAK BELİRLENMESİ

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İŞİN ADI

Zenobiyotiklerin Biyolojik Ayrışabilirliğinin Enzimatik Olarak İncelenmesi

YÜKLENİCİ: İ. Serden Başak
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Dedicated to my parents, my brother and my wife…

FOREWORD

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ABBREVIATIONS

AMES : *Salmonella typhimurium* reversion assay
ASM : Activated Sludge Model
B[a]A : Benzo[a]anthracene
B[a]P : Benzo[a]pyrene
b_H : Endogenous decay rate for X_H (1/day)
CA : Chromosomal aberrations
COD : Chemical oxygen demand
DA : DNA adducts
DM : Dry matter
DW : Dry weight
F&D : Fill and Draw
k_h : Maximum hydrolysis rate for S_H (1/day)
K_S : Half saturation constant for growth of X_H (mgCOD/L)
K_{STS} : Half saturation constant for storage of PHA by X_H (mgCOD/L)
k_{STO} : Maximum storage rate of PHA by X_H (1/day)
K_X : Hydrolysis half saturation constant for S_H (gCOD/gCOD)
OHM/TADS : Oil and Hazardous Materials/Technical Assistance Data System
PAH : Polycyclic Aromatic Hydrocarbon
PHA : Polyhydroxyalkanoate
r_G : Maximum growth rate for growth on PHA for X_H (1/day)
SBR : Sequencing batch reactor
SCE : Sister Chromatid Exchange
S_H : Initial readily hydrolysable COD (mgCOD/L)
SRT : Sludge Retention Time
S_S : Initial readily biodegradable COD
SS : Suspended Solids
UDS : Unscheduled DNA synthesis
VSS : Volatile Suspended Solids
Y_H : Heterotrophic yield coefficient (gCOD/gCOD)
Y_{STO} : Storage yield of PHA (gCOD/gCOD)
μ_{Hmax} : Maximum growth rate for X_H (1/day)
$?O_2$: Total oxygen consumption

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RESPIROMETRIC ANALYSIS OF BENZO[A]ANTHRACENE INDUCED INHIBITION ON PEPTONE BIODEGRADATION

SUMMARY

Xenobiotic is a chemical that is not a normal component of the microorganism in which it is detected. The term 'xenobiotic' also covers chemical, petrochemical compounds, pesticides and plastics. The number of chemical compounds in the market is around 40000 and this number is increasing by 2000 each year. There are more than 100000 xenobiotics. These compounds have been threatening the environment more and more due to their toxic and carcinogenic effects on human and animal health and their high persistency in environment and biological systems. Xenobiotics are being discharged into the environment after being used as raw material and additive for many industrial processes. Therefore, assessment of their toxicity and investigation of their effects on treatment systems have utmost importance. Furthermore, biological degradation studies have been focusing on these compounds due to their low concentrations in the environment which makes their detection very difficult and the unknown effects on biota and biological treatment systems.

In this study, Benzo[a]anthracene (B[a]A) was selected as the model compound and its acute and chronic effects on activated sludge were investigated. B[a]A, as a toxic and carcinogenic polycyclic aromatic hydrocarbon (PAH), is present in cigarette smoke, exhaust gas of diesel engines, fire smoke, commercial solvents, etc. B[a]A is one of the 16 PAHs in the priority pollutants list of US Environmental Protection Agency (USEPA). Acute and chronic effects of B[a]A on activated sludge were determined by using conventional methods, detection of polyhydroxialkaonates (PHA), oxygen utilization rate (OUR), determination of dominant species and dehydrogenase enzyme test. Dehydrogenase enzyme is used for biological degradation of B[a]A by microorganisms in the activated sludge. The acclimation studies were initiated with biomass taken from the Istanbul ISKI Pasaköy Biological Wastewater Treatment Plant and a fill & draw reactor was operated at a hydraulic retention time of 1 day and sludge age of 10 days. Before starting with the acute experiments, the system was fed with synthetic peptone solution (600 mgCOD/L), which is believed to best represent the municipal wastewater for 3 months prior. Different concentrations of B[a]A (0.011, 0.044 ve 0.088 g/L) were used in the experiments for the determination of acute effects. Sequencing Batch Reactor (SBR) operated at two cycles per day was used for the determination of acute effects and a constant B[a]A concentration (0.011 g/L) was added to the system for 21 days. The modeling studies conducted provided experimental data and information on inhibition kinetics described under Modified Activated Sludge Model No. 3 (ASM3) through respirometric tests. Experimental and modelling results revealed that

addition of B[a]A affects the amount of PHA stored in the activated sludge, the rate of storage of PHA and rate of growth on PHA. Another important result is that the added B[a]A during the acute test changed the dominant microorganism species in the activated sludge. In the system with no addition of B[a]A the dominant species was *Entrococcus faecalis*, whereas in the system with addition of B[a]A at steady state, the dominant species were *Bacillus subtilis*, *Bacillus atrophaeus* and *Bacillus cereus*, which are able to biodegrade PAHs. Furthermore, PAH measurements were made for the samples taken from influents of two different wastewater treatment plants on the Asian and European sides of Istanbul. The total PAH concentration in the influent was found to be in the range of 1.3-1.9 mg/L.

PEPTONUN BİYOLOJİK OLARAK AYRIŞTIRILMASI SIRASINDA BENZO[A]ANTHRACENE'NİN SEBEP OLDUĞU İNHİBİSYONUN RESPİROMETRİK OLARAK BELİRLENMESİ

ÖZET

Zenobiyotik, ortaya çıktığı organizmanın normal bileşeni olmayan bir kimyasaldır. Zenobiyotik terimi aynı zamanda kimyasal, petrokimyasal maddeleri, pestisitleri ve plastikleri de içerir. Piyasada bulunan kimyasal maddelerin sayısı 40.000 civarındadır ve bu sayı her sene 2000 kadar artmaktadır. Yaklaşık olarak 100.000'den fazla zenobiyotik bulunmaktadır. Bu bileşikler, insan ve hayvan sağlığını olumsuz yönde etkileyen toksik ve kanserojen etkileri ve çevre ile biyolojik sistemlerdeki yüksek dayanıklılıkları nedeniyle çevreyi giderek daha fazla tehdit etmektedirler. Zenobiyotikler, hammadde ve katkı maddesi gibi pek çok endüstriyel üretim sürecinde kullanılıp, sonrasında çevreye deşarj edilirler. Bu nedenle, toksisitelerinin değerlendirilmesi ve arıtma sistemleri üzerindeki etkilerinin araşırılması büyük önem taşır. Sentetik ve toksik kökenleri, yüksek miktarlarda üretilmeleri, kanserojen etkileri ve çevrede varlıklarını uzun süre korumalarından dolayı oldukça ilgi görmektedirler. Ayrıca, çevrede zorlukla belirlenebilen düşük konsantrasyonları, canlılar ve biyolojik arıtma sistemleri üzerindeki tam olarak bilinmeyen ve toksik etkileri, biyolojik ayrışma çalışmalarının bu bileşikler üzerine odaklanmasına neden olmaktadır.

Bu çalışmada, Benzo[a]anthracene (B[a]A), model zenobiyotik olarak seçilmiş ve B[a]A'nın aktif çamur sistemi üzerine etkileri akut ve kronik olarak incelenmiştir. B[a]A, toksik ve karsinojen bir polisiklik aromatik hidrokarbon (PAH) olup sigara dumanında, özellikle dizel araçların eksoz dumanında, yangın sonucu çıkan dumanlarda, ticari solventlerde vb. bulunmaktadır. B[a]A, Amerika Çevresel Koruma Ajansı (USEPA) tarafından öncelikli kirleticiler listesinde yer alan 16 PAH'tan biridir. Aktif çamur üzerine B[a]A'nın akut ve kronik etkileri, konvansiyonel yöntemler, PAH, oksijen tüketim hızı (OTH), polihidroksialkonat (PHA), baskın tür tayini ve dehidrogenaz enzim testi uygulanarak belirlenmiştir. Dehidrogenaz enzimi, aktif çamurda bulunan mikroorganizmalar tarafından B[a]A'nın biyolojik olarak parçalanmasında kullanılmaktadır. Aklimasyon çalışmaları İSKİ Paşaköy İleri Biyolojik Atıksu Arıtma Tesisi'nden alınan biyokütle ile başlatılmış, hidrolik bekletme süresi 1 gün olan doldur-boşalt reaktörler çamur çaşı 10 gün olarak işletilmiştir. Akut deneylere başlamadan önce sistem 3 ay süre ile organik madde olarak evsel atıksuyu en iyi temsil ettiği düşünülen sentetik pepton karışımı (600 mgKOİ/L) ile beslenmiştir. Akut etkinin belirlenmesi amacıyla farklı B[a]A konsantrasyonları (0,011, 0,044 ve 0,088 g/L) deneylerde kullanılmıştır. Kronik etkinin belirlenmesi için günde iki çevrim ile çalıştırılan ardışık kesikli reaktör (AKR) sistemi kullanılmış ve sabit B[a]A konsantrasyonu (0,011 g/L) sisteme 21 gün boyunca ilave edilmiştir. Yürütülen modelleme çalışması, respirometrik testler

aracılığıyla Modifiye Edilmiş Aktif Çamur Modeli No. 3'teki (ASM 3) inhibisyon kinetikleri hakkında deneysel veri desteği ve bilgi sağlamıştır. Eklenen B[a]A'nın, aktif çamurdaki depolanan PHA miktarlarını, PHA depolama hızlarını ve PHA üzerinden çoğalma hızlarını etkilediği deneysel ve model sonuçlarıyla ortaya konulmuştur. Bir diğer önemli sonuç ise kronik test süresince eklenen B[a]A ile aktif çamurda bulunan baskın mikroorganizma türlerinin sistemde değiştiğidir. B[a]A ilavesiz sistemde *Entrococcus faecalis* türü baskın iken B[a]A ile beslenen kararlı sistemde ise PAH'ları ve B[a]A'yı biyolojik olarak parçalayabilen türler olan *Bacillus subtilis*, *Bacillus atrophaeus*, *Bacillus cereus* türlerinin baskın olduğu belirlenmiştir. Ayrıca, İstanbul'un Avrupa ve Anadolu yakalarında bulunan iki farklı atıksu arıtma tesisi giriş atıksularında PAH ölçümleri gerçekleştirilmiştir. Giriş atık suyunda PAH konsantrasyonu 1,3-1,9 mg/L aralığında bulunmuştur.

1. INTRODUCTION

Biodegradation of xenobiotic compounds are of great concern in recent years. Sources of these compounds are chemicals, which are commercially available in increasing amounts, and mainly industrial activities that end up with discharges to the environment after they are used as raw materials or additives.

Because of their synthetic and toxic origin, production in high amounts, carcinogenetic effects, and persistence in the environment, they receive considerable attention. Studies on the biodegradability of such substances receive high attention from the scientific community focusing on the facts that they are not easily detected in the environment due to their low concentration, and it is possible that they may have unknown and toxic effects on organisms and biological treatment systems.

The aim of the study is to investigate the enzymatic effect of a xenobiotic named Benzo[a]anthracene (B[a]A), which is a PAH. The PAHs can also enter the environment from the spilling of mineral or tar oils, which can pollute soil and water. PAHs are present in air as vapors or stuck to the surfaces of small solid particles. They can travel long distances before they return to earth via rainfall or particle settling. Some PAHs evaporate into the atmosphere from surface waters. However, most stick to solid particles and settle to the bottoms of rivers or lakes. The number of benzene rings makes also the biodegradation possible to be faster than PAHs with more than four rings.

The PAHs in activated sludge mainly originate from; human waste, household disposal, as well as from rainfall which drains airborne pollutants and road surface dirt. Their presence in activated sludge is of great concern due to the possible bioavailability of these toxic compounds.

Acute and chronic effect of the B[a]A were investigated. For the investigation of the acute effect, Fill&Draw (F&D) reactor was operated with different B[a]A loads. For the investigation of the chronic effect, Sequencing Batch Reactor (SBR) was operated with a fixed B[a]A load. Respirometric method was applied to investigate the effects observed from both of the F&D and SBR operations. Additionally, the storage compound PHA was measured. Kinetic and stoichiometric coefficients were determined with ASM 3 model Aquasim software was used.

1.1 Purpose of the Thesis

In this study, activated sludge, taken from a biological treatment plant in Istanbul, was used for the studying a selected xenobiotic, namely, B[a]A from Fluka. Activated sludge operated at a sludge age of 10 days and fed with peptone synthetic wastewater having similar characteristics of domestic sewage. This process was performed to investigate the reference behavior of activated sludge without B[a]A addition to wastewater. Acclimation to B[a]A was monitored through respirometric studies. In parallel, dehydrogenase enzymatic test, quantitative analyses of PAH, storage products, chemical oxygen demand, and some other conventional parameter analysis were measured. One sample from Day 21 (without B[a]A) and one sample from Day 21 with B[a]A were taken for species identification with FAME method after steady state was established during the operation of the SBR. Furthermore, 16 individual PAH analysis were performed with HPLC in for samples taken from two different treatment plants in Istanbul.

2. XENOBIOTICS AND PAHS

2.1 Definition of Xenobiotics

A Xenobiotic is a chemical (or, more generally, a chemical mix) which is not a normal component of the organism which is exposed to it [1]. Xenobiotics are understood as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans. The term xenobiotic is derived from the Greek words ????? (xenos) = foreigner, stranger and ß??? (bios, vios) = life, plus the Greek suffix for adjectives -t????, -?, -? (tic) [2]. Xenobiotic can also cover substances, which are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal diet.

Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators. The xenobiotic term includes not only drugs and carcinogens but also chemicals, petrochemicals, pesticides, and plastics. It is generally used for phenolic compounds, dioxins, polychlorinated biphenyls and as their effect on the biota. The number of chemicals, which are commercially available, is about 40000 with an increase of 2000 number in each year. There are approximately more than 100000 xenobiotics. These compounds are of rising environmental concern; due to their toxic and carcinogenic effects; adverse health effects to humans, animals; high persistence in the environment and biological systems. Xenobiotics are used in many industrial production processes as raw materials or additives and end up in waste streams, which are eventually discharged into the environment. Thus, the assessment of their toxicity and the evaluation of

their effects on the treatment systems are of great significance. Difficulty in determination of xenobiotics exists due to very low concentrations in the environment [3-4].

2.2 Sources of Xenobiotics

Xenobiotics are introduced to the environment by either domestic discharges or industrial discharges. Xenobiotics originate from variety of sources including industrial discharges, pesticide applications, formation of unintentional by-products through low temperature combustion and herbicide production, pharmaceuticals and personal care products, use of household chemicals, rainfall runoff, water from atmospheric washout, traffic emissions and erosion of building materials [3, 5]. PAH wastewater is one of the important sources of xenobiotics in the environment [6].

2.3 Definition of PAH

Polycyclic aromatic hydrocarbons (PAHs), which are a class of xenobiotic, are unique contaminants in the environment because they are generated continuously by the inadvertently incomplete combustion of organic matter [7-8]. PAHs include 70 natural and anthropogenic organic compounds constituted by aromatic rings, ranging between two and seven, and mainly derived from petroleum activities. In the 20^{th} century, there was a great increase of anthropogenic production of PAHs by combustion of fossil fuel. Beside of predominating anthropogenic sources e.g. road traffic and combustion of fossil fuels, there are also natural sources, e.g. volcanic eruptions and forest fires. PAHs consist of fused benzene rings in linear, angular or clustered arrangements and contain by definition only carbon and hydrogen atoms. However, nitrogen, sulfur and oxygen atoms may readily substitute in the benzene rings to form heterocyclic aromatic compounds, which are commonly grouped with the PAHs [8].

Due to their toxicity, 16 PAHs were listed by U.S. Environmental Protection Agency (USEPA) as priority pollutants, which should be monitored in aquatic and terrestrial ecosystems. 16 PAHs listed by USEPA shown in Figure 2.1 [9].

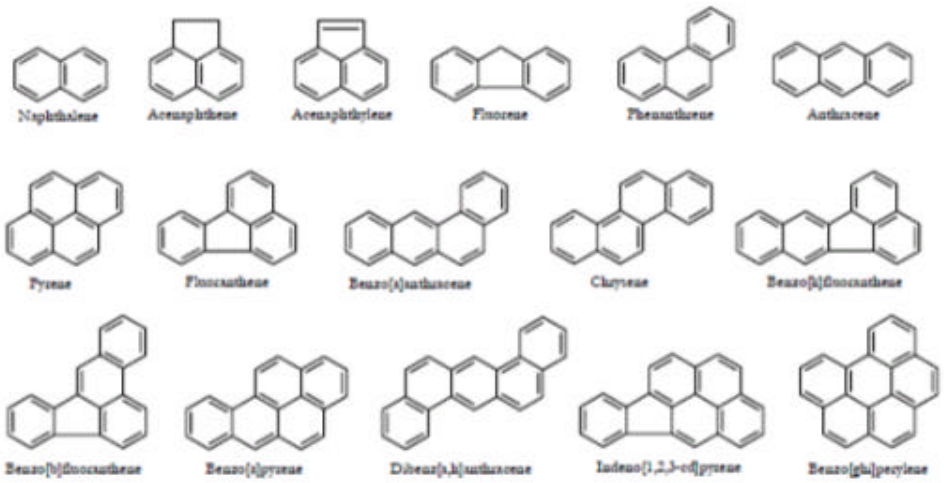


Figure 2.1 : Structures of the 16 US-EPA PAHs [6].

Furthermore, PAHs substituted with alkyl groups are normally found together with the PAHs in the environment. The whole group of PAHs and related compounds are sometimes referred to as polycyclic aromatic compounds (PACs). PAHs have been thoroughly studied due to their toxicity, persistency and environmental prevalence [10-12].

The synonyms, chemical formula, CAS registries, EPA hazardous waste codes and OHM/TADS were given in Table 2.1.

Table 2.1 : The synonyms, chemical formula, CAS registries, EPA hazardous waste codes and OHM/TADS properties of PAHs [6-7, 13-14].

	Synonym(s)	Chemical formula	CAS registry	EPA hazar-dous waste	OHM/TADS
Acenapthene	1,2-Dihydroacenaphthylene 1,8-dihydroacenapthaline 1,8-ethylenenapthalene 1,2-dihydroacenapththylene	$C_{12}H_{10}$	82-29-9	No data	8200126
Acenaphthylene	Cyclopenta[d,e]naphtlhalene	$C_{12}H_6$	208-96-8	No data	No data
Anthracene	Anthracin, Green oil, Tetra olive NZG, Antracene oil	$C_{14}H_{10}$	120-12-7	No data	82001222
B[a]A	Benz[a]anthracene, 1,2-benzanthracene, Benzo[b]phenanthrene, 2,3-benzophenanthrene, tetraphene	$C_{18}H_{12}$	56-55-3	U018	8200123
B[a]P	Benzo[d,e,f]chrysene, 3,4-benzopyrene, 3,4-benzpyrene, Benz[a]pyrene, BP	$C_{20}H_{12}$	50-32-8	U022	No data
Benzo[b]fluoranthene	3,4-Benz[e]acephenanthrylene, 2,3-benzfluoranthene, 3,4-benzfluoranthene, Benzo[e]fluoranthene	$C_{20}H_{12}$	205-99-2	No data	8200124
Benzo[k]fluoranthene	8,9-Benzfluoranthene, 8,9-benzofluoranthene, 11,12-benzofluoranthene, 2,3,1,8-binaphthylene, Dibenzo[b,j,k]fluorene	$C_{20}H_{12}$	207-08-9	No data	8200125
Benzo[g,h,i]perylene	1,12-Benzoperylene BPE	$C_{22}H_{12}$	191-24-2	No data	No data
Benzo[j]fluoranthene	10,11-Benzofluoranthene, Benzo-12,13-fluoranthene, Dibenzo[a,j,k]-fluorene, 7-8,benzofluoranthene, B[j]F	$C_{20}H_{12}$	205-82-3	No data	No data
Crysene	1,2-Benzophenanthrene, Benzo[a]-phenanthrene, 1,2-Benzphenanthrene, Benz[a]phenanthrene, 1,2,5,6-dibenzonaphthalene	$C_{18}H_{12}$	218-01-9	U050	No data
Fluoranthene	Ortho,Biphenylene methane, Diphenylenemethane, 2,2-methylene biphenyl, 2,3-benzidene	$C_{13}H_{10}$	86-73-7	No data	No data
Phenanthrene	Phenanthren	$C_{14}H_{10}$	85-01-8	No data	No data
Pyrene	Benzo[d,e,f]phenanthrene 8-pyrene	$C_{16}H_{10}$	129-00-00	No data	No data
Dibenz[a,h]anthracene	Dibenz[a,h]anthracene, DB[a,h]A, DBA, Dibenz[a]anthracene	$C_{22}H_{14}$	53-70-3	U063	No data

Table 2.1 : (contd.) The synonyms, chemical formula, CAS registries, EPA hazardous waste codes and OHM/TADS properties of PAHs [6-7, 13-14].

	Synonym(s)	Chemical formula	CAS registry	EPA hazardous waste	OHM/TADS
Indeno[1,2,3-c,d]pyrene	Indenopyrene, IP, Ortho-phenylene, 1,10-[orthophenylene]pyrene 1,10-[1,2-phenylene]pyrene 2,3-orto-phenylene pyrene	$C_{22}H_{12}$	193-39-5	U137	No data
Naphthalene	Albocarbon; Camphor Tar; Dezodorator; Mighty 150 Mighty RD1; Moth Balls; Moth flakes; Naftalen;	$C_{10}H_8$	91-20-3	No data	No data

The increase in the hydrophobicity and electrochemical stability is associated with an increase in the number of benzene rings and angularity of a PAH molecule. The high molecular weight (HMW) PAHs are more persistent and recalcitrant than the low molecular weight (LMW) PAHs. The stability and distribution of the PAHs in the natural environment is influenced by the configuration of the aromatic rings, physico-chemical properties [12, 15-16].

LMW PAHs are much more water soluble and volatile than their HMW relatives, while the HMW PAHs show higher hydrophobicity than the LMW compounds. The difference in hydrophobicity is also reflected by the octanol-water partitioning coefficient (K_{OW}) and soil organic carbon coefficient (K_{OC}) shown in Table 2.2. These physico-chemical properties largely determine the environmental behavior of PAHs, and indicate that transfer and turnover will be more rapid for LMW PAHs than for the heavier PAHs [12].

Table 2.2 : Chemical and physical properties of the 16 US-EPA PAHs [12,17].

	Number of rings	Molecular weight	Water solubility (mg/L)	Vapor Pressure (Pa)	Log K_{OW}	Log K_{OC}
Naphthalene	2	128	31	$1.0x10^{-2}$	3.37	-
Acenaphthylene	3	152	16	$9.0x10^{-1}$	4.00	1.40
Acenaphthene	3	154	3.8	$3.0x10^{-1}$	3.92	3.66
Fluorene	3	166	1.9	$9.0x10^{-2}$	4.18	3.86
Phenanthrene	3	178	1.1	$2.0x10^{-2}$	4.57	4.15
Anthracene	3	178	0.045	$1.0x10^{-3}$	4.54	4.15
Pyrene	4	202	0.13	$6.0x10^{-4}$	5.18	4.58
Fluoranthene	4	202	0.26	$1.2x10^{-3}$	5.22	4.58
B[a]A	4	228	0.011	$2.8x10^{-5}$	5.91	5.30
Chrysene	4	228	0.006	$5.7x10^{-7}$	5.91	5.30
Benzo[b]fluoranthene	5	252	0.0015	-	5.80	5.74
Benzo[k]fluoranthene	5	252	0.0038	$5.2x10^{-5}$	6.00	5.74
B[a]P	5	252	0.0038	$7.0x10^{-7}$	5.91	6.74
Dibenzo[a,h]anthracene	6	278	0.0006	$3.7x10^{-10}$	6.75	6.52
Indeno[1,2,3-cd]pyrene	6	276	0.00019	-	6.50	6.20
Benzo[g,h,i]perylene	6	276	0.00026	$1.4x10^{-8}$	6.50	6.20

2.4 Sources of PAHs

PAHs enter the environment mostly as releases to air from volcanoes, forest fires, residential wood burning, coal tar, creosote, and exhaust from automobiles and trucks. They can also enter surface water through discharges from industrial plants and wastewater treatment plants, and they can be released to soils at hazardous waste sites if they escape from storage containers. PAHs enter also human through food sources.

In many studies, the sources of PAHs in various environmental samples have been qualitatively determined by the various molecular diagnostic ratios. For example, a widely used concentration ratio is the anthracene (Ant)/[Ant + phenanthrene (Phe)], and if this is lower than 0.10, it is taken as indicative of non-burnt fossil fuel, whereas if this ratio exceeds 0.10, this suggests combustion sources. Similarly, many other ratios exist (Table 2.3), and are used to reveal petrogenic or pyrogenic origin, fuel or wood combustion or traffic related sources [18- 20].

Table 2.3 : Characteristic PAH molecular diagnostic ratios [18, 20].

	Petrogenic	Pyrogenic
Ant/(Ant + Phe)	< 0.1	> 0.1
B[a]A/(B[a]A + Chr)	< 0.2	> 0.35
Flt/(Flt + Pyr)	< 0.4	> 0.4
IP/(IP + BPE)	< 0.2	> 0.2
	Fuel combustion	Grass/coal/wood combustion
Flt/(Flt + Pyr)	0.4-0.5	> 0.5
IP/(IP + BPE)	0.2-0.5	> 0.5
	Non-traffic	Traffic
B[a]P/BPE	< 0.6	> 0.6

Some researchers used these ratios for the characterization of sources of PAHs ending in the sewage sludge [20- 22]. The researchers of the present correspondence claim that such use is not appropriate based on two main reasons: (1) The mixing and homogenization that takes place during the transportation of wastewaters; (2) The treatment that the wastewaters undergo at the various treatment steps. These two parameters will be discussed to prove the afore-mentioned statement about the improper use of PAH molecular diagnostic ratios in sewage sludges. Wastewater mixing and homogenization AWWTP may receive municipal, domestic, or industrial wastewater, effluents from small business (e.g. car washing) or medical facilities, plus street runoff, drainage water, wet and dry deposition from the atmosphere.

Furthermore, big WWTPs may receive effluents from entire cities, thus from places that are very far from each other, and where different PAH sources may exist [20, 23].

The release of PAHs into the environment is widespread since these compounds are ubiquitous products of incomplete combustion. PAHs have been detected in a wide variety of environmental samples, including air, soil, sediments, water, oils, tars and foodstuffs. Industrial activities, such as processing, combustion and disposal of fossil fuels, are usually associated with the presence of PAHs at highly contaminated sites. PAH contamination on industrial sites is commonly associated with spills and leaks from storage tanks and with the conveyance, processing, use and disposal of these fuel/oil products [15, 24-37].

2.4.1 Air

The primary natural sources of airborne PAHs are forest fires and volcanoes. The residential burning of wood is the largest source of atmospheric PAHs; releases are primarily the result of inefficient combustion and uncontrolled emissions. Other important stationary anthropogenic sources include industrial power generation, incineration the production of coal tar, coke, and asphalt; and petroleum catalytic cracking [6].

Environmental tobacco smoke, unvented radiant and convective kerosene space heaters, and gas cooking and heating appliances may be important sources of PAHs in indoor air. Stationary sources account for about 80% of total annual PAH emissions; the rest are from mobile sources. The most important mobile sources of PAHs are vehicular exhaust from gasoline and diesel-powered engines [6, 12, 16].

2.4.2 Water

Peat, coal, and petroleum are relatively rich in complex assemblages of PAHs. These compounds reach aquatic environments through Municipal wastewater discharge, urban storm water runoff, runoff from coal storage areas, effluents, from wood treatment plants and other industries, oil spills, and petroleum pressing. In 1987, it was estimated that spilled petroleum contributes $1.7x10^5$ tones of PAH to aquatic environments each year. This source overwhelms all others in terms of global inputs [6, 38].

The explosion in the Deepwater Horizon on 20 April 2010 was the largest marine oil spill in the history of the petroleum industry. On15 July 2010, the leak was stopped by capping the gushing wellhead after releasing about 4.9 million barrels ($780\times103\ m^3$) of crude oil. It was estimated that 53000 barrels per day (8400 m^3/d) were escaping from the well just before it was capped. It is believed that the daily flow rate diminished over time, starting at about 62000 barrels per day (9900 m^3/d) and decreasing as the reservoir of hydrocarbons feeding the gusher was gradually depleted [39].

2.4.3 Wastewaters and sewage sludge

Industrial and domestic wastewaters are rich in PAH. Secondary sewage treatment removes some PAH, but most are released to aquatic environments through sewage treatment plant outfalls. It was noted that untreated, raw sewage contains 100 to 500 ppb total PAHs and sewage sludge contains 200 to 1750 ppb and storm water runoff from urban areas and highways accounted for 71% of the high molecular weight PAHs [38].

2.4.4 Soil

Most of the PAHs in soil are believed to result from atmospheric deposition after local and long-range transport. The presence of PAHs in the soil of remote regions from any industrial activity supports this contention. Other potential sources of PAHs in soil include sludge disposal from public sewage treatment plants, automotive exhaust, irrigation with coke oven effluent, leachate from bituminous coal storage sites, and use of soil compost and fertilizers. The principal sources of PAHs in soils along highways and roads are vehicular exhausts and emissions from wearing of tires and asphalt. PAHs may also be released to soils at concentrations above background and landfill sites and industrial sites, including creosote production, wood-preserving, and coking plants. Soils at the sites of former manufactured gas plants are also heavily contaminated with PAHs [6].

2.4.5 Food sources

Foods normally containing microgram quantities of PAHs. Foods found to contain minute quantities of B[a]A, benzo[j]fluoranthene, B[a]P, dibenz[a,h]anthracene, or indeno[1,2,3-cd]pyrene include: smoked, barbecued, or

charcoal-broiled foods, vegetables and vegetable oils, margarines, roast coffee and coffee powders, fresh sausages, cereals, grains, flour, breads, meats, seafood, fruits, processed foods, and beverages. PAHs have been detected at low levels in some drinking water supplies as well as in fresh and seawater in the United States [41].

2.5 Sources of B[a]A

B[a]A is found in gasoline and diesel exhaust, cigarette smoke and smoke condensate, amino acid, fatty acid, and carbohydrate pyrolysis products, coal tar and coal tar pitch, asphalt, soot and smoke, wood smoke, coal combustion emissions, commercial solvents, waxes, mineral oil, and creosote [13].

2.6 Toxicity of PAHs

PAHs are considered as important environmental pollutants since many of the compounds in this group are of major concern to environmental agencies and researchers worldwide due to their mutagenic, toxic, genotoxic and carcinogenic properties depending upon the number and configuration of the benzene rings and the presence and position of their substituents [12, 14, 24, 41-44].

The lipophilicity, environmental persistence and genotoxicity increase as the molecular size of PAHs increases up to 4 or 5 fused benzene rings and toxicological concern shifts towards chronic toxicity, primarily carcinogenesis (Figure 2.2) [16].

PAHs have : (1) chronic health effects (carcinogenicity); (2) microbial recalcitrance; (3) high bioaccumulation potential; and (4) low removal efficiencies in traditional treatment processes. Higher molecular weight four -to seven-ring PAHs are highly mutagenic and carcinogenic, and lower molecular weight two -to three-ring PAHs, although less mutagenic, can be highly toxic. Carcinogenic PAHs refer to fluoranthene, pyrene, B[a]A, chrysene, benzo[b]luoranthene, benzo[k]fluoranthene, B[a]P, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene. Non-carcinogenic PAHs refer to naphthalene, acenaphthylene, acenapthene, fluorene, anthracene and phenanthrene [45]. In many cases the parent compounds are relatively inert, but the metabolites exert toxicity. Low molecular weight PAHs, dominant in fossil fuel assemblages, are more labile and readily volatilize into the atmosphere from the air/water interface [46].

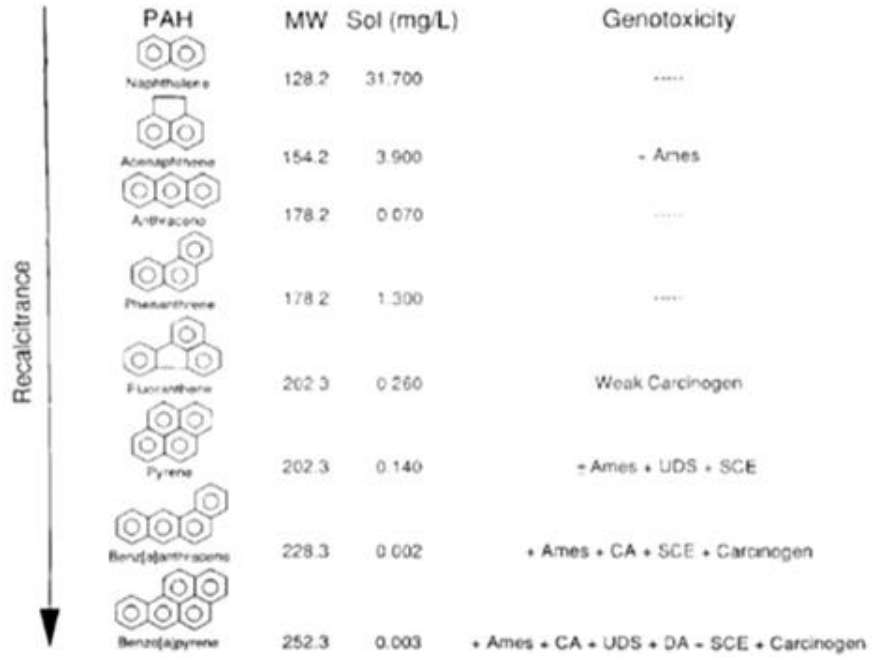


Figure 2.2 : Chemical structures and toxicological characteristics of PAHs [16].

Many PAHs contain a 'bay-region' as well as 'K-region', both of which allow metabolic formation of bay- and K-region epoxides, which are highly reactive. Carcinogenicity has been demonstrated by some of these epoxides. Therefore, many PAHs are considered to be environmental pollutants that can have a detrimental effect on the flora and fauna of affected habitats, resulting in the uptake and accumulation of toxic chemicals in food chains, some instances and serious health problems and/or genetic defects in humans [47-48].

PAH mixtures lead to tumors of the respiratory tract after inhalation and to skin tumors after dermal application [49]. It has long been known that PAHs can have serious deleterious effects to human health, with the physician John Hill first recognizing the link between the use of snuff and nasal cancer in 1761 [50].

The relative toxicity of PAHs can be measured using LD_{50} values (the lethal dose in 50% of cases). These are expressed as milligrams of toxic material per kilogram of the subject's body weight that will cause death in 50% of cases. It is important to specify the route by which the toxic material was administered to the test animal (such

as oral or intraperitoneal), and the animal upon which the toxic material was tested (i.e. rat, mouse). The LD_{50} values of some representative PAHs were given in Table 2.4 [52].

Table 2.4 : LD_{50} values of some representative PAHs toxicity of end-products [50].

Material	LD_{50} value (mg/kg)	Test subject	Exposure route
Naphthalene	533-710	Male/female mice respectively	Oral
Phenanthrene	750	Mice	Oral
Anthracene	> 430	Mice	Intraperitoneal
Fluoranthene	100	Mice	Intravenous
Pyrene	514	Mice	Intraperitoneal
B[a]P	232	Mice	Intraperitoneal

Acute toxicity causes observable physiological lesions and is usually measured by mortality. PAH can interact with cells in several ways to cause toxic responses. Potentially impacted and important intracellular organelles include lysosomes, which contain strong enzymes important in intracellular digestion of complex organic molecules and in the immune response. The lower molecular weight, unsubstituted PAH compounds, containing 2 or 3 rings, such as naphthalene, fluorene, phenanthrene and anthracene have significant acute toxicity to some organisms, whereas the higher molecular weight, four to seven ring aromatics, do not. However, these heavier molecules contain numerous potentially carcinogenic and mutagenic intermediates [51].

For aquatic organisms, acute toxicity is established in test of generally 48 to 96 hrs, sometimes up to 168 hrs (7 days). Chronic toxicity is more difficult to measure than acute toxicity. Chronic stress causes reduced scope for growth and reduced reproductive capacity, which can have long term consequences for populations of aquatic species. In addition to direct physiological stress, there is a potential for the high molecular weight PAHs to form carcinogenic, mutagenic and teratogenic compounds during metabolism by crustaceans and vertebrates [51].

An overview of freshwater and marine toxicity testing in an ecotoxicological perspective for selected groups of PAH was given in Table 2.5.

Table 2.5 : Comparison of ecotoxicity threshold concentrations (µg/L) for PAH compounds [51].

Compound	Ecotoxicity threshhold (µg/L)
Naphthalene	10
Acenaphthylene	60
Fluorene	125
Anthracene	6.6
Phenanthrene	8
Pyrene	91
Fluoranthene	38
B[a]A	5
B[a]P	5
Benzo[k]fluoranthene	0.2

2.7 Toxicity of B[a]A

The quantum mechanical calculations predicted that at least one of the metabolically possible diol epoxides of each of the highly carcinogenic hydrocarbons should be highly reactive and that "bay region" diol epoxides of weak or noncarcinogenic aromatic hydrocarbons should be generally less reactive than the corresponding diol epoxides of the more carcinogenic hydrocarbons. In 1997, it was synthesized derivatives of B[a]A in order to test the predictions of the calculations [52]. The calculated ΔE_{deloc} values for formation of carbonium ions from the respective diol epoxides (using the model previously discussed) differ greatly, with the 3,4-diol-l,2-epoxide ("bay region" epoxide) being predicted to be the most reactive. The experimental approach involved, first, synthesis of the dihydrodiol derivatives of B[a]A and an examination of their ability to be metabolically activated to mutagenic species (presumably diol epoxides). The results of the metabolic activation confirmed the expectations of the calculations. The metabolites of B[a]A 3,4-dihydrodiol caused more than ten times as many mutations in *S. typhimurium* strain TA 100 as did metabolites from the other dihydrodiols. Secondly, it was felt important to synthesize the diol epoxide derivatives of the dihydrodiols, since similar rates of conversion of the dihydrodiols to the diol epoxides cannot be assumed, and knowledge of the intrinsic mutagenicity of the diol epoxides toward S. typhimurium strain TA 100 was desired. Thus far, the diastereomeric diol epoxides derived from B[a]A 3,4-dihydrodiol, B[a]A 8,9-dihydrodiol and B[a]A 10,11-dihydrodiol have been synthesized, although the corresponding derivatives of B[a]A 1,2- dihydrodiol have eluded preparation. The results of the testing of the

mutagenicity of the B[a]A diol epoxides were gratifying [53]. The diol epoxides derived from B[a]A 8,9- and 10,11-dihydrodiols had only 3-7% of the mutagenicity of the diol epoxides derived from B[a]A 3,4-dihydrodiol. Further, the differences in mutagenicity between the structural isomers were much greater than the differences in mutagenicity between the diastereomeric pairs of diol epoxides (in which the oxirane oxygen can be either cis- or trans- to the benzylic hydroxyl group). The latter results suggest that the neglect of stereochemistry that is inherent in the perturbational molecular orbital calculations may not be a serious deficiency. An attempt to correlate the relative approximate carcinogenic potency of the parent hydrocarbons with the highest value calculated for ΔE_{deloc} for potential diol epoxide metabolites of the hydrocarbon was fairly successful, but some exceptions were observed that emphasize the limitations inherent in such an approach. For example, the highest value of ΔE_{deloc} for dibenzo[a,h]anthracene is 0.738ß whereas the highest value of ΔE_{deloc} for B[a]A is 0.766ß, although it is known that dibenzo[a,h]anthracene is a more potent carcinogen than is B[a]A. Whether the different biological activities are a consequence of metabolism differences, with dibenzo[a,h]anthracene being converted to a greater extent to the (calculated) critical diol epoxide or whether the calculations are deficient are questions that remain to be answered. Further, the "bay region" diol epoxide of benzo[a]naphthacene is calculated to be exceptionally reactive (ΔE_{deloc} = 0.846ß), but the parent hydrocarbon is non carcinogenic. Again, it is possible either that little, or no, metabolism occurs to produce the "bay region" diol epoxide, or that the calculations are deficient. Fortunately, these and similar questions are amenable to experimental test [52, 54].

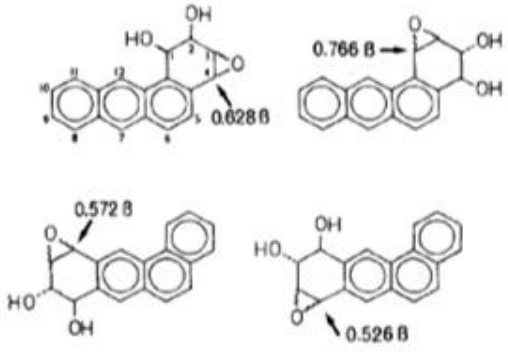


Figure 2.3 : Calculated ?E_{deloc} values for formation of carbonium ions from the respective B[a]A diol epoxides [52].

The EC_{50}, LC_{50}, EC_{10} and EC_{20} values of selected PAH, B[a]A, for the acute effect on different species were given in Table 2.6.

Table 2.6 : The toxicity of B[a]A [55-56].

LC_{50} and EC_{50} values measured with aquatic bioassays		**mg/L**
Microtox (EC_{50}—30 min)		0.056
Daphnia (EC_{50}—48 h)		0.14
Ceriodaphnia (EC_{50}—7 d)		0.022
Algae (EC_{50}—3 d)		0.010
Daphnia (EC_{50} - 96 h)		0.01
Pimephales promelas (LC_{50} - 65 h)		0.0018
In the contaminated soil, corresponding to the concentration inducing 50% lethality (LC_{50}) of invertebrates, and the effective concentrations reducing by 10% invertebrate reproduction (EC_{10}) or by 20% plant growth (EC_{20})		**mg/kg**
Collembola (28 d)	LC_{50} (survival)	25
	EC_{10} (reproduction) (14 d)	7
Earthworm	LC_{50} (survival) (28 d)	170
	EC_{10} (cocoon)	10
Plant (17 d)	EC_{20} (growth)	25

2.8 PAH Risk Assessment

Overall, there are two primary approaches to risk assessment of PAH fractions. The first method is called Individual PAH method (IPM) or toxic equivalency factor (TEF). This approach first estimates the potency of B[a]P and then express the environmental levels of other PAH as "B[a]P equivalents". In order to estimate the potency of a PAH fraction of a mixture, the total number of B[a]P equivalents of the mixture is multiplied by the potency for B[a]P. The result is numerically equivalent to summing up risks attributable to individual PAH in the mixture (Table 2.7). An alternative approach (Whole Mixture Model or WMM) estimates the potency of a PAH fraction of the mixture as a whole. The model assumes that the potency of the PAH fraction of a mixture is proportional to the B[a]P content of the mixture. The potency of the fraction is given by the product of the B[a]P content of the mixture and the typical potency of the PAH fraction of the mixture [57].

Table 2.7 : Comparison of relative potencies of PAH developed by different agencies. Relative potency of an individual PAH is the ratio of its potency of B[a]P [16, 57-60].

PAH	MOE [58]	CEPA [59]	USEPA [16]	CalEPA [60]
Anthracene	0.28			0.1
B[a]A	0.014		0.145	0.1
B[a]P	1.0			
Benzo[b]fluoranthene	0.11	0.06	0.167	0.1
Benzo[e]pyrene	0.0			0.01
Benzo[g,h,i]perylene	0.012			0.01
Benzo[j]fluoranthene	0.045	0.05		0.1
Benzo[k]fluoranthene	0.037	0.04	0.020	0.1
Chrysene	0.026		0.0044	0.01
Dibenzo[a,h]anthracene	0.89		1.11	
Dibenzo[a,h]pyrene	1.2			
Fluoranthene				0.01
Indeno[1,2,3-cd]pyrene	0.067	0.12	0.055	0.1
Pyrene	0.0			0.1

2.9 Fate of PAHs

The persistence of PAHs in the environment is dependent on a variety of factors, such as the chemical structure of the PAH, the concentration and dispersion of the PAH and the bio availability of the contaminant. In addition, environmental factors such as soil type and structure, pH and temperature and the presence of adequate levels of oxygen, nutrients and water for the activity of the pollutant-degrading microbial community will control the time that PAHs persist in the environment. The schematic representation of environmental fate of PAHs given in Figure 2.4 [47].

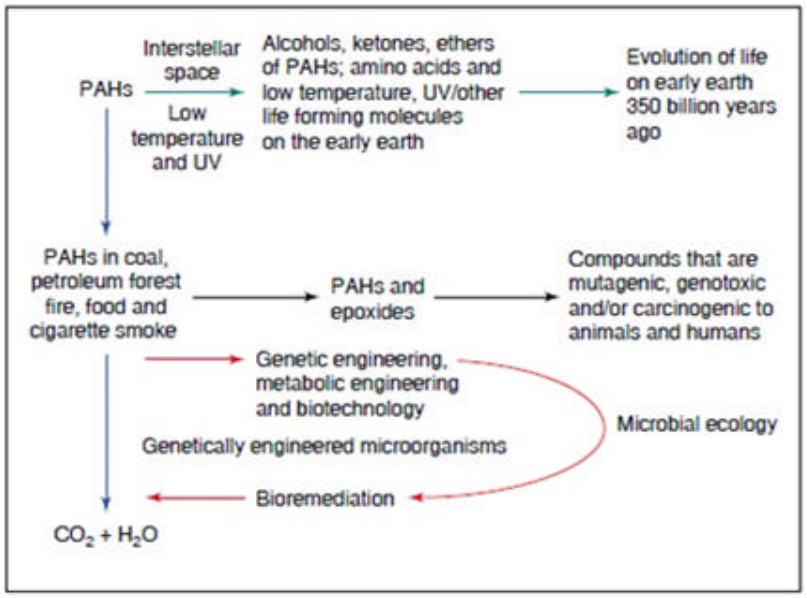


Figure 2.4 : Fate, toxicity and remediation of PAHs from the environment [47].

PAHs are degraded by photo-oxidation and chemical oxidation [61], but biological transformation is probably the prevailing route of PAH loss [24, 62]. The most important degradative processes for PAHs in the marine environment are photooxidation, chemical oxidation, and biological transformation by bacteria and animals [51].

2.9.1 Air

PAHs are present in the atmosphere in the gaseous phase or sorbed to particulates. The phase distribution of PAHs in the atmosphere is important in determining their fate because of the difference in rates of chemical reactions and transport between the two phases. The phase distribution of any PAH depends on the vapor pressure, the atmospheric temperature, the concentration and the affinity. In general, PAHs having two to three rings (naphthalene, acenaphthene, acenaphthylene, anthracene, fluorene, phenanthrene) are present in air predominantly in the vapor phase. PAHs that have four rings (fluoranthene, pyrene, chrysene, B[a]A) exist both in the vapor and particulate phase, and PAHs having five or more rings (B[a]P, benzo[g,h,i]perylene) are found predominantly in the particle phase. The ratio of particulate to gaseous PAHs in air samples collected in Antwerp, Belgium, was 0.03 for anthracene, 0.49 for pyrene, 3.15 for summed B[a]A and chrysene, and 11.5 for summed benzo[a]fluoranthene and benzo[b]fluoranthene [6].

The semivolatile nature of the LMW PAHs means that they exist in the atmosphere partly as vapors and are therefore highly susceptible to atmospheric degradation processes. Similarly, in aqueous environments, the LMW PAHs are partly dissolved, making them highly available for various degradation processes. The HMW PAHs, on the other hand, are primarily associated with particles in the atmosphere and water, and are therefore less available for degradation [9].

2.9.2 Water

In water, PAHs evaporate, disperse into the water column, become incorporated into bottom sediments, concentrate in aquatic biota, or experience oxidation and biodegradation [51].

PAH compounds tend to be removed from the water column by volatilization to the atmosphere, by binding to suspended particles or sediments, or by being accumulated

by or sorbed onto aquatic biota. The transport of PAHs from water to the atmosphere via volatilization will depend on the Henry's law constants (Hs) for these compounds. The low molecular weight PAHs have Henry's law constants in the range of 10^{-3}-10^{-5} atm-m^3/mol; medium molecular weight PAHs have constants in the 10^{-6} range; and high molecular weight PAHs have values in the range of 10^{-5}-10^{-8}. Compounds with values ranging from 10^{-3} to 10^{-5} are associated with significant volatilization, while compounds with values less than 10^{-5} volatilize from water only to a limited extent. Half-lives for volatilization of B[a]A and B[a]P (high molecular weight PAHs) from water have been estimated to be greater than 100 hours. Lower molecular weight PAHs could be substantially removed by volatilization if suitable conditions (high temperature, low depth, high wind) were present. Half-lives for volatilization of anthracene (a low molecular weight PAH) of 18 hours in a stream with moderate current and wind, versus about 300 hours in a body of water with a depth of 1 meter and no current. Even for PAHs susceptible to volatilization, other processes, such as adsorption, photolysis or biodegradation may become more important than volatilization in slow-moving, deep waters [6].

2.9.3 Treatment plants

In case a persistent xenobiotic is the sole carbon source in a system, it is not able to be utilized by biomass. Biomass remains intact with the xenobiotic without growth corresponding to prolonged lag period. The compound is not able to be degraded until there is a biochemical adjustment and induction. These processes constitute acclimation procedure. During acclimation procedure, biomass initially has no degradation capacity. As the time proceeds, it gains gradually acquisition capacity of degrading the persistent organic compound. The contact time of biomass and the xenobiotic have an important role in acclimation period [50].

In mixed cultures, acclimation to an organic compound can be either a result of the selection of specialized organisms. Moreover, it was shown that, the acclimation capacity was related to the genetic information within biomass. In soil, the degradability of organopollutants is depended on several factors including essential substrate supply such as C, N, P, S, favorable state of external conditions including O_2, H_2O, pH, temperature, and bioavailability of the organopollutants [50].

2.9.4 Sediment and soil

Microbial metabolism is the major process for degradation of PAHs in soil environments. Photolysis, hydrolysis, and oxidation generally are not considered to be important processes for the degradation of PAHs in soils [6].

Another study that assessed the fate of several PAHs, which included naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, B[a]A, benzo[b]fluoranthene, dibenz[a,h]anthracene, B[a]P, dibenzo[a,i]pyrene and indeno[l,2,3-c,d]pyrene, in two soils concluded that abiotic degradation (photolysis and oxidation) accounted for mean losses of 13, 8.3, and 15.8% loss in case of naphthalene, anthracene, and phenanthrene, respectively. No significant abiotic loss was observed for the other PAHs [6, 14].

PAHs can biodegrade or accumulate in aquatic organisms. PAHs in soil can volatilize, undergo abiotic degradation (photolysis and oxidation), biodegrade, or accumulate in plants. PAHs in soil can also enter groundwater and be transported within an aquifer [6].

2.10 Regulations of PAHs

The PAH concentration limitations in sewage sludge were not released and enforced in North America but limitations were available for drinking water. In Table 2.8 was given the drinking water regulations for North America.

Table 2.8 : Drinking water regulations in EU and America [13, 49, 51].

Country	Limitations
North America	**EPA** Clean Air Act Mobile Source Air Toxics: Polycyclic Organic Matter listed as a Mobile Source Air Toxic for which regulations are to be developed NESHAP: Polycyclic Organic Matter (POM) listed as a Hazardous Air Pollutant (HAP) Urban Air Toxics Strategy: Polycyclic Organic Matter identified as one of 33 HAPs that present the greatest threat to public health in urban areas Clean Water Act Effluent Guidelines: Listed as a Toxic Pollutant Water Quality Criteria: Based on fish/shellfish and water consumption = 0.0038 µg/L (B[a]A, B[a]P, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, indeno[1,2,3,-cd]pyrene); based on fish/shell-fishconsumption only = 0.018 µg/L (B[a]A, B[a]P, benzo[b]fluoranthene, benzo[k]fluoranthene, dibenzo[a,h]anthracene, and indeno[1,2,3-cd]pyrene) Comprehensive Environmental Response, Compensation, and Liability Act Reportable Quantity (RQ) = ranges from 1 lb to 5,000 lb for the various PAHs

Table 2.8 : (contd.) Drinking water regulations in EU and America [13, 49, 51].

Country	Limitations
North America	Emergency Planning and Community Right-To-Know Act Toxics Release Inventory: All 15 are listed substances subject to reporting requirements Resource Conservation and Recovery Act Numerous specific PAHs listed as a Hazardous Constituents of Waste Listed Hazardous Waste: Waste codes in which listing is based on specific PAHs -U018, U022, U063, U064, U137 Safe Drinking Water Act Maximum Contaminant Level (MCL) = 0.0002 mg/L (B[a]P) **FDA** Maximum permissible level in bottled water = 0.0002 mg/L (B[a]P) **ACGIH Guidelines** Threshold Limit Value - Time-Weighted Average Limit (TLV-TWA) = as low as possible (B[a]A, benzo[b]fluoranthene, B[a]P)
Alaska	Has a water quality standard of 10 µg/L total aromatic hydrocarbons (AH) that includes mono-cyclic hydrocarbons
EU	Drinking water directive : 0.010 g/L B[a]P

While it encourages the use of sewage sludge, the EU Directive 86/278/EEC regulates its use to prevent harm to the environment, in particular to soil. In order to improve the long-term protection of Community soils the Commission is currently working on some aspects of the Directive in the light of new scientific evidence and technological progress [63]. In Table 2.9 was shown limit values for concentrations of organic compounds in sludge of different countries and as suggested in the third draft of the "Working paper on sludge" [49].

Table 2.9 : Standards for concentrations of organic contaminants in sewage sludge in different countries of the EU [49].

	AOX (mg/kg DM)	**PAH**[1] (mg/kg DM)	**PCB** (mg/kg DM)	**PCDD/F** (mg TEQ/kg DM)
EU 2000	500	6	0.8	100
Denmark	-	3	-	-
Sweden	-	3	0.4	-
Lower Austria	500	-	0.2	100
Germany	500	-	0.2	100

[1]Sum of 9 PAHs

The maximum concentration of 11 main PAH compounds in sewage sludge could not exceed 6 mg/kg of dry matter if the sludge is designated to be applied on agricultural land [64].

German threshold value for 15 PAHs (without naphthalene) 0.20 µg/L soil-water based on the soil-water pathway for seepage water in soil and 16 PAHs for the soil matrix 200 µg/kg [49, 65-66].

Table 2.10 gives the French guide values for concentrations of PAH and for the maximum cumulated input over a period of 10 years.

Table 2.10 : The French guide values for concentrations of PAH and for the maximum cumulated input over a period of 10 years [49].

Compound	Concentrations in sludge to be used in agriculture at a rate of no more than 30 tons/ha/10a (mg/kg DW)	Maximum permissible cumulated input on pasture soils per hectare in 10 years (g/ha DW)
Fluoranthene	4	60
Benzo(b)fluoranthene	4	60
Benzo(k)fluoranthene	4	60
Benzo(g,h,i)perylene	4	60
B[a]P	1.5	20
Indeno(1,2,3-cd)pyrene	4	60

In 1995, a working group of the Danish Ministry of Environment and Energy identified organic chemical residues, for which limit values should be elaborated [49, 67-69].

The PAH concentrations in sewage sludges in relation to the limits of the 3rd Draft of the EU-Initiative are as such:

UK: All samples above EU limit, even those WWTPs for which there was 0% trade effluent and purely rural, domestic wastewater [70].

Norway: The PAH content was low in most sewage sludge samples and well below the Swedish and Danish standards [71].

On the grounds that a soil limit value for B[a]P is set in respect to the pathway soil - plant, the introduction of a limit value for soil concentrations of B[a]P into the EU-Initiative is recommended. A regulation seems important, because there will be atmospheric deposition as well as introduction of B[a]P to agricultural soils via sludge application for years to come [49].

2.11 Treatment

Sewage sludges are residues generated at centralized wastewater treatment plants (WWTPs) as a result of the treatment of wastes released from a variety of sources including homes, industries, medical facilities, street runoff and businesses The use of these sludges as soil amendments is widely practiced in the U.S., where more than

60% of the 6.2 million dry metric tons (MT) of sludge produced annually are applied to land. Since 1991 when ocean dumping was banned, both the quantity produced and the percentage land-applied have increased [72-73].

Denmark, Luxembourg and Germany are expected to have the highest sewage sludge production per population equivalent. Germany, United Kingdom, France and Spain will probably still be the countries which use the highest amounts of sewage sludges in agriculture (> 500.000 t/a), with Ireland, Finland and United Kingdom reusing the highest percentage of their sludges in agriculture (> 70%). The population density in the EU in 1995 was given in Figure 2.5 [49].

Under the Clean Water Act, (CFR Section 405 (d)), the rules regarding the concentration of pollutants permitted in land-applied sewage sludges in the U.S. are mandated to be protective of human health and the environment [73].

(yellow: <50, red: >500 inhabitants per km^2)

Figure 2.5 : Population density in the EU in 1995 [49].

In Table 2.11 was given the area population and sewage sludge production of EU member states in the year 2005.

Table 2.11 : Area population and sewage sludge production in the year 2005 [49, 73].

	Area	Population		Sludge destination in 2005 (1000 t DM/a)			Relative sludge production
	(km^2)	Million	Density	Total	Reuse	%	kg/person/a
FIN	338 000	5.1	15	160	115	72	31
S	450 000	8.9	20	-	-	-	-
IRL	70 000	3.7	53	113	84	74	31
E	504 782	39.4	78	1088	589	54	28
GR	131 957	10.5	80	99	7	7	9
A	88 945	8.1	91	196	68	35	24
F	550 000	60.4	110	1172	765	65	19
P	92 072	10.8	117	359	108	30	33
DK	43 094	5.3	123	200	125	63	38
L	2 586	0.4	166	14	9	64	35
I	301 263	57.6	191	-	-	-	-
D	356 854	82	230	2786	1391	50	34
UK	242 500	58.6	242	1583	1118	71	27
B	30 158	10.2	338	160	47	29	16
NL	41 864	15.8	377	401	110	27	25

The chemical composition of municipal sewage sludge can vary greatly, depending on the composition of wastewater, and applied wastewater and sludge treatment processes. As sewage sludge sequesters hydrophobic compounds, concentrations of pollutants in this material reflect the flow of chemicals in a contemporary society [74-75]. Sources of pollutants in urban wastewater (UWW) that become subsequently enriched in sewage sludge. Before disposal or recycling, sludge is subject to undergo one or several treatment processes such as thickening, dewatering, stabilization, disinfection and thermal drying, in order to reduce water content, biodegradability and improve hygienic properties. Apart from the enrichment of above-mentioned constituents of agricultural value (organic matter, nitrogen, phosphorus, potassium, and to a lesser extent, calcium, magnesium and sulfur), sewage sludge is significantly enriched in organic pollutants, trace metals and pathogens. The EC study performed by ICON formulates the type and loads of both organic and metal pollutants in wastewater (sewage) treatment systems and consequently in sewage sludge as a complex function of:

a. size and type of conurbation (commercial, residential, mixed);

b. plumbing and heating systems;

c. domestic and commercial product formulation and use patterns;

d. dietary sources and feces;

e. atmospheric quality, deposition and run-off;

f. presence and type of industrial activities;

g. use of metals, and other materials in construction;

h. urban land use;

i. traffic type and density;

j. urban street cleaning;

k. maintenance practices, for collecting systems and storm water control;

l. accidental releases.

The pollutants that through the wastewater treatment process accumulate in sewage sludge, thus posing a potential risk to the environment, represent three major groups:

m. potentially toxic elements (PTEs) that include heavy metals: Cd, Cr(III) and Cr(VI), Cu, Hg, Ni, Pb, Zn, Ag, platinum group metals (PGMs) and metalloids (As, Se);

n. organic pollutants (PAHs, PCBs etc.);

o. pathogens [49, 74, 76].

PAHs are known for their adverse and cumulative effects at low concentration. In particular, the PAHs accumulate in sewage sludge during wastewater treatment, and may thereafter contaminate agricultural soils by spreading sludge on land. Therefore, sludge treatment processes constitute the unique opportunity of PAH removal before their release in the environment [77].

2.11.1 Wastewater treatment

During the wastewater treatment process (especially for activated sludge WWTPs), the fate of hydrophobic organic compounds (HOCs) is governed by their physico-chemical properties, the design and the operating conditions of the WWTP. The HOCs removal mechanisms that take place during the treatment process are advection, sorption, volatilization, air-stripping and biotransformation. Sorption on the particulate matter is the main removal mechanism that takes place in the primary sedimentation tank and is theoretically removing all HOCs. For very lipophilic chemicals, like PAHs, this removal should be quasi quantitative. Especially for

chemicals with similar lipophilicity (octanol-water partition coefficients, K_{OW}), like Ant and Phe (log K_{OW} of 4.54 and 4.46), the sorption should occur in the same extent [20, 78-80].

In Danish sludges the concentrations of PAHs (sum of 9 PAHs) were typically below 3 mg/kg (DM). It was reported that the concentrations of PAHs in UK sewage sludges in the range of 1-10mg PAH/kg, which is significantly higher than the normal range of concentrations found in agricultural soils. In the study of Norwegian sludges, it was founded PAH concentrations below the Swedish and Danish standards of 1997 in most samples. There were large monthly variations in most treatment plants and hence the authors suggest that one single sample is not sufficient to evaluate the level of toxic organics in sewage sludge [49, 71, 81].

2.11.2 The levels of total PAHs in wastewater treatment plants

PAHs reported as a serious problem in the world. Several researchers investigated the amount of PAHs in sewage sludge from WWTPs located different countries. The amount of total PAHs in different countries was shown in Table 2.12.

Table 2.12 : Total PAHs found in sewage sludge from WWTPs located different countries.

Countries	Number of Samples	Range (DW)	References
Several locations (China)	11	1.4-33 (mg/kg)	[19]
Area of Paris (France)	16	21 700-30 900 (ng/g)	[22]
Danish (1995) ?18PAH	20	< 0.01-8.5 (mg/kg)	[49]
Danish (1993-94) ? 18PAH	9	0.42-2.4 (mg/kg)	[49]
Norway	36	0.7-30 (mg/kg)	[49]
Parts of Germany ? 6PAH	124	0.4-12.83 (mg/kg)	[49]
Parts of Germany ? 16PAH	88	0.25-16.28 (mg/kg)	[49]
Upper Silesia (Poland)	17	300-40 000 (ng/g)	[82]
Several Locations (Canada)	18	1000-28 700 (ng/g)	[83]
Several Cities of Kuwait	16	1600-6200 (ng/g)	[84]
Fusina (Italy)	16	1200-1400 (ng/g)	[85]
Area of Thessaloniki (Greece)	13	7500 (ng/g)	[86]
Several locations (Catalonia, Spain)	16	2500-5500 (ng/g)	[87]
Jedez de la Frontera (Cadiz, Spain)	16	1900-6800 (ng/g)	[88]
UK	24	67 000-370 000 (ng/g)	[89]
Area of Madrid (Spain)	27	1800-6400 (ng/g)	[90]
Several locations (China)	12	33.73-87.50 (mg/kg)	[91]

2.11.3 The levels of B[a]A in wastewater treatment plants

Several researchers focused the amount of B[a]A in sewage sludge from WWTPs located different countries. The amounts of B[a]A in different countries sewage sludge and wastewater were given in Table 2.13. The highest values were from China and Mediterranean countries.

Table 2.13 : The B[a]A found in sewage sludge and wastewater from WWTPs located different countries.

Countries	B[a]A	References
China	0.013 – 8.3 mg/kg (dry sludge in 11 different WWTP)	[19]
Italy	3.3 – 51.2 ng/L (Municipal WW) 59 – 421 ng/L (SS / act. sludge)	[90]
China	0.18 - 4.82 mg/kg (dry sludge in 12 different WWTP)	[91]
Greece	50 ng/L (Municipal WW)	[92]
France	0.001 – 0.667 mg/kg (dry sediment)	[93]
Spain	0.157 mg/kg (WWTP1/May) 0.184 mg/kg (WWTP1/June) 0.08 mg/kg (WWTP1/July) 0.125 mg/kg (WWTP2) 0.101 mg/kg (WWTP3/May- Secondery Treatment) 0.096 mg/kg (WWTP3/June - Secondery Treatment) 0.064 mg/kg (WWTP3/July- Secondery Treatment) 0.155 mg/kg (WWTP4 - Secondery Treatment) 0.029 mg/kg (WWTP5 - Primary Treatment) 0.030 mg/kg (WWTP6 - Primary Treatment)	[94]
France	3.70 mg/kg (dry sludge)	[95]
Spain	0.17 mg/kg (February /Primary Treatment) 0.65 mg/kg (February /Secondary Treatment) nd. (February /digested-dehydrated sludge samples) 0.05 mg/kg (May /Primary Treatment) 0.06 mg/kg (May /Secondary Treatment) 0.06 mg/kg (May/digested-dehydrated sludge samples)	[96]
Spain	nd. – 0.170 mg/kg (dry sludge / Primary Treatment) nd. – 0.618 mg/kg (dry sludge / Secondary Treatment) nd. - 0.088 mg/kg (dry sludge/ digested-dehydrated sludge samples) nd. -1.115 mg/kg (dry sludge/ compost)	[97]
USA	nd – 99 mg/kg (dry sludge /literature)	[98]
Umman	39 ng/L (influent /WWTP1) nd. (effluent /WWTP1) nd. (stream /WWTP1) 0.002 mg/kg (dry sludge /WWTP1) 51 ng/L (influent /WWTP2) 19 ng/L (effluent /WWTP2) nd. (stream /WWTP2) nd. (sludge/WWTP2) 10 ng/L (influent /WWTP3) 7 ng/L (stream /WWTP3) 0.0007 mg/kg (dry sludge /WWTP3)	[99]
Spain	0.157 mg/kg (dry sludge - May/WWTP1) 0.184 mg/kg (dry sludge - June/WWTP1) 0.096 mg/kg (dry sludge - May/WWTP2) 0.064 mg/kg (dry sludge - June/WWTP2)	[100]
Morocco (2008)	0.0062 mg/kg (dry sludge)	[101]

nd. Not detected

2.12 Treatment Alternatives of PAHs

2.12.1 Composting

Soils and sediments can be treated for PAH contamination both by in situ and ex situ methods. Landfarming is an in situ treatment for soils, which focuses upon stimulating

the indigenous microorganisms in the soil by providing nutrients, water and oxygen. For example, a pilot-scale landfarming treatment of PAH-contaminated soil from a wood-treatment facility was achieved by inoculum of *Pseudomonas aeruginosa* (strain 64). Aeration was provided by tilling of the soil. The workers found that 86% of total PAHs were removed from the soil over 1 year, including a reduction in high molecular weight PAHs such as B[a]A and B[a]P (79.5% and 11.3% respectively). Biopiling of soil and the treatment of soil in bioreactors are ex-situ treatments that are less cost effective than in situ treatment, however ex situ treatment benefits from being more subject to monitoring and control [49, 102-104].

Composting, which is also an ex-situ treatment for PAH-contaminated soil, is an aerobic process whereby microorganisms degrade organic materials which results in thermogenesis and the generation of organic and inorganic compounds. Composting in bioreactors is a popular option because it is possible to exert greater control over parameters such as temperature and oxygen supply during the composting process and a variety of organic materials as bulking agents can be used. However, it is often not feasible to remediate contaminated groundwater ex situ due to the costs involved with abstraction and shipping of the contaminated water, and the fact that much of the contamination will be sorbed within the aquifer [49, 105].

2.12.2 Enricher

Full secondary treatment to control trace amounts of PAHs and other potentially hazardous compounds that may be contained in the combined domestic /industrial wastewater, as opposed to conventional pollutants such as oxygen- demanding materials and suspended solids. Therefore, it is proposed that existing biological treatment facilities be reevaluated as a treatment technology for hazardous wastewater [106].

A modification of the activated sludge process, called an enricher reactor system, was evaluated for its ability to enhance treatment of hazardous wastewater. Enricher reactors were used to produce biomass to biodegrade a specific compound or class of compounds. This biomass was then used to inoculate or seed continuous-flow reactors, which are typical of many existing activated sludge plants. The goal of inoculation or bioaugmentation was to produce enhanced biodegradation in the continuous-flow reactors. The term enricher is used, as opposed to enrichment,

because the enricher reactor is used to modify or enrich the microbial culture in another reactor, the continuous-flow reactor (for example, activated sludge process) [106].

2.12.3 Bioremediation

Bioremediation, which is also referred to as bioreclamation and biorestoration, can be described as 'the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state' [49, 107]. Bioremediation seems to be the most promising method for the removal of these toxic pollutants using microbial populations [14]. The main principle of this technique is to remove pollutants from the natural environment and/or convert the pollutants to a less harmful product using the indigenous microbiological community of the contaminated environment [49]. In any of these applications, bioremediation is complicated by two factors. First is the need to biodegrade a complex mixture of contaminants with varying ring number and molecular weight. For example, sediment contamination from manufactured gas plant site soils typically include 2-, 3-, 4-, and 5-ringed PAHs (along with non-aromatic hydrocarbons). Second, contaminants must be made available to the degrading consortia [108].

Bioremediation strategies are developed to promote the microbial metabolism of contaminants, by adjusting the water, air and nutrient supply. This is accomplished by the biostimulation (the addition of a bulking agent such as wood chips and/or nutrients such as N/P/K) and bioaugmentation (often an inoculum of microorganisms with known pollutant transformation abilities) of the contaminated environment Bioremediation of PAH-contaminated soils, sediments, and water can be accomplished in a variety of ways, e.g.in situ treatment or ex-situ methods such as biopiling and composting [49].

Waste can also be treated in bioreactors, though this can be more costly than in situ technologies. It is important for bioremediation to be comparable in cost and success to physical and chemical treatments of contaminated land, such as landfilling, incineration, and beach-surface bioremediation of oil spills, and soil washing. The applicability of bioremediation can be variable, but this is generally due to unfavorable site, therefore a thorough understanding of site conditions will allow optimization of bioremediation and subsequently more effective results. In commercial

situations, bioremediation of PAH contaminated soils is not typically carried out when the site contains significant amounts of PAHs that have more than four rings as the low percentage removal of PAHs of this molecular weight and the time taken for successful reduction in PAH concentrations is not economically viable [49]. Bioremediation has been shown to be effective in remediating soils contaminated with low molecular weight PAHs [24, 109-111]. Bioremediation using naturally occurring microorganisms was a major mechanism of removing oil from the Exxon Valdez oil spill in Prince William Sound, Alaska [47]. The method used is normally nutrient addition and aeration by frequent turning of contaminated soil. Total PAH levels during a bioremediation trial are generally reduced from approximately 3000 mg to 1000 mg total PAHs, per kg [61].

2.12.4 Extracellular enzyme addition

PAHs treatment could be based on the use of white rot fungi, which are known to degrade a great variety of complex compounds due to their complex enzymatic system [112]. Lignin Peroxidase (LiP) and Manganese Peroxidase (MnP) are extracellular peroxidases produced by white rot fungi and the onset of their production is associated to secondary metabolism conditions in response to nutrient depletion. In particular, manganese and nutrient nitrogen have been shown to have strong regulating effects [113-114]. The ligninolytic system is nonselective, consequently other aromatic substrates, such as PAHs are potentially oxidised and biodegraded by white rot fungi. The catalytic action of these enzymes generates more polar and water-soluble metabolites, such as quinones, which are more susceptible to further degradation by indigenous bacteria present in soils and sediments. The potential of using more complex enzymes such as ligninolytic enzymes produced by white rot fungi, which require specific environmental conditions for the activation of their catalytic cycle [115-117].

2.13 Degradation of PAHs

PAHs are considered among the most difficult POP to be treated because of their highly stable physical– chemical characteristics and frequent occurrences [118-119]. Low molecular weight PAHs are removed from the water column by evaporation, microbial oxidation, and sedimentation. Higher molecular weight PAH, including

B[a]P, are removed primarily by photooxidation and sedimentation [51]. Different approaches have been studied for the removal of PAHs from contaminated soils or solid wastes in the past few years: biodegradation, chemical and photodegradation; moreover, some extraction methods using surfactants have been successfully applied for remediation of PAH-contaminated soils or sediments (Figure 2.6) [16, 119-125]. Several environmental factors are known to influence the capacity of indigenous microbial populations to degrade PAHs. The interactions among environmental factors such as temperature, pH, soil gas oxygen concentrations, oxidation reduction potential and the presence of other substrates often control the feasibility of biodegradation [45, 126]. These processes may also be utilized for remedial purposes. However, the degradation may result in a variety of transformation products some of which could potentially accumulate [12].

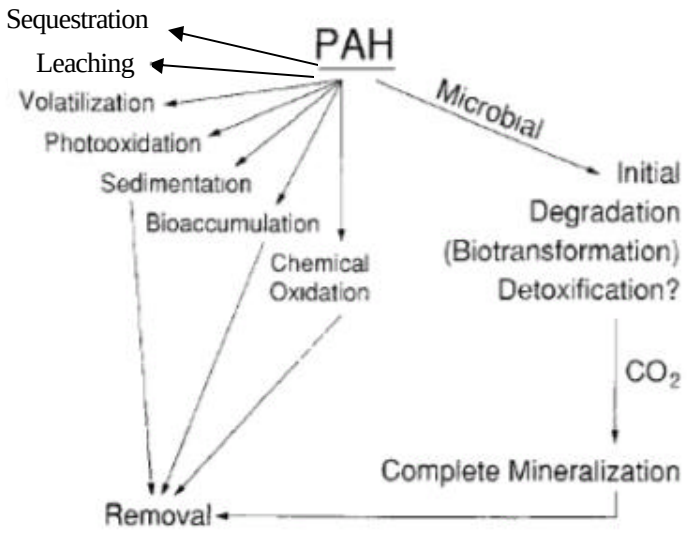


Figure 2.6 : Schematic representation of the environmental fate of PAHs [16, 24].

There are also a few studies for PAH removal form sludge: anaerobic PAH degradation in sludge, biodegradation in aerobic processes treating urban sludge [102, 127].

2.13.1 Biological degradation

Microorganisms, such as bacteria and fungi, may transform the PAHs to other organic compounds or to inorganic end products such as carbon dioxide and water. The latter process has been referred to as mineralization. Some PAH-degrading microorganisms, primarily bacteria, are capable to use the PAHs as a carbon and

energy source, and may thus transform the contaminants into molecules that can enter the organisms' central metabolic pathways. Other microorganisms have the capacity to degrade PAHs, while living on a widely available substrate. Such cometabolism does not always result in growth of the microorganism, and sometime the cosubstrate, i.e. the PAH, is only transformed into another compound without any apparent benefit for the organism. This may lead to partial degradation, if no enzyme capable of transforming the metabolite is available. For PAHs, the contribution of the cometabolic degradation processes increases as the number of rings in the PAH-molecule increases, since far fewer microorganisms are capable of using the HMW PAHs as carbon and energy sources [12, 128-131].

2.13.2 Chemical degradation

PAHs in soil are also degraded through abiotic processes. Oxidation reactions are the most important in this context, although photochemical reactions may contribute significantly to the degradation on the surface of soils. In addition, most of the oxidants that commonly initiate the oxidation reactions in the environment, i.e. singlet oxygen ($^1O_2$), organic peroxides, hydrogen peroxide, ozone and radicals such as alkoxy radicals (RO•), peroxy radicals (RO_2•) and hydroxyl radicals (HO•), are directly or indirectly generated through photochemical processes. However, some can also be produced from inorganic salts and oxides, especially those of iron and manganese. Chemical oxidation reactions involving hydroxyl radicals, generated from hydrogen peroxide, and ozone, have been most widely studied. Hydroxyl radicals are strong, relatively unspecific oxidants that react with aromatic compounds, such as PAHs, at near diffusion-controlled rates (i.e., $k_{OH} > 10^9$ $M^{-1}s^{-1}$)by abstracting hydrogen atoms or by addition to double bonds. The ozone molecule may attack double bonds directly, but it can also form reactive hydroxyl radicals by decomposing water. The reaction pathways that follow are very complex, and numerous intermediates are formed. The final reaction products include, for both oxidants, a mixture of ketones, quinones, aldehydes, phenols and carboxylic acids [12, 132-143]. Photochemical degradation of PAHs often involves the same oxidative species that are produced during the pure chemical oxidation of PAHs, i.e. oxygen, hydroxyl radicals and other radicals. Consequently, the reaction products include similar complex mixtures of ketones, quinones, aldehydes, phenols and carboxylic acids [12, 132, 141, 144-146].

2.13.3 Reactivity

During chemical reactions, PAHs are largely transformed into other PACs, i.e. they do not lose their aromatic character. The aromaticity is conserved since the non-aromatic hydrocarbons are much richer in energy. Thus, considerable amounts of energy are required to change an aromatic compound into a non-aromatic. The electron distribution over the PAH molecule determines the positions of the molecule that are most reactive. For addition reactions, Whelands's concept of localization energy has proven useful. The localization energy (Lu) is the energy required to isolate a p-electron at the center of u of a PAH from the remaining p-system. In this process, the attacking species may be a nucleophile, an electrophile or a radical. A number of reactivity indices have been developed that reflect the localization energy, one of which is Dewar's reactivity number, Nu. The magnitude of Nu reflects the localization energy and the reactivity at a certain position of an aromatic compound. The smaller the value of Nu the lower the activation energy and the greater the reaction rate of an addition at the center of u. Nu values for a number of aromatic compounds are given in Figure 2.7. The values shown in this figure indicate that benzene should be the least reactive species. For naphthalene, higher reactivity is expected for the 1-position than the 2-position. Similarly, anthracene and phenanthrene should preferably react at the 9,10-positions, although the reactivity of phenanthrene should be markedly lower than that of anthracene. Thus, the localization energy concept may be helpful in clarifying the mechanisms of PAH-degradation and PAH-derivative formation [12].

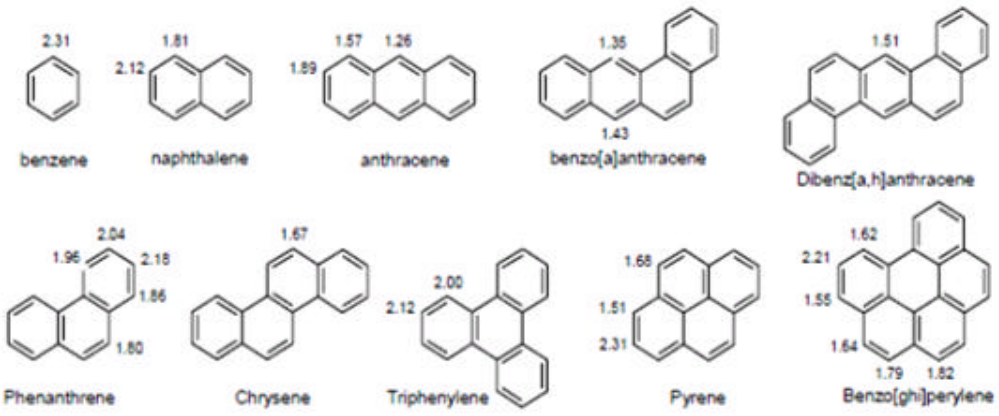


Figure 2.7 : Electron localization energies for benzene and selected 16 US-EPA PAHs [12].

2.13.4 Transformation products

The ultimate result of a degradation process is total mineralization of the organic contaminants, resulting in carbon dioxide, water and other inorganic compounds. However, as shown above for PAHs, both biological and chemical degradation processes may produce a variety of other compounds that may include temporary intermediates and compounds that are resistant to further degradation. Such dead-end products could potentially accumulate during a remedial process if no mechanism is available for their further degradation. This may happen if, for instance, the original contaminant has been transformed co-metabolically, and no microorganisms is present that could metabolize the transformation product, or if a chemical oxidizing agent has been added in insufficient quantity or strength. From the literature and pathways described above, the dead-end products appear to be primarily carbonyl compounds, such as ketones, quinones, dicarboxylic acid anhydrides and coumarins, while carboxylic acids and phenols seems to be more easily degraded or transformed. The higher stability of the carbonyl compounds is also indicated by the fact that these compounds are found more frequently than the hydroxylated and carboxylated compounds in environmental samples, including contaminated soil. This could be due in some cases to restriction in the analytical methods used, since the hydroxylated and carboxylated compounds are more polar and more difficult to analyze. However, similar results have been obtained with methods carefully developed for all these classes of compounds. Consequently, the studies this thesis is based upon focused on the carbonylic PAH-derivatives, which will be referred to as oxygenated PAHs (oxy-PAHs) in the following sections. Another motive for studying the oxy-PAHs is that many of these compounds are toxic and mutagenic. Some are even more toxic than their parent PAHs. For example, anthracene-9,10-dione and phenanthrene-9,10-dione have been shown to inhibit the growth of duckweed, *Lemna gibba*, and the marine bacterium *Photobacterium phosphoreum*. Furthermore, 7H-benz[de]anthracene-7-one, 4-oxapyren-5-one and several benzofluorenones, B[a]P quinones and pyrene quinones have all shown mutagenic activities in biological assays, such as the Ames test. In contrast to the unsubstituted PAHs some of these oxy-PAHs do not require metabolic activation to exhibit their mutagenic properties, but may react with DNA directly. Further, oxy-PAHs have also been identified as the predominant class of compounds in the most mutagenic

fractions obtained during bioassay-based chemical fractionation of various environmental samples [12, 86, 146-159].

2.14 Biodegradation of Xenobiotics

Many xenobiotic compounds simply pass through wastewater treatment systems without significant reduction due to intrinsic limitations. The limitations include lack of cell uptake, lack of proper enzymes initiating attack to these compounds, and thermodynamically unfavorable reactions. Hence, acclimation of microorganisms to the compounds is essential. Acclimation of microbial community involves selection of microorganisms containing existing enzymes and pathways or development of new catabolic pathways for the removal of xenobiotics [3].

When the capacity of catabolic metabolism is considered, the dominance of a particular strain degrading the pollution can be observed rarely. Thus, uneven fraction of the biomass has a potential to degrade a xenobiotic. The degradation capacity changes depended on the degree of induction of catabolic enzymes that enable microorganisms to degrade these compounds. However, the degree of induction is not known. Thus, the kinetic measurements in mixed cultures are based on "black box" approach. Kinetic parameters are selected for the total biomass not for its active part. In case of substrate mixture utilization, the cells grow not only with single carbon source leading to high probability of partially induced catabolic enzymes [3, 160].

2.15 Biodegradation of PAHs

PAHs biodegradation may occur both aerobically and anaerobically [8, 77]. The metabolism of PAHs by pure cultures of microorganisms and cometabolic transformations by mixed microbial communities have been studied for almost 80 years, however little information is avaible for mixed cultures. Biodegradation mechanisms, both procaryotic and eucaryotic, require the presence of molecular oxygen to initiate enzymatic attack on the PAH rings [16].

The biological treatment of soils contaminated with PAH should be an efficient, economic, and versatile alternative to physicochemical treatment, because it offers potential advantages such as the complete destruction of the pollutants, lower

treatment cost, greater safety, and less environmental disturbance [161]. It is well established that many individual PAHs are degraded by bacteria [16, 24, 108, 141, 162-165]. The list of some PAHs degrading bacteria were given in Table 2.14.

Table 2.14 : PAH degrading bacteria [24].

PAH	Organism
Naphthlene	*Acinetobacter calcoaceticus, Alcaligenes denitrificans, Mycobacterium sp., Pseudomonas sp., P. putida, P. fluorescens, Sp. paucimobilis, Brevundimonas vesicularis, Burkholderia cepacia, Comamonas testosteroni, Rhodococcus sp., Corynebacterium renale, Moraxella sp., Streptomyces sp., B. cereus, P. marginalis, P. stutzeri, P. saccharophila, Neptunomonas naphthovorans, Cycloclasticus sp.*
Acenaphthene	*Beijernickia sp., P. putida, P. fluorescens, Bu. cepacia, Pseudomonas sp., Cycloclasticus sp., Neptunomonas naphthovorans, Alcaligenes eutrophus, Alcaligenes paradoxus*
Phenanthrene	*Aeromonas sp., A. faecalis, A. denitrificans, Arthrobacter polychromogenes, Beijernickia sp., Micrococcus sp., Mycobacterium sp., P. putida, Sp. paucimobilis, Rhodococcus sp., Vibrio sp., Nocardia sp., Flavobacterium sp., Streptomyces sp., S. griseus, Acinetobacter sp., P. aeruginosa, P. stutzeri, P. saccharophila, Stenotrophomonas maltophilia, Cycloclasticus sp., P. fluorescens, Acinetobacter calcoaceticus, Acidovorax dela®eldii, Gordona sp., Sphingomonas sp., Comamonas testosteroni, Cycloclasticus pugetii, Sp. yanoikuyae, Agrobacterium sp., Bacillus sp., Burkholderia sp., Sphingomonas sp., Pseudomonas sp., Rhodotorula glutinis, Nocardioides sp., Flavobacterium gondwanense, Halomonas meridiana*
Anthracene	*Beijernickia sp., Mycobacterium sp., P. putida, Sp. paucimobilis, Bu. cepacia, Rhodococcus sp., Flavobacterium sp., Arthrobacter sp., P. marginalis, Cycloclasticus sp., P. fluorescens, Sp. yanoikuyae, Acinetobacter calcoaceticus, Gordona sp., Sphingomonas sp., Comamonas testosteroni, Cycloclasticus pugetii*
Fluoranthene	*A. denitrificans, Mycobacterium sp., P. putida, Sp. paucimobilis, Bu. cepacia, Rhodococcus sp., Pseudomonas sp., Stenotrophomonas maltophilia, Acinetobacter calcoaceticus, Acidovorax delafieldii, Gordona sp., Sphingomonas sp., P. saccharophilia, Pasteurella sp.*
Pyrene	*A. denitrificans, Mycobacterium sp., Rhodococcus sp., Sp. paucimobilis, Stenotrophomonas maltophilia, Acinetobacter calcoaceticus, Gordona sp., Sphingomonas sp., P. putida, Bu cepacia, P. saccharophilia*
Chyrsene	*Rhodococcus sp., P. marginalis, Sp. paucimobilis, Stenotrophomonas maltophilia, Acinetobacter calcoaceticus, Agrobacterium sp., Bacillus sp., Burkholderia sp., Sphingomonas sp., Pseudomonas sp., P. saccharophilia*
B[a]A	*A. denitrificans, Beijernickia sp., P. putida, Sp. paucimobilis,Stenotrophomonas maltophilia, Agrobacterium sp., Bacillus sp., Burkholderia sp., Sphingomonas sp., Pseudomonas sp., P. saccharophilia*
Dibenz[a,h]anthracene	*Sp. paucimobilis, Stenotrophomonas maltophilia*

Microorganisms were found to degrade PAHs via either metabolism or cometabolism. Cometabolism is important for the degradation of mixtures of PAHs and high-molecular-weight PAHs. In contrast, several PAHs, especially 2- to 4-ring PAHs, have been known for years to be growth substrates for bacteria. Although the metabolism of PAHs by a bacterial pure culture in anaerobic conditions has been reported, most attention has been paid to metabolism of PAHs by aerobic bacteria, and various bacteria capable of utilizing PAHs have been investigated. The biochemical and biotechnological studies of this subject have been reviewed. The common biochemical pathways for the bacterial metabolism of PAHs have been well investigated [161].

PAHs are a carbon and energy source [108]. However it has been reported, three *Burkholderia cepacia* strains isolated from soil grew on pyrene at concentrations of up to 1000 mg/L and also degraded fluoranthene and B[a]A as sole carbon and energy sources. Pseudomonas organism, strain HL7b and was reported to degrade fluoranthene, but not as a sole carbon and energy source [131].

2.15.1 Microbial metabolism

The basis of these mechanisms is the oxidation of the aromatic ring, followed by the systematic breakdown of the compound to PAH metabolites and/or carbon dioxide. Anaerobic metabolism of PAHs is thought to occur via the hydrogenation of the aromatic ring. The principal mechanism for the aerobic bacterial metabolism of PAHs is the initial oxidation of the benzene ring by the action of dioxygenase enzymes to form cis-dihydrodiols. These dihydrodiols are dehydrogenated to form dihydroxylated intermediates, which can then be further metabolised via catechols to carbon dioxide and water [12, 24, 50].

Bacteria trigger the initial oxidation by incorporating both the atoms of molecular oxygen catalyzed by a dioxygenase [46]. The dioxygenase that catalyses these initial reactions is a multicomponent enzyme system. The initial ring oxidation is usually the rate-limiting step in the biodegradation reaction of PAHs. cis-Dihydrodiols are rearomatised through a cw-dihydrodiol dehydrogenase to yield a dihydroxylated derivative. Further oxidation of the cw-dihydrodiols leads to the formation of catechols, which are substrates for other dioxygenases that bring about enzymatic cleavage of the aromatic ring. Catechol can be oxidised via two pathways. The ortho pathway involves cleavage of the bond between carbon atoms of the two hydroxyl groups to yield cis,cis-muconic acid. On the other hand, the meta pathway involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent carbon atom with a hydroxyl group (Figure 2.8) [16, 24, 44, 128, 166].

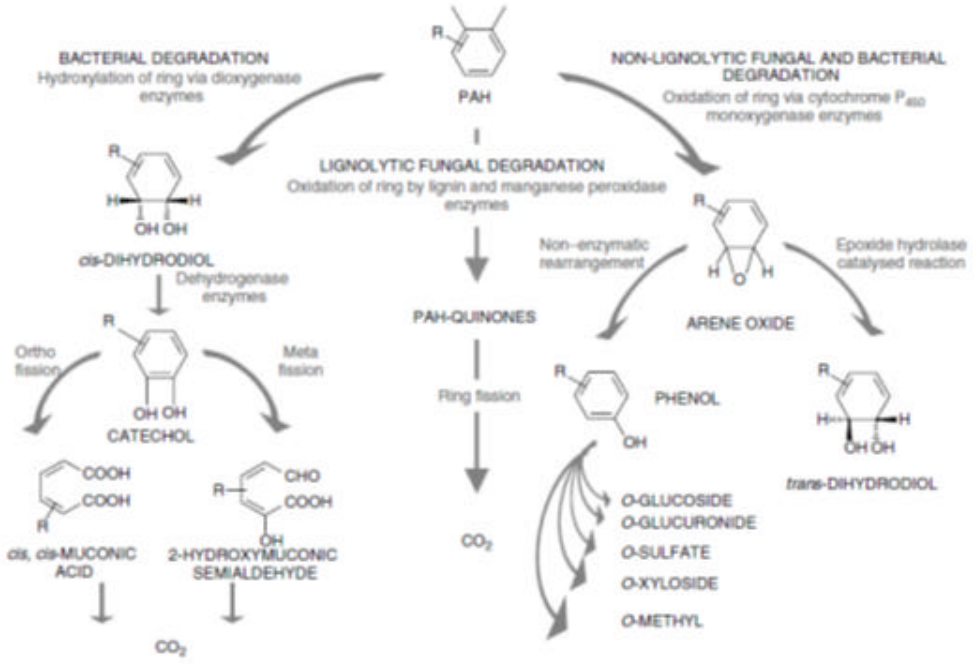


Figure 2.8 : The three main pathways for PAH degradation by fungi and bacteria [12, 16, 24, 50].

Hydroxylation of benzenoid aromatic compounds always involves the incorporation of molecular oxygen. Procaryotic microorganisms utilize dioxygenase enzymes to incorporate both atoms of molecular oxygen into the aromatic nucleus to form cis-dihydrodis. cis-dihydrodiolsare stereoselectively dehydrogenated by cis-dihydrodiol dehydrogenases, which rearomatize the benzene nucleus to form dihydroxylated intermediates. Subsequent enzymatic fission of the aromatic ring by procaryotic organisms is also catalyzed by highly regio- and stereoselective dioxygenases. As a prerequisite for this reaction, the aromatic ring must contain two hydroxyl groups placed either ortho or para to each other. If the hydroxyl moieties are ortho to each other, oxygenolytic ring cleavage can occur either between the two hydroxyl groups, by ortho or intradiol cleaving dioxygenases, or adjacent to the two hydroxyl groups, by metaor extradiol cleaving dioxygenases [16]. Ring cleavage results in the production of succinic, fumaric, pyruvic and acetic acids and aldehydes, all of which are utilized by the microorganism for the synthesis of cellular constituents and energy. A by-product of these reactions is the production of carbon dioxide and water [24].

2.15.2 The effecting factors of PAHs

Microbial degradation of PAHs in aquatic and terrestrial ecosystems is influenced strongly by a wide variety of abiotic and biotic factors which include: temperature, pH, soil type, aeration, nutrients, depth, diffusion, microbial adaptations, bioavailability, previous chemical exposure, water availability, sediment toxicity, physico-chemical properties of the PAH, concentration of the PAH and seasonal factors [16, 50, 51, 166].

2.15.3 Chemotaxis

To enhance the degradation of toxic compounds in the environment, different strategies can be considered with chemotaxis as one of the potential means to achieve this goal. From the biodegradation aspect in natural environment, microorganisms that have degradation capability and also show chemotaxis towards a compound would be more efficient for bioremediation than non-chemotactic microorganisms. Although chemotaxis is a phenomenon that has been known for some time (and there are several reports in the literature regarding chemotaxis of *E. coli*, *Salmonella*, *P. aeruginosa*, *P. putida*, *Bacillus cereus*, *Myxococcus sp.*, *Rhizobium sp.* and *Azospirillum sp.*), it is a complex process in which bacterial cells detect temporal changes in the concentrations of specific chemicals, respond behaviourally to these changes and then adapt to the new concentration of the chemical stimuli. Chemotaxis can be positive (i.e. the microorganism migrate towards the compound) or negative (i.e. microorganisms swim away from the compound). Both cases require concentration gradients of the attractant or repellent for a chemotactic response to occur [47, 167].

2.15.4 Interactions in PAH mixtures

A complication in evaluating the biodegradation kinetics of PAHs is the possibility of substrate interactions. Within a mixture of PAHs, the substrate interactions include negative effects, which involve inhibition and/or diauxic effects; positive effects include enhancement and cometabolism; or no effect at all. To understand the interactions that take place in mixtures, positive and negative effects that occur within the mixtures must be evaluated. Since the underlying goal of biodegradation is to reduce the concentration of the contaminants to an acceptable level, enhanced

degradation would be preferred. The interactions not only indicate the effects that occur for PAHs, but also reveal underlying mechanisms of enzymatic activity involved in the transformation of different compounds in a mixture. The negative effects are due to competitive inhibition of multiple substrates or other means of retarding the degradation of one substrate in the presence of another. Competitive inhibition lowers the affinity of the enzyme. In competitive inhibition, multiple substrates are transformed by a common enzyme system. Similar or identical enzyme systems may catalyze the degradation of compound(s), which may be structurally similar. Multisubstrate competitive inhibition captures the effect of two converse processes; enhanced degradation as result of an augmentation in the biomass population and retarding rates of degradation as a result of competition for the substrate. In 1995, it was demonstrated that competitive metabolism between naphthalene and phenanthrene where phenanthrene degradation was inhibited by naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, and fluorene. The study further suggested that competitive metabolism may be commonly encountered among PAH-degrading organisms. Positive effects result in the enhanced degradation of the substrate as a result of proliferation of the biomass growth on multiple substrates and enzyme induction. The presence of a suitable substrate affects the fortuitous degradation of other PAHs. Phenanthrene degradation by several strains decreased due to lack of preexposure to other PAH compounds. However, the responses to PAH induction are strain specific and cannot be extended for all environmental media. It was observed enhanced degradation of PAHs as a result of pre-exposure to other aromatic hydrocarbons. In previous studies, naphthalene and phenanthrene enhanced the degradation of each of the other PAHs through cross acclimation. However, the presence of simultaneously occurring PAHs did not impact the degradation of individual PAHs. It may be reasonable to conclude that the interactions and effects encountered in a multisubstrate system are a function of the microbial community, the type of culture (mixed versus pure) and the physiological state of the community at the time of the experiment [44, 92, 168-174].

2.16 Activated Sludge Modelling (ASM)

In 1982 the International Association on Water Pollution Research and Control (IAWPRC), as it was then called, established a Task Group on Mathematical Modelling for Design and Operation of Activated Sludge Processes. At that time modelling of activated sludge processes had been a discipline for about 15 years [175]. Purpose of activated sludge modelling can be stated as to design, control, organize treatment plants, and optimize operational conditions. With respect to intended use of models such as design and control, structure of them differs. Although models are useful tools, which are simplifying the complicated processes, they are never true. This is because they are based on assumptions, depended on wastewater characterization in addition to the lack of knowledge in the microbiology of treatment plants [3, 176].

In wastewater treatment plant design, deterministic models are used. However, for controlling purposes black-box type models are also used [176]. It is commonly agreed that design and operation of treatment plants are based on reliable experimental data, mechanistic description of kinetic processes and material balances. Multi-component modelling of activated sludge is a common approach which reaction kinetics is evaluated by means of multiple parameters. In this approach, defined COD fractions are useful for understanding particulate matter, its fractions, kinetic and stoichiometric coefficients which are responsible for biodegradation leading to better understanding of biological treatment [3, 177, 178].

2.16.1 ASM 1

Most of the models are based on the IAWQ Activated Sludge Model No.1, which is called ASM 1 [177]. The ASM 1 was well received and has been widely used as a basis for further model development. The direct use of the ASM1 for modelling has been almost nil, but ASM 1 has been the core of numerous models with a number of supplementary details added in almost every case. It was especially the matrix notation, which was introduced together with ASM 1 that facilitated the communication of complex models and allowed the concentration of discussions on essential aspects of biokinetic modelling [175].

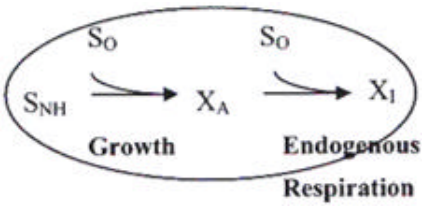
2.16.2 ASM 2

At the time of publication of the ASM 1, biological phosphorus removal was already being used in a (limited number) of full-scale treatment plants. The theoretical status of the processes was such that the Task Group at that time did not enter into the modelling of it. Nevertheless, from the mid- 1980s to the mid- 1990s the biological phosphorus removal processes grew very popular and at the same time the understanding of the basic phenomena of the process was increasing. Thus in 1995 the Activated Sludge Model No. 2 was published. This model included nitrogen removal and biological phosphorus removal. In 1994, when the ASM 2 was finished, the role of denitrification in relation to biological phosphorus removal was still unclear, so it was decided not to include that element. However, the development in research was fast, and denitrifying PAOs (phosphorus accumulating organisms) were needed for simulation of many results from research and practice. Because of this, the ASM 2 model was expanded in 1999 into the ASM 2d model, where denitrifying PAOs were included. Although the models might not have been heavily needed for nitrogen removal processes, the complexity of the combined nitrogen and phosphorus removal processes makes the models important for design and control purposes [3].

2.16.3 ASM 3

The models have grown more complex over the years, from ASM 1, including nitrogen removal processes, to ASM 2, including biological phosphorus removal processes and to ASM 2d including denitrifying PAOs. In 1998 the Task Group decided to develop a new modelling platform, the ASM 3, in order to create a tool for use in the next generation of activated sludge models. The ASM 3 is based on recent developments in the understanding of the activated sludge processes, among which are the possibilities of following internal storage compounds, which have an important role in the metabolism of the organisms [175]. The basic processes that are involved in ASM 3 is shown in Figure 2.9. The substrate flow is given as storage, growth, and maintenance [3].

Nitrifiers



Heterotrophs

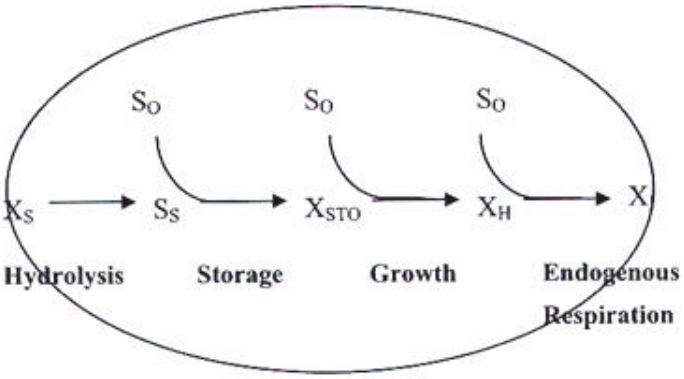


Figure 2.9 : Processes for heterotrophic organisms in ASM 3 [3].

According to metabolic model of storage compounds, PHB, acetate is taken by microorganism and it is converted into acetyl-CoA. Acetyl-CoA is used for biosynthesis, as energy source and in the storing processes. Famine conditions forces biomass to hydrolyze acetyl-CoA. On the other hand, glucose is taken up and used for the production of Glucose-6-phosphate. It is converted into glycogen and used for biomass synthesis. Glucose-6-phosphate is also an intermediate in catabolic reactions. When the external substrate is over, glycogen is used for the synthesis of Glucose-6-phosphate. Aerobic storage of readily biodegradable substrate is the main process that differs from ASM 1. Under transient loading, heterotrophic bacteria can store organic matter, S_S in the form of polyhydroxyalkanoate (PHA). S_S is first stored in the biomass and converted to internal storage polymers in an energy requiring process. The storage products X_{STO} are used in aerobic heterotrophic growth process when there is not external substrate in the environment. In aerobic endogenous respiration process, all forms of biomass loss are involved including decay, endogenous respiration, lysis, predation motility etc. The respiration process of storage products is the other main process that is similar to endogenous respiration in ASM 1 that emphasize both storage products and biomass decay. In ASM 3, conversion of X_S to S_S is also involved. Moreover, description of hydrolysis is the same that expresses the same surface reaction kinetics. In ASM 3 substrate

consumption rate is given as follows. k_{STO} is maximum rate of storage [M COD.(M Cell COD. T)$^{-1}$]:

$$\frac{dS_S}{dt} = k_{STO} \frac{S_S}{K_S + S_S} X_H \quad \textbf{(2.1)}$$

The storage product formation rate is given in equation 2.1 where Y_{STO} is storage yield [M COD.(M COD. T)$^{-1}$]. Y_{STO} reflects the stoichiometric amount of substrate converted into storage products followed by utilization for growth:

$$\frac{dX_{STO}}{dt} = Y_{STO}\, k_{STO} \frac{S_S}{K_S S_S} X_H \quad \textbf{(2.2)}$$

Growth of biomass under both feast and famine conditions is described depended on storage polymer concentration and half saturation constant of storage respectively:

X_{STO} [M COD L^{-3}], and K_{STO} [M COD (M COD^{-1})]

$$\frac{dX_H}{dt} = \mu_H \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H \quad \textbf{(2.3)}$$

Decay rate of storage products is given depended on heterotrophic yield:

$$\frac{dX_{STO}}{dt} = \frac{\mu_H}{Y_H K_{STO}} \frac{X_{STO}/X_H}{1 + X_{STO}/X_H} X_H \quad \textbf{(2.4)}$$

The process of endogenous decay is given as a function of endogenous decay rate of heterotrophs, b_H and X_H :

$$\frac{dX_H}{dt} = b_H X_H \quad \textbf{(2.5)}$$

Respiration of storage products is a function of endogenous respiration rate of storage products, b_{STO} and X_{STO} [T^{-1}] [3]:

$$\frac{dX_{STO}}{dt} = b_{STO} X_{STO} \quad \textbf{(2.6)}$$

The simplified matrix representation of ASM 3 is given in Table 2.15.

Table 2.15 : Simplified matrix representation of ASM 3 for organic carbon removal [3].

Component→	1	2	3	4	5	6	7	Process Rate
Process↓	S_0O_2	S_S	S_I	X_S	X_I	X_H	X_{STO}	$ML^{-3}T^{-1}$
Hydrolysis		$(1-f_{SI})$	$-f_{SI}$	-1				$k_h \frac{X_S/X_H}{(K_X+X_S/X_H)} X_H$
Storage of S_S	$-(1-Y_{STO})$	-1					Y_{STO}	$k_{STO} \frac{S_S}{K_{STO}+S_S} X_H$
Growth on X_{STO}	$-\frac{(1-Y_H)}{Y_H}$					1	$-\frac{1}{Y_H}$	$\tilde{\mu}_H \frac{X_{STO}/X_H}{(K_{STO}+X_{STO}/X_H)} X_H$
Endogenous Respiration	$-(1-f_I)$				f_I	-1		$b_H X_H$
Respiration of X_{STO}	-1						-1	$b_{STO} X_{STO}$
Parameter, ML^{-3}	O_2	COD	COD	COD	COD	Cell COD	COD	

3. MATERIAL & METHOD

3.1 Reactor Operation

Activated sludge was taken from Pasaköy Wastewater Treatment Plant and was fed with OECD (Table 3.1) solution having 700 mgCOD/L. The Fill&Draw (F&D) reactor was used, which had a working volume of 15 L. In addition, SBR, which had a working volume 13 L operated with 500 mgCOD/L OECD solution. All other macro and micronutrients were added in sufficient quantities for biological growth. The peptone mixture for the F&D consist peptone water (Acumedia 7365) and Merck meat extract (1.03979.0500), for the SBR peptone G (Acumedia 7182) and Acumedia meat extract (7228). The temperatures of systems were kept constant at 20 °C. Dissolved oxygen concentration in the reactors was also kept at minimum of 3 mg/L. The reactors were operated at a sludge age of 10 days and a hydraulic retention time of one day for F&D reactor. The systems were operated until steady state was reached. After that the effect of B[a]A on activated sludge was investigated. During the experiments sufficient amount of macro and micronutrients were added to the solutions. In order to prevent PAH contamination, only glassware lab equipment were used. All glassware were burned in 500 ºC before use.

Table 3.1 : Composition of OECD nutrient solution [179].

Compound	Feed concentration (g/L)
Peptone	16
Meat extract	11
Urea	3
NaCl	0.7
$CaCl_2.2H_2O$	0.4
$MgSO_4.7H_2O$	0.2
K_2HPO_4	2.8

3.1.1 Fill & draw reactors

Activated sludge taken from Pasaköy Wastewater Treatment Plant were fed with 700 mgCOD/L peptone mixture for about 3 months before any experiments were conducted. The F&D reactor had a volume of 15 liters. The reactor was operated at a sludge age of 10 days. The average SS and VSS concentrations were 1805 mg/L and 1475 mg/L, respectively. The adaption process of activated sludge to peptone mixture was shown in Figure 3.1.

Figure 3.1 : Peptone mixture adaption process.

3.1.2 Sequencing batch reactor

The activated sludge used for the Sequencing Batch Reactor (SBR) was taken from the F&D reactor operated for 8 months. The SBR had 13 liter volume and fed at two cycles in a day with the 500 mgCOD/L peptone mixture. The reactor was operated at a sludge age of 10 days. The volume of wasted sludge from the completely mixed reactor was 1.5 L per day. The SBR reactor had, per cycle, 9 hours of aeration, 30 minutes of activated sludge wastage, 60 minutes of settling, 30 minutes of supernatant wastage, 30 minutes of idling, and 30 minutes of filling. The design details of SBR were given in Figure 3.2. As the peptone mixture is sensitive to temperature, an icebox was used to keep it cold (Figure 3.3). The SBR was operated only with feeding the peptone mixture for 21 days, then with peptone mixture+B[a]A (0.011 g/L) for 21 days and finally 7 days again without B[a]A. Since the B[a]A could be oxidized via light, the reactor and water + B[a]A mixture reactors were sealed (Figure 3.3). To avoid interaction of other PAHs only glass reactors were used.

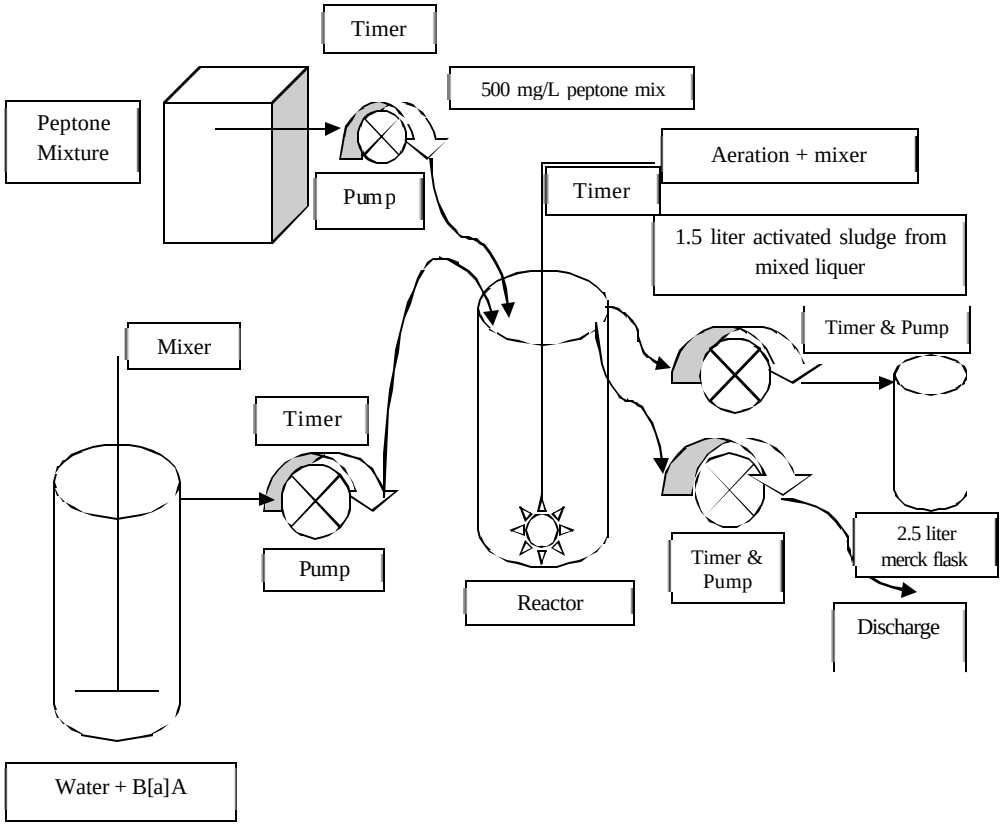


Figure 3.2 : Design details for SBR.

Figure 3.3 : Sealed reactors and icebox for peptone mixture.

3.2 PAH Survey for Treatment Plants in Istanbul

Two different domestic treatment plants in Istanbul were selected for measuring detailed PAH concentration. The first selected plant named WWTP1 is operated since 2000 and has 500000 m^3/day treatment capacity [180].

The second selected plant named WWTP2 is operated since 1997 and services Kagithane, Beyoglu, Sisli, Besiktas and Sariyer districts. This treatment plant is a pre-treatment plant and the collected wastewaters pumped from the coast 350 m away and 70 m deep undercurrent of Bosporus. Because the WWTP2 has no biological treatment unit, the wastewaters were taken from both treatment plants influents [180].

3.3 Analysis

3.3.1 Conventional parameters

Suspended solids, volatile suspended solids, COD and pH analysis were performed in order to monitor and control reactor operation. In the experiments, pH, SS and VSS analysis were performed as defined in the Standard Methods [181]. The COD samples were filtered through 0.45 µm membrane filters and performed as described in the method proposed by ISO 6060 [182]. pH measurements were performed by a 520Aplus pH meter.

3.3.2 Respirometric analysis

The respirometric analysis were conducted with relevant biomass seeding alone to obtain endogenous oxygen uptake rate (OUR) level of biomass. Samples with desired S_O/X_O ratios are added to the reactor and the OUR data was monitored and measured as mg/L.h. Experimental studies are conducted by using activated sludge operated at the sludge age of 10 days. AppliTek Ra-Combo respirometer was used for the respirometric analysis (Figure 3.4). A nitrification inhibitor (Formula 2533TM, Hach Company) was used to prevent any possible interference induced by nitrification.

Figure 3.4 : AppliTek Ra-Combo respirometer.

3.3.3 Enzyme analysis

It is well known that dehydrogenases are a group of intra cellular enzymes that are involved in microbial oxidoreductase metabolism. These enzymes have been frequently used as an index of microbial activity in wastewater and soil samples (Table 3.2) [183].

Table 3.2 : Redox processes in soil and sludge sample [183].

Reaction	Redox potential pH = 7.0 : 1 atm
$4H^+ + 4e^- + O_2 \Leftrightarrow 2H_2O$	E_0 = +800 mv
TTC + $2H^+ \Leftrightarrow$ TPF	E_0 = +490 mv

Determination of dehydrogenase activity in activated sludges or soil sample is usually based on the use of tetrazolium salts as artificial electron acceptors, which are reduced by microbial activity to red colored formazans, can be determined spectrophotometrically. By choosing appropriate conditions the natural H^+ / e^- acceptors of the microorganisms of soil or sludge samples are replaced by the redox dye triphenyl tetrazolium chloride. Respiration of the microorganisms causes a reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF), which is red and insoluble in water. TTC is significantly reduced only by the aerobic cytocrome system [184]. The measurement of TTC in the activated sludge reveals the effect of PAHs on bacteria [185-186].

Many different methods to measure TTC in activated sludge in the literature are given. However, the selected method is given below:

Dehydrogenase activity in activated sludge was determined by reduction of TTC. 5 mL TTC (5 gL^{-1}) and 2 mL glucose solution (0.1 $molL^{-1}$) were added to 5 mL activated sludge, shaken at 200 rpm for 20 min (Thermo Orbital Shaker) and incubated at 37 ºC for 12 h (Heraeus B6042). 1 mL of concentrated sulfuric acid was added to stop the deoxidization reaction in the flask. 5 mL methylbenzene was added to extract the triphenyl formazan formed in the reaction mixture, and the sample was shaken at 200 rpm for 30 min. The flask was stand stilled on the table for 3 min and then the reaction mixture was centrifuged at 4000 rpm for 5 min. The absorbance of the supernatant was checked at 492 nm with an UV spectrometer (Pharmacia LKB Novaspec II, Spectro) [185-186]. The color differences between the samples were given in Figure 3.5.

The enzyme analyses were performed parallel with respirometric analysis and the samples taken at the beginning and end of respirometer.

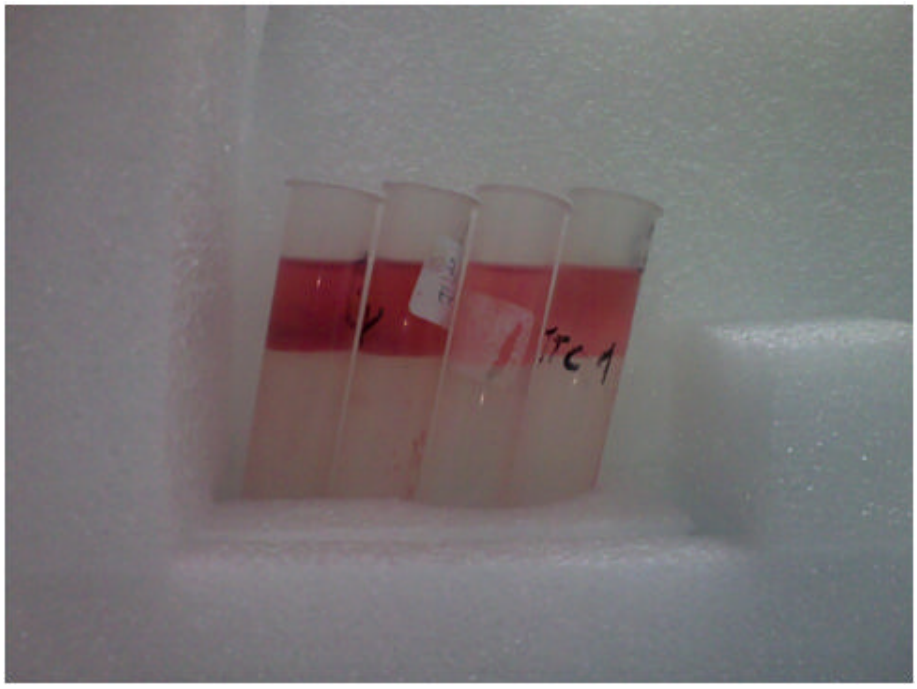


Figure 3.5 : Different colors for different samples.

3.3.4 PAH analysis

All HPLC analyses were performed using Thermo Surveyor HPLC with PDA (DAD) Detectorand with Macherey-Nagel Nucleosil C_{18} column. The column dimensions was 50x4 mm. Separation was achieved by isocratic elution in acetonitrile: water (70:30), with a pressure 25-30 bar and flow rate of 1 mL/min. The PAH recovery was 107.3 %. The sequence of 16 EPA PAHs for one injection was shown in Figure 3.6.

1. Naphthalene
2.Acenaphthylene
3.Acenaphthene
4. Fluorene
5. Phenantrene
6.Anthracene
7.Fluoranthene
8. Pyrene
9.B[a]A
10.Chrysene
11.Benzo[b]fluoranthene
12.Benzo[k]fluoranthene
13.B[a]P
14.Dibeenz[ah]anthracene
15.Benzo[ghi]perylene
16.Indeno[1,2,3-cd]pyrene

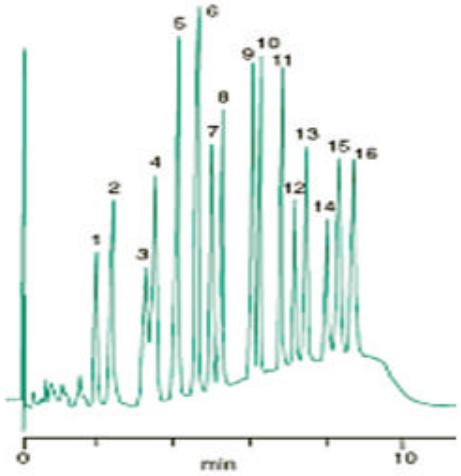


Figure 3.6 : The sequence of 16 EPA PAHs [187].

Dichloromethane extraction method was used for wastewater and supernatant. 500 mL wastewater was extracted with 3x30 mL dichloromethane in a separating funnel. The organic layer were combined and dried with Na_2SO_4 and filtered through glasswool. The samples were concentrated by rotary evaporator (Figure 3.7) to ≈ 2.5 mL and the rest were concentrated to dryness in a gentle N_2 flow.

Figure 3.7 : Rotary evaporator.

The concentration redisoluted in 0.5 mL acetonitrile (CH_3CN) [187]. The brown glass vials were used to keep the samples.

The samples from activated sludge were collected and freeze-dried until the analysis begin. To prevent high amount of solvents discharges, a new method named ultrasonic extraction procedure applied. 0.5-1 g of dried sludge sample was transferred to 50 mL glass flasks and 50 mL of methanol were added. The flasks were shaken for 5 min and sonicated for 20 min. Bandelin Sonopuls HD2200 sonicator with TT13 sonicator plate used for the extraction (Figure 3.8).

Figure 3.8 : Bandelin Sonopuls sonicator.

The liquid phase was filtered through a 0.45 µm glass fibre membrane filter (Whatman, Mainstone, UK) and cleaned-up by solid phase extraction. Prior to use, SPE cartridges were conditioned using 3mL of methanol and 3 mL of deionized water at a flow-rate of about 3 $mLmin^{-1}$. Then, an aliquot of 10 mL of the filtered sludge extract was transferred to the SPE cartridge at a flow-rate of about 3 $mL\ min^{-1}$ using a vacuum manifold system connected to a vacuum pump. Fractions of 1 and 5 mL of the extract were separately evaporated to dryness by a gentle nitrogen stream and the residues were dissolved in 0.5 mL of acetonitrile, respectively [188-189].

The activated sludge was washed with water twice, and respirometer analysis were performed. At the end of respirometer analysis, the activated sludge was settled. After settling, samples taken from settled activated sludge and from supernatant.

3.3.5 FAME analysis

To identify the microbial species during the steady state period in the SBR and the possible changes after B[a]A loading, Fatty Acid Methyl Ester (FAME) analysis were used [190]. The analysis contracted to the Scientific and Technological Research Council of Turkey, Marmara Research Center (TÜBITAK-MAM). The liquid mixture samples were taken in Day 21 from SBR was fed with peptone mixture and Day 21 from SBR was fed with peptone mixture + B[a]A. The samples were kept at -20 ^{o}C with glycerol until the analyses were performed.

3.3.6 PHA analysis

Polyhydroxyalkanoates (PHA) is one of the mostly observed storage polymer in activated sludge. The commonly known component are polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and 3-hydroxy-2-methylvalerate (3H2MV). For the analyses, 25-30 mL samples taken in to tubes from mixed liqueur. Each tubes having a few drops of formaldehyde. The samples centrifuged (SED Model 5X Centrifuge) and the liquid phase discarded. 4.5-5 ml phosphate buffer added and then the samples were centrifuged again before vortex. Collected sludge pellets in a sample tube were freezed at -20 ^{o}C. The pellets freeze-dried (ThermoSavant, ModulyoD Freeze dryer, Figure 3.9) for at least 48 hours at -50^{o}C. The freeze-dried sludge pellets crushed. 20-30 mg with a microgram balance weighed and recorded exact amount. The weighed amount of sample in glass tubes with PTFE lined screw caps (Schott GL18 Max 200^{o}C) putted. 3 standards each 2-3 mg for each series of 15 samples prepared.

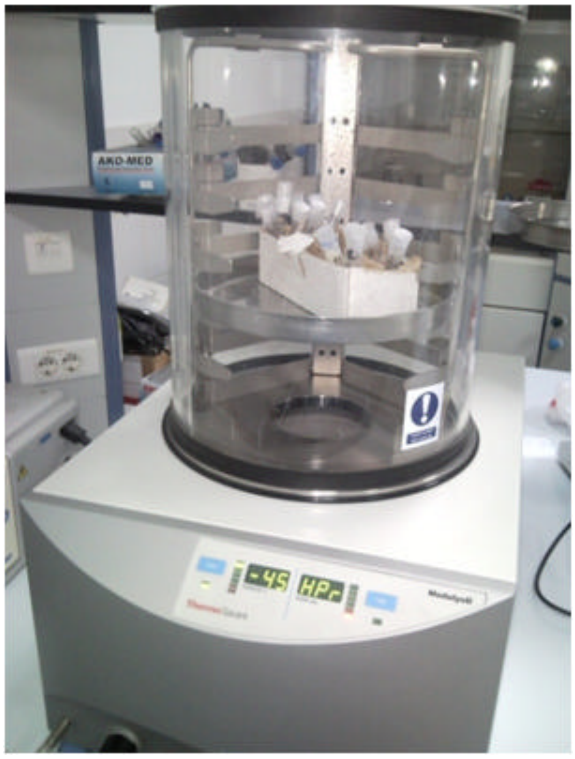


Figure 3.9 : ThermoSavant, ModulyoD freeze dyer.

50 µL of internal standards to each sample and standard pipetted. 1.5 mL of the acid mixture in each sample and standard added. 1.5 mL 1,2 dichloroethane is added to each standard and sample. The samples and standards boiled for 2 hours at 100 ^{o}C and shaked every 15 minutes. After cooling down, 3 mL distilled water added and shaked vigorously for 10 min by vortex. The samples centrifuged for 5 min. at 2500-3000 rpm. Waited for phase separation for a few minutes. Around 1 mL from the lower organic phase taken. Glasswool and add sodium sulfate in blue pipette tips (0.5 mL) putted. 1 mL organic phase filtered from sodium sulfate column [191].

The samples were analyzed by Agilent 6890N gas chromatograph.3-hydroxybutyricacid-co-3-hydroxyvaleric acid, PHV and caproic acid sodium salt was used as standards for PHB, PHV and 3H2MV measurements, respectively. COD equivalents of the measured components were calculated according to conversion ratios of 1.38 mg COD/mg PHB, 1.63 mg COD/mg PHV, and 1.82 mg COD/mg 3H2MV.

4. RESULTS & DISCUSSION

4.1 Fill & Draw Reactor Results – Acute Effect of B[a]A

To determine the acute effect of B[a]A on activated sludge, respirometric analyses were performed. Apart from the control set, three different B[a]A loads which were 0.011 g/L, 0.044 g/L, 0.088 g/L, respectively were applied. To increase the solubility of B[a]A in water, acetone was used as solvent. In addition, three sets with acetone including peptone mix.+acetone, peptone mix.+acetone+0.011 g/L B[a]A and peptone mix.+acetone+0.044 g/L, were applied. Duplicate sets; Set 1.1, Set 1.2 and Set 31, Set 3.2 had the same conditions. However, the Set 1.2 and Set 3.2 had also PHA results besides the respirometric results. The experiments were started with an initial feed having a total COD of 300 mg/L. The details of sets were given in Table 4.1.

Table 4.1 : The details of F&D reactor sets.

Sets	Details
Set 1.1	peptone mix. 300 mg COD/L
Set 1.2	peptone mix. 300 mg COD/L
Set 2	peptone mix. 300 mg COD/L + 0.011g/L B[a]A
Set 3.1	peptone mix. 300 mg COD/L + 0.044 g/L B[a]A
Set 3.2	peptone mix. 300 mg COD/L + 0.044 g/L B[a]A
Set 4	peptone mix. 300 mg COD/L + 0.088 g/L B[a]A
Set 5	peptone mix. 300 mg COD/L + 1.5 mL/L acetone
Set 6	peptone mix. 300 mg COD/L + 1.5 mL/L acetone + 0.011 g/L B[a]A
Set 7	peptone mix. 300 mg COD/L + 1.5 mL/L acetone + 0.044 g/L B[a]A

4.1.1 Conventional parameters

As conventional parameters; COD removal, pH, SS and VSS were measured. For all sets; the COD removal was measured 85 % and the pH was kept 6.9, initial SS was 1000 mg/L and initial VSS was 800 mg/L.

4.1.2 Respirometer results

For each set, details given above, one respirometric analysis was performed. The respirometric results for Set 1 to Set 7 with different conditions are shown in Figure 4.1. In addition, ? O_2 for all sets were calculated.

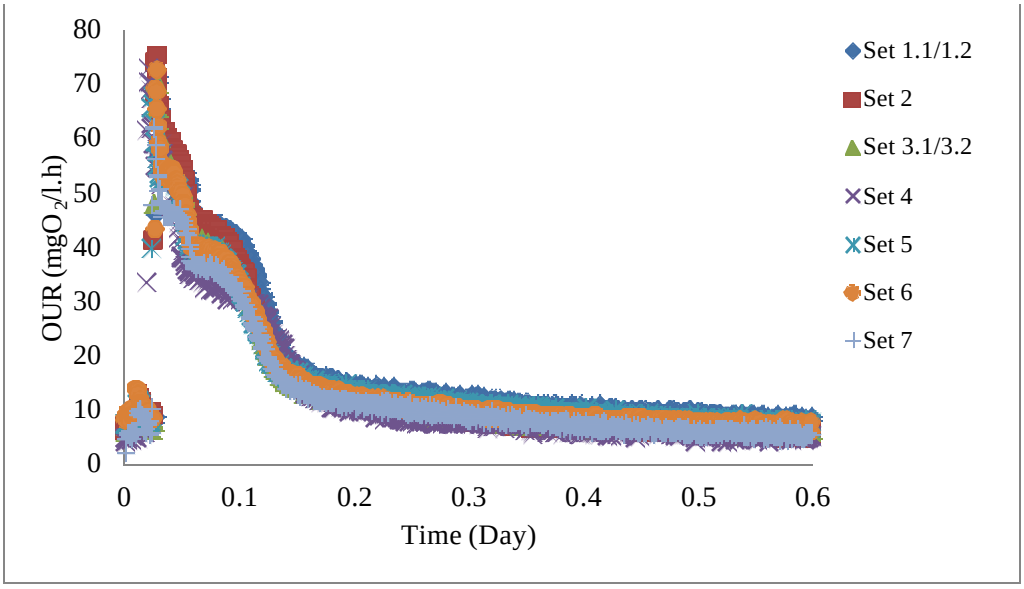


Figure 4.1 : The respirometric results of Set 1.1 – 7.

The results, which are shown in Figure 4.1, are divided in two groups to present the details. The respirometric results of the sets conducted without acetone are shown in Figure 4.2. The $?O_2$ was calculated for all sets separately. The $?O_2$ of Set 1.1 and Set 1.2 were both 122 mgCOD/L. The initial b_H levels for both sets were 8.45 mgO_2/L.h. The OUR peaks after feeding with peptone mixture drop from 71.5 mgO_2/L.h to b_H level in 0.45 days (10 hours).

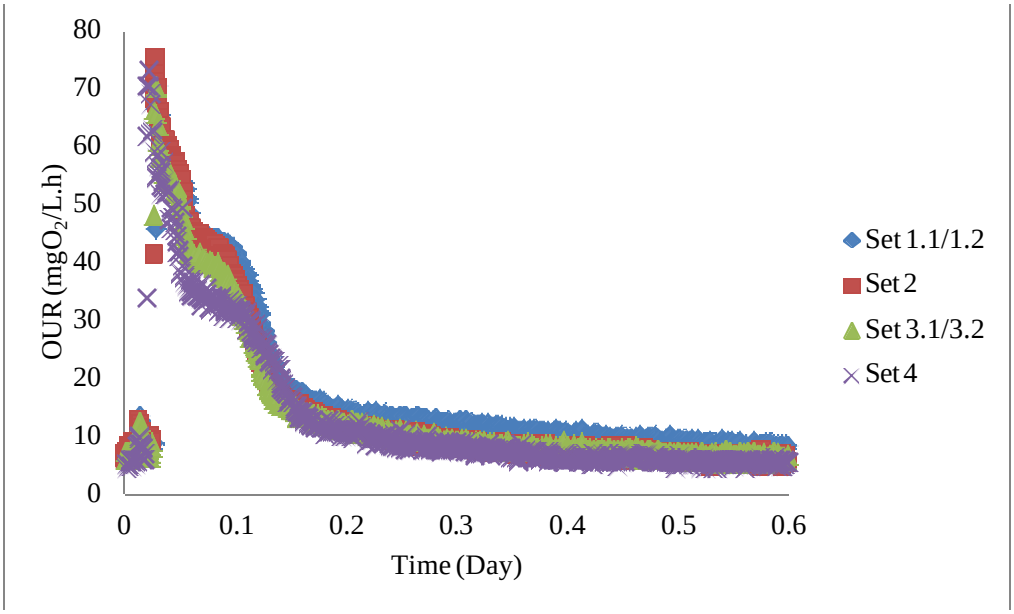


Figure 4.2 : The respirometric results of Set 1.1 – 4.

The $?O_2$ of Set 2 was 97 mgCOD/L. The initial b_H level was 8.78 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A drop from 75.1 mgO_2/L.h to b_H level in 0.26 days (6.2 hours). The $?O_2$ of Set 31 and 3.2 were 93 mgCOD/L. The initial b_H levels were 8.27 mgO_2/L.h for both sets. The OUR peaks after feeding with peptone mixture and B[a]A drop from 70.4 mgO_2/L.h to b_H level in 0.28 days (6.7 hours). The $?O_2$ of Set 4 was 91 mgCOD/L. The initial b_H level was 8.31 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A drop from 73 mgO_2/L.h to b_H level in 0.26 days (6.2 hours). The $?O_2$ from Set 2 to Set 4, drop with B[a]A concentration from 97 mgCOD/L to 91 mgCOD/L. In Figure 4.3 are shown the respirometric results of the sets with acetone.

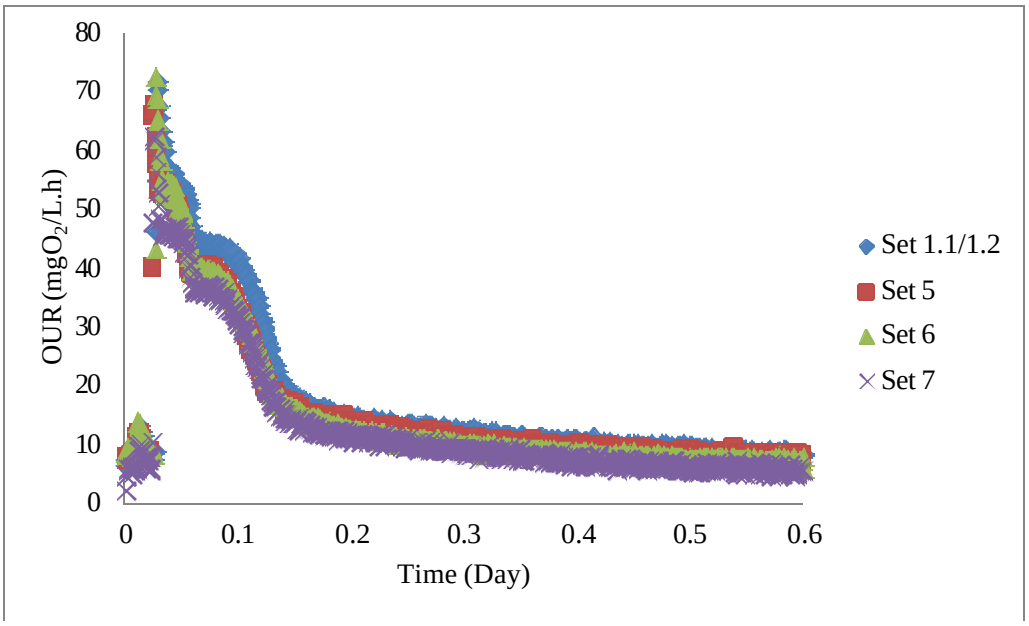


Figure 4.3 : The respirometric results of the control Set 1.1/1.2 and Set 5 – 7.

The $?O_2$ of Set 5 was 96 mgCOD/L. The initial b_H level was 8.8 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A drop from 67.82 mgO_2/L.h to b_H level in 0.4 days (9.5 hours). The $?O_2$ of Set 5 was very close to Set 2. However, the drop of peak after feeding, needed as time as Set 1. The $?O_2$ of Set 6 was 85 mgCOD/L. The initial b_H level was 9.1 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A drop from 72.3 mgO_2/L.h to b_H level in 0.24 days (≈6 hours). The $?O_2$ of Set 7 was 84 mgCOD/L. The initial b_H level was 8

mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A drop from 62.23 mgO_2/L.h to b_H level in 0.28 days (6.7 hours). The results showed that the effect of B[a]A were inhibited from acetone. Because of that, two sets without acetone addition were selected for PHA sampling to show the effect of B[a]A on the storage mechanism. First set was the control set (Set 1.2) and the second set was the set with 0.044 mg/L B[a]A (Set 3.2) addition. The respirometric results for Set 1.2 and Set 3.2 are shown in Figure 4.4.

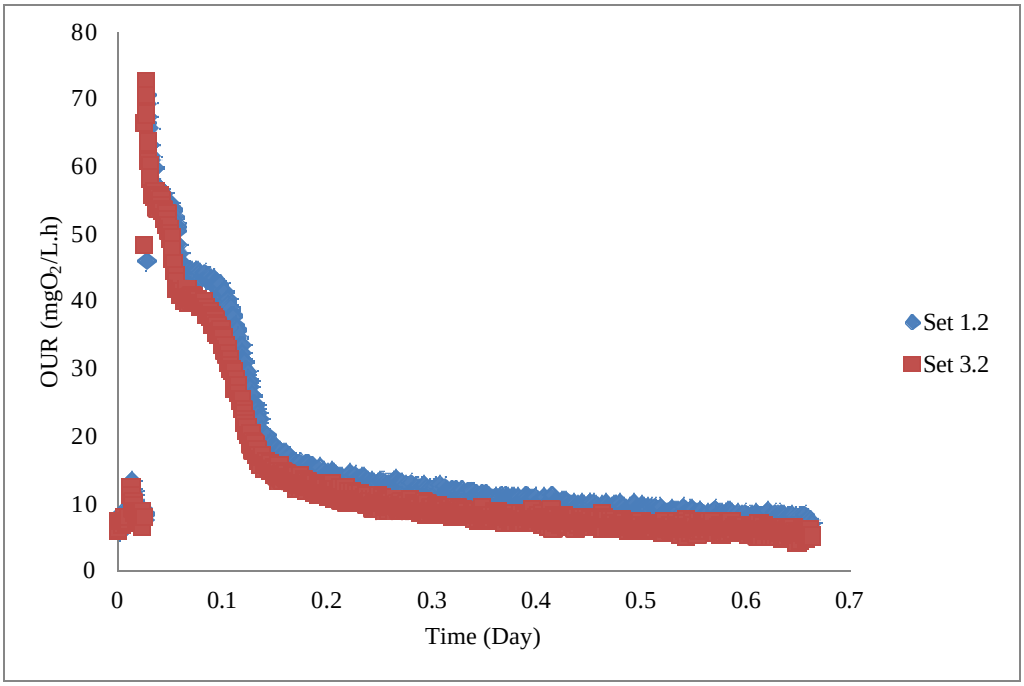


Figure 4.4 : Respirometer results for Set 1.2 and Set 3.2.

4.1.3 Modelling results

All sets, from Set 1.1 to Set 7, were modeled with Aquasim software. Modified ASM3 model, which includes dual initial readily biodegradable COD, was used. Matrix representation of the model structure for simultaneous growth and storage is shown in Table 4.2.

Table 4.2 : F&D, Matrix representation of the model structure for simultaneous growth and storage.

Process	S_{O2}	S_{S1}	S_{S2}	S_H	X_H	X_{STO}	X_P	Rate equation $M L^{-3}T^{-1}$
Growth of X_H for S_{S1}	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$			1			$\mu_{H1}\frac{S_{S1}}{K_{S1}+S_{S1}}X_H$
Growth of X_H for S_{S2}	$-\frac{1-Y_H}{Y_H}$		$-\frac{1}{Y_H}$		1			$\mu_{H2}\frac{S_{S2}}{K_{S2}+S_{S2}}X_H$
Storage of PHA	$-(1-Y_{STO})$	-1	-1			Y_{STO}		$k_{STO}\frac{S_S}{K_S+S_S}X_H$
Hydrolysis of S_H		1	1	-1				$k_h\frac{S_{H1}/X_H}{K_X+S_{H1}/X_H}X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$				1	$-\frac{1}{Y_H}$		$r_G X_{STO}$
Decay of X_H	$-(1-f_P)$				-1		f_P	$b_H X_H$
Parameter, ML^{-3}	O_2	COD	COD	COD	COD	Cell COD	COD	

The measured OUR data is showed with the circles in the model results. The model data is shown with the line. The modeled graphic of the control Set 1.1 and Set 1.2 are given in Figure 4.5.

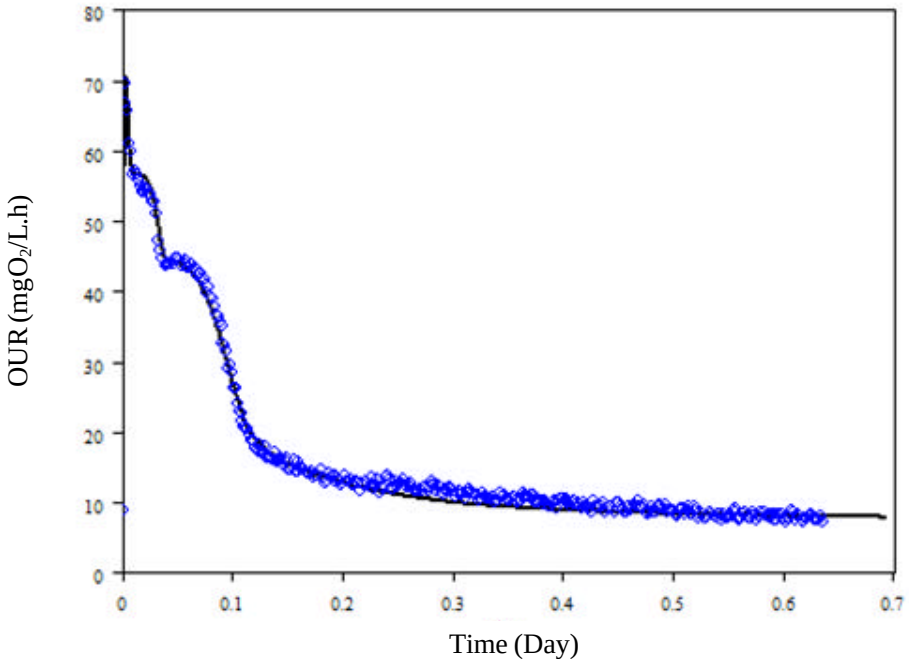


Figure 4.5 : Set 1.1 and Set 1.2, control set modelling results.

The PHA model result for Set 1.2 is given in Figure 4.6.

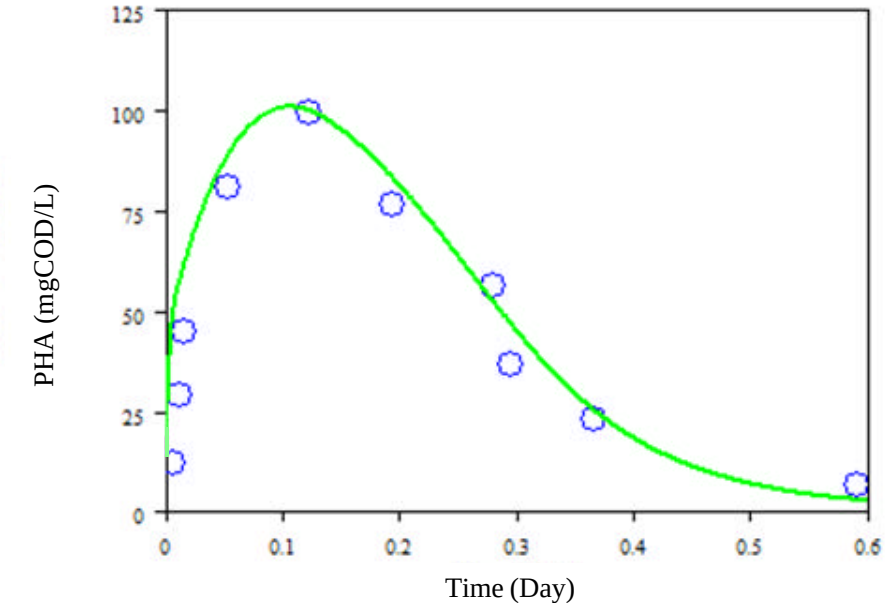


Figure 4.6 : Set 1.2, (control) PHA modelling result.

The modeled graphic of the Set 2 with peptone mixture and 0.011 g/L B[a]A addition is given in Figure 4.7.

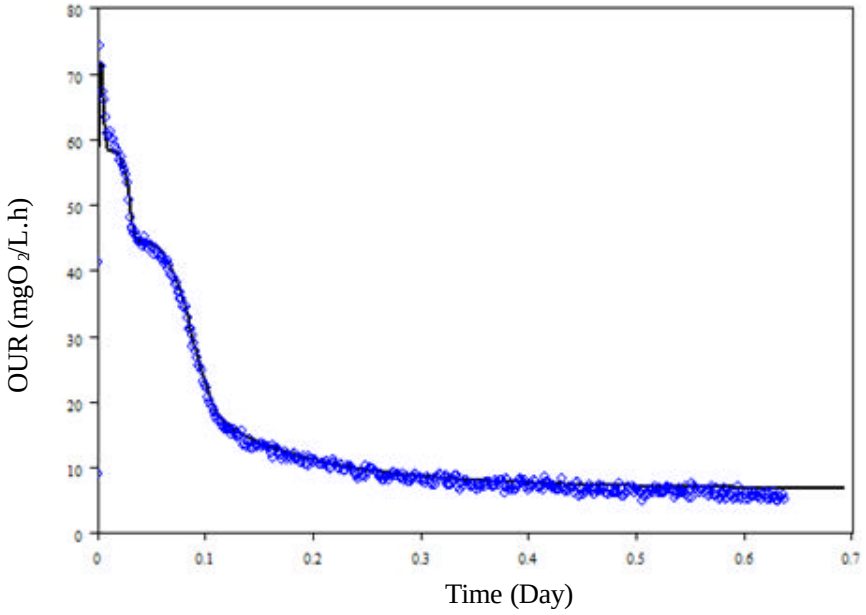


Figure 4.7 : Set 2, respirometer modelling result.

The modeled graphics of the Set 3.1 and 3.2, which include peptone mixture and 0.044 g/L B[a]A addition is given in Figure 4.8.

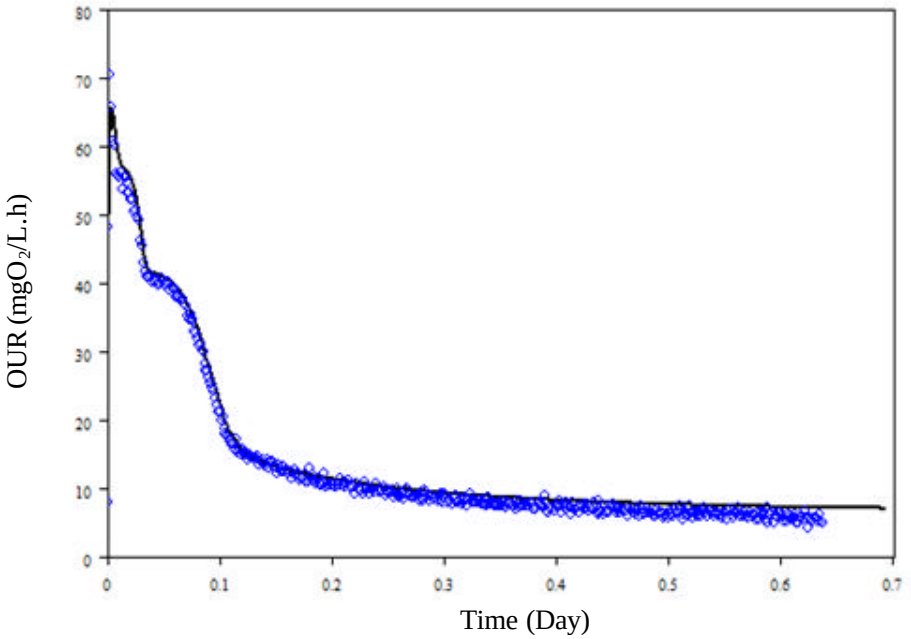


Figure 4.8 : Set 3.1 and 3.2, respirometer modelling results.

The PHA model result for Set 3.2 is given in Figure 4.9.

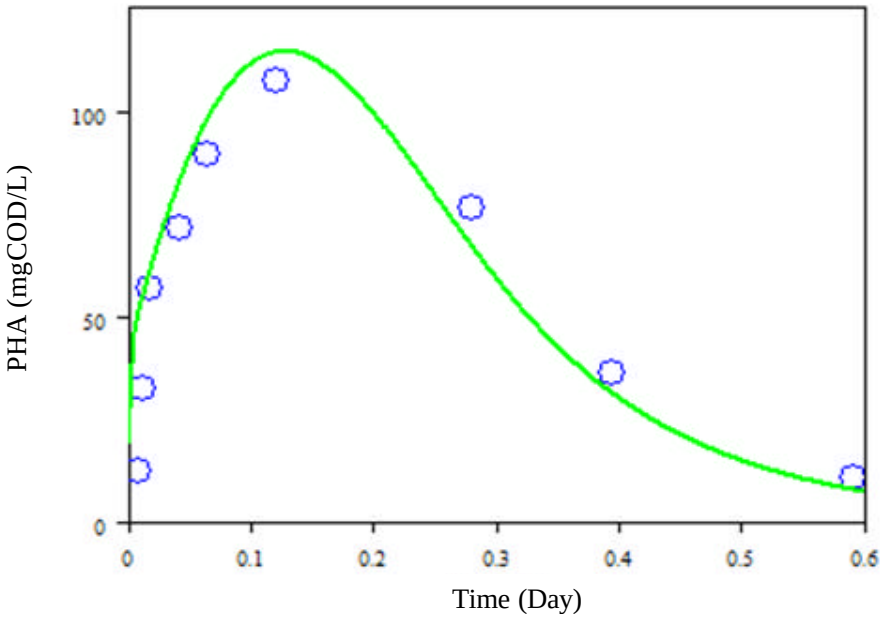


Figure 4.9 : Set 3.2, PHA modelling result.

The modeled graphic of the Set 4 which includes peptone mixture and 0.088 g/L B[a]A addition is given in Figure 4.10.

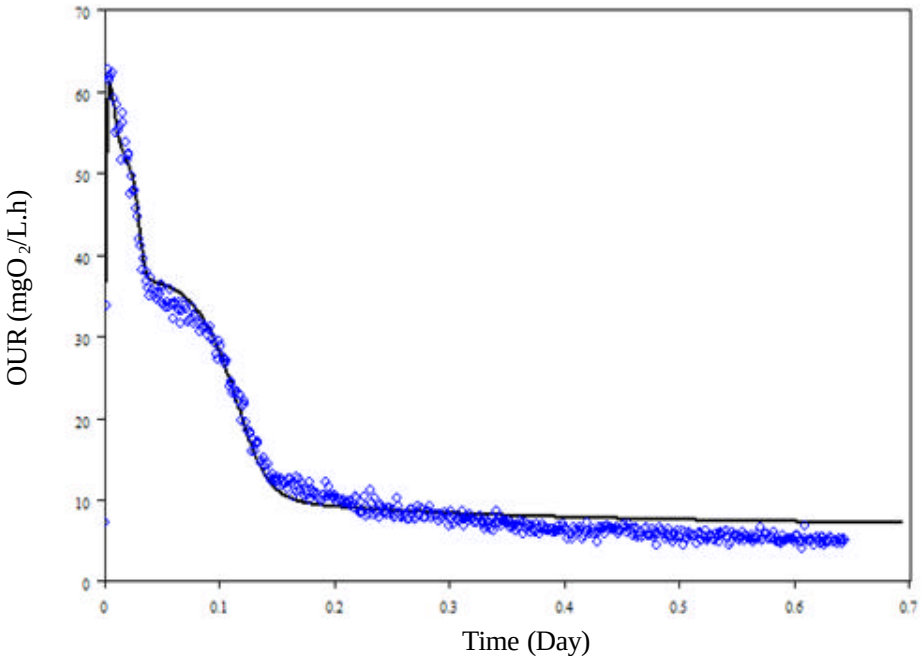


Figure 4.10 : Set 4, respirometer modelling result.

After modelling sets with control and different B[a]A loads, the sets with acetone were modeled. In Set 5, peptone mix. and acetone were used as control set. The modeled graphic of the Set 5 is given in Figure 4.11.

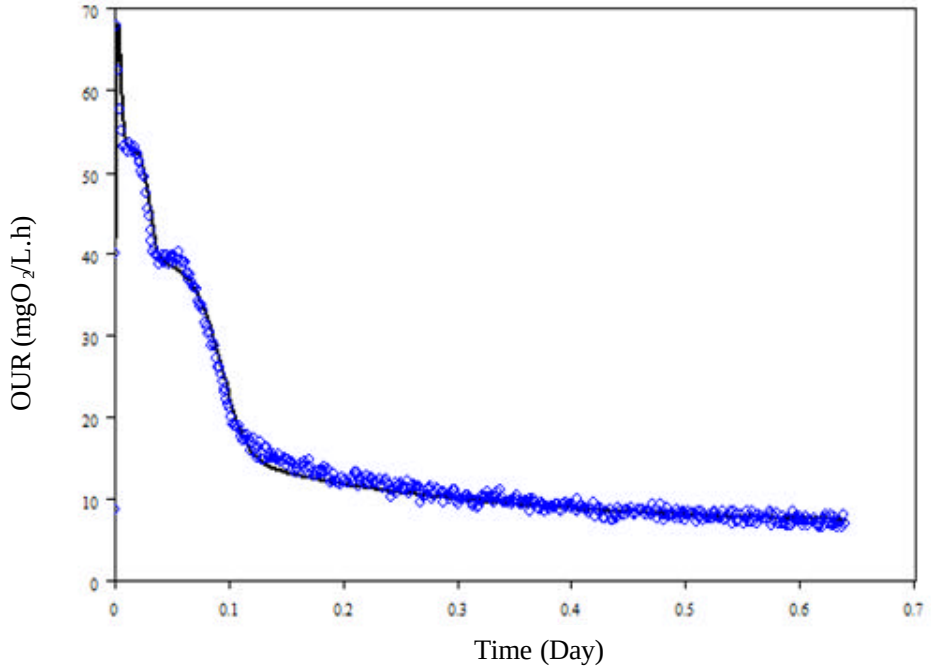


Figure 4.11 : Set 5, peptone mix. + acetone control respirometer result.

The modeled graphic of the Set 6 which includes peptone mixture, acetone and 0.011 g/L B[a]A addition is given in Figure 4.12.

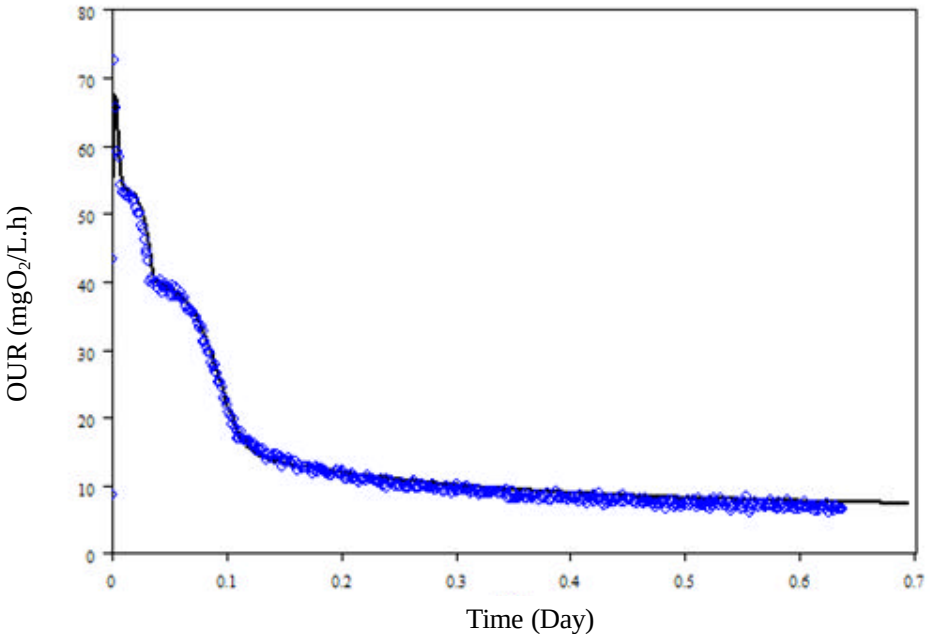


Figure 4.12 : Set 6, respirometer modelling result.

The modeled graphic of the Set 7 which includes peptone mixture, acetone and 0.044 g/L B[a]A addition is given in Figure 4.13.

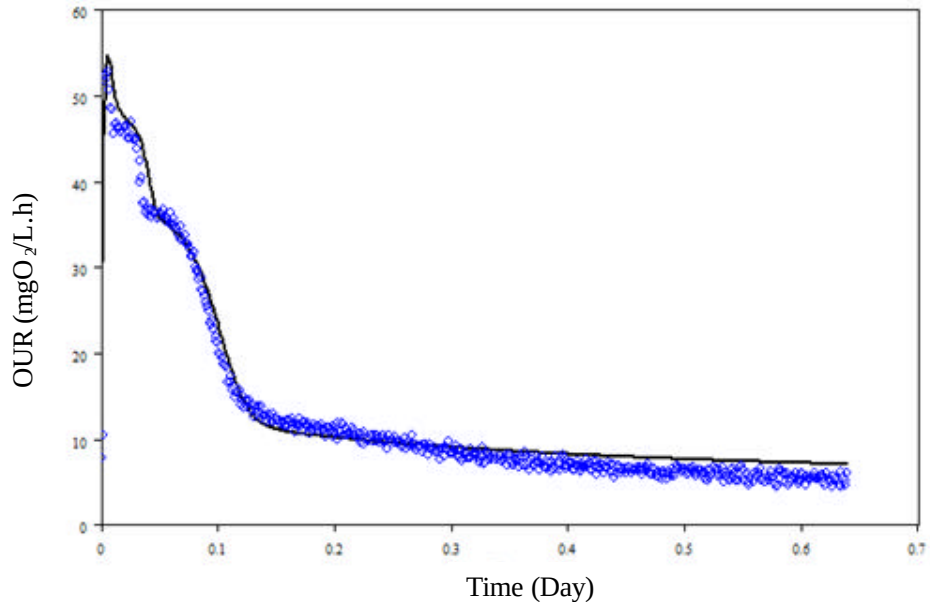


Figure 4.13 : Set 7, respirometer modelling result.

The kinetic parameters were determined based on modelling results of the Sets 1.1 - 7. The calculated kinetic parameters are given in Table 4.3.

Table 4.3 : The kinetic parameters of the Set 1.1 – Set 7.

	Set 1.1/1.2	**Set 2**	**Set 3.1/3.2**	**Set 4**	**Set 5**	**Set 6**	**Set 7**
B[a]A (g/L)	-	0.011	0.044	0.088	-	0.011	0.044
Acetone (1.5 mL/L)	-	-	-	-	+	+	+
Maximum growth rate for X_{H1}, μ_{H1}	4	4	4	4	4	4	4
Maximum growth rate for X_{H2}, μ_{H2}	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Half saturation constant for growth of X_{H1}, K_{S1}	12	12	12	12	12	12	12
Half saturation constant for growth of X_{H2}, K_{S2}	3	3	3	3	3	3	3
Maximum hydrolysis rate for S_H, k_h	3.12	3.3	3.04	2.23	3.05	3.12	3.12
Hydrolysis half saturation constant for S_H, K_X	0.029	0.029	0.029	0.029	0.029	0.029	0.029
Maximum storage rate of PHA, k_{STO}	10	8	8	1	7.6	7.6	7.6
Maximum growth rate for growth on PHA, r_G	6.9	6.86	4.8	4.3	3.54	3.54	3.54
Endogenous decay rate for X_H, b_H	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Heterotrophic yield coefficient, Y_H	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Storage yield of PHA, Y_{STO}	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Initial Readily Biodegradable COD, S_{S1}	8	8	10	14	8	8	8
Initial Readily Biodegradable COD, S_{S2}	34	34	34	38	34	34	34
Initial active Heterotrophic biomass, X_H	950	950	950	1080	950	950	950

Maximum storage rate of PHA by X_H (k_{STO}) from Table 4.2 showed almost the same value for Set 2 and Set 5. The effect of acetone overshadowed the effect of B[a]A. Because of that, the effect of B[a]A could not be determinate by Set 6 and Set 7. The B[a]A addition had an adverse effect on maximum hydrolysis rate for S_H (k_h), on maximum storage rate of PHA by X_H (k_{STO}) and on maximum growth rate for growth on PHA for X_H (r_G). The adverse effects of B[a]A had a positive correlation with the increasing loads. The B[a]A had an acute effect on activated sludge, which affects PHA storage mechanism.

4.2 SBR Results – Chronic Effect of B[a]A

Fourteen different sets were performed to observe the chronic effect of B[a]A on activated sludge. Six of fourteen sets were selected which had also respirometric and PHA data for modelling. The details of selected sets are given in Table 4.4.

Table 4.4 : The details of Set 8 – Set 13.

Sets	Days	Details	COD
Set 8	Fill and Draw	Fill and Draw, without B[a]A addition	700 mg COD/L
Set 9	Day 21	SBR, steady state, without B[a]A addition	500 mg COD/L
Set 10	Day 1 B[a]A	SBR, with B[a]A addition	500 mg COD/L
Set 11	Day 3 B[a]A	SBR, with B[a]A addition	500 mg COD/L
Set 12	Day 21 B[a]A	SBR, steady state, with B[a]A addition	250 mg COD/L
Set 13	Day 5	SBR, without B[a]A addition	250 mg COD/L

4.2.1 Conventional parameters

The SS and VSS results of F&D and SBR are shown in Table 4.5. In order to prevent possible O_2 limitation in respirometer, the amount of activated sludges from Set 12, Set 13 and Day -7 were 750 mL instead of 1500 mL. S_O/X_O ratios were also shown in Table 4.5.

Table 4.5 : The SS, VSS and So/Xo amounts of F&D and SBR.

Day	Set No.	B[a]A addition	SS	VSS	S_O/X_O
F&D	Set 8	-	1660	1500	0.46
1		-	1520	1430	0.35
3		-	1500	1210	0.41
5		-	1735	1555	0.32
10		-	2160	1960	0.25
14		-	2295	2065	0.24
21	Set 9	-	2260	2125	0.23
1	Set 10	+	1220	1200	0.41
3	Set 11	+	1385	1375	0.36
12		+	1830	1815	0.27
15		+	2340	2280	0.22
21	Set 12	+	1675*	1660	0.15
-5	Set 13	-	1650*	1640	0.15
-7		-	1545*	1535	0.16

*half biomass was added

To analyze COD removal, samples were taken at the end of the cycles. The COD removal results are shown in Table 4.6. The average COD removal of SBR in steady state was 87 %. The B[a]A addition decreased the COD removal in first 12 days. With Day 15, the COD removal increased again to the 87 %.

Table 4.6 : COD removal of F&D and SBR.

Day	Set No.	B[a]A Addition	COD removal (%)
F&D	Set 8	-	80.0
1		-	79.17
3		-	83.5
5		-	85.3
10		-	87.0
14		-	86.5
21	Set 9	-	87.2
1	Set 10	+	80.50
3	Set 11	+	82.67
12		+	84.17
15		+	86.67
21	Set 12	+	86.50
-5	Set 13	-	85.80
-7		-	85.70

For all sets, pH values were measured between 6.8 and 6.9.

4.2.2 PAH results

4.2.2.1 PAH results for SBR

The PAH results of SBR are shown in numbers in Table 4.7 and graphical presentation is shown in Figure 4.14. Since the amount of B[a]A addition was higher than water solubility, some of B[a]A attached to the reactor wall. In addition, activated sludge was washed with water twice. These could explain the reason why a decrease in the B[a]A level was observed both in the activated sludge and the supernatant. However, the higher amount of B[a]A addition, results in higher B[a]A level in the supernatant and in the activated sludge. The detection of B[a]A in the supernatant is an evidence of desorption from the activated sludge.

Table 4.7 : B[a]A amounts in activated sludge and supernatant from SBR.

Day	B[a]A addition	Set No.	Supernatant (B[a]A mg/L)	Activated Sludge (B[a]A mg/kg)
Day 3	+	Set 11	11.7	129.35
Day 12	+		37.1	163.53
Day 15	+		61.67	199.89
Day 21	+	Set 12	62	208.84
Day -5	-	Set 13	4.17	167.70
Day -7	-		4.76	108.97

It was found on Days -5 and -7, that the amount of B[a]A in supernatant decreased sharply. Nevertheless, the amount of B[a]A in activated sludge decreased slowly.

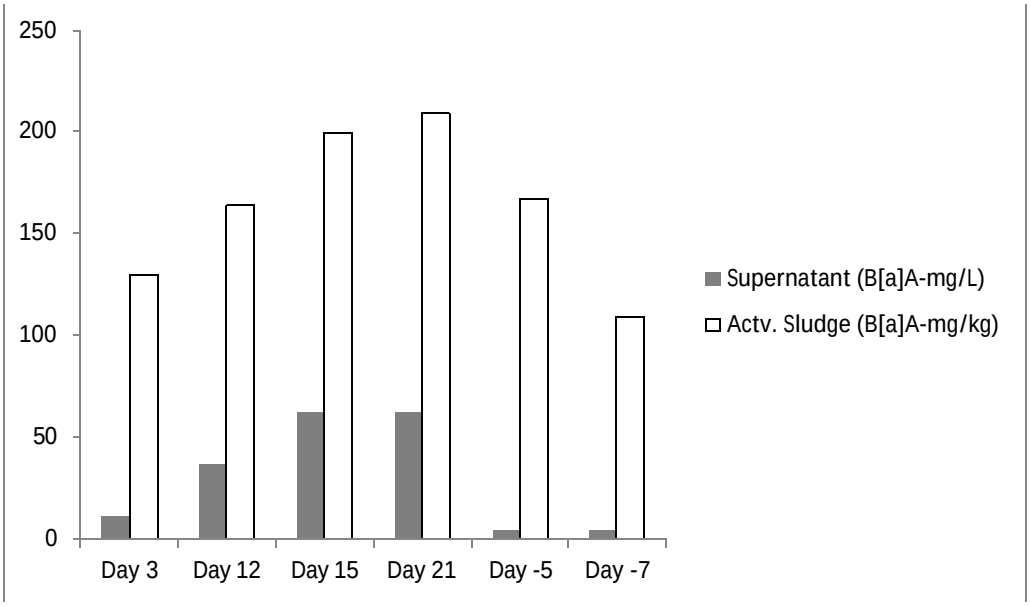


Figure 4.14 : B[a]A amounts in activated sludge and supernatant from SBR.

4.2.2.2 PAH results for treatment plants in Istanbul

The PAH results of WWTP1 and WWTP 2 treatment plants were given in Table 4.8. The samples were taken from treatment plants influents. The first samples taken end of December (2009) and second samples were taken end of February (2010). The amount of two samples (A and B) from WWTP1 and WWTP2 were close. The number of PAHs and the amount of total PAHs from WWTP1 was higher than WWTP2.

Table 4.8 : The PAH amounts (mg/L) in WW of treatment plants in Istanbul.

WWTP 1 - A	**mg/L**
Acenaphthylene	0.25
Anthracene	0.039
Benzo[a]anthracene	0.13
Benzo[a]pyrene	0.58
Chrysene	0.014
Fluoranthene	0.24
Indeno[1,2,3-c,d] pyrene	0.031
Pyrene	0.62
SPAH	**1.904**

Table 4.8 : (contd.) The PAH amounts (mg/L) in WW of treatment plants in Istanbul.

WWTP 2 - A	**mg/L**
Acenaphthylene	0.93
Anthracene	0.067
Benzo[a]anthracene	0.39
Benzo[a]pyrene	0.12
Phenanthrene	0.023
Pyrene	0.32
SPAH	**1.85**

WWTP 1 - B	**mg/L**
Benzo[a]anthracene	0.509
Benzo[a]pyrene	0.21
Naphthalene	0.58
SPAH	**1.299**

WWTP 2 - B	**mg/L**
Anthracene	0.035
Benzo[a]anthracene	0.36
Benzo[a]pyrene	0.884
Fluoranthene	0.027
Fluorene	0.017
SPAH	**1.323**

The PAH amounts in activated sludge is the most important concern in Europe, because the activated sludge is being used as fertilizer in agriculture and therefore the researcher focused mainly on PAH. However, WWTP2 had no biological treatment, only the influents of both treatment plants were investigated. The amounts of PAHs varied from 11 to 474 ng/L in raw runoff water in the literature [192]. Other researchers found SPAH concentration in the raw influent as 309.8 g/d in summer and 419.1 g/d in winter [193]. One reason of the high amounts is that they were found in the influents of the treatment plants receiving flows from combined collectors in Istanbul. The other reason could be the sampling periods. In winter, the citizens of Istanbul could prefer to use stoves instead of environmental friendly natural gas, for any reasons. The individual PAHs founded in Istanbul, except naphthalene, had minimum three benzene rings, which proved the sources of PAHs were anthropogenic.

4.2.3 Enzyme results

The dehydrogenase enzyme test results are given in Table 4.9. The active biomass was calculated through dividing the biomass to the activity. The enzyme test result was divided to the active biomass and multiplied with 10^3. The enzyme results are expressed mg/g in active biomass. According to Table 4.9, the dehydrogenase enzyme level was 0.05 mg/g average without B[a]A addition at the beginning of respirometer test. The average enzyme level measured at the end of respirometer tests was 0.08 mg/g. On Day 14 and 21, the enzyme levels at the end of respirometer were higher than the beginning. The increase of enzyme level at the respirometer end was an evidence of adaptation of activated sludge to SBR.

During the B[a]A addition period (Day 1 and Day 3 with B[a]A), the average enzyme levels at the respirometer start and at the end were 0.22 mg/g and 0.19 mg/g, respectively. The enzyme levels on Days 12 and 15 with B[a]A could not be determined because of the O_2 limitation. The enzyme level of Day 21 at the respirometer start and at the end were 0.29 mg/g and 0.94 mg/g, respectively. High level of dehydrogenase enzyme was determined also 7 days of operation without B[a]A addition. This result indicated that the B[a]A effect continued during this period. The previous studies had proven that the PAH addition into the soil and activated sludge increased the dehydrogenase enzyme activity in microbial community [194-196]. This study had similar results with previous studies.

Table 4.9 : Dehydrogenase enzyme results of SBR.

Day	Set No.	B[a]A addition	Enzyme-mg/L (Start)	Enzyme mg/L (End)	VSS	Active Biomass (VSS)	Activity (%)	Enzyme mg/g (Start)	Enzyme mg/g (End)
1		-	0.046	0.030	1430	715	0.50	0.06	0.04
3		-	0.049	0.031	1210	605	0.52	0.08	0.05
5		-	0.042	0.050	1555	778	0.52	0.05	0.06
10		-	0.040	0.068	1960	1078	0.55	0.04	0.06
14		-	0.044	0.151	2065	1177	0.57	0.04	0.13
21	Set 9	-	0.039	0.125	2125	1232	0.58	0.03	0.10
1	Set 10	+	0.090	0.145	1200	704	0.59	0.13	0.21
3	Set 11	+	0.272	0.149	1375	845	0.61	0.32	0.18
12		+	0.317	0.159	*	*	*	*	*
15		+	0.380	0.163	*	*	*	*	*
21	Set 12	+	0.346	1.131	1660	1197	0.72	0.29	0.94
-5	Set 13	-	0.412	0.935	1640	1197	0.73	0.34	0.78
-7		-	0.466	1.004	1535	1190	0.73	0.39	0.90

* not calculated because of the O_2 limitation

4.2.4 FAME results

The samples for FAME analysis taken from SBR at steady state. The other parameter such as dehydrogenase enzyme, SS and VSS were shown whether the SBR at steady state. The FAME analysis has a disadvantage, which the analyze gives the dominant species, not the whole species.

The FAME analysis results were given in Table 4.10. Set 9contained subgroups of *Entrococcus faecalis*, which is normally found in activated sludge. The genus *Enterococcus* is comprised of Gram-positive, microaerophilic cocci (round), which are not motile and occur in chains or pairs. The genus is defined by a combination of antigenic, haemolytic, and physiological characteristics and was formerly part of the genus *Streptococcus* [197-199].

Set 12 with B[a]A, contained *Bacillus* species, which were capable to degrade B[a]A [24, 200]. *Bacillus* is a genus of Gram-positive rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzymecatalase. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera. The cell wall of *Bacillus* is a structure on the outside of the cell that forms the second barrier between the bacterium and the environment, and at the same time maintains triangle shape and withstands the pressure generated by the cell's turgor. The cell wall is composed ofteichoic and teichuronic acids. Therefore, *Bacillus* species is persist against the environmental changes and xenobiotics [201-202].

Table 4.10 : FAME analysis results.

Day	Set No.	Species
Day 21	Set 9	*Entrococcus faecalis* – subgroup A
		Entrococcus faecalis – subgroup B
		Entrococcus faecalis – subgroup D
Day 21 with B[a]A	Set 12	*Bacillus subtilis*
		Bacillus atrophaeus
		Bacillus cereus – subgroup A

The dehydrogenase enzyme level in Set 12 (Day 21 with B[a]A) was the highest observed enzyme level. In Set 12, the second FAME sample was taken and result of the FAME analysis showed that the dominant species were different than in Set 9. The dominant species, *Bacillus*, are capable of biodegrading PAHs including B[a]A as mentioned before. During the biodegradation process, enzyme activity levels were increased. If other species were available as dominant species, the biodegradation process would not occur, so the enzyme level would not increase to the levels which could be observed in this study.

4.2.5 Respirometric results

Before starting the operation of SBR, one set F&D respirometer (Set 13) experiment was performed for comparing with the SBR. The SBR reached steady state in 14 days. Day 21 was selected for the comparison. After 21 days, B[a]A (0.011 g/L) was added into the reactor at each cycle and the chronic effect of B[a]A was observed. All respirometric sets had also PHA samples. The details from selected days were given in Table 4.3.The respirometric results were given in Figure 4.15.

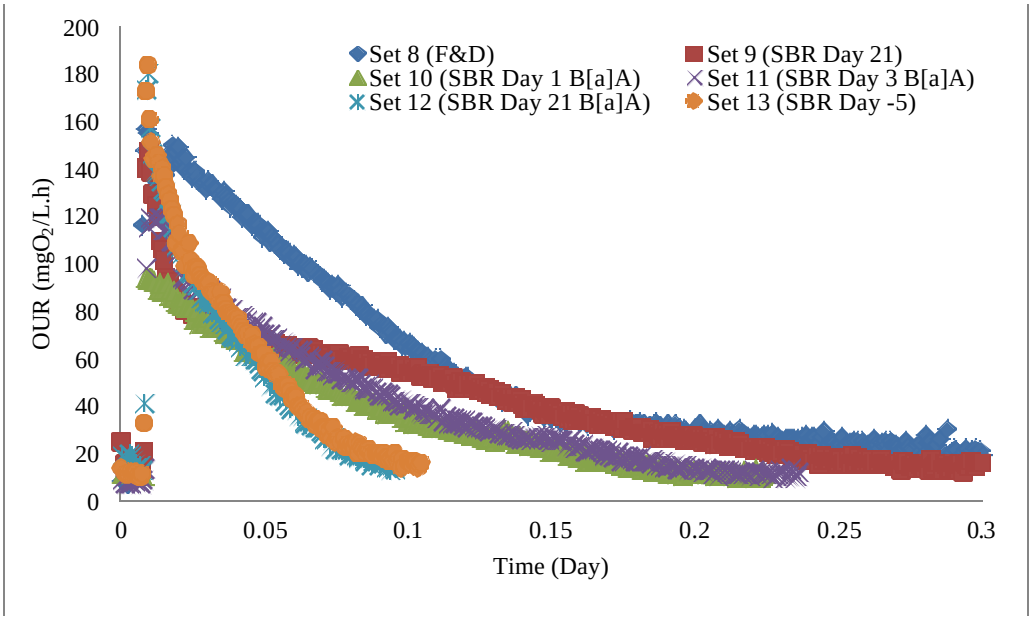


Figure 4.15 : OUR data of selected days for modelling

The Set 1 and Set 8 were respirometric results from F&D reactors with the same activated sludge and operated under the same conditions. However, the respirometric profiles of both sets were not the same, which was attributed to the use of the different peptone and meat extract.

The respirometric results of Set 8 and Set 9 are given in Figure 4.16. The difference between F&D reactor and SBR under steady state conditions are shown in Figure 4.16.

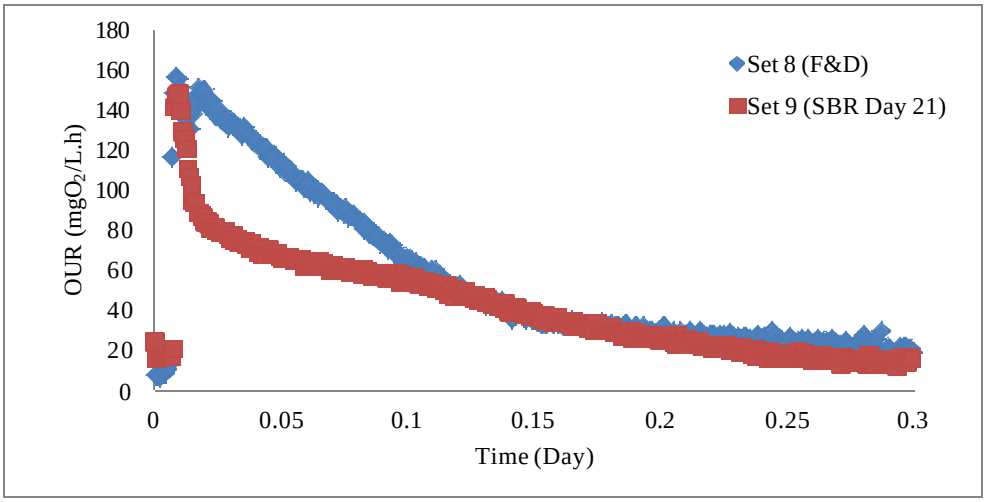


Figure 4.16 : OUR data of Set 8 and Set 9.

The respirometric results of Set 9, Set 10, Set 11 and Set 12 were given in Figure 4.17.

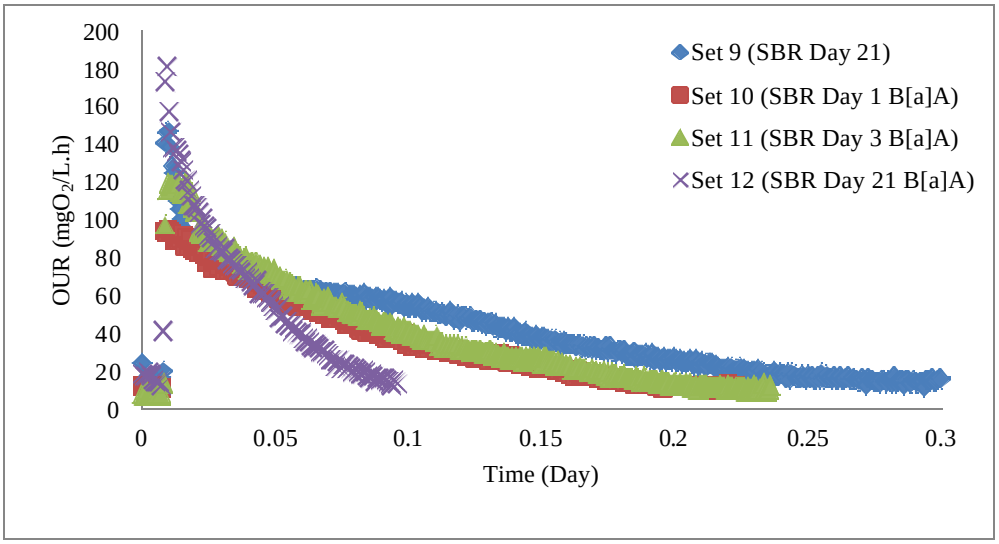


Figure 4.17 : OUR data of Set 9, Set 10, Set 11 and Set 12.

In Figure 4.17 was shown the differences between the Day 21 without B[a]A (Set 9) addition and Day 1 (Set 10), Day 3 (Set 11), Day 21 (Set 12) with B[a]A addition in SBR. The respirometric results of Set 9, Set 12 and Set 13 were given in Figure 4.18.

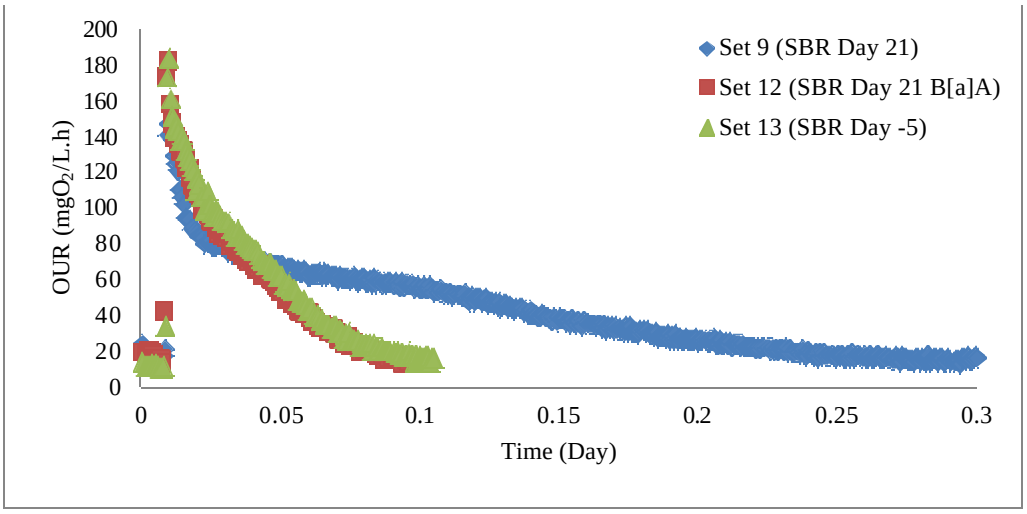


Figure 4.18 : OUR data of Set 9, Set 12 and Set 13.

In Figure 4.18 was shown the differences between Day 21 (Set 9) without B[a]A addition, Day 21 (Set 12) with B[a]A addition and Day -5 (Set 13) without B[a]A addition. The similarity of Set 12 and Set 13 pointed that the effect of B[a]A persists in Day 5 without B[a]A.

4.2.6 Modelling results

All sets, from Set 8 to Set 13, were modeled with Aquasim software. Modified ASM 3 model was used. Matrix representation of the model structure for simultaneous growth and storage was shown in Table 4.11.

Table 4.11 : SBR, Matrix representation of the model structure for simultaneous growth and storage.

Process	S_{O2}	S_S	S_H	X_H	X_{STO}	X_P	Rate equation $ML^{-3}T^{-1}$
Growth of X_H for S_S	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$		1			$\hat{m}_{H1}\frac{S_S}{K_S+S_S}X_H$
Hydrolysis of S_H		1	-1				$k_h\frac{S_{H1}/X_H}{K_X+S_{H1}/X_H}X_H$
Storage of PHA	$-(1-Y_{STO})$	-1			Y_{STO}		$k_{STO}\frac{S_S}{K_S+S_S}X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$			1	$-\frac{1}{Y_H}$		$r_G X_{STO}$
Decay of X_H	$-(1-f_P)$			-1		f_P	$b_H X_H$
Parameter, ML^{-3}	O_2	COD	COD	COD	Cell COD	COD	

The measured OUR and PHA data were shown with the circles in the model results. The model data showed with the lines.

The calculated ?O_2 of Set 8 was 295 mgCOD/L. The initial b_H level was 12.6 mgO_2/L.h. The OUR peak after feeding with peptone mixture dropped from 156.8 mgO_2/L.h to b_H level in 0.6 days (≈14 hours). The measured maximum PHA level was 121.7 mgCOD/L. The modelling results of respirometric and PHA data were showed in Figure 4.19.

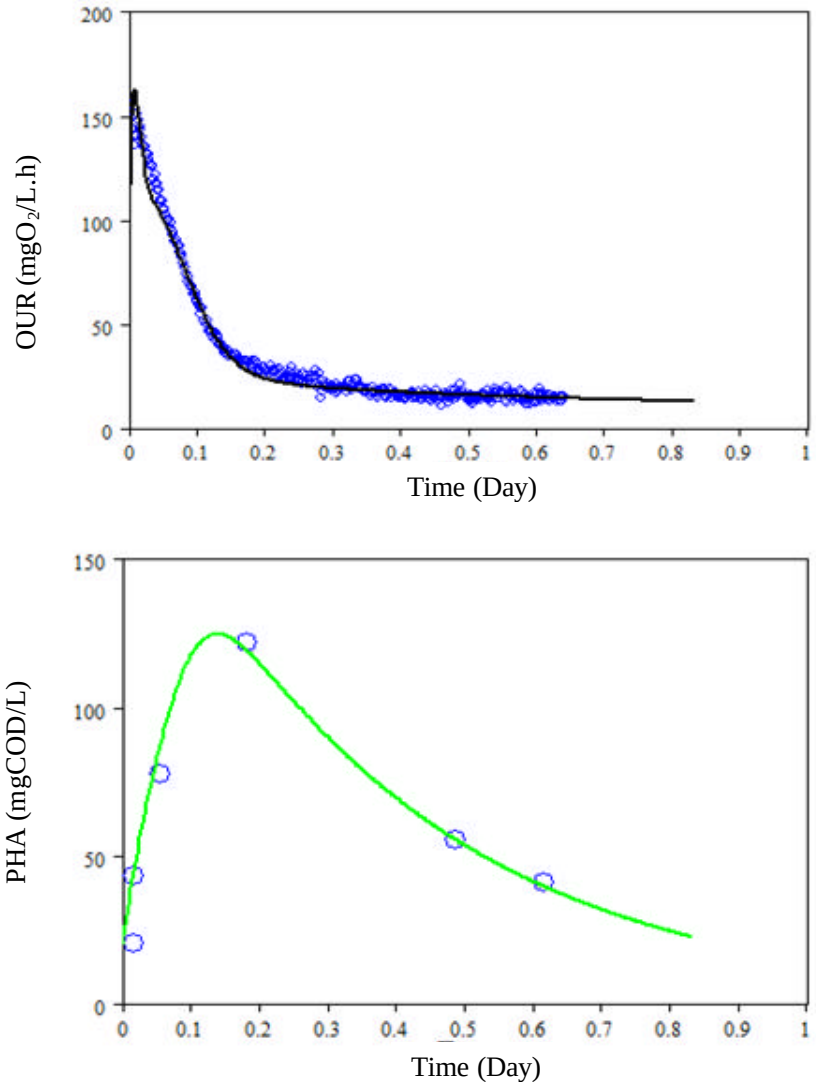


Figure 4.19 : Set 8, F&D respirometer and PHA modelling

The calculated $?O_2$ of Set 9 was 182 mgCOD/L. The initial b_H level was 16.2 $mgO_2/L.h$. The OUR peak after feeding with peptone mixture dropped from 147.1 $mgO_2/L.h$ to b_H level in 0.26 days (≈6 hours). The measured maximum PHA level was 55.6 mgCOD/L. The modelling results of respirometric and PHA data were shown in Figure 4.20.The effect of SBR was shortening the time that needed drop of OUR peak to b_H level. In addition, the maximum PHA level was decreased from 121.7 mgCOD/L to 55.6 mgCOD/L. The $?O_2$ evaluated from the area under OUR curve by 10 sludge ages, peptone mixture fed and as SBR operated mixed culture, as defined previous study [203], was calculated as 227 mgCOD/L, which was a nearly result founded by Set 9 as 182 mgCOD/L.

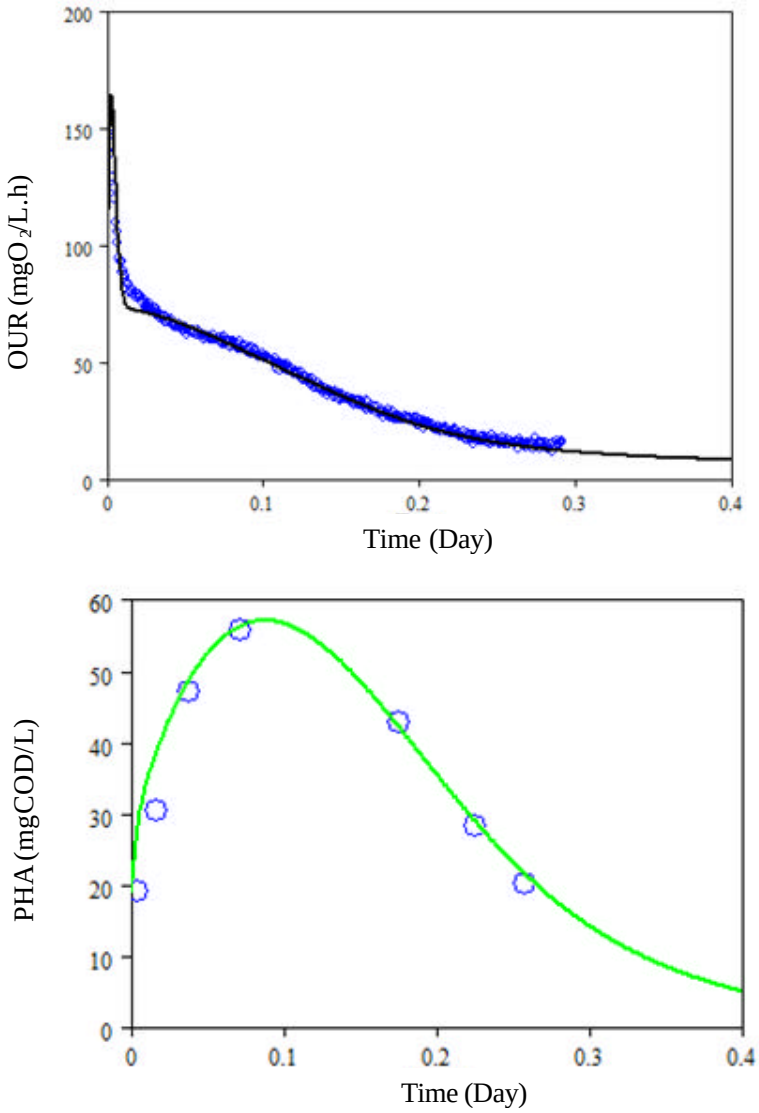


Figure 4.20 : Set 9, Day 21 respirometer and PHA modelling.

The calculated $?O_2$ of Set 10 was 166 mgCOD/L. The initial b_H level was 10.2 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A addition dropped from 94.6 mgO_2/L.h to b_H level in 0.22 days (≈5.2 hours). The measured maximum PHA level was 48.3 mgCOD/L. The modelling results of respirometric and PHA data were shown in Figure 4.21.

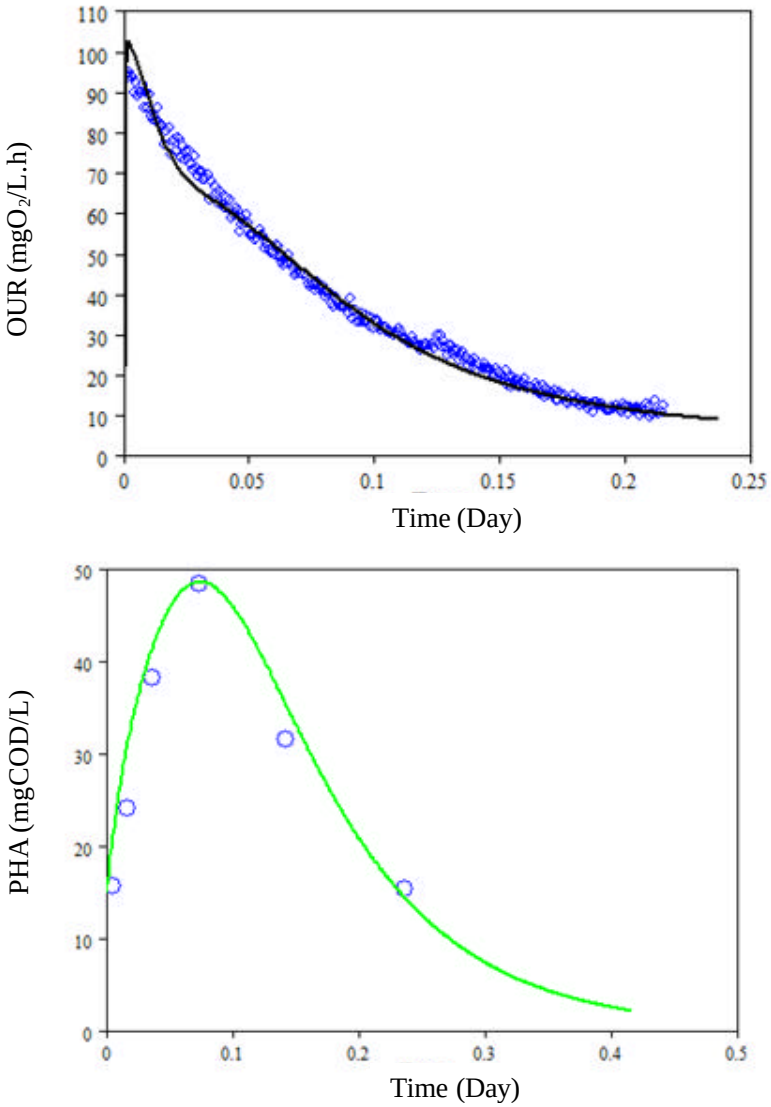


Figure 4.21 : Set 10, Day 1 B[a]A respirometer and PHA modelling.

The calculated $?O_2$ of Set 11 was 173 mgCOD/L. The initial b_H level was 10.2 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A addition dropped from 120.7 mgO_2/L.h to b_H level in 0.23 days (≈5.5 hours). The measured maximum PHA level was 53.3 mgCOD/L. The modelling results of respirometric and PHA data were shown in Figure 4.22.

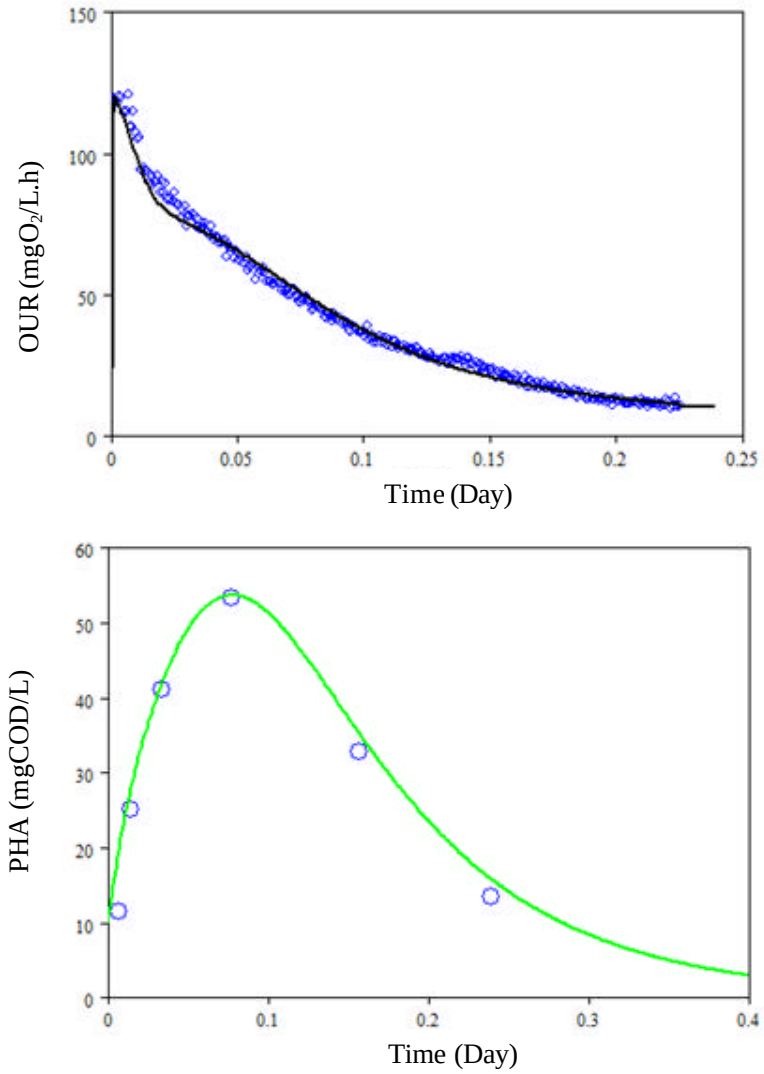
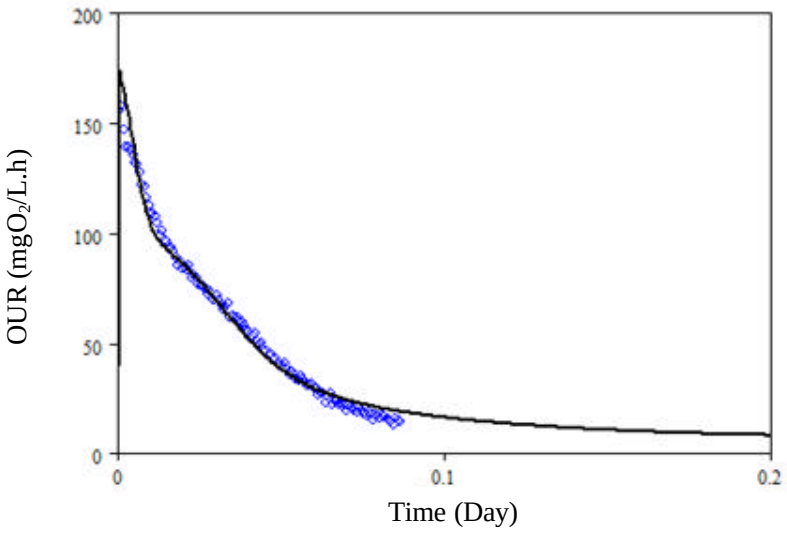


Figure 4.22 : Set 11, Day 3 B[a]A respirometer and PHA modelling

The calculated $?O_2$ of Set 12 was 94 mgCOD/L. The initial b_H level was 13.9 mgO_2/L.h. The OUR peak after feeding with peptone mixture and B[a]A addition dropped from 157.4 mgO_2/L.h to b_H level in 0.08 days (≈2 hours). The measured maximum PHA level was 44.6 mgCOD/L. The modelling results of respirometric and PHA data were shown in Figure 4.23.



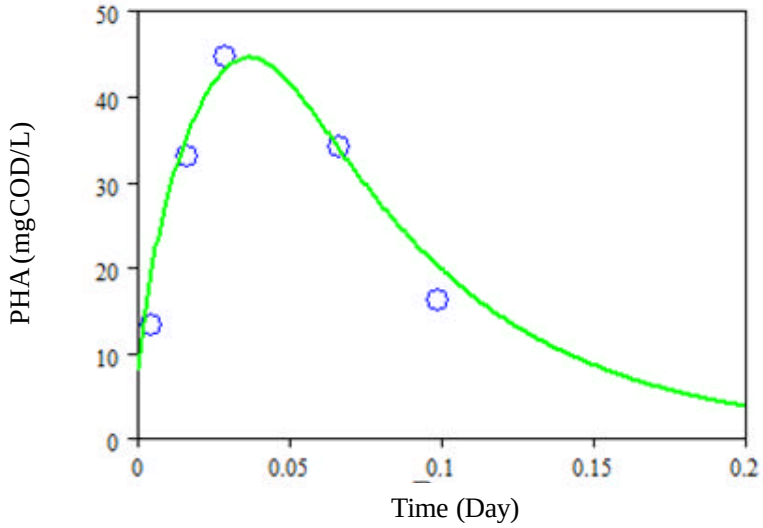


Figure 4.23 : Set 12, Day 21 B[a]A respirometer and PHA modelling

The calculated $?O_2$ of Set 13 was 100 mgCOD/L. The initial b_H level was 13.4 mgO_2/L.h. The OUR peak after feeding with peptone mixture dropped from 172.6 mgO_2/L.h to b_H level in 0.08 days (≈2 hours). The measured maximum PHA level was 48 mgCOD/L. The modelling results of respirometric and PHA data were shown in Figure 4.24.

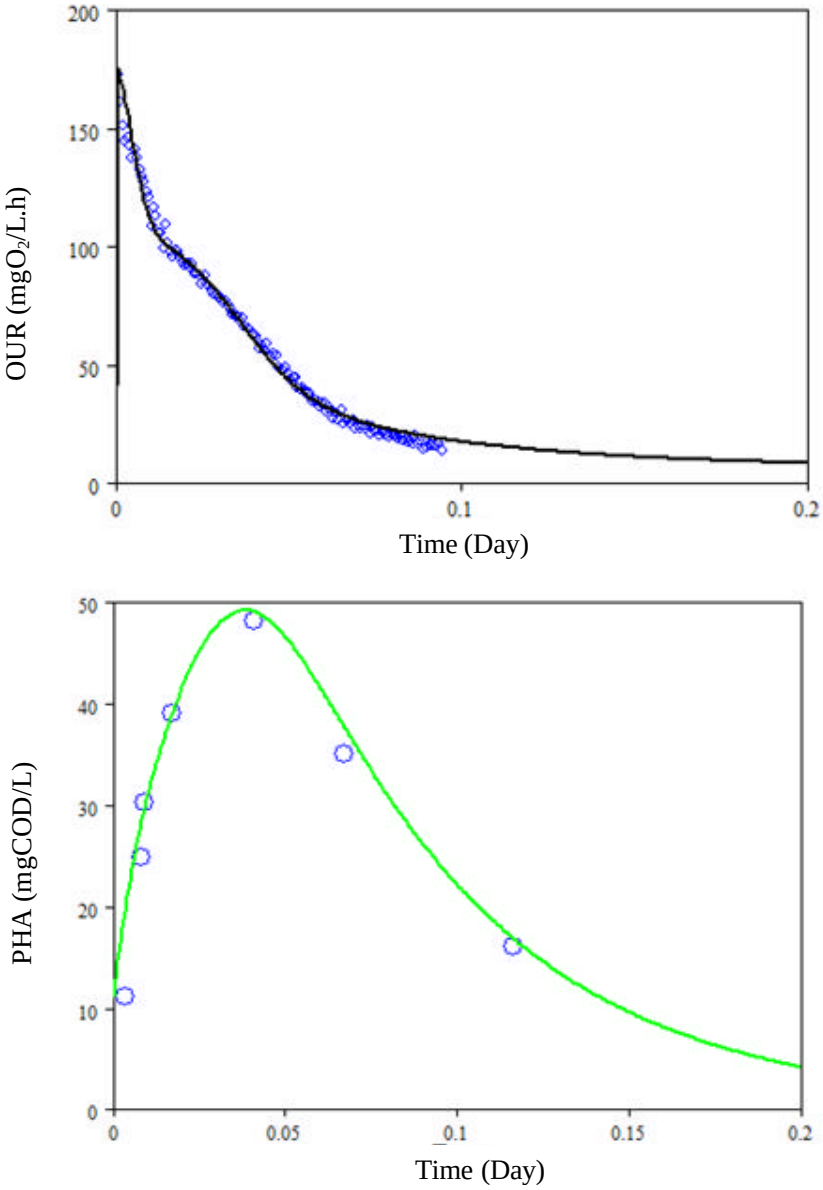


Figure 4.24 : Set 13, Day 5 without B[a]A addition, respirometer and PHA modelling.

The kinetic modelling results from Set 8 and Set 9 were shown in Table 4.12.

Table 4.12 : Set 8 and Set 9 modelling results.

Model Parameter & State Variables	**Set 8 (F&D)**	**Set 9 (Day 21)**
B[a]A addition	-	-
Maximum growth rate for X_H, μ_{Hmax}	6.3	5.5
Half saturation constant for growth of X_H, K_S	28	23
Maximum hydrolysis rate for S_H, k_h	8.3	6
Hydrolysis half saturation constant for S_H, K_X	0.26	0.34
Maximum storage rate of PHA, k_{STO}	1.6	2
Maximum growth rate for growth on PHA, r_G	1.7	7
Endogenous decay rate for X_H, b_H	0.2	0.2
Heterotrophic yield coefficient, Y_H	0.65	0.65
Half Saturation Constant for Storage of PHA by X_H, K_{STS}	9.5	9.5
Storage yield of PHA, Y_{STO}	0.8	0.8
State Variables		
Added Initial Biodegradable Peptone COD	700	500
Initial Biodegradable Peptone COD	784	510
Initial Readily Biodegradable COD, S_S	71	45
Initial Readily Hydrolysable COD, S_H	713	465
Initial PHA Concentration	20.27	18.83
Initial active Heterotrophic biomass, X_H	1400	1750
Volatile Suspended Solids	1500	2125
Activity (%)	50	58

Maximum growth rate (μ_{Hmax}), half saturation constant for growth of X_H (K_S), maximum hydrolysis rate for S_H (k_h), hydrolysis half saturation constant for S_H (K_X), maximum storage rate of PHA by X_H (k_{STO}) and maximum growth rate for growth on PHA for X_H (r_G) were all affected from the SBR conditions. The values for μ_{Hmax}, K_S, k_h decreased and the values for K_X, k_{STO}, r_G increased when the operation of the reactor was switched from F&D mode to the SBR mode. It was seen that the researchers operated F&D and SBR using *E. coli* as pure culture. They found that parameters changed with the changing reactor conditions [204]. Also in this study, employing a mixed culture, the parameters showed difference with the changing reactor conditions. The SBR operating conditions increased also the activity from 50% to 58%.

The kinetic modelling results from Set 9, Set 10, Set 11 and Set 12 were showed in Table 4.13.

Table 4.13 : Set 9, Set 10, Set 11 and Set 12 modelling results.

Model Parameter & State Variables	**Set 9 (Day 21)**	**Set 10 (Day 1)**	**Set 11 (Day 3)**	**Set 12 (Day 21)**
B[a]A addition	-	+	+	+
Maximum growth rate for X_H, μ_{Hmax}	5.5	5.5	5.5	5.5
Half saturation constant for growth of X_H, K_S	23	23	23	23
Maximum hydrolysis rate for S_H, k_h	6	8	8	6
Hydrolysis half saturation constant for S_H, K_X	0.34	0.25	0.25	0.063
Maximum storage rate of PHA, k_{STO}	2	2	2	2.5
Maximum growth rate for growth on PHA, r_G	7	7	7	11
Endogenous decay rate for X_H, b_H	0.2	0.2	0.2	0.2
Heterotrophic yield coefficient, Y_H	0.65	0.65	0.65	0.65
Half Saturation Constant for Storage of PHA by X_H, K_{STS}	9.5	9.5	9.5	9.5
Storage yield of PHA, Y_{STO}	0.8	0.8	0.8	0.8
State Variables				
Added Initial Biodegradable Peptone COD	500	500	500	250
Initial Biodegradable Peptone COD	510	370	430	250
Initial Readily Biodegradable COD, S_S	45	45	45	45
Initial Readily Hydrolysable COD, S_H	465	325	225	205
Initial PHA Concentration	18.83	15.67	11	13.06
Initial active Heterotrophic biomass, X_H	1750	1000	1200	1700
Volatile Suspended Solids	2125	1200	1375	1660
Activity (%)	58	58	61	72

In order to prevent O_2 limitation, the regular amount of biomass used was added in half to the Set 12. Due to this fact, for this set, initially added biodegradable peptone was 250 mgCOD/L instead of 500 mgCOD/L. The substrate intake was affected by the B[a]A addition in Set 10 and Set 11. In addition, maximum hydrolysis rate for S_H (k_h) andhydrolysis half saturation constant for growth of X_H (K_X) were affected by the B[a]A addition. The parameters; maximum growth rate for growth on PHA for X_H (r_G) and maximum growth rate of PHA by X_H (k_{STO}), were only different from other sets on Day 21 with B[a]A addition. The difference between Set 10 and Set 11 was only the activity of activated sludge. Also in Set 12, FAME analysis proved the change of species.

The kinetic modelling results from Set 9, Set 12 and Set 13 are shown in Table 4.14

Table 4.14 : Set 9, Set 12 and Set 13 modelling results.

Model Parameter & State Variables	**Set 9 (Day 21)**	**Set 12 (Day 21)**	**Set 13 (Day -5)**
B[a]A addition	-	+	-
Maximum growth rate for X_H, μ_{Hmax}	5.5	5.5	5.5
Half saturation constant for growth of X_H, K_S	23	23	23
Maximum hydrolysis rate for S_H, k_h	6	6	5.72
Hydrolysis half saturation constant for S_H, K_X	0.34	0.063	0.055
Maximum storage rate of PHA, k_{STO}	2	2.5	2.5
Maximum growth rate for growth on PHA, r_G	7	11	11
Endogenous decay rate for X_H, b_H	0.2	0.2	0.2
Heterotrophic yield coefficient, Y_H	0.65	0.65	0.65
Half Saturation Constant for Storage of PHA by X_H, K_{STS}	9.5	9.5	9.5
Storage yield of PHA, Y_{STO}	0.8	0.8	0.8
State Variables			
Added Initial Biodegradable Peptone COD	500	250	250
Initial Biodegradable Peptone COD	510	250	270
Initial Readily Biodegradable COD, S_S	45	45	45
Initial Readily Hydrolysable COD, S_H	465	205	225
Initial PHA Concentration	18.83	13.06	12.01
Initial active Heterotrophic biomass, X_H	1750	1700	1700
Volatile Suspended Solids	2125	1660	1660
Activity (%)	58	72	72

In order to prevent O_2 limitation, the regular amount of biomass used was added in half to the Set 13. Due to this fact, for these sets, initial biodegradable peptone was not 500 mg COD/L. Set 12 and Set 13 had shown the same activity level, which was higher than other sets. The Set 13 had lower hydrolysis half saturation constant for growth of X_H (K_X) value than Set 12 although the other values were equal. The effect of B[a]A could be seen after 5 days of operation without B[a]A addition.

The dehydrogenase enzyme level increased by B[a]A addition in Day 3 (Set 11). The maximum enzyme level was reached in Day 21 (Set 12). Nevertheless, the enzyme activity indicated that the effect of B[a]A continued in Day -5 (Set 13) and in Day -7.

5. CONCLUSIONS

In this study, it was found that the operating conditions of a reactor such as F&D or SBR affects the values of the kinetic parameters attained in the biological systems.

The acute test showed that the B[a]A addition had an adverse effect on maximum hydrolysis rate for S_H (k_h), on maximum storage rate of PHA by X_H (k_{STO}) and on maximum growth rate for growth on PHA for X_H (r_G). As the B[a]A loads were increased, also the adverse effects of B[a]A on PHA storage mechanism on activated sludge were increased.

The chronic test showed that for 21 day operation with a constant B[a]A load affects the maximum hydrolysis rate for S_H (k_h), hydrolysis half saturation constant for growth of X_H (K_X) and the PHA storage mechanism. It was observed that the dehydrogenase enzyme and O_2 uptake rate increased with operation time. The FAME analysis showed that the dominant species changed with B[a]A addition in the chronic test.

The presence of trace PAHs in activated sludge is an important concern in countries, which use the activated sludge in land applications; such as fertilizers. Application of activated sludges as fertilizers can lead to PAHs accumulation in the soils, as well as their migration to the food chain. The EU countries have a standard for PAHs in sewage sludge. Other developed countries, such as USA, have standards for PAHs in drinking water. As Turkey is currently under the EU accession process, related regulations on activated sludge has been transposed to domestic regulations by the Turkish authority. Adaption of PAH regulation is also important for Turkey as agriculture is practiced wide-spread and the uses of activated sludge as fertilizers in agriculture will be continued for economical reasons.

The PAHs in wastewater treatment plants in Istanbul/Turkey were first investigated in this study. The values of SPAHs ranged between 1.29 to 1.90 mg/L in the influents. Because the limit proposed in the draft directive of the EU is only for the activated sludge (6 $mgkg^{-1}$), a comparison could not be made with PAH values from the Istanbul WWTPs influents.

The PAH levels in Turkey seas was investigated in previously studies. However, unfortunately, there is not enough data for municipal wastewater treatment plants. This study is a first step to create a database for municipal wastewater treatment plants. Nevertheless, the treatment plants have to be observed for more than one season. The season, which was selected for this study, was winter. The amounts of SPAHs in influents were ranging between 1.29 to 1.90 mg/L. These amounts will probably be lower in summer season.

The measurement with HLPC in activated sludge has showed the accumulation and desorption properties of B[a]A. This result is an evidence how toxic can be the B[a]A in the environment.

The PAH measurement with HPLC or GC in wastewater and activated sludge is an expensive method, needing high investments. In addition, the solvents, which were used in these methods, are expensive and not environmental friendly. However, the methods are critical to measure the amounts. Using these methods periodically will also be very expensive.

Dehydrogenase enzyme is an enzyme, which is increase by the xenobiotic biodegrading. Measuring dehydrogenase enzyme is a good alternative to monitor the changes in treatment plant. It is cost effective due to very low initial investment. The application is also very simple and fast. The method is sensitive for xenobiotics. This study proved that dehydrogenase enzyme tests supplies the requirements to observe the changes in activated sludge with the addition B[a]A. Under the steady state condition, the activated sludge in SBR had almost the constant level of dehydrogenase enzymes. With the addition B[a]A into the SBR, the changes could be measured after the first day of operation. It was found that with the B[a]A addition the dehydrogenase enzyme level increase.

One important effect of B[a]A in acute and chronic tests was the change of kinetic parameters, especially the storage kinetics of PHA. In addition, the PHA measurements were showed that the B[a]A addition decrease the PHA storage. The other important effect was observed in the chronic test. The change of species has occurred and *Bacillus* species were found to be the dominant species, which is resistance to the PAHs.

PAHs can enter in to the treatment plants through exhaust gases, car oil and wash services, rainfalls, forest fires etc. Even these intrusions will be not permanent; it can adversely impact the activated sludge process. It is important to monitor the full-scale wastewater treatment plant for operation and management purposes. Dehydrogenase enzyme test gives this opportunity.

PAH levels in the treatment plants in Turkey shall be measured for four seasons in order to create a database. If in any treatment plants, the PAH levels are permanently high, a new process can be applied such as using different microorganism such as *Bacillus* species, which are capable of biodegrading PAHs and resisting to PAHs. Extracellular enzyme addition can be an another option to decrease PAH levels. A new process can help to decrease the levels in the effluent, to operate easily and to control the operation of treatment plant.

REFERENCES

[1] **Url-1** <*http://msds.chem.ox.ac.uk/glossary/xenobiotic.html*>, accessed at 07.06.2010.

[2] **Url-2** <*http://en.wikipedia.org/wiki/Xenobiotic*>, accessed at 12.07.2010.

[3] **Katipoglu, T.,** 2007: Evaluation of acclimation and inhibitory impact of 2,6-Dibenzoic acid on the biodegradation of peptone under aerobic conditions, *Master Thesis*, ITU, Istanbul.

[4] **Schmidt-Bleek, F., and Haberland, W.,** 1980: The yardstick concept for the hazard evaluation of substances, *Ecotoxico. Environ. Safet.*, **4**, 455.

[5] **Ross, P., and Birnbaum, L.,** 2003: Integrated human and ecological risk assessment: A case study of persistent organic pollutant risk to human and wildlife.*Human and Ecological Risk Assessment*, **9**, 303–324.

[6] **U.S. Deparment of Health and Human Services**, 1995: Public Health Service, Agency for Toxic Substances and Disease Registry, *Toxicolocigal Profile for Polycyclic Aromatic Hydrocarbons*, p. 1-487. US Government Printing Office, USA.

[7] **Anders, R. J., Lukas Y. W., and Hauke H.,** 2005: Principles of microbial PAH-degradation in soil, *Environmental Pollution.*,**133,** 71-84.

[8] **Andrea, G., Loredana, S., Francesco, P., Raffaele, C., and Giuseppe, B.,** 2005: Sequencing batch reactor performance treating PAH contaminated lagoon sediments, *Journal of Hazardous Materials.*, **B119**, 159-166.

[9] **Howsam, M., and Jones, K. C.,** 1998: Sources of PAHs in the environment. In: Neilson AH (ed). Anthropogenic compounds. PAHs and related compounds, Springer, Berlin, Germany.

[10] **Blumer, M.,** 1976: Polycyclic aromatic compounds in nature. *Scientific American.*, **234**, 3, 35-45.

[11] **Kanaly, R. A., and Harayama, S.,** 2000: Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria, *J. Bacteriol.*, **182**, 2059-2067.

[12] **Url-3** <*umu.diva-portal.org/smash/get/diva2:143820/FULLTEXT01*>, accessed at 04.06.2010.

[13] **Url-4** <*http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s150pah.pdf*>, accessed at 04.06.2010.

[14] **Wattiau, P., Springael, D., Agathos, S. N., and Wuertz, S.,** 2002: Use of the pAL5000 replicon in PAH-degrading mycobacteria: application for strain labelling and promoter probing, *Appl Microbiol Biotechnol.,* **59**, 700-705

[15] **Wilson, S. C., and Jones, K. C.,** 1993: Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs) - A review. *Environ. Pollut.,* **81**, 229-249.

[16] **Cerniglia, C. E.,** 1992: Biodegradation of polycyclic aromatic hydrocarbons, *Biodegradation.,* **3,** 351-368.

[17] **Mackay, D., Shiu, W. Y., and Ma, K. C.,** 1992: Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals: *Polynuclear aromatic hydrocarbons, polychlorinated dioxins and dibenzofurans.* Lewis Publishers, Chelsea, Michigan, USA.

[18] **Brandli, R., Bucheli, T. D., Kupper, T., Mayer, J., Stadelmann, F. X., and Taradellas, J.,** 2007: Fate of PCBs, PAHs and their source characteristic ratios during composting and digestion of source-separated organic waste in full-scale plants, *Environ. Pollut.*. doi:10.1016/j.envpol.2006.11.021.

[19] **Cai, Q. Y., Mo, C. H., Li, Y. H., Zeng, Q. Y., Katsoyiannis, A., Wu, Q. T., and Ferard, J. F.,** 2007: Occurrence and assessment of polycyclic aromatic hydrocarbons in soils from vegetable fields of the Pearl River Delta, South China, *Chemosphere.,* **68**, 159-168.

[20] **Athanasios, K., Eleni, T., and Quan-Ying, C.,** 2007: On the use of PAH molecular diagnostic ratios in sewage sludge for the understanding of the PAH sources. Is this use appropriate? *Chemosphere.,* **69**, 1337-1339.

[21] **Dai, J., Xu, M., Chen, J., Yang, X., and Ke, Z.,** 2007: PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China, *Chemosphere.,* **66**, 353-361.

[22] **Blanchard, M., Teil, M. J., Ollivon, D., Legenti, L., and Chevreuil, M.,** 2004: Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewaters and sewage sludges from the Paris area (France), *Environ. Res.,* **95**, 184-197.

[23] **Karvelas, M., Katsoyiannis, A., and Samara, C.,** 2003: Occurrence and fate of heavy metals in the wastewater treatment process, *Chemosphere.*, **53,** 1201-1210.

[24] **Albert, L. and Juhasz, R. N.,** 2000: Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of Benzo[a]pyrene, *International Biodeterioration & Biodegradation*., **45,** 57-88.

[25] **Freeman, D. J., and Cattell, F. C. R.,** 1990: Wood burning as a source of atmospheric polycyclic aromatic hydrocarbons, *Environmental Science and Technology.,* **24,** 1581-1585.

[26] **Sexton, K., Liu, K., Hayward, S. B., and Spengler, J. D.,** 1985: Characterization and source apportionment of wintertime aerosol in a wood-burning community, *Atmospheric Environment*, **19,** 1225-1236.

[27] **Greenberg, A., Darack, F., Harkov, R., Lioy, P., and Daisey, J.,** 1985: Polycyclic aromatic hydrocarbons in New Jersey: a comparison of winter and summer concentrations over a 2-year period, *Atmospheric Environment.,* **19,** 1325-1339.

[28] **Jones, K. C., Stratford, J. A., Tidridge, P., Waterhouse, K. S., and Johnston, A. E.,** 1989: Polynuclear aromatic hydrocarbons in an agricultural soil: long-term changes in profile distribution, *Environmental Pollution.,* **56,** 337-351.

[29] **Jones, K. C., Stratford, J. A., Waterhouse, K. S., Furlong, E. T., Giger, W., Hites, R. A., Schaffner, C., and Johbston, A. E.,** 1989: Increases in the polynuclear aromatic hydrocarbon content of an agricultural soil over the last century, *Environmental Science and Technology.,* **23,** 95-101.

[30] **Jones, K. C., Stratford, J. A., Waterhouse, K. S., and Vogt, N. B.,** 1989: Organic contaminants in Welsh soil: Polynuclear aromatic hydrocarbons, *Environmental Science and Technology.,* **23,** 540-550.

[31] **Youngblood, W. W., and Blumer, M.,** 1975: Polycyclic aromatic hydrocarbons in the environment: homologous series in soils and recent marine sediments, *Geochimica et Cosmochimica Acta.,* **39,** 1303-1314.

[32] **Laflamme, R. E., and Hites, R. A.,** 1978: The global distribution of polycyclic aromatic hydrocarbons in recent sediments, *Geochimica et Cosmochimica Acta.,* **42,** 289-303.

[33] **Shiaris, M. P., and Jambard-Sweet, D.,** 1986: Polycyclic aromatic hydrocarbons in surficial sediments of Boston Harbour, MA, USA, *Marine Pollution Bulletin.,* **17,** 469-472.

[34] **Cerniglia, C. E., and Heitkamp, M. A.,** 1989: Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment, p. 41–68. In U. Varanasi (ed.). *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment.* CRC Press, Inc., Boca Raton, Fla.

[35] **Nishioka, M., Chang, H. C., and Lee, V.,** 1986: Structural charactristics of polycyclic aromatic hydrocarbon isomers in coal tars and com bustion products, *Environmental Science and Technology.,* **20,** 1023-1027.

[36] **Lijinsky, W.,** 1991: The formation and occurence of polynuclear aromatic hydrocarbons associated with food, *Mutation Research.,* **259,** 251-262.

[37] **Dipple, A., and Bigger, C. A. H.,** 1991: Mechanism of action of food-associated polycyclic aromatic hydrocarbon carcinogens, *Mutation Research.,* **259,** 263-276.

[38] **Eisler, R.,** 1987: U.S. Fish and Wildlife Service, Polycyclic Aromatic Hydrocarbon Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. Final ed. Patuxent Wildlife Research Center, Laurel, MD 20708: U.S. Department of the Interior; *Contaminant Hazard Reviews*, Report No. 11. p. 81.

[39] **Url-5** <*http://en.wikipedia.org/wiki/Deepwater_Horizon_oil_spill*>, accessed at 05.08.2010.

[40] **IARC,** 1984: Polynuclear Aromatic Compounds, Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron and Steel Founding. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, 34, Lyon, France: *International Agency for Research on Cancer*, **3,** p. 219.

[41] **Baumard, P., Budzinski, H., Garrigues, P., Dizer, H., and Hansen, P. D.,** 1999: Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the western Baltic Sea: occurrence, bioavailability and seasonal variations, *Marine Environmental Research.*, **47,** 17-47.

[42] **USEPA,** 1993: Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons. *EPA/600/R-93/089*, July 1993.

[43] **Sepic, E., Bricelj, M., and Leskovsek, H.,** 1997: Biodegradation studies of polyaromatic hydrocarbons in aqueous media, *Journal of Applied Microbiology*, **227**, 83, 561-568.

[44] **Anuradha, M. D.,** 2005: Biodegradability of select polycyclic aromatic hydrocarbon (PAH) Mixtures, *Master Thesis,* Texas A&M University, USA.

[45] **Hurst, C.J., Sims, R. C., Sims, J. L., Sorensen, D. L., McLean, J. E., and Huling, S.,** 1995: Polycyclic Aromatic HydrocarbonBiodegradation as a Function of OxygenTension in Contaminated Soil, *Proceedings of the 10th Annual Conference on Hazardous Waste Research*, Manhattan, Kansas, USA, May 23-24. pp. 20-32.

[46] **Humphries, L. F.,** 2006: Effects of polycyclic aromatic hydrocarbon exposure on three life stages of freshwater mussels (Bivalvia: Unionidae), *Master Thesis*, Raleigh, NC, USA.

[47] **Sudip, K. S., Om, V. S., and Rakesh K. J.,** 2002: Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, *Trends in Biotechnology*, **20**(6), 243-248.

[48] **Goldman, R., Enewold L., Pellizzari E., Beach, J. B., Bowman, E. D., Krishnan, S. S., and Shields, P. G.** 2001: Smoking increase carcinogenic polycyclic aromatic hydrocarbons in human lung tissue, *Cancer Res.*, **61,** 6367–6371.

[49] **Url-6** <*http://ec.europa.eu/environment/waste/sludge/pdf/organics_in_sludgs.pdf*>, accessed at 05.06.2010

[50] **Selina, M, B., and Ian S.,** 2005: Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions, *J Chem Technol Biotechnol.,* **80,** 723–736.

[51] **Brooks, K. M.,** 1997: Computer Model and Assessment of the Potential Environmental Risks Associated With Creosote Treated Wood Products Used in Aquatic Environments, *Literature Review*, pp. 138, WA 98368, USA.

[52] **Roland, E. L., and Donald M. J.,** 1977: Metabolic Activations of Polycyclic Hydrocarbons Structure-Activity Relationships, Arch, *Toxicol.,* **39,** 1-6.

[53] **Lehr, R. E., Schaefer-Ridder, M., and Jerina, D. M.,** 1977: Synthesis and properties of the vicinal trans dihydrodiols of anthracene, phenanthrene, and Benzo(a)anthracene, *J. Org. Chem.,* **42,** 736.

[54] **Lehr, R. E., Schaefer-Ridder, M., and Jerina, D. M.,** 1977: Synthesis and reactivity of diol epoxides derived from non-K-region trans-dihydrodiols of Benzo(a)anthracene, *Tetrahedron Lett.,* 539-542.

[55] **Eoma, I. C., Rast, C., Veber, A. M., and Vasseur, P.,** 2007: Ecotoxicity of a polycyclic aromatic hydrocarbon(PAH)-contaminated soil, *Ecotoxicology and Environmental Safety*, **67**, 190–205.

[56] **Url-7** *<http://www.irmm.jrc.be/html/reference_materials_catalogue/catalogue/attachements/BCR-271_msds.pdf>*, accessed at 11.08.2010.

[57] **Url-8** *<http://www.toronto.ca/health/pdf/cr_appendix_b_pah.pdf>*, accessed at 09.07.2010

[58] **MOE (Ministry of the Environment),** 1997: Scientific Criteria Document for Multimedia Standards Development. Polycyclic Aromatic Hydrocarbons (PAH). *Part 1: Hazard Identification and Dose-Response Assessment*.pp.269, Ministry of the Environment, Toronto, Ontario.

[59] **CEPA (Canadian Environmental Protection Act),** 1994: Polycyclic Aromatic Hydrocarbons. Environment Canada and Health Canada, Ottawa, Ontario. EN40-215/42E, ISBN : 0-662-22209-1, Canada.

[60] **Url-9** *<http://www.arb.ca.gov/toxics/id/summary/bap.pdf>* accessed at 07.07.2010.

[61] **Shiaris, M. P.,** 1989: Phenanthrene mineralisation along a natural salinity gradient in an urban estuary, Boston Harbor, MA, *Microbial Ecology.,* **18,** 135-146.

[62] **Mueller, J. G., Chapman, P. J., and Pritchard, P. H.,** 1990: Action of a fluoranthene-utilising bacterial community on polycyclic aromatic hydrocarbon components of creosote, *Applied and Environmental Microbiology.,***55,** 3085-3090.

[63] **Marmo, L.,** 2000: Sewage sludge and the Community waste strategy.-in: Langenkamp H. & L. Marmo (eds.) (2000): *Workshop on Problems Around Sewage Sludge (NO) Italy Proceedings* - European Commissions Joint Research Center, EUR 19657 EN, 242, p. 17-24. 18-19 November Italy.

[64] **Zheng, X-J., Blais, J-F., Mercier, G., Bergeron, M., and Drogui**, **P.,** 2007: PAH removal from spiked municipal wastewater sewage sludge using biological, chemical and electrochemical treatments, *Chemosphere*, **68**, 1143–1152.

[65] **BMU** (Bundesumweltministerium), 1999: Organische Schadstoffe bei der Klärschlammverwertung. *Bericht des Bundesumweltministeriums über die Notwendigkeit der Festlegung zusätzlicher Regelungen*.-Abfall-brief, 4/99, 10-13. Bonn, Germany.

[66] **UM** (Umweltministerium Baden-Württemberg Hrsg), 1996: Vierte Verwaltungsvorschrift des Umweltministeriums zum Bodenschutzgesetz über die Ermittlung und Einstufung von Gehalten organischer Schadstoffe im Boden (VwV Organische Schadstoffe).-*Gemeinsames Amtsblatt des Landes Baden-Württemberg* (GABL), Nr. 2, 87-94, Stuttgart, Germany.

[67] **DK-EPA**, 1996: Analysis parameters for the assessment of the use of wastewater sludge on farmland, *Working Report from DK-EPA* **No. 19**, København, Denmark.

[68] **DK-EPA**, 1996: Ecological assessment of the use of wastewater sludge on farmland, *Working Report from DK-EPA* **No.43,** København, Denmark.

[69] **Madsen, T., Kristensen P., Samsö-Petersen, L., Törslöv, J. and Rasmussen J. O.,** 1997: Application of sludge on farmland - quality objectives, level of contamination and environmental risk assessment.-*Specialty conference on management and fate of toxic organics in sludge applied to land*. Copenhagen, 30 April - 2 May.

[70] **Jones, K. C., and Northcott G. L**., 2000: Organic Contaminants in Sewage Sludges: A Survey of UK Samples and a Consideration of their Significance.-*Final Report to the Department of the Environment*, Transport and the Regions. Water Quality Division, UK.

[71] **Paulsrud, B., Wien, and Nedland, K. T.,** 1998: A survey of toxic organics in Norwegian sewage sludge, compost, and manure. *8th Annual International Conference of the FAO ESCORENA Network of Recycling of Agricultural, Municipal and Industrial Residues in Agriculture* (Formerly Animal Waste Management), May 26–28, Rennes, France.

[72] **U.S. Environmental Protection Agency,** 1999: Biosolids generation, use and disposal in the United States. *Municipal and Industrial Solid Waste Division, Office of Solid Waste*, EPA530-R-99-009, p. 74.USA.

[73] **Ellen, Z. H., Summer, R. O., Matthew, H., and Anthony, H.,** 2006: Organic chemicals in sewage sludges, *Science of the Total Environment*, **367,** 481-497.

[74] **Twardowska, I., Schramm, K-W., and Berg, K.,** 2004: Sewage sludge, Solid Waste: Assessment, Monitoring and Remediation. 4, p. 239-295, *Waste Management Serie*, Elsevier Ltd, Netherlands.

[75] **Hale, R. C., La Guardia, M. J., Harvey, E., Gaylor, M. O., Ciparis, S., Elizabeth, B. O., Jacobs, M., Mainor, M., and Lioy, P. J.,** 2002: Sewage sludges as a sink and source of polybrominated diphenyl ethers: a multinational comparison. *SETAC 23rd Annual Meeting in North America,* November 2002, Salt Lake City, SETAC Office, Pensacola, FL, (Abstract Book, p. 39).

[76] **Url-10** *<http://ec.europa.eu/environment/waste/sludge/pdf/sludge_pollutants_xsum.pdf>*, accesed at 11.05.2010

[77] **Eric, T., and Dominique, P.,** 2006: Successful Treatment of Low PAH-Contaminated Sewage Sludge in Aerobic Bioreactors, *ESPR – Environ Sci & Pollut Res*., **13**(3), 170 – 176.

[78] **Byrns, G.,** 2001: The fate of xenobiotic organic compounds in wastewater treatment plants, *Water Res.*, **35**(10), 2523-2533.

[79] **Katsoyiannis, A., Zouboulis, A., and Samara, C.,** 2006: Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: model predictions against experimental values, *Chemosphere.*, **65,** 1634–1641.

[80] **Huckins, J. N., Petty, J. D., Orazio, C. E., Lebo, J. A., Clark, R. C., Gibson, V. L., Gala, W. R., and Echols, K. R.,** 1999: Determination of uptake kinetics (sampling rates) by liquid-containing semipermeable membrane devices (SPMDs) for polycyclic aromatic hydrocarbons (PAHs) in water, *Environ. Sci. Technol.*, **33,** 3918–3923.

[81] **Wild, S. R., Harrad, S. J., and Jones, K. C.,** 1994: The influence of sewage sludge applications to agricultural land on human exposure to polychlorinated dibenzo-p-dioxins (PCDDs) and -furans (PCDFs). - *Environmental Pollution*, **83**(3), 357-369.

[82] **Bodzek, D., Janoszka, B., Dobosz, C., Warzecha, L., and Bodzek, M.,** 1997: Determination of polycyclic aromatic compounds and heavy metals in sludges from biological sewage treatment plants, *J. Chromatogr. A.*, **774,** 177-192.

[83] **Kohli, J., Lee, H.-B., and Peart, T. E.,** 2006: Organic Contaminants in Canadian Municipal Sewage Sludge. Part II. Persistent Chlorinated Compounds and Polycyclic Aromatic Hydrocarbons, *Water Qual. Res. J. Can.*, **41,** 47-55.

[84] **Helaleh, M. I. H., Al-Omair, A., Nisar, A., and Gevao, B.,** 2005: Validation of various extraction techniques for the quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludges using gas chromatography-ion trap mass spectrometry, *J. Chromatogr. A.*, **1083,** 153-160.

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

[86] **Mantis, I., Voutsa, D., and Samara, C.,** 2005: Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods, *Ecotoxicology and Environmental Safety*, **62**(3), 397-407.

[87] **Perez, S., Guillamon, M., and Barcelo, D.,** 2001: Quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludge from wastewater treatment plants. *J. Chromatogr. A.*, **938**, 57-65.

[88] **Villar, P., Callejon, M., Alonso, E., Jimenez, J. C., and Guiraun, A.,** 2006: Temporal evolution of polycyclic aromatic hydrocarbons (PAHs) in sludge from wastewater treatment plants: Comparison between PAHs and heavy metals**,** *Chemosphere.*, **64,** 535-541.

[89] **Stevens, J. L., Northcott, G. L., Stern, G. A., Tomy, G. T., and Jones, K. C.,** 2003: PAHs, PCBs, PCNs, Organochlorine Pesticides, Synthetic Musks, and Polychlorinated n-Alkanes in U.K. Sewage Sludge· Survey Results and Implications, *Environ. Sci. Technol.*, **37,** 462-467.

[90] **Sanchez-Brunete C., Miguel E., and Tadeo J. L.,** 2007: Analysis of 27 polycyclic aromatic hydrocarbons by matrix solid-phase dispersion and isotope dilution gas chromatography-mass spectrometry in sewage sludge from the Spanish area of Madrid, *Journal of Chromatography A*, 1148 , 219–227.

[91] **Hua, L., Wu W-X., Liu, Y-X., Tientchen, C. M., and Chen, Y-X.,** 2008: Heavy Meta,ls and PAHs in Sewage Sludge from Twelve Wastewater Treatment Plants in Zhejiang Province, *Biomedical and Environmental Sciences.*, **21,** 345-352.

[92] **Manoli, E., and Samara, C.,** 1996: Polycyclic Aromatic Hydrocarbons in Wastewaters and Sewage Sludge: Extraction and Clean-up for HPLC Analysis with Fluorescence Detection, *Chromatographia*., **43**(3/4), 135-142.

[93] **Sarrazin, L., Diana, C., Schembri, T., and Rebouillon, P.,** 2004: HPLC determination of polycyclic aromatic hydrocarbons (PAHs) in the littoral urban sewage area of Cortiou (Marseille, France), *Intern. J. Environ. Studies.*, **61**(4), 413-425.

[94] **Pérez, S., Guillamón M., and Barceló D.,** 2001: Quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludge from wastewater treatment plants, *Journal of Chromatography A.*, **938,** 57–65

[95] **Trably, E., and Patureau, D.,** 2006: Successful treatment of low PAH contaminated sewage sludge in aerobic bioreactors, *Environ. Sci. Pollut. Res.*, **13,** 170-176.

[96] **Santos, J. L., Aparicio, I., and Alonso, E.,** 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,** 102-109.

[97] **Aparicio, I., Santos, J. L., and Alonso, E.,** 2009: Limitation of the concentration of organic pollutants in sewage sludge for agricultural purposes: A case study in South Spain. *Waste Manag*., **29**(5), 1747-1753.

[98] **Harrison, E. Z., Oakes, S. R., Hysell, M., and Hay, A.,** 2006: Organic chemicals in sewage sludges, *Science of the Total Environment.,* **367,** 481-497.

[99] **Jirie, A., Hussain, H., and Lintelmann, J.,** 2000: Determination Of Polycyclic Aromatic Hydrocarbons In Wastewater, Sediments, Sludge And Plants In Karak Province, Jordan, *Water, Air, and Soil Pollution.,* **121,** 217-228.

[100] **Pérez, S., Farré, M. l., García, M. J., and Barceló, D.,** 2001: Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contrubiton to its toxicity in the ToxAlert® 100 bioassay, *Chemosphere.,* **45,** 705-712.

[101] **Hafidi M. M., Amir S., Jouraiphy, A., Winterton, P., El Gharous, M., Merlina, G., and Revel, J. C.,** 2008: Fate of polycyclic aromatic hydrocarbons during composting of activated sewage sludge with green waste, *Bioresource Technology.,* **99,** 8819-8823.

[102] **Straube, W. L., Nestler, C. C., Hansen, L. D., Ringleberg, D., Pritchard, P. H., and Jones-Meehan, J.,** 2003: Remediation of polyaromatic hydrocarbons (PAHs) through landfarming with biostimulation and bioaugmentation, *Acta Biotechnol.,* **23,** 179-196.

[103] **Alexander, M.,** 1995: How toxic are toxic chemicals in soil? *Environ Sci. Technol.,* **29,** 2713-2717.

[104] **Lundstedt, S., Haglund, P., and Öberg, L.,** 2003: Degradation and formation of polycyclic aromatic compounds during bioslurry treatment of an acid aged gasworks soil, *Environ Toxicol Chem.,* **22,** 1413-1420.

[105] **Semple, K. T, Reid, B. J., and Fermor, T. R.,** 2001: Impact of composting strategies on the treatment of soils contaminated with organic pollutants, *Environ.Pollut.,* **112,** 269-283.

[106] **Lynne, J., Cardinal, M., and Stenstrom, K.,** 1991: Enhanced biodegradation of polyaromatic hydrocarbons in the activated sludge process, *Research Journal Water Pollution Control Federation,* **63**(7), 950-957.

[107] **Mueller, J. G., Devereux, R., Santavy, D. L., Lantz, S. E., Willis, S. G., and Pritchard, P. H.,** 1997: Phylogenetic and physiological comparisons of PAH-degrading bacteria from physiologically diverse soils, *Antonie Leeuwenhoek*., **71,** 329-343.

[108] **Hughes, J. B., Beckles, D. M., Chandra, S. D., and Ward, C. H.,** 1997: Utilization of bioremediation processes for the treatment of PAH-contaminated sediments, *Journal of Industrial Microbiology & Biotechnology.*, **18,** 152-160.

[109] **Mueller, J. G., Lantz, S. E., Blattmann, B. O., and Chapman, P. J.,** 1991: Bench-scale evaluation of alternative biological treatment process for the remediation of pentachlorophenol and creosote contaminated materials: solid phase bioremediation, *Environmental Science and Technology.*, **25,** 1045-1055.

[110] **Kastner, M., and Mahro, B.,** 1996: Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost, *Applied Microbiology and Biotechnology.*, **44,** 668-675.

[111] **Banerjee, D. K., Fedorak, P. M., Hashimoto, A., Masliyah, J. H., Pickard, M. A., and Gray, M. R.,** 1995: Monitoring the biological treatment of anthracene-contaminated soil in a rotating-drum bioreactor, *Applied Microbiology and Biotechnology.*, **43,** 521-528.

[112] **Field, J. A., de Jong, E., Feijoo, G., and de Bont, J. A. M.,** 1992: Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white-rot fungi, *Appl. Environ. Microbiol.*, **58**, 2219–2226.

[113] **Bumpus, J. A., and Steven, D. A.,** 1987: Biodegradation of environmental pollutants by the white rot fungus Phanerochaete chrysosporium: involvement of the ligning degrading system, *Bioessays*, **6**, 166–170.

[114] **Kirk, T. K., and Farrell, R. L.,** 1987: Enzymatic combustion: the microbial degradation of lignin, *Annu Rev Microbiol.*, **41,** 465–505.

[115] **Field, J. A., Vledder, R. H., van Zeist, J. G., and Rulkens, W. H.,** 1996: The tolerance of lignin peroxidase and manganese-dependent peroxidase to miscible solvents and the in vitro oxidation of anthracene in solvent:water mixtures, *Enzyme Microb. Technol.*, **18**, 300–308.

[116] **Meulenberg, R., Rijnaarts, H. H. M., Doddema, H. J., and Field, J. A.,** 1997: Partially oxidized polycyclic aromatic hydrocarbons show an increased bioavailability and biodegradability. *FEMS Microbiol. Lett.*, **154**, 45-49.

[117] **Eibes, G., Lu-Chau, T., Feijoo, G., Moreira, M. T., and Lema, J. M.,** 2005: Complete degradation of anthracene by Manganese Peroxidase in organic solvent mixtures. *Enzyme and Microbial Technology*, **37**, 365–372.

[118] **Volkering, F., and Breure, A.M.,** 2003: Biodegradation and general aspects of Bioavailability. In: Douben, P.E.T., (ed.). *PAH: An Ecotoxicological Perspective*. Wiley, New York, NY.

[119] **Chang, B. V., Shiung, L. C., and Yuan, S. Y.,** 2002: Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil, *Chemosphere.*, **48,** 717-724.

[120] **Flotron, V., Delteil, C., Bermond, A., and Camel, V.,** 2003: Remediation of matrices contaminated by polycyclic aromatic hydrocarbons: Use of Fenton's reagent, *Polycyc. Aromat. Compd.,* **23,** 353-376.

[121] **N'Guessan, A. L., Levitt, J. S., and Nyman, M. C.,** 2004: Remediation of benzo(a)pyrene in contaminated sediments using peroxy-acid, *Chemosphere.,* **55,** 1413–1420.

[122] **Miller, J. S., and Olejnik, D.,** 2001: Photolysis of polycyclic aromatic hydrocarbons in water, *Water Res.,* **35,** 233-243.

[123] **Li, J.-L., and Chen, B.-H.,** 2002: Solubilisation of polyaromatic hydrocarbons by non-ionic surfactants, *Chem. Eng. Sci.,* **57,** 2825-2835.

[124] **Zhou, W. J., and Zhu, L. Z.,** 2005: Solubilization of polycyclic aromatic hydrocarbons by anionic-nonionic mixed surfactant. Coll. Surf. A: *Physicochem. Eng. Aspects.,* **255,** 145-152.

[125] **Wattiau, P.,** 2002: Microbial aspects in PAH bioremediation., eds. Agathos, S., Reineke, W., Focus on biotechnology. *Biotechnology for the environment: Strategy and fundamentals*. 3A, p. 69–89, Kluwer, Dordrecht.

[126] **Fairey J. L., and Loehr**, **R. C.,** 2003: Total and Volatile Polyaromatic Hydrocarbon Losses during Aerobic Slurry Phase Biotreatment, *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, 182-189.

[127] **Christensen, N., Batstone, D. J., He, Z., Angelidaki, I., and Schmidt, J. E.,** 2004: Removal of polycyclic aromatic hydrocarbon (PAHs) from sewage sludge by anaerobic degradation, *Water Sci. Technol.,* **50,** 237-244.

[128] **Cerniglia, C. E.,** 1984: Microbial degradation of polycyclic aromatic hydrocarbons, *Adv. Appl. Microbiol.,* **30,** 31-71.

[129] **Gibson, D. T.,** 1993: Biodegradation, biotransformation and the Belmont, *J. Ind. Microbiol.,* **12,** 1-12.

[130] **Heitkamp, M. A., and Cerniglia, C. E.,** 1988: Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil-field, *Appl. Environ. Microbiol.,* **54,** 1612-1614.

[131] **Kanaly, R. A., and Harayama, S.,** 2000: Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria, *J. Bacteriol.,* **182,** 2059-2067.

[132] **Kochany, J., and Maguire, R. J.,** 1994: Abiotic transformations of polynuclear aromatic hydrocarbons and polynuclear aromatic nitrogen heterocycles in aquatic environments, *Sci. Total Environ.,* **144,** 17-31.

[133] **Neilson, A. H.,** 1994: Organic chemicals in the aquatic environment; *Distribution, persistency and toxicity*. Lewis Publishers, Boca Raton, Florida, USA.

[134] **Berg, M.V. D., Meent, D. V. D., Peijnenburg, W. J .G. M., Sijm, D. T. H. M., Struijs, J., and Tas, J. W.,** 1995: Transport, accumulation and transformation processes, Eds. van Leeuwen, C.J., and Hermens J.L.M., *Risk assessment of chemicals: An introduction.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

[135] **Haag, W. R., and Yao, C. C. D.,** 1992: Rate constants for reaction of hydroxyl radicals with several drinking water contaminants, *Environ. Sci. Technol.,* **26,** 1005-1013.

[136] **Legrini, O., Oliveros, E., and Braun, A. M.,** 1993: Photochemical processes for water treatment, *Chemical Reviews.,* **93,** 671-698.

[137] **Gurol, M. D., and Singer, P. C.,** 1982: Kinetics of ozone decomposition: A dynamic approach, *Environ. Sci. Technol.,* **16,** 377-383.

[138] **Yao, J. J., Huang, Z. H., and Masten, S. J.,** 1998: The ozonation of pyrene: Pathway and product identification, *Water Res.,* **32,** 3001-3012.

[139] **Yao, J. J., Huang, Z. H., and Masten, S. J.,** 1998: The ozonation of Benz[a]anthracene: Pathway and product identification, *Water Res.,* **32,** 3235-3244.

[140] **Lee, B. D., Iso, M., and Hosomi, M.,** 2001: Prediction of Fenton oxidation positions in polycyclic aromatic hydrocarbons by Frontier electron density, *Chemosphere.,* **42,** 431- 435.

[141] **Rivas, F. J., Beltran, F. J., and Acedo, B.,** 2000: Chemical and photochemical degradation of acenaphthylene. Intermediate identification, *Journal of Hazardous Materials.,* **75,** 89-98.

[142] **Reisen, F., and Arey, J.,** 2002: Reactions of hydroxyl radicals and ozone with acenaphthene and acenaphthylene, *Environ. Sci. Technol.,* **36,** 4302-4311.

[143] **Koeber, R., Bayona, J. M., and Niessner, R.,** 1997: Analysis of ozonolysis products of Benzo[a]pyrene with capillary gas chromatography mass spectrometry and liquid chromatography mass spectrometry, *International Journal of Environmental Analytical Chemistry.,* **66,** 313-325.

[144] **Barbas, J. T., Sigman, M. E., and Dabestani, R.,** 1996: Photochemical oxidation of phenanthrene sorbed on silica gel, *Environ. Sci. Technol.,* **30,** 1776-1780.

[145] **David, B., and Boule, P.,** 1993: Phototransformation of hydrophobic pollutants in aqueous medium 1. PAHs adsorbed on silica, *Chemosphere.,* **26,** 1617-1630.

[146] **Mallakin, A., Dixon, D. G., and Greenberg, B. M.,** 2000: Pathway of anthracene modification under simulated solar radiation, *Chemosphere.,* **40,** 1435-1441.

[147] **Freeman, H. M., and Harris, E. F.,** 1995: Hazardous Waste Remediation.Innovative treatment technologies, Technomic Publishing Company, Lancaster, Pennsylvania, USA.

[148] **Akimoto, Y., Aoki, T., Nito, S., and Inouye, Y.,** 1997: Oxygenated polycyclic aromatic hydrocarbons from MSW incinerator fly ash, *Chemospher.*, **34,** 263-273.

[149] **Alsberg, T., Stenberg, U., Westerholm, R., Strandell, M., Rannug, U., Sundvall, A., Romert, L., Bernson, V., Pettersson, B., Toftgard, R., Franzen, B., Jansson, M., Gustafsson, J. A., Egeback, K. E., and Tejle, G.,** 1985: Chemical and biological characterization of organic material from gasoline exhaust particles, *Environ. Sci. Technol.,* **19,** 43-50.

[150] **Brooks, L. R., Hughes, T. J., Claxton, L. D., Austern, B., Brenner, R., and Kremer, F.,** 1998: Bioassay-directed fractionation and chemical identification of mutagens in bioremediated soils, *Environ. Health Perspect.,* **106,** 1435-1440.

[151] **Casellas, M., Fernandez, P., Bayona, J. M., and Solanas, A. M.,** 1995: Bioassay-directed chemical-analysis of genotoxic components in urban airborne particulate matter from Barcelona (Spain), *Chemospher.,* **30,** 725-740.

[152] **Eriksson, M., Dalhammar, G., and Borg-Karlsson, A. K.,** 2000: Biological degradation of selected hydrocarbons in an old PAH/creosote contaminated soil from a gasworks site, *Appl. Microbiol. Biotechnol.,* **53,** 619-626.

[153] **Fernandez, P., and Bayona, J. M.,** 1992: Use of off-line gel-permeation chromatography normal-phase liquid-chromatography for the determination of polycyclic aromaticcompounds in environmental-samples and standard reference materials (air particulate matter and marine sediment), *Journal of Chromatography.,* **625,** 141-149.

[154] **Fernandez, P., Grifoll, M., Solanas, A. M., Bayona, J. M., and Albaiges, J.,** 1992: Bioassaydirected chemical analysis of genotoxic components in coastal sediments, *Environ. Sci. Technol.,* **26,** 817-829.

[155] **Meyer, S., Cartellieri, S., and Steinhart, H.,** 1999: Simultaneous determination of PAHs, hetero-PAHs (N, S, O), and their degradation products in creosote-contaminated soils. Method development, validation, and application to hazardous waste sites, *Anal. Chem.,* **71,** 4023-4029.

[156] **Moyano, E., and Galceran, M. T.,** 1997: Determination of oxy-, nitro- and hydroxypolycyclic aromatic hydrocarbons in atmospheric aerosol samples, *Quim. Anal. (Barcelona).,* **16,** 159-164.

[157] **Huang, X. D., Dixon, D. G., and Greenberg, B. M.,** 1993: Impacts of UV-radiation and photomodification on the toxicity of PAHs to the higher plant *Lemna gibba* (Duckweed), *Environ. Toxicol. Chem.,* **12,** 1067-1077.

[158] **Mallakin, A., McConkey, B. J., Miao, G. B., McKibben, B., Snieckus, V., Dixon, D. G., and Greenberg, B. M.,** 1999: Impacts of structural photomodification on the toxicity of environmental contaminants: Anthracene photooxidation products, *Ecotoxicol. Environ Safety.*, **43,** 204-212.

[159] **McConkey, B. J., Duxbury, C. L., Dixon, D. G., and Greenberg, B. M.,** 1997: Toxicity of a PAH photooxidation product to the bacteria Photobacterium phosphoreum and the duckweed Lemna gibba: Effects of phenanthrene and its primary photoproduct, phenanthrenequinone, *Environ. Toxicol. Chem.*, **16,** 892-899.

[160] **Kovarova-Kovar, K and Egli, T.,** 1998: Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics, *Microbiology and Molecular Biology Reviews.*, **62,** 646-666

[161] **Hiroshi, H., and Toshio, O.,** 2003: Genetics of Polycyclic Aromatic Hydrocarbon Metabolism in Diverse Aerobic Bacteria, *Biosci. Biotechnol. Biochem.*, **67**(2), 225-243.

[162] **Davies, J. I., and Evans, W. C.,** 1964: Oxidative metabolism of naphthalene by soil Pseudomonads - The ring fission mechanism, *Biochem J.*, **91,** 251–261.

[163] **Mueller, J. G., Chapman, P. J., and Pritchard, P. H.,** 1989: Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote, *Appl. Environ. Microbiol.*, **55,** 3085-3090.

[164] **Geerdink, M. J., Loosdrecht, M. C. M. V., and Luyben, K. C. A. M.,** 1996: Model for microbial degradation of nonpolar organic contaminants in a soil slurry reactor, *Environ. Sci. Technol.*, **30,** 779-786.

[165] **Hunter, R. D., Ekunwe, S. I. N., Dodor, D.E., and Ekunwe, H-M. H. L.,** 2005: *Bacillus subtilis* is a Potential Degrader of Pyrene and Benzo[a]pyrene. *Int. J. Environ. Res. Public Health*., **2(2)**, 267–271.

[166] **Atlas, R. M.,** 1982: Microbial Degradation of Petroleum Hydrocarbons: An Environmental Perspective, *Microbiological Reviews.*, **45**, 180-209.

[167] **Adler, J.,** 1975: Chemotaxis in bacteria, *Annu. Rev. Biochem.*, **44,** 341-356.

[168] **Guha, S., Peters, C. A., and Jaffé, P. R.,** 1999: Multisubstrate biodegradation kinetics of naphthalene, phenanthrene, and pyrene mixtures, *Biotech. Bioengr.*, **65,** 491-499.

[169] **Beckles, D. M., Ward, C. H., and Hughes, J. B.,** 1998: Effects of mixtures of PAHs and sediments on fluoranthene biodegradation patterns, *Environ. Toxicol. Chem.*, **17,** 1246-1251.

[170] **Molina, M., Araujo, R., and Hodson, R. E.,** 1999: Cross-induction of pyrene and phenanthrene in a Mycobacterium sp. isolated from PAHs contaminated river sediments, *Can. J. Microbiol.*, **45,** 520-529.

[171] **Stringfellow, W. T., and Aitken, M. D.,** 1995: Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene–degrading Pseudomonad, *Appl. Environ. Microbiol.,* **61,** 357-362.

[172] **Knightes, C. D.,** 2000: Mechanisms governing sole-substrate and multi-substrate biodegradation kinetics of polycyclic aromatic hydrocarbon, *PhD Thesis* Dissertation. Princeton University, Princeton, NJ.

[173] **Bauer, J. E., and Capone, D. G.,** 1988: Effects of co-occurring aromatic hydrocarbons on degradation of individual aromatic hydrocarbons in marine sediment slurries, *Appl. Environ. Microbiol.,* **54,** 1649-1655.

[174] **Luning Prak, D. J., and Pritchard, P. H.,** 2002: Degradation of PAHs dissolved in Tween 80 surfactant solutions by Sphingomonas paucimobilis EPA505, *Can. J. Microbiol.,* **48,** 151-158.

[175] **Url-11** *<http://www.iwapublishing.com/pdf/contents/isbn1900222248_contents.pdf>*, accessed at 03.08.2010.

[176] **Jordening, H-J, and Winter, J. (Eds),** 2005: Environmental Biotechnology: *Concepts and Applications*, Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany.

[177] **Henze, M., Grady, C. P. L. Jr., Gujer, W., Marais, G v. R., and Matsuo, T.,** 1987: Activated sludge model No.1, lA *WPRC Science and Technical Report No.1*, IA WPRC, London, UK.

[178] **Orhon, D., Artan, N.,** 1994: *Modelling of activated sludge systems*. Lancaster PA, Technomic Press. USA.

[179] **ISO 8192,** 1995: Water quality. Test for the inhibition of oxygen consumption by activated sludge. International Standard.

[180] **Url-12** *<http://www.iski.gov.tr/web/statik.aspx?KID=1000388>*, accessed at 01.08.2010.

[181] **Standard Methods**, 1998: Examination of Water and Wastewater.

[182] **ISO 6060,** 1989: Water quality. Determination of the chemical oxygen demand. *International Standard.*

[183] **Fleschin, S., Bunaciu A., and Scripcariu, M**., 2004: Enzymatic Marker For Toxicity of Contaminated Soils. *Chimie, Anul XIII (serie noua)*, **1-2**, 45-48.

[184] **Alef, K., and Nannipieri, P.,** 1999: Methods in Applied Soil *Microbiology and Biochemistry*, p. 576, Academic Press. Eastbourne, UK.

[185] **Casida, L. E., Klein, D., and Santoro, T.,** 1964: Soil dehydrogenase activity. *Soil Sci.,* **98**, 371–376.

[186] **Lv, Z., Yao, Y., Lv, Z., and Min, H.,** 2008: Effect of tetrahydrofuran on enzyme activities in activated sludge. *Ecotoxicology and Environmental Safety.*,**70**, 259–265.

[187] **Manoli, E., and Samara, C.,** 1996: Wastewaters and sewage sludge: Extraction and clean-up for HPLC analysis with fluorescence detection, *Chromatographia.*, **43**(3/4), 135-142.

[188] **Miege C., Dugay, J., and Hennion, M. C.,** 2003: Optimization, 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,** 87–97.

[189] **Santos, J. L., Aparicio, I., and Alonso, E.,** 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**, 102–109.

[190] **Assih, A., Ouattara, A. S., Thierry, S., Cayol, J. L., Labat, M., and Macarie, H.,** 2002: *Stenotrophomonas acidaminiphila sp*. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor, *Int J. Syst. Evol. Microbiol*., **52**, 559-568.

[191] **Beun, J. J., Pale tta, F., van Loosdrecht, M. C. M. and Heijnen, J.J.,** 2000: Stoichiometryand kinetics of poly-ß-hydroxybutyrate metabolismin aerobic, slow growing activated sludge cultures. *Biotechnol. Bioeng*., **67**(4), 379–389.

[192] **Legret, M., Nicollet, M., Miloda, P., Colandini V. and Raimbault, G.,** 1999: Simulation of heavy metal pollution from stormwater infiltration through a porous pavement with reservoir structure. *Water Science and Technology*, **39**, 119-125.

[193] **Wlodarczyk-Makula, M.,** 2005: The loads of PAHs in wastewater and sewage sludge of municipal treatment plant. *Polycyclic Aromatic Compounds*, **25**, 183–194.

[194] **Url-13** *<http://repository.lib.ncsu.edu/dr/bitstream/1840.4/1843/1/NC-WRRI-299.pdf>*, accessed at 30.07.2010.

[195] **Shen, G., Lu, Y., Zhou, Q., and Hong, J.,** 2005: Interaction of polycyclic aromatic hydrocarbonsand heavy metals on soil enzyme. *Chemosphere*, **61**, 1175–1182.

[196] **Baran, S., Bielinska, J. E., and Oleszczuk, P.,** 2004: Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons. *Geoderma*, **118**, 221–232.

[197] **Url-14** *<http://www.food-info.net/uk/bact/enter.htm>*, accessed at 30.07.2010.

[198] **Url-15** *<http://en.wikipedia.org/wiki/Enterococcus_faecalis>*, accessed at 30.07.2010.

[199] **Url-16** *<http://ijs.sgmjournals.org/cgi/content/full/58/8/1844>*, accessed at 30.07.2010.

[200] **Hunter, R. D., Ekunwe, S. I. N., Dodor, D. E., Hwang H-M., and Ekunwe L.,** 2005: Bacillus subtilis is a Potential Degrader of Pyrene and Benzo[a]pyrene, *Int. J. Environ. Res. Public Health*, **2(2)**, 267–271.

[201] **Url-17** *<http://www.food-info.net/uk/bact/bacer.htm>*, accessed at 02.08.2010.

[202] **Url-18** *<http://en.wikipedia.org/wiki/Bacillus>*, accessed at 02.08.2010.

[203] **Orhon, D., and Okutman, D.,** 2003: Respirometric assessment of residual organic matterfor domestic sewage. *Enzyme Microb. Tech.*, **32**, 560–566.

[204] **Insel, G., Celikyilmaz, G., Ucisik-Akkaya, E., Yesiladali, K., Cakar, Z. P., Tamerler, C., and Orhon, D.,** 2007: Respirometric Evaluation and Modelling of Glucose Utilization by *Escherichia coli* Under Aerobic and Mesophilic Cultivation Conditions. *Biotechnology and Bioengineering*, **96**(1), 94-105.

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- **Basak, S**., Okay, O., Karacik, B., Henkelmann, B., Schramm, K-W., 2010: Complementary chemical and biological determination of dioxin-like compounds in sediments of Istanbul Strait.*Fresenius Environmental Bulletin*, **19**(7), 1245-1253.

- **Basak, S**., Ünal Şengör, G. F., Telli Karakoç, F., 2010: The Detection of Potential Carcinogenic PAH Using HPLC Procedure in Two Different Smoked Fish, Case Study : Istanbul / Turkey, *Turkish Journal of Fisheries and Aquatic Sciences*, **10**(3), 351-355.

- Okay, O. S., Karacık, B., **Basak, S.**, Henkelmann, B., Bernhöft, S., Schramm, K-W., 2009: PCB and PCDD/F in sediments and mussels of the Istanbul strait (Turkey).*Chemosphere*, **76**(2), 159-166.

- Okay, O. S., Morkoç, E., Pekey, H., **Basak, S.,** Baykal, B., 2008: Metals in the Surface Sediments of Istanbul Strait (Turkey). *J. Environ. Sci. Health, Part A.*, **43**(14), 1725-1734.

- Insel, G., Sözen, S., **Başak, S.**, Orhon, D., 2006: Optimization of Nitrogen Removal for Alternating Intermittent Aeration-Type Activated Sludge System:A New Process Modification.*J. Environ. Sci. Health, Part A*, **41**(9), 1819-1829.

- Karl, H., **Basak, S**., Ziebell, S., Quast, P., 2005: Changes of the Iodine Content in Fish during Household Preparation and Smoking, *Deutsche Lebensmittel-Rundschau*, **101**(10), 431-436.

Papers in Peer-Reviewed National Journals

- Sengör, G.F., **Basak S.**, 2006: Su Ürünlerinin Dumanlanmasi ve Polisiklik Aromatik Hidrokarbonlar ile Ilgili Yasal Düzenleme, *Food, World Publication A.S, Globus Publishing House*, **9**(9), 63-67.

- Sengör, G.F., **Basak, S.,** 2004: Vitamin Contents of Seafood and Importance of Nutrition, *Food, World Publication A.S, Globus Publishing House*, **9**(3), 64-71.

- **Basak, S.**, Topçu E., Yilmaz T., 1999:Kirleticilerin Su Ürünlerine Etkisi, *I.Ü. Müh. Fak. Denizcilik K. Dergisi*, **8**, 32-36.

Conference Papers

- Okay, O.S., Karacik, B., **Basak, S.,** Tütüncü, O., 2007: The effects of pollution in Istanbul Strait.*First Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE)*, 24-28 June, 2007 Skiathos, Greece.

- Sözen, S., Teksoy, S., **Basak , S.,** 2005: Autonomous Desalination System as a Tool for Management Of Water Scarcity Problem In Mediterranean Countries, *13th International Symposium of MESAEP*, 08-12 October, 2005 Thessalonica, Greece.

- Sözen, S., Teksoy, S., **Basak, S.,** Çiggin, A., Seibert, U., Vogt, G., 2005: Conceptual Approach for the Desalination Practices with Renewable Energies in Turkey. *3rd Dubrovnik Conference on Sustainable Development of Energy, Water and Environment Systems*, 6-10 June, 2005 Dubrovnik, Croatia.

- Sözen, S., Teksoy, S., **Basak, S.**, Çiggin, A., 2005: Autonomous Desalination as an Alternative Approach to Decentralized Water Management. *1st Zer0-M Conference on Sustainable Water Management* 15-16 March, 2005 Istanbul, Turkey.

- Telli Karakoç, F., Karakas, D., Tolun, L., **Basak, S.,** 2002: Fingerprint analysis of source of the PAHs in Izmit Bay. *Second International Conference, Oceanography of the Eastern Mediterranean and Blacksea, Similarities and Differences of the Two Interconnected Basins*, 14-18 October 2002, Ankara, Turkey.

- **Basak , S.,** Sengör, G.F., Telli Karakoç, F., 2007: Türkiye'deki Üç Farkli Füme Balikta Potansiyel Karsinojenik Polisiklik Aromatik Hidrokarbonlarin (PAH) HPLC Yöntemi ile Belirlenmesi. *Ulusal Su Günleri*, 16-18 May 2007, Antalya, Türkiye.

- Çavus, E., **Basak , S.,** Çokgör, E., Orhon, D., 2004: Agir Metallerin Biyolojik Sistemlere Etkilerinin Respirometrik Olarak Degerlendirilmesi Üzerine Bir Çalisma: Nikel Örnegi. *9. Endüstriyel Kirlenme Kontrolü (EKK) Sempozyumu*, 4-6 June 2004, Istanbul, Turkey.