İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EVALUATION OF ACCLIMATION AND INHIBITORY IMPACT OF 2,6-DIHYDROXYBENZOIC ACID ON THE BIODEGRADATION OF PEPTONE UNDER AEROBIC CONDITIONS

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2,6 DİHİDROKSİBENZOİK ASİTİN AKLİMASYON VE İNHİBİTÖR ETKİSİNİN AEROBİK ŞARTLAR ALTINDA

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ABBREVIATIONS

ASM : Activated Sludge Model

OMW : Olive Mill Wastewater

2,6 DHBA : 2,6 dihydroxybenzoic acid

ASM : Activated Sludge Model

VSS : Volatile Suspended Solids

SS : Total Suspended Solids

SRT : Sludge Retention Time

TOC : Total Organic Carbon

DOC : Dissolved organic carbon

VFA : Volatile suspended solids

TKN : Total Kjeldahl Nitrogen

TON : Total Organic Nitrogen

PHA : Polyhydroxyalkanoate

COD : Chemical oxygen demand

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$\mathbf{b_H}$: Endogenous decay coefficient
$\mathbf{D_e}$: Total elimination of test compound
$\mathbf{D_s}$: Primary elimination of test compound
$\mathbf{D_t}$: Degradation of test compound
$\mathbf{f_{ES}}$: Fraction of soluble inert product
$\mathbf{f_{EX}}$: Fraction of inert particulate metabolic product
$\mathbf{f_I}$: Fraction of inert particulate metabolic product
$\mathbf{f_{SI}}$: Fraction of soluble inert COD generated in biomass decay
$\mathbf{k_h}$: Maximum spesific hydrolysis rate for S_H
$\mathbf{k_{hx}}$: Maximum spesific hydrolysis rate for X_S
$\mathbf{K_{NH}}$: Ammonium saturation as nutrient
$\mathbf{K_O}$: Half saturation coefficient of oxygen
$\mathbf{K_S}$: Half saturation constant of substrate
$\mathbf{K_{STO}}$: Half saturation coefficient of storage
$\mathbf{k_{STO}}$: Maximum storage rate
$\mathbf{K_X}$: Half saturation coefficient for S_H
$\mathbf{K_{XX}}$: Half saturation coefficient for X_S
OUR : Oxygen utilization rate
$\mathbf{S_H}$: Rapidly hydrolysable COD
$\mathbf{S_{HCO}}$: Bicarbonate concentration
$\mathbf{S_I}$: Soluble inert COD
$\mathbf{S_{NH}}$: Ammonia concentration
$\mathbf{S_O}$: Dissolved oxygen concentration
$\mathbf{S_S}$: Soluble readily biodegradable COD
$\mathbf{X_A}$: Autotrophic biomass
$\mathbf{X_H}$: Heterotrophic biomass
$\mathbf{X_I}$: Particulate inert COD
$\mathbf{X_P}$: Inert particulate product
$\mathbf{X_S}$: Slowly hydrolysable COD
$\mathbf{X_{STO}}$: Storage products
$\mathbf{Y_H}$: Heterotrophic yield
$\mathbf{Y_{H1}}$: Heterotrophic yield coefficient for direct growth
$\mathbf{Y_{H2}}$: Heterotrophic yield coefficient for growth on stored polymers
$\mathbf{Y_{STO}}$: Storage yield
$\boldsymbol{\theta_X}$: Sludge age
$\boldsymbol{\rho_{cB1}}$: COD/DOC concentration in blank at 3h±30 min
$\boldsymbol{\rho_{cBt}}$: COD/DOC concentration in blank at time t
$\boldsymbol{\rho_{cT0}}$: DOC concentration in test suspension at time t_0
$\boldsymbol{\rho_{cT1}}$: COD/DOC concentration in test suspension at 3h±30 min
$\boldsymbol{\hat{\mu}_H}$: Maximum growth rate for heterotrophs

$\hat{\mu}_{H1}$: Maximum growth rate for heterotrophs on S_S

$\hat{\mu}_{H2}$: Maximum growth rate for heterotrophs on X_{STO}

ρ_{cTt} : COD/DOC concentration in test suspension at time t

ρ_S : Concentration of test compound in test suspension at time t

ρ_T : Concentration of test compound in abiotic control at time t

SUMMARY

In this study, acclimation of activated sludge taken from a biological treatment plant to 2,6 dihydroxybenzoic acid which is generated from olive mill wastewater was investigated. Inherent biodegradation of 2,6 dihydroxybenzoic acid was observed in a inherent biodegradation test. Activated sludge from a treatment plant was acclimated to a synthetic peptone mixture, and operated at high and low sludge ages. Acclimation of activated sludge from the same origin to 2,6 dihydroxybenzoic acid with peptone mixture was performed and monitored through respirometric studies as well as conventional parameters. Results were evaluated in comparison with peptone acclimated systems. Inhibitory effects of 2,6 dihydroxybenzoic acid to peptone degradation was observed for different sludge ages. However, acclimation of activated sludge to this compound was achieved after a certain operating period. The different sludge ages on the acclimation process was evaluated in terms of degradation of peptone and 2,6 dihydroxybenzoic acid. The only peptone fed systems of different sludge ages were evaluated in terms of kinetic characteristics estimated by using a multi-component model.

ÖZET

Bu çalışmada, bir biyolojik arıtma tesisinden alınan aktif çamurun zeytin yağı endüstrisi kaynaklı 2,6 dihidroksibenzoik asite aklimasyonu incelenmiştir. 2,6 dihidroksibenzoik asitin biyolojik arıtılabilirliği bir biyolojik ayrışma testi ile desteklenmiştir. Bir arıtma tesisinden alınan aktif çamur pepton içerikli sentetik atıksuya aklime edilerek yüksek ve düşük çamur yaşlarında çalıştırılmıştır. Aynı nitelikteki aktif çamura sentetik atıksu ile 2,6 dihidroksibenzoik asit aklimasyonu birlikte uygulanmış; respirometrik çalışmalar ve konvansiyonel parametrelerle sistem izlenmiştir. 2,6 dihidroksibenzoik asitin, farklı çamur yaşlarında pepton giderimi üzerinde inhibitör etkisi olduğu görülmüştür. Belirli bir işletim periyodu sonunda aktif çamurun bu karışıma aklimasyonu gözlenmiştir. Bu maddenin aklimasyonu, farklı çamur pepton ve 2,6 dihidroksibenzoik asit giderimleri kullanılarak değerlendirilmiştir. Sadece pepton ile beslenen sistemlerde çok bileşenli modeller kullanılarak farklı çamur yaşlarının etkisi kinetik özellikler kapsamında değerlendirilmiştir.

1. INTRODUCTION

1.1. Aim

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. Moreover, their low concentration, which is difficultly determined in the environment, possibility of unknown and toxic effects on organisms and biological treatment systems cause biodegradation studies to focus on these compounds.

The aim of the study is to investigate biodegradation of a xenobiotic named 2,6 dihyroxybenzoic acid generated in high amounts in olive mill industry. Additionally, sludge ages of 2 and 10 days and acclimation period effects will be investigated together with respirometric evaluation in detailed.

1.2. Scope

In this study, acclimation of activated sludge taken from Paşaköy Wastewater Treatment Plant will be used for the biodegradation studies of a selected xenobiotic, named 2,6 dihydroxybenzoic acid from olive mill wastewater. Inherent biodegradation of 2,6 dihydroxybenzoic acid was investigated and supported by an inherent biodegradation test. Activated sludge with a certain sludge age was acclimated to Peptone synthetic wastewater having similar characteristics of domestic sewage. The acclimation process was performed to investigate the reference behavior of activated sludge without 2,6 dihydroxybenzoic acid addition to wastewater. Acclimation to 2,6 dihydroxybenzoic acid was monitored through

respirometric studies. In parallel chemical oxygen demand, dissolved organic carbon, 2,6 dihydroxybenzoic acid and some other conventional parameter analysis were measured. The acclimation process and experimental studies were repeated for activated sludge in a rapidly growing system having smaller sludge age, which is 2 days. The evaluation of different sludge ages on the acclimation process was performed by using multi-component model.

2. LITERATURE REVIEW

2.1. Xenobiotics

2.1.1 Definition of Xenobiotics

Xenobiotics are chemicals, which are not produced normally by organisms. The term is the combination of Greek words; *xenos* and *bios*. They are not synthesized normally in an organism resulting in no expectation to be present in living organisms. Xenobiotics are introduced to the environment artificially and are defined as substances, which are foreign to life (Schmidt Bleel *et al*, 1989). A naturally produced compound can be a xenobiotic when it is taken up by a living organism. The chemicals found in much higher concentrations are also defined as xenobiotics.

The xenobiotic term includes not only drugs and carcinogens but also chemicals, petrochemicals, pesticides, and plastics as stated by Schmidt and Haberland in 1989. It is generally used for phenolic componds, dioxins, polychlorinated biphenyls and as their effect on the biota. The number of chemicals, which are commercially available, is about 40,000 with an increase of 2000 number in each year (1989). 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 and animals, and 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.

2.1.2. 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 (Ross and Birnbaum, 2003). Olive mill wastewater is one of the important sources of xenobiotics in the environment.

2.1.3. Fate of Xenobiotics

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 (Singleton, 1994; Buitron and Gonzalez, 1996; Mangat and Elefsiniotis, 1999).

In mixed cultures, acclimation to an organic compound can be either a result of the selection of specialized organisms or as reported (Wiggings *et al*., 1987; Hu et al., 1996; Hu et al., 1998) in literature. Moreover, it was shown that, the acclimation capacity was related to the genetic information within biomass (Buitron and Gonzalez, 1996; Buitron *et al.,* 1998).

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 (Jördening *et al.*, 2005).

2.1.4. Aerobic Biodegradation of Xenobiotics

Aerobic biodegradation is the combination of biologically catalyzed reductions and they suggest two major processes such as growth and cometabolism. By growth process, organic pollutants are used as sole carbon source and electron donors for the generation of energy. This process results in mineralization of organic compounds. Cometabolism process is defined as the metabolism of an organic compound in the presence of a growth substrate, which microorganisms use as primary carbon source (Jördening *et al.,* 2005).

Rapid and complete biodegradation of majority of organic pollutants are possible under aerobic conditions. Microorganisms involved in biodegradation under aerobic conditions have some characteristics. These microorganisms should access chemical compounds, which are related to properties of themselves. Oxidative processes are the initial intracellular attack on organic pollutants involving activation and incorporation of oxygen by oxygenases and peroxidases. Furthermore, degradation pathways results in intermediates of central intermediary metabolites, which are present in tricarboxylic acid cycle. Acetyl CoA, succinate, pyruvate and sugars are examples of central metabolites for biosynthesis (Jördening *et al.,* 2005).

Furthermore, one of the important mechanisms in the breakdown of many compounds is the removal of the halogen atoms. The removal process is defined as dehalogenation involving several mechanisms, by which halogen atoms are removed from the molecules. Dehalogenation of compounds is possible under either anaerobic conditions developed as reductive dehalogenation or aerobic conditions. However, some compounds including chlorinated benzenes appear to be only dehalogenated under aerobic conditions. Pesticides and halogenated 1 and 2 carbon compounds are examples of reductive dehalogenation. Some of the compounds can be degraded under both conditions (Jördening *et al.,* 2005).

Although many bacteria are able to metabolize organic pollutants in soil from polluted areas, which include aliphatic and aromatic hydrocarbons, polycyclic aromatic hydrocarbons and chlorinated compounds, mixed bacterial community has the most powerful ability of degradation of organics in soil (Jördening *et al.,* 2005).

The bacterial species that are able to degrade organic pollutants are found among both gram-positive and gram-negative bacteria. *Pseudomonas spp.,* such as *Pseudomonas putida and Pseudomonas fluorescens* (Houghton *et al.,* 1994), and *Comomonas, Burkhoderia and Xanthomonas* genera, *Alcaligenes sp., Flavabacrerium/Cytophaga* group are gram-negative bacteria degrading organic pollutants. *Rhodococcuc spp.* such as *Nocardia spp., Mycobacterium spp., Corynebacterium spp*., and *Arthrobacter spp., Bacillus spp.* are organisms present in gram-positive bacteria, which are capable of degradation.

2.1.5. Anaerobic Biodegradation of Xenobiotics

Anaerobic biodegradation of xenobiotic compounds has been subject to extensive research during the last years. Many anaerobic bioreactors and remediation systems have been developed to effectively eliminate harmful impacts of xenobiotics on the environment. There are several reasons explaining the importance of anaerobic biodegradation of xenobiotic compounds. High-energy costs of aerobic processes due to oxygen supply to the systems, and high sludge production compared to anaerobic systems are reasons of operating anaerobic systems. Moreover, some xenobiotic compounds such as tetrachloroethylene, polychlorinated biphenyls (PCBs), and nitro-substituted aromatics can be biodegraded by only anaerobic bacteria. In some cases, anaerobic degradation is required prior to aerobic processes. (Zhang and Bennett, 2005).

2.1.6. Acclimation of Microorganisms to Xenobiotics

Many xenobiotic compounds simply pass through wastewater treatment systems without significant reduction due to intrinsic limitations as stated by Hu *et al.* (2005). 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.

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 (Kovarova-Kovar *et al.,* 1998).

Degradation of most of the pollutants are achieved or they are eliminated as a result of cell amount increase in the environment. On the other hand, the enzymes are induced in potential degraders of the chemical for biodegradation. Both of the factors influence the time required which varies from minutes to hours (Kovarova-Kovar *et al.,* 1998).

In order to examine the effect of acclimation on biodegradation of selected synthetic organic chemicals including benzoate and related aromatic compounds, 3-nitrobenzoate, 4-chlorobenzoate, 4-chlorophenol and 2,4-dichlorophenol by unacclimated and acclimated biomass some of the studies were conducted by Hu *et al*. (2005). The results indicated that the biomass acclimation to benzoate, 3-nitrobenzoate and 4-chlorophenol were possible with high biodegradation rates after long-term exposure in sequencing batch reactors. Moreover, some biodegradation of structurally similar synthetic organic compounds such as 4-chlorobenzoate and 2,4-dichlorophenol were achieved. It was concluded that biodegradation of only benzoate was possible by unacclimated biomass as many degradation pathways existed and biodegradation capability was linked to its presence in domestic sewage. The biomass from the treatment plants can be considered that they are acclimated to benzoate. Thus, biomass acclimation is crucial for the degradation of xenobiotic compounds (Hu *et al.*, 2005).

2.2. Olive Oil Industry

Olive oil is produced from olive trees in numerous small plants, which operate seasonally. Olive trees yields 15-40 kg olive oil production per year. The worldwide olive oil production is reported 2546306 tons in 25000 olive mills in 2002. Olive oil production is distributed to Mediterranean region with 97%, European Union countries with 80–84% and to Middle East, the USA, Argentina and Australia (Paraskeva *et al.*, 2006). Olive oil production of Turkey is reported 850000 t in 2005 (FAOSTAT, 2007).

2.2.1. Olive Mill Wastewater

Discontinuous press or centrifugation method is used for olive oil production (Paraskeva *et al.*, 2006). The production process results in an aqueous phase. In Mediterranean countries, generation of olive mill wastewater increases in each year that is more than 30 million m^3 (Fiestas and Borja, 1992). This aqueous phase is combination of water content of fruit, water used in washing and production process of original olives. Olive mill wastewater is an aqueous effluent that includes high organic fraction such as sugars, tannins, acids, pectins, lipids, phenols and polyphenols and other organic compounds (Balice *et al.*, 1990; Hamdi, 1993).

Phenolic compounds are classified into three groups such as mono-cyclic aromatic molecules, including hydroxylated-and/or methoxylated-benzoic acids, phenylacetic acid and phenylpropenoic acid (Ronchero *et al*., 1974; Ehaliotis *et al.,* 1999). After polymerization of phenolic compounds, the molecular weight of these compounds increases. These polymerized forms are recalcitrant to biodegradation, which cause toxicity of OMW (Capasso *et al*., 1995; Beccari *et al*., 1996; Martirani *et al*., 1996).

The wastewater generation of olive milling process is between 0.5–1.5m^3 per 1000 kg of olives, which is closely related to the processes applied. The centrifugation process produces 1–1.5 m^3 wastewater per 1000 kg of olives. The discontinuous process produces less wastewater with higher concentration such as 0.5–1 m^3 wastewater per 1000 kg compared to its alternative. (Hamdi *et al.,* 1996, Paixao *et al.,* 1999, Rinaldi *et al.*, 2003).

Olive mill wastewater characteristics vary depended on the method of extraction, type and maturity of olives, region of origin, climatic conditions and

cultivation/processing methods. Wastewater from olive oil industry has a strong organic content as given in Table 2.1 (Niaounakis *et al.,* 2004). Its high lignin and tannin content give dark color to the wastewater. Long-chain fatty acids, phenolic compounds including simple phenols and flavonoids, or polyphenols which are resulted from polymerisation of the simple phenols constitute major characteristic of OMW. Since these compounds are toxic and present with recalcitrant compounds, treatment is imperative (Paixao *et al.,* 1999; Paixao *et al.,* 2002; Rinaldi *et al.,* 2003).

Table 2.1: Olive Mill Wastewater Characteristics

Parameter	**Value**
COD	45-170 g/L
BOD_5	35–110 g/L
SS	1–9 g/L
Phenolic compounds (Phenols, flavonoids, polyphenols)	0.5–24 g/L
Color	52270–180000 Pt-Co units
pH	3.9–5.1
TOC	53.32–74.9 g/L
TKN	0.94–1.30 g/L
Phenolic acids	0.3–0.66 g/L
Tannins	3.8–9.68 g/L
Pectins	1.83–4.67 g/L

2.2.2. Treatment Strategies

Applications of olive mill wastewater treatment include discharge into nearby rivers, lakes or seas, storage/evaporation in lagoons, and disposal. However, these applications result in environmental problems such as water body and soil pollution, odor and underground seepage (Paraskeva *et al.*, 2006). In addition, seasonal production of olive oil in small enterprises and family businesses cause unaffordable on site-treatment of OMW.

Since biological processes are able to remove organic matter and nutrients by environmentally friendly, reliable, and cost-effective manner, they have been used for treatment of wastewaters. However, the selection of the microorganisms employed and in their adaptation to treating olive mill wastewater is important because phenolic substances present in OMW are inhibitory to microorganisms (Niaounakis *et al.*, 2004, Caputo *et al.*, 2003, Mantzavinos *et al.*, 2005). Aerobic treatment is one of the commonly used technologies in OMW treatment for which acclimatization period for the microorganisms is required. Studies on activated sludge treatment reported COD removal rates of 80–85% and HRTs in the range of 20–25 days. There are studies, which are focused on aerobic degradation of OMW in completely mixed batch activated sludge reactors after the adaptation of microorganism. Benitez *et al.*, reported that (1997, 1999) 58–68% removals of COD was achieved in case 65–98 gr COD/L was fed to the systems. The COD removal efficiency was given as 80% in the presence of 22 gr COD/L where 90% phenol was observed. BOD removal of 45–77% for retention times of 2,5–4,5 days was reported in literature (Velioglu *et al.*, 1992) Similar results were seen in wetland systems (Del Bubba *et al* 2004).

2.3. Biodegradation of Phenolic Compounds

Organic compounds are utilized by either unity of catabolic or cometabolic processes by microorganisms. In catabolic concepts, some manmade compounds are similar to natural compounds that enable microorganisms to degrade these chemicals. These manmade aromatic compounds include benzene, phenol, toluene, aniline, phenanthrene, benzoate, *p*-hydroxybenzoate, alkylphenol, *m*-Nitrobenzoate,

phthalate. Aromatic compounds are converted into natural intermediates of degradation such as catechol and protecatechuate (Jördening *et al*., 2005).

Biodegradation of aromatic compounds in catabolic processes is possible by regulating metabolism of bacteria. Exposure to aromatic or structurally related compounds enables induction and synthesis of sufficient amount of key enzymes needed for degradation for these chemicals (Ramos *et al*, 1997). The group of reactions involved in aromatic substrate degradation are hydroxylation, oxygenolytic ring cleavage, isomerization, and hydrolysis (Jördening *et al*., 2005).

Biodegradation of phenolic compounds is reported to be possible by aerobic bacteria. The biodegradation pathway of phenolic compounds can be either through hydroxylation of aromatic ring or by *ortho* or *meta* cleavage pathway. There are enzymes such as dioxygenases that catalyze the incorporation of both atoms of dioxygen into their substrates. The most important properties of these enzymes are that they are widely distributed in nature and are involved in both anabolic and catabolic processes (Harayama *et al.,* 1992).

One of the important catabolic process involved in phenolic compound degradation is catecholic metabolite formation which is the typical substrate of the latter reaction. In this process hydroxyl substituents are possessed on two adjacent carbon atoms followed by cleave reactions. Cleavge is generally catalyzed by metalloenzymes of one of two functional classes. Intradiol dioxygenases cleave *ortho* to the hydroxyl substituents and typically depend on nonheme Fe(III) (Harayama *et al.,* 1992). *cis,cis-muconate* which is a product of *ortho* cleavage is transformed into an enol-lactone form and hydrolyzed to oxoadipate. Oxoadipate, which is a dicarboxylic acid, is activated by transfer to CoA. Formation of succinate and acetyl-CoA is observed by thiolytic cleavage (Jördening *et al*., 2005).

In contrast, extradiol dioxygenases cleave *meta* to the hydroxyl substituents and typically depend on nonheme Fe(II). Although these distinctions may appear to be minor, they are in fact a manifestation of enzymes that have completely different structures and exclusively utilize different mechanisms (Harayama *et al.,* 1992). *meta* cleavage yields 2-hydroxymuconic semialdehyde. It is converted to formate,

acetaldehyde, and pyruvate by hydrolytic enzymes. Products of *ortho* and *meta* cleavage are metabolized in central metabolism of bacteria (Jördening *et al*., 2005).

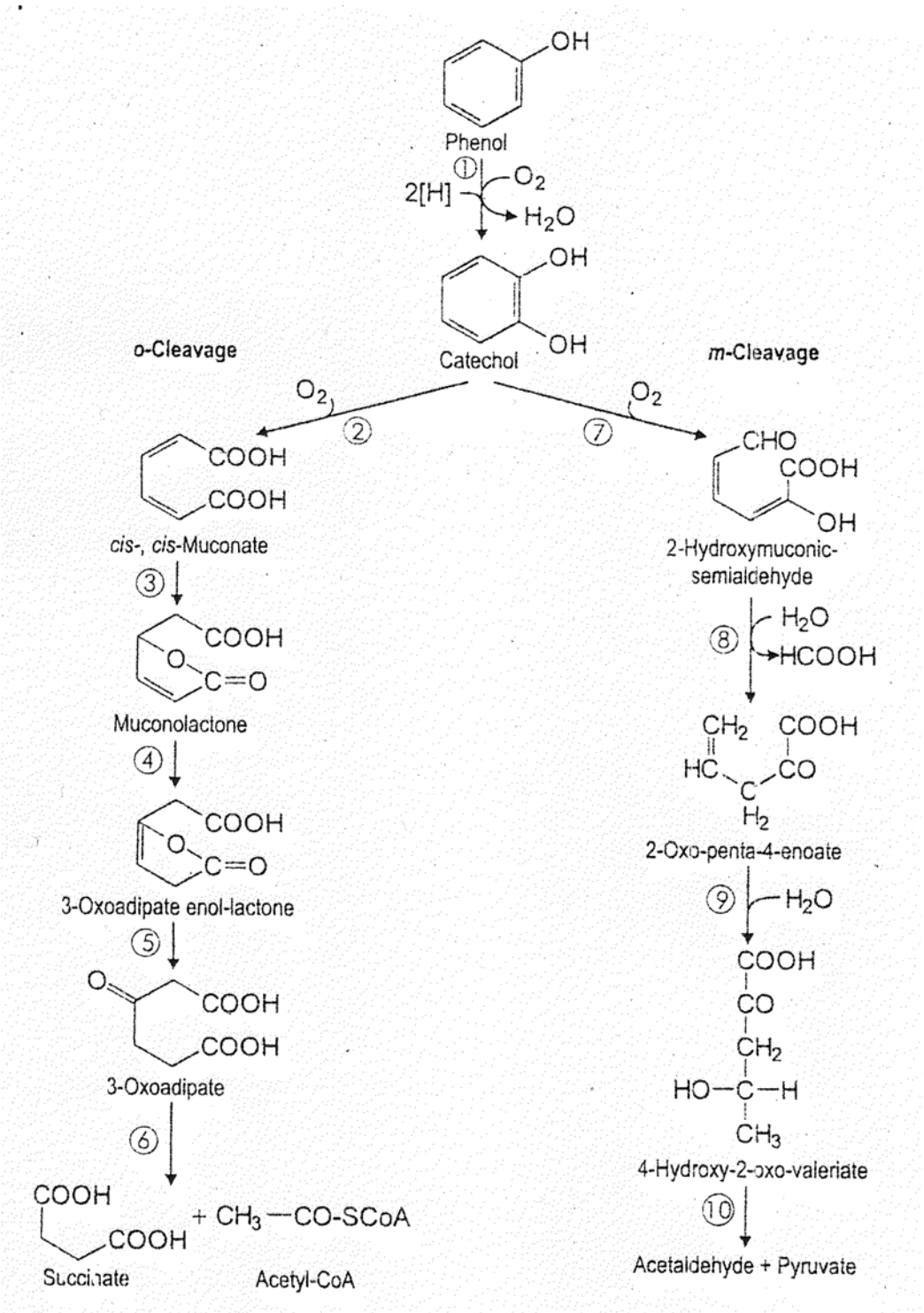

Figure 2.1: Alternative Pathways for Aerobic Degradation of Aromatic Compounds (Jördening *et al*., 2005).

Cometabolic degradation constitutes the alternative degradative pathways for many organopollutants. Cometabolism is the transformation process of a substance when there is a growth substrate. In the presence of a particular substrate, microorganisms are able to oxidize a second substrate, cosubstrate. Other type of microorganisms in mixed cultures utilizes oxidized form of cosubstrate. The enzymes and cofactors such as hydrogen donors for oxygenases that are involved in cometabolism are key factors in this mechanism (Jördening *et al*., 2005).

Pseudomonas sp. and rhodococci are among the bacteria that have high degradation potential because of inducible enzymes and substrate specifity they have (Jördening *et al*., 2005). *Pseudomonas sp.* CF600 is one of the organisms utilizing phenols and methyl-substituted phenols. It uses phenol, cresols, or 3,4-dimethylphenol (3,4-dmp) as the sole carbon and energy source by converting them into catechol (Powlowski *et al.,* 1994).

2.3.1. Inhibition Effect of Phenolic Compounds

Biodegradation kinetics of phenol and mixture of substrates including glucose and pentachlorophenol were studied by Autenrieth *et al*. (1991) in short term and long term experiments with large SRTs. The results indicate that phenol concentrations grater than 50 mg/L, inhibit the biodegradation rates. Moreover, pentachlorophenol cometabolism in the presence of phenol was reported.

Inhibition effects of phenols and phenolic compounds are reported on specified bacterial strains. Although *Pseudomonas putida* (MTCC 1194) is able to degrade phenol in water in the range of 100±1000 ppm, the inhibition effects are observed above the concentration of 500 ppm (Bandyopadhyay *et al*., 1998).

There are studies on kinetics of pure cultures. Reardon, *et al.* studied the kinetics of *Pseudomonas putida* F1 growing on benzene, toluene, phenol, and their mixtures (2000). The comparison of mathematical models pointed out that these aromatics are each able to act as carbon and energy sources for this strain. The Monod model results indicated that phenol caused a small degree of substrate inhibition on *P. putida* F1. The rate of consumption of one substrate was affected by the presence of the others in mixture experiments which enzymatic catabolism pathways were the

same. It was shown that phenol had a little effect on the degradation of other substrates.

2.3.2. Substrate Utilization of Mixed Cultures

Monod kinetics, which were used for pure cultures and single substrates are used for describing mixed cultures for defining their behavior in the presence of single or complex substrates. (Kovarova-Kovar *et al.,* 1998). In case of mixed cultures, the growth parameters involved in kinetics represent the overall values of different and many types of strains. Moreover, they are affected by the concentration of different substrates and microbial composition. Thus, kinetic parameters such as $\hat{\mu}_H$ and K_S are not constants but are variables reflecting physiological state of the strains responsible for growth and substrate consumption (Kovarova-Kovar *et al.,* 1998).

The presence of contaminants in mixtures may cause many problems since some of the compounds in the mixture can inhibit the removal or degradation of another compound (Egli, 1995). The impact of constituents of mixtures was observed in many fields of degradation, including toxic chemical mixtures, wastewater treatment, and fermentation. Moreover, the chemicals may affect each other in a positive or negative way. In case of homologous carbon and energy sources presence, biodegradation of the compound can be positive similar to increased growth at low substrate concentrations (McCarty *et al.,* 1984; Schmidt and Alexander, 1985). In addition, induction of degradative enzymes is possible (Alvarez and Vogel, 1991). Many researchers reported negative effects of mixed substrates. Chang *et al.* stated that competitive inhibition is an example of negative interaction (1993). Bartels *et al.,* also reported the formation of toxic intermediates by nonspecific enzymes.

2.3.3. Biodegradation of 2,6-Dihydroxybenzoic acid

2,6 dihydroxybenzoic acid is one of phenolic compounds in olive mill wastewater. It has been reported several studies for the degradation of this compound in pure cultures as given in Table 2.2. These studies mainly focused on biodegradation in anaerobic conditions. Studies show that aerobic biodegradation of this compound is possible by a certain strain of organisms in case it is exposed to this compound through conversion to resorcinol. It is followed by aromatic nucleus cleavage and beta cleavage (Kluge *et al.,* 1990).

It is reported that an enzyme is present in *Rhizobium* sp. strain MTP-10005 of bacteria found in river waters. The enzyme synthesized in case of induction by 2,6 dihydroxybenzoic acid and it catalyzes decarboxylation of this chemical (Masahiro *et al.*, 2004). However, it is not reported the biodegradation of this compound in mixed cultures.

Table 2.2: Studies of 2,6 Dihydroxybenzoic Acid on Pure Cultures

Organism	**Condition**	**Result**	**Reference**
Clostridium sp. Campylobacter sp.	Anaerobic	Degradation with 2,6 DHBA deacarboxylase when the cells are grown on this substrate. Conversion to resorcinol, aromatic nucleus reduction, beta cleavage.	Kluge *et al., 1990*
Trichosporon cutaneum KUY-6A	Anaerobic	No growth on 2,6 DHBA	Hasegawa *et al*., 1990
Ligniolytic cultures of *Phanerochaete chrysosporium*		% 41 degradation	Leatham *et al.*, 1983
Ralstonia sp. LD 35 *P. putida DSM 1868*	Aerobic	% 0 degradation	Gioia et al., 2001
Ralstonia sp. strain AV5BG strain AV1A strain AV2A strain AV6C	Aerobic	% 100 degradation without intermediate production which is peculiar to AV5BG strain	Gioia et al., 2002

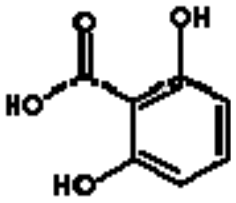

Figure.2.2: Molecular Structure of 2,6 Dihydroxybenzoic Acid

2.4. Biodegradation Tests

Biodegradation tests are used for gaining information about degradability of chemicals which may be used for hazard assessment or risk assessment. Hazard or risk assessment, and aquatic hazard classification, are normally based on data obtained from standardized ready biodegradability tests. In addition to ready biodegradability tests, there are also other types of tests simulating biodegradation in water, aquatic sediments, and soil that may also be used for these purposes. By using the data of sewage treatment plant simulation, inherent biodegradability, anaerobic biodegradability, biodegradability in seawater and abiotic transformation tests, environmental hazard potential or risk can be assessed (OECD, 2005).

Ready biodegradability tests are conducted in order to find out if the ultimate biodegradation of the substance, which means degradation of the substance to carbon dioxide, biomass, water, and other inorganic substances like ammonia, is possible in most environments including biological sewage treatment plants (ISO 9888, 1999).

Using favorable conditions, the tests of inherent biodegradability are designed to assess whether the chemical has any potential for biodegradation under aerobic conditions. They possess a high capacity for degradation to take place, and in which biodegradation rate or extent is measured. The test procedures (TS EN ISO 9888) allow prolonged exposure of the test substance to microorganisms and a low ratio of test substance to biomass, which offers a better chance to obtain a positive result compared to tests for ready biodegradability. Moreover, microorganisms that have previously been exposed to the test substance, which resulted in adaptation, leads to a significant increase of the degradation rate. Inherent biodegradability can be measured by specific analysis such as primary biodegradation or by non-specific

analysis such as ultimate biodegradation. A substance yielding a positive result in a test of this type may be classified as inherently biodegradable, which, preferably, should be qualified by one of the terms with pre-adaptation or without pre-adaptation as appropriate. Because of the favorable conditions employed in these tests, rapid biodegradation in the environment of inherently biodegradable chemicals cannot generally be assessed.

When the results point out that inherent, ultimate biodegradability occur, it indicates that the substance has a potential for degradation under favorable conditions, e.g. in well-operated sewage treatment plants. When a negative result is obtained in a test of inherent biodegradability, it may lead to a preliminary conclusion of environmental persistence and to an evaluation of potential adverse effects of transformation products (TS EN ISO 9888, 1999).

2.5. Activated Sludge Modeling

The effluent quality of treatment plants have been improved by focusing on operational conditions and design. However, it has become a complicated procedure pointing out the necessity of dynamic models (Jördening *et al.,* 2005).

Purpose of activated sludge modeling 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 (Jördening *et al.,* 2005).

In general, deterministic models are used in wastewater treatment plant design. There are also black-box type models that are used for controlling purposes (Jördening *et* al., 2005). 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 modeling of activated sludge is a common approach which reaction kinetics is evaluated by means of multiple parameters (Henze *et al.,* 1997, Orhon *et al.,* 1994).

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 (Henze *et al*., 1997, Orhon *et al.,* 1994).

Most of the models are based on the IAWQ Activated Sludge Model No. 1, which is called ASM1 (Henze *et al.,* 1987). This model includes components such as kinetic and stoichiometric parameters involved in basic processes. It has been improved based on the complexity of design by adding processes and components to this model and new models are introduced. ASM2, ASM3 are examples of activated sludge models (Jördening *et al.,* 2005).

2.5.1. Activated Sludge Model No. 1

The model has some processes that it enables calculation of oxygen consumption, ammonia, and nitrate in tanks of treatment plants and in effluents. In addition, mixed liquor suspended solids, solids retention time and sludge production can also be assessed by using this type of models (Jördening *et al*., 2005).

In the multi component model, COD is selected as the most suitable parameter for defining the carbon sources as it provides a link between electron equivalents in the organic substrate, biomass, and oxygen utilized. Organic carbon removal can be modeled by using Activated Sludge Model No. 1 (ASM1) and Activated Sludge No. 3 (ASM3), which involve different processes.

In Activated Sludge Model 1 (ASM1), COD is divided into fractions based on its solubility, biodegradability, biodegradation rate, and viability for biomass. The main COD fractions are defined as soluble (S), and particulate (X) COD. They are further divided into non-biodegradable fraction and biodegradable fraction. The non-biodegradable fraction is biologically inert and passes through an activated sludge system in an unchanged form. The inert soluble organic matter (S_I) leaves the system at the same concentration as it enters. Inert particulate organic matter (X_I) in the influent wastewater is removed with particulate organic matter produced via decay processes by sludge wastage (Orhon *et al.,* 1994).

Growth, decay, and hydrolysis are basic processes which are involved in ASM 1. The basic relationship is given in Figure 2.3. According to the model, carbon removal is slightly coupled with nitrification. Heteretrophs utilize organic matter directly or after hydrolysis process whereas autotrophic bacteria utilize ammonia for their growth. Decay of organisms results in particulate matter formation which in turn can be utilized for growth following hydrolysis.

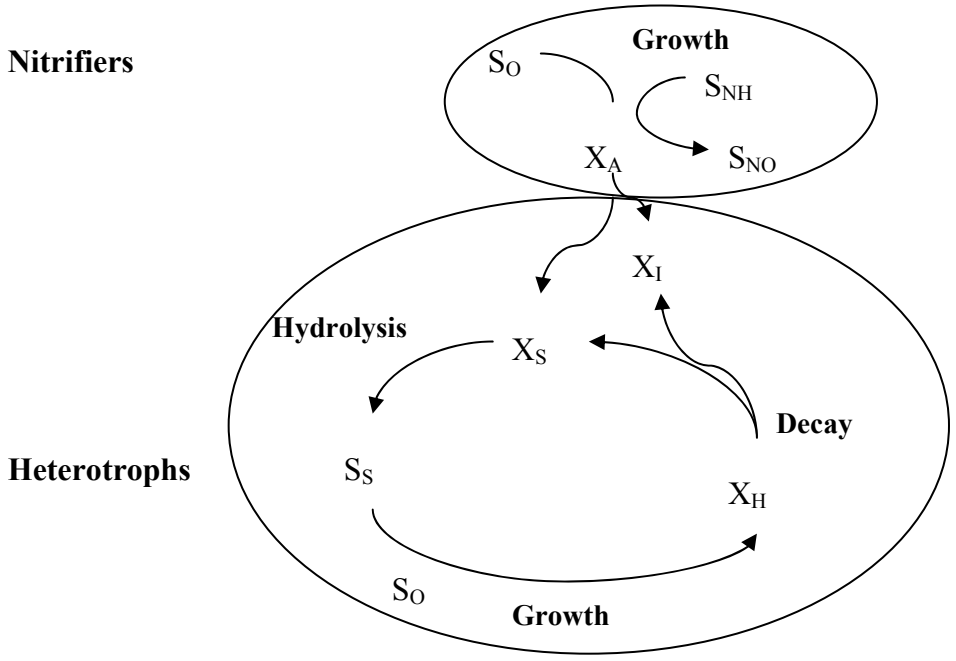

Figure 2.3: Processes for Heterotrophic and Nitrifying Bacteria in ASM 1 (Jördening *et al.*, 2005)

The biodegradable matter is divided into soluble readily biodegradable, (S_S) and slowly biodegradable substrate (X_S). Some of the X_S is assumed soluble. S_S is assumed to be simple organic matter that is utilized by heterotrophic organisms for growth. On the other hand, regeneration of slowly biodegradable particulate matter on nonviable biomass is observed in death-regeneration model while the rest of it is converted to inert particulate product (X_P). On the contrary, X_S consists of relatively complex molecules that require enzymatic breakdown prior to utilization to S_S such as hydrolysis. Heterotrophic biomass X_H and autotrophic biomass X_A are generated by growth on S_S or by growth on ammonia nitrogen S_{NH}. The biomass is lost via the

decay process and converted to some other particulate components (Orhon *et al*., 1994).

In endogenous decay model, S_S is utilized in only growth process. In addition, generation of inert particulate products is linked to the active biomass decay, which a fraction of biomass (f_{EX}) turns into inert particulate products, X_P. These products do not go any further reaction and accumulate in the system until they are removed by sludge wastage. On the other hand, soluble inert product formation is assumed through decay of a fraction of biomass (f_{ES}) (Orhon *et al*., 1994).

The decrease of biomass can be given as (McKinney, 1962):

$$\frac{dX}{dt} = \frac{dX_H}{dt} + \frac{dX_P}{dt} \qquad \textbf{(3.1)}$$

b_H is defined as the endogenous decay coefficient. The change in active biomass is expressed as:

$$\frac{dX_H}{dt} = -b_H X_H \qquad \textbf{(3.2)}$$

Generation rate of particulate inert products are given as follows :

$$\frac{dX_P}{dt} = f_{EX} \frac{dX_H}{dt} \qquad \textbf{(3.3)}$$

When the maximum growth rate of heteretrophs and half saturation constant of substarte are defined as $\hat{\mu}_H$ and, K_S respectively, biodegradation rate of S_S which is directly used in growth is given as follows:

$$\frac{dS_S}{dt} = \frac{\hat{\mu}_H}{Y_H} \frac{S_S}{(K_S + S_S)} X_H \qquad \textbf{(3.4)}$$

The decay associated soluble inert product formation rate can be given as follows:

$$\frac{dS_P}{dt} = f_{ES} b_H X_H \qquad \textbf{(3.5)}$$

Where K_X and k_h are maximum spesific hydrolysis rate and half saturation coefficient for hydrolysis of slowly biodegradable substrate, hydrolysis of this fraction to S_S is given as:

$$\frac{dX_S}{dt} = k_h \frac{X_S / X_H}{(K_X + X_S / X_H)} X_H \qquad \textbf{(3.6)}$$

Matrix representation of basic relationships between process components of endogenous model is given in Table 2.3. The S_I and X_I components are not included in the matrix since they do not go through biochemical processes.

Table 2.3: Simplified Matrix Representation of ASM1 Involving Endogenous Decay

Component→	1	2	3	4	5	6	Process Rate
Process↓	S_S	X_S	X_H	X_P	S_P	S_0	$ML^{-3}T^{-1}$
Growth	$-\frac{1}{Y_H}$		1			$-\frac{(1-Y_H)}{Y_H}$	$\hat{\mu}_H \frac{S_S}{(K_S + S_S)} X_H$
Hydrolysis	1	-1					$k_h \frac{X_S / X_H}{(K_X + X_S / X_H)} X_H$
Decay			-1	f_{EX}	f_{ES}	$-(1 - f_{EX} - f_{ES})$	$b_H X_H$
Parameter, ML^{-3}	COD	COD	Cell COD	COD	COD	O_2	

2.5.2. Activated Sludge Model No. 3

Activated Sludge Model No 3 (ASM3) is one of the multi component models involving carbon and nitrogen removal with additional substrate storage process to ASM1.

In literature, it is reported that microorganisms are able to accumulate and store polymers in both mixed and pure cultures. In the presence of dynamic conditions, substrate is converted into internal storage products (van Loosdrecht *et a.l,* 1997). The storage products are used for the growth of heterotrophic biomass (Mahone *et*

al., 1999). The basic processes that are involved in ASM3 is shown in Figure 2.4. The substrate flow is given as storage, growth, and maintenance.

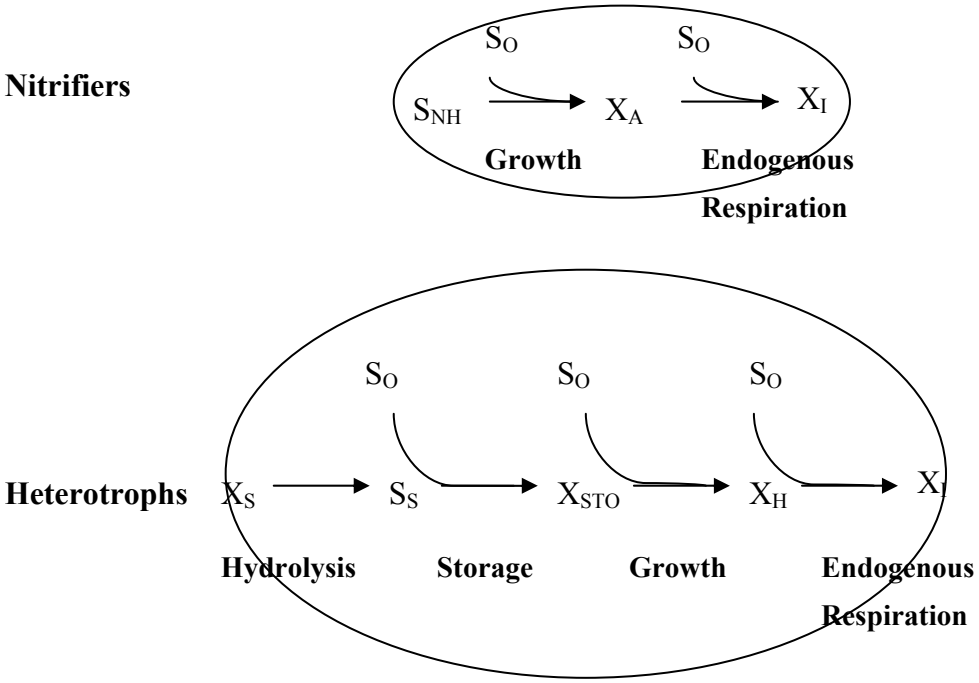

Figure 2.4:. Processes for Heterotrophic Organisms in ASM 3

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 ASM1. 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 ASM1 that emphasize both storage products and biomass decay. In ASM3, 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 ASM3 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 storege 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.

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

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}].

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

For the assessment of stoichiometric and kinetic constants, respirometric studies are proposed associated with ASM1. The simplified matrix representation of ASM3 is given in Table 2.4.

Table 2.4 Simplified matrix representation of ASM3 for organic carbon removal

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	$-\frac{1}{Y_H}$	$\hat{\mu}_H \frac{X_{STO}/X_H}{(K_{STO}+X_{STO}/X_H)} X_H$
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	

The modification of ASM3 model is proposed by Karahan (2004) involving additions to carbon removal process. Storage and primary growth on readily biodegradable substrate processes are introduced to the model. The reaction rates of the processes are based on Monod kinetics with limitations on the growth of ammonia nitrogen and bi-carbonate. In case of primary substrate presence, the secondary growth process is inhibited in the system. In remaining processes, stored products are used surface reaction kinetics are applied in the processes.

Table 2.5: Matrix Representaion of Modified ASM3 Structure (Karahan, 2004)

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				$k_h \frac{X_S/X_H}{(K_X+X_S/X_H)} X_H$
Aerobic Storage of COD	$-\frac{1-Y_{STO}}{Y_{STO}}$	$-\frac{1}{Y_{STO}}$					Y_{STO}	$k_{STO} \frac{S_O}{K_O+S_O} \frac{S_S}{K_S+S_S} X_H$
Growth on S_S	$-\frac{(1-Y_{H1})}{Y_{H1}}$	$-\frac{1}{Y_{H1}}$				1		$\hat{\mu}_{H1} \frac{S_O}{K_O+S_O} \frac{S_{NH}}{K_{NH}+S_{NH}} \frac{S_{HCO}}{K_{HCO}+S_{HCO}} \frac{S_S}{K_S+S_S} X_H$
Growth on X_{STO}	$-\frac{(1-Y_{H2})}{Y_{H2}}$					1	$-\frac{1}{Y_{H2}}$	$\hat{\mu}_{H2} \frac{S_O}{K_O+S_O} \frac{S_S}{K_S+S_S} \frac{S_{NH}}{K_{NH}+S_{NH}} \frac{S_{HCO}}{K_{HCO}+S_{HCO}} \frac{X_{STO}/X_H}{(K_{STO}+X_{STO}/X_H)} X_H$
Endogenous Respiration	$-(1-f_I)$				f_I	-1		$b_H \frac{S_O}{K_O+S_O} X_H$
Respiration of X_{STO}	-1						-1	$b_H \frac{S_O}{K_O+S_O} X_{STO}$
Parameter, ML^{-3}	O_2	COD	COD	COD	COD	Cell COD	COD	

3. MATERIALS AND METHODS

3.1. Reactor Operation

Activated sludge taken from Paşaköy Wastewater Treatment Plant used for acclimation purposes. Activated sludge was acclimated by feeding OECD (Table 3.1) solution having 500 mgCOD /L in fill & draw reactors, which had a working volume of 3 or 4 L. A phosphate salt was introduced as both a source of phosphorus for the microorganisms and to maintain a stable pH. All other macro and micronutrients were added in sufficient quantities for biological growth. The temperatures of systems were kept constant at 20 ^{O}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. The system was operated until steady state were reached. After the acclimation period, fate and effect of 2,6 dihydroxybenzoic acid to activated sludge was investigated. During the experiments sufficient amount of macro and micronutrients were added to the solutions. TS EN ISO 9888 Biodegradation Test (Zahn Wellens Biodegradation Test) and respirometric tests were performed for these purposes.

Table 3.1: Composition of OECD Nutrient Solution (ISO 8192, 1999)

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

3.2. Analytical Techniques

Suspended solids, COD and pH analysis were performed in order to monitor and control reactor operation. In the experiments, total and dissolved organic carbon, volatile fatty acids, glycogen, PHA, pH parameters and nitrogen were performed as defined in the Standard Methods (1998). On the other hand, COD samples were filtered through 0, 45 μm membrane filters and performed as described in the method proposed by ISO 6060 (1986). pH measurements were performed by a 520Aplus pH meter. Respirometric tests were also performed with Applitek RA respirometer with PC connection for overall evaluation and modeling purposes. Determination of 2,6 dihydroxybenzoic acid were performed using Lambda 25UV/VIS spectrometer at 306,17 nm (Davey *et al*., 2000). Calibration curve used for the determination of 2,6 DHBA is given in Figure 2.1.

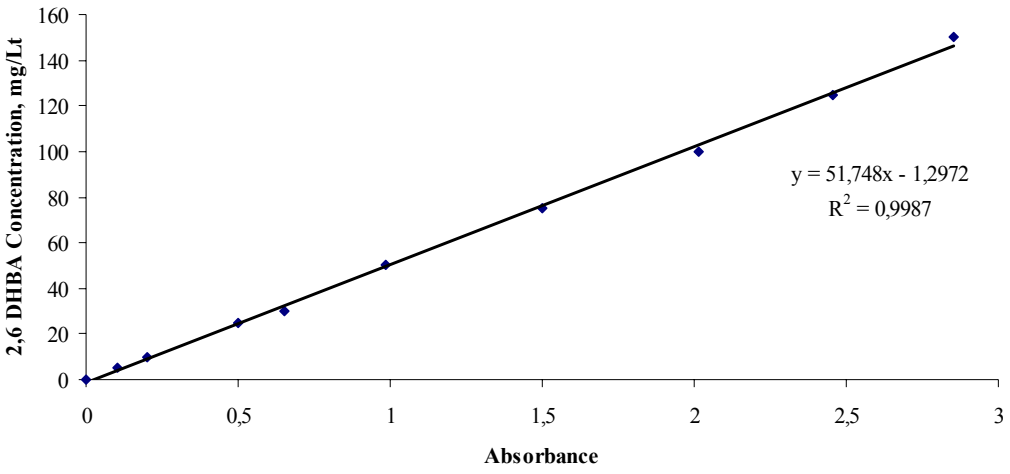

Figure 3.1: Calibration Curve for Determination of 2,6 DHBA

Glycogen and glucose analysis were performed using a BIORAD HPX87H column in Shimiadzu HPLC Systems, equipped with a Shimiadzu SCL-10A vp system controller, a LC-10A vp pump, a DGU-14A degaser, a SPD-10A vp UV-Vis detector. RID-10A refractive index ditector, SIL-10AD vp autoinjector, CTO-10AC vp oven, Class-VP software. Volatile fatty acids were analyzed with GC (Agilent 6890 N) using a flame ionization detector (FID) with a HP-FFAP capillary column

having the inner diameter and length of the column of 0,53 mm and 10 m respectively.

3.2.1. Biodegradation Test

For the determination of 2,6 dihydroxybenzoic acid biodegradation, Zahn Wellens Test as standard method was applied in this study (OECD 302 B Biodegradation Test, Zahn Wellens Test, ISO 9888). In the biodegration tests, peptone mixture acclimated sludge was used. Loading rate (S_0/X_0) was selected as 0.5 g COD/g VSS. 2,6 dihydroxybenzoic acid having 500 mgCOD/lt was fed in the reactor.

3.2.1.1. Principle of TS EN ISO 9888

This method is specified for the evaluation in aqueous medium of ultimate biodegradability of organic compounds from water at given concentrations. In addition, primary biodegradability and the total elimination from water is also evaluated (TS EN ISO 9888).

Biodegradation above 20% of measured DOC removal or COD may be regarded as evidence of inherent, primary biodegradability, whereas biodegradation above 70% of measured as DOC removal or COD may be regarded as evidence of inherent, ultimate biodegradability. Test duration and biodegradation rates are not very strict (TS EN ISO 9888).

$$D_t = \left[1 - \frac{\rho_{cTt} - \rho_{cBt}}{\rho_{cT1} - \rho_{cB1}}\right] \times 100 \qquad \textbf{(2.1)}$$

D_t = Biodegradation
ρ_{cT1} = concentration (mg/lt) of DOC/COD in the test measured at 3h±30min
ρ_{cTt} = concentration (mg/lt) of DOC/COD in the test suspention at time t
ρ_{cB1} = concentration (mg/lt) of DOC/COD in the blanks measured at 3h±30 min
ρ_{cBt} = concentration (mg/lt) of DOC/COD in the blanks at time t

$$D_e = \left[1 - \frac{\rho_{cTt} - \rho_{cBt}}{\rho_{cT0}}\right] \qquad \textbf{(2.2)}$$

D_e = Total elimination

ρ_{CT0} = DOC concentration (mg/lt) at time t_0 in the test suspension

$$D_S = \frac{\rho_S - \rho_T}{\rho_S} \times 100 \quad \textbf{(2.3)}$$

D_S = Primary degradation

ρ_T = concentration of test compound (mg/lt) at time t in the test supension

ρ_S = concentration of test compound (mg/lt) at time t in the abiotic control

3.2.1.2. Experimental Procedure

A mixture containing the test substance, mineral nutrients and a relatively large amount of activated sludge in aqueous medium was agitated and aerated at 20-25°C in the dark or in diffuse light for up to 28 days. Blank controls, containing activated sludge and mineral nutrients but no test substance, were run in parallel. The biodegradation process was monitored by determination of DOC and COD in filtered samples taken at defined time intervals. The ratio of eliminated DOC and COD was corrected for the blank, after each time interval, to the initial DOC value was expressed as the percentage biodegradation at the sampling time. The percentage biodegradation was plotted against time to give the biodegradation curve. Specific analysis of the test substance were performed.

3.2.2. Respirometric Analysis

The respirometric tests were conducted with relevant acclimated biomass seeding alone to obtain endogenous oxygen uptake rate (OUR) level of biomass. Samples with desired S_0/X_0 ratios are added to the reactor and the OUR data was monitored. Experimental studies are conducted by using activated sludge operated at the sludge age of 10 days and 2 days. The summary of respirometric studies is given in Table 3.2.

Experiments representing the same conditions in respirometric tests were conducted in parallel. VSS was found between 1700-2000 mg/L in the experiments. The monitored data for experiment was detailed in Table 3.3.

Table 3.2: Experimental Conditions Conducted

Run	Substrate Type	Sludge Age (day)	Acclimation Period (day)
Run 1.1	peptone mixture (500 mg COD/L)	10	Control
Run 1.2	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	10	0.
Run 1.3	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	10	4.
Run 1.4	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	10	15.
Run 1.5	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	10	30.
Run 1.6	2,6 dihydroxybenzoic acid (500 mg COD/L)	10	30
Run 2.1	peptone mixture (500 mg COD/L)	2	Control
Run 2.2	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	2	0
Run 2.3	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	2	2.
Run 2.4	peptone mixture (500 mg COD/L) + 2,6 dihydroxybenzoic acid (500 mg COD/L)	2	4.

Table 3.3: Monitored Data for Experimental Runs

Time	pH	SS/VSS	COD Total	COD Filtered	TOC Total	TOC Filtered	TKN Total	TKN Filtered	TON Filtered	VFA	PHA	Glycogen	Glucose
-10 min		x	x	x	x	x	x	x	x	x	x	x	x
5 min			x	x	x	x			x	x	x	x	x
20min	x		x	x	x				x	x	x	x	x
60 min	x		x	x	x	x	x	x	x	x	x	x	x
90 min			x	x					x	x	x	x	x
120 min	x		x	x	x	x	x	x	x	x	x	x	x
150 min			x	x					x	x	x	x	x
180 min			x	x					x	x	x	x	x
240 min	x		x	x	x	x	x	x	x	x	x	x	x
300 min	x		x	x					x		x	x	x
430 min	x		x	x	x	x	x	x	x	x	x	x	x
24 h	x	x	x	x	x	x	x	x	x	x	x	x	x

4. RESULTS AND DISCUSSION

The averaged reactor analysis results operated at SRT of 10 days at steady state condition for the peptone mixture reactor in a period of approximately 5 months were represented in Figure 4.1. The peptone mixture reactor characteristics at steady state conditions was given in Table 4.1

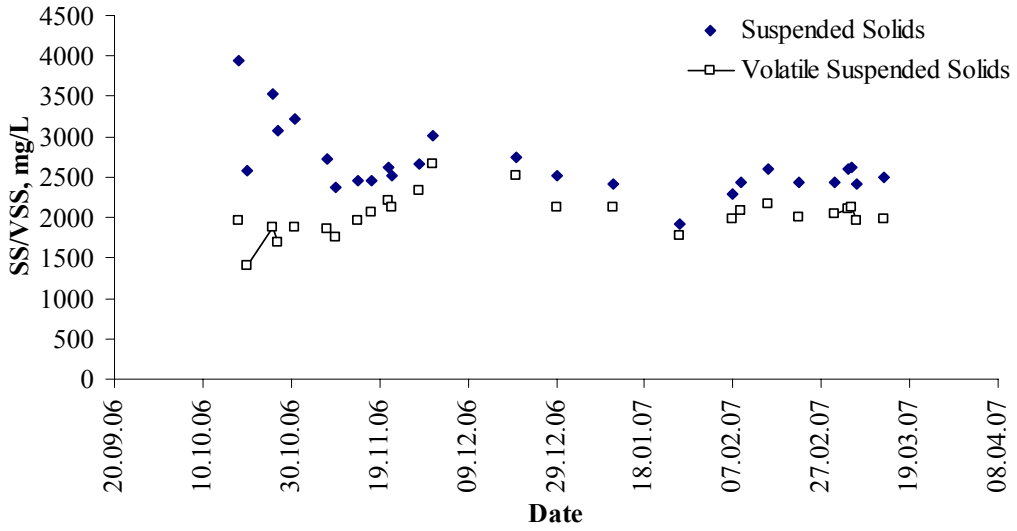

Figure 4.1: Monitoring Results of the Peptone Mixture Reactor (SRT=10 days)

Table 4.1: The Peptone Mixture Reactor Characteristics at Steady State

Substrate Type	**S_0/X_0**	**SS**	**VSS**	**VSS/SS**	**COD_{inf}**	**COD_{eff}**	**Removal Efficiency**
	mgCOD/ mgVSS	mg/L	mg/L	mgVSS/ mgSS	mg/L	mg/L	%
Peptone mixture	0,25	2500	2050	0,83	500	32	0,94

Experiments representing the same conditions in respirometric tests were conducted in parallel for the peptone mixture and 2,6 dihydroxybenzoic acid mixture. The loading ratio (So/Xo) were aproximately 2000 mg VSS/L for all runs.

Eleven experiments were performed to evalute of the sludge age effect on acclimation period of 2,6 dihydroxybenzoic acid. Oxygen uptake rate measurements together with parameters given in Table 4.1 were conducted on peptone mixture alone and mixture of 2,6 dihydroxybenzoic acid and peptone mixture for sludge of 10 days.

Figure 4.2-4.5 illustrate the data obtained for Run 1.1.

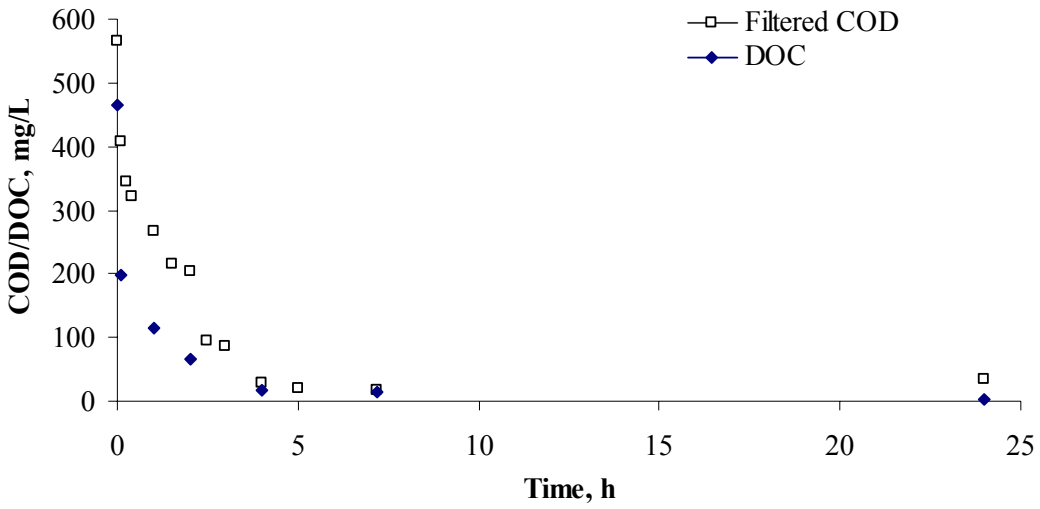

Figure 4.2: Filtered COD and DOC Concentrations versus Time (Run 1.1)

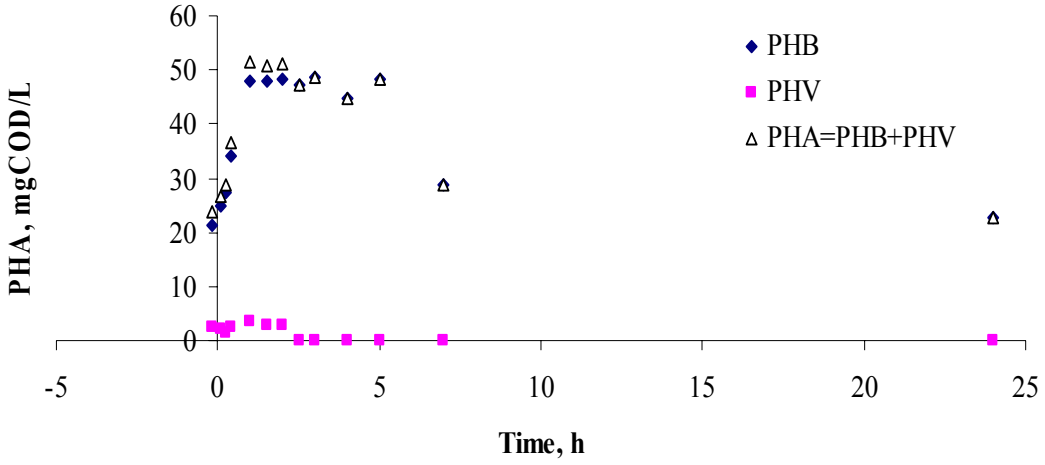

Figure 4.3: PHA versus Time (Run 1.1)

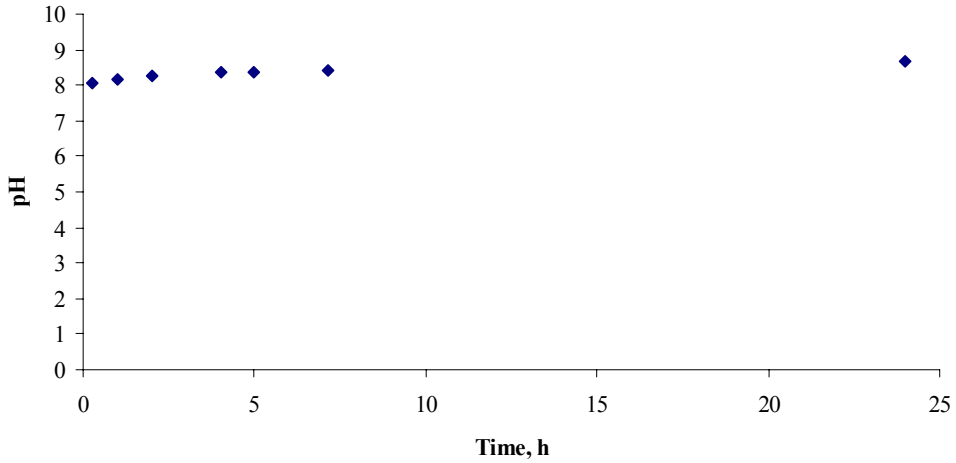

Figure 4.4: pH versus Time (Run 1.1)

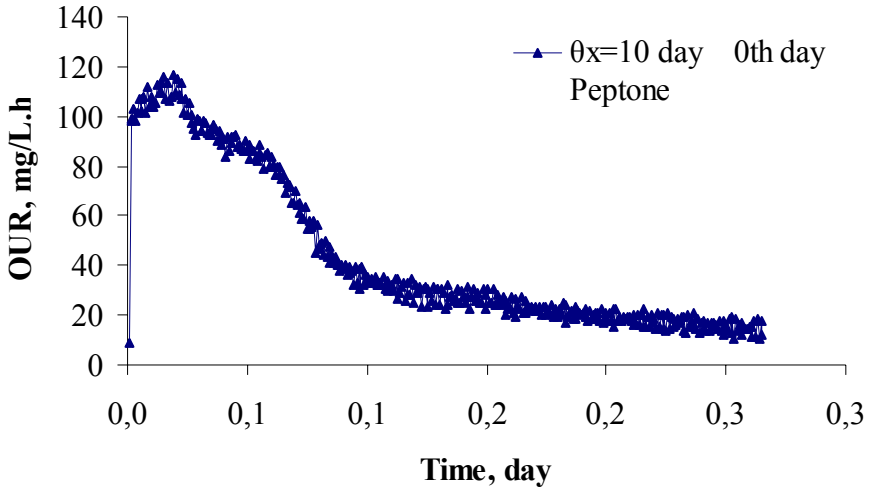

Figure 4.5: OUR Data versus Time (Run 1.1)

After the peptone mixture reactor acclimation, the peptone mixture and 2,6 DHBA addition was started by using the peptone mixture acclimated sludge for sludge age of 10 days and a hydraulic retention time of 1 days. SS, VSS and COD results were given in Figure 4.6.

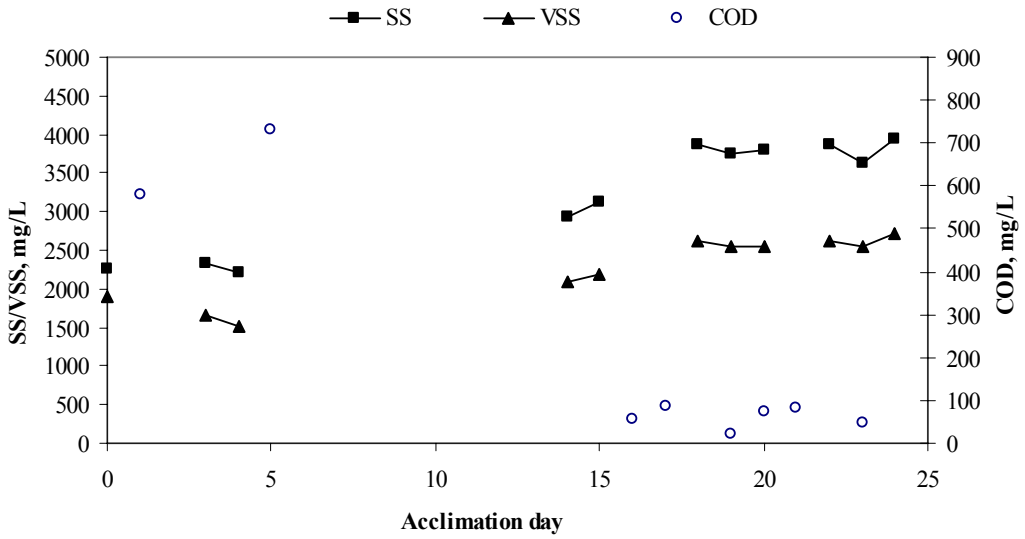

Figure 4.6: Monitoring Results of the Peptone Mixture and 2,6 DHBA Reactor

Run 1.2 representing first time addition of 2,6 DHBA was performed with 500 mgCOD/L peptone mixture and 500 mg COD/L 2,6 DHBA by using the peptone mixture acclimated sludge. Run 1.2 results are illustrated in Figure 4.7-4.11.

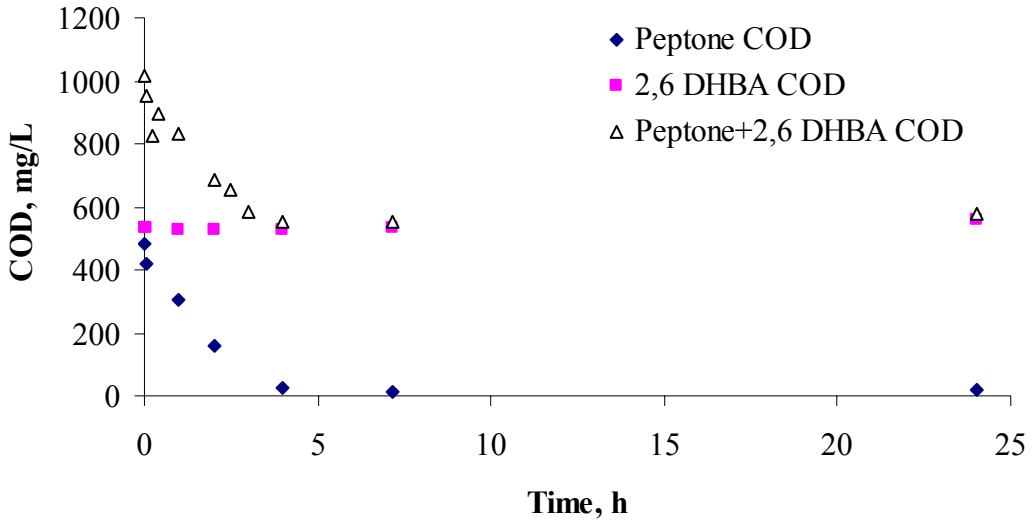

Figure 4.7: COD Concentration versus Time (Run 1.2)

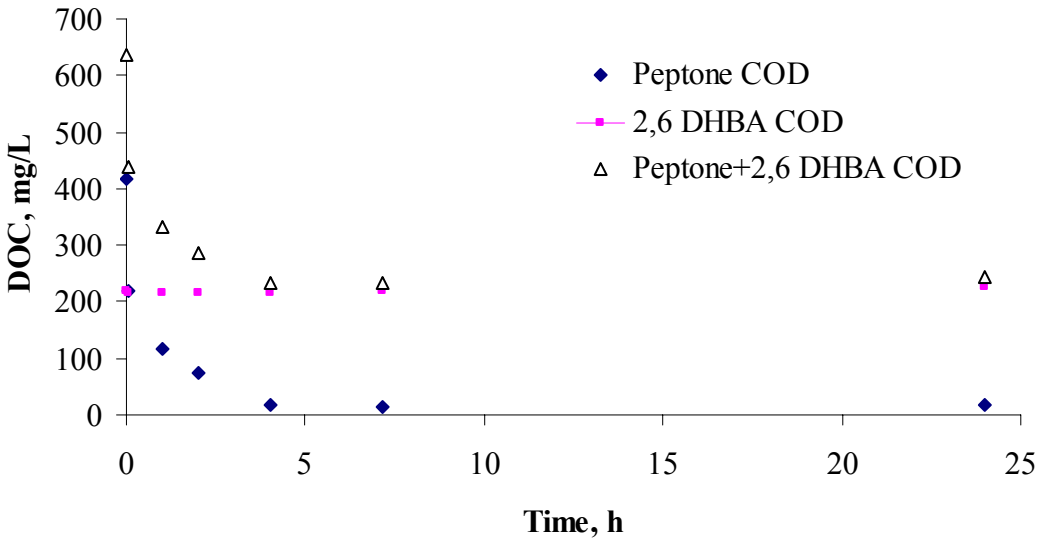

Figure 4.8: DOC Concentration versus Time (Run 1.2)

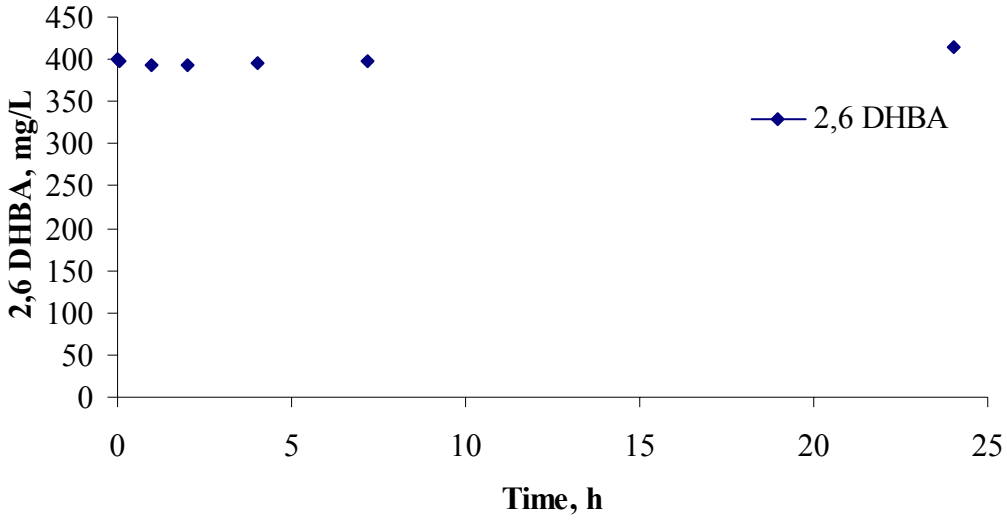

Figure 4.9: 2,6 DHBA Concentration versus Time (Run 1.2)

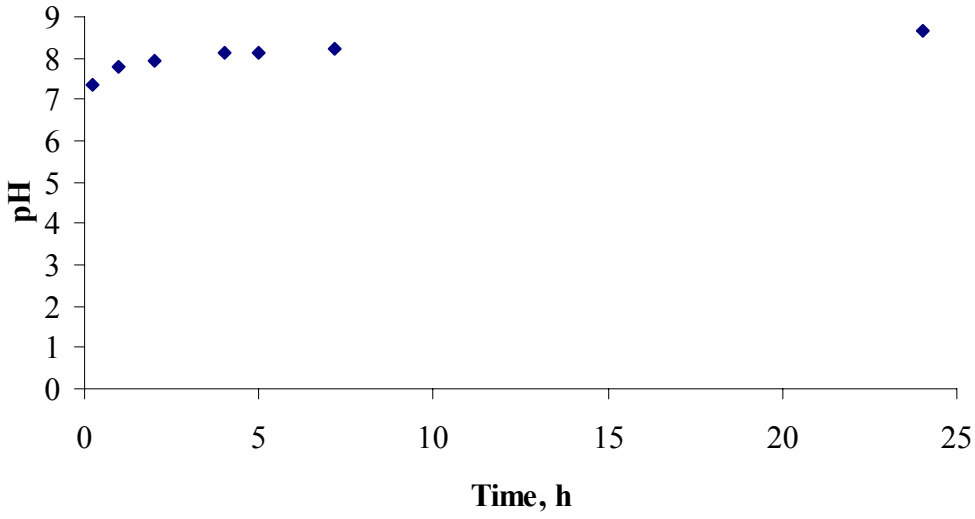

Figure 4.10: pH versus Time (Run 1.2)

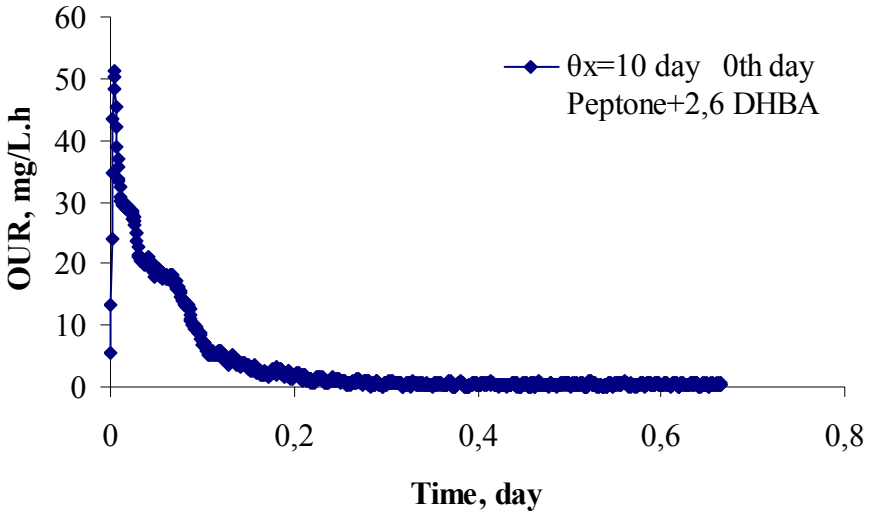

Figure 4.11: OUR Data versus Time (Run 1.2)

Addition of 2,6 DHBA with peptone mixture resulted in decrease of maximum oxygen uptake rate from 115 mg/L.h to 51 mg/L.h. The results indicate that 2,6 DHBA had an inhibitory effect of 56% on peptone mixture acclimated activated sludge. However, the trend of the OUR profile remained the same.

2,6 DHBA removal did not observed. However, the trend of peptone COD degradation did not change leading to decrease in COD degradation efficiency. Storage of PHA were also observed which is about 20 mg COD/L before the addition of peptone and 2,6 DHBA. PHA storage increased to 50 mg COD/L during the experiment and decreased to its initial concentration. pH was about 8 during a one day period with a slight increase.

Run 1.3 experiment representing forth day of 2,6 DHBA addition was conducted with 500 mg COD/L peptone mixture and 500 mg COD/L 2,6 DHBA by using the peptone mixture and 2,6 DHBA acclimated sludge. Run 1.3 results are given in Figure 4.12-16.

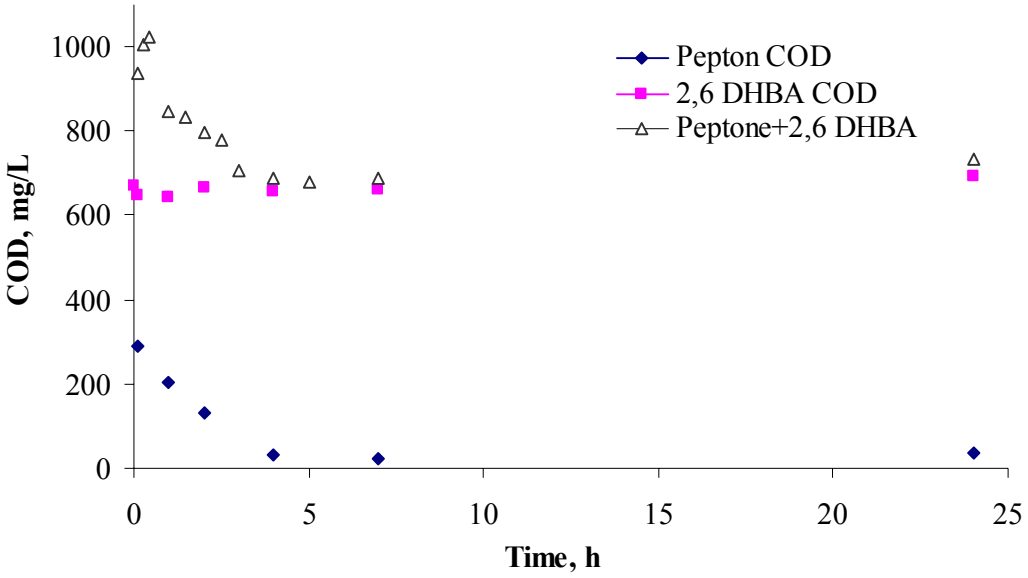

Figure 4.12: COD Concentration versus Time (Run 1.3)

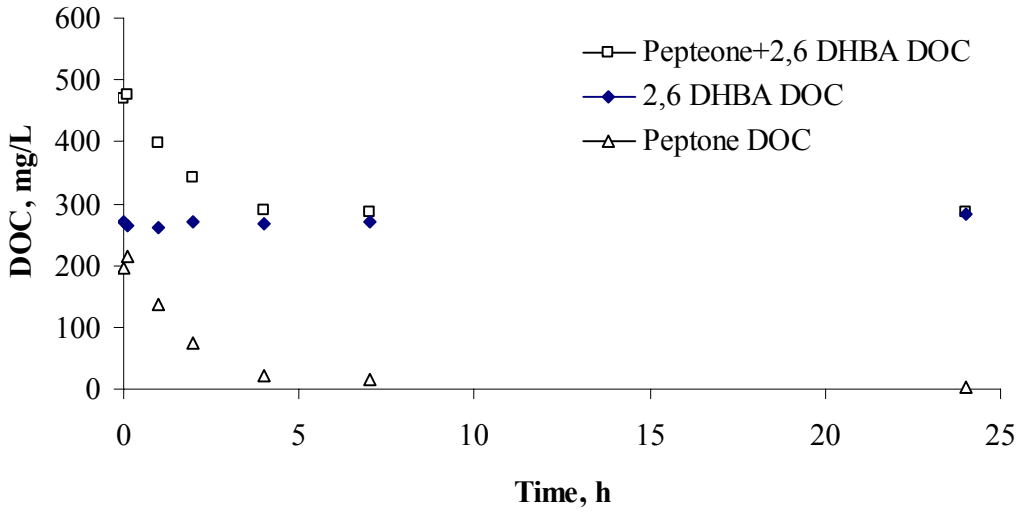

Figure 4.13: DOC Concentration versus Time (Run 1.3)

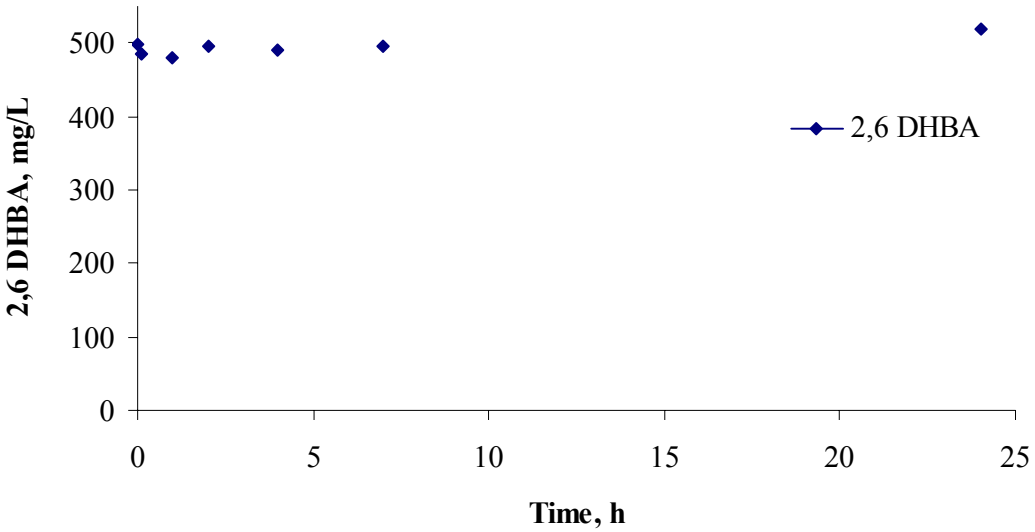

Figure 4.14: 2,6 DHBA Concentration versus Time (Run 1.3)

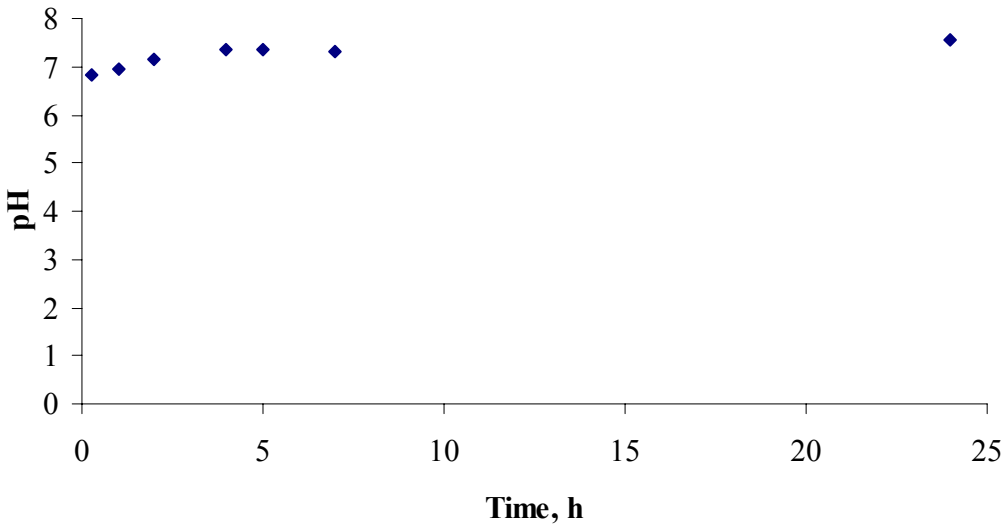

Figure 4.15: pH versus Time (Run 1.3)

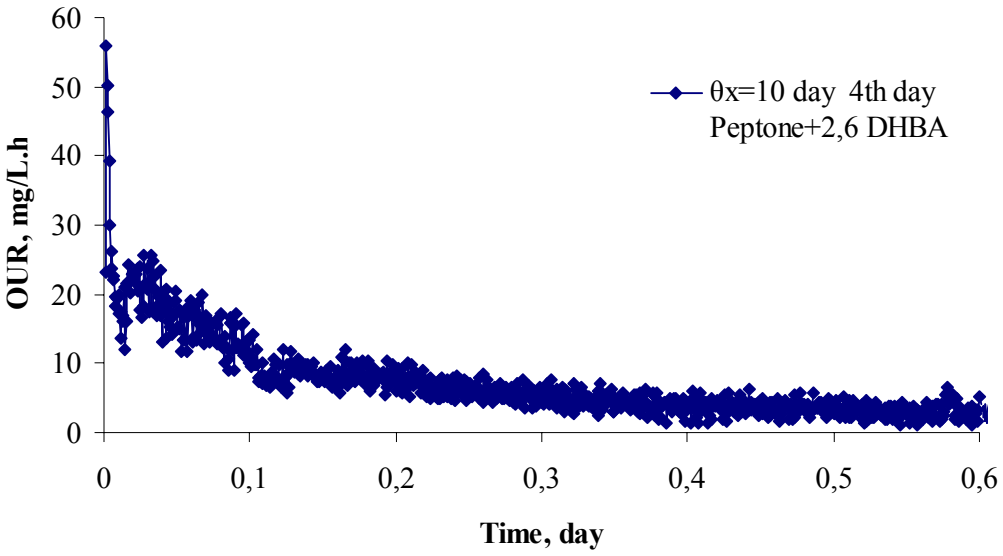

Figure 4.16: OUR Data versus Time (Run 1.3)

Oxygen uptake rate decreased to 55 mg/L.h on the forth day of acclimation indication inhibition of 50%. On the other hand, the trend of the OUR profile had changed.

2,6 DHBA removal did not observed. The trend of peptone COD degradation did not change leading to decrease in COD degradation efficiency. DOC experiments supported the decreased in COD concentration. pH was about 7 during a one day period with a slight increase.

Run 1.4 representing fifteenth day of 2,6 DHBA addition was conducted with 500 mg COD/L peptone mixture and 500 mg COD/L 2,6 DHBA by using the peptone mixture and 2,6 DHBA acclimated sludge. Run 1.4 results are illustrated in Figure 4.17-21.

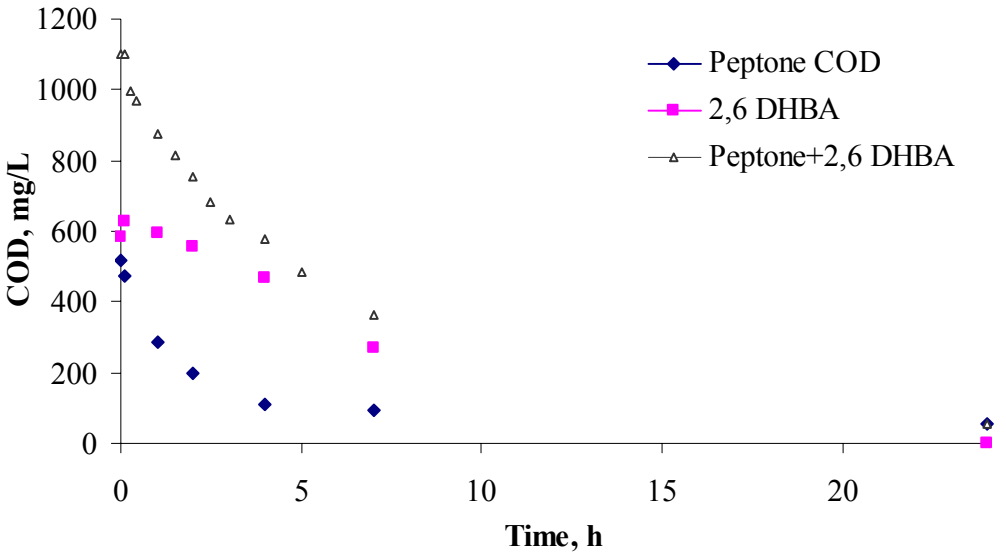

Figure 4.17: COD Concentration versus Time (Run 1.4)

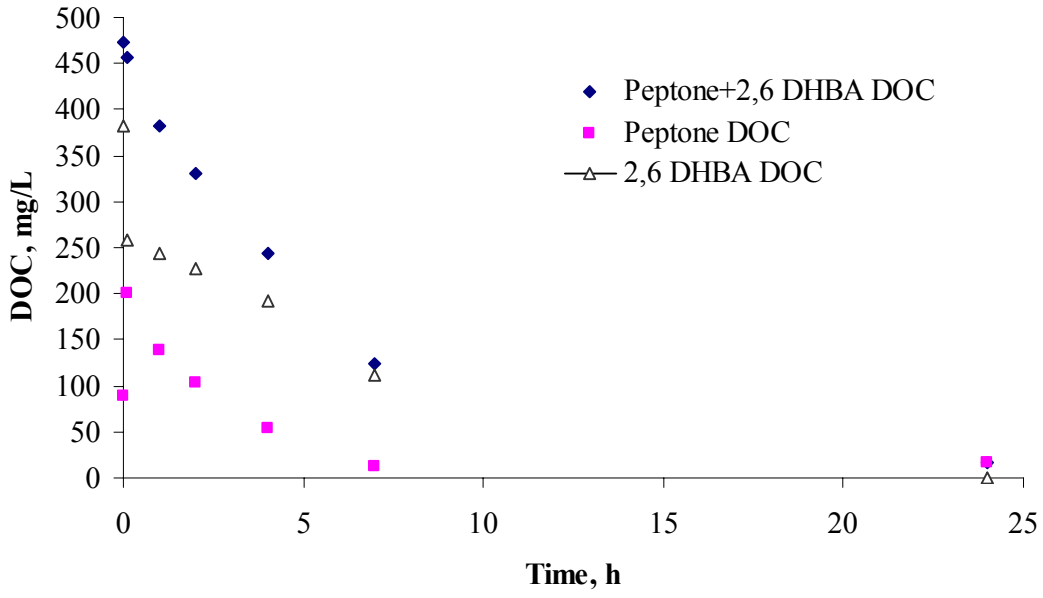

Figure 4.18: DOC Concentration versus Time (Run 1.4)

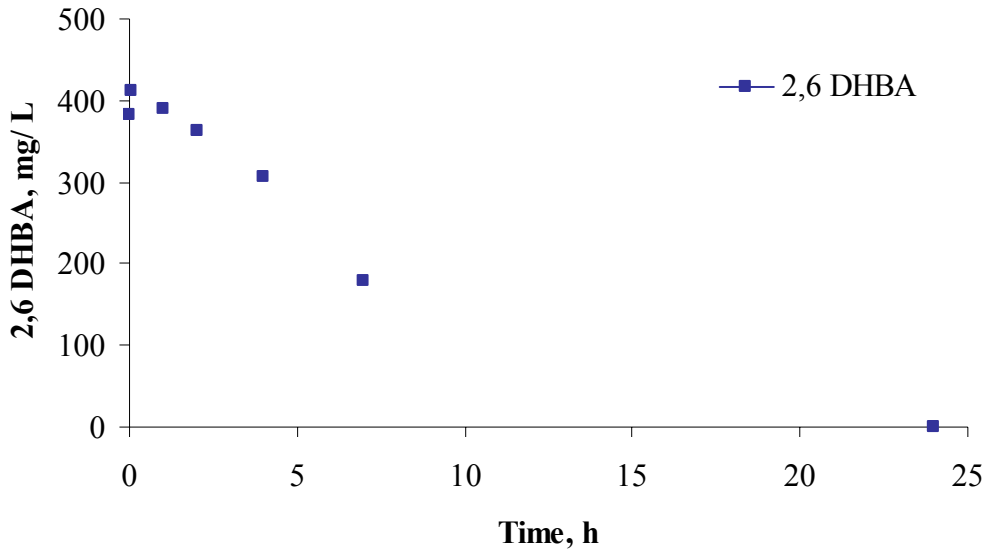

Figure 4.19: 2,6 DHBA Concentration versus Time (Run 1. 4)

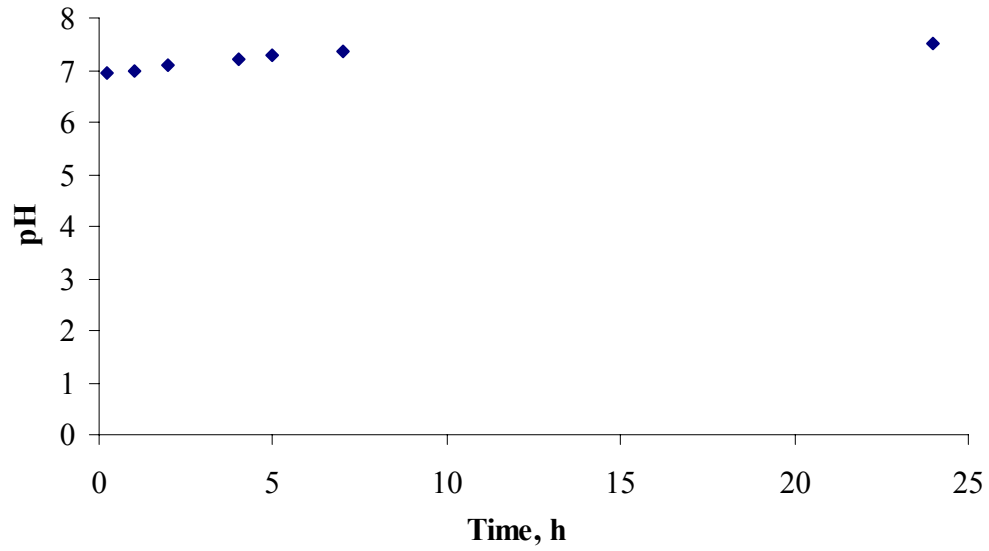

Figure 4.20: pH versus Time (Run 1.4)

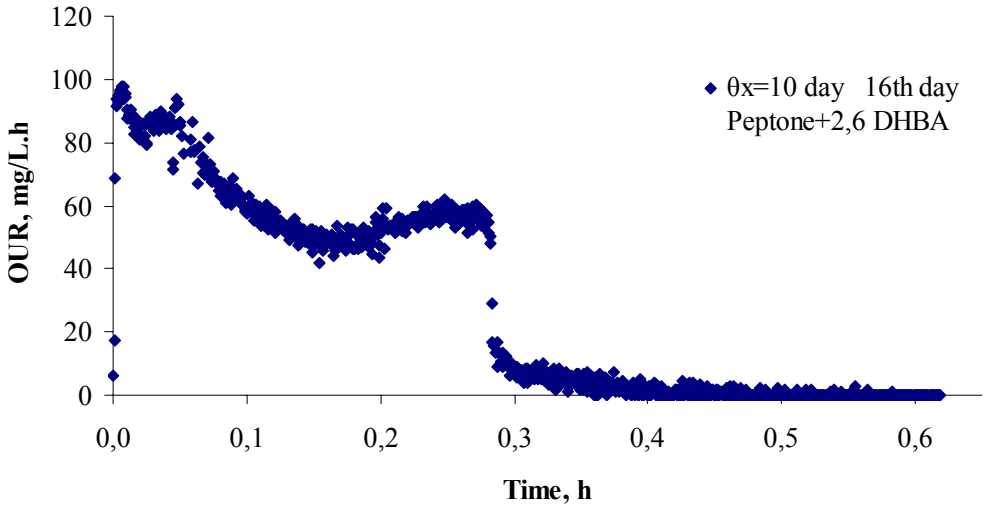

Figure 4.21: OUR Data versus Time (Run 1.4)

Oxygen uptake rate increased to 100 mg/L.h on the fifteenth day of acclimation and also OUR profile also changed.

2,6 DHBA removal was observed during hydraulic retention time with an efficiency of 100%. The trend of peptone COD degradation did not change. DOC experiments supported the decreased in COD concentration. pH was about 7 with a slight increase during the experiment.

Run 1.5 representing thirtieth day of 2,6 DHBA addition was conducted with 500 mgCOD/L peptone mixture and 500 mg COD/L 2,6 DHBA by using the peptone mixture and 2,6 DHBA acclimated sludge. Run 1.5 results are illustrated in Figure 4.22-25.

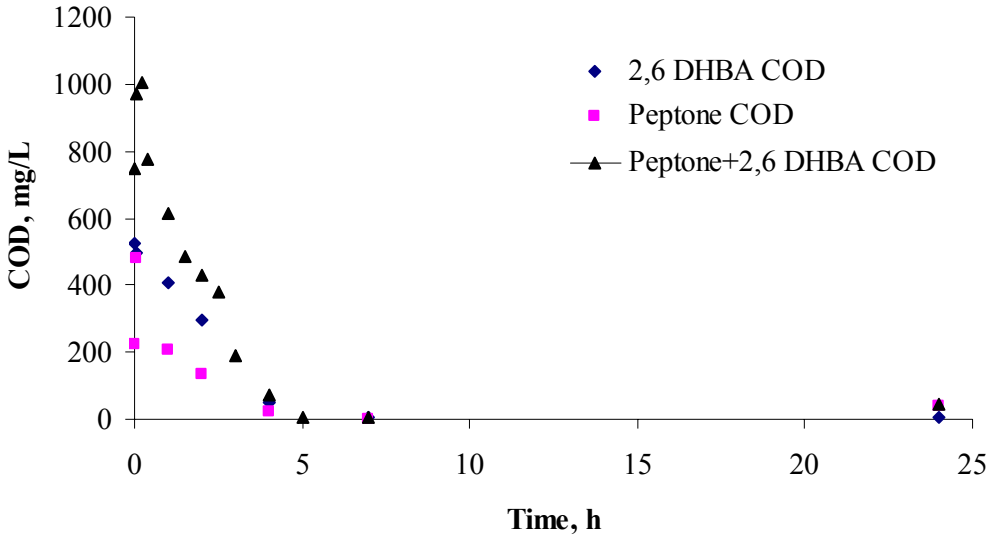

Figure 4.22: COD Data versus Time (Run 1.5)

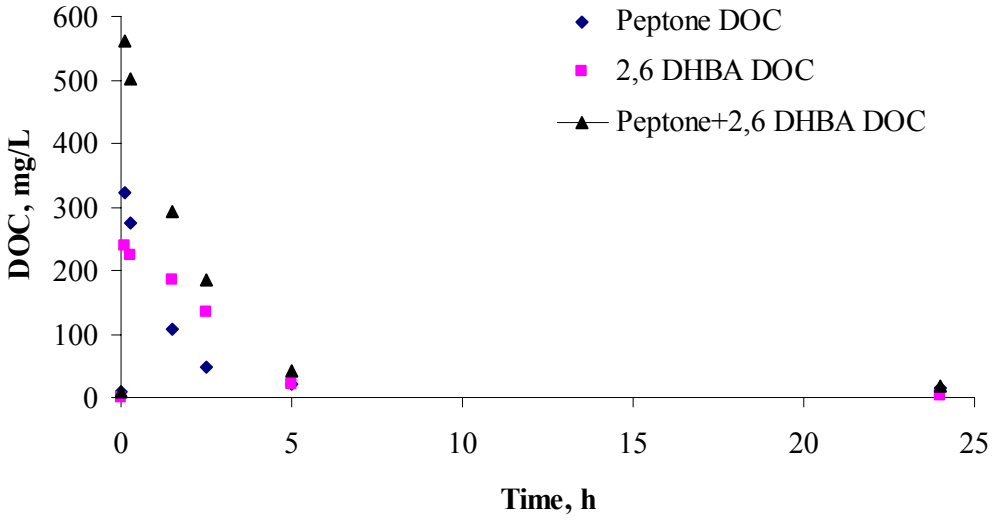

Figure 4.23: DOC Concentration versus Time (Run 1.4)

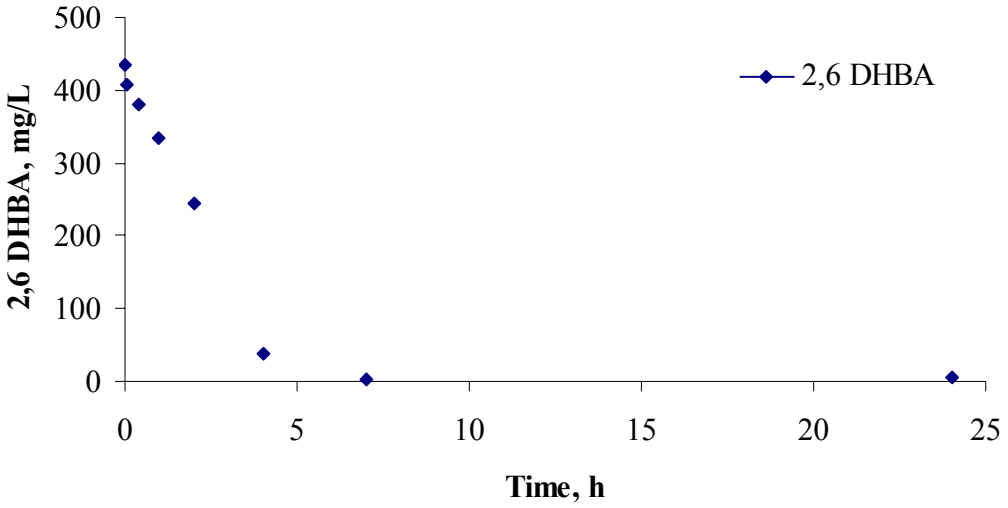

Figure 4.24: 2,6 DHBA Concentration versus Time (Run 1.5)

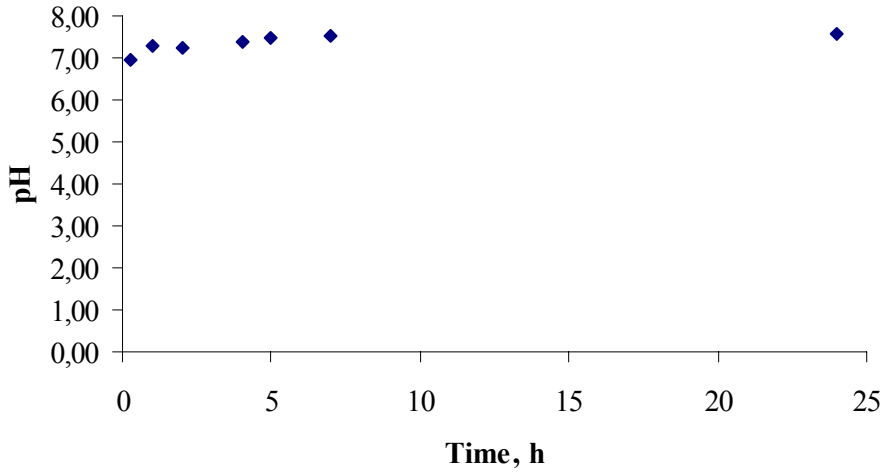

Figure 4.25: pH versus Time (Run 1.5)

Run 1.6 thirtieth day of 2,6 DHBA addition was conducted with only 500 mg COD/L 2,6 DHBA by using the peptone mixture and 2,6 DHBA acclimated sludge. Run 1.6 results are illustrated in Figure 4.26.

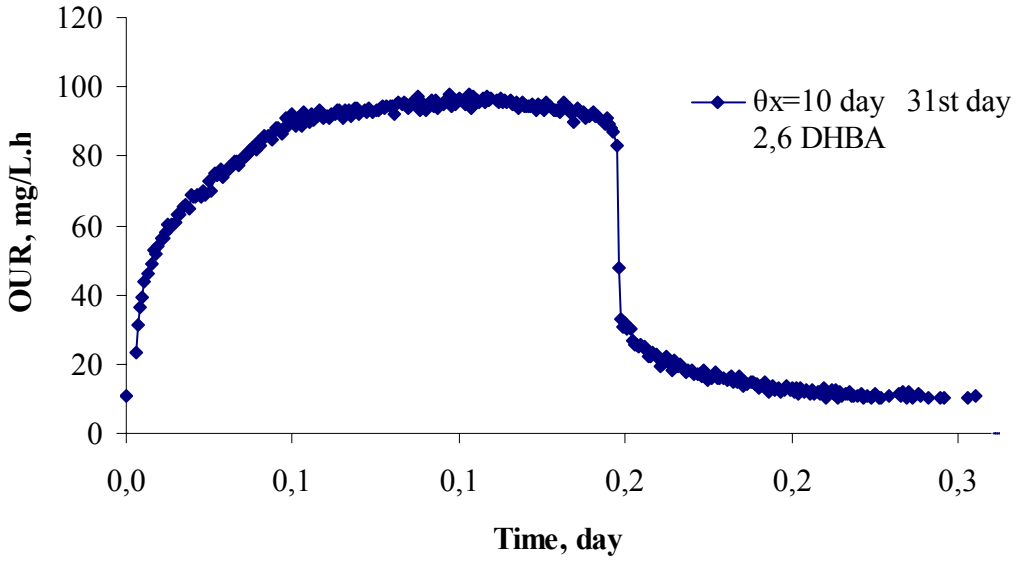

Figure 4.26: OUR Data versus Time (Run 1.6)

Oxygen uptake rate was about 100 mg/L.h on the thirtieth day of acclimation in case only 2,6 DHBA was applied. The trend of the OUR profiles obtained were completely different from the addition of peptone and 2,6 DHBA mixture.

2,6 DHBA removal efficiency was about 100%. The trend of peptone COD degradation did not change. DOC experiments supported the decrease in COD concentration. pH was about 7 with a slight increase during the experiment.

The averaged reactor analysis results operated at SRT of 2 days at the steady state conditions in a period of approximately 1 month is presented in Figure 4.28. The peptone mixture reactor characteristics under steady state conditions for SRT of 2 days are given in Table 4.3.

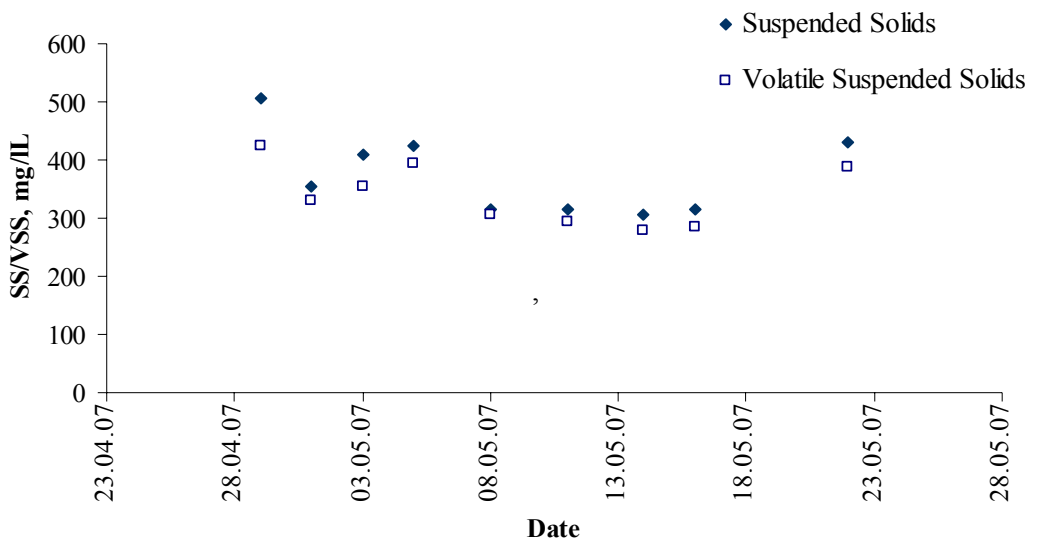

Figure 4.27: Monitoring Results of the Peptone Mixture Reactor (SRT=2 days)

Table 4.2: Characteristics of the Peptone Mixture Reactor under Steady State Conditions

Substrate Type	**S_0/X_0** mgCOD/ mgVSS	**SS** mg/L	**VSS** mg/L	**VSS/SS** mgVSS/ mgSS	**COD_{inf}** mg/L	**COD_{eff}** mg/L	**Removal** %
Peptone mixture	0,25	375	360	0,90	500	10	0,98

Figure 4.29-4.30 illustrate the data obtained for Run 2.1.

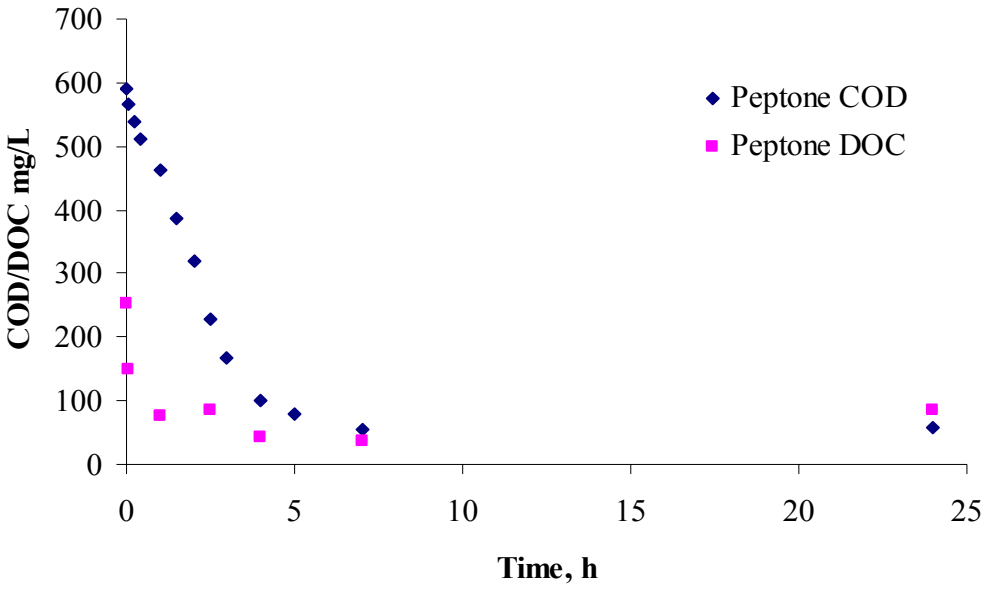

Figure 4.28: COD and DOC Concentration versus Time (Run 2.1)

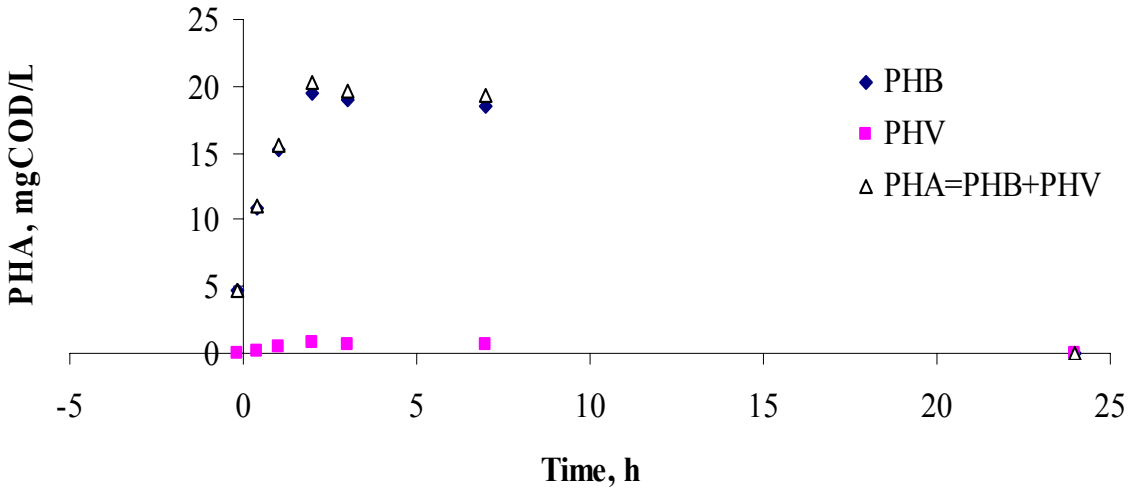

Figure 4.29: PHA versus Time (Run 2.1)

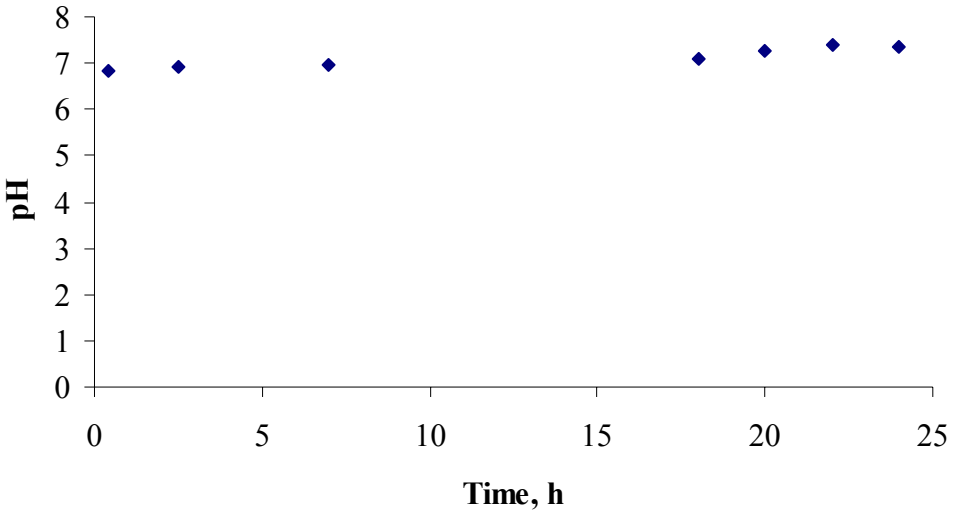

Figure 4.30: pH versus time (Run 2.1)

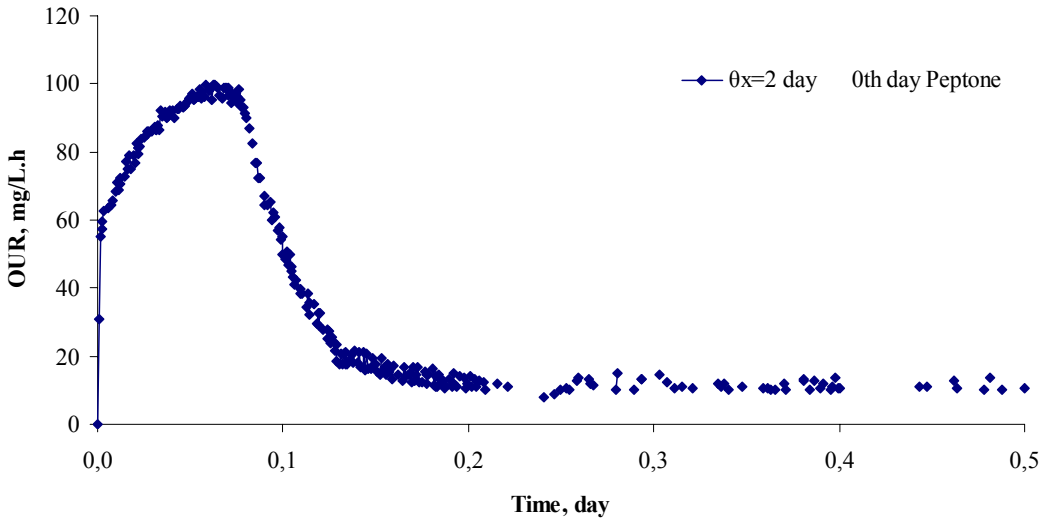

Figure 4.31: OUR Data versus Time (Run 2.1)

After the peptone mixture reactor acclimation of 2 days, the peptone mixture and 2,6 DHBA addition was started by using the peptone mixture acclimated sludge for sludge age of 2 days and a hydraulic retention time of 1 days. SS, VSS and COD results for 4 day period are given in Figure 4.33.

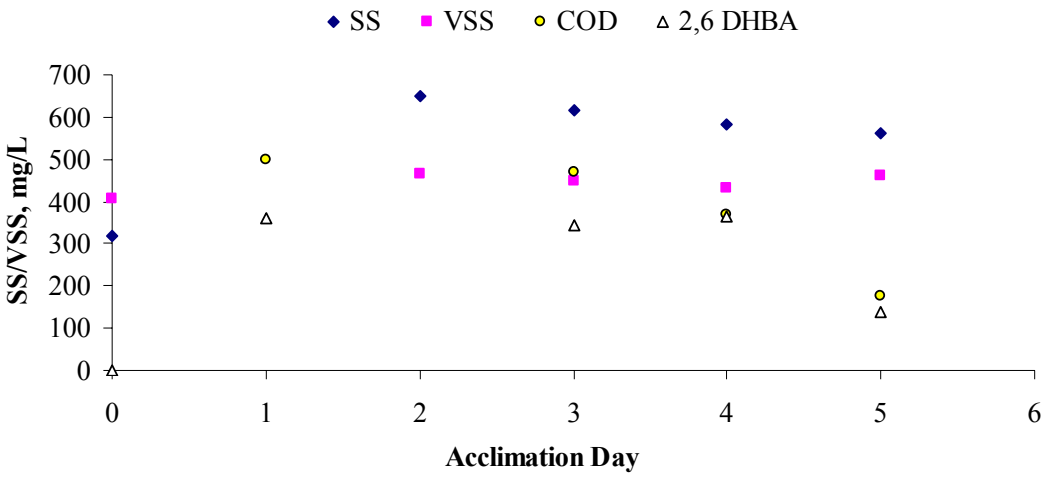

Figure 4.32: Monitoring Results of the Peptone Mixture Reactor (θx=2 days)

Run 2.2 presenting first time addition of 2,6 DHBA was performed with 500 mg COD/L peptone mixture and 500 mg COD/L by using the peptone mixture sludge. Run 2.2 results are illustrated in Figure 4.34-4.38.

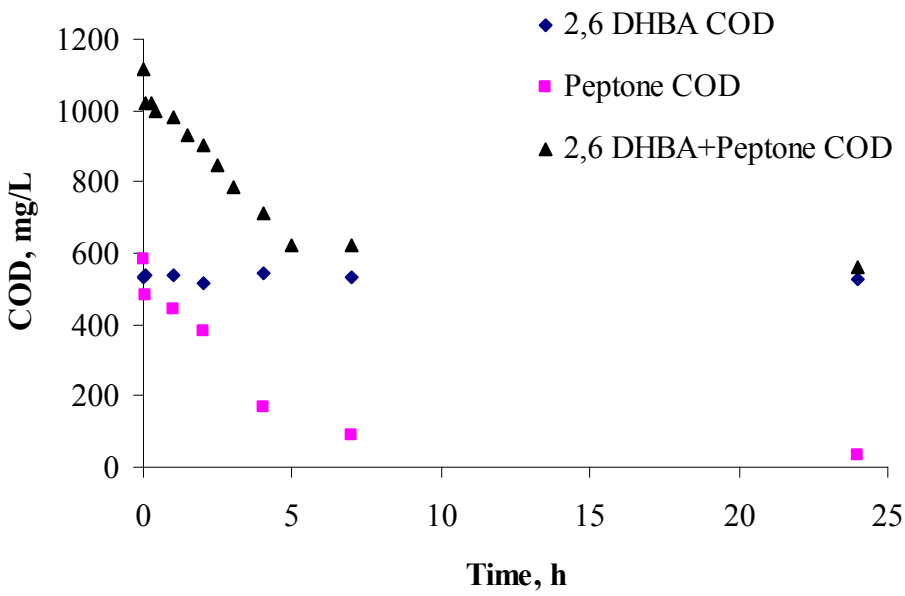

Figure 4.33: COD Concentration versus Time (Run 2.2)

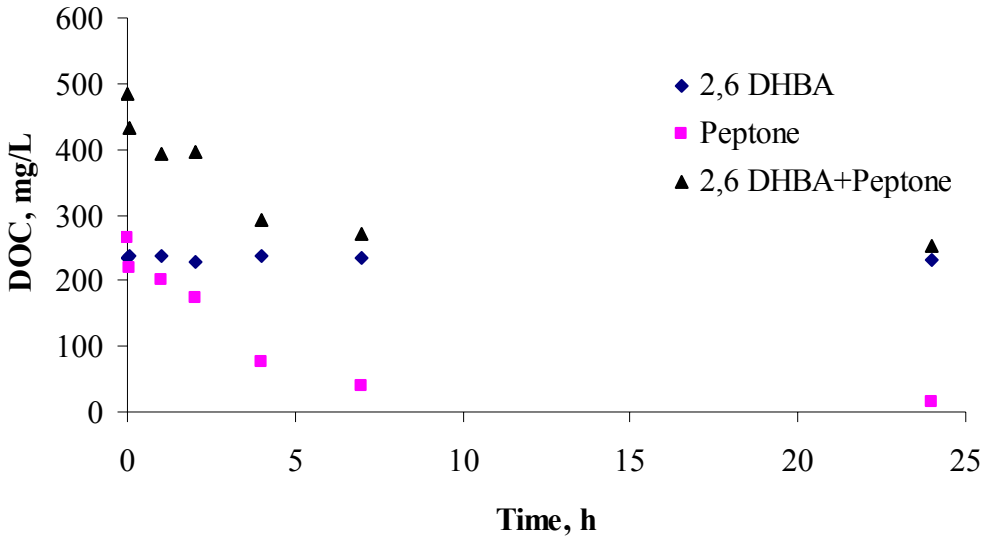

Figure 4.34: DOC Concentration versus Time (Run 2.2)

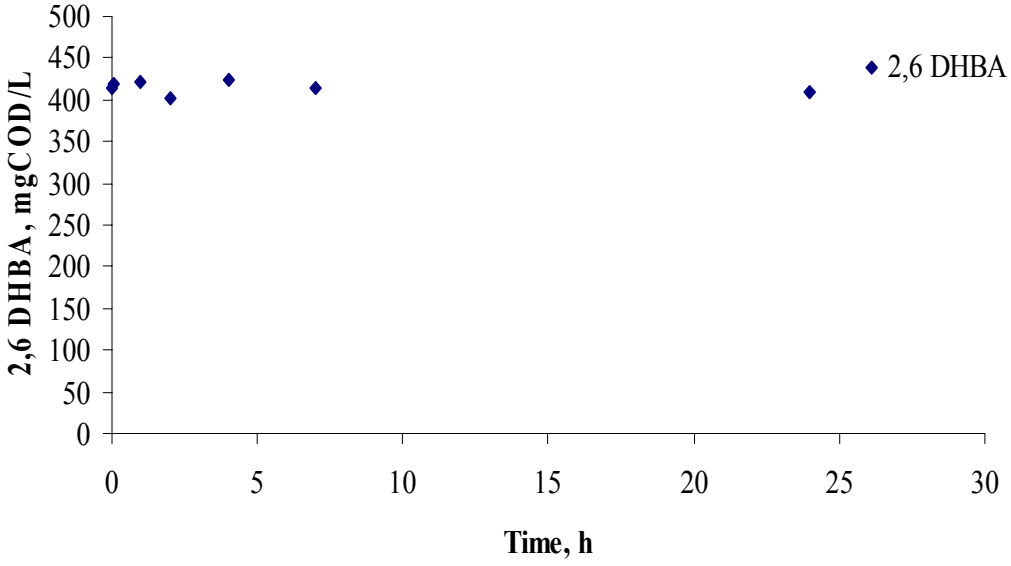

Figure 4.35: 2,6 DHBA Concentration versus Time (Run 2.2)

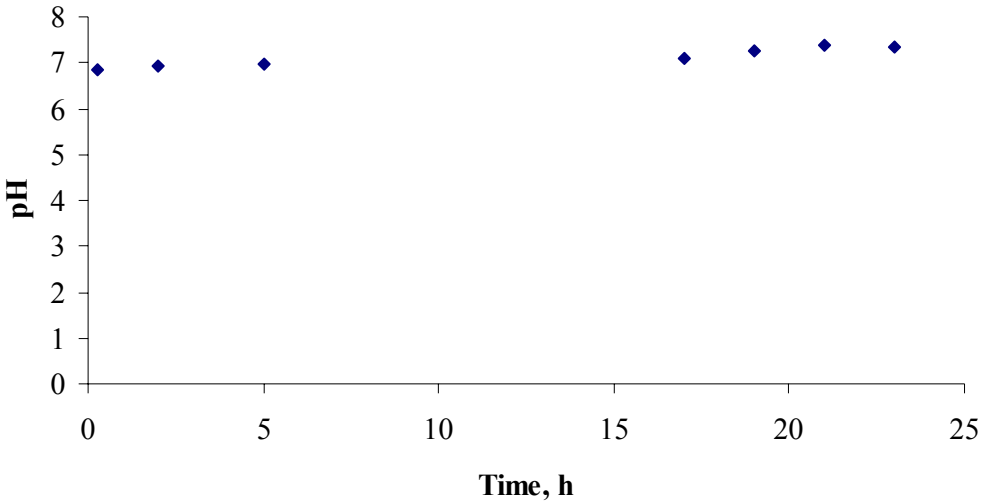

Figure 4.36: pH versus Time (Run 2.2)

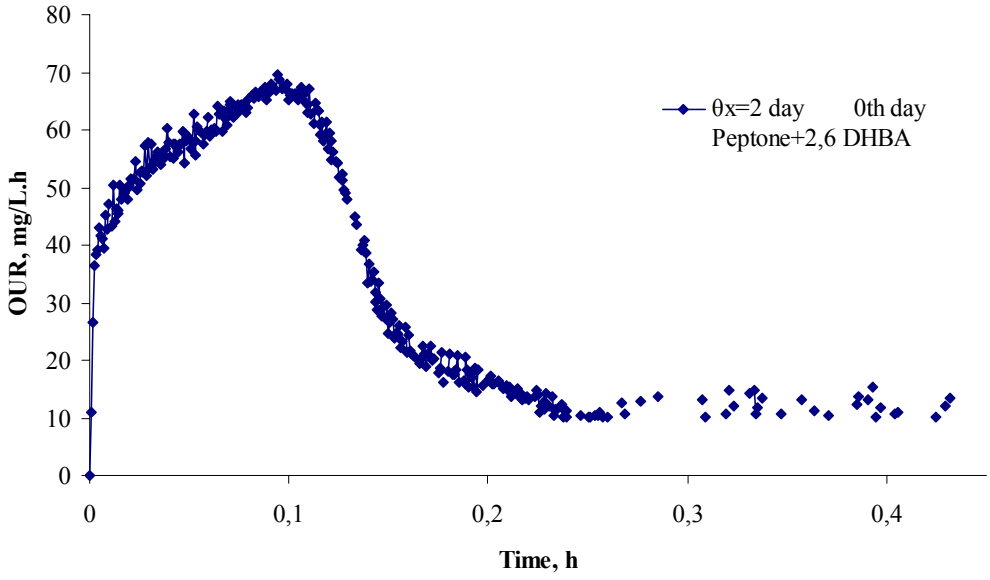

Figure 4.37: OUR Data versus Time (Run 2.2)

Addition of 2,6 DHBA with peptone mixture resulted in decrease of maximum oxygen uptake rate from 100 mg/L.h to 70 mg/L.h. The results indicate that 2,6 DHBA had an inhibitory effect of 30% on peptone mixture acclimated activated sludge whereas the trend of the profile remained the same.

2,6 DHBA removal did not observed. However, the trend of peptone COD degradation did not change leading to decrease in COD degradation efficiency. Storage of PHA were also observed which is about 5 mg COD/L before the addition of peptone and 2,6 DHBA. PHA storage increased to 20 mg COD/L during the experiment and decreased to its initial concentration. pH was about 7 during a one day period with a slight increase.

Run 2.3 presenting second day of acclimation was performed with 500 mg COD/L peptone mixture and 500 mg COD/L 2,6 DHBA. Run 2.2 results are illustrated in Figure 4.39-4.44.

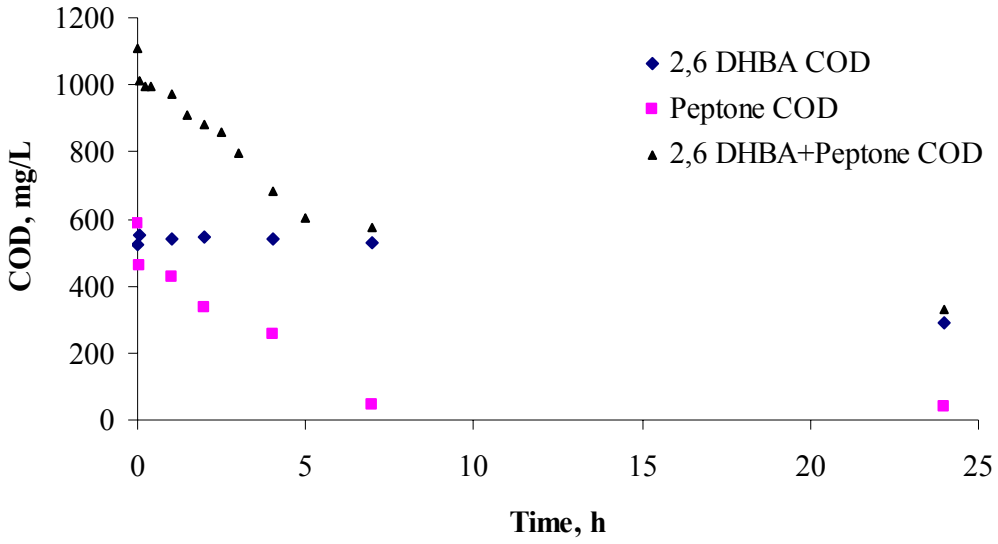

Figure 4.38: COD Concntration versus Time (Run 2.3)

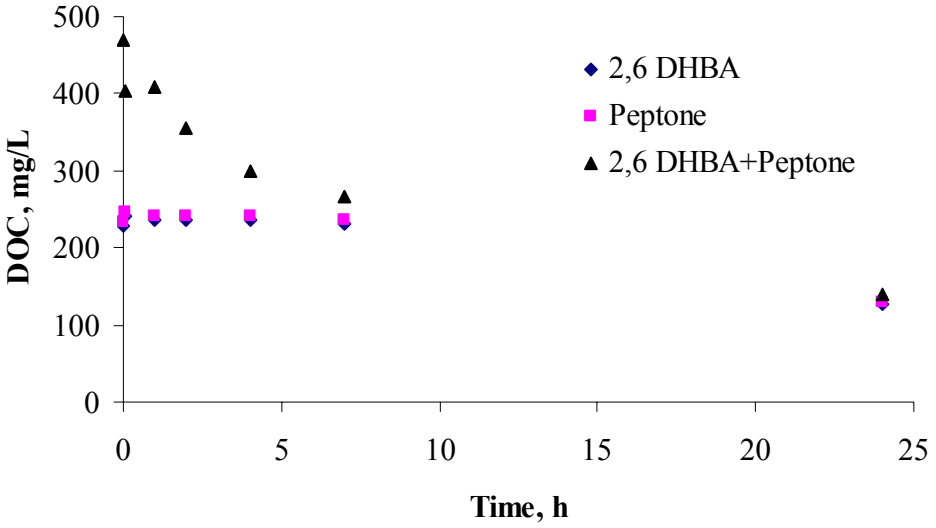

Figure 4.39: DOC Concentration versus Time (Run 2.3)

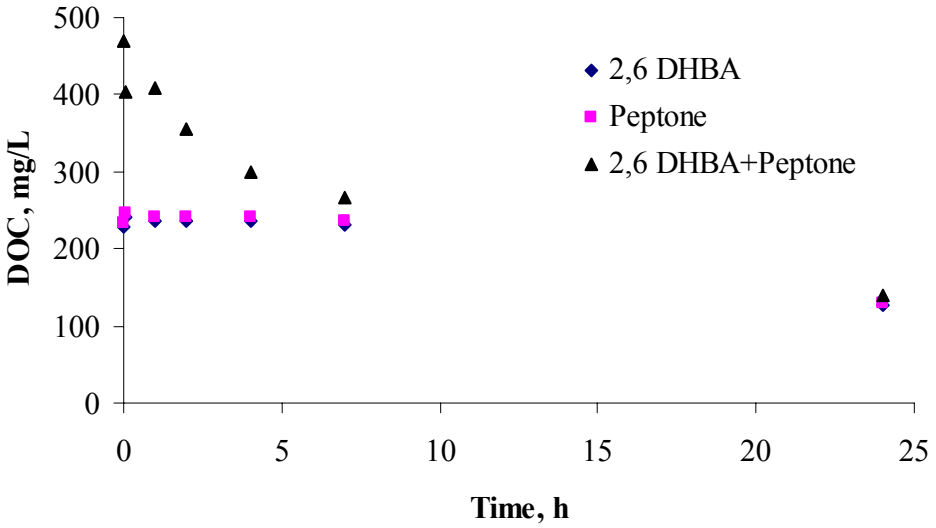

Figure 4.40: DOC Concentration versus Time (Run 2.3)

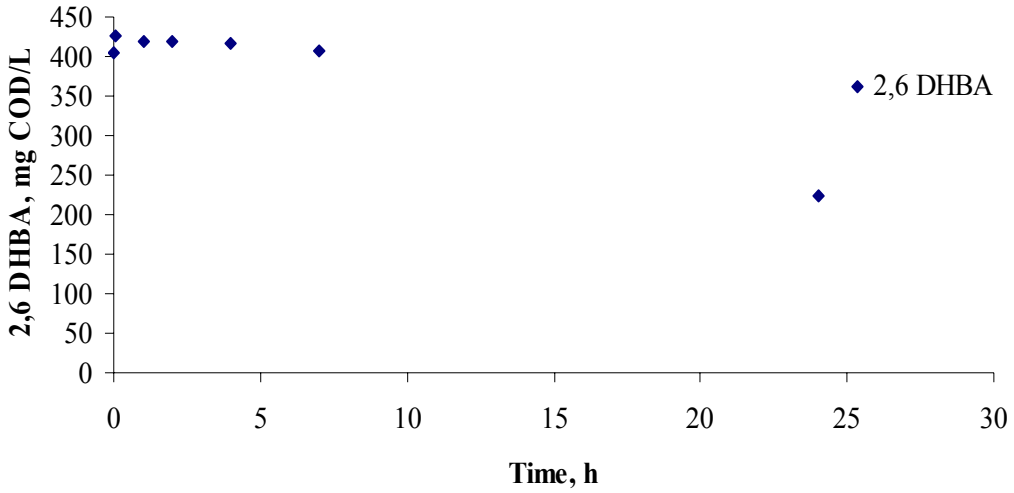

Figure 4.41: 2,6 DHBA Concentration versus Time (Run 2.3)

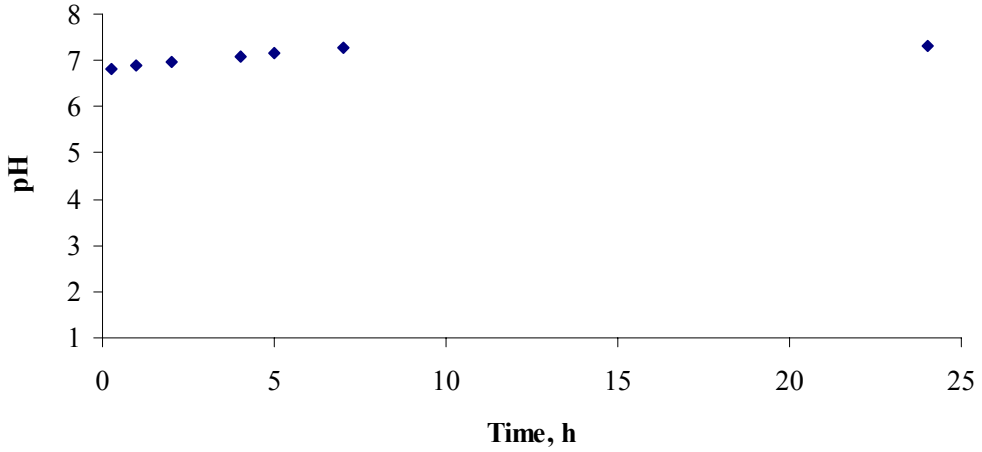

Figure 4.42: pH versus Time (Run 2.3)

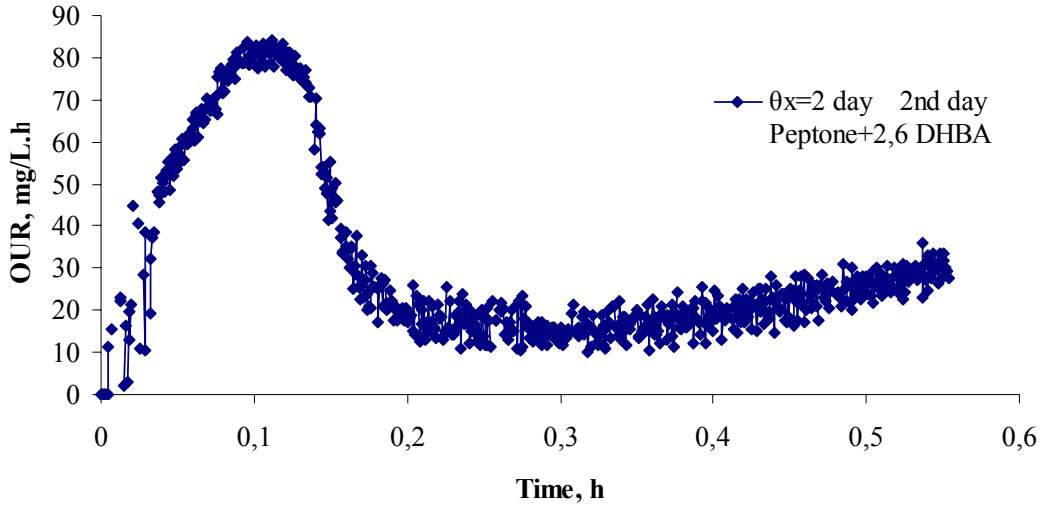

Figure 4.43: OUR Data versus Time (Run 2.3)

Addition of 2,6 DHBA with peptone mixture resulted in decrease of maximum oxygen uptake to 85 mg/L.h. The results indicate that 2,6 DHBA had an inhibitory effect of 15% on peptone mixture acclimated activated sludge and the trend of the profile had changed.

2,6 DHBA removal was observed. The removal efficiency was about 55%. The trend of peptone COD degradation has changed leading to increase in COD degradation efficiency. pH was about 7 with a slight increase during the experiments.

Run 2.4 presenting forth day of acclimation was performed with 500 mg COD/L peptone mixture and 500 mg COD/L 2,6 DHBA. Run 2.2 results are illustrated in Figure 4.45-4.49.

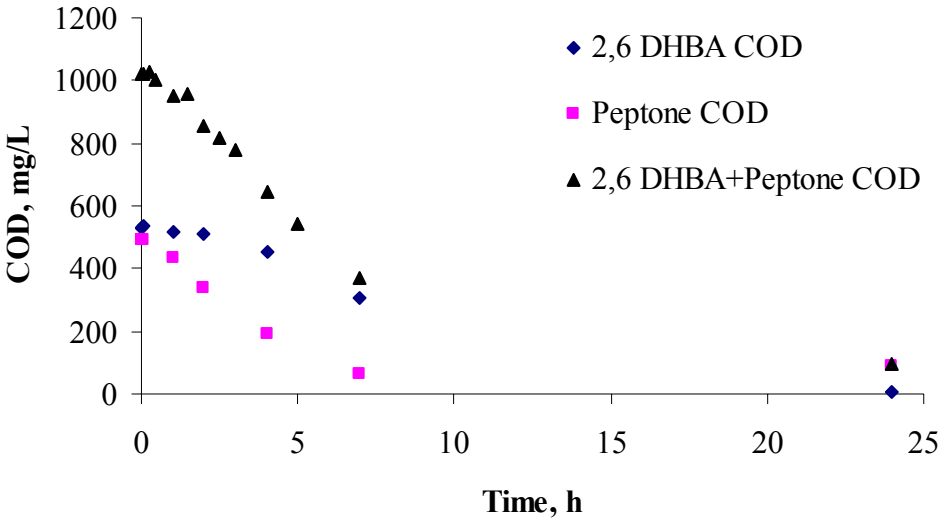

Figure 4.44: COD Concentration versus Time (Run 2.4)

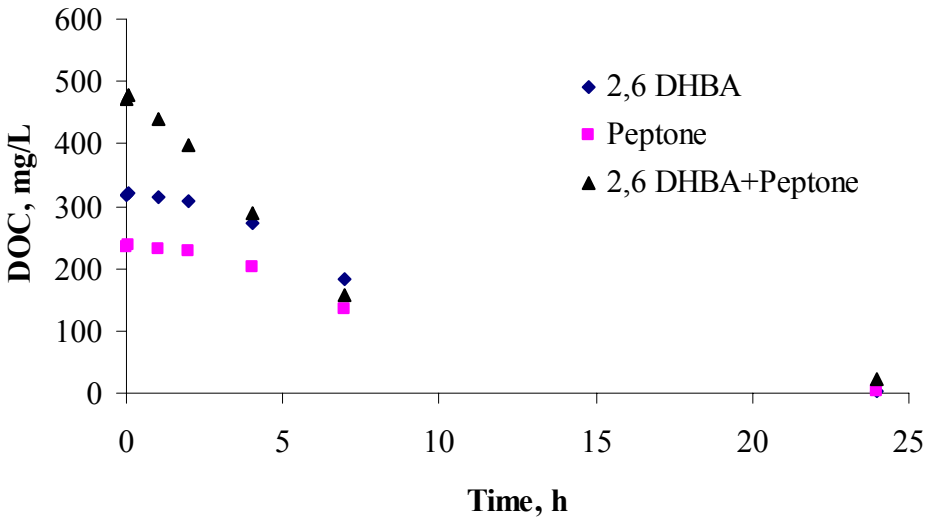

Figure 4.45: DOC Concentration versus Time (Run 2.4)

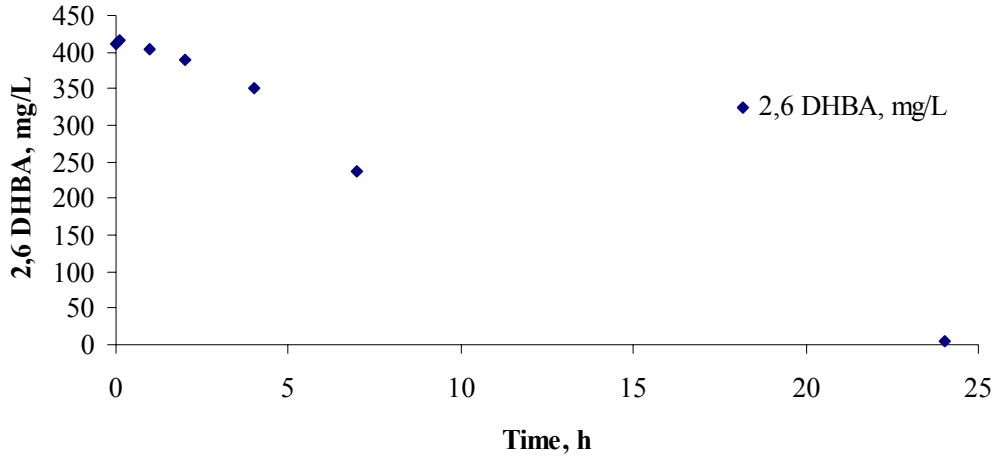

Figure 4.46: 2,6 DHBA Concentration versus Time (Run 2.4)

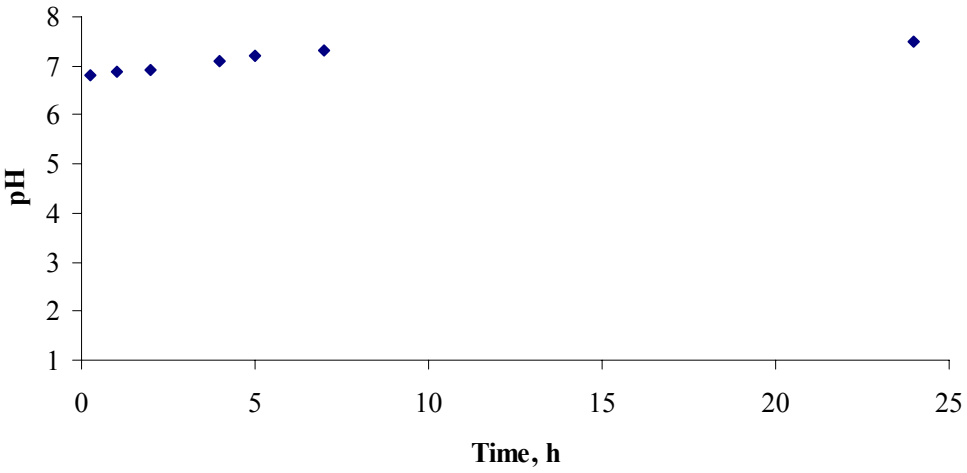

Figure 4.47: pH versus Time (Run 2.4)

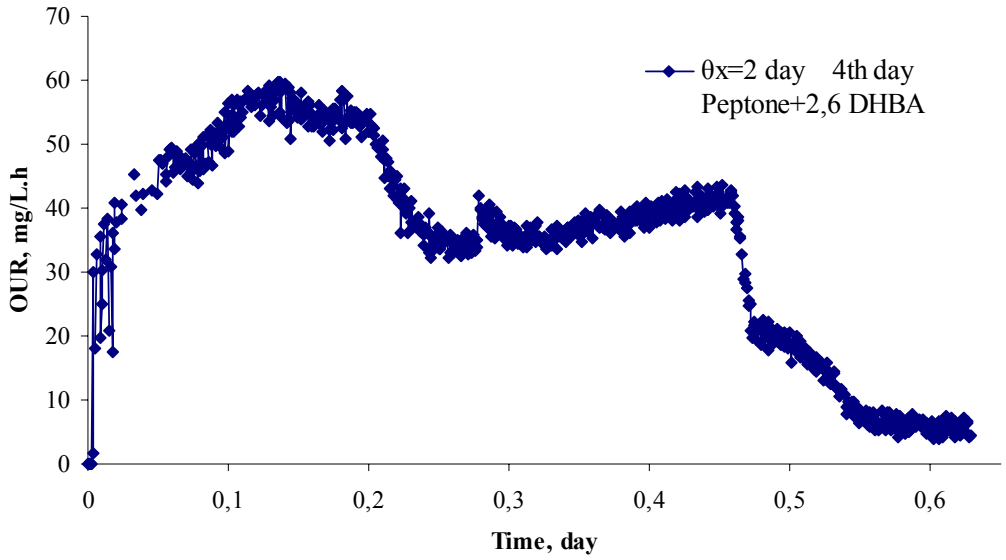

Figure 4.48: OUR Data versus Time (Run 2.4)

Addition of 2,6 DHBA with peptone mixture resulted in maximum oxygen uptake of 60 mg/L.h on the forth day of acclimation. The OUR profile also changed and became similar to OUR profile on fifteenth day of acclimation for SRT of 10 days.

2,6 DHBA removal was observed. The removal efficiency was about 100%. The trend of peptone COD degradation changed leading to increase in COD degradation efficiency. pH was about 7 with a slight increase during the experiments.

Zahn Wellens Biodegradation Test (TS EN ISO 9888, 1999) results are given in Table 4.2, Figure 4.49-4.51.

Table 4.3: Results of Biodegradation Test (TS EN ISO 9888)

t (day)	pH	COD (mg/L)	2,6 DHBA (mg/L)	Total Degradation (%)	Biodegradation (%)
0,00	7,49	446	387	0	
0,05	8,02	331	385	27	0
1	7,97	305	336	34	9
5	8,23	17	3	98	97
7	8,36	7	1	100	100

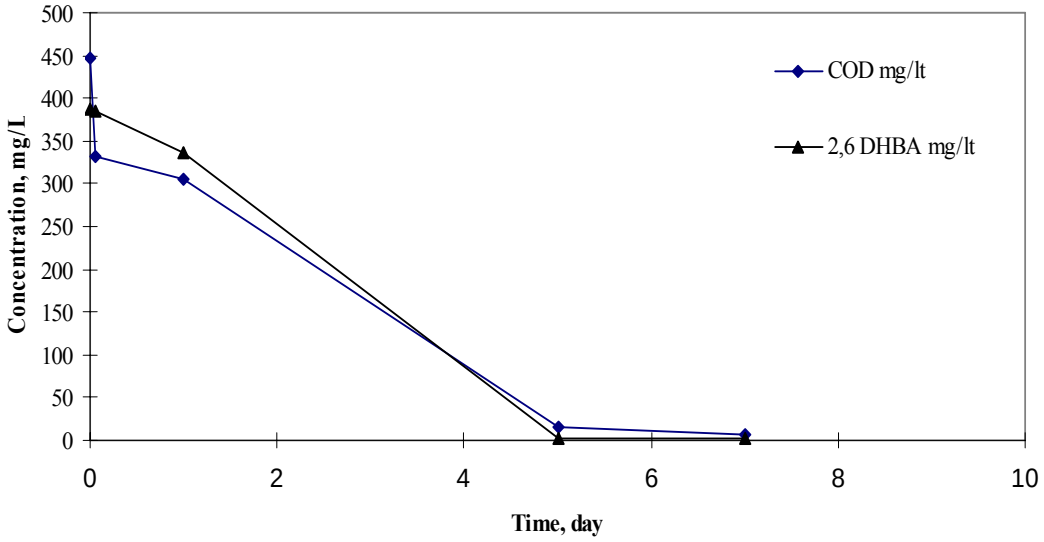

Figure 4.49: COD versus Time for Biodegradation Test (TS EN ISO 9888)

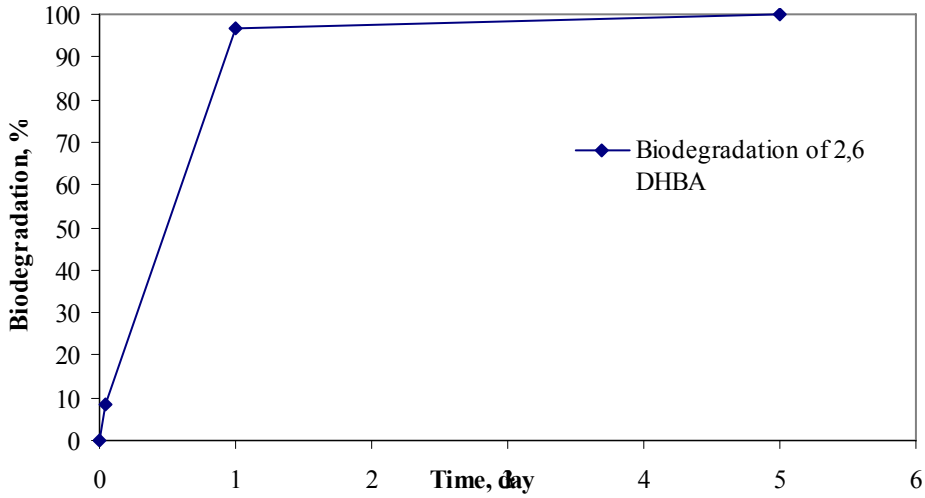

Figure 4.50: Biodegradation of 2,6 DHBA (TS EN ISO 9888)

Biodegradation test results showed that 2,6 DHBA can be totally biodegraded after 7 day with a unacclimated sludge and 2,6 DHBA has no vitalization.

Modeling results of Run 1.1 according to ASM1 and ASM3 are given with experimental data in Figure 4.51-4.52.

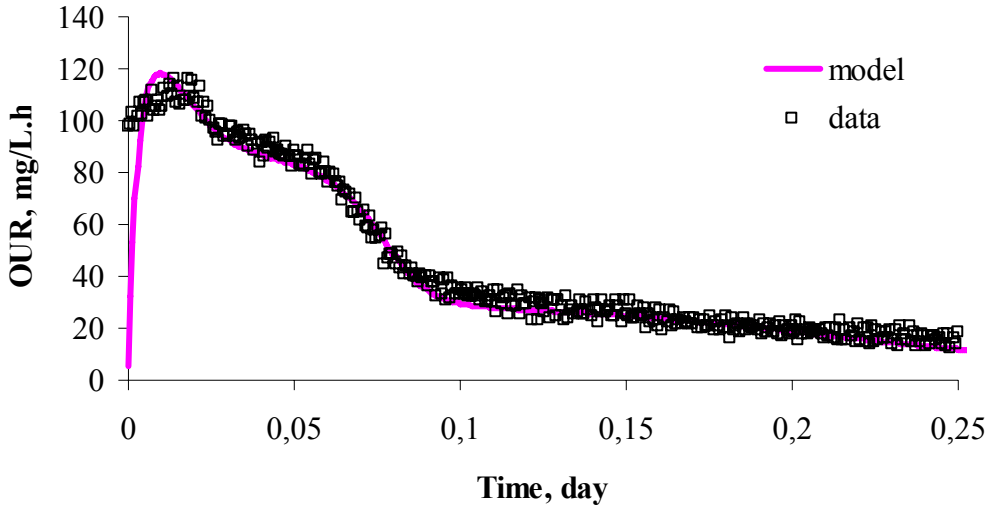

Figure 4.51: ASM1 Simulation (Run 1.1)

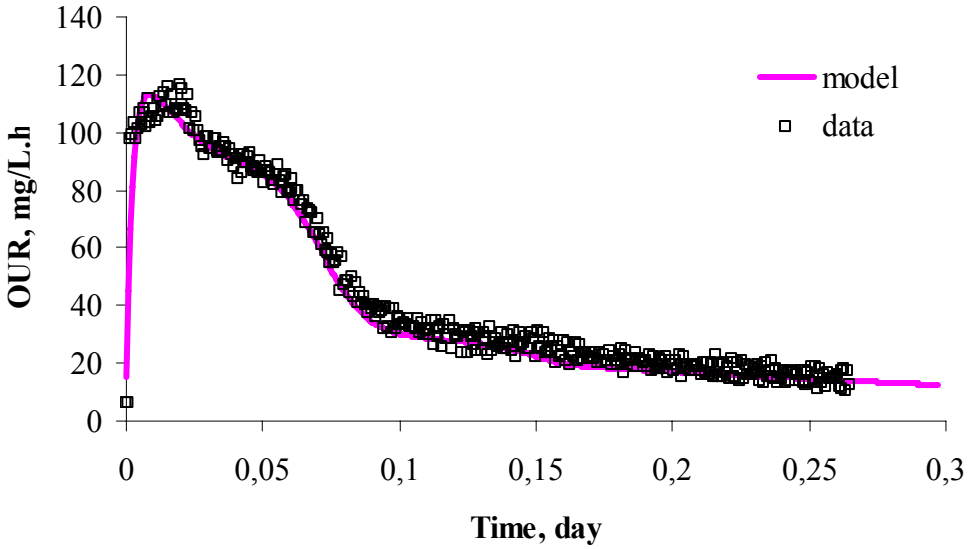

Figure 4.52: ASM3 Simulation of (Run 1.1)

Model simulation results of Run 2.1 according to ASM1 and ASM3 are given with date in Figure 4.53-4.54.

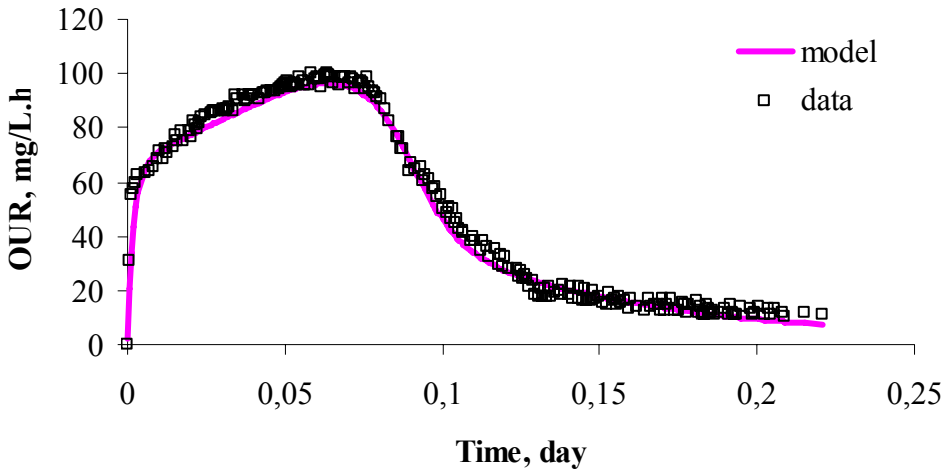

Figure 4.53: ASM1 Simulation of (Run 2.1)

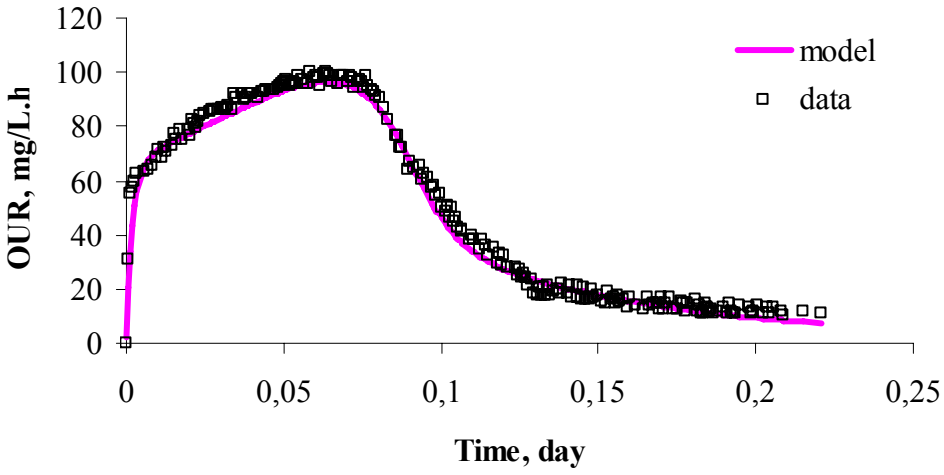

Figure 4.54: ASM3 Simulation (Run 2.1)

The model simulation results based on ASM1 and ASM3 of Run 1.1 and Run 2.1 are given in Table 4.5 and 4.6.

Table 4.4: Estimated Dual Hydrolysis Model Parameters for Run 1.1 and Run 2.1 (ASM No:1)

Model Parameters & Components		**SRT**		
		10 day	**2 day**	**10 day***
Growth Kinetics	$\hat{\mu}_H$, day^{-1}	5	12,7	5
	K_S , mgCOD/l	20	20	23
Hydrolysis Kinetics	k_h , day^{-1}	3,2	9,1	1,78
	k_{hx} , day^{-1}	1,3	10	0,48
	K_{XS} , grCOD/grcellCOD	0,02	0,08	0,03
	K_{XX} , grCOD/grcellCOD	0,04	0,4	0,03
	b_H , day^{-1}	0,13	0,24	0,05

Assumptions: Y_H =0,6 grcellCOD/grCOD * Insel *et al.,* 2007

Modeling results of ASM1 for SRT of 10 and 2 days indicate that maximum heterotrophic growth rate is 5 day^{-1} for SRT of 10 days whereas it was found 12,7 day^{-1} for SRT of 2 days.

The estimated kinetic parameters for SRT of 10 days were similar to reported values by Insel *et al.* (2007). K_S and $\hat{\mu}_H$ values for both of the operating conditions were found 20 and 5 gr COD/gr cellCOD respectively. These values are in the range of typical values of domestic sewage, which are reported as 6 day^{-1} for $\hat{\mu}_H$ and 20 gr COD/gr cellCOD for K_S (Orhon *et al.,* 1994). Simulation estimations for $\hat{\mu}_H$ changed depended on the sludge age. In experiments conducted at SRT of 2 days, a considerable increase of $\hat{\mu}_H$ and b_H was observed to 12,7 day^{-1} and 0,24 day^{-1}. However, endogenous decay rate for SRT=10 day was observed 0,13 day^{-1}.

Increase in $\hat{\mu}_H$ points out the relationship of physiological state of microbial cultures. As it was reported by recent studies, $\hat{\mu}_H$ level is a function of r-RNA level of the cells and the activity of the transporting enzymes involved in substrate utilization. r-RNA level increase stimulate protein synthesis mechanism leading to increase in maximum growth rate (Kavarova-Kovar and Egli, 1998). Moreover, transport enzymes are also affected, which in turn increases its affinity, lower K_S values, to the substrate available. Half saturation constant for the hydrolysis of slowly and rapidly hydrolysable substrate also increased based on model simulation when compared to the parameters estimated for SRT of 10 days. In this operating condition, increasing hydrolysis rate may result in available substrate for both growth and hydrolysis process although affinity of enzymes (K_S) remained constant for growth and or decreased (K_X) for hydrolysis involved in S_S utilization.

Table 4.5: Estimated Model Parameters for Run 1.1-Run 2.1. (ASM No:3)

Model Parameters & Components		**SRT**		
		10 day	**2 day**	**10 day***
Growth Kinetics	$\hat{\mu}_H$, day^{-1}	7	9,2	6,7
	K_S , mgCOD/l	20	20	23
	k_{STO} , day^{-1}	2	2	1
	b_{STO} , day^{-1}	0,5	0,5	2,5
Hydrolysis Kinetics	k_h , day^{-1}	6,5	9,8	6,1
	k_{hx} , day^{-1}	2,0	5,3	-
	K_{XS} , gr COD/grcellCOD	0,05	0,11	0,22
	K_{XX} , grCOD/grcellCOD	0,2	0,2	-
	b_H , day^{-1}	0,2	0,2	0,05

Assumptions: Y_H =0,6 grcellCOD/grCOD, Y_{STO} =0,8 grCOD/grCOD

*Insel *et al.*, 2007

Modeling results of ASM3 based on data obtained from PHA analysis for SRT of 10 and 2 days indicate that maximum heterotrophic growth rate is 7 day^{-1} for SRT of 10 days whereas it was found 9,2 day^{-1} for SRT of 2 days.

The estimated kinetic parameters for SRT of 10 days were similar to reported values by Insel *et al.* (2007). K_S and $\hat{\mu}_H$ values for both of the operating conditions were found 20 and 5 respectively as in the case of ASM1 simulation.

Simulation estimations for $\hat{\mu}_H$ changed depended on the sludge age. In experiments conducted at SRT of 2 days, a considerable increase of $\hat{\mu}_H$ and b_H was observed to 9,2 day^{-1} and 0,2 day^{-1}. However, endogenous decay rates for SRT=10 day was observed 0,05 day^{-1}.

Half saturation constant for the hydrolysis of slowly and rapidly hydrolysable storage compounds also increased based on model simulation when compared with the parameters estimated for SRT of 10 days. In this operating condition, increasing hydrolysis rates may result in available substrate for both growth and storage process although affinity of enzymes, K_S, and K_X involved in S_S utilization remained constant or decreased.

5. CONCLUSION

2,6 DHBA is one of the phenolic compounds which is present in high concentrations in olive mill wastewater. Although it is reported as non-biodegradable in the tests which pure cultures were studied, biodegradability test results indicate that 2,6 DHBA is ultimately biodegradable.

Respirometric experiments conducted in parallel to batch tests showed a useful information about the inhibitory effect of 2,6 DHBA and it was followed by acclimation of activated sludge to this compound. The only peptone fed reactor operated with an efficiency of 94% COD removal. The inhibition of 2,6 DHBA when it is fed with peptone mixture was 56%. The removal of chemical compound was not observed for the first and forth day of acclimation. Acclimation of activated sludge to 2,6 DHBA was observed on the fifteenth day of acclimation period resulting in changes in OUR profiles. The period of thirty days also supported the acclimation of activated sludge to 2,6 DHBA.

Similar experiments conducted on activated sludge systems operated with SRT of 2 days showed 30% inhibition of oxygen uptake rate, which is lower than obtained for high sludge retention time. The acclimation of the system to 2,6 DHBA was observed on the second day of acclimation period and 100% removal was achieved on the forth day of acclimation with a similar OUR profile obtained in higher sludge ages.

Peptone degradation trend and efficiency of the system did not change during hydraulic retention time for SRT of 10 and 2 days although peptone degraded slowly compared to control experiments. Both of the models simulated led to estimation of higher maximum growth rates for the systems operated with sludge age of 2 days, when the two systems are compared in terms of modeling results. Storage compound

analysis supported that these systems stored small amount of PHA and used available substrate for their growth resulting in high maximum growth rates.

Model simulation estimations proved the dependence of kinetic parameters to physiological state of microbial cultures, feeding pattern of substrates and culture history. In small sludge ages, high amount of substrate was available. Thus, the increase of r-RNA level in the cell led to increase in protein synthesis of microbial cells. They caused increase in maximum growth rate, although affinity of enzymes to readily biodegradable substrate remained the same and increased for hydrolysable substrate.

In conclusion, 2,6 DHBA has an inhibitory impact on peptone degradation which cause decrease on peptone degradation rate but does not change removal efficiency at both high and low sludge ages. Acclimation to 2,6 DHBA is possible in the presence of available substrate, peptone mixture which may be a result of cometabolism.

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