İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EVALUATION OF NONYLPHENOL ETHOXYLATE AEROBIC BIODEGRADATION

M.Sc. Thesis by
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Department : Environmental Engineering

Programme : Environmental Biotechnology

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

NONİL FENOL ETOKSİLATIN AEROBİK PARÇALANMASININ DEĞERLENDİRİLMESİ

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Ocak 2011

FOREWORD

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ABBREVIATIONS

AE : Alkyl Ethoxylates
AES : Alkyl Ethoxy Sulphates
APEO : Alkylphenol Ethoxylates
AS : Alkyl Sulphates
ASM : Activated Sludge Model
CMC : Critical Micelle Concentration
COD : Chemical Oxygen Demand
EDC : Endocrine Disrupting Chemical
EU : European Union
HPLC : High Performance Liquid Chromatography
HRT : Hydraulic Retention Time
LAS : Linear Alkylbenzene Sulphonates
NP : Nonylphenol
NPEO : Nonylphenol Ethoxylate
OP : Octylphenol
OPEO : Octylphenol Ethoxylate
OUR : Oxygen Uptake Rate
PHA : Polyhydroxyalkanoate
QAC : Quaternary Ammonium Compounds
sCOD : Soluble Chemical Oxygen Demand
SRT : Sludge Retention Time
SS : Total Suspended Solids
VSS : Volatile Suspended Solids
WE : Western Europe
WWTP : Wastewater Treatment Plant

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EVALUATION OF NONYLPHENOL ETHOXYLATE AEROBIC BIODEGRADATION

SUMMARY

Nonylphenol ethoxylate (NPEO) is a non-ionic surfactant that belongs to Alkylphenol Ethoxylate (APEO) class, has a very wide usage area in industry used in the production of a variety of industrial products as detergents, emulsifiers, solubilizers, dispersing and wetting agents. APEOs are biodegraded and removed in both aerobic and anaerobic conditions easily and because of that they were not attracted attention till 1984. However, it is understood that their primary biodegradation leads to the formation of metabolites known for their inherent biorecalcitrance, potential toxicity and endocrine disrupting behaviour. Therefore, it became more significant to investigate their biodegradation kinetics.

In this study, nonylphenol ethoxylates, a non-ionic surfactant, acute and chronic effects were evaluated for an activated sludge composed of heteretrophic biomass. Activated sludge was acclimated to peptone mixture and acute effects of NPEO for specified concentrations were investigated via respirometric analyses. Chemical oxygen demand (COD) and NPEO change within the system were analysed. NPEO change was determined via high performance liquid chromatography (HPLC).

Then the sludge is acclimated to NPEO for a specified period and respirometric analyses were examined with the acclimated activated sludge for the investigation of the chronic effects of the chemical. Activated Sludge Model No:1 was used for modeling the respirometric analyses. Results were evaluated in terms of kinetic and stoichiometric coefficients estimated by using a multi-component model and optimum treatment conditions of the chemical is gained to the literature.

NONİL FENOL ETOKSİLATIN AEROBİK PARÇALANMASININ DEĞERLENDİRİLMESİ

ÖZET

Nonil fenol etoksilat (NPEO) bir non-iyonik yüzey aktif madde olup, Alkilfenol etoksilatlar (APEO) sınıfına tabidir ve endüstride çok geniş bir kullanım alanı bulunmaktadır. Madde, deterjan, emulsifiyer, yayıcı ve ıslatıcı maddelerin gerek üretiminde gerekse üretim aşamasında kullanılmaktadır. APEO'lar hem aerobik hem anaerobik ortamlarda kolayca parçalanırlar ve giderilirler bu yüzden de 1984 tarihine kadar dikkat çekmemişlerdir. Fakat birinci biyolojik parçalanması sonucu ortaya çıkan ve daha toksik daha kalıcı ve endokrin bozucu olan metabolitlere dönüştüğü anlaşılmıştır. Bu yüzden de maddelerin biyolojik parçalanma kinetiğinin araştırılması daha önemli hale gelmiştir.

Bu çalışma, alkyl fenol etoksilat sınıfına dahil olan bu nonil fenol etoksilatın hetetrofik biyokütleden oluşan aktif çamur üzerindeki akut ve kronik etkisini değerlendirmeyi amaçlamıştır. Pepton çözeltisiyle aklime edilmiş aktif çamur üzerindeki akut etkisi respirometre ile yapılan analizler sonucu değerlendirilmiştir. Respirometrik analizlerin yanı sıra kimyasal oksijen ihtiyacı (KOİ) değişimi ve NPEO değişimi izlenmiştir. NPEO giderimi alınan numunelerin yüksek basınçlı sıvı kromatografisi (HPLC) ile analizi sonucu bulunmuştur.

Daha sonra sistem bir süre NPEO'ya alıştırılmış ve bu süreçte yine respirometre ile analizler yapılarak maddenin kronik etkisi incelenmiştir. Akut çalışmalar sırasındaki gibi KOİ ve NPEO giderimi analiz edilmiştir. Respirometrik analizlerin değerlendirilmesi Aktif Çamur Modeli No:1'e göre yapılmıştır. Sonuçlar, çok bileşenli model kullanılarak tahmin edilen kinetik ve stokiyometrik katsayılarla değerlendirilmiş ve nonil fenol etoksilatın optimum arıtma koşulları için literature kazandırılmıştır.

1. INTRODUCTION

1.1 Purpose of the Thesis

Alkylphenol ethoxylates are non-ionic surfactants that have very wide usage area in both industry and household activities. Due to being used in the houses daily, the biodegration of the chemical gets more and more important. Actually it is seen that the chemical in the wastewater is removed both in aerobic and anaerobic conditions. However, it is realised recently that after primary biodegradation the alkylphenol ethoxylates go into more hazardous, toxic and endocrine disrupting metabolites. Therefore, it is understood that the removal of the chemical is not the ultimate wated result for biological treatment of the chemical. Consequently, the usage and treatment should get more importance in all over the world. Although there are alternatives to the chemicals, because of economical reasons, unfortunately their usages getting wider day by day in so many countries. Degrading to more hazardous metabolites is not only formed in nature but also in biological treatment plants. Therefore, it is getting more important to investigate the kinetic and stoichiometric coefficients for the systems treating the chemical.

The aim of the study is to investigate biodegradation of nonylphenol 10 ethoxylate, NP-10. Moreover, acute and chronic effects will be searched and analyses done with respirometry will be modeled via AQUASIM and kinetic and stoichiometric coefficients will be estimated for optimum treatment conditions according to ASM1.

1.2 Background

In this study, activated sludge taken from Paşaköy Wastewater Treatment Plant was used for the biodegradation studies of nonylphenol ethoxylate, a non-ionic surfactant. System was acclimated to peptone synthetic wastewater and acute effects of the surfactant were evaluated. Then, the system was acclimated to the surfactant and chronic effects were investigated during the acclimation period. Acute effects of 4 different concentration of nonylphenol ethoxylate were examined with respirometric analysis and chemical oxygen demand and nonylphenol ethoxylate were measured in

parallel. Nonylphenol ethoxylate was determined via HPLC. Thereafter, same analyses were examined during the acclimation period. The inhibition and biodegradation mechanism were performed by using multi-component model.

2. LITERATURE REVIEW

2.1 Surfactants

Surfactants are diverse groups of chemicals that are designed to have cleaning or solubilisation properties with lowering the surface tension of a liquid and lowering the interfacial tension between two liquids. They generally consist of a polar head group (either charged or uncharged), which is well solvated in water, and a nonpolar hydrocarbon tail, which is not easily dissolved in water. Thus, surfactants combine hydrophobic and hydrophilic properties in one molecule. Synthetic surfactants are economically important chemicals. They are widely used in household cleaning detergents, personal care products, textiles, paints, polymers, pesticide formulations, pharmaceuticals, mining, oil recovery and pulp and paper industries (Di Corcia, 1998).

2.1.1 Types of surfactants

There are 3 main kinds of surfactants, anionics, if there is an anion in the structure; cationics, if there is a cation in the structure and non-ionics if there is no ionic structure. The types of most widely used surfactants and their common names used are illustrated in Table 2.1. Linear alkylbenzene sulphonates (LAS), alkyl ethoxy sulphates (AES), alkyl sulphates (AS), alkylphenol ethoxylates (APEO), alkyl ethoxylates (AE), and quaternary ammonium compounds (QAC) are the commonly used commercial surfactants. Especially, LAS, QAC, and APEO are the most extensively studied surfactants (Ying, 2006).

2.1.2 Usage area and amount of surfactants

Linear alkylbenzene sulphonates (LAS) are the most popularly used synthetic anionic surfactants. It has been extensively used for over 30 years with an estimated global consumption of 2.8 million tons in 1998.

Table 2.1: Most widely used surfactants (Ying, 2006).

Class	Common name
Anionic Surfactants	Linear alkyl benzene sulphonates (LAS)
	Alcohol ether sulphates (Alkyl ethoxy sulphates) (AES)
	Alcohol sulphates (AS)
Non-ionic Surfactants	Alkylphenol ethoxylates (APEO)
	Nonylphenol ethoxylates (NPEO)
	Octylphenol ethoxylates (OPEO)
	Alcohol ethoxylates (AE)
Cationic Surfactants	Quaternary ammonium compounds (QAC)
	Alkyl trimethyl ammonium halides (TMAC)
	Alkyl dimethyl ammonium halides (DMAC)

Commercially available products are very complex mixtures containing homologues with alkyl chains ranging from 10 to 14 carbon units . Furthermore, since the phenyl group may be attached to any internal carbon atom of the alkyl chain, each homologue contains 5–7 positional isomers (Ying, 2006).

Quaternary ammonium-based surfactants (QAC) are molecules with at least one hydrophobic hydrocarbon chain linked to a positively charged nitrogen atom, the other alkyl groups being mostly short-chain substituents such as methyl or benzyl groups. The major uses of this group of cationic surfactants are as fabric softeners and antiseptic agents in laundry detergents as well as other industrial uses. The most widely used active ingredient in fabric softeners has been dihydrogenated tallow dimethyl ammonium chloride (DTDMAC) until recently. However, the replacement of DTDMAC by ester cationic surfactants such as diethyl ester dimethyl ammonium chloride (DEEDMAC) has recently begun in Europe (Ying, 2006).

Alkylphenol ethoxylates (APEO) constitute a large portion of the nonionic surfactant market. The worldwide production of APEOs was estimated at 500,000 tons in 1997 with 80% of nonylphenol ethoxylates (NPEO) and 20% of octylphenol ethoxyalyes (OPEO).

Concern has increased recently about the wide usage of APEO because of their relatively stable biodegradation products nonylphenol (NP) and octylphenol (OP). NP and OP have been demonstrated to be toxic to both marine and freshwater species, and to induce estrogenic responses in fish (Ying, 2006).

2.1.3 Chemistry of surfactant

A fundamental property of surfactants is their ability to form micelles in solution. This property is due to the presence of both hydrophobic and hydrophilic groups in each surfactant molecule. It is the formation of micelles in solution that gives surfactants their detergency and solubilisation properties. When dissolved in water at low concentration, surfactant molecules exist as monomers. At higher concentrations, the system's free energy can be reduced by the aggregation of the surfactant molecules into clusters (micelles) with the hydrophobic groups located at the center of the cluster and the hydrophilic head groups towards the solvent. The concentration at which this occurs is known as the critical micelle concentration (CMC) (Haigh, 1996). Nonionic surfactants have lower CMC levels than anionic and cationic surfactants.

At concentrations above the CMC level, surfactants have the ability of solubilise more of hydrophobic organic compounds than would be dissolve in water alone. The effectiveness of surfactants in solubilising water insoluble or poorly soluble compounds is dependent on the sorbed compounds, the environmental media and the surfactant.

Surfactants may affect the mobility and degradation of hydrophobic organic compounds in soil or sediment (Edwards et al., 1994; Tiehm, 1994). Aronstein et al. (1991) found that the extent of phenanthrene biodegradation was markedly increased at nonionic surfactant concentrations of 10 Ag/kg soil in both a mineral and organic soil, despite lack of desorption enhancement in the organic soil. Ying et al. (2005) also found that small percentages (> 1%) of surfactants in water could mobilize triazines in the contaminated soils, which have been stabilized by activated carbon. In sewage sludge amended soils, there are many other hydrophobic organic compounds except surfactants at high concentrations.

These surfactants may interact with those hydrophobic compounds. Kile and Chiou (1989) studied the effect of anionic, cationic and nonionic surfactants on the water solubility of DDT and trichlorobenzene. As would be expected, the solubility was enhanced when the surfactant was present at concentrations greater than the critical micelle concentration. There was also a solubility enhancement at surfactant concentrations less than the CMC levels. However, the studies by Edwards et al. (1994) found that surfactants below CMC enhanced the sorption uptake of hydrophobic organic pollutants due to the formation of hemimicelles. At higher concentrations, the same surfactants in micellar form remobilized those hydrophobic compounds already adsorbed by solubilisation. The concentration of surfactants required to mobilize contaminants is significantly above those normally found in sewage sludge (Sweetman et al., 1994).

2.1.4 Sorption of surfactants

Once surfactants enter the environment through sewage discharge into surface water, pesticide application or sludge disposal on land, they undergo many processes such as sorption and degradation. Knowledge of the processes involved in distributing these surfactants among ecosystem compartments is essential to an understanding of their behavior in the environment.

Sorption of a surfactant onto sediment/soil depends on many factors including its physiochemical properties, sediment nature and environmental parameters. The information from sorption process of a surfactant can be used to estimate the distribution of the surfactant in different environmental compartments (sediment/ soil and water).

Sorption data can also be used to estimate the bioavailability of the surfactant. Furthermore, sorption has a significant influence on the degradation of the surfactant in the environment.

Sorption can be described by using sorption isotherms. The commonly used Freundlich equation defines a nonlinear relationship between the amount sorbed and the equilibrium solution concentration:

$$S = K_f C^n \quad \text{.....(2.1)}$$

where S is the concentration of a surfactant sorbed by the solid phase (mg/kg); K_f is the Freundlich sorption coefficient (L/kg); C is the equilibrium solution concentration (mg/L) and n is a power function related to the sorption mechanism. When the value of n is unity, we have the simplest linear isotherm:

$S = K_dC$.....(2.2)

where K_d is the sorption coefficient (L/kg). The parameter K_d is frequently used to characterise the sorption of a chemical in sediment/soil and is an important parameter governing the partitioning and mobility of the chemical in the environment. Sorption of some chemicals especially those nonpolar compounds closely depends on organic matter in the sediment/soil. Therefore, the organic carbon sorption coefficient (K_{oc}) is often used to describe the sorption of those compounds on sediment/soil.

Due to their chemical features, surfactant molecules may sorb directly onto solid surfaces or may interact with sorbed surfactant molecules. The sorption mechanism is dependent on the nature of the sorbent and the surfactant concentration. At low concentrations, the surfactant molecules may be sorbed to a mineral surface or clean sediment that has very few sorbed surfactant molecules, and sorption may occur mainly due to van der Waals interactions between the hydrophobic and hydrophilic moieties of the surfactant and the surface.

There are no significant sorbate–sorbate interactions at the low concentrations. As the surfactant concentration increases, active sorption sites on solid surface become less and less available, and more and more hemimicelles form. At higher concentrations, such sorption may entail the formation of more structured arrangements including the formation of monomer surfactant clusters on the surface or a second layer, for which these arrangements may be governed mainly by interactions between hydrophobic moieties of the surfactant molecules. Therefore, two stage sorption isotherms have been reported for nonionic surfactants NPEO and AE and anionic LAS although the sorption behavior is different for nonionic and anionic surfactants (Ying, 2006).

The sorption of LAS on natural soils had two stages: linear and exponentially increasing isotherms (Ou et al., 1996).

At low LAS concentration (< 90 Ag/mL), the sorption isotherms were linear and Kd ranged from 1.2 to2.0. At high levels (> 90 μg/mL), cooperative sorption was observed and the sorption amount of LAS increased exponentially with the increasing of LAS concentration in solution. This enhanced sorption of LAS on soils was also observed by Fytianos et al. (1998). Under real soil environment or aquatic environment where LAS levels are rather low, the LAS sorption ability of a soil or sediment is very weak. In contrast, the sorption of a nonionic surfactant reached a maximum on the solid surface when the solution is near or just at the critical micelle concentration of the surfactant. The decreased sorption of non-ionic surfactants (APEOs and AEs) on sediment at higher concentrations was observed. A Langmuir isotherm as described by the following equation provides a reasonable fit to the sorption data

$$S = S_{max}K_lC/(1 + K_lC) \quad \text{.....(2.3)}$$

where S is the sorbed concentration of the surfactant on the solid surface (mg/kg), S_{max} is the maximum sorbed concentration (mg/kg), C is the aqueous phase surfactant concentration (mg/L), and Kl is the Langmuir constant (L/kg).

Surfactant concentrations in the environment are normally at low concentration range below the critical micelle concentration (CMC) of the surfactant. Surfactant sorption onto environmental sorbents (sediment or soil) is mostly Freundlich type.

Anionic surfactant LAS had much lower Kd values than nonionic surfactants APEOs and AEs. However, cationic surfactants tend to adsorb strongly onto sediment/soil (Haigh, 1996). Sorption coefficients of AEs on suspended sediment increased with increasing alkyl and ethoxylate chain lengths (Kiewiet et al., 1996).

The dominant influence of the alkyl chain suggests a hydrophobic sorption mechanism. Ferguson et al. (2001) investigated the partitioning of APEO metabolites to suspended solids in Jamaica Bay, New York, and found that log Koc values did not vary greatly among the APEO metabolites and were 5.39 for NP, 5.18 for OP, 5.46 for NPEO1, 5.18 for NPEO2 and 4.87 for NPEO3, respectively.

John et al. (2000) measured sorption coefficients (Kd) of NPEO3-13 homologues onto native sediment, organic-free sediment, kaolinite, silica, and sewage sludge and found that Kd values for native sediment decreased progressively from 1460 L/kg for NPEO3 to 450 L/kg for NPEO10, then increased again slightly for higher homologue

In contrast, Kd values for organic-free sediment (230–590 L/kg) or kaolinite (190–490 L/kg) increased steadily from NPEO3 to NPEO13. Adsorption to sewage sludge was very strong with Kd values ranged from 12,000 to 33,000 L/kg. These data indicated that interactions with organic matter were important in controlling sorption of Aps and short ethoxylate APEOs. However, as the level of AP ethoxylation increased, association with mineral surfaces became the dominant contributor to APEO sorption (Ying, 2006).

When a chemical in sewage effluent is discharged into the environment, it distributes into the different phases such as water, air, sediment and biota, and equilibrium is formed depending on the properties of the chemical and the phases. Therefore, the water to biota transfer is of critical importance because we are principally concerned with adverse effects on biota.

The process involving the direct transfer of a chemical from water to biota is described as bioconcentration. At equilibrium, bioconcentration is characterised by the bioconcentration factor (BCF), the ratio between the concentration in biota, CB, and the concentration in water, Cw.

Since a surfactant has to be taken up into an organism before it can elicit an effect, the processes and factors influencing uptake are relevant when assessing the environmental risk. Lipophilic compounds are the organics most likely to bioaccumulate. Mackay (1982) has demonstrated that the lipid phase in biota is the dominant phase for their accumulation. Lipophilicity, or hydrophobicity, measured as the octanol to water partition coefficient (K_{ow}) has identified as the driving force for bioconcentration. Bioconcentration increases with increasing K_{ow} value (Ying, 2006).

2.1.5 Biodegradation of surfactants

Balson and Felix described biodegradation as the destruction of a chemical by the metabolic activity of microorganisms. When reviewing the literature concerning the degradation of surfactants it is apparent that studies figures for primary and ultimate biodegradation.

Primary degradation can be defined as to have occurred when the structure has changed sufficiently for a molecule to lose its surfactant properties. Ultimate degradation is said to have occurred when a surfactant molecule has been rendered to CO_2, CH_4, water, mineral salts and biomass (Scott and Jones, 2000).

LAS are generally regarded as biodegradable surfactants. Very high levels of biodegradation (97 - 99%) have been found in some WWTP using aerobic processes. In contrast, APEO are less biodegradable and values of 0-20% have been quoted based on oxygen uptake and 0-9% based on spectroscopic techniques (Scott and Jones, 2000).

The mechanism of breakdown of LAS involves the degradation of the straight alkyl chain, the sulphonate group and finally the benzene ring. The breakdown of the alkyl chain starts with the oxidation of the terminal methyl group (ω-oxidation) through the alcohol, aldehyde to the carboxylic acid as follows.

The reactions are enzyme catalysed by alkane monooxygenase and two dehydrogenases. The carboxylic acid can then undergo β-oxidation and the two carbon fragment enters the tricarboxylic acid cycle as acetylCo-A. It is at this stage that problems arise with branched alkyl chains, a side chain methyl group or a gem-dimethylbranched chain cannot undergo β-oxidation by microorganisms and must be degraded by loss of one carbon atom at a time (α-oxidation) (Scott and Jones, 2000).

Whichever mechanism prevails the breakdown product of the LAS is sulphite which can be oxidised to sulphate in the environment. The loss of the alkyl and the sulphonate group from LAS leaves either phenylacetic or benzoic acids. Microbial oxidation of phenylacetic acid can result in fumaric and acetoacetic acids and benzene can be converted to catechol (Scott and Jones, 2000).

Studies on the biodegradation of LAS and other surfactants by biofilms of bacterial populations isolated from riverine and estuarine sites have been reported. A study of biodegradation of a range of anionic surfactants at a river site (river Ely, South Wales, UK) located near a sewage treatment plant outfall has been made. Experiments were conducted in the laboratory using a population of bacteria isolated from river stone biofilms.

Water collected at the outflow (BO), upstream (BU) and downstream (BD) of the site was incubated with the isolated bacteria. It was found that the reciprocal half-life (or `die-away' time) for biodegradation of surfactants followed the sequence alkyl sulphates > alkyl ethoxy sulphates > secondary linear alkyl sulphates > primary alkane sulphonates > LAS, and that `die-away' time of surfactants depended on the site in the sequence BO > BD > BU. The ability of bacterial species in the population to biodegrade sulphonated surfactants was less widely distributed than the ability to biodegrade sulphate ester surfactants (Scott and Jones, 2000).

In a study of LAS biodegradation by bacterial cultures originating from an estuarine site (Krka river estuary, Croatian Mid Adriatic region; a highly stratified karstic estuary) it was found that the rate of biodegradation depended on the origin of the culture, temperature and the structure of the alkylbenzene group. Cultures isolated from the freshwater layer of the river had a greater ability to degrade LAS than those from the underlying saline water layer. Degradation rates were faster for the longest alkyl chain LAS (in this study C13), and slower for LAS isomers having the sulphophenyl group situated in the middle of the alkyl chain.

The complete biodegradation of surfactants requires a consortium of bacteria due to the limited metabolic capacities of individual microorganisms. The opportunity for commensalism and synergism to develop exists in a consortium. Such interactive effects lead to more effective biodegradation than is possible by any individual microorganism (Scott and Jones, 2000).

The biodegradation of LAS requires a four membered consortium, three members of which oxidise the alkyl chain but synergism amongst the four members was essential for mineralisation of the aromatic ring (Scott and Jones, 2000).

A large amount of surfactant is associated with sewage sludge solids. However, LAS are not biodegraded by either mesophilic or thermophilic anaerobic digestion. Various estimates of the load of LAS and APEO in a typical wastewater treatment plant and their subsequent fate have been given. Although there are wide ranges in some of the values, e.g. values quoted for the load of LAS n treatment plants range from 3 to 21 mg/L^3, it is clear that significant amounts of surfactant are transported into the environment from treatment plants. estimate a discharge of over 100 kg day/L of anionic surfactants and approximately 300 kg day/L of cationic surfactants from a 90U106 gal day/L wastewater treatment plant**.** It should also be noted that the presence of surfactants in water at concentrations below and above the critical micelle concentration can also lead to the solubilisation of other oil-soluble pollutants such as DDT and trichlorobenzene.

The problem of the analysis of surfactants in the aqueous environment has recently been reviewed by Lukaszewski. With the introduction of new types of surfactants developed to replace ethoxylates such as alkyl polyglucosides it is necessary to have methods for their detection and for the detection of their breakdown products as well as improving existing methods for specific determination of different classes of anionic and non-ionic surfactants. The behaviour of LAS in sewage by using direct UV absorption spectra deconvolution has been described (Scott and Jones, 2000).

The biodegradation of LAS is effected by a number of factors amongst which are the concentration of dissolved oxygen, complexing with cationic surfactants, the formation of insoluble calcium and magnesium salts, the presence of other organic contaminants and the effect of LAS on the pH during aerobic degradation. In sewage- contaminated groundwater the rates of LAS biodegradation increase with dissolved oxygen concentration and the longer alkyl chain homologues (C12 and C13) are preferentially biodegraded. However, the removal of LAS was found to be 2^3 times greater under laboratory conditions than in field tracer studies. The formation of LAS complexes with cationic surfactants (alkyltrimethylammonium chloride (TM) and dialkyldimethylammonium chloride (DM)) leads to complex adsorption onto river sediments.

The adsorption of complexes which form with molar ratios of LAS to cationic surfactant in the range 1:1 to 6:1 (with TM) and 1:1 to 2:1 (with DM) obey the Freundlich adsorption isotherm. The rates of biodegradation as measured over 14 days for 1:1 and 2:1 complexes relative to the rate of biodegradation of LAS (taken as 100%) were as follows: 2LAS:TM (56%), LAS:TM (36%), 2LAS:DM (31%) and LAS:DM (29%). The kinetics of biodegradation of LAS and other organic matter by mixed bacterial cultures as used in activated sludge treatment can be affected by LAS at high concentrations (s20 mg l31). This arises as a consequence of LAS decreasing the pH during aerobic degradation. The highest rates of biodegradation are found for the longest alkyl chain homologues.

The biodegradation of APEO by bacteria in seawater polluted with urban sewage is brought about by bacteria of the Pseudomonas genus of marine origin. Few other species of Gram-negative bacteria are able to degrade APEO with nine-ten ethoxy groups. Pseudomonas strains degrade only down to four or five ethoxy groups, although other species of bacteria which are unable to degrade the long chain APEO are able to degrade the APEO with four or five ethoxy groups down to the two ethoxy group compounds (Scott and Jones, 2000).

2.2 Nonylphenol Ethoxylate

Nonylphenol ethoxylate belongs to that APEO class. They are known as surfactants because they decrease surface tension and clean by concentrating between non-mixable interfaces, such as oil and water. There are different types of APEOs, such as NPEOs and octylphenol ethoxylates (OPEOs). Because OPEOs and NPEOs are in the same family, they have similar chemical properties. NPEOs are used more than OPEOs – both are toxic, estrogenic, and more difficult to degrade than other cleaning agents (Hoponick, 2005).

NPEO has a nonyl structer on the phenol group forms the hydrophobic head and the hydrophilic tail formed by ethoxylate chain on the para position as seen in Figure 2.1.

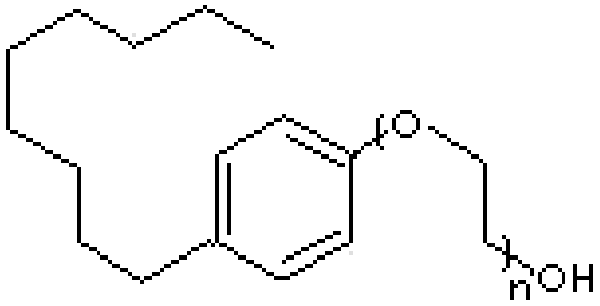

Figure 2.1: Chemical structure of NPEO.

Nonylphenol (NP), chemical structure shown in Figure 2.2, is used in the production of NPEO cleaning agents, also known as surface active agents or surfactants. NPEOs have been commercially synthesized for almost 50 years (Hoponick, 2005).

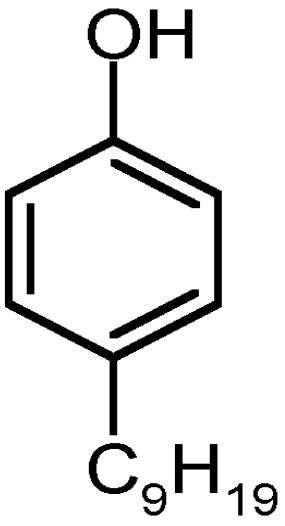

Figure 2.2: Chemical structure of NP.

2.2.1 Usage area and amount of NPEO

NPEOs are produced for high-volume use in many industrial sectors, including industrial laundering, textile processing, pulp and paper processing, paint and resin formulation, oil and gas recovery, steel manufacturing, pest control and power generation.

NPEOs are also utilized in the production and formulation of many commercially sold products: as an industrial and commercial detergent, as an emulsifier in wax for fruit and vegetables, as a polymer resin in plastic food packaging and polyethylene plastic, in cosmetic products (such as skin cream, deodorant, makeup, hair dye, and shampoo), and even in spermicides (Hoponick, 2005).

The largest quantity of NPEOs is used in cleaning products, especially detergents. Of the 260 million pounds of NP used in 2004, 80% was used as a surfactant. In general 37% of NPEO metabolites enter the aquatic ecosystem. Based on this data, nearly 77 million pounds of NPEO-based cleaning agents entered U.S. waterways in 2004 (Hoponick, 2005).

The EU production of NP was, according to the EU risk assessment, 73,500 tonnes in 1997. Around 78,500 tonnes of NP were used in Europe in 1997. Most of this was manufactured in Europe. NP is used almost exclusively as an intermediate in the production of other chemicals, with some 60 % (47,000 tonnes) used to make NPEOs and the remainder to make other NP-derivatives. The EU-production of NPEO has likewise been estimated to 118,000 tonnes in 1997. Around 77,600 tonnes of NPEO were used in Europe in 1997. Depending on their precise make-up (i.e. chain length), NPEOs may be used as emulsifiers, dispersive agents, surfactants and/or wetting agents. In certain applications, NPEOs are also used for the other properties they confer. Given their versatility, NPEOs are used in a wide range of industry sectors. The most important sector is the industrial and institutional cleaning sector (including domestic cleaning) which consumes some 30 per cent of the NPEO used in the EU. Other sectors, which use significant amounts of NPEO include emulsion polymerisation, textiles, ‘captive use’, i.e. use in the chemical industry e.g. synthesis of nonylphenol ether sulphates and nonylphenol ether phosphates (9 per cent) and leather. NP burden associated with ‘other niche markets & unaccounted for’ are mostly unknown. A part of this is attributable to Personal Domestic (personal care products) (Draft guidance document on NPEO, 2002)

Table 2.2: Uses of NPEO in WE in 1997 (Draft Guidance Document, 2002).

Category	NPEO usage	%NPEO usage
Industrial & institutional & domestic cleaning	23	29.6
Other niche markets & Unaccounted for	12.6	16.2
Emulsion polymerisation	9	11.6
Textile auxiliaries	8	10.3
Leather auxiliaries	6	7.7
Agriculture	5	6.4
Paints	4	5.2
Metal industry	2	2.6
Pulp & paper	1	1.3

2.2.2 Monitoring amount

Surface water: Due to the industry led voluntary agreement, and partly the Swiss ban, the use of nonylphenol ethoxylates in domestic detergents in most European countries will have reduced in recent years. Some of the older measurements (notably the data from the Glatt River in Switzerland) may not reflect the current levels of nonylphenol, particularly where the major source was thought to be from nonylphenol ethoxylate use in detergents. In a recent study levels of NPs of around 0.1-0.3 μg/l have been measured in the River Glatt in Switzerland. The corresponding levels in the year 1984, before the Swiss ban on the use of NPEOs in detergents, were 0.3-45 and 0.3-99 μg/l, of NPs and NPEOs, respectively. Levels of NPs of around <1.6-180 μg/l, 0.5-12 μg/l, 0.2-2.7 μg/l, 0.8-2.3 μg/l and 0.6-5.3 μg/l have been measured in six rivers in the United Kingdom (Draft guidance document on NPEO, 2002).

The highest concentration <1.6-180 μg/l, was measured in the River Aire, which received a high input of industrial surfactants from the textile industry (Draft guidance document on NPEO, 2002).

Levels of NPs of around 0.1-0.8 μg/L have been measured in a Finish lake, 1 km from a sewage treatment plant (car washing using NPEO surfactants). Average levels of NPs of around 0.038-0.12 μg/L were measured in the river Main in Germany between the years 1989-1991. Levels of NPs up to 0.14 μg/L have been measured in surface waters in a Channel in the Netherlands. Average levels of NPs of around 0.013 μg/L have been measured in surface waters in Germany in the year 1998. The maximum concentration in rivers in Austria were found to be 0,3 μg/L in years 1998 to 1999 (Draft guidance document on NPEO, 2002).

Sea water: Levels of NPs of around 0.08-3.1 μg/L dissolved NP and 0.09-5.2 μg/L total extractable NP in the Tees estuary in the UK (Draft guidance document on NPEO, 2002).

Ground water: Average levels of NPs of around 0.96 μg/L, 0.40 μg/L, 0.44 μ/L and 0.20 μg/L were found 2.5m, 5m, 7m and 13m, respectively from the River Glatt, due to infiltration of river water to groundwater (Draft guidance document on NPEO, 2002).

Suspended Matter: Levels of NPEOs and NPs of around 0,70-8,0 μg/g dry weight and 0,21-0,62 μg/g dry weight, respectively, have been measured in a Channel in the Netherlands (Draft guidance document on NPEO, 2002).

Sediment: Levels of NPs of around 0.51-5.61 mg/kg have been measured in the River Glatt in Switzerland in the year 1984. Levels of NPs of around 180-890 μg/kg dry weight were found in a Finish lake, close to a sewage treatment plant. Average levels of NPs of around 7.7-9.5 mg/kg dry weight were found in the river Main in Germany in the year 1991. Levels of NPEOs and NPs of around 2,6-5,7 μg/g dry weight and 0,63-1,70 μg/g dry weight, respectively, were found in the Netherlands. A survey of several groups of organic compounds was undertaken in 1995 in 22 estuaries in western Europe. In the Liffey estuary and Schelde, no NP was detected. The highest levels were found in the estuaries of Rijn, Seine, Mersey, Ems and Elbe (Draft guidance document on NPEO, 2002).

NPEO was found at all studied locations with levels varying between 12 and 400 ng/g dry weight. The highest levels were found in the rivers Mersey, Seine, Liffey, Schelde and Rijn. There is a relation between NPEO and NP because all NPEO will end up as NP after degradation (Draft guidance document on NPEO, 2002).

Air : No information available (Draft guidance document on NPEO, 2002).

Municipal wastewater treatment plants: Levels of NPs in municipal wastewater in the Zurich area was 14 µg/L and after treatment 8 µg/L. Level of NP of 467 µg/L and 1000 mg/kg dry weight respectively, were measured in anaerobic sludge digester and in anaerobic digested sludge. Levels of NP in activated sludge was128 mg/kg dry weight. Levels of NPEO´s and NPs of around 2,1-170 µg/L and levels up to 23 µg/L were measured before treatment of municipal wastewater in the Netherlands. After treatment levels fell to 6.1µg/L and up to 1,0 µg/L respectively. Levels of NPEO´s and NP´s of around 0,7-880 µg/L and levels up to 125 µg/L have been measured in sewage sludge. The corresponding data for OPEOs and OPs in sewage sludge were measured in levels up to 28 µg/L and up to 2 µg/L, respectively (Draft guidance document on NPEO, 2002).

Industrial wastewater treatment plants: In Finland, levels of NPs and NPEO of around 100-200 µg/L and 30,000-70,000 µg/L respectively, were measured in untreated wastewater in a sewage treatment plant. After treatment levels were around 4-34 µg/L and 4,600-12,900 µg/L respectively (Draft guidance document on NPEO, 2002).

Levels of NPs of around <1-214 mg/kg dry weight and <1-39 mg/kg dry weight in sewage sludge from domestic wastewater treatment plants and industrial wastewater treatment plants respectively were measured in Eastern Germany between the years 1993 to 1994. Levels of NPEO´s and NPs of levels up to 2.270µg/L and levels up to 400 µg/L have been measured in untreated industrial wastewater in the Netherlands and after treatment levels of around 0,9-15 µg/L and up to 1,2 µg/L, respectively. Levels of NPEO´s and NP´s up to 2.400µg/L and levels up to 2.500µg/L have been measured in sewage sludge. The corresponding data for OPEOs and OPs in sewage sludge were measured in levels up to 50µg/L and up to 24 µg/L, respectively (Draft guidance document on NPEO, 2002).

Sewage sludge: Levels of NPs of around 10 mg/kg have been measured in Germany in the year 1998. The corresponding levels in the year 1989 were 264 mg/kg. Levels of NPs of around 90 mg/kg have been measured in Switzerland in the year 1997. The corresponding levels in the year 1984 were 1010 mg/kg (Draft guidance document on NPEO, 2002).

Concentrations in Biota: Levels of NPs in the range from <0.03 to 1.6 mg/kg dry weight have been measured in fish tissues taken from the Glatt River in Switzerland. Levels of NPs up to 1.2 mg/kg dry weight were found in samples from ducks (muscle) taken from the Glatt River in Switzerland. Levels of NPs and NPEO of 1.0 mg/kg and 9.5 mg/kg dry weight respectively, were measured in kopvoorn liver and in kopvoorn muscle 0.18 mg/kg and 0.31 mg/kg respectively in River Air in Great

Britain and in the Glatt River in Switzerland (Draft guidance document on NPEO, 2002).

Human beings: No information available (Draft guidance document on NPEO, 2002).

2.2.3 Biodegradation of NPEO

Treatment at wastewater treatment plants produces NPEO metabolites that are more toxic, more estrogenic, and more persistent when compared to the original parent compounds. When compared to other surfactants, NPEOs take substantially longer to biodegrade. The intermediary chemicals formed from initial degradation are much more persistent than the original compounds of NPEO ultimate biodegradation occurs slowly. Overall, wastewater treatment decreases the concentration of NPEOs that enter the environment but increases the concentrations of the NPEO metabolites. Environment Canada, Canada's environmental protection agency, estimates that "at least 63% of the total mass of all NP compounds entering wastewater treatment plants is released into the environment." Additionally, the highest concentrations of certain NPEO metabolites entering the environment come from wastewater treatment plants with more treatment. A number of factors can influence the biodegradation of NPEOs. First, NPEOs degrade more quickly when there are higher temperatures. Second, ultimate degradation takes longer in seawater. Third, biodegradation occurs more quickly when water is moving (Hoponick, 2005).

2.2.4 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).

ASM1 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.

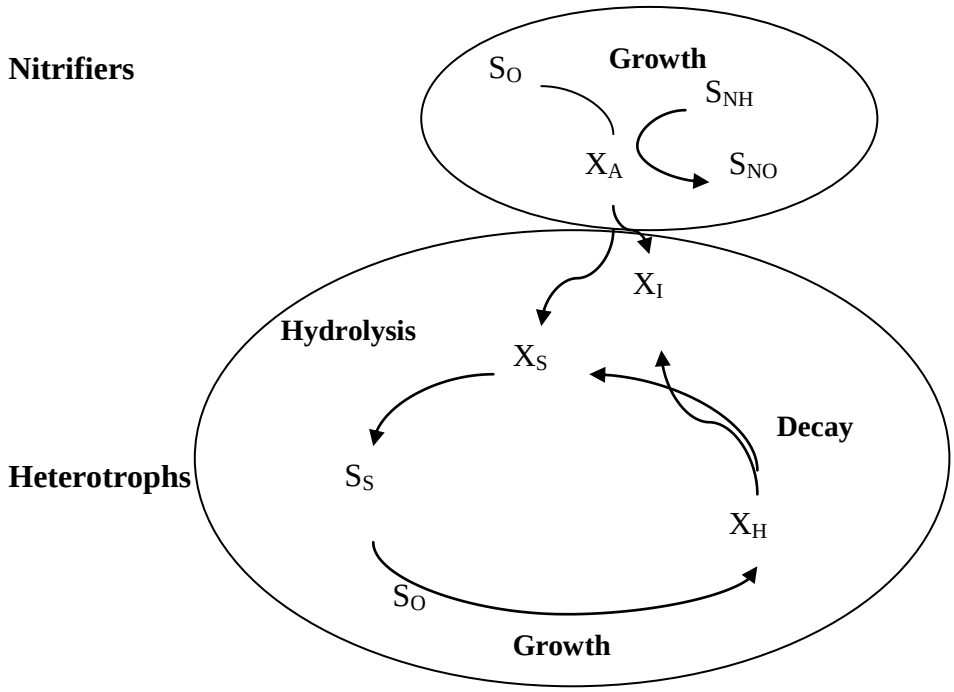

Figure 2.3: Processes for heterotrophic and nitrifying bacteria in ASM1 (Jördening et al., 2005).

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):

$$\frac{dX}{dt} = \frac{dX_H}{dt} + \frac{dX_P}{dt} \quad \text{.....(2.4)}$$

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

$$\frac{dX_H}{dt} = -b_H X_H \quad \text{..... (2.5)}$$

Generation rate of particulate inert products are given as follows :

$$\frac{dX_P}{dt} = f_{EX} \frac{dX_H}{dt} \quad \text{.....(2.6)}$$

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:

$$\frac{dS_S}{dt} = \frac{\hat{\mu}_H}{Y_H} \frac{S_S}{(K_S + S_S)} X_H \quad \text{..... (2.7)}$$

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

$$\frac{dS_P}{dt} = f_{ES} b_H X_H \quad \text{.....(2.8)}$$

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:

$$\frac{dX_S}{dt} = k_h \frac{X_S / X_H}{(K_X + X_S / X_H)} X_H \quad \text{..... (2.9)}$$

Matrix representation of basic relationships between process components of endogenous model is given in Figure 2.4. The S_I and X_I components are not included in the matrix since they do not go through biochemical processes.

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	

Figure 2.4: Simplified matrix representation of ASM1 involving endogenous decay.

2.2.5 Endocrine disrupting effect

Disruption of the endocrine system in living organisms by synthetic organic chemicals has been of great concern in recent years due to the recognition that the environment is contaminated with numerous ‘‘endocrine disrupting compounds’’ (EDCs) that exert hormonal activity (Scott and Jones, 2000). An endocrine disruptor by definition is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action and elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development and behavior (US EPA, 2001).

The primary function of an endocrine system is to transform various exogenous stimuli into chemical messengers and hormones, resulting in appropriate gene expressions and synthesis of proteins and/or activation of already existing tissue-specific enzyme systems. The three major endocrine disruption endpoints are estrogenic (compounds that mimic or block natural estrogens), androgenic (compounds that mimic or block natural testosterone) and thyroidal (compounds with direct and/or indirect impacts on the thyroid). EDCs are linked to a variety of adverse health effects in wildlife, such as hormone-dependent cancers, reproductive system disorders and reduction in reproductive fitness. Endocrine disruptors may be natural or more commonly synthetic, all with very diverse chemical structures (US EPA, 2001).

The most prominent classes of chemicals that contain EDCs are natural estrogens, phytoestrogens, pesticides (methoxychlor), surfactants (nonylphenol), plasticizers (diethylphthalate) and organohalogens (dioxin) (US EPA, 2001).

The majority of EDCs are ubiquitous as they may be present in all compartments of the environment (water, air, soil and sediments) upon imperfect manufacturing processes and/or leaching from final end products. Sources of surface water contamination with EDCs are sewage effluents from domestic and industrial facilities and industrial effluent discharges. The major source of EDCs in domestic sewage is the daily produced male and female hormones and/or ingested synthetic steroids, which are excreted with urine and discharged into municipal systems, where they may be partially removed by biochemical oxidation before being released to surface waters as active estrogens (Gultekin and Ince, 2007).

2.2.6 Bioaccumulation of NPEO metabolites

Most studies suggest that aquatic species bioconcentrate and bioaccumulate NPEO metabolites to a low or moderate degree. Aquatic organisms take in NP compounds faster than they can eliminate them, so NP compounds can bioaccumulate in the body (Hoponick, 2005).

2.2.7 Alternatives of NPEO

According to industry the substitutes in the use area detergents and cleaning agents are mostly alcohol ethoxylates. In terms of environmental risk, alcohol ethoxylates appear to present a clear advantage over NPEOs, chiefly owing to issues of biodegradability. According to industry the substitutes in the use area detergents and cleaning agents for domestic and industrial uses are mixtures of anionic and non-ionic surfactants, such as linear alcohol ethoxylates, fatty acids and derivatives, fatty amines or unsaturated hydrocarbons.

Specifically, alcohol ethoxylates biodegrade more readily than NPEOs in the environment. Furthermore, alcohol ethoxylates tend to degrade fully to carbon dioxide and water in a relatively short time scale, while NPEOs degrade to form NPs, the toxicity and slow biodegradability of which have been identified in the risk assessment. In terms of human health risks, no data have been found which favour either alcohol ethoxylates or NPEOs as a group. Nevertheless, when substituting an NPEO with an alcohol ethoxylate, it is important to look at the toxicity of the specific chemicals under consideration, as toxicity may vary substantially depending on the alkyl chain lengths, chain branching and the degree of ethoxylation. In the binding polymer emulsion of water based paints mostly fatty alcohol ethoxylates, but also esterified linseed oil, different kinds of non-ionic tensides, phosphate esters, and potassium polycarboxylates are used as alternatives to alkylphenolethoxylates according to the paint industry in Sweden (Draft guidance document on NPEO, 2002).

Mostly fatty alcohol ethoxylates are used as alternatives in the polymer emulsion of water based adhesives according to the adhesive industry. According to the adhesives industry, the major difficulties are in replacing NPEOs in acrylic and chloroprene rubber dispersions. Mostly alcohol ethoxylates and other ethoxylates are used as alternatives to NPEOs according to the textile industry. In the leather industry there are available alternatives to APEOs mostly based on fatty alcohol ethoxylates and blends thereof, e.g. mixtures of alcohol ethoxylates or anionic surfactants. Concerning NP/NPEO-containing pesticides, alternatives are available in Sweden, at least for some uses. Developing efforts are also ongoing. It has however not been possible to obtain information on the substitute(s), while the composition information is seen as company property (Draft guidance document on NPEO, 2002).

OPs are known substitutes for NPs in the manufacture of derivatives other than NPEOs. The use of OPs is not expected to yield any reduction in risk over the use of NPs. A Swedish risk assessment of APEOs (KemI Report 1/00) has just been published at the National Chemicals Inspectorate. Since data on other APEOs are scarce, this assessment is focusing on octylphenol and butylphenol. It is stated that octylphenol is one of the most potent APs to produce estrogenic effects in vitro and that estrogenic effects have also been demonstrated in vivo in young rats.

In addition, and according to CEPAD, neither the cost (much higher than NP) of octylphenol, nor its performance nor availability make it suitable as a substitute for NP. It was agreed at the OECD Expert Meeting on NP/NPEOs, hosted by Switzerland on 8-10 November 1999, that some form of exchange of information on substitute chemicals and processes was desirable. A password protected web site has been organised by the OECD Secretariat (Draft guidance document on NPEO, 2002).

2.2.8 Restrictions and Bans

The European Union has effectively banned the use of NPEs in laundry detergents, and Canada has set strict water quality guidelines. In 1987, the U.S. EPA placed NP on its Inerts List 1 for pesticides due to "toxicological concern because human health and/or ecological considerations." NP compounds are not currently regulated under the federal Clean Water Act, but in 2004 the EPA proposed draft water quality criteria for NP "designed to protect aquatic organisms." (Hoponick, 2005).

The EPA's draft criteria are divided into freshwater and saltwater criteria, as NP is more toxic to organisms in saltwater. For freshwater, the draft criterion for short-term exposure to NP is 27.9 parts per billion, and the criterion for long-term exposure is 5.9 parts per billion. For saltwater, the draft criterion for short-term exposure is 6.7 parts per billion, and the criterion for long-term exposure is 1.5 parts per billion.

Canada has set guidelines for NPEOs that are much more protective of aquatic organisms. First of all, Canada's guidelines account for all NPEO metabolites, while the U.S. draft criteria proposes to only regulate NP. Secondly, Canada's freshwater guideline is 1 part per billion, while their saltwater guideline is 0.7 parts per billion.

The EPA has not yet produced final water quality criteria for NP or any other metabolites of NPEOs, but the draft criteria are not stringent enough. The EPA states that the draft criteria are "designed to protect aquatic organisms and their uses." However, the critical studies chosen to reach the draft water quality standard do not take into account more sensitive aquatic organisms, endocrine disrupting effects, or the cumulative effects of NPEO metabolites. The EPA's draft water quality criteria are inadequate, as the EPA proposes criteria only for one of the NPE metabolites, NP. Under the draft water quality criteria, as long as NP is below the water quality standard, aquatic organisms are considered "protected" even though organisms are

also exposed to other NPEO metabolites, which are also toxic and estrogenic. When NPEO metabolites are considered together, organisms that are considered to be protected, according to the draft criteria, may actually be harmed. It does not make logical sense to establish water quality criteria for NP only and set the criteria at a level that may harm certain organisms. The water quality criteria are supposed to protect aquatic organisms. The final water quality criteria must include all NPEO metabolites in order to fully protect aquatic organisms. The EPA needs to implement final water quality criteria before NPEOs can be regulated (Hoponick, 2005).

Procter & Gamble (P&G) is an industrial giant based in the U.S. that produces items ranging from laundry detergent to mascara. P&G recognizes that NPEO metabolites are more toxic and degrade more slowly than other cleaning agents. P&G states that NP compounds "might create long-term concerns for the environment," and furthermore, that alternatives are both available and feasible. P&G acknowledges that "for most products, there are available substitutes, and indirect uses can be avoided." P&G also noted that the European Union has revised its risk assessment for NP compounds, and prohibited the use of NP compounds in consumer products. P&G then stated that "based on these scientific assessments and actions, P&G has eliminated the intentional use of NP and NPEOs in our products," including laundry detergent and other cleaning products (Procter and Gamble, 2005).

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 600 mg COD/L in fill & draw reactors, which had a working volume of 14 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 reactor was operated at a sludge age of 10 days and a hydraulic retention time of 24 hours. The system was operated until steady state conditions were reached. After the acclimation period, acute and chronic effects of NPEO to activated sludge were investigated by performing the respirometric tests.

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 and COD analysis were performed in order to monitor and control reactor operation. The samples taken for COD measurements were filtered through 0.45 μm Millipore membrane syringe filters. COD samples were preserved with H_2SO_4 and H_3PO_4, respectively. COD measurements were performed as described in ISO 6060 method (ISO 6060, 1986). MLSS and MLVSS analysis were performed by using the procedure defined in Standard Methods (1995).

Respirometric tests were also performed with Applitek RA respirometer with PC connection for overall evaluation and modeling purposes. Determination of NPEO was performed using HPLC Agilent 1100 series at conditions given in the Table 3.2 and the calibration curve used for the determination of NPEO is given in Figure 3.1.

Table 3.2: Chromatographic conditions for determination of NPEO via HPLC.

Column Type	C18
Mobile Phase	80/20 (v/v) CH_3OH/H_2O
Mobile Phase Flow Rate	1.1 mL/min
Column Temperature	25 °C
Injection Volume	50 μL
Dedector	DAD, 227 nm

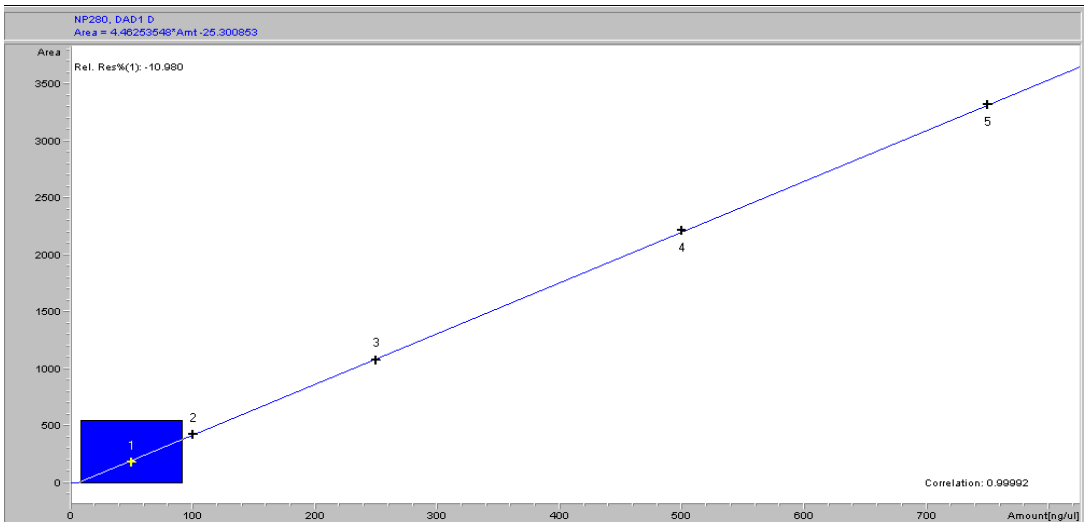

Figure 3.1: Calibration curve for determination of NPEO (10-750 mg/L)

3.2.1 Respirometric analysis for acute effects

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. The summary of respirometric studies for acute effects of different concentrations of NPEO is given in Table 3.3.

Experiments representing the same conditions in respirometric tests were conducted in parallel. By keeping the S/X ratio same with the system, COD and NPEO samples were monitored for experiments.

Table 3.3: Experimental conditions for acute tests for 1500 mg VSS/L system.

Set	Substrate Type and Amount
1	peptone mixture (450 mg COD/L)
2	peptone mixture (450 mg COD/L) + NPEO (225 mg COD/L)
3	peptone mixture (450 mg COD/L) + NPEO(450 mg COD/L)
4	peptone mixture (450 mg COD/L) + NPEO (900 mg COD/L)
5	NPEO (450 mg COD/L)

3.2.2 Respirometric analysis for chronic effects

The 14 L fill & draw reactor was fed with both 450 mg COD/L peptone mixture and 450 mg COD/L NPEO during the acclimation period. MLSS, MLVSS, COD and NPEO amounts were observed and according to the MLVSS results the sludge age of the system was determined. For two sludge age time period respirometric analysis were done according to the plan given in Table 3.4.

Table 3.4: Experimental conditions for chronic tests for 1500 mg VSS/L system.

Set	Substrate Type and Amount	Acclimation Period (day)
1	peptone mixture (450 mg COD/L)	Control
2	peptone mixture (450 mg COD/L) + NPEO (450 mg COD/L)	1.
3	NPEO (450 mg COD/L)	1.
4	peptone mixture (450 mg COD/L) + NPEO(450 mg COD/L)	6.
5	peptone mixture (450 mg COD/L) + NPEO (450 mg COD/L)	13.
6	NPEO (450 mg COD/L)	13.
7	peptone mixture (450 mg COD/L)	13.
8	peptone mixture (450 mg COD/L) + NPEO (450 mg COD/L)	20.

During the experiments shown in Table 3.3 and Table 3.4, COD and NPEO amounts were observed in the samples taken from the reactor operated parallel to the respirometer.

4. RESULTS AND DISCUSSION

After a five month period of acclimation, the peptone mixture reactors suspended solid amounts and soluble COD of effluent values are shown in the Figures 4.1 and 4.2, respectively.

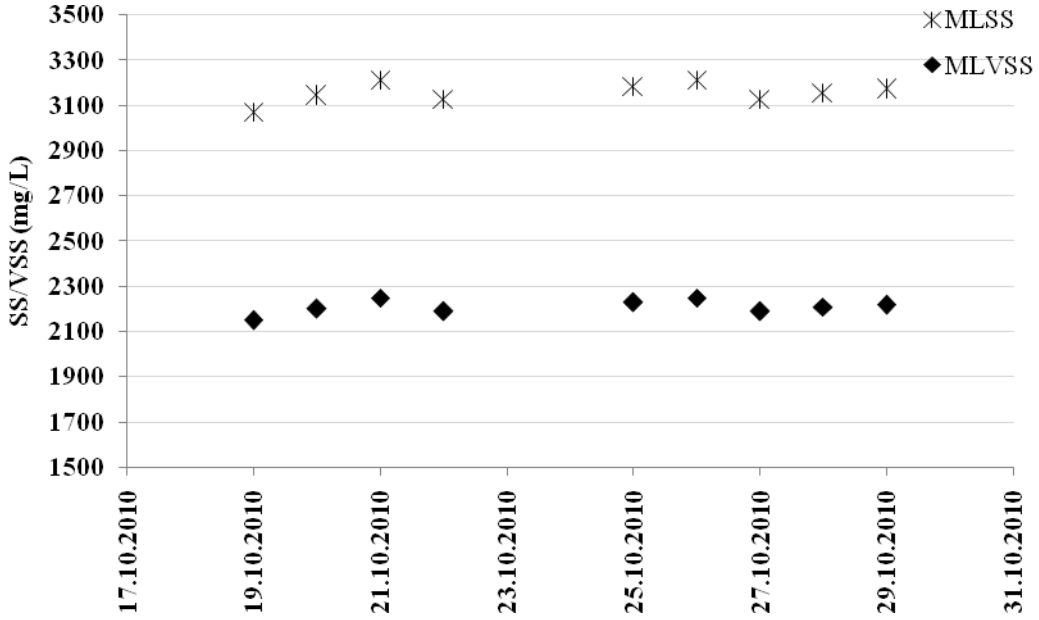

Figure 4.1: Final MLSS-MLVSS results of acclimated peptone mixture system.

As mentioned above the peptone mixture system is operated at 10 day sludge age at 0.3 mg COD/mg VSS ratio. As seen from the Figure 4.1, the MLVSS amount of the peptone mixture system is reached approximately 2200 mg/L (VSS/SS ratio is 0.7) after the hydraulic retention time, 24 h, at steady state conditions.

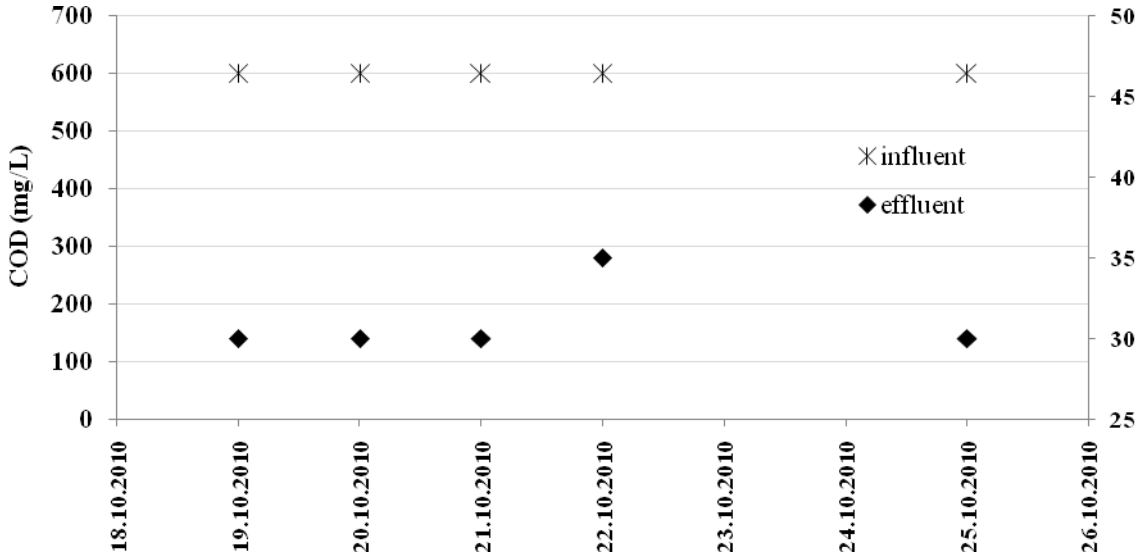

Figure 4.2: Final effluent COD results of acclimated peptone mixture system.

Moreover, five final soluble organic matter content of the effluent data is demonstrated in Figure 4.2 and seen that system fed with 600 mg COD/L is operated with 95% removal efficiency at steady state conditions.

4.1 Acute Results

After reaching the steady state conditions, respirometric tests were performed to figure out the acute effects of nonylphenol ethoxylate. Experiments representing the same conditions in respirometric tests were conducted in parallel for the peptone mixture and different concentrations of NPEO solutions. The volatile suspended solid values were approximately 1500 mg VSS/L for all runs.

After conducting the respirometric analysis for peptone mixture only by keeping the loading ratio same with the original system (0.3 mg COD/mg VSS). Then, 225 mg COD/L, 450 mg COD/L and 900 mg COD/L NPEO solutions were added to system with the peptone mixture same with the first control run, respectively. And finally, only 450 mg COD/L NPEO solution was fed to the system without any peptone mixture.

4.1.1 450 mg COD/L peptone mixture (control)

In the first set, control, respirometric analysis was done for the system and 450 mg COD/L peptone mixture was added for 1500 mg VSS/L system, as mentioned above. The OUR profile is demonstrated in Figure 4.3. As seen from the profile the maximum oxygen uptake rate was reached at nearly 150 mg/L/h when 450 mg COD/L peptone mixture was added to the system.

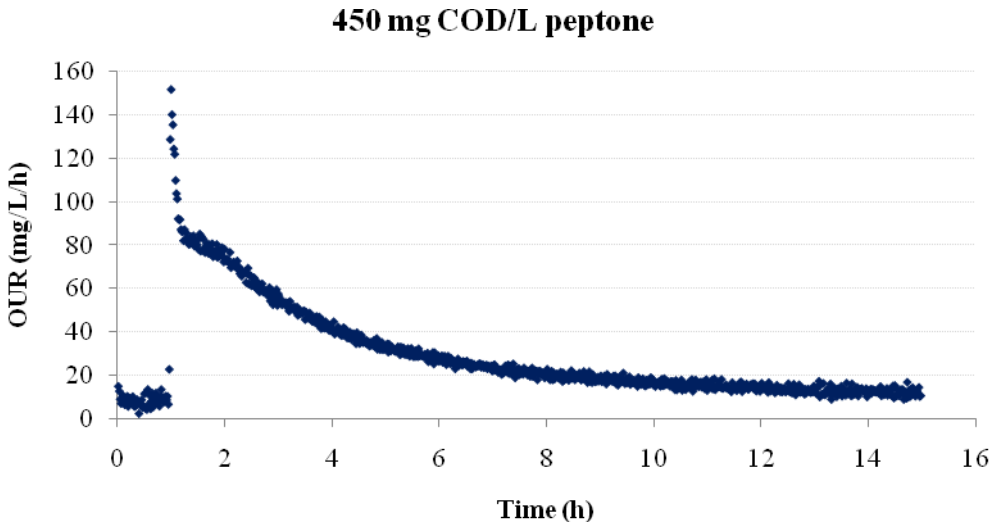

Figure 4.3: OUR data versus time (Acute Set1).

The respirometric analysis for the non-acclimated system, the control analysis was performed by feeding the system only with the peptone mixture by keeping the loading ratio same with the reactor. 3000 mg VSS taken from the reactor was diluted to 2 L in the respirometer.

The reactor conducted parallel to the respirometer was used for monitoring COD and NPEO data. The soluble organic matter removal is demonstrated in the Figure 4.4. As seen from the figure, most of the organic matter given to the system is removed immediately in nearly 4 hours, in the control set of the system.

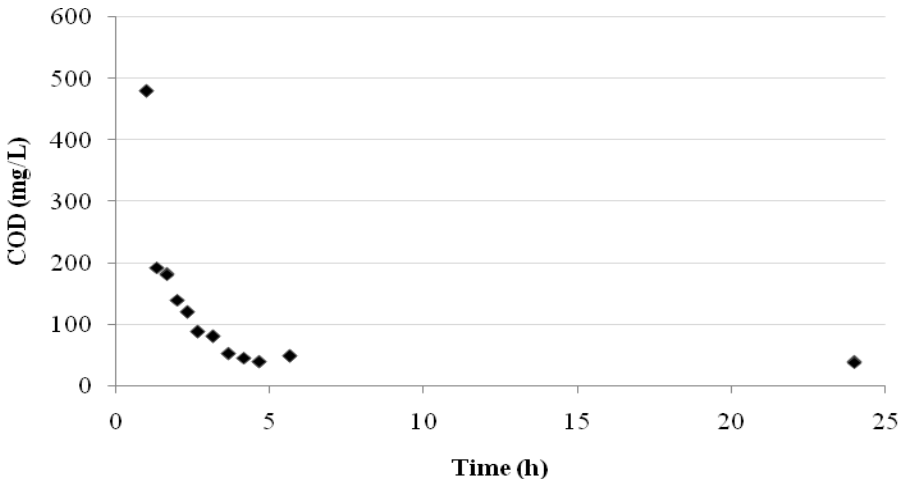

Figure 4.4: COD concentration versus time (Acute Set1).

4.1.2 450 mg COD/L peptone mixture + 225 mg COD/L NPEO solution

After the control set was implemented, same steps were followed for the acute effect of minimum concentration of NPEO solution, 225 mg COD/L. The OUR profile of the second set of acute analysis is illustrated in Figure 4.5.

When 225 mg COD/L NPEO solution was added to the system with the peptone solution, the maximum OUR data decreased to approximately 105 mg/L/h from 150 mg/L/h and a peak was formed differently from set 1. Moreover, the biodegradation rate was decreased and the time required for consumption of biological degradable COD for peptone solution got longer with respect to set 1. Those effects illustrate the inhibition results of the NPEO solution.

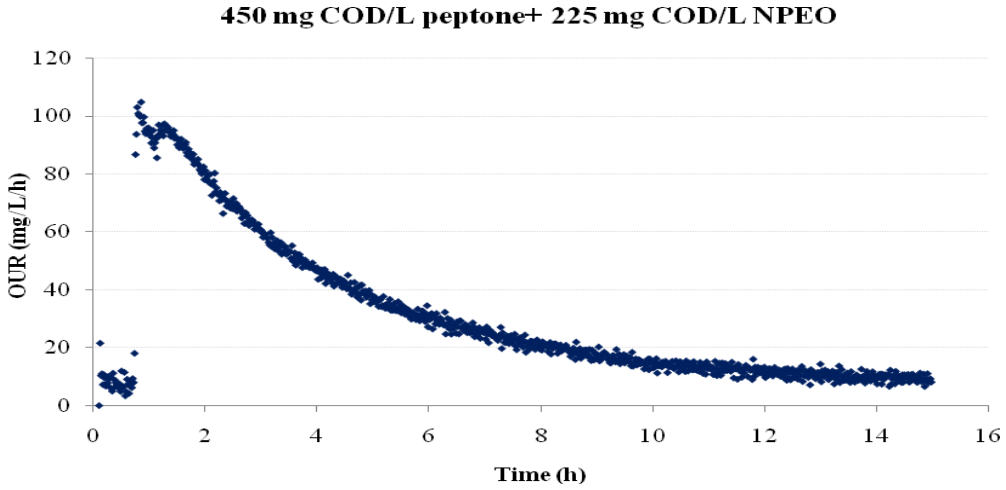

Figure 4.5: OUR data versus time (Acute Set 2).

The change of COD and NPEO concentrations of the experiment are illustrated in Figure 4.6 and 4.7, respectively.

The first value of organic matter at the feeding time is seen approximately 700 mg COD/L. At the end of the hydraulic retention time it is measured as nearly 150 mg COD/L in the reactor. At that time the NPEO concentration is measured nearly 50 mg/L which is equal to nearly 100 mg COD/L.

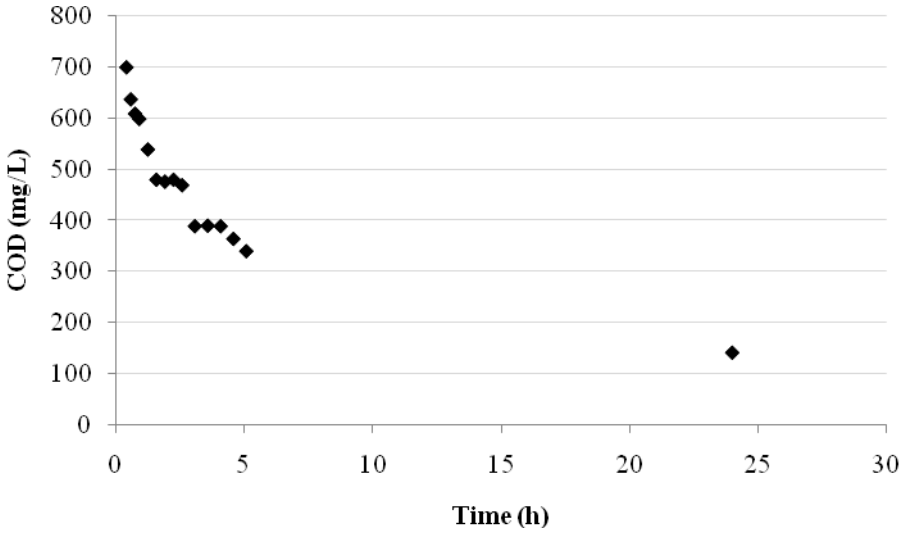

Figure 4.6: COD concentration versus time (Acute Set 2).

The organic matter is removed in longer time with respect to the first set, as defined as inhibition effect of the NPEO.

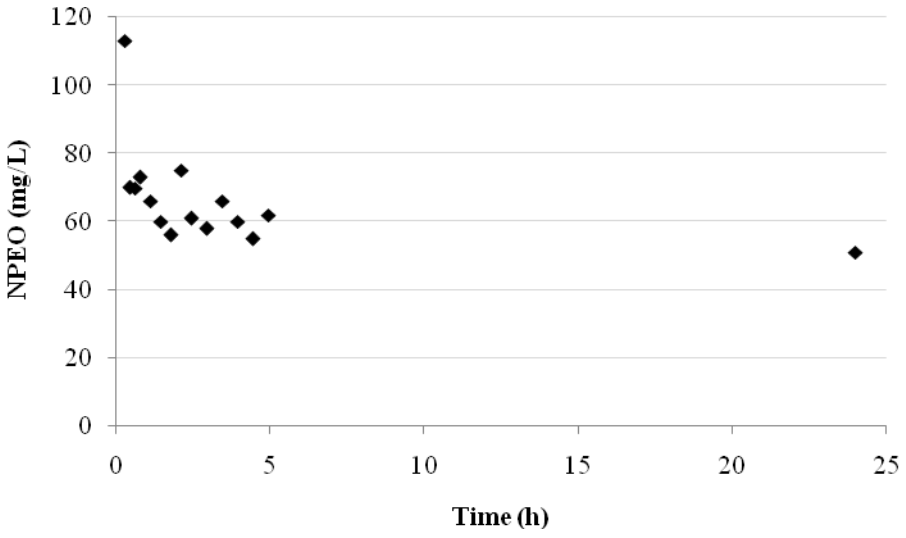

Figure 4.7: NPEO concentration versus time (Acute Set 2).

NPEO concentration was seen nearly 70 mg/L for a 2 hour period and decreased to 50 mg/L, barely. It is known that NPEO has a tendency to be adsorbed by the sludge due to its amphiphilic nature. Therefore, nearly 90 mg COD/L equivalence of the chemical was adsorbed by the sludge immediately.

4.1.3 450 mg COD/L peptone mixture + 450 mg COD/L NPEO solution

In the third set of acute experiments 450 mg COD/L equivalence NPEO solution was added with the peptone mixture. OUR profile is illustrated in the Figure 4.8.

As seen from the profile, the maximum oxygen uptake rate was reached about 140 mg/L/h and the peak was seen after a 4 hour period.

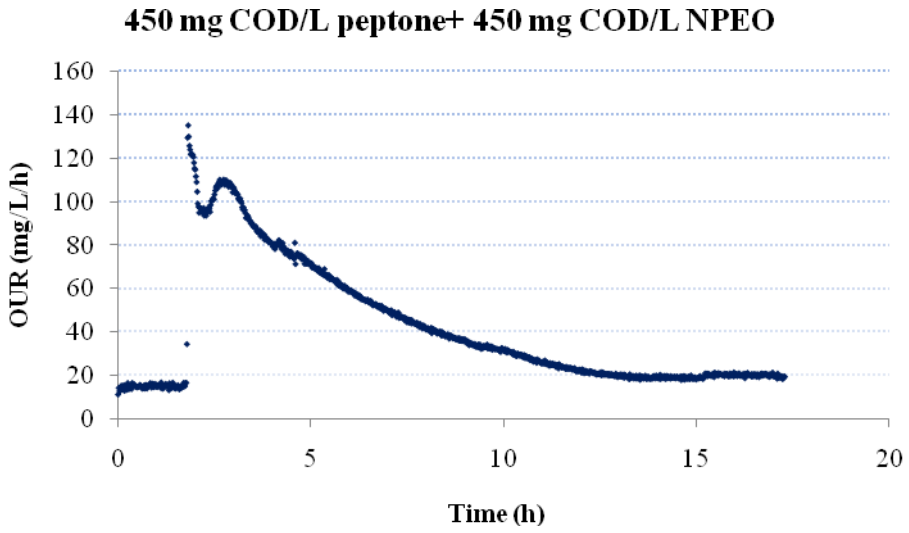

Figure 4.8: OUR data versus time (Acute Set 3).

The change of COD concentration of the experiment is illustrated in Figure 4.9. Organic matter concentration is decreased from 900 mg/L to 200 mg/L COD at the end of the day.

And the NPEO concentration change of the system is illustrated in the Figure 4.10. It is seen that 160 mg COD/L equivalence of NPEO is remained at the end of the day and it is seen nearly at the COD effluent data, 200 mg COD/L.

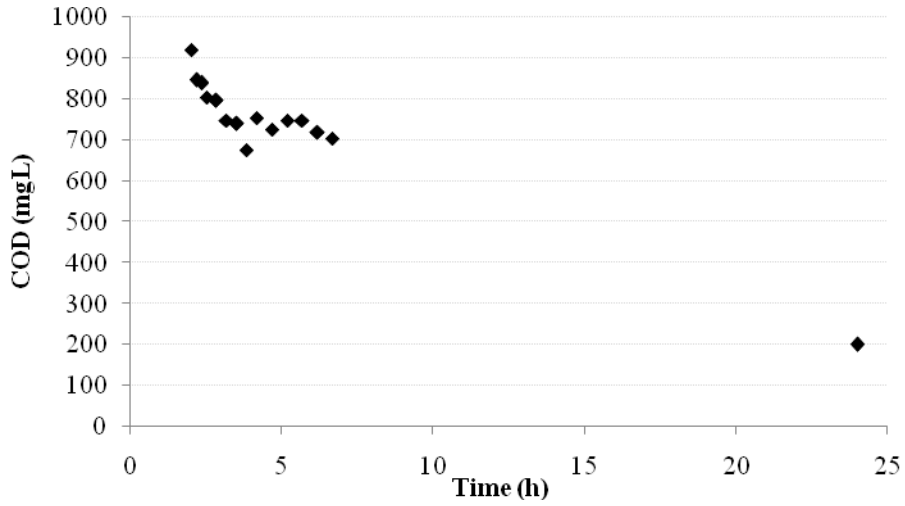

Figure 4.9: COD concentration versus time (Acute Set 3).

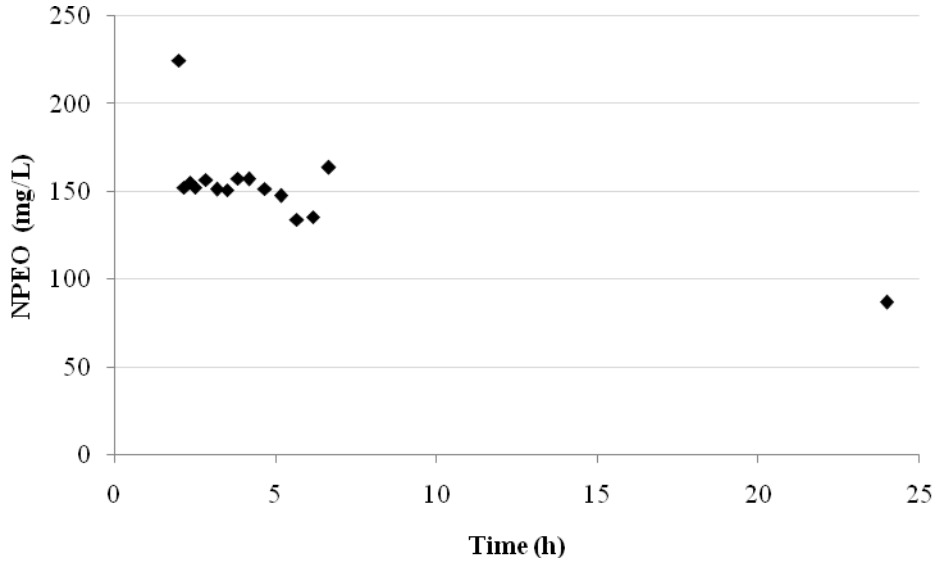

Figure 4.10: NPEO concentration versus time (Acute set 3).

4.1.4 450 mg COD/L peptone mixture + 900 mg COD/L NPEO solution

In the fourth set of acute experiments 900 mg COD/L equivalence NPEO solution was added with the peptone mixture. OUR profile is illustrated in the Figure 4.11.

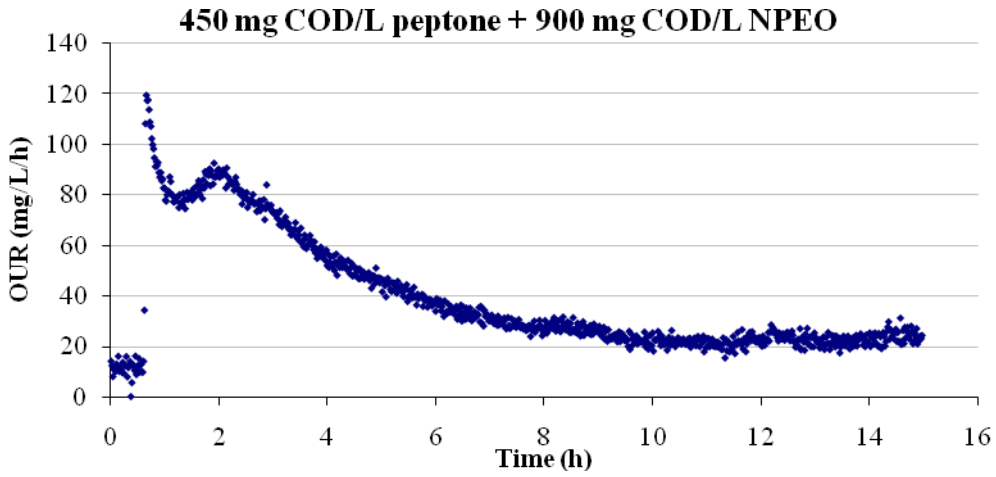

Figure 4.11: OUR data versus time (Acute Set 4).

As seen from the figure, the maximum OUR is reached 120 mg/L/h value and there is become another peak nearly after 3 hours. The organic mater change of the system is given in Figure 4.12 and the NPEO concentration in Figure 4.13 below.

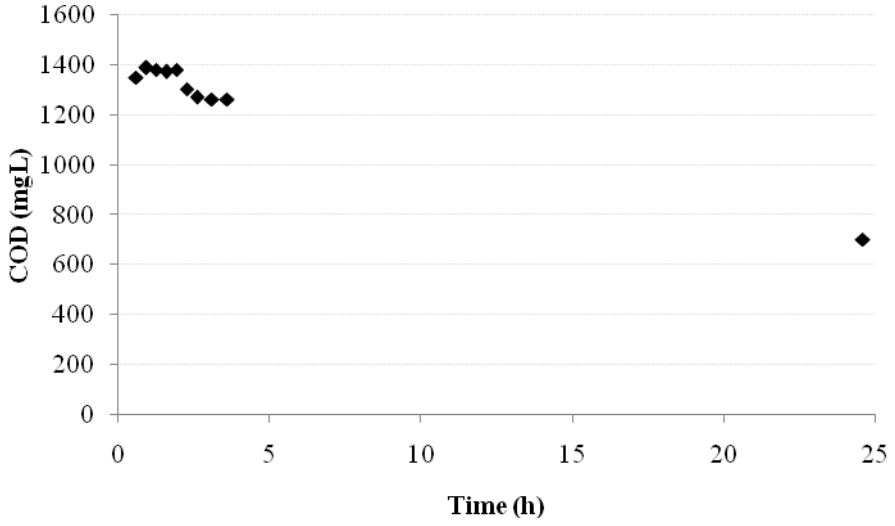

Figure 4.12: COD concentration versus time (Acute Set 4).

The initial COD value, nearly 1400 mg COD/L is decreased to 700 mg COD/L at the end of the day. The NPEO concentration is not changed for a while and it is decreased to nearly 250 mg/L which is equal to nearly 500 mg COD/L. Therefore it can be said that some of the peptone cannot be removed due to the inhibition of that much NPEO given to the system.

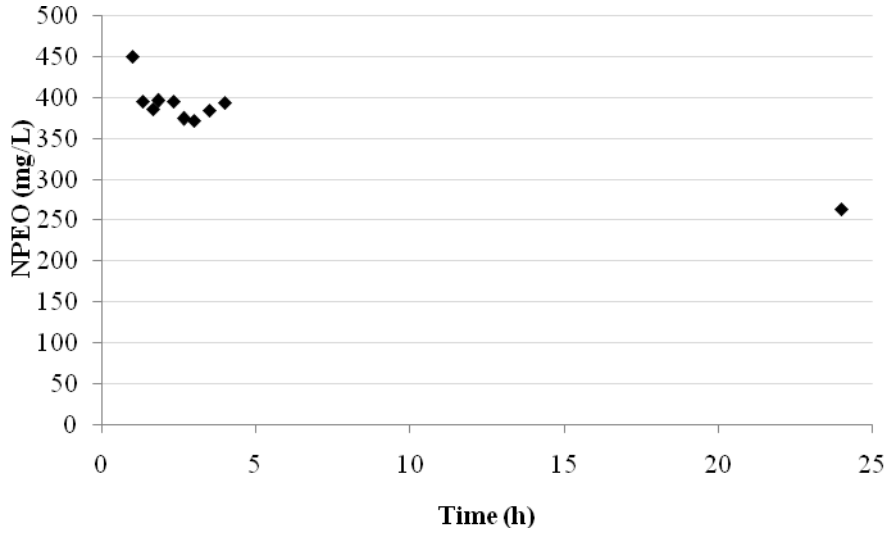

Figure 4.13: NPEO concentration versus time (Acute set 4).

4.1.5 450 mg COD/L NPEO solution

And finally, 450 mg COD/L equivalence NPEO solution was added to the system without any peptone mixture. OUR profile is illustrated in the Figure 4.14.

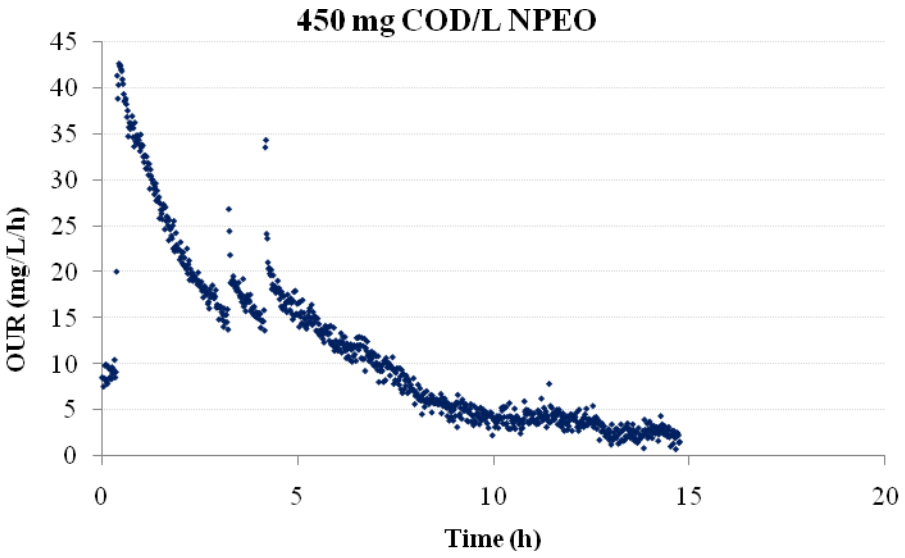

Figure 4.14: OUR data versus time (Acute Set 5).

As seen from the OUR profile, the maximum OUR value is reached up to nearly 43 mg/L/h and profile has peaks in the 4 to 5 hours. The organic matter change of the system is showed in the Figure 4.15 and NPEO concentration change graph is in the Figure 4.16.

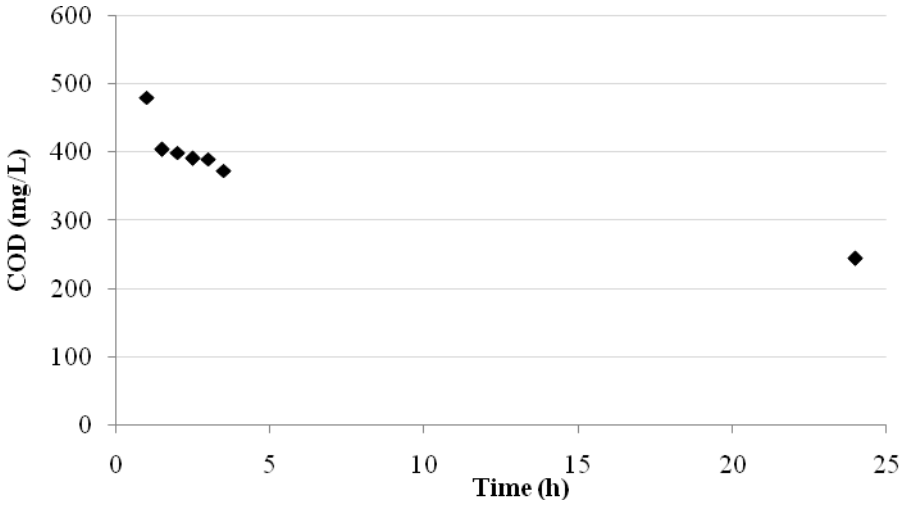

Figure 4.15: COD concentration versus time (Acute Set 5)

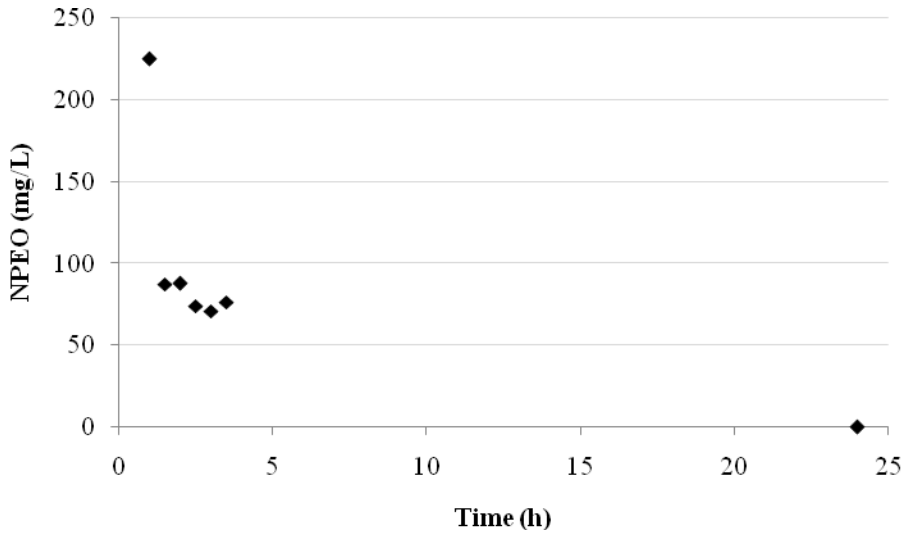

Figure 4.16: NPEO concentration versus time (Acute Set 5)

The organic matter level of the system is decreased from 450 mg COD/L till to 250 mg COD/L. However, the NPEO concentration at the end of the day is seen 0. It can be commented that NPEO is changed to its metabolites and while there is not seen any NPEO in the system the organic matter level is not changed that much. Moreover, adsorption of the chemical by the sludge in the first hour is seen definetly in Figure 4.16.

4.2 Chronic Results

After acute effects of NPEO is searched for different concentrations of the matter, the fill and draw system is acclimated to NPEO. System is set to 1500 mg VSS/L and the feeding amount is adjusted to 450 mg COD/L peptone mixture with 450 mg COD/L NPEO. In the first day the system SS and VSS amount is increased other than biological growth as seen from the Figure 4.17 due to the adsorbed amount of the chemical. Thereafter, in 10 days system was watched and the decreasing trend of SS-VSS values determined and no sludge was removed. After 10 days, system adjusted to chemical and begun to be operated at 10 day sludge age. From the figure it is seen that, at sludge age ten, system has nearly 1500 mg VSS/L.

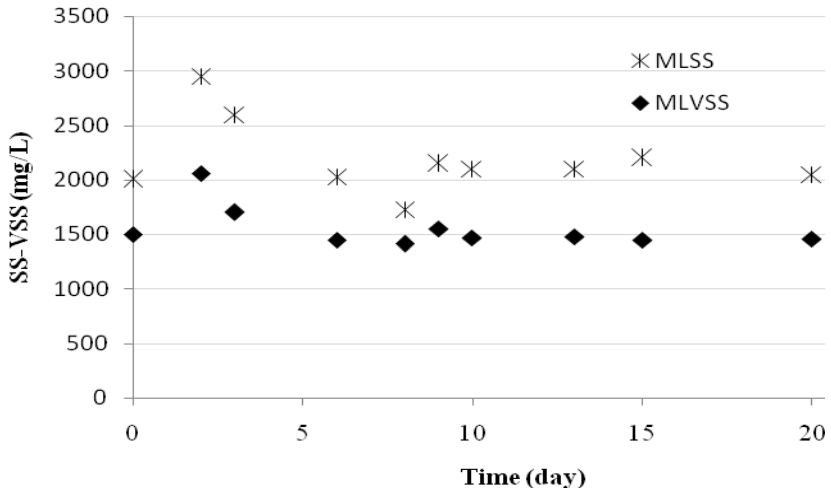

Figure 4.17: MLSS-MLVSS versus time.

Moreover, effluent organic matter content of the system was watched and the results are demonstrated in Figure 4.18. As seen from the graph, influent COD amount is changed according to the remained organic matter a day before and with 900 mg COD/L feeding amount. In the first days it is seen that the effluent quality is much better than the forward searchs. It can be explained that, the chemical was adsorbed by sludge in the first days as mentioned before, then the adsorption capacity of the sludge is decreased and the effluent quality was fixed at nearly 500 mg COD/L after a 10 day period.

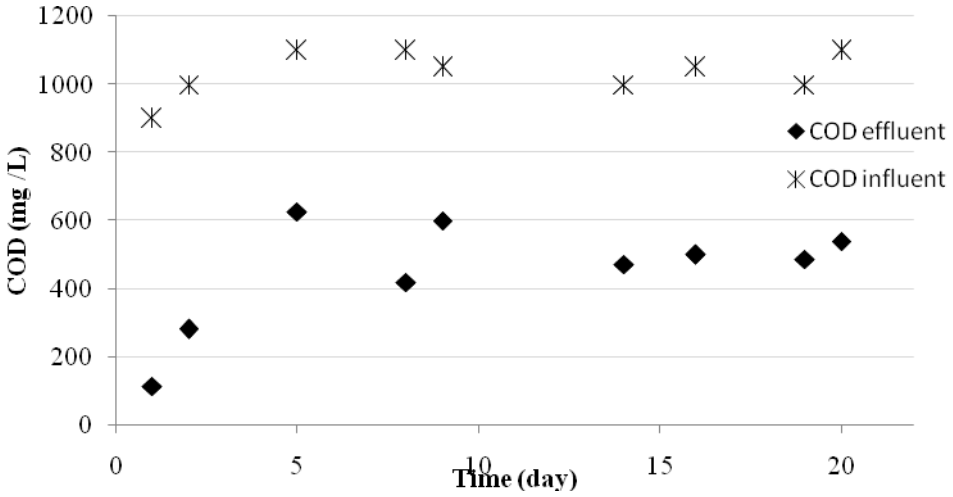

Figure 4.18: COD effluent versus time

The NPEO concentration of the system was observed during 20 day acclimation period via HPLC and the results are showen in Figure 4.19. First day the NPEO amoun in the effluent was observed as 0 and then it increased till to 180 mg/L in 5th day. After that, the amount was going to decrease again and after 15 days there was no NPEO in the effluent observed.

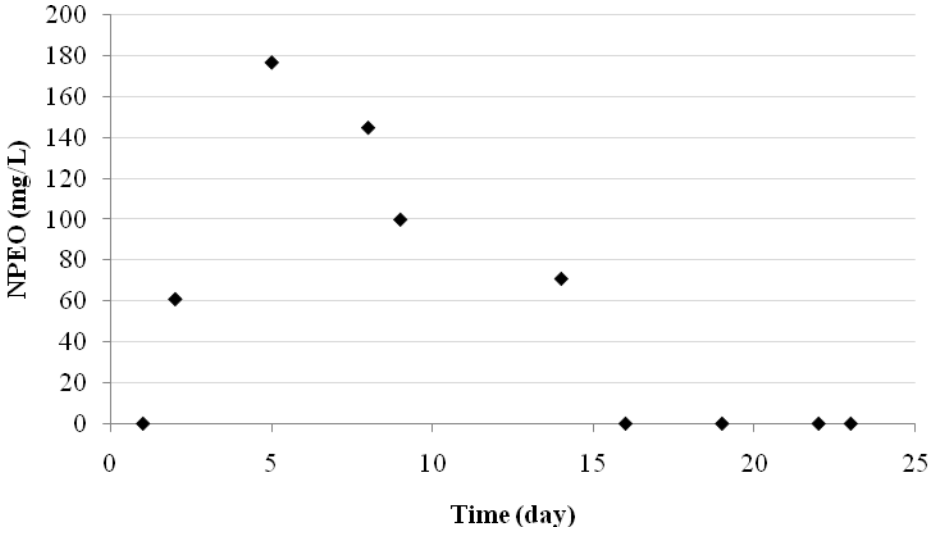

Figure 4.19: NPEO concentration of effluent versus time.

After being adsorbed in the first day, with the decreasing adsorption capacity of the sludge, the amount of the chemical in the effluent increased. Then, sludge acclimated to the chemical began to remove the chemical and after 15 days there was no NPEO

observed in the chemical. However, as mentioned above, organic matter content did not changed that much which means NPEO was biodegraded to its metabolites.

In the first day when the system was fed with 450 mg COD/L peptone mixture, control, the OUR profile was determined as shown in Figure 4.20. During the experimet the samples taken for the analysis of organic matter given in Figures 4.21, for set 1.

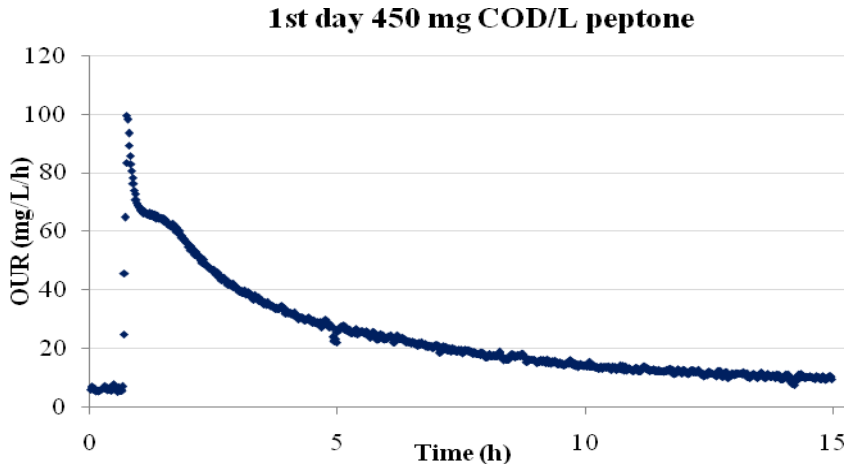

Figure 4.20: OUR data versus time (Chronic Set 1).

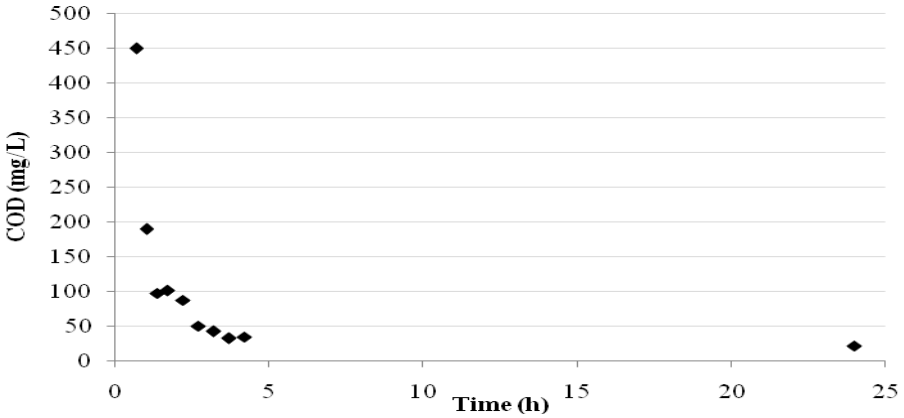

Figure 4.21: COD versus time (Chronic Set 1).

At the same time other respirometric analyses were done with both 450 mg COD/L peptone and 450 mg COD/L NPEO solution and only with 450 mg COD/L NPEO solution. The OUR profile of set 2 is illustrated in Figure 4.22.

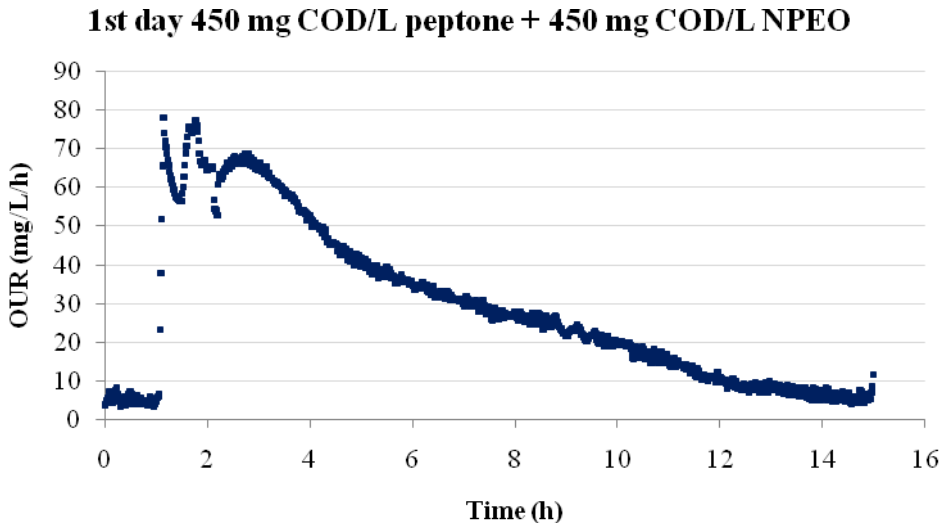

Figure 4.22: OUR data versus time (Chronic Set 2).

The COD removal graph is shown in Figure 4.23 and NPEO concentration change is in Figure 4.24.for set 2.

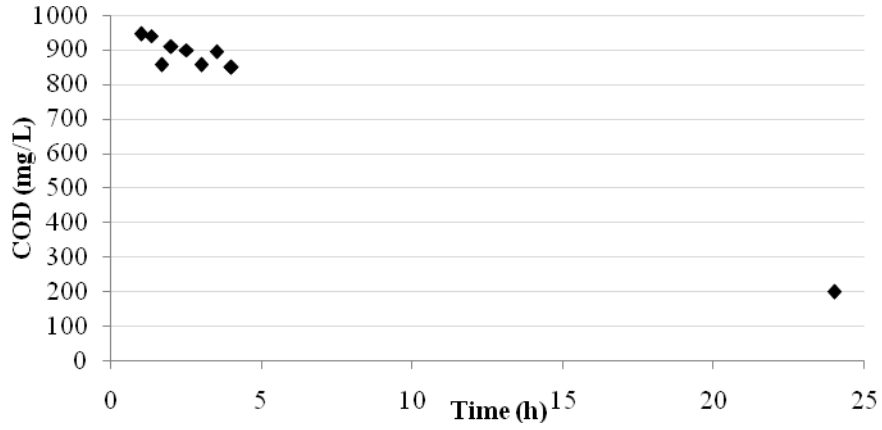

Figure 4.23: COD versus time (Chronic Set 2).

While NPEO concentration decreased to 90 mg/L at the end of the day the COD level decreased to nearly 200 mg/L which can be explained as the NPEO could not been biodegraded and changed to another form in the first day experiment.

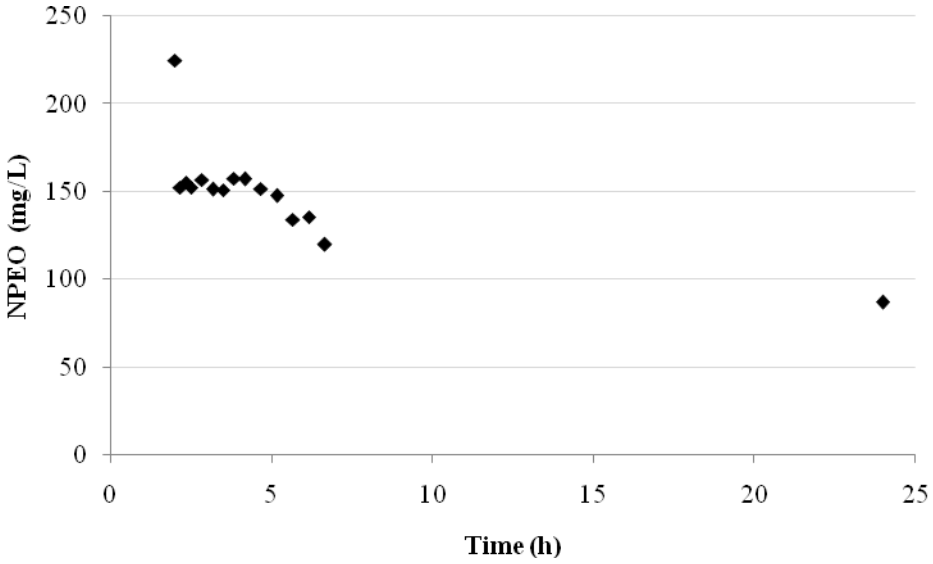

Figure 4.24: NPEO concentration versus time (Chronic Set 2).

Again, in the first day a repirometric analysis was operated by feeding the system only with 450 mg COD/L NPEO. The OUR profile is shown in Figure 4.25.

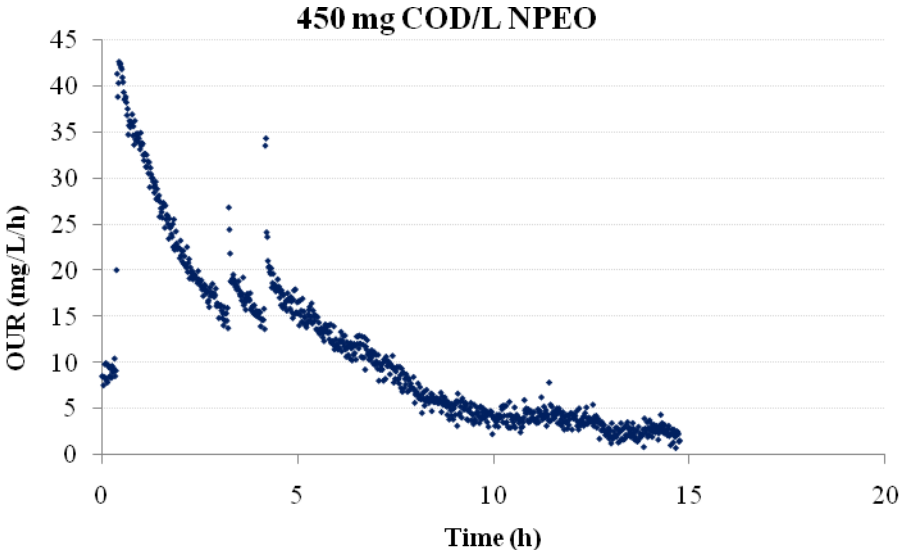

Figure 4.25: OUR data versus time (Chronic Set 3).

The organic matter removal is shown in Figure 4.26 and the change in NPEO concentration is in Figure 4.27. While NPEO was removed totally at the end of the day, organic matter content of the effluent just decreased to nearly 250 mg COD/L.

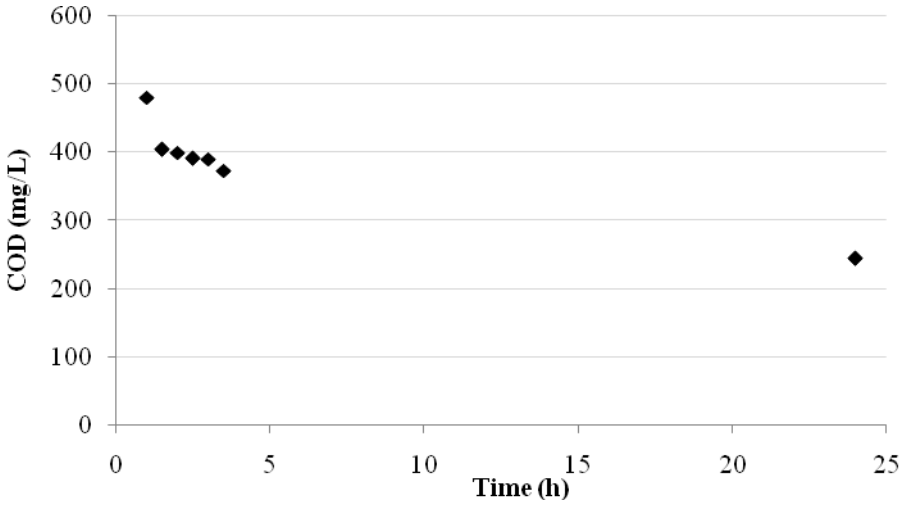

Figure 4.26: COD versus time (Chronic Set 3).

Moreover, the maximum adsorption capacity of the first day sludge is seen from the immediate decrease of NPEO concentration in the first hour of set 3.

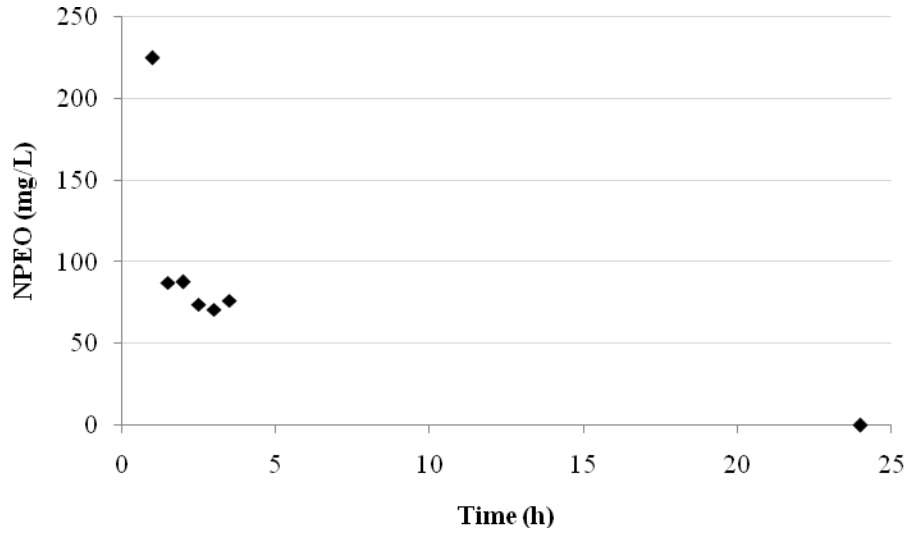

Figure 4.27: NPEO concentration versus time (Chronic Set 3).

6th day of the operation respirometric analysis was experimented and the OUR profile is determined as illustrated in Figure 4.28. While the hydraulysation duration was getting longer, the maximum OUR data obtained was decreased with respect to the first day profile. The inhibition effect of the chemical can be definetelly seen from that characteristics of the OUR profile at the end of the 6th day.

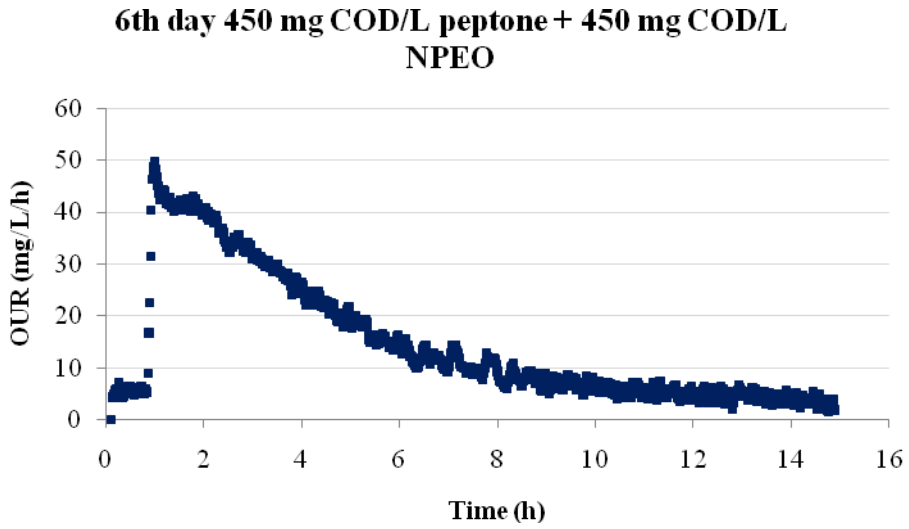

Figure 4.28: OUR data versus time (Chronic Set 4).

The COD change was in set 4 is like in Figure 4.29 and decreased to 400 mg COD/L at the end of the day. On the other hand, NPEO concentration decreased to nearly 200 mg/L whose COD equivalance is 400 mg COD/L and illustrated in the Figure 4.30, below.

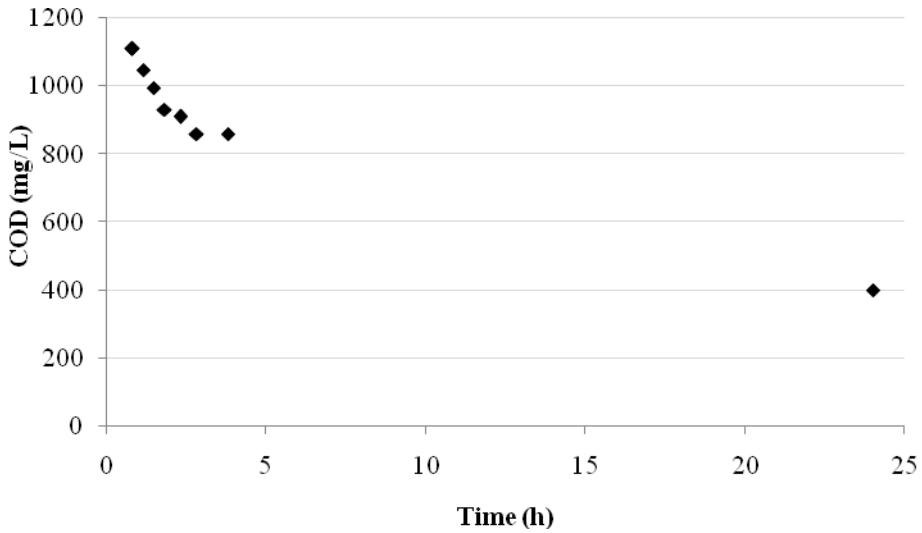

Figure 4.29: COD versus time (Chronic Set 4).

In the 6th day it is seen that the adsorption capacity of the sludge is less than the first day because the change of the concentration is changed slowly.

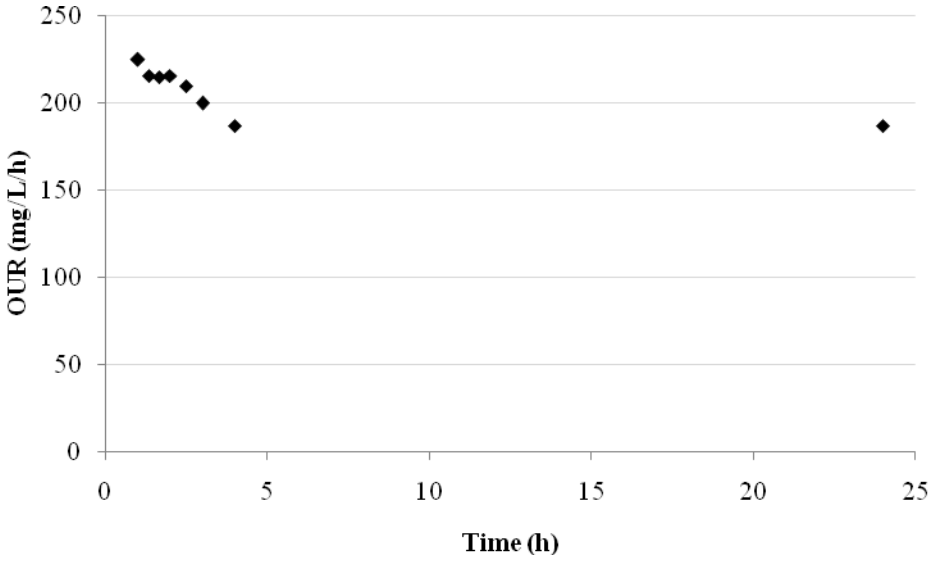

Figure 4.30: NPEO concentration versus time (Chronic Set 4)

In 13th day, respirometric analyses were done with peptone mixture and NPEO solution together, only peptone and only NPEO seperately. The OUR profile of the experiment of the system fed with 450 mg COD/L peptone and 450 mg COD/L NPEO is seen in Figure 4.31. There is a difference in the trend of the profile that a hump was formed between 2nd and 3rd hours of the experiment.

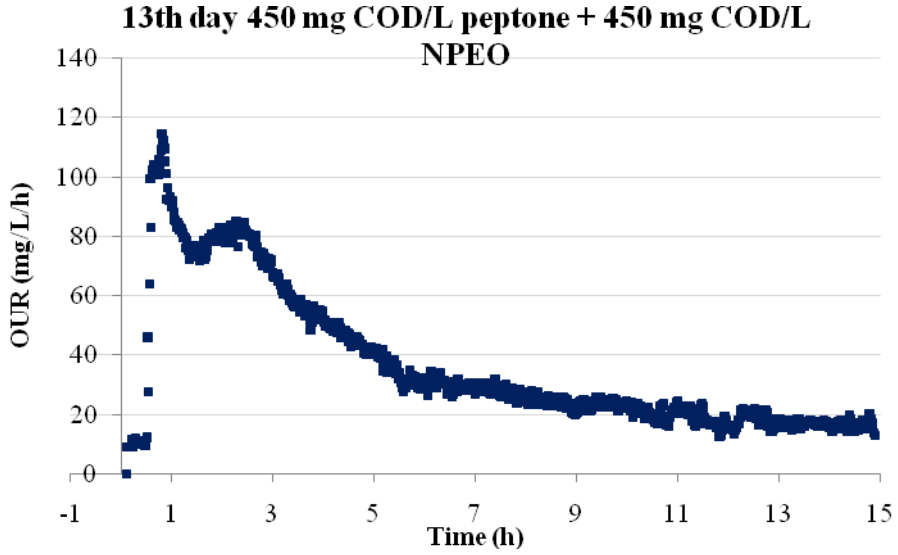

Figure 4.31: OUR data versus time (Chronic Set 5)

The COD and NPEO change of set 5 is illustrated in Figures 4.32 and 4.33, respectively. Organic matter was observed as approximatelly 500 mg COD/L while the NPEO concentration of the effluent is nearly 200 mg/L.

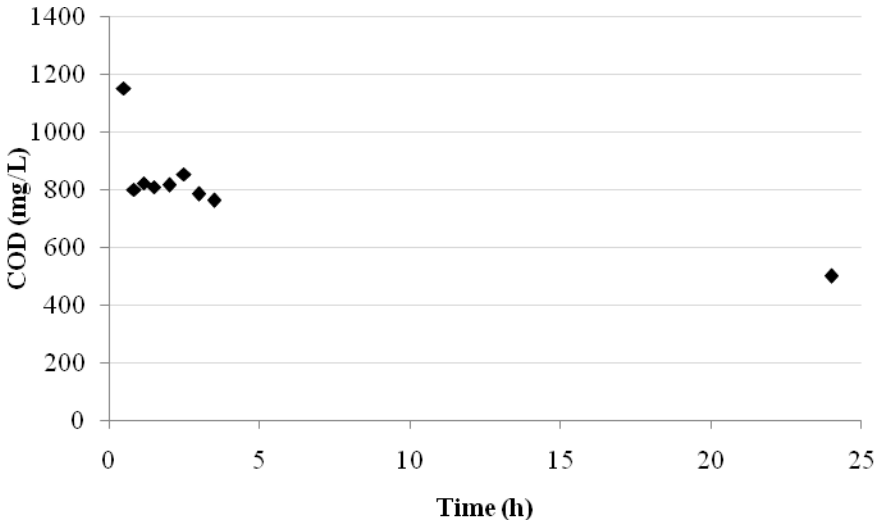

Figure 4.32: COD versun time (Chronic Set 5).

Same as the 6th day experiments any of adsorption effect is seen in 13th day, as well.

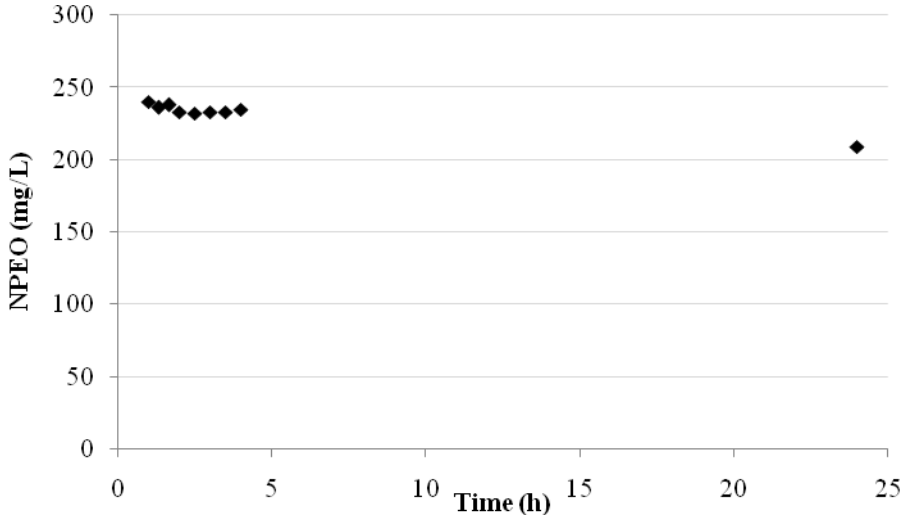

Figure 4.33: NPEO concentration versus time (Chronic Set 5).

In set 6, only 450 mg COD/L NPEO solution was fed to the system and OUR profile was observed as in Figure 4.34.

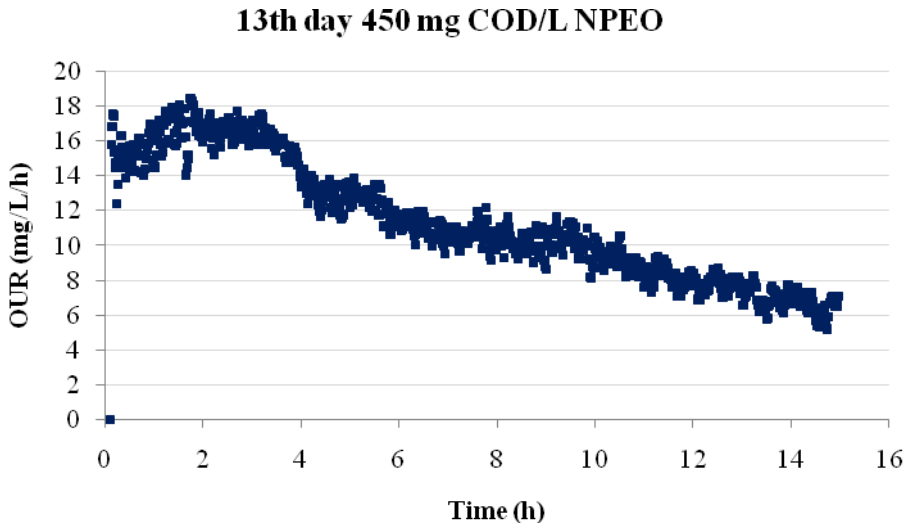

Figure 4.34: OUR data versus time (Chronic Set 6).

The change of organic matter and NPEO concentration is given in Figures 4.35 and 4.36, respectively. In this set, in the effluent there was not any NPEO observed. However, the organic matter content is still high which means NPEO is turned to its metabolites, as seen before.

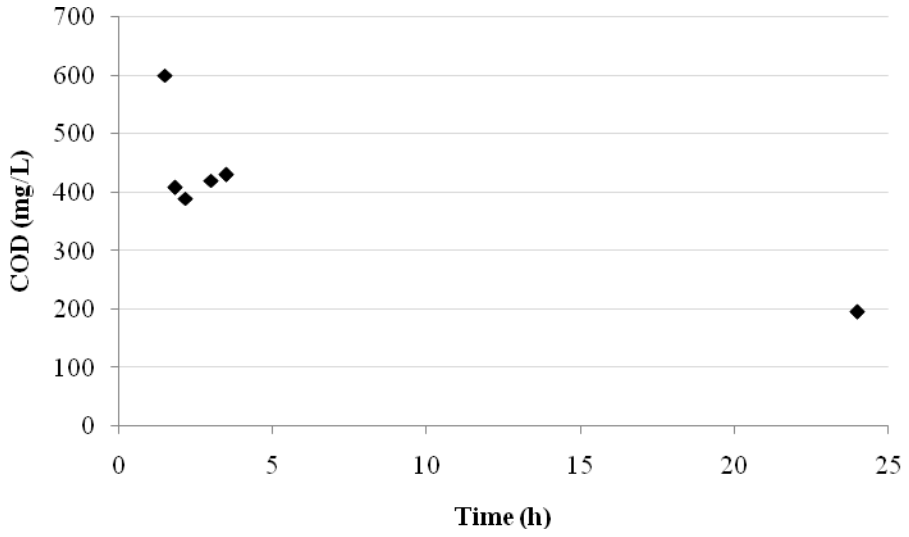

Figure 4.35: COD versus time (Chronic Set 6).

Moreover, the concentration of NPEO was changed slowly at the beginning which shows no adsorption capacity of the sludge is remained.

The OUR profile observed when only 450 mg COD/L peptone mixture was added to the system is seen in Figure 4.37. It is seen that there is a slight change in the

consumption of peptone mixture without any NPEO solution. The hydraulysis time is decreased and the maxmimum OUR value is higher than the previous sets.

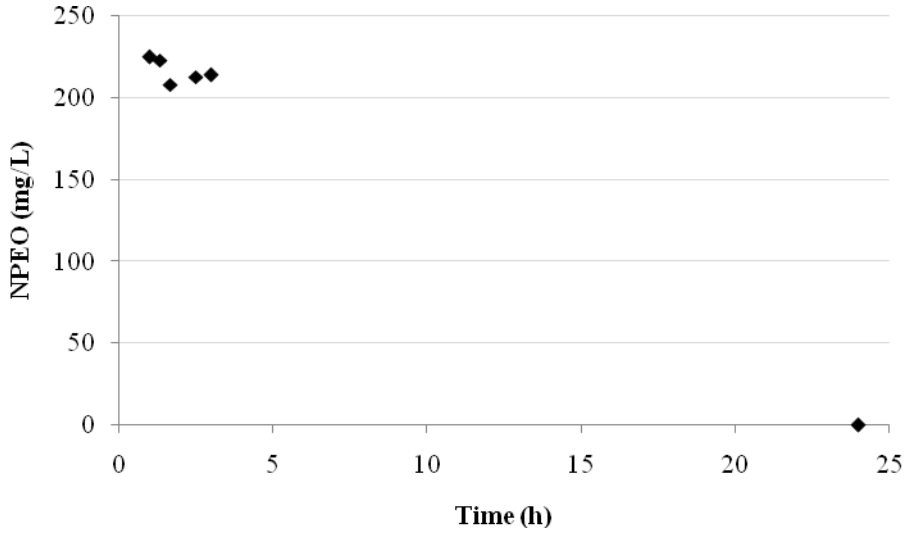

Figure 4.36: NPEO concentration versus time (Chronic Set 6).

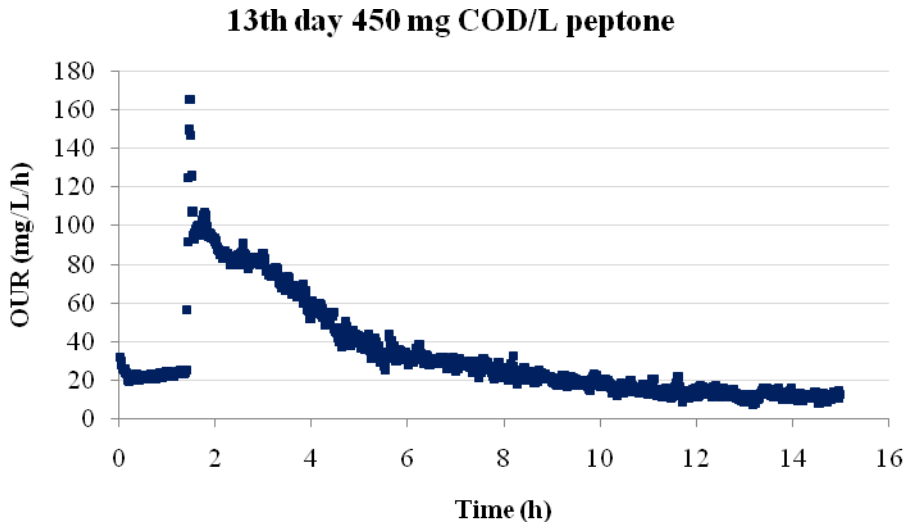

Figure 4.37: OUR data versus time (Chronic Set 7).

The change in organic matter content of 7th set is shown in Figure 4.38, below. Although, only 450 mg COD/L peptone mixture was added to the system the influent COD is seen nearly 700 mg/L, which remains from the day previous. Therefore, the effluent organic matter value is seen nearly 150 mg COD/L. Consequently, that does not mean that the organic matter remains in the effluent is frm peptone, it is from other mid-products can not be removed from the day previous.

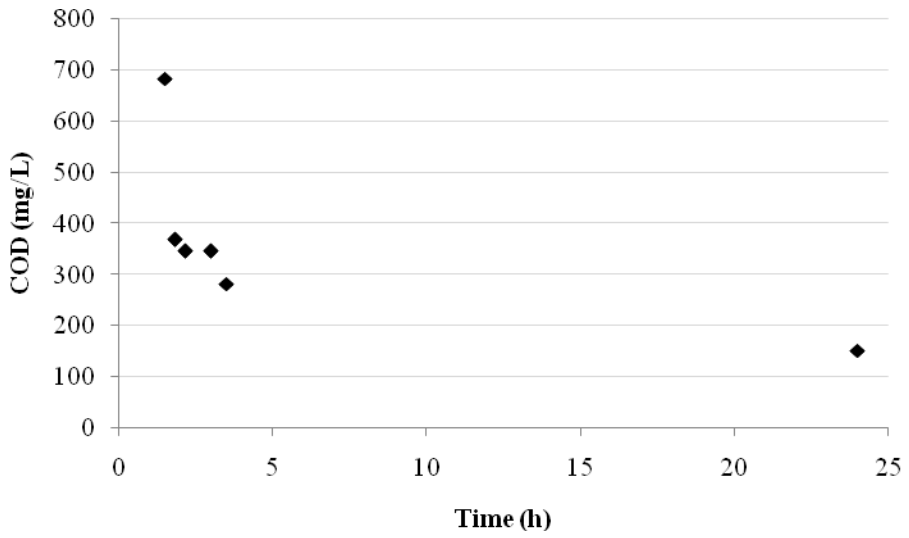

Figure 4.38: COD versus time (Chronic Set 7).

And in 20th day, two sludge age time period is completed, same experiments were done again. The OUR profile of the system fed with 450 mg COD/L peptone and that much COD equivalence NPEO is shown in Figure 4.39.

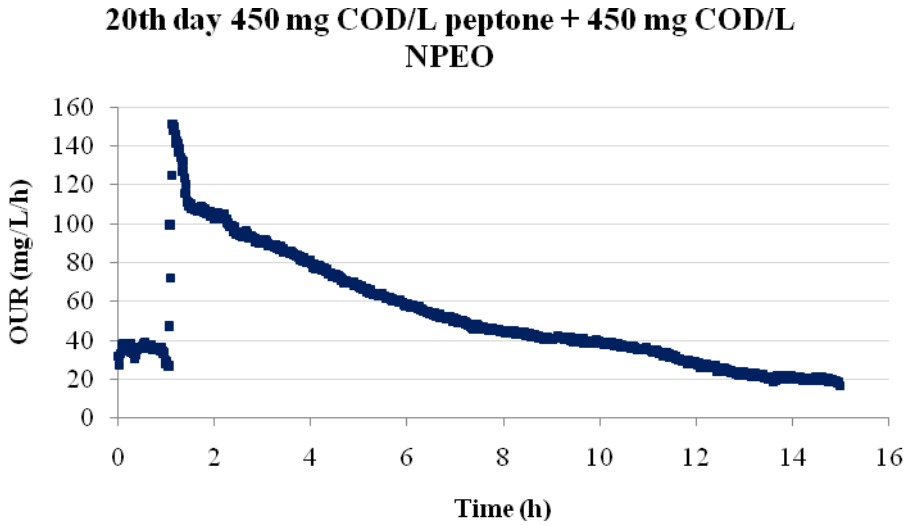

Figure 4.39: OUR data versus time (Chronic Set 8).

The organic matter change and NPEO concentration change datas are given in Figures 4.40 and 4.41, respectively.

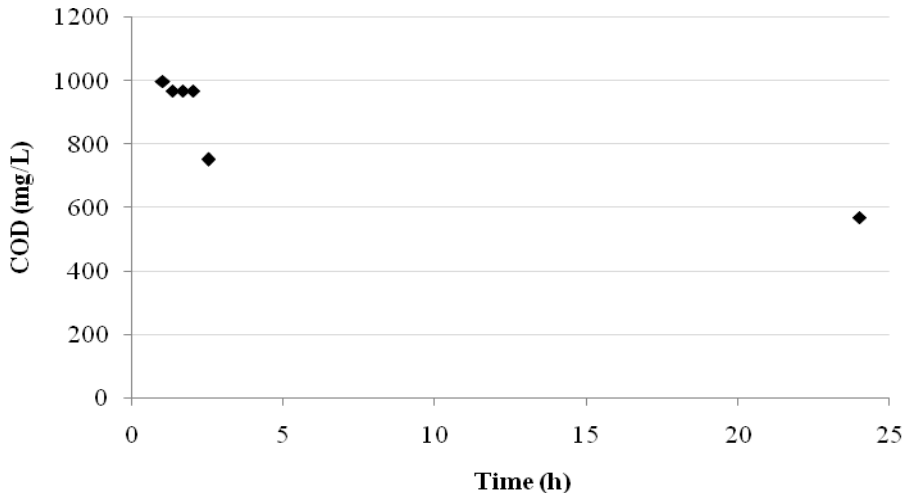

Figure 4.40: COD versus time (Chronic Set 8).

In this final set, the effluent organic matter content was decreased to nearly 600 mg COD/L where the initial organic content was observed nearly 1000 mg COD/L.

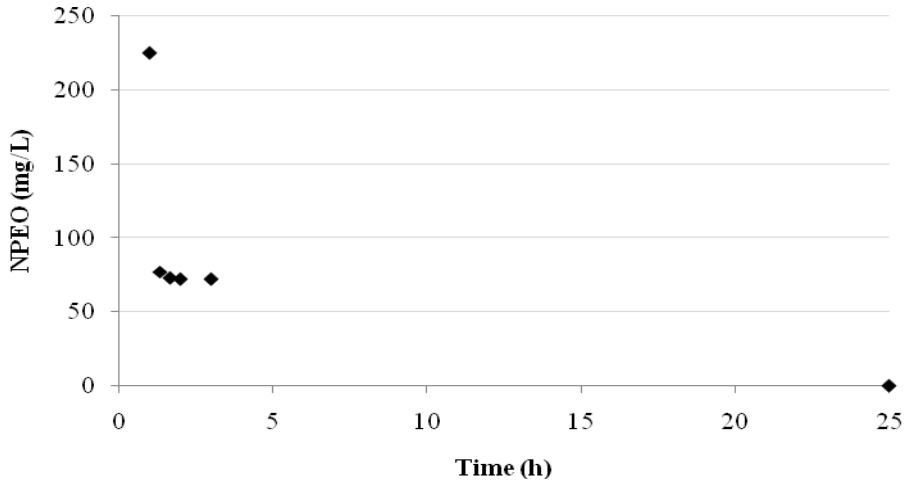

Figure 4.41: NPEO concentration versus time (Chronic Set 8).

Again, there was no NPEO in the effluent observed after 20 day acclimated system at the end of 20^{th} day.

Set 1, 2 and 8 were modelled according to ASM 1 and the model parameters were estimated for those sets. The estimated model parameters were illustrated in Table 4.1. The model and the OUR profiles were shown in Figure 4.42, 4.43 and 4.44 for the sets 1, 2 and 3, respectively.

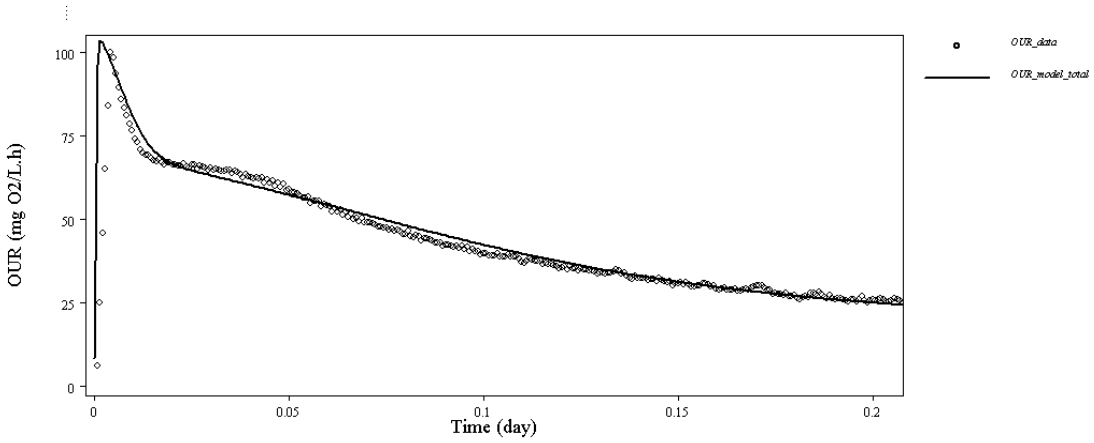

Figure 4.42: ASM1 simulation of chronic set 1.

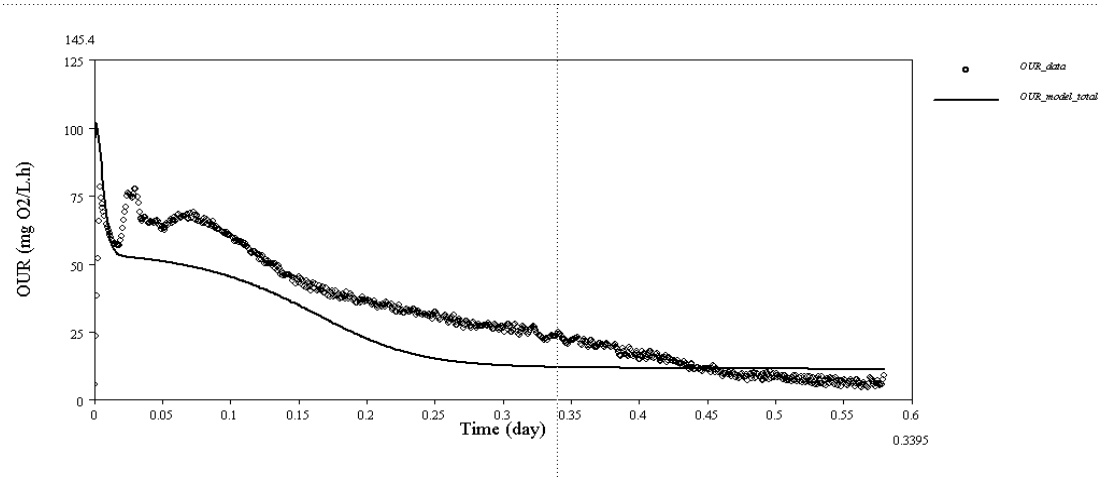

Figure 4.43: ASM1 simulation of chronic set 2.

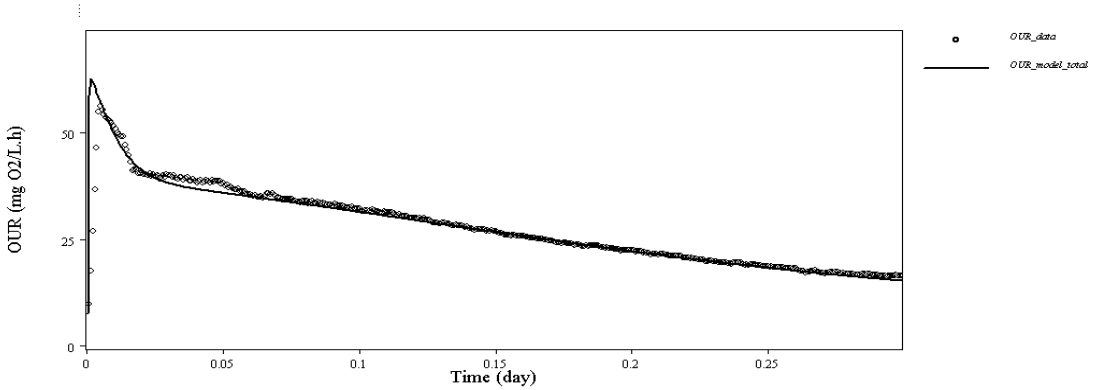

Figure 4.44: ASM1 simulation of chronic set 8.

Table 4.1: Model parameters estimated for Set 1, 2 and 8.

Model Parameters	**Unit**	**Control (Set1)**	**1st day (Set2)**	**20th day (Set8)**
Maximum specific growth rate, μ_{Hmax}	1/day	4.1	4.1	4.1
Half saturation constant for growth, K_s	mg COD/L	10	30	30
Maximum hydrolysis rate for S_{H1}, k_{h1}	1/day	4.67	1	3.6
Hydrolysis half saturation constant for S_{H1}, K_X	g COD/g cell COD	0.22	0.034	0.38
Maximum hydrolysis rate for S_{H2}, k_{h2}	1/day	0.87	1.1	0
Hydrolysis half saturation constant for S_{H2}, K_{XX}	g COD/g cell COD	0.06	0.012	-
Activity	%	56-60	56-60	56-60

Assumption: Heterotrophic yield, Y_H = 0.6 g cell COD/g COD, endogenous decay coefficient, b_H = 0.2 day^{-1}, fraction of inert metabolic product, f_E = 0.2.

Modeling results of ASM1 for the system acclimated to NPEO solution and operated with 10 day sludge retention time for control set, fed only with peptone, 1st day fed with peptone and NPEO and the final 20th day fed with peptone and NPEO indicate that maximum heterotrophic growth rate does not change for all of that three sets. It is estimated as 4.1 day^{-1}.

The heterotrophic yield Y_H, 0.6 g cell COD/g COD, the endogenous decay coefficient b_H, 0.2 day^{-1}, and the fraction of inert metabolic product, f_E, 0.2, are assumed constant for all runs.

The estimated half saturation constant for growth of X_H, K_S, is 10 mg COD/L for the control set. In the first day of feeding the chemical and the final day it is increased to 30 mg COD/L. It can be commented that the affinity to substrate get more difficult for biochemical reactions after feeding the chemical.

The maximum hydraulysis rate of S_{H1}, k_{h1}, is estimated 4.67 day^{-1}, for the control set. For the second set it is decreased to 1 day^{-1} as an inhibition effect. It is commented before that the hydraulisation time gets longer which shows the decreasing of hydraulysation rate as mentioned before. After acclimation of the chemical for about 20 days, the hydraulisation rate increased up to 3.6 day^{-1} again. However, its inhibition effect does not over totally.

Hydraulysis half saturation constant for S_{H1}, K_X, is estimated as 0.22 g COD/g cell COD for the control set. However, it is incresed to 0.34 in the first day and to 0.38 in the final day.

Maximum hydraulysis rate for S_{H2}, k_{h2}, is estimated as 0.87 $day^{-1}$for the control set. Then it is incresed to 1.1 in the first day. Therefore, the hydraulysation time of slowly hydraulysable substrate get faster with respect to control set. On the other hand the value of the model parameter is 0 for the twentieth day. It can be said that all of the substrate in the final set is hydraulysed with the rate of k_{h1}. Cosequaently, hydraulysis half saturation constant for S_{H2}, K_{XX}, cannot be defined for the final set.

The activity of the biomass does not change for all sets modeled and the value is estimated between %56-60.

5. CONCLUSION AND RECOMMENDATIONS

The purpose of study is evaluating the biodegradation of NPEO a non-ionic surfactant. An activated sludge system with heterotrophic biomass acclimated to a synthetic substrate mixture was investigated for acute effects of different concentrations of NPEO solution. The experiments conducted parallel to the respirometer, organic matter removal efficiency and NPEO amounts were monitored.

The NPEO biodegradation and inhibition coefficients will be established by employing respirometry.

Thereafter, to establish the chronic effects of the surfactant the system was begun to fed with 450 mg COD/L peptone mixture and 450 mg COD/L NPEO solution. During the acclimation studies, the respirometric analyses were performed and with the results of the experiments the difference between acclimated and non-acclimated sludge response to chemical was investigated in detail. As observed from the acclimation period, the concentration amount of NPEO is 0 while the organic matter of the effluent is not. That illustrates the transformation of the chemical to different metabolites definitely.

From the non-acclimated studies, the inhibitory effects were observed without examining the activated sludge model from the OUR profiles. According to ASM1, the control run, first day and final day analyses were modelled. And it is seen that the inhibitory effect of the chemical is seen specifically in the first day with decreasing of maximum hydrolysis rate coefficient. Thereafter, with the acclimation period, at the final day, the hydrolysis rate coefficient was observed higher than the first day result. Consequently, the substrate is become slowly biodegradable characteristic with the acclimation.

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