ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

FATE OF SULFAMETHOXAZOLE AND ITS INHIBITORY IMPACT ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT HIGH SLUDGE AGE

M.Sc. THESIS

Aslıhan URAL

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JANUARY 2012

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

AEROBİK ORTAMDA YÜKSEK ÇAMUR YAŞINDA SÜLFAMETOKSAZOL'ÜN AKİBETİ VE ASETATIN BİYOLOJİK AYRIŞABİLİRLİĞİ ÜZERİNE İNHİBİSYON ETKİSİ

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OCAK 2012

Aslıhan URAL, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID **501091813**, successfully defended the **thesis** entitled "**FATE OF SULFAMETHOXAZOLE AND ITS INHIBITORY IMPACT ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT HIGH SLUDGE AGE**" which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 19 December 2011
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To my mother and father,

FOREWORD

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December 2011

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ABBREVIATIONS

ASM : activated sludge model
$b_{H,O2}$: aerobic endogenous respiration rate of X_H (d^{-1})
$b_{STO,O2}$: aerobic respiration rate for X_{STO} (d^{-1})
COD : chemical oxygen demand ($mg.L^{-1}$)
C_S : substrate concentration (C $mol.m^{-3}$)
C_X : biomass concentration (C $mol.m^{-3}$)
DO : dissolved oxygen (mg $O_2.L^{-1}$)
DO_{Total} : total dissolved oxygen (mg $O_2.L^{-1}$)
DO_{Endo} : dissolved oxygen for endogenous respiration (mg $O_2.L^{-1}$)
$DO_{Gro,PHA}$: dissolved oxygen for growth on PHA (mg $O_2.L^{-1}$)
$DO_{Sto+Gro}$: dissolved oxygen for growth and storage (mg $O_2.L^{-1}$)
f_{ES} : fraction of the soluble metabolic products
f_{EX} : fraction of the particulate metabolic products
f_{SI} : production of S_I in hydrolysis
f_{XI} : production of X_I in endogenous respiration
F/M : food to microorganism ratio
$i_{SS,XI}$: SS to COD ratio for X_I (g SS.g COD^{-1})
$i_{SS,XS}$: SS to COD ratio for X_S (g SS.g COD^{-1})
$i_{SS,BM}$: SS to COD ratio for biomass, X_H, X_A (g SS.g COD^{-1})
k_H : hydrolysis rate constant (d^{-1})
k_{STO} : maximum storage rate (d^{-1})
K_{ALK} : half saturation constant for alkalinity for X_H (g $HCO_3^-.m^{-3}$)
K_{O2} : half saturation constant for S_{NO2} (g $O_2.m^{-3}$)
K_S : half saturation constant for substrate S_S (g $COD.m^{-3}$)
K_{SA} : half saturation constant for S_{SA} (g $COD.m^{-3}$)
K_{S1} : half saturation constant for direct microbial growth (mg $COD.L^{-1}$)
K_{S2} : half saturation constant for growth on stored product (mg $COD.L^{-1}$)
K_{STO} : half saturation constant for storage (g $X_{PHB}.gX_H^{-1}$)
K_X : hydrolysis saturation constant (g COD.g COD^{-1})
MBR : membrane bioreactor
MLVSS : volatile suspended solid (mg $COD.L^{-1}$)
m_S : maintenance coefficient for growth on acetate (C mol.C $mol^{-1}.h^{-1}$)
m_P : maintenance coefficient for growth on PHB (C mol.C $mol^{-1}.h^{-1}$)
m_{ATP} : maintenance coefficient based on ATP (mol.C $mol^{-1}.h^{-1}$)
OUR : oxygen uptake rate (mg $OUR.L^{-1}.h^{-1}$)
PABA : para-aminobenzoic acid
PHA : poly-β-hydroxylalkanoate
PHB : poly-β-hydroxybutyrate
S_{ALK} : alkalinity of the wastewater
S_I : inert soluble organic material (mg $COD.L^{-1}$)
S_{NH} : ammonium plus ammonia nitrogen (mg $N.L^{-1}$)
S_{N2} : dinitrogen (mg $N.L^{-1}$)

S_{NO} : nitrite plus nitrate nitrogen (mg $N.L^{-1}$)
So : dissolved oxygen concentration (mg $O_2.L^{-1}$)
S_P : soluble microbial products (mg $COD.L^{-1}$)
S_{S1} : readily biodegradable organic substrate (mg $COD.L^{-1}$)
$S_{Sremoved}$: biodegradable organic substrate removed (mg $COD.L^{-1}$)
S_{SA} : biodegradable portion of antibiotic (mg $COD.L^{-1}$)
SBR : sequencing batch reactor
SMX : sulfamethoxazole
SRT : sludge retention time (d)
SS : suspended solids (mg $SS.L^{-1}$)
TET : tetracycline
WWTP : wastewater treatment plant
X_A : autotrophic organisms (mg $COD.L^{-1}$)
X_{H1} : heterotrophic biomass (mg $COD.L^{-1}$)
X_{HA} : heterotrophic biomass active for antibiotic (mg $COD.L^{-1}$)
X_P : particulate microbial products (mg $COD.L^{-1}$)
X_{PHB} : stored internal PHB (mg $COD.L^{-1}$)
X_S : slowly biodegradable substrate (mg $COD.L^{-1}$)
X_{STO} : internal storage product (mg $CODL^{-1}$)
X_{TS} : total suspended solids (mg $COD.L^{-1}$)
VSS : volatile suspended solids (mg VSS L^{-1})
Y_H : heterotrophic yield coefficient for direct growth (g COD.g COD^{-1})
$Y_{H,O2}$: aerobic yield of heterotrophic biomass (g COD.g COD^{-1})
Y_{OBS} : observed yield (g COD.g COD^{-1})
Y_{PX} : yield of growth on the stored substrate (g COD.g COD^{-1})
Y_{PX}^{max} : maximum yield of biomass on PHB (C mol.C mol^{-1})
Y_{SP} : yield of storage on substrate (g COD.g COD^{-1})
Y_{SP}^{max} : maximum yield of PHB on acetate (C mol.C mol^{-1})
Y_{SX}^{max} : maximum yield of biomass on acetate (C mol.C mol^{-1})
Y_{STO} : storage yield of PHB on acetate (g COD.g COD^{-1})
$Y_{STO,O2}$: aerobic yield of stored product per S_S (g COD.g COD^{-1})
μ : specific growth rate (h^{-1})
μ_H : maximum growth rate of X_H (d^{-1})
μ_{HA} : maximum growth rate of X_{HA} (d^{-1})
μ_{STO} : maximum growth rate of X_H on X_{STO} (d^{-1})
δ : P/O ratio, ATP produced per $NAHDH_2$ oxidized ($mol.mol^{-1}$)

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FATE OF SULFAMETHOXAZOLE AND ITS INHIBITORY IMPACT ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITION AT HIGH SLUDGE AGE

SUMMARY

In this study, acute and chronic effects and biodegradability of one of the major antibiotics, Sulfamethoxazole (SMX) was investigated.

Activated sludge taken from a domestic watewater treatment plant (WWTP) was acclimated to acetate as a main carbon source and operated at a sludge age of 10 days. The influent COD concentration of fill & draw reactor was kept as 400 mg COD/l.

In acute inhibition experiments, biomass taken from the fill & draw reactor and having the same F/M ratio was used as well as fed acetate together with four different pulse feedings of 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of SMX. In acute experiments, the observed antibiotic removal can be neglected. According to kinetic coefficients, maximum storage rate increased; however, endogenous decay rate decreased.

In chronic inhibition experiments, biomass taken from the fill & draw reactor and having the same F/M ratio was used as well as fed acetate together with continuous feeding of 50 mg/L of SMX. In chronic studies, three different types of substrates were conducted in the experiments; mixed substrate (including acetate and antibiotic), only acetate and only antibiotic. Synthetic substrate and SMX acclimation were applied to the same quality activated sludge; the system was monitored by respirometric experiments and conventional parameters. On the fifth day of acclimation, it was observed that the storage process disappeared. After twenty days of operation, acclimation of the activated sludge to this mixture was seen and complete removal of SMX was obtained. Moreover, a higher maximum growth rate and a lower endogenous decay rate were monitored in the chronic experiments.

In the study, relevant kinetic and stoichiometric coefficients were achieved using modified ASM3 pattern.

AEROBİK ORTAMDA YÜKSEK ÇAMUR YAŞINDA SÜLFAMETOKSAZOL'ÜN AKIBETİ VE ASETATIN BİYOLOJİK AYRIŞABİLİRLİĞİ ÜZERİNE İNHİBİSYON ETKİSİ

ÖZET

İlaç etken maddelerinin üretimi ve tüketimi son yıllarda oldukça artmıştır. Henüz çevre üzerindeki riskleri ve etkileri tam olarak bilinemediği için, bu maddelerin varlıkları ve giderimleri en önemli araştırma konularından biri olmuştur. Sık kullanılan bir ilaç türü olan antibiyotiklerin yıllık tüketimleri tüm dünyada yaklaşık 500 ton'dur.

Son yıllarda artan antibiyotik kullanımı hayatımızın her alanında kendini göstermektedir. Özellikle insan ve hayvan sağlığı, balık gibi sucul canlıların sağlığı ve gelişimi, tıbbi atıklar, endüstriyel aktiviteler, antibiyotik ve ilaç üreten endüstriler, gıda sektöründe besinlerin korunması, ev gereçleri, ürünler üzerine spreyleme, çiftlik hayvanlarının üretimi, balık çiftlikleri gibi alanlar antibiyotiklerin temel kaynaklarını oluşturmaktadır. Günümüzde geniş bir kullanım aralığına sahip olan antibiyotikler, insan ve hayvansal tıpta tedavi edici amaçlar için sıklıkla kullanılır. Bu nedenle, tedavi edici antibiyotiklerin kalıcı konsantrasyonları çevrede bulunur.

Veterinerlikte hayvan sağlığı amacıyla kullanılan antibiyotikler tüketiciler tarafından kolay temin edilmesi ve maliyetinin ucuz olmasından dolayı yoğun bir şekilde kullanılmaktadır. Örneğin, kümeslerde ortaya çıkan bazı hastalıklarla mücadele amacı ile tavuk yemine antibiyotiklerin katıldığı bilinmektedir. Bu antibiyotikler, hayvanların eti veya yumurtası ile tüketiciye kadar ulaşabilmektedir. Bu tehlike nedeni ile antibiyotiklerin kullanılması kontrol altında tutulmalıdır. Ancak bilinçsiz ve gereksiz antibiyotik kullanımı sonucunda hem çevresel sorunlar hem de besin zinciri yoluyla canlılarda özellikle insanlarda sağlık problemleri meydana gelmektedir.

Balık çiftliklerinde gerekli sağlığa uygunluk kurallarına uyulmamasından dolayı hastalıklar sıklıkla yaşanmakta ve büyük ekonomik kayıplar ortaya çıkmaktadır. Balıkların bakteriyel hastalıklarının tedavisinde en yaygın yöntem antibiyotik ve diğer bazı kimyasalların kullanılmasıdır. Yeme karıştırılarak balıklara verilen antibiyotiklerin tüketilmeyen kısmı suda çözünür veya zemine çöker. Atık yemle birlikte ortama geçen ilacın bir kısmı doğal ortamdaki balık ve kabuklular tarafından alınır ve bünyede birikerek yüksek konsantrasyonlar oluşturur. Antibiyotiklerin aşırı kullanımı standart antimikrobiyal uygulamalara karşı dirençli patojenlerin gelişimini de teşvik eder. Bunun sonucu olarak da besin zinciri yoluyla insanlar ve diğer canlılar sağlık sorunları yaşamaktadırlar.

Antibiyotikler mikroorganizmaların büyümesini durduran ya da öldüren biyolojik kaynaklı veya sentetik olarak elde edilen çok etkili biyoaktif maddelerdir. Etki tarzlarına ve etkiledikleri mikroorganizmalara göre çok sayıda antibiyotik bulunmaktadır. Mikroorganizmanın hücre duvarını bozmak, protein sentezini bozmak veya mikroorganizmanın ihtiyaç duyduğu maddeleri yok etmek antibiyotiklerin etkileri arasındadır. Antibiyotikler, doğada bakteriler veya mantarlar tarafından üretilir. Bu canlıların antibiyotik üretip bulundukları ortama salma nedenleri, diğer türlerle besin yarışı içinde olmalarıdır. Bu nedenle, bulundukları ortamda kendilerinden başka organizmaların yok olmalarını veya daha fazla büyümelerini engelleyen antibiyotik maddeleri üretirler.

Antibiyotikler etki mekanizmalarına ve kimyasal yapılarına göre sınıflandırılmaktadır. Etki mekanizmalarına göre: Hücre duvarı sentezini engelleyenler, protein sentezini engelleyenler ve nükleik asitlere etki edenler şeklinde sıralanmaktadır. Antibiyotikler etki mekanizmalarına ve kimyasal yapılarına göre 9 ana başlık altında sınıflandırılmaktadır: β-Laktamlar (penisilin, sefalofosporin, monobaktam), Tetrasiklinler (oksitetrasiklin ve tetrasiklinler), Makrolid antibiyotikler (eritromisin), Aminoglikozidler (gentamisin, tobramisin, amikasin), Quinolonlar (siprofsaksin), Linkosamidler (klindamisin), Oksazolidler (linezolid), Sülfa antibiyotikler (sülfisosazol), Sasilik peptidler (vankomisin, polimiksinler). Veterinerlik alanında öncelikli olarak uygulanan antibiyotik sınıfları; tetrasiklin ve sulfamidlerdir. İnsan tedavisinde ise sentetik penisilinler, tetrasiklin ve makrolidler yaygın olarak kullanılır.

Antibiyotikler konvansiyonel biyolojik arıtma proseslerine dirençli oldukları için, alıcı su kaynaklarına tam olarak arıtılamadan deşarj edilmektedirler. Bu nedenle, çıkış sularında ve alıcı ortamlarda birikmektedirler. Endüstride kullanılan prosesler ve kimyasallardan dolayı oluşan atıksular yoğun kirlilik içermekte ve toksik nitelikte olmaktadır. Bu maddeleri içeren ilaç endüstrisi atıksuların arıtımı için fizikokimyasal arıtıma ihtiyaç duyulmaktadır. Çünkü miktarları her geçen gün ekosistemde artan bu ilaç etken maddeleri, doğada antibiyotiğe dirençli patojen organizmaların artışına sebep olmakta ve bu durum halk sağlığı için büyük bir tehdit oluşturmaktadır.

Aslında sorunların temelinde çevrenin korunmayıp yoğun olarak kirletilmesi ve doğal kaynakların bilinçsizce tüketilmesi gelmektedir. Özellikle doğal ortamların evsel, endüstriyel ve tarımsal faaliyetler sonuncunda yok olma aşamasına gelmesi insanları farklı arayışlara yöneltmiştir. Örneğin günümüzde akuatik yaşamın dengesinin bozulması ile balık çiftliklerinin hızla gelişip çoğalması ve bunun sonucu olarak da çevrenin göz ardı edilmesi yine çevre kirliliği olgusunu ortaya çıkarmıştır. Bu nedenle yapılan ya da yapılacak her faaliyette çevre koruma bilincinin ön planda olması gerekmektedir.

Bu çalışmada, önemli antibiyotiklerden biri olan Sülfametoksazol (SM)'ün, aktif çamur sistemlerine olan akut ve kronik etkileri ve biyolojik arıtılabilirliği incelenmiştir.

Bir evsel atıksu arıtma tesisinden alınan aktif çamur ana karbon kaynağı olarak asetata aklime edilerek 10 çamur yaşında çalıştırılmıştır. Doldur-boşalt çalıştırılan sistem kolay ayrışabilir organik olarak asetat ile beslenmiş ve giriş KOİ konsantrasyonu 400 mg KOİ/L olarak belirlenmiştir. Reaktör kararlı dengeye ulaşıncaya kadar ve sonrasında, konvansiyonel parametreler izlenerek kontrol altında tutulmuştur.

Akut inhibisyon deneylerinde, doldur-boşalt reaktörden alınan ve aynı F/M oranına sahip biyokütle kullanılmış ve asetat ile birlikte SM'ün 25 mg/L, 50 mg/L, 100 mg/L ve 200 mg/L'lik dört farklı konsantrasyonu anlık olarak beslenmiştir. Akut inhibisyon deneylerinde gözlenen antibiyotik giderimi ihmal edilebilir düzeydedir. Kinetik katsayılara bakıldığında, maksimum depolama hızının azaldığı; fakat içsel solunum hızının arttığı görülmüştür.

Kronik inhibisyon deneylerinde, doldur-boşalt reaktörden alınan ve aynı F/M oranına sahip biyokütle kullanılmış ve asetat ile birlikte SM'ün 50 mg/L'lik konsantrasyonu sürekli olarak beslenmiştir. Kronik çalışmalarda üç farklı substrat tipi uygulanmıştır: karışık substrat (antibiyotik ile asetat içerir), yalnızca asetat ve yalnızca antibiyotik. Aynı nitelikteki aktif çamura sentetik atıksu ile sülfametoksazol aklimasyonu birlikte uygulanmış; respirometrik çalışmalar ve konvansiyonel parametrelerle sistem izlenmiştir. İlk 5 günlük aklimasyon sürecinde, depolama işleminin ortadan kalktığı görülmüştür. 20 günlük işletim periyodu sonunda aktif çamurun bu karışıma alıştığı gözlenmiş ve SM'ün tamamen giderildiği görülmüştür.

Çalışmadaki ilgili stokiyometrik ve kinetik katsayılar, modifiye edilmiş ASM3 modeli kullanılarak elde edilmiştir.

1 INTRODUCTION

1.1 Purpose of Thesis

Antibiotics are commonly used for either prevention or treatment of diseases caused by microorganisms. Since antibiotics cannot totally be excluded from the environment, it is important to obtain best treatment techniques for elimination of their severe effects on the aquatic and terrestrial ecosystems.

In order to investigate the elimination mechanism of antibiotic substances in activated sludge systems and their effects on these systems, a model substance was chosen in this study. This substance, which is among the abundantly used antibiotic compounds in the world and in Turkey, was chosen to represent one of the major groups of antibiotics. The antibiotic SMX was chosen to represent Sulfonamide group of antibiotics.

Main objectives of the study carried out with the chosen antibiotic can be given as follows:

- Determination of inhibition effects of SMX on the aerobic activated sludge culture under both acute and chronic conditions
- Observation of the effects of the inhibiting substance on the activity of the microbial culture
- Investigation of the biodegradability characteristics of SMX by the aerobic activated sludge culture in SBR
- Identification of the storage ability of microorganisms when readily biodegradable substrate (acetate) and/or SMX was subjected

As a last step of the thesis, the biochemical processes occurring under inhibiting and non-inhibiting conditions were modelled with the aid of the results obtained from experimental studies. Using the data obtained from analyzes, optimum model parameters were developed for optimum treatment of antibiotic containing wastewaters.

1.2 Scope of Thesis

The fate of antibiotics in wastewater treatment systems is of particular concern, because they are likely to exert adverse effects on treatment process efficiency and by-pass treatment systems. This study focused on the acute and chronic effects of a major antibiotic, SMX, on mixed microbial culture. For this purpose, two SBRs were operated at steady state conditions with a sludge retention time (SRT) of 10 d. One of them was "control reactor" fed with the acetate as a sole carbon source for acute experiments and the other one was operated at the same conditions except for the carbon source. Both reactors had the same total volume of 10 l. In the second reactor, namely "antibiotic reactor", biomass was acclimated to selected antibiotic, for chronic experiments. During the acclimation studies, SS and VSS analyses were made and also, samples were collected from these reactors, regularly. The collected samples including soluble COD, SMX and PHA were analyzed for the microbial compositions, treatment efficiencies and substrate storage capacities. The acute and chronic effects of SMX on the activated sludge diversity were investigated by oxygen uptake rate (OUR) measurements.

2 LITERATURE REVIEW

2.1 Antibiotics

2.1.1 Background

Pharmaceuticals in the environment first became a focus of scientific interest and public awareness in the 1970s. Some investigations to prove the existence of pharmaceuticalss in the effluent of wastewater treatment plants (WWTPs) were carried out in the mid 1980s, mainly in Great Britain. These reports triggered more detailed investigations into the occurrence, fate and effects of pharmaceuticals in the environment in Europe, the USA and Canada. Meanwhile, a high awareness about the effects of pharmaceuticals in the environment has grown since the mid 1990s all over the world [1].

Antibiotics are among the most important groups of pharmaceuticals that are persistent to biodegradation and have the tendency to accumulate in the environment [1,2]. They are widely used in human and veterinary medicine as well as the aquaculture for preventing and/or treating microbial infections, while they are used to promote growth of animals in livestock farming. Some antibiotics are also used in growing fruit and in bee keeping [1].

Human beings have used antibiotics since the beginning of the century as this type of synthesized chemicals play a very positive effect in human society in fighting against various diseases [3]. Many antibiotics can be changed structurally by microorganisms in the gut or by enzymes in the bodies of humans and animals, respectively. While some of these antibiotics are largely metabolized before they are excreted, others can only be moderately or poorly metabolized [1].

However, because of their wide application, various antibiotics have been detected in all environmental media, such as WWTP effluents, surface water, seawater, groundwater, soils, and sediments. This wide presence of antibiotics in the environment has brought public and scientific concerns. For example, some of the antibiotics have harmful effects on human metabolism or organisms and thus, disrupt

the balance of the ecological system [3]. The release of the antibiotics to aquatic environment is very important since it may contaminate raw, treated, recycled, irrigation and recreation water. This contamination can cause to irreversible negative effects on ecosystem bacteria such as toxicity and inhibition [4].

For the environmental engineering point of view, the ecological system can damage irreversibly as microorganisms may develop drug-resistance to these antibiotics after long-term contact [3]. Researches about persistence and long-term adverse effects of antibiotics on the environment have been raised after the reports on the undesirable ecological and potentially human health effects of antibiotics even if they present in a very low concentrations. The concentrations in WWTP effluents have been shown to lie in the range of ng/l to µg/l, and in surface waters in ng/l for different countries. Antibiotic compounds, which are mostly polar, are generally present at the parts-per-trillion or low parts-per-billion range [1,5,6].

2.1.2 Sources of antibiotics

Possible irresponsible consumption of antibiotics, which leads to resistant pathogenic microorganisms living in the aquatic environment and soil, causes a great deal of concern regarding the fate of antibacterial compounds in relevant water treatment processes [7]. An important source of pharmaceutical contaminantion is through land application of livestock manure, which contains antibiotics due to the usage of them either as growth promoters or as therapeutic agents to fertilize crops. Furthermore, after heavy rain, veterinary antibiotics may reach surface water due to run-off from the soil, and eventually reach the drinking water supply [1,5]. The presence of antibiotics in the aquatic environment is of particular concern because of fears that they may stimulate dissemination of antibacterial resistance among native bacterial populations. Even though a causal link between chronic exposure to sub-therapeutic concentrations of antibiotics and induction of antibacterial resistance has yet to be proven, singly and multiply antibacterial resistant bacteria have been detected in municipal wastewater effluents, sewage-affected surface water systems, and even in drinking water [8]. Pathways of the antibiotics and their metabolites in the environment are schematically indicated in Figure 2.1 [1].

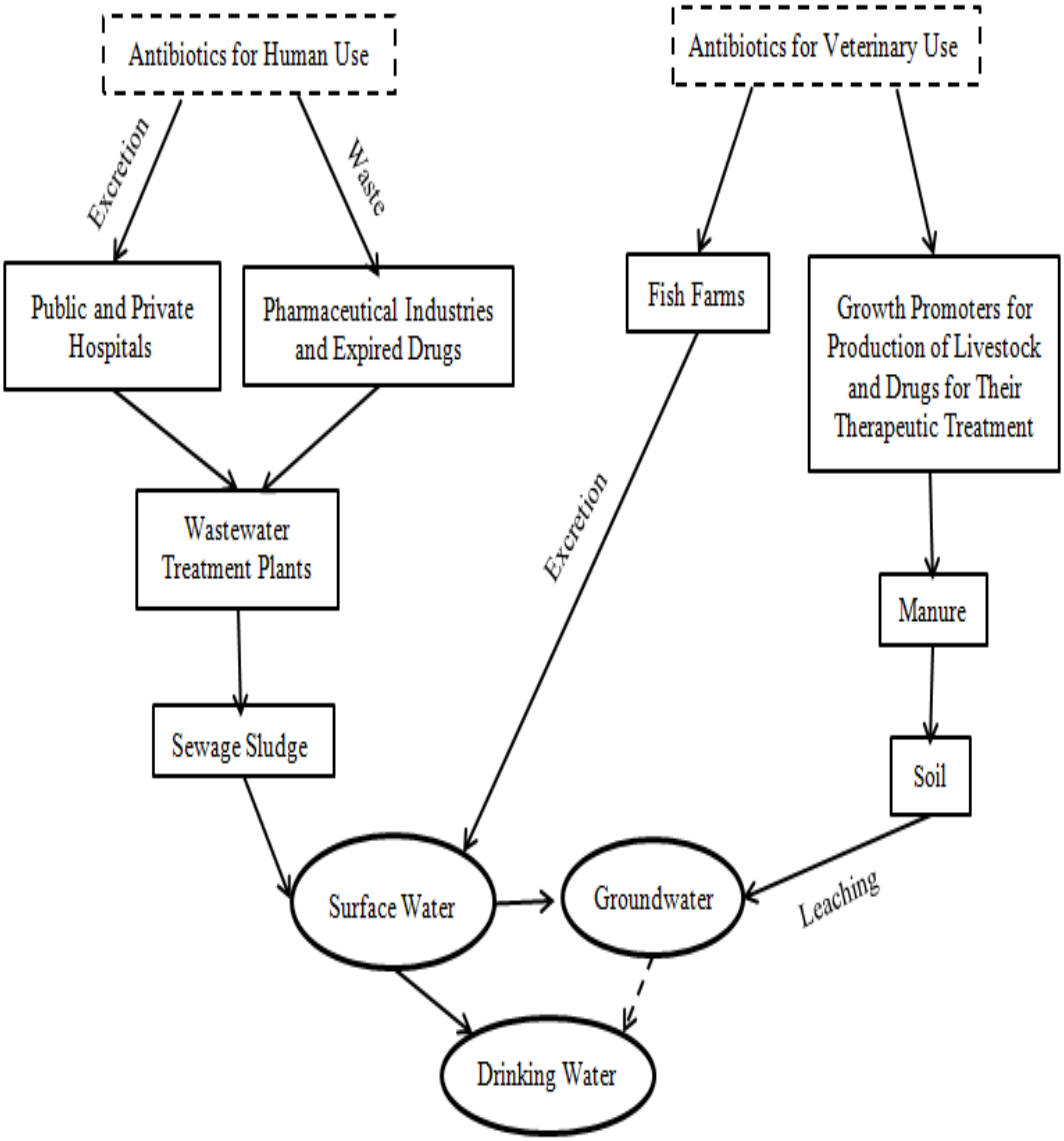
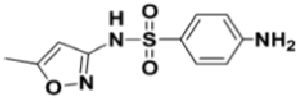
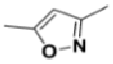
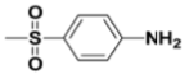
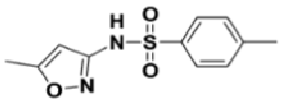


Figure 2.1 : Pathways of antibiotics and their metabolites in the environment.

Antibiotics are a diverse group of chemicals that can be divided into subgroups such as β-lactams, quinolones, tetracyclines, macrolides, sulfonamides etc. [1]. SMX is an important member of the synthetic sulfonamide antibacterials or sulfa drugs, and often prescribed in tandem with antibacterial agent trimethoprim, which does not belong to any specific class as a major first-line clinical option for treatment of various bacterial infections. Sulfonamides have been used extensively for many years in human and veterinary medicines to treat diseases and infections such as urinary tract infections, bronchitis, sinusitis, toxoplasmosis or ear infections, and in feed additives to promote livestock growth and weight gain of food animals [3,5,7]. SMX is a structural analog of para-aminobenzoic acid (PABA) and inhibits bacterial synthesis of dihydrofolic acid, which is important for DNA production, by competing with PABA [9,10].

As summarized in Table 2.1, sulfonamide compounds contain two moieties connected to both sides of the characteristic sulfonamide linkage. Sulfonamides exhibit two acid dissociation constants (pKa1 & pKa2); one involving protonation of the aniline N, and the other corresponding to deprotonation of the sulfonamide NH, as shown in Figure 2.2 [7].

Table 2.1 : Sulfamethoxazole and associated substructure model compounds [7].

Compound	Structure	Mol. Weight	pK_a
Sulfamethoxazole (SMX)		253.28	pK_{a1}=1.7, pK_{a2}=5.6
3,5-dimethylisoxazole (DMI)		97.12	Not Available
4-aminophenyl methyl sulfone (APMS)		171.22	pK_{a1}=1.5
4-methyl-N-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (MMIB)		252.29	Not Available

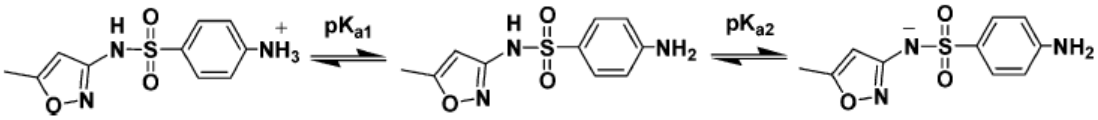


Figure 2.2 : Speciation of sulfamethoxazole.

Because of their wide application, various antibiotics have been detected in all environmental media, such as sewage treatment plant effluents, surface water, seawater, groundwater, soils and sediments [11,12]. The removal rate of SMX in wastewater treatment facilities is lower as compared to other antibiotics including fluoroquinolone and macrolide groups [13]. In addition, SMX showed lower adsorption to sludge, solids, and sediments in comparison to other antibiotics [14-16]. Therefore, a large quantity of SMX will remain in water and could be available to organisms and has higher risks to the environment as compared with other antibiotics. In this context, SMX has been detected in raw influents of WWTPs at concentration level of 118 ng/l in Southeast China, 580 ng/l in Spain, and 520 ng/l in the USA [13,17,18]. Among the target analytes measured, SMX in particular has been repeatedly detected at concentrations of 70-150 ng/l in surface waters and 200-2000 ng/l in secondary wastewater effluents [19-23].

However, certain point sources, such as waste streams and effluents generated by hospitals, may have higher antibiotic concentrations, in order of between 10 to 600 mg/l [24,25].

Another important class of antibiotic among the Tetracyclines group, which are broad-spectrum bactreiostatic antibiotics, is the Tetracycline (TET). This antibiotic is important since it can cause adverse effects on protein biosynthesis by reversibly binding to the 30S ribosomal subunit and inhibiting translation [26,27]. Investigations have shown that TET is present in liquid manure at concentrations up to 20 mg/l, respectively. Moreover, the authors conclude that TET enters the soil in significant concentrations in liquid manure, builds persistent residues and accumulates in the soil [1].

The release of high levels of antibiotics is problematic in at least three ways. First of all, the antibiotics may impair the general treatment efficiency of effluent by killing microorganisms or preventing them from growing. Secondly, the antibiotics are very likely to affect the local environment, including microbial ecosystems. Thirdly, and perhaps most importantly, the release raises serious concerns about an accelerated rate of microbial resistance development [1].

2.1.3 Fate of antibiotics

Over the past decade, a great amount of interest has arisen regarding the occurrence and fate of trace organic contaminants, many of which are ubiquitous in municipal WWTPs. For this reason, many studies about the presence of antibiotics in the environment have focused on transformation and fate of them as they are released, move through wastewater treatment processes, are discharged and transported through surface waters or ground waters, and ultimately result in exposure to human and aquatic life [5,6,9,27].

Very large numbers of antibiotics used in human and veterinary medicine are industrially produced and commercially used, but they are not required to undergo the extensive level of testing for possible environmental impact. They and their metabolites have therefore been subject to many years of unrestricted emission to the environment, directly or via WWTP effluents or sludge [1]. Disposal of antibiotics through manufacturing and packaging facilities in the environment means that many different substances in varied concentrations, products and modes of action have to

be considered [5,28]. In this context, their identification and quantification in the aquatic environment has become an issue in order to develop the analytical techniques needed to quantify their occurrence in effluents, but also to assess their chemical properties, their biodegradability potential, etc. [1,29].

Analysis of the antibiotics in the environment nowadays is mostly done by LC-MS/MS, some by GC/MS-MS. These medical substances have been measured in the effluent from hospitals and domestic WWTPs, as well as in surface waters, groundwaters and drinking waters. Antibiotics have also been detected in the leachate from landfills [1].

The fate of the antibiotics can be divided into three categories; transport, sequestration, and degradation. The predominant fate processes most likely to transform or mineralize the antibiotics, and more permanently remove them from the aquatic environment, include photolysis, hydrolysis, and biodegradation. Antibiotics may also be transferred without degradation and storage, at least temporarily, in other matrices or compartments through processes such as sorption and deposition of particles [1,5].

2.1.4 Antibiotics in wastewaters

Some pharmaceutical substances have been detected in sewage, the effluents of WWTPs, surface waters, manures and soil since the 1980s [1]. Municipal wastewaters contain many organic compounds, among them, active ingredients of pharmaceuticals and personal care products, which are used in large quantities throughout the world. Most of these compounds come either from domestic sewage or from hospital or industrial discharges and enter municipal WWTPs. Generally, antibiotics are absorbed by the organism after intake and are subject to metabolic reactions. However, a significant fraction of the original substances leave human or animal organisms unmetabolized via urine or feces being therefore emitted into raw sewage, sewage sludge or manure [29].

Modern WWTPs can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control. However, urban WWTPs normally receive streams that contain a lot of different trace polluting compounds (synthetic and natural), for which conventional treatment technologies have not been specifically designed [29]. In this context, WWTPs can be considered as an important point source for antibiotic

contamination of surface waters. Consequently, significant fractions of the antibiotics are discharged with the final effluent of the WWTPs into the aquatic environment. Besides, these substances can also imply an important pollution source for the soil if primary and secondary sludge to which they are adsorbed are spread on land [5,30].

It is often assumed that hospitals are the most important source for input of the antibiotics and resistant bacteria into municipal wastewater. Concentrations of antibiotics in municipal sewage and in WWTPs are much lower than in hospital effluent. However, taking into consideration that hospital effluents contribute to less than 1% of the total amount of municipal sewage, it is plausible that hospitals are not the main source for resistant bacteria in municipal sewage. Because of the use of antibiotics in houses, it is probably the general community that is responsible for the main input of resistant bacteria into WWTPs [1].

2.2 Removal Mechanisms of Antibiotics

Although WWTPs are designed to remove pathogens and nutrients from sewage, antibiotics and other emerging chemicals found in wastewater can be incidentally removed, but the elimination is variable. Since, chemicals are moderated in wastewater treatment either through dilution, oxidation by disinfectants, biological degradation or sorption to the solid materials and are settled out of the system [5].

Researchers have focused on the removal of antibiotics by biodegradation in many different systems, including WWTPs, membrane bioreactors (MBRs), sequencing batch reactors (SBRs), sand columns, and constructed wetlands [28]. On the other hand, despite the wide consortium of microorganisms present in the activated sludge, it is unlikely that antibiotics present as micro contaminants in wastewater which can be effectively removed by biodegradation alone. First, the relatively low concentration of antibiotics from other pollutants may be insufficient to induce enzymes that are capable of degrading antibiotics. Second, many of these compounds are bioactive, which can inhibit growth or metabolism of microorganisms. Thus, it is unlikely that antibiotics will be favourable energy or carbon sources of microorganisms. Third, the degree of biodegradation will depend on the nature of each compound and the operating conditions, such as SRT or the combination of anoxic/aerobic steps that could improve the efficiency [1,5].

Wastewater treatment plays an important role in removing contaminants from reclaimed water. However, large variability in removal efficiency was reported for some antibiotics, implying that factors other than compound-specific properties affect removal efficiency [31].

The biological wastewater treatment technique is regarded as one of the most promising treatment approaches for removal of contaminants from wastewater because of its affordable cost and high efficiency [30]. The primary pollutant removal mechanisms in conventional wastewater treatment processes are adsorption onto suspended solids and biodegradation, which mainly takes place in the aerobic treatment. Therefore, it is reasonable to assume that key component in biological WWTPs responsible for the removal of antimicrobial pollutants in the aeration basin containing the microorganisms. Biodegradation and sorption also could take place in other unit processes, such as primary settling, but removal efficiencies are difficult to control at this stage [5]. Their removal efficiencies are influenced, apart from the chemical properties of specific compounds, by microbial activity and environmental conditions [29].

Sorption is an important elimination pathway, which depends on the extent of neutral and ionic species present and the characteristics of the target particles. It may have an impact on the spread and bioavailability of antibiotics in the environment and their removal during wastewater treatment. The ability to estimate the sorption of an antibiotic to solids in various media is critical to understand. The antibiotic TET is known to have a tendency to bind to soil particles or to form complexes with double cations, such as calcium or magnesium. More than 50% of TET can be eliminated by sorption to sewage sludge [1,8,27].

Another kind of abiotic elimination of substances is hydrolysis. For example, hydrolysis rates for TET increase as pH deviates from 7 and as temperature increases. However, sulfonamides are stable against hydrolysis [1].

Removal of SMX is observed in biological treatment systems with a long hydraulic retention time, such as extended aeration, where there is an absence of a readily biodegradable substrate [32]. The removal efficiency for SMX during wastewater treatment in full scale plants is ranged from no removal to greater than 98% removal. In a lab scale SBR, cometabolism of SMX with acetate is observed and it is found

that microorganisms may use the degraded antibiotic as a nitrogen source [9]. The overall removal efficiency of the WWTP in NW Spain is around 60% for SMX [29].

Some antibiotics, such as quinolones, tetracyclines are sensitive to light. If a substance is sensitive towards light, photodegradation may be of major significance in the elimination process. Photodecomposition takes place mainly in surface water due to the presence of light. Since the effectiveness of the processes depends on light intensity and frequency, and the latter relates to the absorption spectrum of a compound, phototransformation may not occur when the compounds are present in turbid water or if the creek, river or lake is shadowed by trees [1].

Respirometry, which is the measurement and interpretation of the biological oxygen consumption rate under well defined experimental conditions, has been commonly used to determine whether a certain compound is toxic or inhibitor or not to a biomass content of a WWTP [33,34]. The toxicity of a certain compound can be assessed by evaluating the OUR profile obtained from a pulse feeding of substrate to endogenous biomass and then comparing the OUR profiles obtained from the pulse feeding consists of the substrate and a toxic or inhibitor compound. However, using this method with biodegradable compounds leads to some difficulties because the toxic consumption implies oxygen consumption as well. Then, respirograms obtained adding the substrate with the possible toxic or inhibitor cannot be reliably compared to the ones obtained adding only substrate. Therefore, it can be really complex to distinguish between the oxygen consumption due to substrate and the oxygen consumption due to the inhibitor, especially when biodegradability rates of inhibitor and substrate are similar [34].

2.3 Enzymatic Effects of Antibiotics on WWTP

Enzymes are very complex and specific catalytic molecules produced by microorganisms, capable of accelerating reaction rate by lowering the activation energy of the biochemical reaction they catalyse [35,36]. In biochemical reactions, the biodegradable wastewater components act as substrates to form an enzyme-substrate complex that is generated by the attachment of substrate on the active site of the specific enzyme molecule. When the biochemical reaction is completed, the active site is liberated and returns to its cyclic function to receive another substrate molecule. Sometimes, enzymatic functions may be retarded or even stopped by

inhibitors which may either compete for or simply block the active sites. A number of pollutants present in wastewater such as nonbiodegradable organics, antibiotics, etc. may exert significant inhibitory action upon biological treatment processes [36].

The mechanism of enzyme kinetics is based on the attachment of the substrate to the enzyme to yield a product. It provides a practical tool to mathematically explain the behavior of microbial cultures under inhibitory effects. The general equation for enzyme-substrate reactions as applicable to biological treatment systems **(2.1)** [35,36]:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad \textbf{(2.1)}$$

where S, E, ES and P represent concentrations of substrate, free enzyme, enzyme-substrate complex and product, respectively. Since the last step (defined by k_3) is considered the controlling step or slower step, the attachment of the substrate to the enzyme is regarded in equilibrium, which is defined by k_1 and k_2. On the basis of these reactions, the rate of formation of the enzyme-substrate complex is expressed as **(2.2)**:

$$\frac{dES}{dt} = k_1 E.S - (k_2 + k_3)ES \qquad \textbf{(2.2)}$$

with the simplifying assumption that the reactions proceed under equilibrium concentrations of the enzyme-substrate complex, the above equation is found as **(2.3)**:

$$E = \frac{(k_2 + k_3)}{k_1}\frac{ES}{S} \qquad \textbf{(2.3)}$$

A mass balance on enzyme species yields **(2.4)**:

$$E = E_0 - ES \qquad \textbf{(2.4)}$$

where E_0 is the initial free-enzyme concentration. If the value of E in Equation **(2.4)** is substituted in Equation **(2.3)**, the resulting expression is:

$$E = E_0 \frac{S}{K_S + S} \qquad \textbf{(2.5)}$$

where K_S is a combination of the kinetic constants for both step **(2.6)**s:

$$K_S = \frac{k_2 + k_3}{k_1} \quad \textbf{(2.6)}$$

Since the activated complex decomposition is assumed as a slow process, k_3 is small and therefore K_S approximately defines the equilibrium or affinity coefficient [35]. On the other hand, the rate of substrate utilization is defined as **(2.7)**:

$$\frac{dS}{dt} = -k_1 S.E + k_2 ES \quad \textbf{(2.7)}$$

or

$$\frac{dS}{dt} = -k_3 ES \quad \textbf{(2.8)}$$

Substituting the value of ES in Equation **(2.5)** into the Equation **(2.8)** yields **(2.9)**:

$$\frac{dS}{dt} = -V_M \frac{S}{K_S + S} \quad \textbf{(2.9)}$$

where $V_M = k_3 E_0$, a constant which defines the maximum rate of substrate utilization.

An analogy between the above Michaelis-Menten equation and the Monod equation may be established with the assumption that the total enzyme concentration is proportional to the active biomass concentration in the reactor **(2.10)** [36]:

$$V_M = k_3 E_0 = k_m X \quad \textbf{(2.10)}$$

The capacity for yielding a product, in the mechanism described in Equation **(2.1)**, may be lost when there is an interfering substance interacting either with the same specific site of the enzyme – competitive inhibition. Another possibility is the interaction of the inhibitor with the activated complex – uncompetitive inhibition [35].

2.4 Inhibition Effects of Antibiotics on WWTPs

Since biological processes deal with living organisms, they are subject to upset by inhibitory or toxic agents. Inhibition is caused either by chemical or physical agents, such as pH, temperature, oxidation-reduction potential of the medium, etc. On the other hand, many substances that are present in wastewater exert inhibitory effects on the microorganisms in a WWTP. In the context of biological wastewater treatment,

inhibition is defined as impairment of the enzymatic system of the living cell or direct damage to the cell structure, resulting in the slowing down of the cell activity [35].

pH and temperature, the two most common variations within a process, can lead to severe inhibitory conditions since all living systems are rather limited in their operating range. The effect of these parameters or any other, on the microbial activity is non-monotonous in nature. This means there are values for these parameters where the activity is at its optimum. Outside these values, the system does not perform at its peak that is interpreted as inhibition. On the other hand, the presence of inhibitory substances should be taken into account in terms of their adverse effects on the biomass activity. One clear manifestation of the activity is the oxygen consumption rate in aerobic processes, which is recognized as respirometric rate. OUR profiles are comprehended for the evaluation of substrate removal mechanisms. Respirometric test for inhibitory environments or for a specific substance is based on the measurement of a standard decrement in respirometric rate: the difference in respiration relative to a non-inhibitory situation [35].

When a microorganism is subject to ever increasing concentrations of an inhibitory substance, its activity, which is measured by the substance degradation rate or growth rate, diminishes until it reaches a point when all activity ceases. This point, i.e. the concentration level of the inhibitor, depends on the previous exposure of the microorganism to the same compound. The cell has the ability for adaptation, as it is capable of developing its enzymatic machinery to a point at which the inhibitor can also be used as substrate; perhaps at the expense of cell energy reserves. As the adaptation progresses, the microorganism is capable of tolerating higher concentrations and still perform close to the non-inhibiting condition. Obviously, time is important when dealing with exposure; there are short-term and long-term exposures, and the latter is useful for adaptation, especially if the concentration is low at the beginning [35].

Today, wastewater management is greatly complicated by the presence of biologically resistant substances such as heavy metals, antibiotics, etc. commonly called inhibitors. This becomes critically important in developing local pretreatment programs. In these programs, the effect of an incompatible pollutant on a treatment plant receiving both domestic and industrial wastewater from a community must be

evaluated from three perspectives: impact on the treatment plant, impact on the receiving water, and impact on sludge. The limit for that pollutant can then be set to ensure that all objectives are met. The most stringent of the three will determine the influent limit to be used for the pollutant [36].

The most important part of this management scheme is to derive a numerical value that would give an appropriate indication of the inhibitory effect. The expected indication is not only a certain inhibitory response, but it should also reflect a safety factor. In practice, the common way to reach this numerical limit is through empirical observations and evaluations. To calculate a discharge limit that will prevent inhibition of an activated sludge process, the traditional way is to check if an inhibition or upset condition exists (e.g., settling characteristics, level of mixed liquor suspended solids, etc.). If an upset condition is noted, the concentration of the pollutants most likely to cause the irregularity are determined and compared with the likely inhibitory threshold concentrations of the same pollutants [36].

A major achievement in trying to understand and utilize the concept of inhibition would be to describe it physically and mathematically, in terms of simple and meaningful parameters, and incorporate this model into general mass balance expressions of the activated sludge process. This approach inevitably defines a set of kinetic constants or operational parameters specifically related to the inhibition mechanism, and its merit chiefly relies upon experimental methods to quantify these constants and parameters. In other words, this approach is useful only if it is used in conjunction with experiments that would yield accurate and uniformly consistent results for its kinetic constants [36].

The mathematical models of the complex interactions of inhibitors with the microbial community in activated sludge systems are of practical significance if they can be evaluated within the framework of substrate removal kinetics. This approach is adopted in a number of studies defining the effect of inhibitors in terms of enzyme-catalyzed reactions [36].

There are three classic forms of inhibition including competitive, noncompetitive, and uncompetitive inhibition. In addition, the mixed case exists, sharing characteristics of competitive and uncompetitive inhibitors. Substrate inhibition is also reported in literature as a noncompetitive type of inhibition [35,36].

2.4.1 Competitive inhibition

Competitive inhibition defines the case where substrate, S, and inhibitor, I, compete for the same enzymatic site; the inhibitor can take the place of the substrate to form an enzyme inhibitor complex (EI), which cannot undergo further reactions with the substrate. When incorporated into the basic enzyme kinetics described by Equation **(2.1)**, competitive inhibition involves the following additional reaction **(2.11)** [36]:

$$E + I \rightleftharpoons EI \qquad \textbf{(2.11)}$$

Since the inhibitor (I) occupies the same site as the substrate, the result is a modified affinity, i.e. the apparent decrease in the forward reaction of the equilibrium in Equation **(2.1)**. This concept defines a dissociation constant, K_I **(2.12)** [35,36]:

$$K_I = \frac{E.I}{EI} \qquad \textbf{(2.12)}$$

The mass balance for enzyme components yields **(2.13)**:

$$ES = E_0 - E - EI \qquad \textbf{(2.13)}$$

Substitution of the value of EI in Equation **(2.12)** and the value of E in Equation **(2.3)** into the above mass balance permit to define ES in terms of the initial free-enzyme concentration, E_0 **(2.14)**:

$$ES = \frac{E_0 S}{K_S\left(1 + \frac{I}{K_I}\right) + S} \qquad \textbf{(2.14)}$$

This changes the basic rate Equation **(2.8)** into the following form **(2.15)**:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}\frac{S.X}{K_S\left(1 + \frac{I}{K_I}\right) + S} \qquad \textbf{(2.15)}$$

It is noted that Equaiton **(2.15)** differs from the Monod expression only in term $1+1/K_I$ which affects the value of K_s, so that an "apparent" K'_s is measured for competitive inhibition **(2.16)** [36]:

$$K_S' = K_S\left(1 + \frac{I}{K_I}\right) \qquad \textbf{(2.16)}$$

A Lineweaver-Burk plot for this case is presented in Figure 2.3 and shows effects of competitive inhibition on kinetic parameters [35].

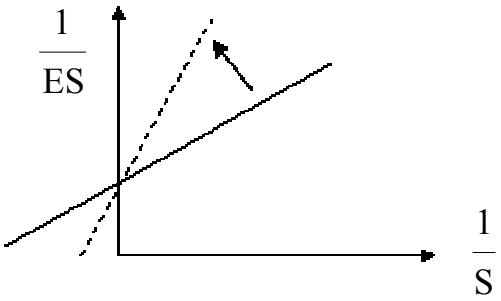


Figure 2.3 : Competitive inhibition.

2.4.2 Noncompetitive inhibition

In noncompetitive inhibition, the inhibitor does not compete for the active site; once attached somewhere else on the enzyme, it changes its composition and prevents the product from being formed. In this case, the inhibition reaction cannot be reversed by raising the substrate concentration. The substrate is then unable to prevent the combination of the inhibitor with the enzyme. The magnitude of inhibition will accordingly depend on I and K_1, and it will not be influenced by S or ES. The mathematical interpretation of the preceding statement gives in Equation **(2.17)** [35,36]:

$$E_0' = E_0 - EI \qquad \textbf{(2.17)}$$

This expression implies that the inhibitor blocks a portion of the initially present enzyme concentration, E_o, so that only E_o is practically available for substrate. Then, Equation **(2.18)**:

$$K_I = \frac{E_0'.I}{EI} \qquad \textbf{(2.18)}$$

and combining Equations **(2.17)** and **(2.18)**, EI is defined **(2.19)**:

$$EI = \frac{E_0 I}{K_I + I} \qquad \textbf{(2.19)}$$

For the substrate-enzyme interaction the following mass balance applies **(2.20)**:

$$E_0' = E_0 - EI = E + ES \qquad \textbf{(2.20)}$$

Using Equations **(2.3)** and **(2.19)**, this mass balance equation can be arranged to define ES **(2.21)**:

$$ES = \frac{E_0\left(\frac{K_I}{I+K_I}\right)S}{K_S + S} \qquad \textbf{(2.21)}$$

Since there is no competition, the affinity of the substrate is not modified, and as a consequence, K_S remains constant; however, E_o decreases in the presence of I. The corresponding rate expression for substrate then becomes **(2.22)**:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}\left(\frac{K_I}{I+K_I}\right)\frac{SX}{K_S+S} \qquad \textbf{(2.22)}$$

It is clear from Equation **(2.22)** that system will behave as if it contained less enzyme in the presence of a noncompetitive inhibitor, and consequently the maximum growth rate, $\hat{\mu}$ will be apparently reduced to a lower level **(2.23)** [35,36]:

$$\hat{\mu}' = \hat{\mu}\left(\frac{K_I}{I+K_I}\right) \qquad \textbf{(2.23)}$$

The slope of Lineweaver-Burk plot (Figure 2.4) increases with rising inhibitor concentration in this case [35].

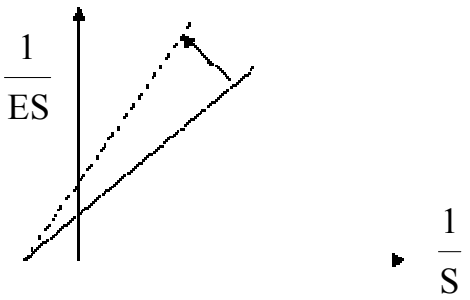


Figure 2.4 : Noncompetitive inhibition.

2.4.3 Uncompetitive inhibition

In this type of inhibition, the inhibitor attaches to the active complex formed by the substrate, blocking the product formation. Both parameters are modified by the presence of the inhibitor, K_S and E_o decrease. Then, the kinetic expression takes the following form **(2.24)**:

$$ES = \frac{E_0 S}{K_S + S\left(1+\frac{I}{K_I}\right)} \qquad \textbf{(2.24)}$$

The Lineweaver-Burk representation for this case is given in Figure 2.5 [35].

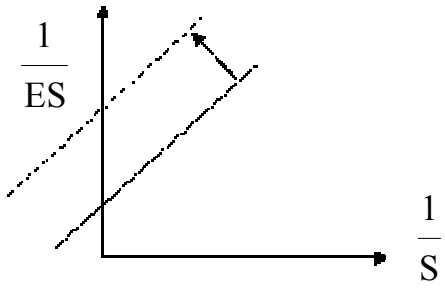


Figure 2.5 : Uncompetitive Inhibition.

2.4.4 Mixed inhibition

In this case, both parameters change E_o decreases and K_S increases, while the overall affinity decreases. This model is written as **(2.25)**:

$$ES = \frac{E_0 S}{K_S\left(1+\frac{I}{K_I}\right)+S\left(1+\frac{I}{K_I'}\right)} \qquad \textbf{(2.25)}$$

The Lineweaver-Burk representation of mixed model is shown in Figure 2.6, which is similar to the competitive inhibition model [35].

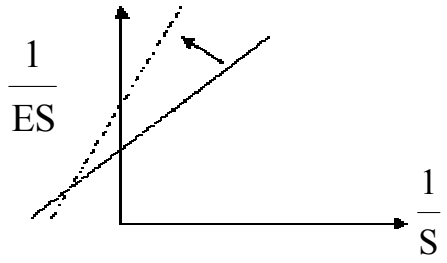


Figure 2.6 : Mixed inhibition.

2.4.5 Substrate inhibition

Substrate inhibition is described on the basis of the assumption that some substrates have the ability to bind with the enzyme-substrate complex as well as with the free enzyme at high concentrations. This leads to the formation of a substrate-enzyme-substrate complex (SES) which cannot undergo further reaction and does not yield the product (P). This can be seen as a special case of uncompetitive inhibition, whereby the same substrate inactivates the enzyme. The following reactions may be used to represent this mechanism **(2.26)** [35,36]:

$$ES + S \overset{K_{IS}}{\rightleftharpoons} SES \qquad \textbf{(2.26)}$$

where

$$K_{IS} = \frac{ES.S}{SES} \qquad \textbf{(2.27)}$$

By using the approach previously outlined, the following rate expression can be developed **(2.28)**:

$$\mu_{IS} = \hat{\mu} \frac{S}{\left(K_S + S + \frac{S^2}{K_{IS}} \right)} \qquad \textbf{(2.28)}$$

where μ_{IS} is the specific growth rate under substrate inhibition [36].

This expression is generally known as Haldane's equation that is of little practical value for activated sludge modelling, which primarily uses substrate removal kinetics at low concentrations. It is the only one of the similar models, shown in Table 2.2.

Table 2.2 : Model equations for substrate inhibition kinetics [36].

Equation No.	Kinetic Model
1	$\mu_I = \hat{\mu} \frac{S}{(K_S + S)\left(1 + S/K_{IS}\right)}$
2	$\mu_I = \hat{\mu} \frac{S(1 + S/K_S)}{K_S + S + S^2/K_{IS}}$
3	$\mu_I = \hat{\mu} \frac{S}{K_S + S + \left(S^2/K_{IS}\right)\left(1 + S/K\right)}$
4	$\mu_I = \hat{\mu} \frac{S}{K_S + S} e^{-S/K_{IS}}$
6	$\mu_I = \hat{\mu}\left(e^{-S/K_{IS}} - e^{-S/K_S}\right)$
7a	$\mu_I = \hat{\mu} \frac{S}{K_S + S}$ when $S < S^*$[1]
7b	$\mu_I = \hat{\mu} \frac{S}{K_S + S} - K_{IS}(S - S^*)$ when $S < S^*$
8	$\mu_I = \hat{\mu} \frac{S}{K_S + S}\left(1 - \frac{S}{S_m}\right)^{\alpha 2}$

[1] S^* = Treshold substrate concentration below which there is no substrate inhibition.

[2] S_m = Substrate concentration above which growth is completely inhibited.

Another modelling approach is presented for substrate inhibition, based upon the fact that sewage is not just a single substrate, but a mixture of substrate, and a minor

fraction of this mixture may be expected to exhibit inhibiting properties. Generally, this can be reflected by **(2.29)**:

$$S = S_S + S_I \tag{2.29}$$

where S is the total substrate concentration in sewage, and S_S and S_I its noninhibiting and inhibiting fractions respectively. This concept is introduced into the following model **(2.30)**:

$$ES + S_I \overset{K_{IS}}{\rightleftharpoons} SES_I \tag{2.30}$$

Proper manipulation of the model equations yields **(2.31)**:

$$\mu_{IS} = \hat{\mu} \frac{S}{K_S + S + \frac{S.S_I}{K_{IS}}} \tag{2.31}$$

Equation **(2.31)** represents a very different biochemical mechanism for substrate inhibition in sewage treatment. It implies that the validity of using a single substrate approximation for substrate inhibition is more and more questionable for higher mixed liquor substrate concentrations and for lower inhibiting substrate fractions. This equation is also identical in format to the one derived for product inhibition because of the following reaction **(2.32)**:

$$ES + P \overset{K_P}{\rightleftharpoons} ESP \tag{2.32}$$

For low substrate concentrations, where $S \ll K_S$, the rate expression for substrate utilization in the absence of inhibitors is reduced to Equation **(2.33)**:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{YK_S} SX \tag{2.33}$$

The use of this equation modifies expressions **(2.15)** and **(2.22)**, given for competitive and noncompetitive inhibitions respectively, into one single Equation **(2.34)**:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{YK_S} \frac{K_I}{K_I + I} S.X \tag{2.34}$$

Similarly, at high substrate concentrations where $S \gg K_S$, the acceptable approximation may be written as **(2.35)**:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y} X \qquad \textbf{(2.35)}$$

which indicates that for $S \gg K_S$ the impact of competitive inhibition may be ignored and that of noncompetitive inhibition may be reformulated as **(2.36)** [36]:

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y} \frac{K_I}{K_I + I} X \qquad \textbf{(2.36)}$$

2.5 Modelling of Activated Sludge

A model can be defined as a purposeful and often simplified representation of a system of interest [35]. The use of dynamic models in activated sludge processes has become more widespread. Various mathematical models have already been developed to describe the process kinetics of microorganisms in an activated sludge process during the aerobic biodegradation of slowly as well as readily biodegradable substrates. Proper understanding of substrate removal mechanisms is very essential for modeling purposes. A multicomponent approach to the modelling of activated sludge provides the correct assessment of the viable biomass in the reactor as well as particulate inert fractions for the evaluation of the amount of sludge to be wasted from the system [36-38].

Activated Sludge Model No. 1 (ASM1), which is generally accepted as state-of-the-art, presented by the IAWQ Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment Processes is capable of simulating organic matter and nitrogen removal in different activated sludge processes [35,38].

In addition to ASM1, Activated Sludge Model No. 3 (ASM3) includes storage of organic substrates as a new process. ASM3 proposed that all readily biodegradable substrate is firstly stored inside the cells and then storage products are reused for growth without addition of external substrate; however, biochemical modelling approaches accepted that growth and storage occur simultaneously [38,39]. Composition of biomass both in ASM3 and biochemical approach is shown in Figure 2.7.

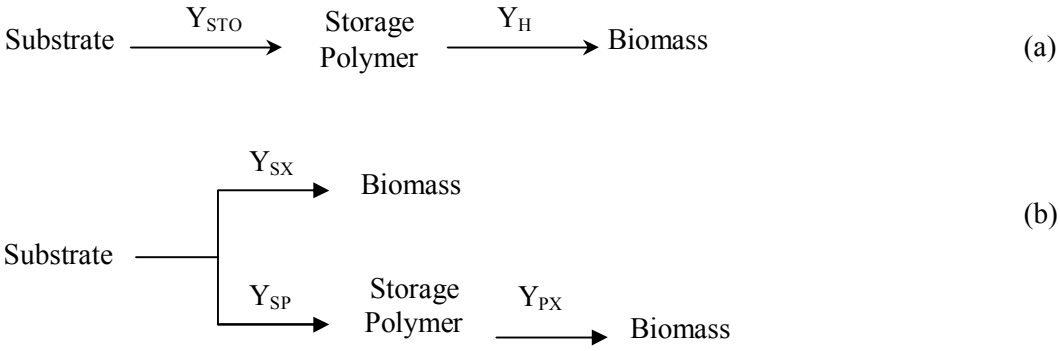


Figure 2.7 : (a) Composition of biomass in ASM3 (b) Composition of biomass in biochemical approach.

In case of simultaneous growth and storage, there should be three different yield coefficients that are the yield for growth of biomass on substrate (Y_{SX}), the yield of storage on substrate (Y_{SP}) and the yield of growth on the stored substrate (Y_{PX}). If there is no simultaneous storage and growth, it is not necessary to define the yield for growth on external substrate [38].

2.5.1 Activated Sludge Model No.1

Organik carbon removal can be modeled by using ASM1 and ASM3, which involve different processes. In ASM1, biomass is considered to grow solely on the external substrate present and the oxygen consumption after the external substrate depletion is explained with decay process. However, ASM1 could not explain the "tail" part of OUR profile that occurs during biodegradation of readily biodegradable substrates in batch experiments [36,37].

In 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 nonbiodegradable 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 [36].

Growth, decay and hydrolysis are basic processes which are involved in ASM1. According to the model, carbon removal is slightly coupled with nitrification.

Heterotrophs utilize organic matter directly or after hydrolysis process whereas autotropic bacteria utilize ammonia for their growth. Decay of organisms results in particulate matter formation which in turn can be utilized for growth following hydrolysis [39].

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, and 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 growth on ammonia nitrogen (S_{NH}). The biomass is lost via the decay process and converted to some other particulate components [39].

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 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}) [36].

The decrease of biomass can be given as **(2.37)** [40]:

$$\frac{dX}{dt} = \frac{dX_H}{dt} + \frac{dX_P}{dt} \qquad \textbf{(2.37)}$$

b_H is defined as the endogenous decay coefficient. The change in active biomass is expressed as **(2.38)**:

$$\frac{dX_H}{dt} = -b_H X_H \qquad \textbf{(2.38)}$$

Generation rate of particulate inert products are given in Equation **(2.39)**:

$$\frac{dX_P}{dt} = -f_{EX} X_H \qquad \textbf{(2.39)}$$

When the maximum growth rate of heterotrophs and half saturation constant of substrate are defined as μ_H and K_S respectively, biodegradation rate of S_S which is directly used in growth is given in Equation **(2.40)**:

$$\frac{dS_S}{dt} = \frac{\mu_H}{Y_H} \frac{S_S}{(K_S + S_S)} X_H \quad \textbf{(2.40)}$$

The decay associated soluble inert product formation rate can be given in Equation **(2.41)**:

$$\frac{dS_P}{dt} = f_{ES} b_H X_H \quad \textbf{(2.41)}$$

Where K_X and k_h are maximum specific hydrolysis rate and half saturation coefficient for hydrolysis of slowly biodegradable substrate, hydrolysis of this fraction to S_S is given in Equation **(2.42)**:

$$\frac{dX_S}{dt} = k_h \frac{X_S}{(K_X + X_S / X_H)} X_H \quad \textbf{(2.42)}$$

2.5.2 Activated Sludge Model No.3

ASM3 has been proposed by IAWQ Task Group to establish the new concept in activated sludge modeling. While components and processes are similar to other activated sludge models, ASM3 includes storage process as well as products, and attains to estimate the oxygen consumption, sludge production, nitrification, and denitrification of activated sludge systems. In addition, ASM3 contains nitrogen and alkalinity limitation for growth of microorganisms with description of ammonification kinetics by assuming constant N and COD ratio [41].

The hydrolysis process has been very important in predicting the electron acceptor under aerobic and anoxic conditions. Whereas the quantification of the kinetic parameters for hydrolysis process was difficult, ASM3 simplified hydrolysis process by only one process independent of the electron acceptor. The model has also included the differences in decay rates of nitrifiers under aerobic and anoxic conditions [41].

2.5.2.1 Components and processes of the model

ASM3 integrated seven soluble and six particulate components to characterize the wastewater and activated sludge. Soluble and particulate components were distinguished by 0.45 μm membrane filtration [41].

The first component is dissolved oxygen (So), which can be directly measured and is subject to gas exchange. S_I is a part of influent and may be produced by the hydrolysis of X_S. S_S is first taken up by heterotrophic organisms and stored as internal storage products (X_{STO}), then used for growth, according to ASM3. S_S and S_I are approximately equal to the total soluble COD determined by filtration from 0.45 μm membrane filters. Ammonium plus ammonia nitrogen (S_{NH}), dinitrogen (S_{N2}) and nitrite plus nitrate nitrogen (S_{NO}) is given as fractions of nitrogen. Alkalinity of the wastewater (S_{ALK}) is used to approximate the conservation of ionic charge in biological reactions [41].

X_I is a part of influent and may be produced as a result of biomass decay. Bacteria cannot directly use the colloidal and particulate organic substrates, because they have high molecular weights. Therefore, X_S must undergo hydrolysis before it is used by the biomass. The products of the hydrolysis are assumed to be readily biodegradable or soluble inert substrate. It must be reminded that all X_S is contained in the influent and not involved in the filtrate of 0.45 μm membrane filters in ASM3. X_H may grow both aerobically and anoxically. Hydrolysis does not consume any electron acceptor in ASM3. Active biomass is responsible for the hydrolysis of X_S and storage process. X_{STO}, which is only defined as a functional component required for modeling but is not directly identifiable chemically consists of poly-β-hydroxylalkanoate (PHA), glycogen etc. X_A oxidize the ammonium directly to nitrate, which is not considered in ASM3. Last component considered in model is the total suspended solids (X_{TS}) that may be used for the modeling of volatile suspended solids (VSS) by some special ratios. There are twelve processes taken into account in ASM3 [41].

1. Hydrolysis: All of the slowly biodegradable substrates in the influent is converted to available soluble substrates. This process differs from ASM1 by being independent from electron acceptor.
2. Aerobic storage of readily biodegradable substrate: All readily biodegradable substrate is assumed to be stored as X_{STO}, and then, ready to be used for the

growth of biomass. This conversion requires energy produced by aerobic respiration in the form of ATP.

3. Anoxic storage of readily biodegradable substrate: This process is similar to aerobic storage, but energy requirement of this process is supplied by anoxic respiration. Due to less amount of denitrifiers, the process rate is naturally smaller than aerobic storage rate.
4. Aerobic growth of heterotrophs: X_{STO} are assumed to be used as substrate for the growth of heterotrophs.
5. Anoxic growth of heterotrophs: This process is identical with aerobic growth, but it is based on anoxic respiration.
6. Aerobic endogenous respiration: This process, which is explained as all forms of biomass loss and energy requirements being not related to growth consists of decay, maintenance, endogenous respiration, lysis, predation, motility and death.
7. Anoxic endogenous respiration: This process is similar to aerobic endogenous respiration, but it occurs at a slower rate. Especially protozoa predation is considerably less active under anoxic conditions than under aerobic conditions.
8. Aerobic respiration of storage products: X_{STO} decay together with biomass like endogenous respiration.
9. Anoxic respiration of storage products: Denitrifying conditions are applied for the similar process of aerobic respiration of X_{STO}.
10. Aerobic growth of autotrophs: Nitrifiers oxidize ammonium directly to nitrate; nitrite, as an intermediate component, is not considered.
11. Aerobic endogenous respiration for autotrophs: Apart from description for nitrifiers, it takes place similar to aerobic endogenous respiration.
12. Anoxic endogenous respiration for autotrophs: It is similar to aerobic endogenous respiration for autotrophs, but it occurs at a slower rate.

2.5.2.2 Process stoichiometry

The net (true) yield of X_H produced per unit of S_S removed in ASM3 is found by Equations **(2.43)**:

$$Y_{net,O2} = Y_{STO,O2}.Y_{H,O2} \quad \textbf{(2.43)}$$

All stoichiometric parameters of ASM3 are identified together with their units and a typical value in Table 2.3 [41].

Table 2.3 : Typical stoichiometric and composition parameters for ASM3.

Symbol	Characterization	Value	Units	
f_{SI}	Production of S_I in hydrolysis	0	g COD_{SI}/(g COD_{XS})	
f_{XI}	Production of X_I in endogenous respiration	0.2	g COD_{XI}/(g COD_{XBM})	
$Y_{STO,O2}$	Aerobic yield of stored product per S_S	0.85	g COD_{XSTO}/(g COD_{SS})	The values below are suggested if XSS is used to model VSS rather than SS:
$Y_{H,O2}$	Aerobic yield of heterotrophic biomass	0.63	g COD_{XH}/(g COD_{XSTO})	
$i_{SS,XI}$	SS to COD ratio for X_I	0.75	g SS/(g COD_{XI})	0.75 g VSS/(g COD_{XI})
$i_{SS,XS}$	SS to COD ratio for X_S	0.75	g SS/(g COD_{XS})	0.75 g VSS/(g COD_{XS})
$i_{SS,BM}$	SS to COD ratio for biomass, X_H, X_A	0.9	g SS/(g COD_{XBM})	0.75 g VSS/(g COD_{XBM})

2.5.2.3 Process kinetics

In relation to removal of all soluble compounds, the kinetic expressions of ASM3 are relied on switching functions, which are hyperbolic or saturation terms, Monod equations, S/(K+S). Similarly, the switching functions are affected by particulate compounds with the ratio of X_{STO}/X_H and X_S/X_H. All kinetic parameters of ASM3 are given in Table 2.4. The units and typical values at 10°C and 20°C for these kinetic parameters are identified in Table 2.5 [41].

Interpolation of kinetic parameters k for different temperatures is proposed with Equation **(2.44)**:

$$k(T) = k(20°C).\exp\left(\theta_T.(T - 20°C)\right) \quad \textbf{(2.44)}$$

where θ_T (in °C) may be found from **(2.45)**:

$$\theta_T = \frac{\ln\left(k(T_1)/k(T_2)\right)}{T_1 - T_2} \quad \textbf{(2.45)}$$

Table 2.4 : Kinetic rate expressions ρ_j for ASM3 All $\rho_j \geq 0$.

j	Process	Process rate equation ρ_j, for all $\rho_j \geq 0$
1	Hydrolysis	$k_H \cdot \frac{X_S / X_H}{K_X + X_S / X_H} . X_H$
Heterotrophic organisms, aerobic and denitrification		
2	Aerobic storage of COD	$k_{STO} \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_S}{K_S + S_S} . X_H$
3	Aerobic growth	$\mu_H \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \cdot \frac{X_{STO} / X_H}{K_{STO} + X_{STO} / X_H} . X_H$
4	Aerobic endogenous respiration	$b_{H,O2} \cdot \frac{S_O}{K_O + S_O} . X_H$
5	Aerobic respiration of PHA	$b_{STO,O2} \cdot \frac{S_O}{K_O + S_O} . X_{STO}$

According to ASM3, readily biodegradable substrate (S_S) is best determined from bioassay in wastewater. Kinetics of the storage and growth processes are related to rapid uptake of oxygen after addition of wastewater to biomass. Therefore, the yield of storage process must be used to relate oxygen uptake to substrate consumption. This can be simulated with batch experiment for estimation of S_S in ASM3 by Equation **(2.46)**:

$$S_S(batch) = \int \Delta r_{S_S} . dt = \frac{\upsilon_{S_S}}{\upsilon_{SO_2}} \int \Delta r_{SO_S} . dt = \int \frac{\Delta r_{SO_2} . dt}{1 - Y_{O_2,STO}} \qquad \textbf{(2.46)}$$

Table 2.5 : Typical values of kinetic parameters for ASM3.

Symbol	Characterization	Temperature 10°C	Temperature 20°C	Units
k_H	Hydrolysis rate constant	2	3	g COD_{XS}/(g.COD_{XH}).d
K_X	Hydrolysis saturation constant	1	1	g.COD_{XS}/(g.COD_{XH})
Heterotrophic organisms X_H, *aerobic and denitrifying activity*				
k_{STO}	Storage rate constant	2.5	5	g COD_{SS}/(g.COD_{XH}).d
K_{O2}	Saturation constant for S_{NO2}	0.2	0.2	g O_2/m^3
K_S	Saturation constant for substrate S_S	2	2	g COD_{SS}/m^3
K_{STO}	Saturation constant for X_{STO}	1	1	g.COD_{XSTO}/(g.COD_{XH})
μ_H	Heterotrophic maximum growth rate of X_H	1	2	d^{-1}
$b_{H,O2}$	Aerobic endogenous respiration rate of X_H	0.1	0.2	d^{-1}
$b_{STO,O2}$	Aerobic respiration rate for X_{STO}	0.1	0.2	d^{-1}

2.5.3 Metabolic models

A metabolic model is based on the principle that the metabolism of organism is composed of a limited number of metabolic pathways which results in more or less constant energy requirement and ultimately constant stoichiometry [42]. Metabolic pathways are described with internal reactions that are combined with reaction stoichiometry and degree of reduction balances to derive the linear equation for description of involved metabolism.

Since biological wastewater treatment processes generally occur under dynamic conditions, microorganisms have experience rapidly changing conditions of availability of nutrients. The response of microorganisms to feast/famine conditions is regularly the accumulation of storage polymers like PHA and glycogen. A metabolic model was proposed that as a carbon source, acetate is aerobically stored as PHB under dynamic conditions. This metabolic model reduces the number of unknown parameters and describes the observed kinetics of PHB formation and consumption by selected microorganism [42].

The substrate uptake rate will be larger than required for growth when carbon source is given periodically. It is observed that the fast uptake of substrate results in the formation of $NADH_2$ which is consumed by oxidative phosphorylation and leading to ATP formation. If the energy requirement for growth is limited, ATP will accumulate by resulting in $NADH_2$ accumulation in turn. This explains that the production of a more reduced storage polymer PHB (requires $NADH_2$) is more likely to be compared with the production of glycogen (leads to $NADH_2$ formation) [42].

In this metabolic model, acetate limited continuous culture of *Paracoccus pantotrophus sp.* was used in order to observe the metabolism of a microorganisms capable of producing and consuming which was described by seven internal reaction in Figure 2.8. Biomass is assumed to contain two different parts as an active biomass compartment ($1-f_{PHB}$ = capable of reproduction and growth) and PHB fraction (f_{PHB} = used as storage for carbon and energy) [42].

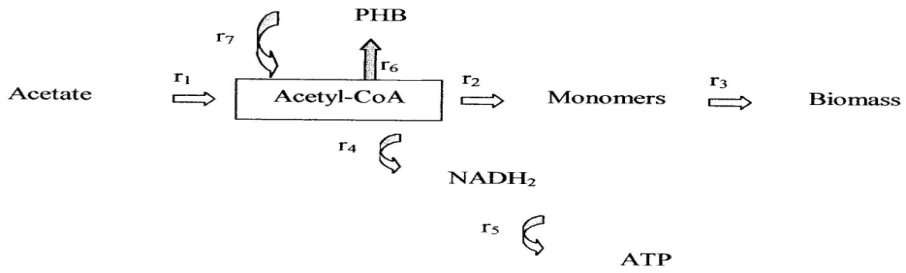


Figure 2.8 : Schematic representation of the metabolism of an organism capable of forming and consuming PHB [42].

Synthesis of Acetyl-CoA from Acetate, (r_1): Acetate is taken into cell by active transport, Acetyl-P is formed and at last, conversion to Acetyl-CoA is completed.

Synthesis of biomass monomers from Acetyl-CoA, (r_2): This is the first part of the synthesis of active biomass.

Polymerization of biomass precursors and maintenance, (r_3): The biomass precursors are polymerized into active biomass and thus, biomass formation is completed.

Carbon source catabolism, (r_4)

Oxidative phosphorylation, (r_5): ATP is produced from $NADH_2$.

Synthesis of the storage product PHB from Acetyl-CoA, (r_6): The substrate is used for the synthesis of both biomass and PHB.

Synthesis of Acetyl-CoA from PHB, (r_7): In the absence of acetate, the microorganisms utilize the intracellular accumulated PHB as carbon and energy source. The storage polymer is hydrolyzed and converted into Acetyl-CoA [42].

The metabolic model results in two equations describing the conversion processes for feast and famine phases. For the feast period, the linear equation describing acetate uptake, biomass growth, PHB production and maintenance is defined as in Equation **(2.47)** [42]:

$$(-r_S) = \frac{1}{Y_{SX}^{\max}} r_X + \frac{1}{Y_{SP}^{\max}} r_P + m_S C_X \qquad \textbf{(2.47)}$$

where,

$$Y_{SX}^{\max} = \frac{4\delta - 2}{4.13\delta + 4.32} \qquad \textbf{(2.48)}$$

$$Y_{SP}^{\max} = \frac{4\delta - 2}{4.5\delta} \quad \textbf{(2.49)}$$

$$m_S = \frac{m_{ATP}}{2\delta - 1} \quad \textbf{(2.50)}$$

Similarly, PHB consumption, biomass growth, and maintenance are expressed with Equation **(2.51)** for the famine period:

$$(-r_P) = \frac{1}{Y_{PX}^{\max}} r_X + m_P C_X \quad \textbf{(2.51)}$$

where,

$$Y_{PX}^{\max} = \frac{4.5\delta - 0.5}{4.13\delta + 4.32} \quad \textbf{(2.52)}$$

$$m_P = \frac{m_{ATP}}{4.5\delta - 0.5} \quad \textbf{(2.53)}$$

The linear equations contain two unknown parameters namely, the ratio between ATP produced and electrons transferred from $NADH_2$ to an electron acceptor (δ) and the ATP consumption due to maintenance processes (m_{ATP}). Substition of $r_X=\mu.C_X$, $r_S=q_S.C_X$, and $r_P=q_P.C_X$ followed by division by the biomass concentration, C_X gives the following Equation **(2.54)**:

$$(-q_S) = \frac{1}{Y_{SX}^{\max}} \mu + \frac{1}{Y_{SP}^{\max}} q_P + m_S \quad \textbf{(2.54)}$$

From earlier experiments, it was observed that maximum yield of biomass on acetate, $Y_{SX}^{max}=0.45$ (C-mol/C-mol) and maintenance coefficient for growth on acetate $m_S=0.038$ (C-mol/C-mol.h) [43]. The P/O ratio, $\delta=1.84$ (mol ATP/mol $NADH_2$) and the ATP maintenance coefficient, $m_{ATP}=0.102$ (mol ATP/C-mol.h) were calculated by using Equations **(2.48)** and **(2.50)**. Maximum yield of PHB on acetate was found as $Y_{SP}^{max}=0.648$ (C-mol/C-mol) by using Equation **(2.49)**. Substituting these yield coefficients in Equation **(2.54)** gives Equation **(2.55)**:

$$-q_S = 2.22\mu. + l.544 q_P + 0.038 \quad \textbf{(2.55)}$$

From the carbon balance and the degree of reduction balance relations for the specific carbon dioxide production rate (q_C) and for the specific oxygen consumption rate (q_O) can be derived as a function of μ and q_P in Equations **(2.56)** and **(2.57)**:

$$q_C = (-q_S) - \mu - q_P = 1.22\mu + 0.544q_P + 0.038 \quad \textbf{(2.56)}$$

$$(-q_O) = 1.188\mu + 0.419q_P + 0.038 \quad \textbf{(2.57)}$$

Stoichiometry of the kinetic model for the PHB production can be based upon Equations **(2.55)-(2.57)**.

Maximum yield of biomass on PHB, Y_{PX}^{max} =0.653 [C-mol/C-mol] and maintenance coefficient for growth on PHB, m_P=0.0131 [C-mol/C-mol.h] were calculated for the famine phase. The linear Equaiton **(2.58)** describes PHB consumption and growth:

$$(-q_P) = \frac{1}{Y_{PX}^{\max}}\mu + m_P \quad \textbf{(2.58)}$$

$$-q_P = 1.532\mu + 0.0131 \quad \textbf{(2.59)}$$

From the carbon balance and the degree of reduction balance relations for the specific carbon dioxide production rate (q_C) and for the specific oxygen consumption rate (q_O) can be derived in Equations **(2.60)** and **(2.61)**:

$$q_C = 0.532\mu + 0.0131 \quad \textbf{(2.60)}$$

$$(-q_O) = 0.693\mu + 0.0147 \quad \textbf{(2.61)}$$

Stoichiometry of the kinetic model for the PHB consumption can be based upon Equations **(2.59)-(2.61)**.

It was observed from experimental and modelling results that, the substrate uptake rate reaches its maximum value immediately after the pulse addition. However, the growth rate is influenced only by maximal growth rate, and slowly decreases during growth on PHB presumably being related to the reduction in PHB amount [42]. In these cases, the storage polymers act as a buffer for the substrate that is taken up but not directly used for growth. The more the microorganisms are able to store during the feast period and subsequently use it for growth, the more they have a competitive

advantage [42,44]. Therefore, for WWTPs it can be discussed that under dynamic conditions microorganisms accumulate more substrate and outcompete organisms that do have lower substrate uptake rates.

Another metabolic model for PHB metabolism in aerobic, slow growing activated sludge culture has been proposed for definition of relation between the PHB storage and activated sludge models. The metabolic model is the same as described by van Aalst-van Leeuwen *et al*., (1997) with the exception of biomass composition. It is found that bacteria can balance their growth rate with respect to dynamic substrate feeding, using the PHB metabolism and regulate the substrate uptake rate. Therefore, bacteria can compete effectively for substrate. While acetate is taken up with maximum specific substrate uptake rate, which is dependent on the SRT, PHB is produced with a constant rate in the feast period. The difference in specific growth rate between the feast and famine period reduces with increasing SRT. Biomass growth on PHB leads to only 4-10% lower biomass yield than the direct utilization of acetate for growth. On the other hand, PHB synthesis process consumes 66 to almost 100% of the acetate by increasing the COD need compared to a situation without storage, the rest of the acetate is used for growth and maintenance [45].

3 MATERIALS AND METHODS

3.1 Experimental Approach

In order to assess the biological treatability of the chosen antibiotics and verify the change in the kinetic parameters, such as growth rate, a laboratory scale batch reactor was operated under aerobic condition. The system had a total reactor volume, V_T, of 10 l, where 5 l functioned as the stationary volume, V_0, holding settled biomass and the remaining 5 l as the fill volume, V_F, corresponding to a V_0/V_F ratio of 1.0. It was started using the activated sludge taken from a domestic WWTP as inoculum. Firstly, microbial culture was acclimated in the fill and draw reactor at a sludge retention time of 10 d with readily biodegradable substrate. The acetate was chosen as sole readily biodegradable carbon source.

After the acclimation of biomass to acetate as a sole carbon source, fate and effect of the SMX on activated sludge was investigated. The acclimated microbial community taken from the reactor was primarily used as the biomass seed for acute inhibition studies in respirometric experiments. All the experiments involved a number of batch reactors initially inoculated with the same microbial culture from the main reactor and started with and without antibiotic additions (control).

The impact of the selected antibiotic at different concentrations was evaluated with the respirometric tests for generating the OUR profiles corresponding to selected initial conditions. To inhibit nitrification, 1.04 g Allyl Thio Urea (ATU) was added to the batch reactor of the respirometer. In the acute inhibition studies, four runs were applied for SMX as 25 mg/l, 50 mg/l, 100 mg/l and 200 mg/l.

As a second step, after the acute tests were completed, an antibiotic reactor was set up in addition to the control reactor (Figure 3.1). In order to determine the effect of continuous exposure to antibiotic SMX, the chronic inhibition studies were carried out by feeding the mixed culture with combination of the acetate and the antibiotic compound. During the chronic inhibition studies, the influent SMX concentration was applied as 50 mg/l.

Figure 3.1 : Picture of the SBRs, both control (right) and antibiotic (left).

3.2 Reactor Operation

The batch reactor were operated until it reached steady state conditions, which was monitored by suspended solids (SS), volatile suspended solids (VSS) and COD parameters. At the steady state conditions, VSS concentration was kept in the range of 1500-1700 mg VSS/l. The sodium acetate was used as synthetic substrate and the initial carbon concentration was kept as 400 mg COD/l. By the way, the substrate solution was prepared to contain in one litter; 95 g of sodium acetate. Besides the organic carbon source, all other macro and micro nutrients were supplied for the nutritional needs of the cells and to provide enough buffer capacity to the reactor so that pH is kept at a range of 6.5–7.5. The composition of the nutrients fed to the system is given Table **3.1** [46].

Table 3.1 : Composition of sodium acetate solution, solution A and solution B.

Compound	Feed Concentration (g/L)
Acetate solution	
CH_3COONa	95
Solution A	
KH_2PO_4	160
K_2HPO_4	320
NH_4Cl	120
Solution B	
$MgSO_4.7H_2O$	15
$FeSO_4.7H_2O$	0.5
$ZnSO_4.7H_2O$	0.5
$MnSO_4.H_2O$	0.41
$CaCl_2.2H_2O$	2.65

During each daily feeding period, reactor was settled for 0.5 h (t_s) and decanted until 5 l (V_0). Aeration was continuously provided and the oxygen concentration in the reactor was kept at minimum of 2 mg/l to maintain aerobic conditions. Furthermore, the temperature of system was kept at around 20± 1°C.

3.3 Analytical Procedures

The SBR was monitored and controlled with regular measurements of SS, VSS, COD and PHA. All analyses were conducted in duplicate. Since the formation of PHB from the central metabolite acetyl-CoA, is the main storage polymer, PHA measurements mainly consisted of PHB, and therefore the results of experiments in fact represent PHB as storage compound.

In the experiments, pH was kept in the range of 6.5–7.5, suitable for biological activity. pH measurements were performed by an Orion 520 Aplus pH meter. Temperature of all experiments was maintained at 20±1°C. Samples, which were taken for soluble COD and antibiotic, were filtered through Millipore membrane filters with a pore size of 0.45 μm to separate the bacterial cells from the liquid. PHB samples were taken from complete mixing via clipped pipette. COD samples were preserved with sulfiric acid (H_2SO_4), PHB samples with sodium hypochlorite (NaClO) solution and antibiotic samples without any addition.

COD measurements were performed as described in the method proposed by ISO6060 [47]. The Millipore AP40 glass fiber filters were used for SS and VSS measurements that were performed as defined in Standard Methods [48].

In order to determine the storage properties of the activated sludge, PHB amounts in the sludge were assessed according to gas chromatographic (GC) method, which is characterized by high accuracy and excellent reproducibility, permitting determinations as low as 10-5 g/l. The basis of the method is to depolymerise and dehydrate PHB by H_2SO_4. PHB samples were taken 8 ml into centrifuge tubes containing 2 ml NaClO solution for preventing the biological activity. The tubes were centrifuged 30 min at 5000 rpm and after discarding supernatant, the centrifuged samples were suspended in a mixture of 2 ml methanol acidified with H_2SO_4 (3% v/v). The retained biomass pellets were put into glass tubes by adding 2 ml of chloroform to each. The mixture in the tubes were subject to extraction and

hydrolization at 100°C for 4h and subsequently cooled back to room temperature. After cooling, 1 ml of deionised water was added to each sample and the samples were shaken vigorously for 10 min. to remove free acids in resulting organic phase. The two phases were allowed to separate for 24 h. After phase separation, the organic phase was transferred directly into standard 2 ml vials and measured by Agilent 6890N GC. Within the linear range of the detector used on GC apparatus, the results of this method depend neither on the volume of the cell suspension nor on the PHB content of the cells; the maximum standard deviation was ± 0.5% [49].

3.4 Biodegradation Test

Since the main aim of this study was to enlighten the treatment mechanism and the effects of antibiotic compound on activated sludge systems, antibiotic concentrations were chosen higher than that of in domestic wastewaters. Determination of SMX was performed by using High Performance Liquid Chromatography (HPLC) at 280 nm.

Measurement methodology for antibiotic compounds has 3 steps, which were sampling, sample preparation and preservation (filtering, pH adjustment and prevention of photo-degradation). For the measurement in water phase, the SMX samples filtered through 0.45 μm Milipore membrane filters were transferred to HPLC vials.

The adsorped part of SMX onto activated sludge was also investigated. Separation of the antibiotic from the activated sludge on which it may be absorbed was applied by ultrasonication process via an ultrasonicator. Ultrasonication resulted in release of SMX residues to the liquid phase from which SMX samples were collected. Then, the samples were centrifuged for 10 min at 5000 rpm. The supernatant was filtered through 0.45 μm Milipore membrane filters and analyzed by HPLC. For specific SMX analyses, Novapak C18 column was used with HPLC. Mobile phase composition was 30:70 v/v methanol-water mixture [50]. Mobile phase was acidified with phosphoric acid (0.1%) at pH 2.5 and flowed with 1 ml/min. Injection volume was 40 μl. Calibration curve used for the determination of SMX with Diode Array Detector at 280 nm is given in Figure 3.2.

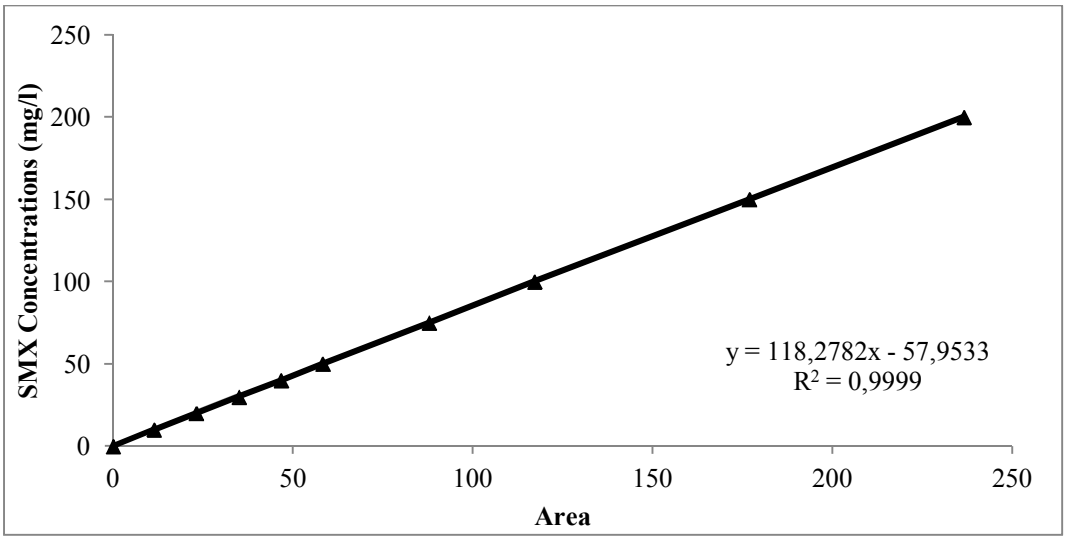


Figure 3.2 : Calibration curve for determination of SMX.

In the biodegration tests, acetate solution acclimated sludge was used. Organic loading rate (OLR) was kept as same with the main reactor throughout the respirometric analysis.

3.5 Respirometric Analysis

OUR equipment, also known as respirometer, can range from a basic system which requires manual data collection and calculation to more advanced commercial systems where calculations are automated. Regardless of the level of automation, the OUR equipment has essential components which include a test chamber to add aerated mixed liquor/substrate, a stirring mechanism, a dissolved oxygen (DO) probe, and a DO analyzer.

Respirometric tests were conducted to determine the degradation profile of the readily biodegradable substrate (acetate) and latter both acute and chronic inhibition effects of the chosen antibiotics on the control sludge. The respirometric tests were conducted with relevant acclimated biomass seeding alone to obtain endogenous OUR level of biomass. Samples with same OLR with main reactor were added to the batch reactor and the OUR data was monitored. For monitoring the OUR, part of the contents of the batch reactor (volume 400-500 ml) was sucked out to the airtight DO monitoring chamber. The DO decrease in the DO monitoring chamber was monitored with a pre-calibrated DO probe and the output was recorded. This operation was continuously performed to obtain the DO profile decreasing with time. The OUR was calculated by the measuring the slope of the DO decrease on the plot.

OUR measurements were performed with an Applitek RA-Combo-1000 continuous respirometer. In all respirometric experiments, twelve samples were taken with respect to time till the end of the experiment. Time durations and monitored data for experimental runs were given in Table 3.2.

Table 3.2 : Monitored data for experimental runs.

Time	pH	SS/VSS	COD filtered	SMX filtered	SMX in the sludge	PHB
-20 min	X	X	X	X	X	X
15 min			X	X		X
30 min			X	X		X
45 min			X	X		X
60 min			X	X		X
80 min			X	X		X
100 min			X	X		X
120 min			X	X		X
160 min			X	X		X
200 min			X	X		X
240 min			X	X		X
24 h			X	X	X	X

The summary of acute respirometric studies performed with SMX is given in Table 3.3. In these experiments, F/M ratio was again kept constant as in the antibiotic reactor, but both VSS and substrate concentrations were diluted by 1/2 ratio.

Table 3.3 : Influent conditions for acute respirometric analyses.

Set No.	Experiment	MLVSS (mg/l)	Initial acetate (mg COD/l)	Initial SMX (mg COD/l)	Initial SMX (mg SMX/l)
Set 1	Control	900	246	-	-
Set 2	SMX-25	1000	200	34	25
Set 3	SMX-50	800	200	69	50
Set 4	SMX-100	930	200	138	100
Set 5	SMX-200	900	200	276	200

The chronic effect of SMX on the treatment efficiency and bacterial behaviour was evaluated with performing the respirometric analysis throughout 30 d from the start up of the chronic acclimation to mixture of 70 mg COD/l of SMX and 200 mg COD/l of acetate. The influent concentrations of the chronic respirometric test were summarized in Table 3.4.

Table 3.4 : Influent conditions for chronic respirometric analyses.

Set No.	Day No.	Substrate type	MLVSS (mg/l)	Initial acetate (mg COD/l)	Initial SMX (mg COD/l)	Initial SMX (mg SMX/l)
Set 3	1	Acetate + Antibiotic	800	200	69	50
Set 6	5	Acetate + Antibiotic	880	200	69	50
Set 7.1	10	Acetate + Antibiotic	865	200	72	50
Set 7.2	10	Acetate	865	200	-	-
Set 7.3	10	Antibiotic	865	-	72	50
Set 8	15	Acetate + Antibiotic	820	200	72	50
Set 9.1	20	Acetate + Antibiotic	820	50	70	50
Set 9.2	20	Acetate	820	50	-	-
Set 9.3	20	Antibiotic	820	-	70	50
Set 10	25	Acetate + Antibiotic	800	50	71	50
Set 11.1	30	Acetate + Antibiotic	850	50	71	50
Set 11.2	30	Acetate	850	50	-	-
Set 11.3	30	Antibiotic	850	-	71	50

4 RESULTS AND DISCUSSION

4.1 Acclimation Studies of Control Reactor

Acclimation studies were run for each feeding pattern. For this purpose, total suspended solids (SS) and volatile suspended solids (VSS) content of biomass were measured. After the acclimation period, both SS and VSS concentrations became constant with time. The results of these measurements were given in Figure 4.1.

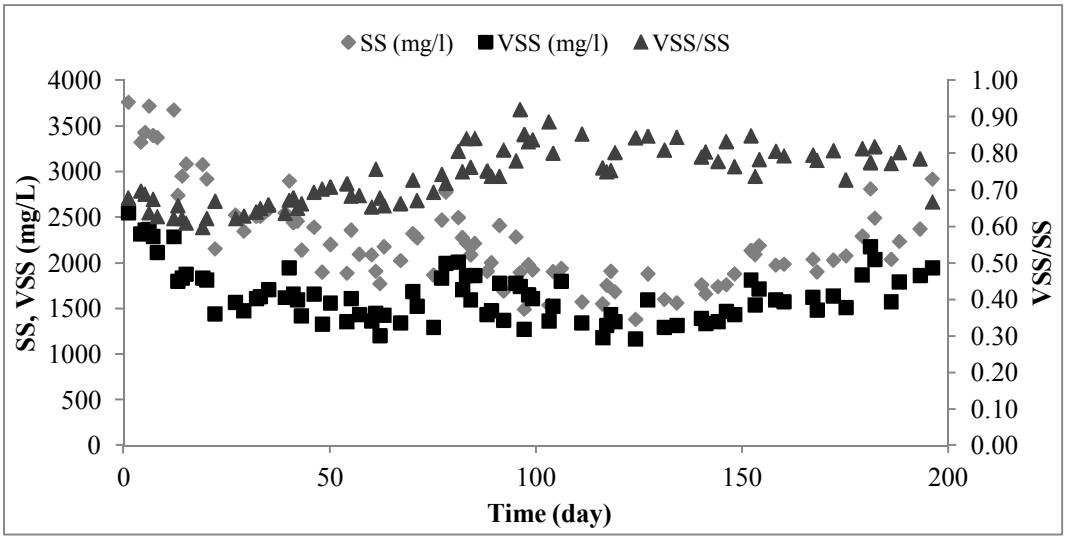


Figure 4.1 : SS, VSS results of the control reactor during the acclimation period.

SBR operation reached to steady state after 90 days, approximately 10 sludge ages. At the steady state condition, average SS and VSS concentrations were found as 2260 mg SS/l and 1650 mg VSS/l respectively. According to these results, 70-75% average VSS/SS ratio was found. Soluble COD effluent concentration during the acclimation period was given in Figure 4.2. When the steady state condition prevailed, effluent COD results became in the range of 30-45 mg COD/l.

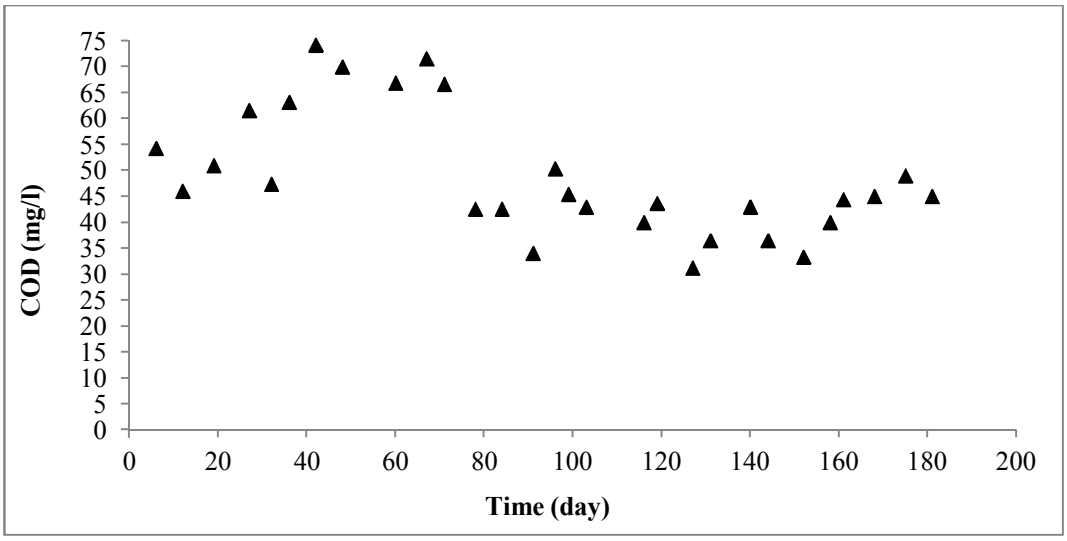


Figure 4.2 : Soluble COD effluents of the control reactor during the acclimation period.

4.2 Experimental Results

First the performance control reactor was determined during several cycles by using only acetate as substrate under aerobic conditions, and then, the batch tests were applied in order to investigate the acute and chronic inhibition effects of SMX. In these experiments, F/M ratio was kept constant, but both VSS and substrate concentrations were diluted by 1/2 ratio. Therefore, the acetate concentration was 200 mg COD/l and the VSS concentration was approximately 800-900 mg VSS/l in the experiments. OUR profile and soluble COD profile of the control reactor were given in Figure 4.3 and Figure 4.4, respectively.

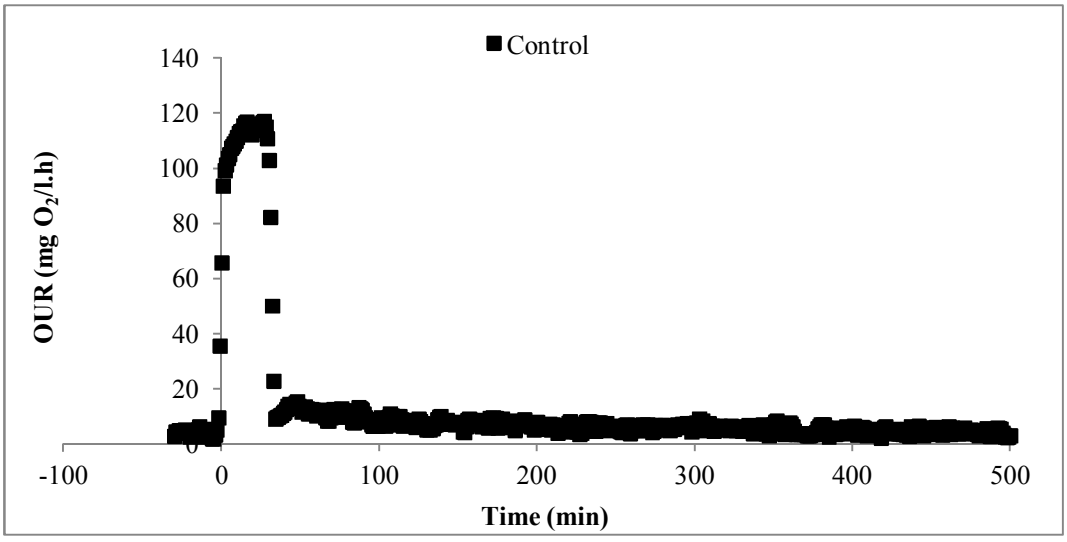


Figure 4.3 : OUR profile of the control experiment versus time (Set 1).

The shoulder on the OUR curve observed from the beginning of the famine period would normally be associated with utilization of storage products as substrate according to principles of ASM3 [24]. Since only acetate was added in this experiment, this shoulder was presumably due to the stored PHB. Total area under the OUR curve, which gives total dissolved oxygen concentration (DO_{Total}), was calculated as 218 mg O_2/l including a 156 mg O_2/l dissolved oxygen concentration used for endogenous respiration (DO_{Endo}).

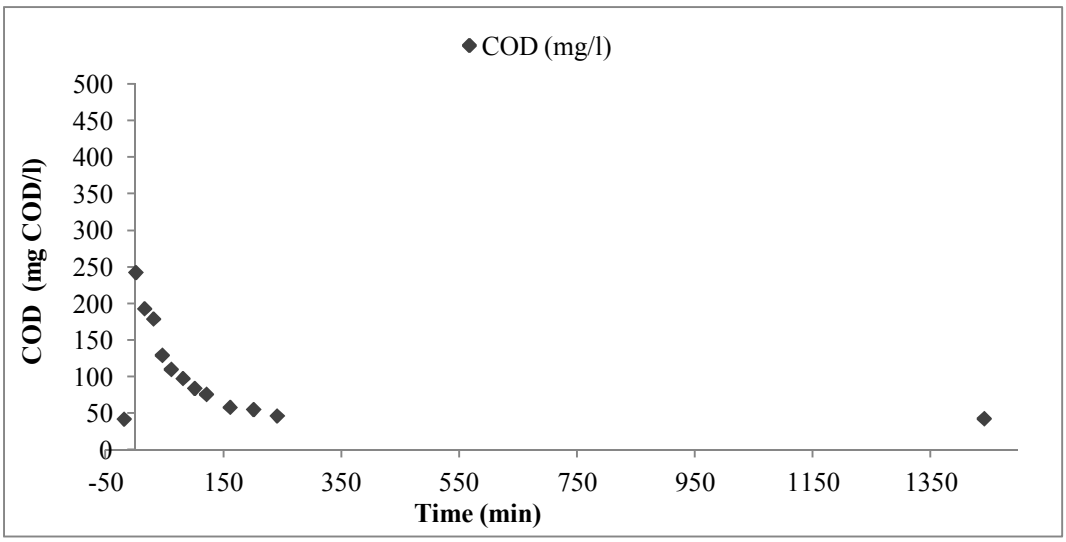


Figure 4.4 : Soluble COD profile of the control experiment versus time.

4.2.1 Acute experiments

The acute experiments conducted by pulse feeding of the SMX were done with sludge acclimated to instant acetate feeding in the beginning of each cycle, under aerobic conditions. Four different concentrations of SMX, which were 25 mg/l, 50 mg/l, 100 mg/l and 200 mg/l were applied to observe the acute inhibition effects of the antibiotic. OUR profile of the first acute experiment (Set 2) was given in Figure 4.5.

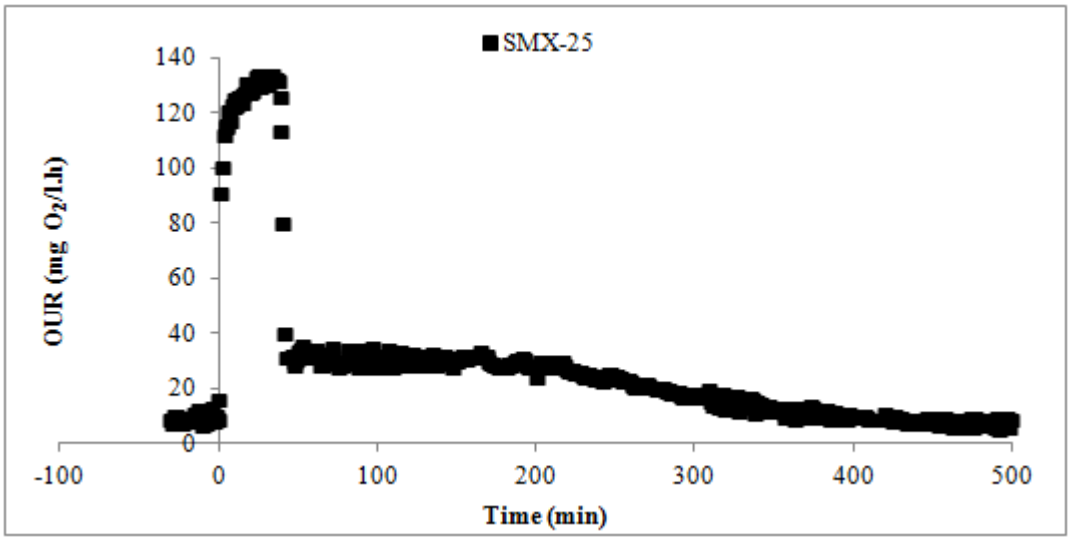


Figure 4.5 : OUR profile versus time (Set 2).

In this experiment, DO_{Total} and DO_{Endo} were calculated as 263 mg O_2/l and 108 mg O_2/l, respectively. Since there was not SMX removal in the acute experiments, the removal of acetate was also calculated by substracting the COD of SMX from total soluble COD. Soluble COD & SMX profiles of the acute experiment, SMX-25 were given in Figure 4.6.

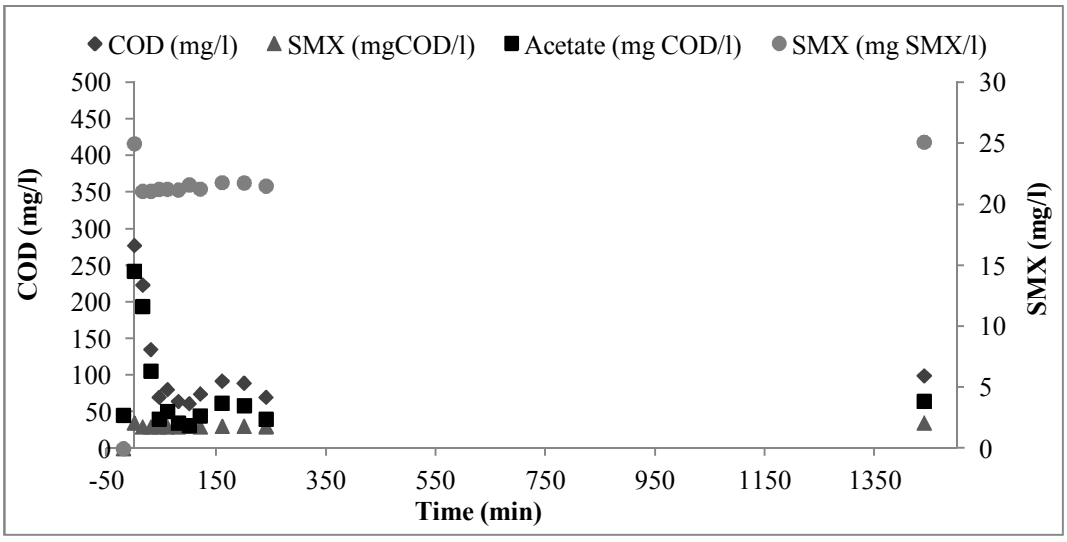


Figure 4.6 : Soluble COD & SMX profiles of Set 2 (SMX-25 mg/l).

OUR profile and soluble COD profile of the second acute experiment (Set 3) were given in Figure 4.7 and Figure 4.8, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 182 mg O_2/l and 96 mg O_2/l.

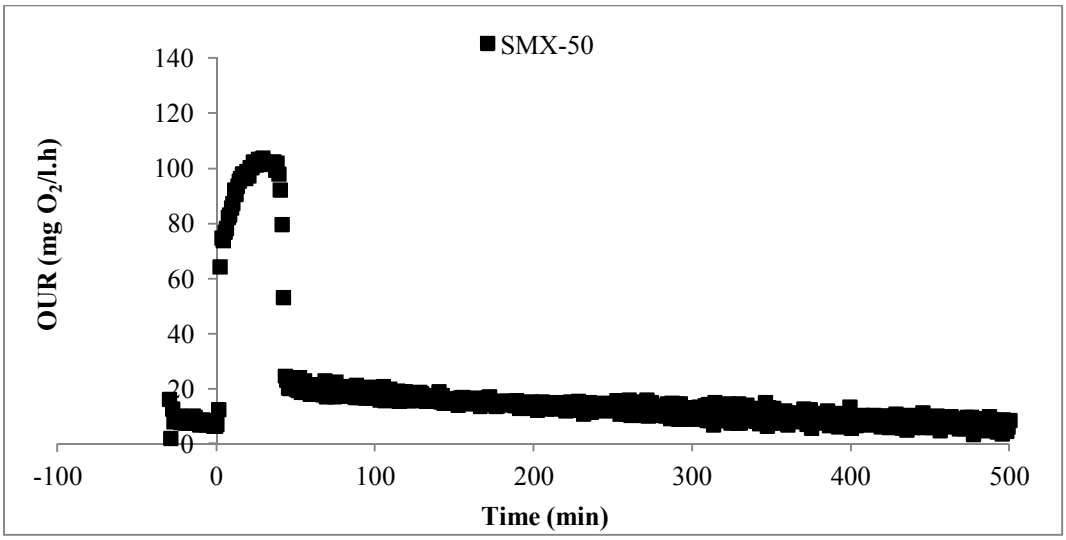


Figure 4.7 : OUR profile versus time (Set 3).

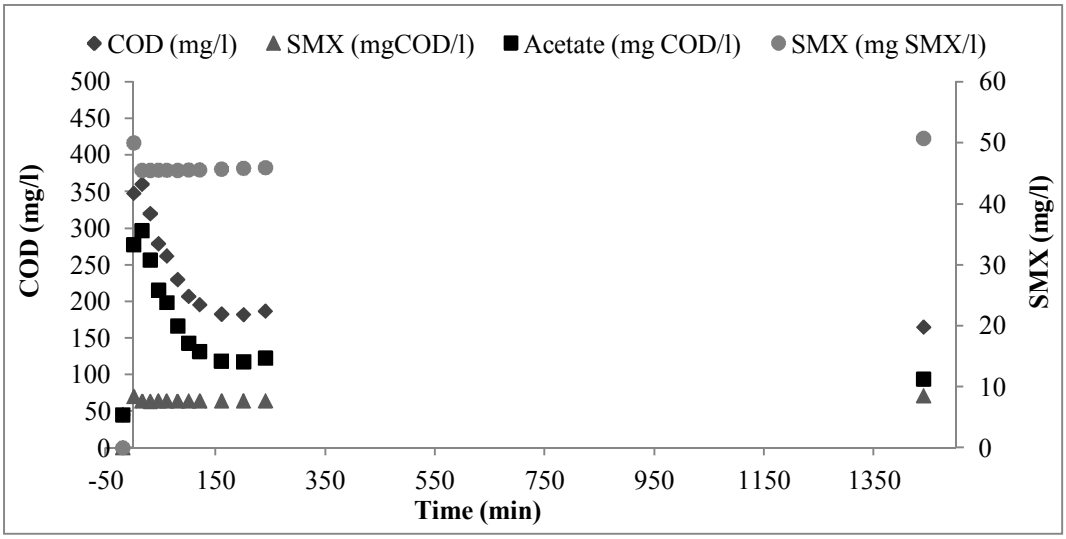


Figure 4.8 : Soluble COD & SMX profiles of Set 3 (SMX-50 mg/l).

OUR profile and soluble COD profile of the third acute experiment (Set 4) were given in Figure 4.9 and Figure 4.10, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 211 mg O_2/l and 84 mg O_2/l.

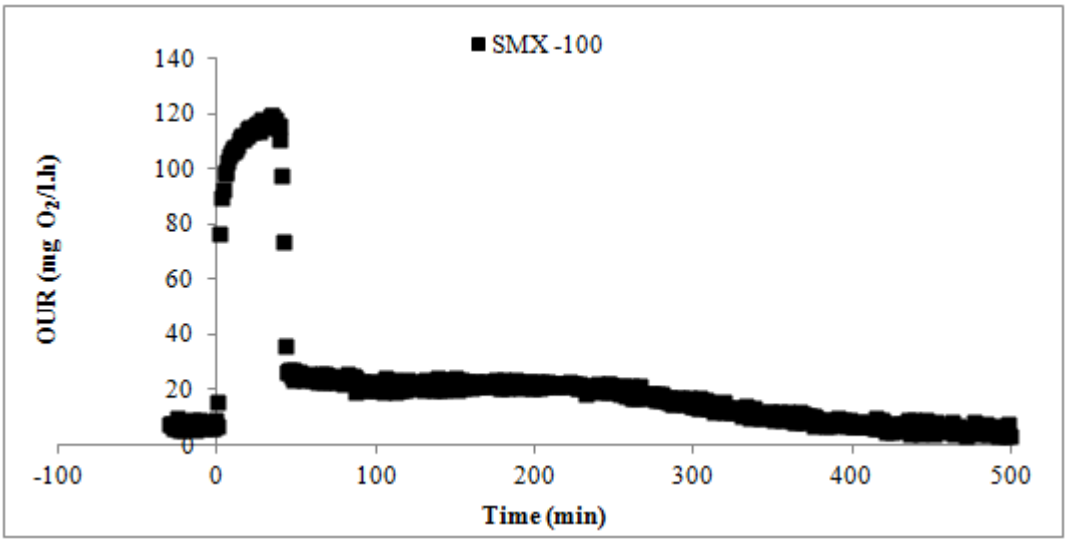


Figure 4.9 : OUR profile versus time (Set 4).

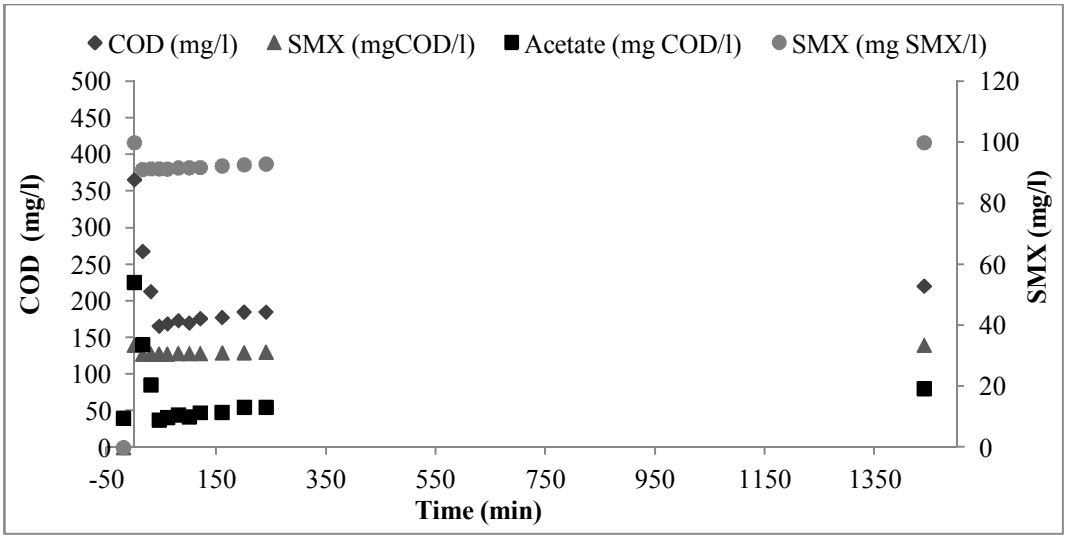


Figure 4.10 : Soluble COD & SMX profiles of Set 4 (SMX-100 mg/l).

OUR profile and soluble COD profile of the last acute experiment (Set 5) were given in Figure 4.11 and Figure 4.12, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 270 mg O_2/l and 149 mg O_2/l.

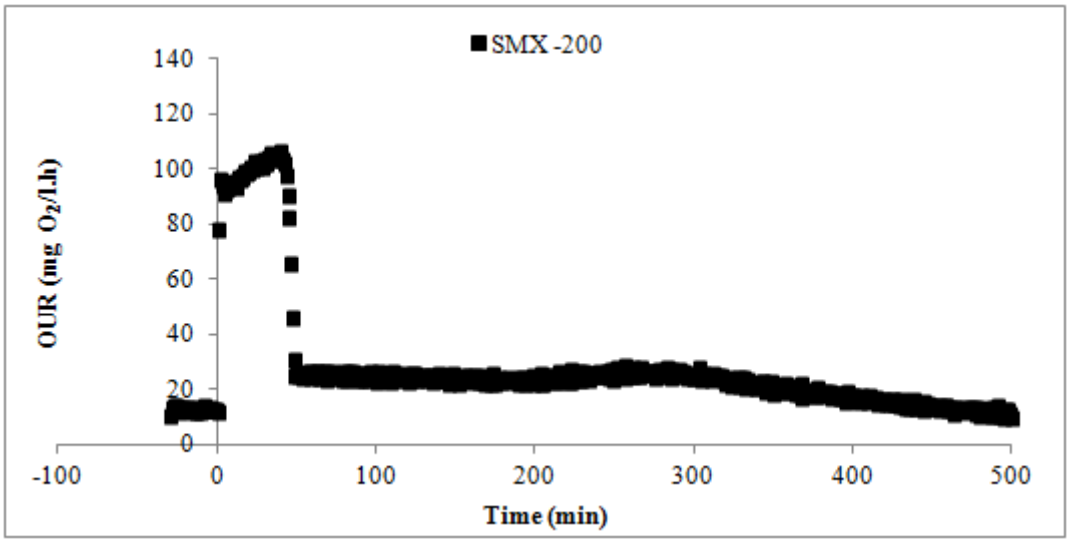


Figure 4.11 : OUR profile versus time (Set 5).

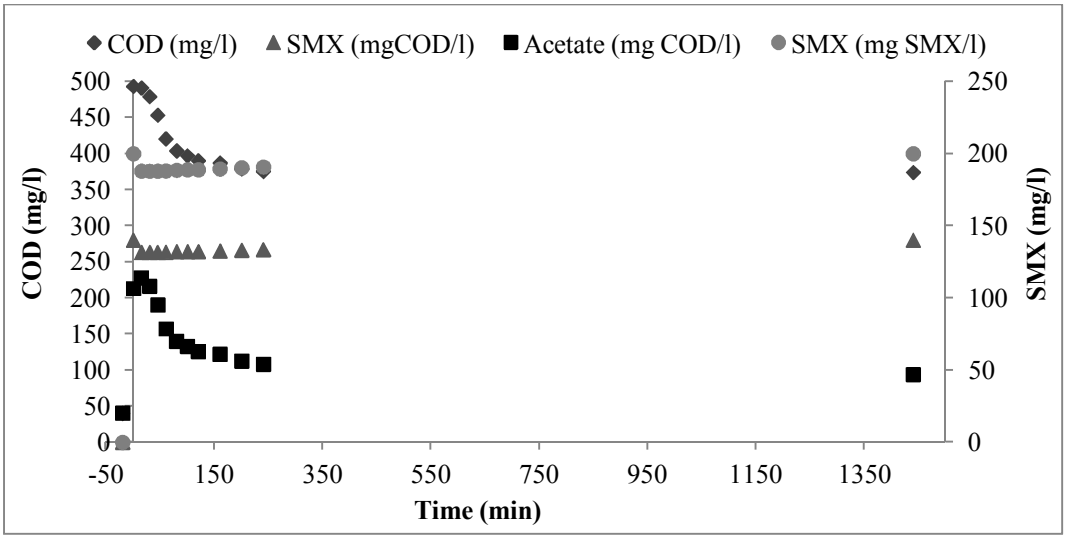


Figure 4.12 : Soluble COD & SMX profiles of Set 5 (SMX-200 mg/l).

Comparison of all acute experiments with respect to the OUR profiles was given in Figure 4.13. As shown in figure, the OUR curves decreased by increasing the given SMX concentration.

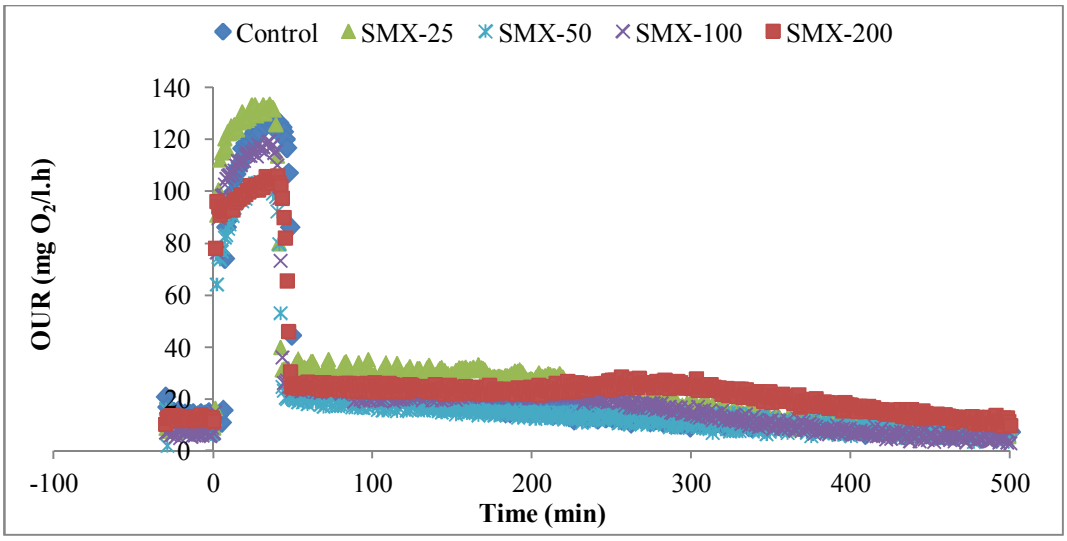


Figure 4.13 : Comparison of OUR profiles of all acute experiments with the control.

4.2.2 Chronic experiments

In order to investigate chronic inhibition effecs of the SMX, a second SBR was set up and fed by the antibiotic in addition with the acetate. A 50 mg SMX/l (approximately 70 mg COD/l) was given to the reactor with the same amount of acetate (400 mg COD/l). Performance of the antibiotic reactor was followed by monitoring SS and VSS concentrations as well as by taking COD and SMX samples regularly. The results of these measurements were given in Figure 4.14 and Figure 4.15, respectively.

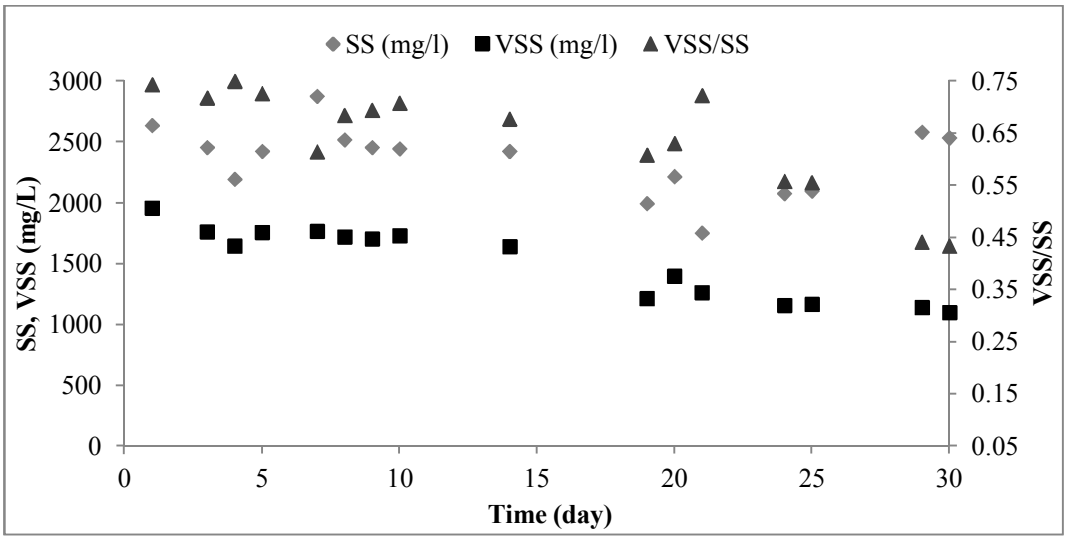


Figure 4.14 : SS, VSS results of the antibiotic reactor versus time.

After the fifteenth day, the VSS concentration was started to decrease and therefore, the VSS/SS ratio decreased.

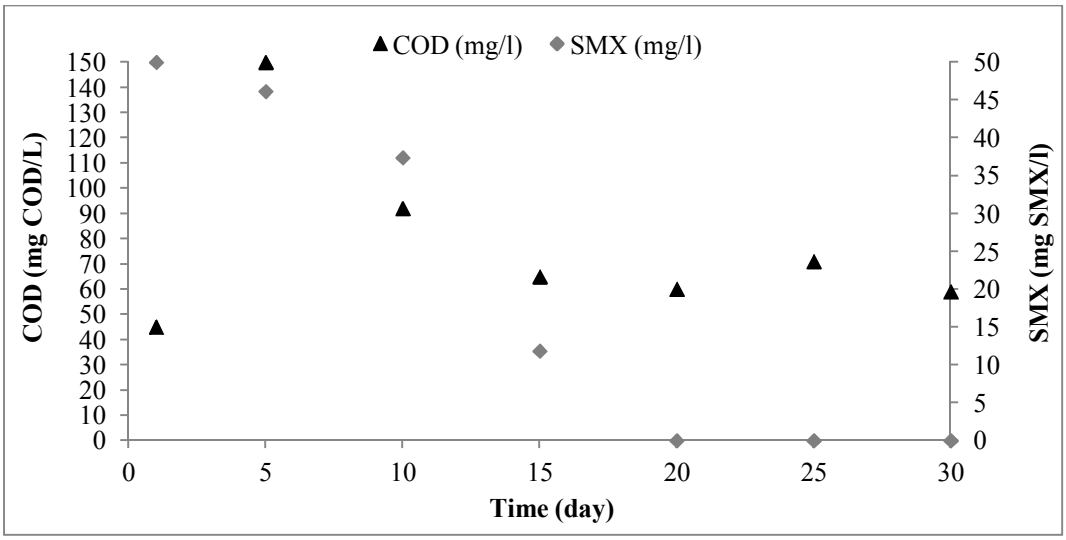


Figure 4.15 : COD & SMX results of the antibiotic reactor versus time.

Several batch tests were conducted by using both acetate and antibiotic (called as mix), only acetate and only antibiotic as substrate under aerobic conditions. In these experiments, F/M ratio was again kept constant as in the antibiotic reactor, but both VSS and substrate concentrations were diluted by 1/2 ratio. The chronic experiments were applied as;

- on the first day of antibiotic feeding ➔ one experiment with mixed substrate
- on the fifth day of antibiotic feeding ➔ one experiment with mixed substrate
- on the tenth day of antibiotic feeding ➔ three experiments with mixed substrate, only acetate and only SMX
- on the fifteenth day of antibiotic feeding ➔ one experiment with mixed substrate
- on the twentieth day of antibiotic feeding ➔ three experiments with mixed substrate, only acetate and only SMX
- on the twenty fifth day of antibiotic feeding ➔ one experiment with mixed substrate
- on the thirtieth day of antibiotic feeding ➔ three experiments with mixed substrate, only acetate and only SMX

OUR profile and soluble COD profiles including SMX data of the first day experiment, in which the acute experiment SMX-50 (Set 3) was taken, were given in

Figure 4.16 and Figure 4.17, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 182 mg O_2/l and 96 mg O_2/l.

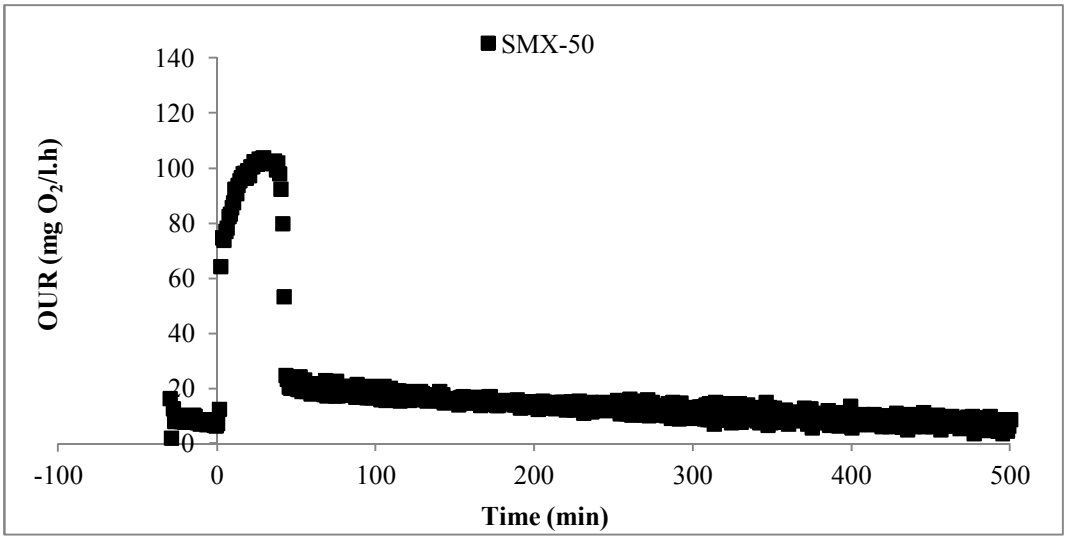


Figure 4.16 : OUR profile versus time (Set 3).

At the beginning of the chronic experiments, SMX removal was not observed as in the acute experiments. Therefore, both acetate and SMX concentrations in mg COD/l were also given in soluble COD profiles.

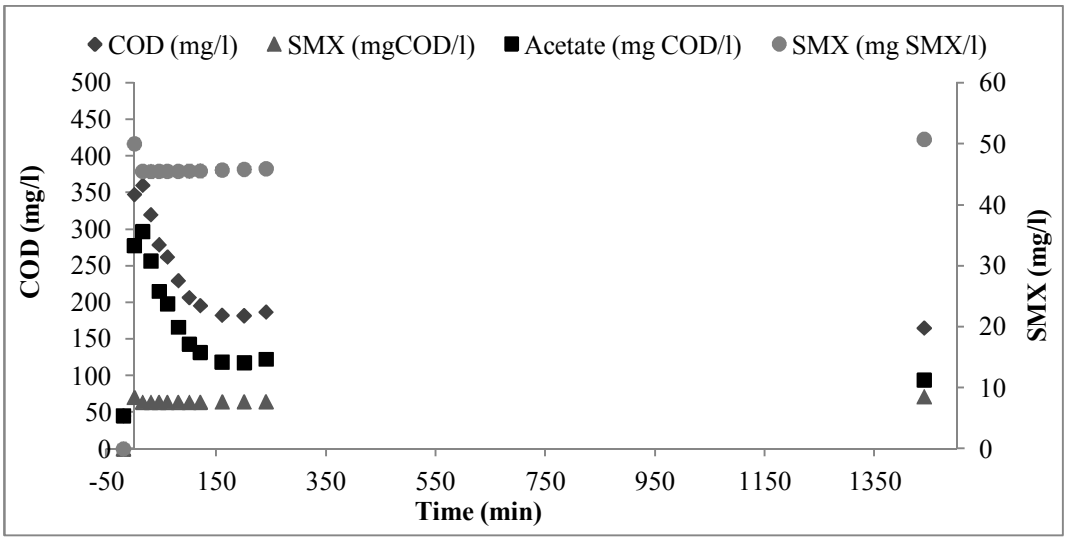


Figure 4.17 : Soluble COD & SMX profiles of Set3.

OUR profile of fifth day experiment was given in Figure 4.18. In this experiment, DO_{Total} and DO_{Endo} were calculated as 96 mg O_2/l and 34 mg O_2/l. Soluble COD and SMX profiles of Set 6 was given in Figure 4.19. The measured SMX concentrations were higher than the fed to the reactor (50 mg SMX/l) due to the accumulation of antibiotic through five days.

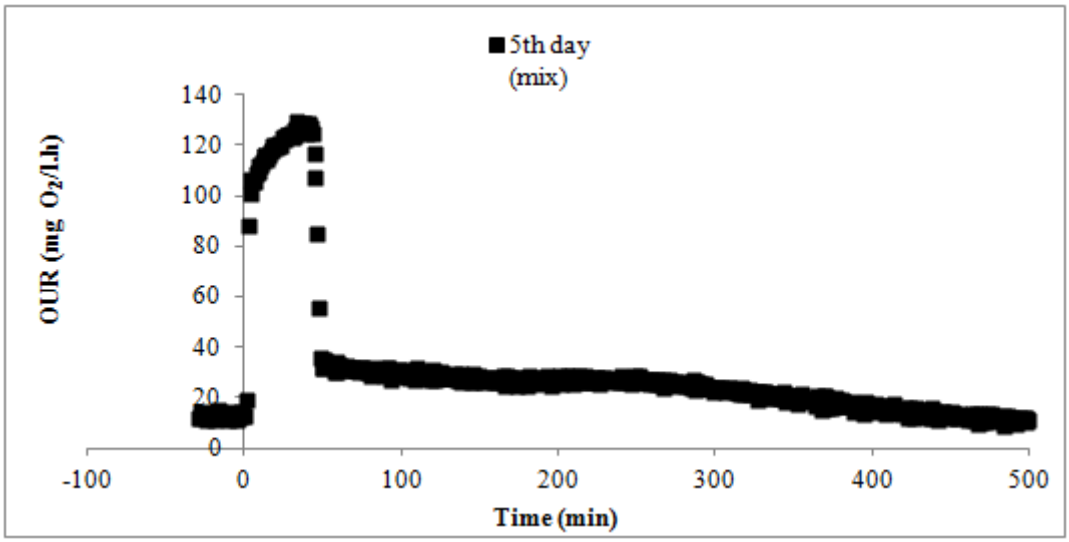


Figure 4.18 : OUR profiles versus time (Sets 6).

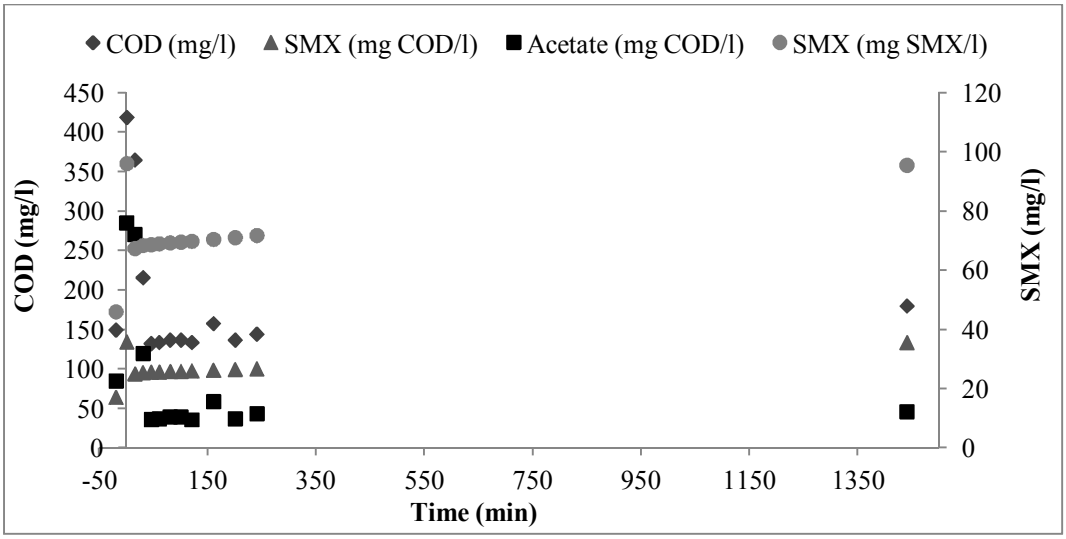


Figure 4.19 : Soluble COD & SMX profiles of Set 6.

OUR profiles of tenth day experiments were given in Figure 4.20. As shown in figure, OUR curve of Set 7.1 was lower than OUR curve of Set 7.2. DO_{Total} and DO_{Endo} were calculated as 109 mg O_2/l and 34 mg O_2/l for Set 7.1; 119 mg O_2/l and 31 mg O_2/l for Set 7.2; 45 mg O_2/l and 45 mg O_2/l for Set 7.3. The same oxygen consumption showed that the biomass did only endogenous respiration in Set 7.3.

Soluble COD and SMX profiles of Set 7.1, Set 7.2 and Set 7.3 were given in Figure 4.21, Figure 4.22 and Figure 4.23, respectively.

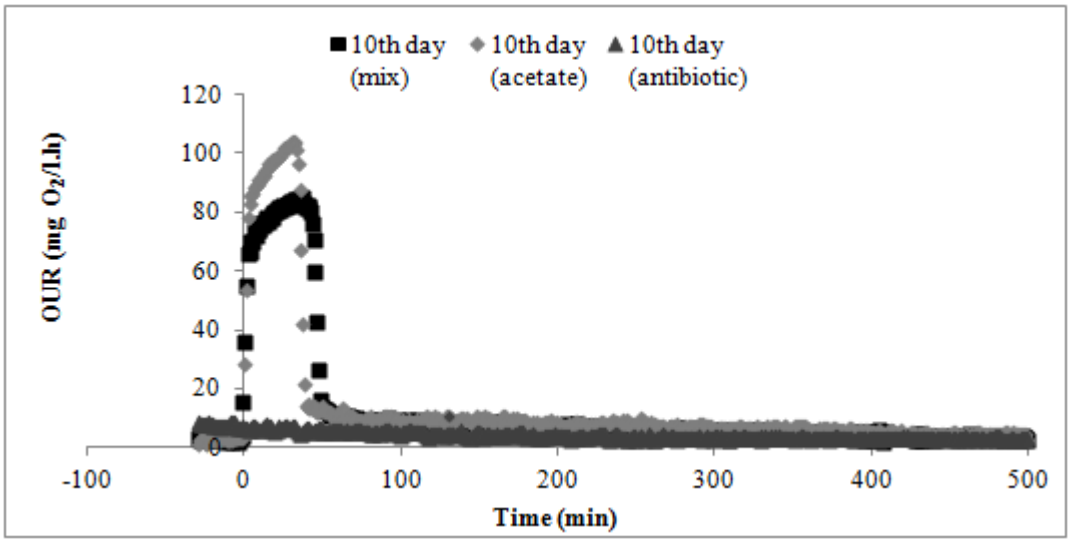


Figure 4.20 : OUR profile versus time (Set 7.1, Set 7.2 & Set 7.3).

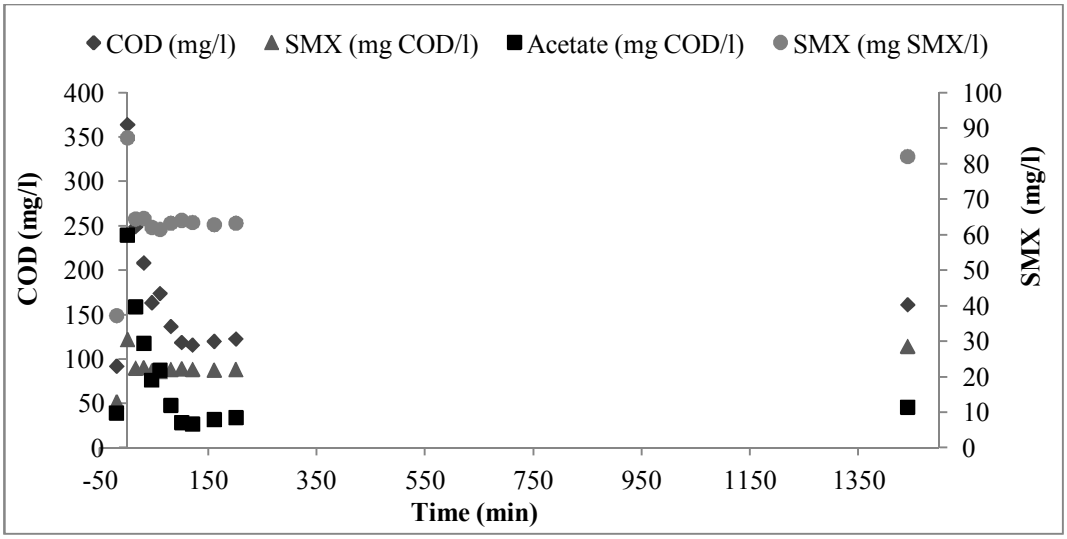


Figure 4.21 : Soluble COD & SMX profiles of Set 7.1.

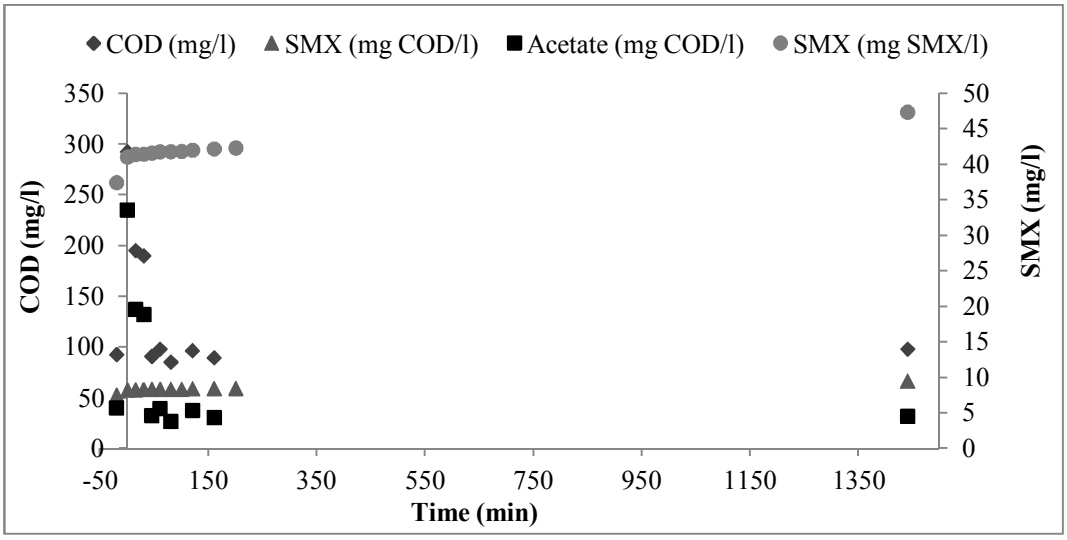


Figure 4.22 : Soluble COD & SMX profiles of Set 7.2.

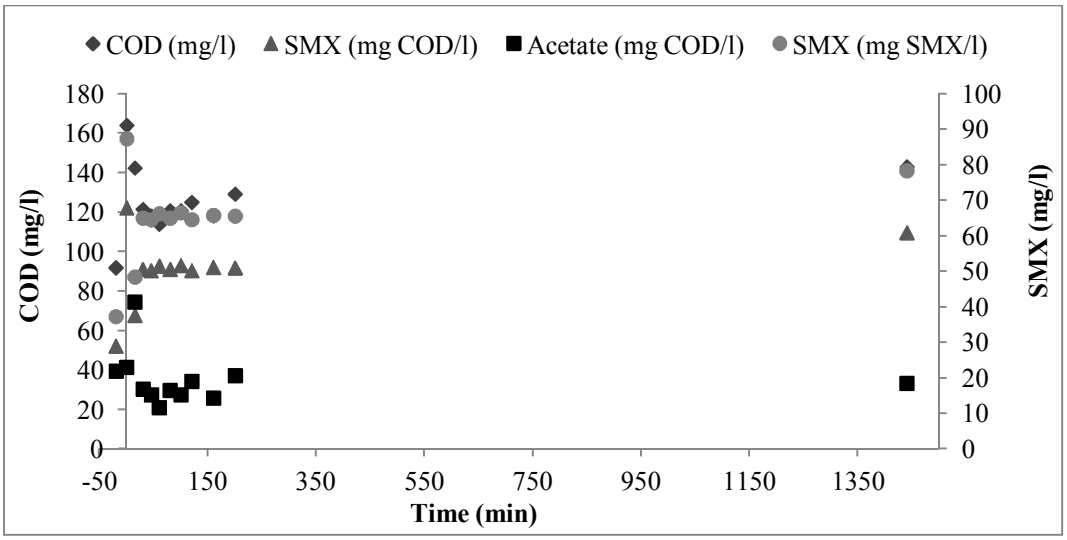


Figure 4.23 : Soluble COD & SMX profiles of Set 7.3.

OUR profile and soluble COD profiles including SMX data of Set 8 were given in Figure 4.24 and Figure 4.25, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 119 mg O_2/l and 35 mg O_2/l. On the fifteenth day, approximately 20% of SMX removal was observed as shown in Figure 4.25.

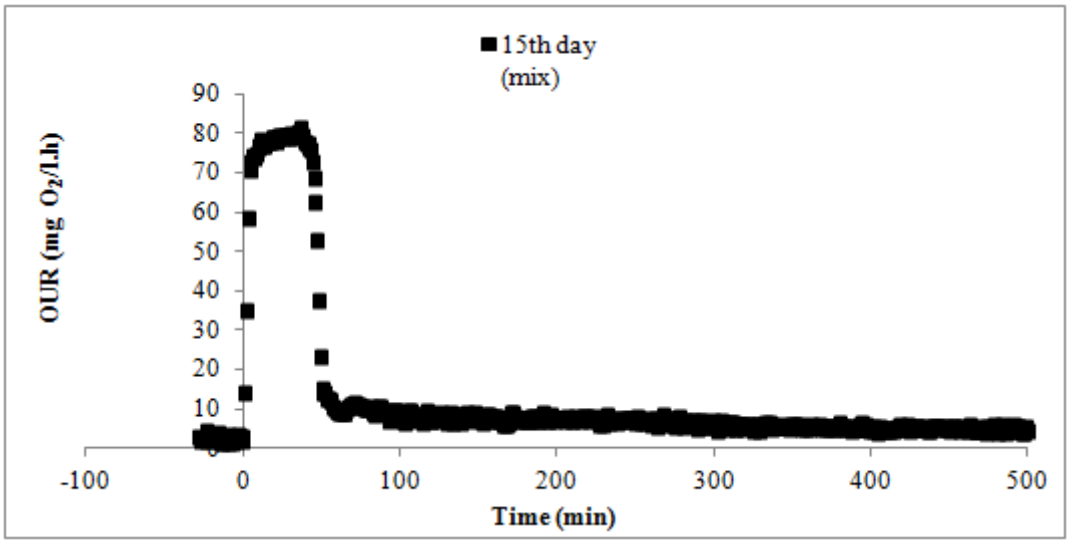


Figure 4.24 : OUR profile versus time (Set 8).

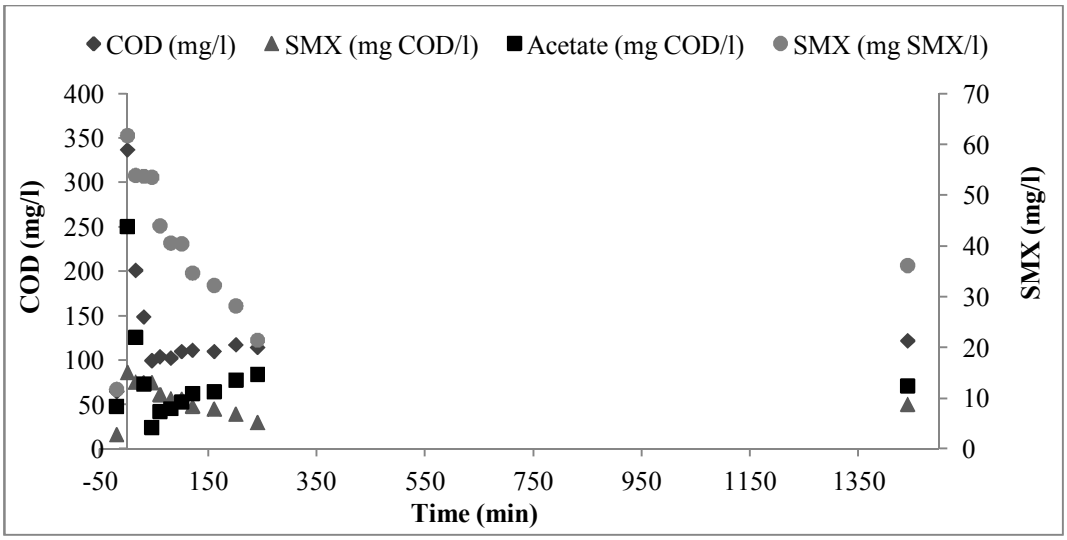


Figure 4.25 : Soluble COD & SMX profiles of Set 8.

OUR profiles of three experiments (Set 9.1, Set 9.2 & Set 9.3) done on the twentieth day were given in Figure 4.26. As shown in figure, OUR curves of Set 9.1 and Set 9.2 were close to each other. DO_{Total} and DO_{Endo} were calculated as 51 mg O_2/l and 17 mg O_2/l for Set 9.1; 31 mg O_2/l and 14 mg O_2/l for Set 9.2; 26 mg O_2/l and 9 mg O_2/l for Set 9.3.

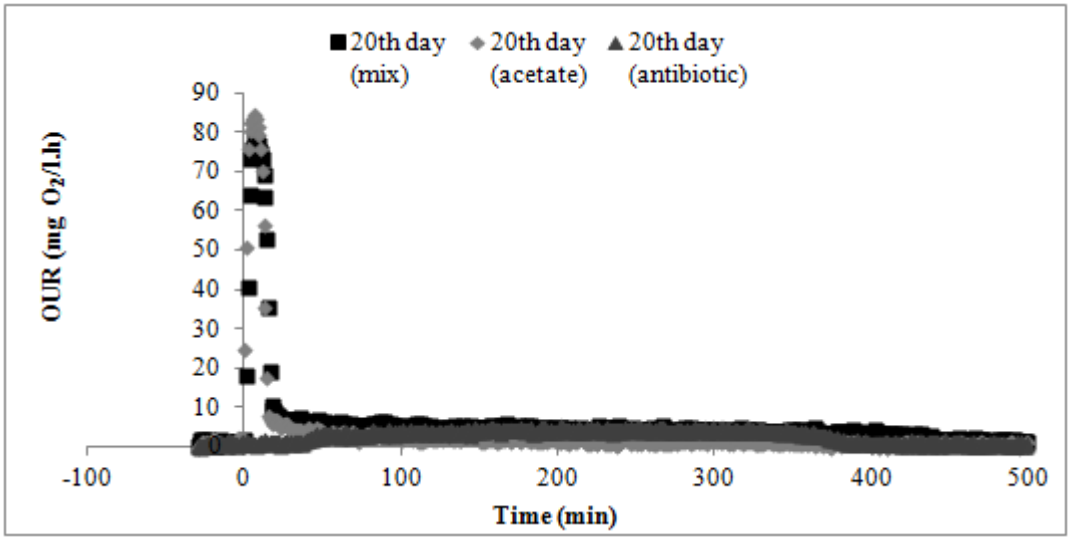


Figure 4.26 : OUR profiles versus time (Set 9.1, Set 9.2 & Set 9.3).

Soluble COD and SMX profiles of Set 9.1, Set 9.2 and Set 9.3 were given in Figure 4.27, Figure 4.28 and Figure 4.29, respectively. On the twentieth day, SMX removal increased with respect to the previous experiments, as shown in Figure 4.27. Furthermore, in Set 9.2 and Set 9.3 effluent SMX concentrations decreased up to zero.

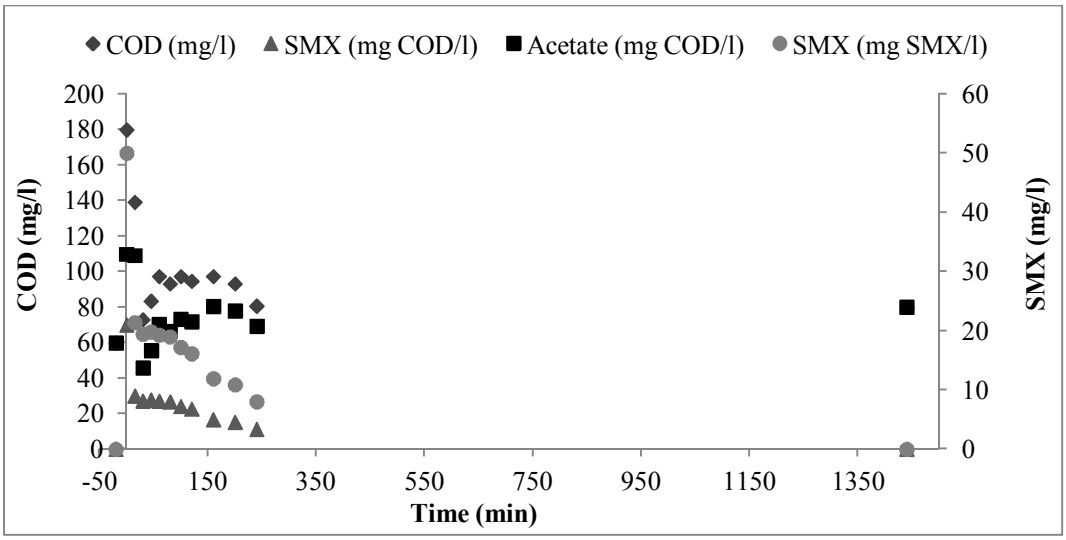


Figure 4.27 : Soluble COD & SMX profiles of Set 9.1.

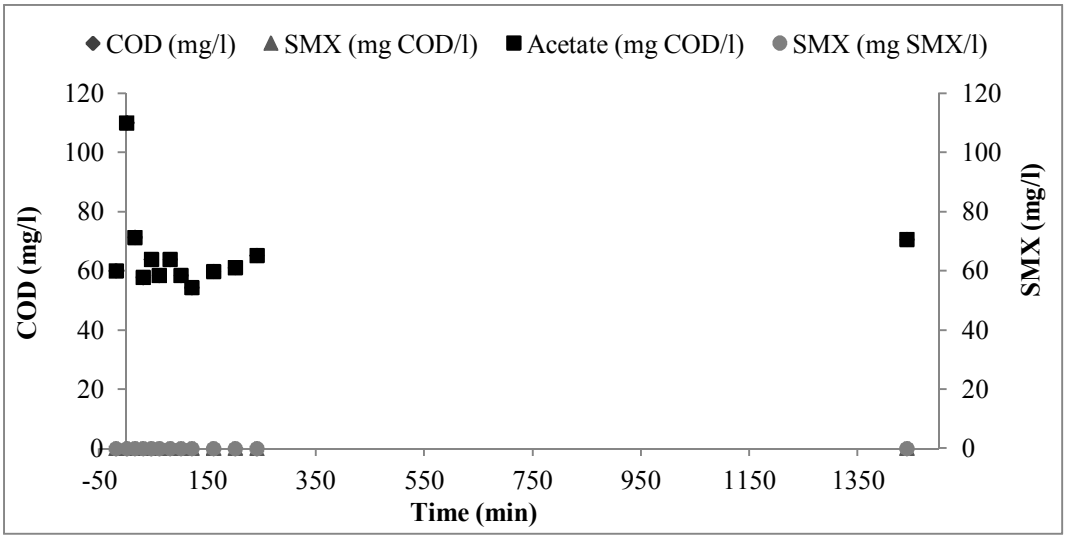


Figure 4.28 : Soluble COD & SMX profiles of Set 9.2.

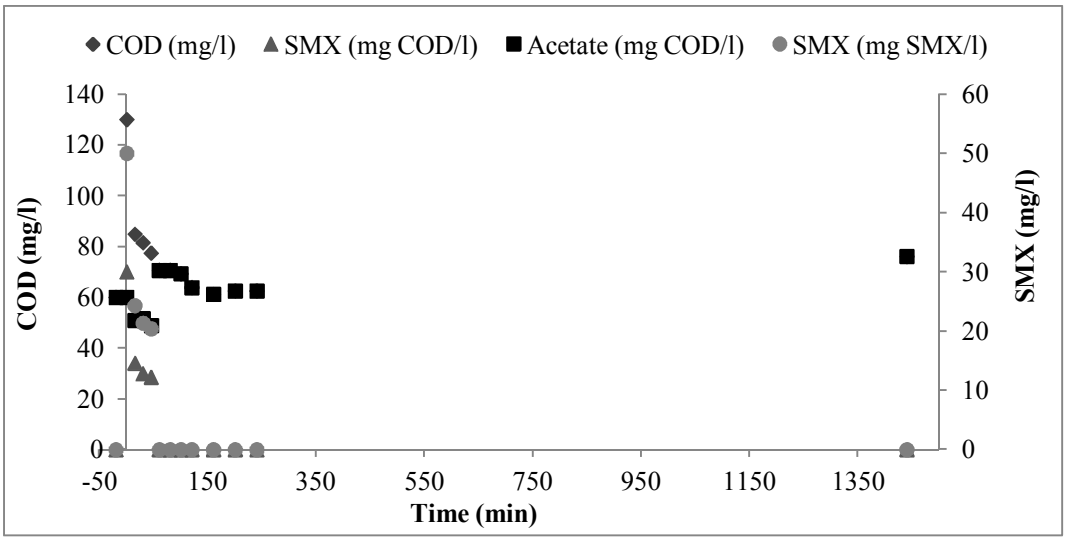


Figure 4.29 : Soluble COD & SMX profiles of Set 9.3.

OUR profile and soluble COD profiles including SMX data of Set 10 were given in Figure 4.30 and Figure 4.31, respectively. In this experiment, DO_{Total} and DO_{Endo} were calculated as 59 mg O_2/l and 21 mg O_2/l.

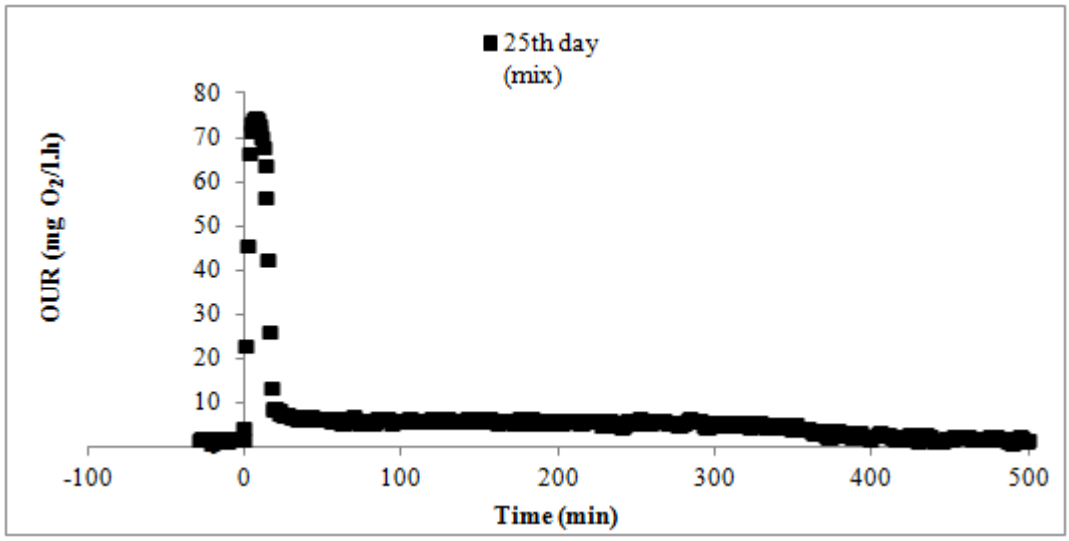


Figure 4.30 : OUR profile versus time (Set 10).

Since a complete SMX removal was observed on the twentieth day, after that SMX decreased to zero in the following experiments.

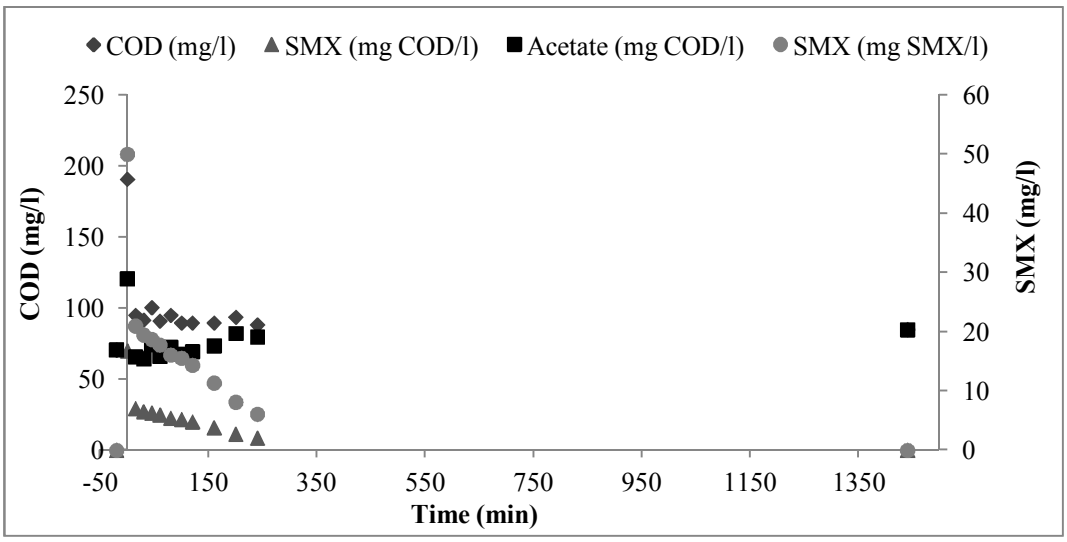


Figure 4.31 : Soluble COD & SMX profiles of Set 10.

OUR profiles of three experiments (Set 11.1, Set 11.2 & Set 11.3) done on the thirtieth day were given in Figure 4.32. As shown in figure, OUR curves of Set 11.1 was lower than OUR curve of Set 11.2. DO_{Total} and DO_{Endo} were calculated as 64 mg O_2/l and 32 mg O_2/l for Set 11.1; 76 mg O_2/l and 29 mg O_2/l for Set 11.2; 113 mg O_2/l and 63 mg O_2/l for Set 11.3.

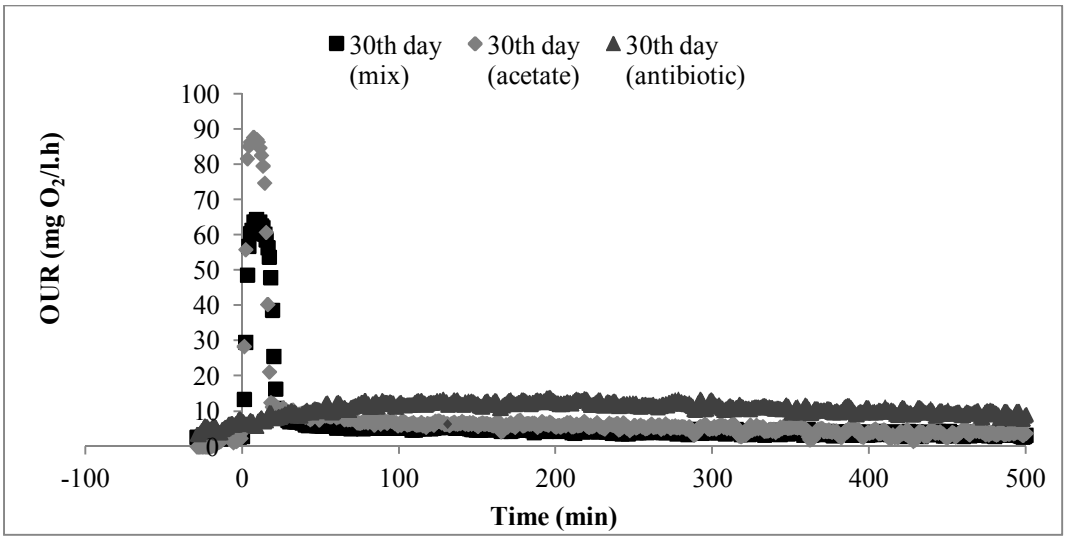


Figure 4.32 : OUR profiles versus time (Set 11.1, Set 11.2 & Set 11.3).

Soluble COD and SMX profiles of Set 11.1, Set 11.2 and Set 11.3 were given in Figure 4.33, Figure 4.34 and Figure 4.35, respectively.

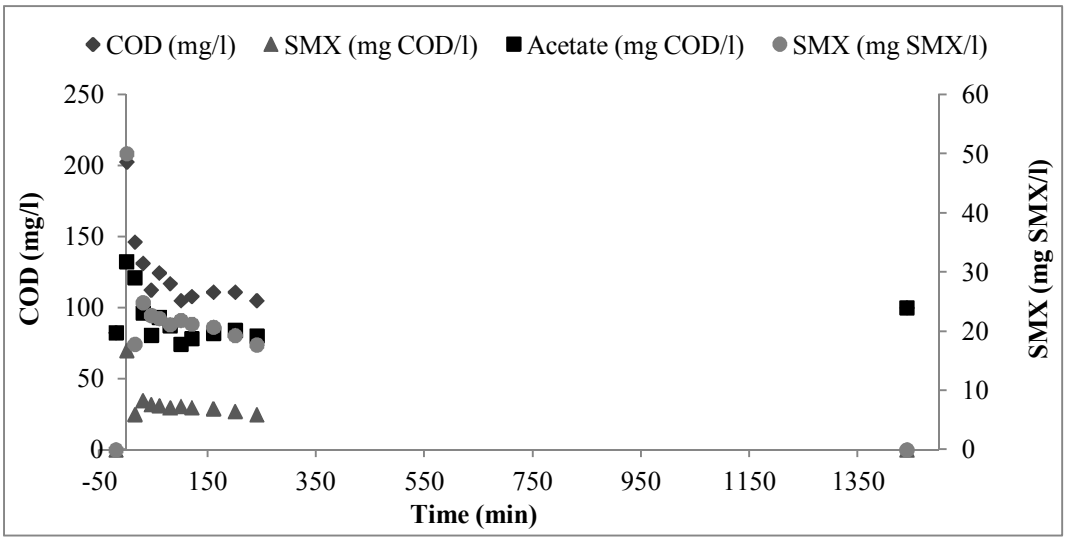


Figure 4.33 : Soluble COD & SMX profiles of Set 11.1.

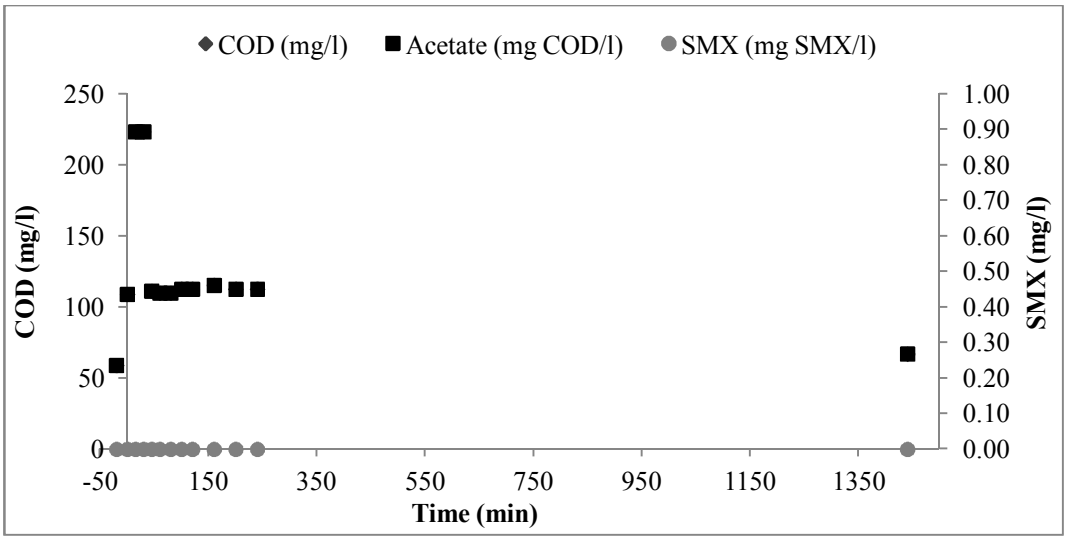


Figure 4.34 : Soluble COD & SMX profiles of Set 11.2.

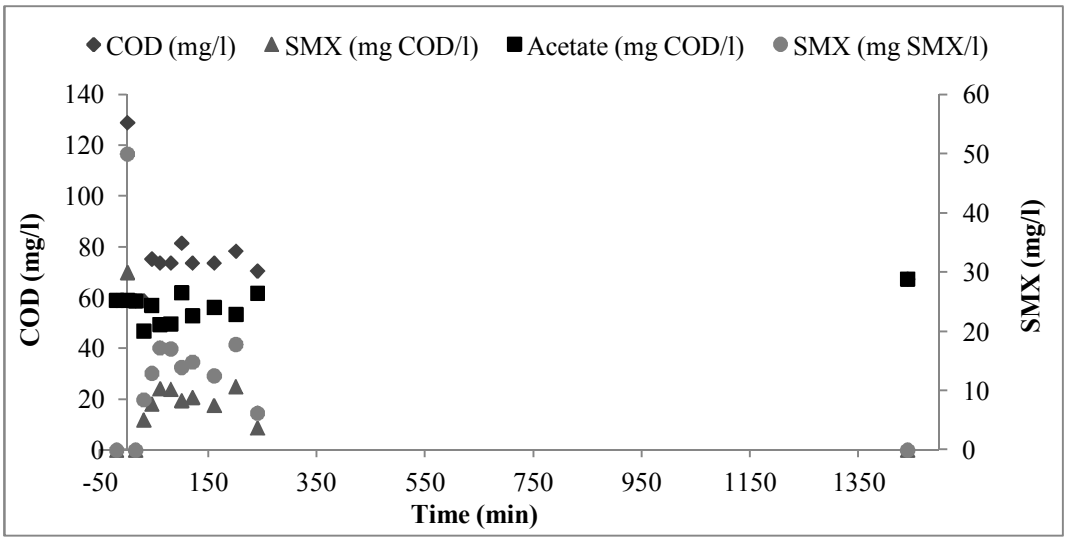


Figure 4.35 : Soluble COD & SMX profiles of Set 11.3.

Comparison of the OUR profiles of all chronic experiments (with mixed substrate, with only acetate and with only antibiotic) were indicated in Figure 4.36, Figure 4.37 and Figure 4.38, respectively.

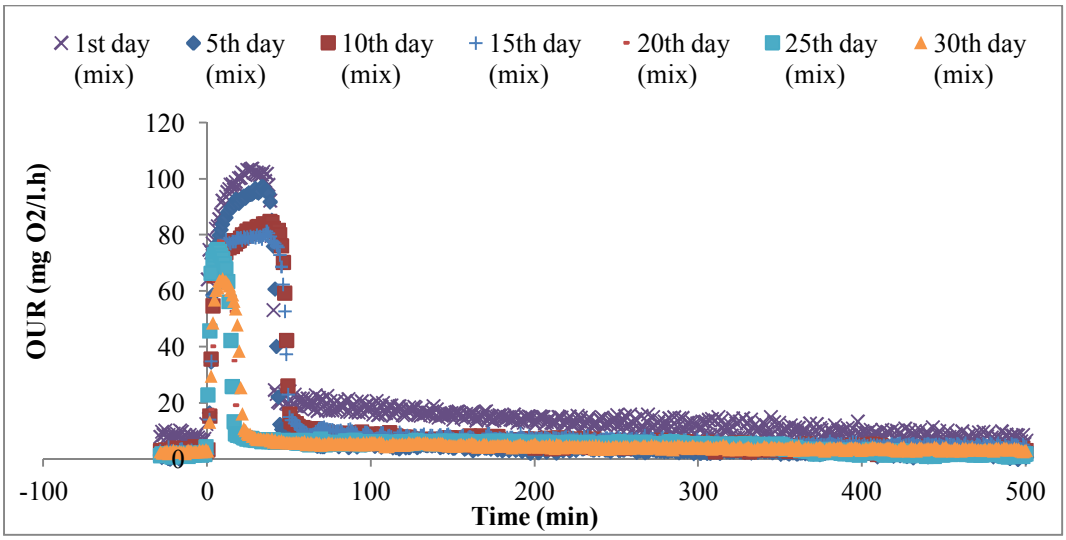


Figure 4.36 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate).

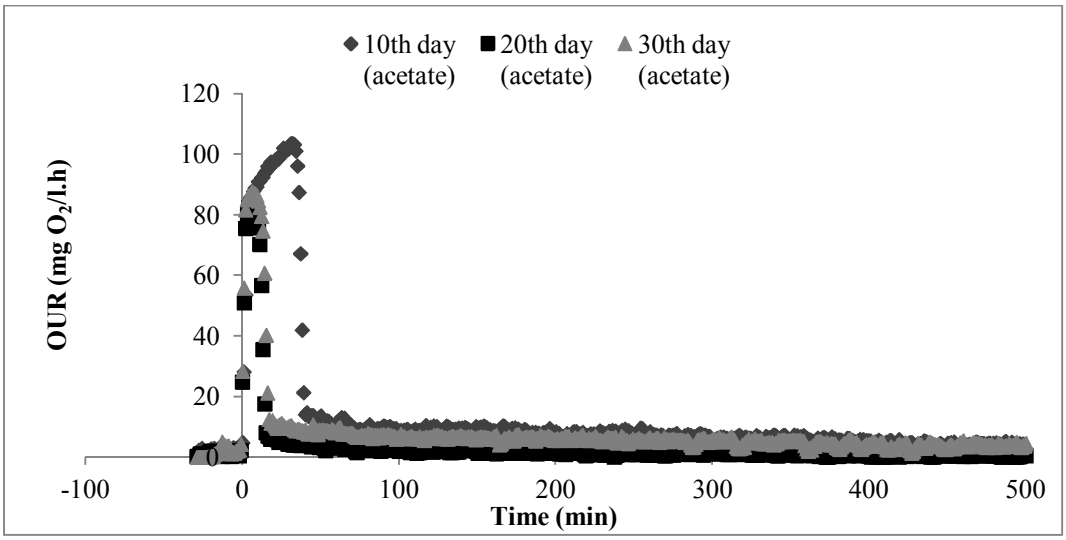


Figure 4.37 : Comparison of the OUR profiles of all chronic experiments (with only acetate).

The trend of the OUR profiles obtained in only antibiotic fed experiments were completely different from the addition of only acetate and mixed substrate. Although there was not any antibiotic removal on the acclimation period of ten days, SMX biodegradation was observed on the twentieth day experiment as shown in Figure **4.38**.

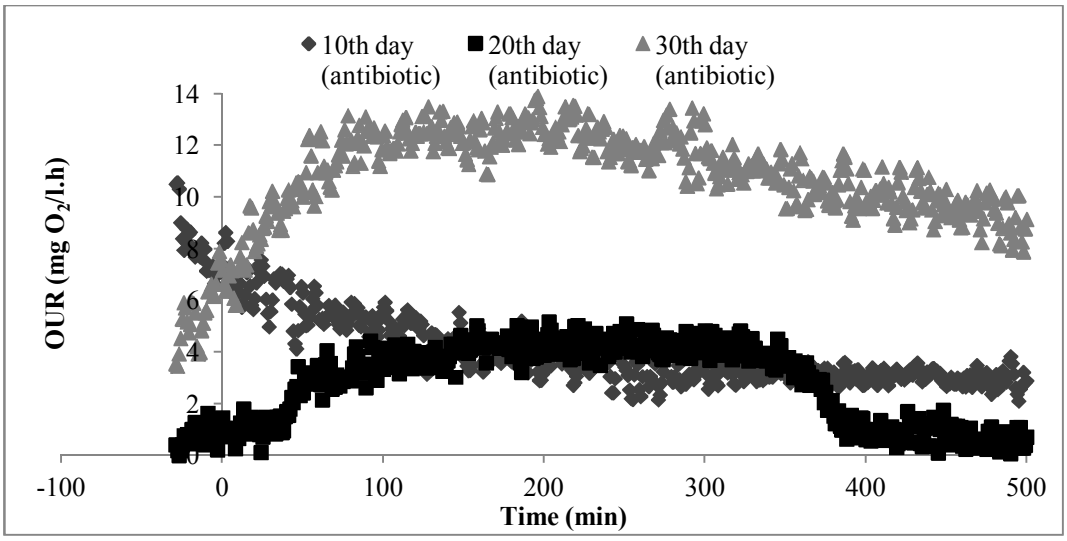


Figure 4.38 : Comparison of the OUR profiles of all chronic experiments (with only SMX).

4.3 Modelling of Experimental Results

A mechanistic model involving model components and kinetic parameters was developed for both acetate and SMX. The AQUASIM simulation program was used in modeling studies. This model was calibrated with related experimental data such as OUR and PHB. Biodegradation characteristics and kinetics of acetate and SMX were estimated. The evaluation of calibrated model indicated that a group of microorganisms adjusted their enzymatic tools for the utilization of SMX at high sludge age (10 days). The matrix representation of modified ASM3 structure was indicated in Table **4.1**.

Table 4.1 : Matrix representation of modified ASM3 structure [51].

Process	S_S	S_O	X_P	X_H	X_{STO}	Rate Equation
Aerobic storage	-1	$-(1-Y_{STO})$			Y_{STO}	$k_{STO}\frac{S_S}{K_S+S_S}\frac{S_O}{K_O+S_O}X_H$
Aerobic growth on S_S	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$		1		$\mu_H\frac{S_S}{K_S+S_S}\frac{S_O}{K_O+S_O}X_H$
Aerobic growth on X_{STO}		$-\frac{1-Y_H}{Y_H}$		1	$-\frac{1}{Y_H}$	$\mu_{STO}\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}\frac{S_O}{K_O+S_O}X_H$
Aerobic Decay		$-(1-f_{EX})$	f_{EX}	-1		$b_H\frac{S_O}{K_O+S_O}X_H$

Model simulation results of Set 1 according to modified ASM3 were indicated in Figure **4.39** and Figure **4.40**, respectively.

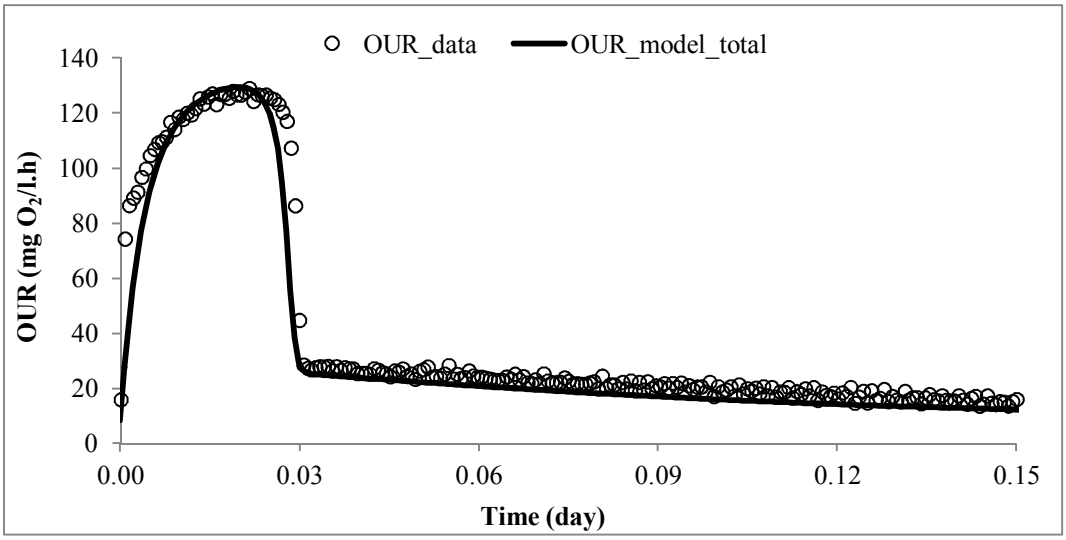


Figure 4.39 : Model simulation of OUR data for Set 1.

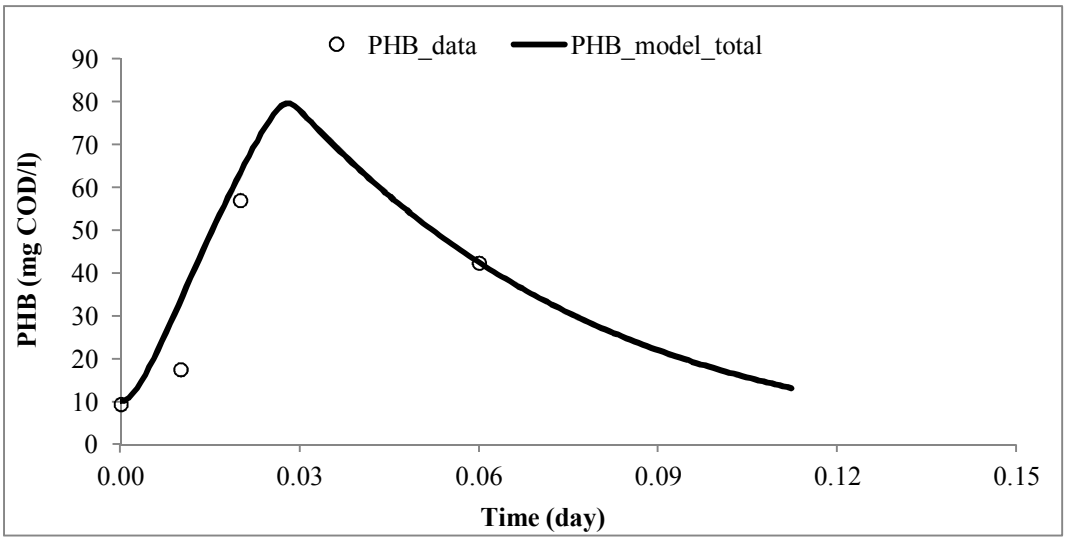


Figure 4.40 : Model simulation of PHB data for Set 1.

In the acute experiments, model simulation was applied to all acute experimental results. Since there were not PHB samples in Set 2, model simulation of PHB data could not be done. Model simulation result of OUR data for Set 2 was shown in Figure **4.41**.

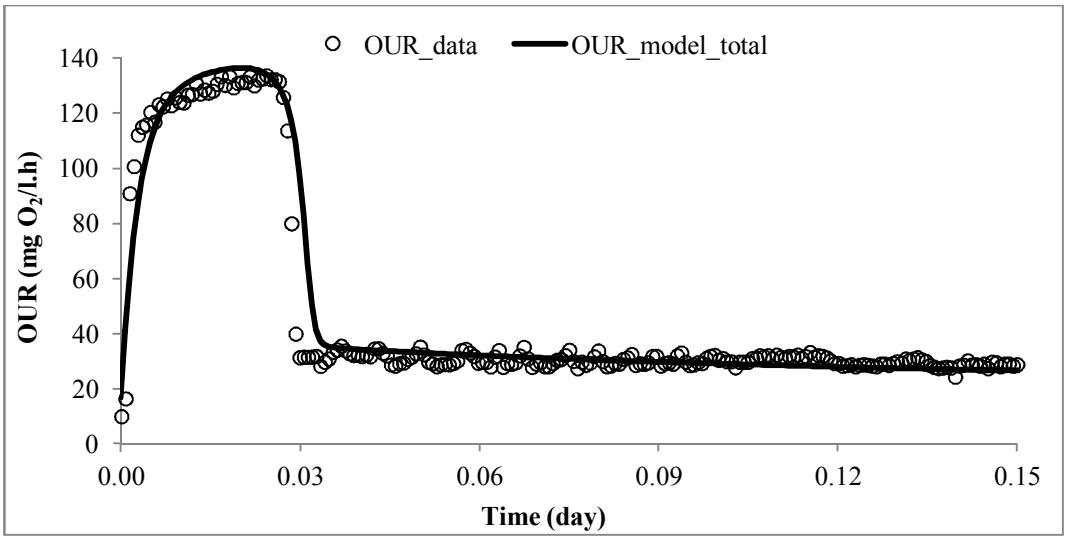


Figure 4.41 : Model simulation of OUR data for Set 2.

Model simulation results of OUR data and PHB data for Set 3 were indicated in Figure **4.42** and Figure **4.43**, respectively.

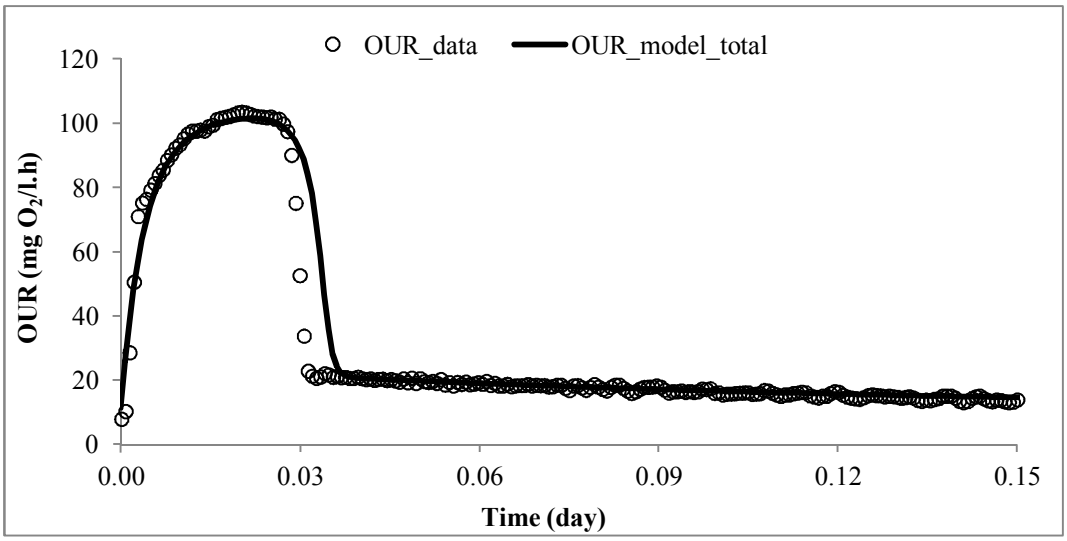


Figure 4.42 : Model simulation of OUR data for Set 3.

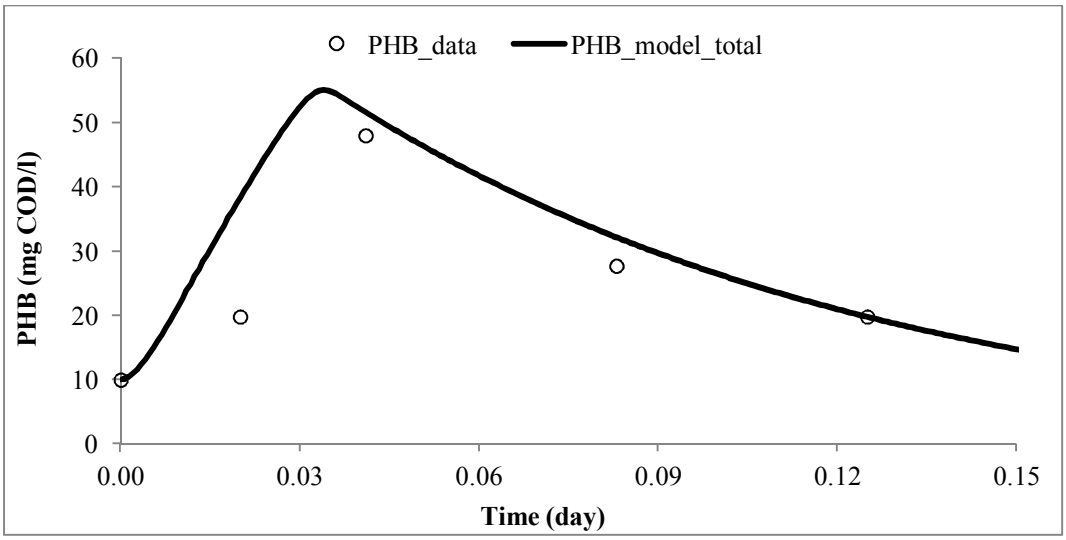


Figure 4.43 : Model simulation of PHB data for Set 3.

Model simulation results of OUR data and PHB data for Set 4 were illustrated in Figure **4.44** and Figure **4.45** respectively.

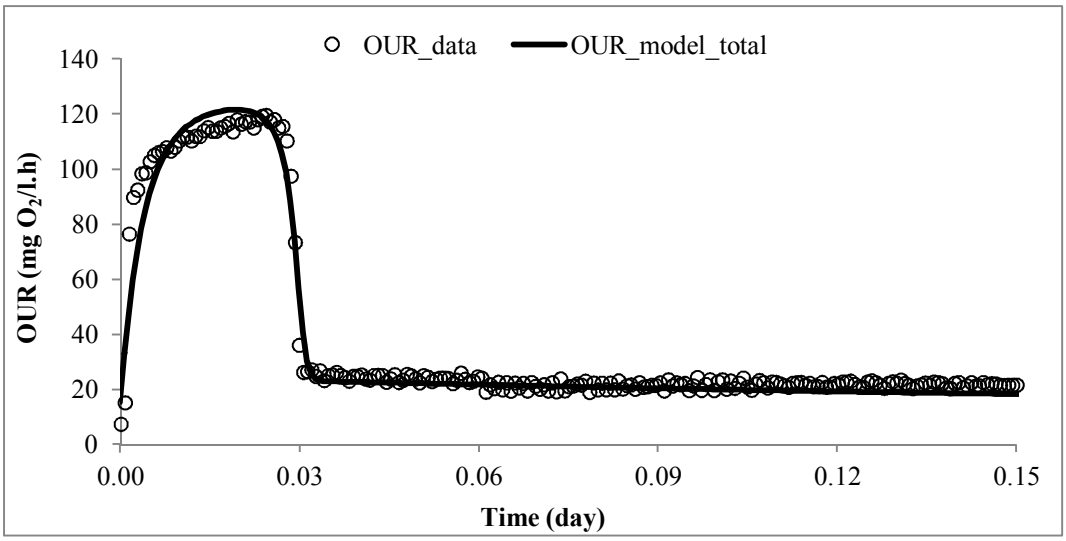


Figure 4.44 : Model simulation of OUR data for Set 4.

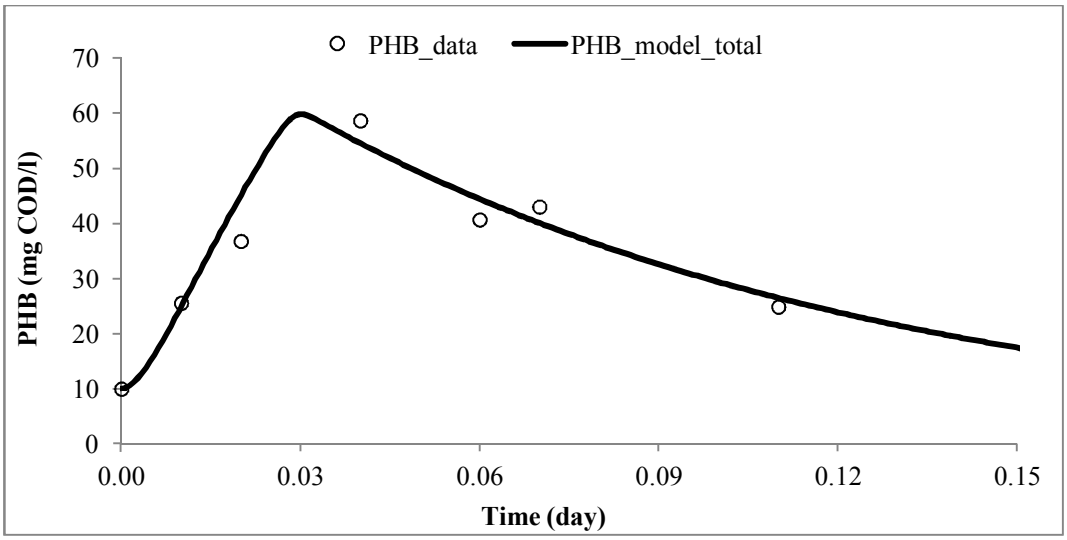


Figure 4.45 : Model simulation of PHB data for Set 4.

Since there were not PHB samples in Set 5, model simulation of PHB data could not be done. Model simulation result of OUR data for Set 5 was given in Figure **4.46**.

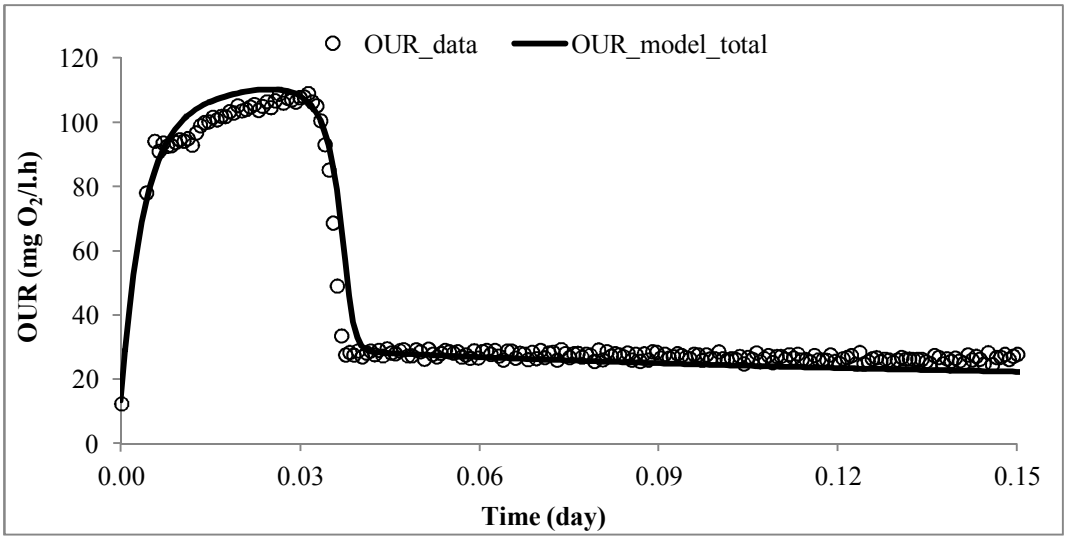


Figure 4.46 : Model simulation of OUR data for Set 5.

Modelling results of modified ASM3 indicated that maximum heterotrophic growth rate (μ_H), maximum growth rate on the stored products (μ_{STO}), and half saturation constant for readily biodegradable substrate (K_S) were the same for all acute experiments with control experiment. According to the simulation results, endogenous decay rate (b_H) was 0.2 d^{-1} for the control experiment whereas it was found 0.45 d^{-1} for the acute experiments. Half saturation constant for storage (K_{STO}) also increased in the acute experiments based on model simulation, when compared

to the value in the control experiment. On the other hand, a considerable decrease of maximum storage rate (k_{STO}) was observed from 5.7 d^{-1} to 3 d^{-1} in the acute experiments. The model simulation results based on modified ASM3 of Set 1, Set 2, Set 3, Set 4 and Set 5 were given in Table **4.2**.

Table 4.2 : Results of model calibration for the acute SMX experiments.

Model Parameters	**Unit**	**Control**	**Acute SMX-25**	**Acute SMX-50**	**Acute SMX-100**	**Acute SMX-200**
μ_H	1/day	3.5	3.5	3.5	3.5	3.5
K_S	mg COD/l	6.0	6.0	6.0	6.0	6.0
b_H	1/day	0.2	0.45	0.45	0.45	0.45
k_{STO}	1/day	5.7	3.0	3.0	3.0	3.0
μ_{STO}	1/day	3.5	3.5	3.5	3.5	3.5
K_{STO}	mg COD/l	0.3	0.6	0.6	0.7	0.7
f_{ES}	-	0.05	0.05	0.05	0.05	0.05
f_{EX}	-	0.15	0.15	0.15	0.15	0.15
State variables	**Unit**					
Total biomass	mg VSS/l	900	1050	800	930	900
X_H	mg COD/l	900	1125	850	1025	900
Activity	%	71	75	75	77	71
X_{STO}	mg COD/l	10	12	10	11	10
S_{S1}	mg COD/l	230	230	200	220	220

Assumptions: Y_H = 0.66 g COD/g COD and Y_{STO} = 0.8 g COD/g COD [52,53].

In the chronic experiments, only the first day mix (Set 3); fifteenth day mix (Set 8); twentieth day mix, acetate and antibiotic (Set 9.1, Set 9.2 & Set 9.3) were simulated by the modified ASM3 model.

Model simulation results of Set 3 were represented in Figure **4.42** and Figure **4.43,** respectively.

Since there were not PHB samples in Set 8, model simulation of PHB data could not be done. Model simulation result of Set 8 was shown in Figure **4.47**.

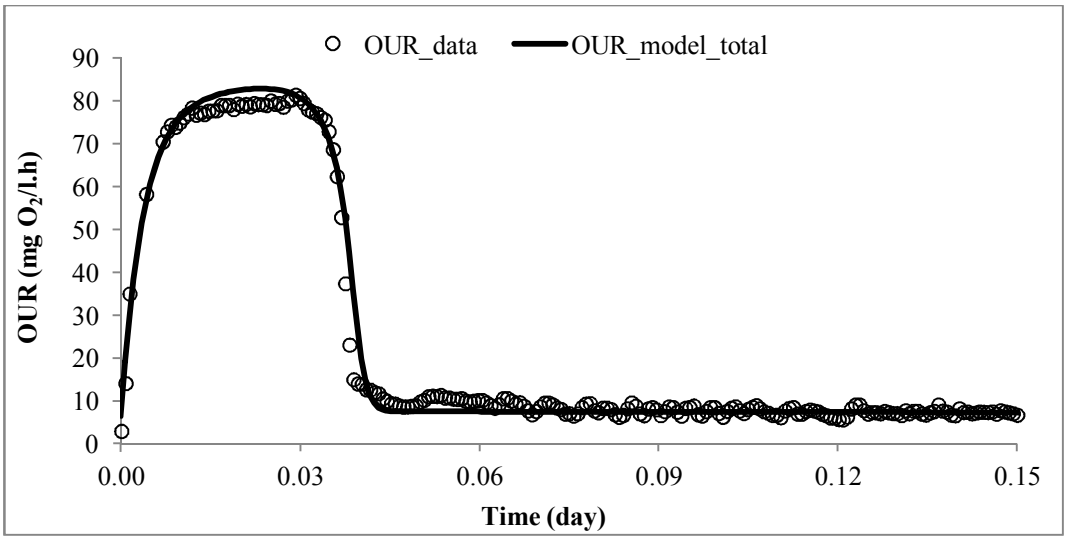


Figure 4.47: Model simulation of OUR data for Set 8.

Model simulation results of Set 9.1, Set 9.2 and Set 9.3 were illustrated in Figure **4.48**, Figure **4.49** and Figure **4.50**, respectively. Since these experiments did not have PHB samples, model simulation of PHB data could not be done.

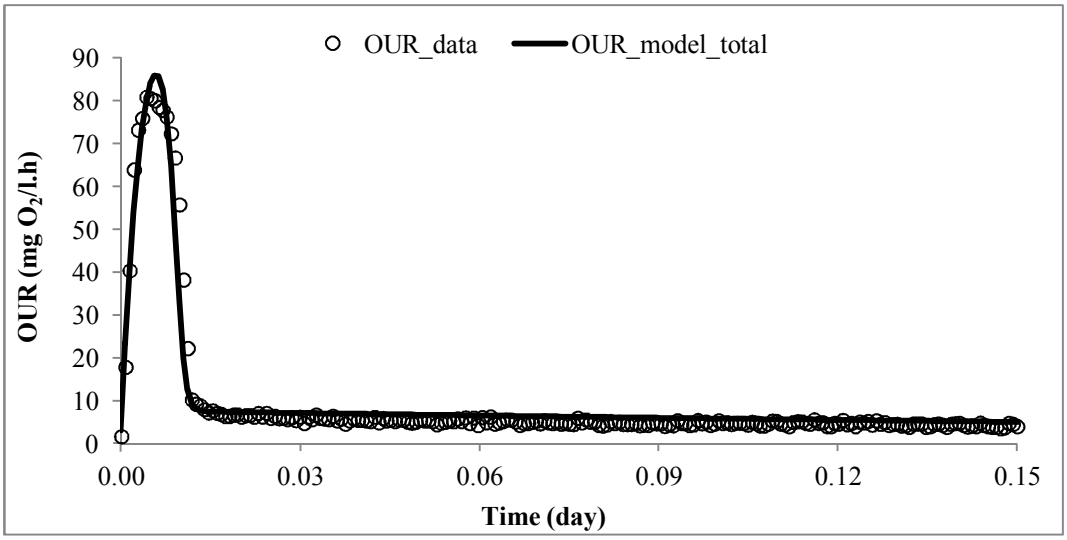


Figure 4.48: Model simulation of OUR data for Set 9.1.

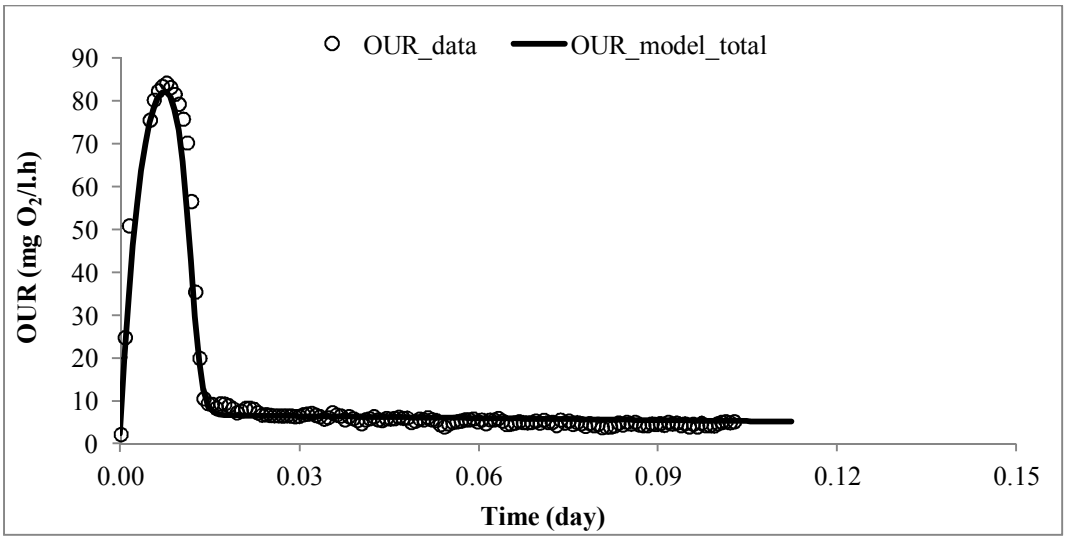


Figure 4.49: Model simulation of OUR data for Set 9.2.

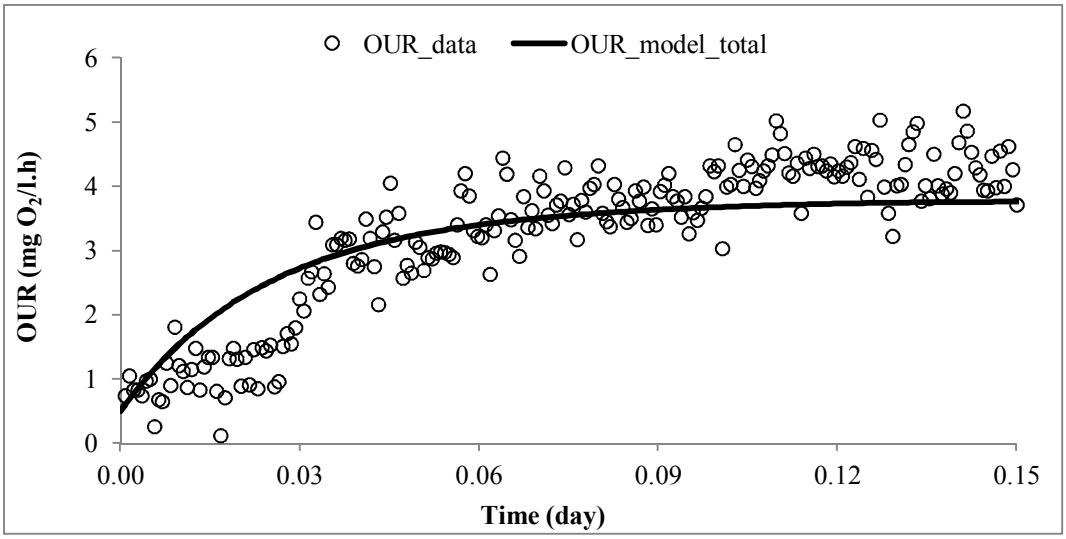


Figure 4.50: Model simulation of OUR data for Set 9.3.

Modelling results of modified ASM3 indicated that maximum heterotrophic growth rate was found the same with the control experiment on the first day of acclimation, and increased from 3.5 to 5 d^{-1} on the fifteenth day of acclimation. According to the simulation results, half saturation constant for readily biodegradable substrate was the same for all acetate including chronic experiments. Furthermore, state variables including active biomass fractions and the set of model parameters for the twentieth day experiment were derived from three interrelated OUR experiments conducted on the synthetic substrate (acetate), the SMX and the synthetic substrate/SMX mixture so that each model calibration step yielded its own values. As a result, a new microbial group capable of metabolizing the antibiotic (X_{HA}), a half saturation constant for antibiotic (K_{SA}) and a maximum growth rate of antibiotic degrading

heterotrophs (μ_{HA}) were estimated in the model simulation. Both K_{SA} and μ_{HA} decreased with respect to K_S and μ_H. Maximum storage rate also decreased from 5.7 to 3 d^{-1} on the first day of acclimation. On the other hand, half saturation constant for storage increased on the first day of acclimation when compared to the value in the control experiment. It was observed that the storage process disappeared with the acclimation of activated sludge to SMX. For this reason, μ_{STO}, k_{STO}, K_{STO} and Y_{STO} were not estimated in both the fifteenth and the twentieth day experiments. The model simulation results based on modified ASM3 of Set 3, Set 8, Set 9.1, Set 9.2 and Set 9.3 were given in Table **4.3**.

Table 4.3 : Results of model calibration for the chronic SMX experiments.

Model Parameters	**Unit**	**Control**	**1st day (mix)**	**15th day (mix)**	**20th day (mix)**	**20th day (antibiotic)**	**20th day (acetate)**
μ_H		3.5	3.5	5	5	-	5
K_S	mg COD/l	6	6	6	6	-	6
μ_{HA}	1/day	-	-	-	1	1	-
K_{SA}	mg COD/l	-	-	-	3	3	-
b_H	1/day	0.2	0.45	0.28	0.1	0.1	0.1
k_{STO}	1/day	5.7	3	-	-	-	-
μ_{STO}	1/day	3.5	3.5	-	-	-	-
K_{STO}	mg COD/l	0.3	0.6	-	-	-	-
f_{ES}	-	0.05	0.05	0.05	0.05	0.05	0.05
f_{EX}	-	0.15	0.15	0.15	0.15	0.15	0.15
State Variables	**Unit**						
Total biomass	mg VSS/l	900	800	820	820	820	820
X_H	mg COD/l	900	850	700	900	-	750
X_{HA}	mg COD/l	-	-	-	-	150	-
Activity	%	71	75	60	77	13	64
X_{STO}	mg COD/l	10	10	-	-	-	-
S_{S1}	mg COD/l	230	200	200	40	-	40
S_{SA}	mg COD/l	-	70	70	70	70	-
Removal Efficiency	**Unit**						
$S_{S1removed}$ for Acetate	%	93	80	64	83	-	83
$S_{SAremoved}$ for Antibiotic	%	-	-	20	71	71	-

Assumptions: Y_H = 0.66 g COD/g COD and Y_{STO} = 0.8 g COD/g COD [52,53].

5 CONCLUSIONS AND RECOMMENDATIONS

Sulfamethoxazole is one of the extensively used antibiotic compounds which is present in high concentrations in both domestic and municipal wastewaters. Although sulfonamides are reported as stable against hydrolysis process, biodegradability test indicate that SMX is ultimately biodegradable.

Respirometric experiments displayed a significant information about the inhibitory impact of SMX, and it was followed by acclimation of activated sludge to this compound. The only acetate fed reactor operated with an efficiency of 90-93% COD removal. The removal of the antibiotic compound was not observed on the tenth day of acclimation. Acclimation of activated sludge to SMX was observed on the fifteenth day of acclimation period resulting in 20% of SMX removal. On the twentieth day of acclimation, complete removal was achieved.

Model simulation estimations proved the dependence of kinetic parameters to physiological state of microbial cultures and feeding pattern of substrates. In the acute experiments, SMX caused decrease in maximum storage rate; however, endogenous decay rate increased. In the chronic experiments, as biomass was acclimating the SMX, the storage process disappeared. On the other hand, higher maximum growth rate and lower endogenous decay rate were observed.

As a consequence, SMX has an inhibitory impact on acetate degradation which cause decrease on acetate degradation rate. Acclimation to SMX is possible in the presence of available substrate, acetate which may be a result of cometabolic degradation.

REFERENCES

[1] **Kümmerer K.** (2008). *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*. Springer-Verlag, Berlin Heidelberg.

[2] **Chrencik J.E., Orans J., Moore L.B., Xue Y., Peng L., Collins J.L., Wisely G.B., Lambert M.H., Kliewer S.A., Redinbo M.R.** (2005). Structural disorder in the complex of human PXR and the macrolide antibiotic rifampicin. *Molecular Endocrinology*, **19**, 1125-1134.

[3] **Zhang X., Pan B., Yang K., Zhang D., Hou J.** (2010). Adsorption of sulfamethoxazole on different types of carbon nanotubes in comparison to other natural adsorbents. *Journal of Environmental Science and Health*, Part A, **45**, 1625-1634.

[4] **Rang H.P., Dale M.M., Ritter J.M.** (1999). *Pharmacology.* Churchill Livingstone: Edinburgh.

[5] **Aga D.S.** (2008). *Fate of Pharmaceuticals in the Environment and in Water Treatment Systems*. CRC Press.

[6] **Carballa M., Omil F., Lema J.M., Llompart M., Garcia C., Rodriguez I., Gomez M., Ternes T.** (2005). Behaviour of pharmaceuticals and personal care products in a sewage treatment plant of northwest Spain. *Water Science & Technology*, **52**, 29-35.

[7] **Dodd M.C., Huang C.H.** (2004). Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: Kinetics, mechanisms, and pathways. *Environ. Sci. Technol.*, **38**, 5607-5615.

[8] **Alexy R., Kümpel T., Kümmerer K.** (2004). Assessment of degradation of 18 antibiotics in the Closed Bottle Test. *Chemosphere*, **57**, 505-512.

[9] **Drillia P., Dokianakis S.N., Fountoulakis M.S., Kornaros M., Stamatelatou K., Lyberatos G.** (2005). On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. *J. Hazard. Mater.*, **122**, 259-265.

[10] < http://trimethoprim.net/ >, accessed at 09.08.2011.

[11] **Nikolaou A., Meric S., Fatta D.** (2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. *Anal. Bioanalytical Chem.,* **387**,1225-1234.

[12] **Kemper N.** (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indic.*, **8**, 1-13.

[13] **Xu W.H., Zhang G., Li X.D., Zou S.C., Li P., Hu Z.H., Li J.** (2007). Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Research*, **41**, 4526-4534.

[14] **Drilla P., Stamatelatou K., Lyberatos G.** (2005). Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere*, **60**, 1034-1044.

[15] **Stein K., Ramil M., Fink G., Sander M., Ternes T.A.** (2008). Analysis and sorption of psychoactive drugs onto sediment. *Environ. Sci. Technol.*, **42**, 6415-6423.

[16] **Xu W.H.H., Zhang G., Wai O.W.H., Zou S.C.C., Li X.D.D.** (2009). Transport and adsorption of antibiotics by marine sediments in a dynamic environment. *J. Soil. Sediment.,* **9**, 364-373.

[17] **Carballa M., Omil F., Lema J.M., Llompart M., Garcia-Jares C., Rodriguez I., Gomez M., Ternes T.** (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research*, **38**, 2918-2926.

[18] **Yang S., Carlson K.H.** (2004). Solid-phase extraction-high-performance liquid chromatography-ion trap mass spectrometry for analysis of trace concentrations of macrolide antibiotics in natural and wastewater matrices. *J. Chromatogr. A*, **1038**, 141-155.

[19] **Ternes T.A.** (2001). Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. *Trends in Analytical Chemistry*, **20**, 419-434.

[20] **Kolpin D.W., Furlong E.T., Meyer M.T., Thurman E.M., Zaugg S.D., Barber L.B., Buxton H.T.** (2002). Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. *Environ. Sci. Technol.*, **36**, 1202-1211.

[21] **Harting C., Storm T., Jekel M.** (1999). Detection and identification of sulphonamide drugs in municipal wastewater by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A*, **854**, 163-173.

[22] **Miao X.S., Bishay F., Chen M., Metcalfe C.D.** (2004). Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada. *Environ. Sci. Technol.,* **38**, 3533-3541.

[23] **Renew J.E., Huang C.H.** (2004). Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A*, **1042**, 113-121.

[24] **Hernando M.D., Mezcua M., Fernandez-Alba A.R., Barcelo D.** (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, **69**, 334-342.

[25] **Gulkowska A., Leung H.W., Taniyasu S., Yamashita N., Yeung LWY, *et al.*** (2008). Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. *Water Research*, **42**, 395-403.

[26] **Gartiser S., Urich E., Alexy R., Kümmerer K.** (2007). Ultimate biodegradation and elimination of antibiotics in inherent tests. *Chemosphere*, **67**, 604-613.

[27] **Kim S., Eichhorn P., Jensen J.N., Weber A.S., Aga A.** (2005). Removal of antibiotics in wastewater: Effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environ. Sci. Technol.*, **39**, 5816-5823.

[28] **Onesios K.M., Yu J.T., Bouwer E.J.** (2009). Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. *Biodegradation*, **20**, 441-466.

[29] **Omil F., Carballa M., Lema J.M., Llompart M., Garcia-Jares C., Rodriguez I., Gomez M., Ternes T.** (2004). Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research*, **38**, 2918-2926.

[30] **Ren H., Lei G., Ding L., Wang F., Zhang X.** (2010). A full-scale biological treatment system application in the treated wastewater of pharmaceutical industrial park, *Bioresource Technology*, **101**, 5852-5861.

[31] **Takada H., Kiri K., Shinohara H., Tanishima T., Nakada N.** (2006). Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Research*, **40**, 3297-3303.

[32] **Gotvajn A.Z., Zagorc-Končan J., Tišler T.** (2007). Pretreatment of highly polluted pharmaceutical waste broth by wet air oxidation. *Journal of Environmental Engineering*, **133**, 89-94.

[33] **Vanrolleghem P.A., Spanjers H., Petersen B., Ginestet P., Takacs I.** (1999). Estimating (Combinations of) Activated Sludge Model No. 1 Parameters and Components by Respirometry. *Water Science and Technology*, **39**, 195-214.

[34] **Guisasola A., Baeza J.A., Carrera J., Casas C., Lafuente J.** (2003). An off-line respirometric procedure to determine inhibition and toxicity of biodegradable compounds in biomass from an industrial WWTP. *Water Science and Technology*, **48**, 267-275.

[35] **Henze M., van Loosdrecht M.C.M., Ekama G.A., Brdjanovic D.** (2008). *Biological Wastewater Treatment Principles, Modelling and Design.* IWA Publishing UK.

[36] **Orhon D., Artan N.** (1994). *Modelling of Activated Sludge Systems.* Technomic Publication, USA.

[37] **Hoque M.A., Aravinthan V., Pradhan N.M.** (2009). Assessment on activated sludge models for acetate biodegradation under aerobic conditions. *Water Science and Technology*, **60**, 983-994.

[38] **Koch G., Kühni M., Gujer W., Siegrist H.** (2000). Calibration and Validation of Activated Sludge Model No. 3 for Swiss Municipal Wastewater. *Water Research*, **34**, 3580-3590.

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

[40] **MicKinney R.E.** (1962). Mathemathics of Complete-Mixing Activated Sludge. *J. SED. Proc. ASCE*, **88**, 87-95.

[41] **Gujer W., Henze M., Takahashi M. and van Loosdrecht M.** (1999). Activated Sludge Model No. 3. *Water Science and Technology*, **29**, 183-193.

[42] **van Aalst-van Leeuwen M.A., van Loosdrecht M.C.M., Heijnen J.J.** (1997). Kinetic modelling of poly(β-hydroxybutyrate) production and consumption by Paracoccus pantotrophus under dynamic substrate supply. *Biotechnology and Bioengineering*, **55**, 773-782.

[43] **Pot M., van Loosdrecht M.C.M., Heijnen J.J.** (1996). Effect of substrate addition on polymer storage in nitrification/denitrification SBR processes. *Water Research*, (Submitted).

[44] **Carta F., Beun J.J., van Loosdrecht M.C.M., Heijnen J.J.** (2001). Simultaneous storage and degradation of PHB and glycogen in activated sludge cultures. *Water Research*, **35**, 2693-2701.

[45] **Beun J.J., Paletta F., van Loosdrecht M.C.M, Heijnen J.J.** (2000). Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures. *Biotechnology and Bioengineering*, **67**, 496-507.

[46] **O'Connor J.T.,** (1971). *Environmental engineering unit operations and unit processes, Laboratory manual*. Association of Environmental Engineering Professors, U.S.A.

[47] **ISO 6060** (1986). *Water quality-determination of the chemical oxygen demand*. International Standards Organization, Switzerland.

[48] **American Public Health Association** (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed.; APHA: Washington, DC.

[49] **Braunegg G., Sonnleitner B., Lafferty R.M.** (1978). A rapid gas chromatographic method for the determination of pol-β-hydroxybutyric acid in microbial biomass. *Applied Microbiology and Biotechnology*, **6**, 29-37.

[50] **Beltran F.J., Aguinaco A., Garcia-Araya J.F., Oropesa A.** (2008). Ozone and photocatalytic processes to remove the antibiotic sulfamethoxazole from water. *Water Research*, **42**, 3799-3808.

[51] **Cokgor E.U., Insel G., Katipoglu T., Orhon D.** (2011). Biodegradation kinetics of peptone and 2,6-dihydroxybenzoic acid by acclimated dual microbial culture. *Bioresource Technology*, **102**, 567-575.

[52] **Karahan-Gul O., Artan N., Orhon D., Henze M., van Loosdrecth M.C.M.** (2002). Respirometric assessment of storage yield for different substrates. *Water Science and Technology*, **46**, 345-352.

[53] **Insel G., Yavasbaya A., Ozcan O., Cokgor E.U.** (2012). Modeling of aerobic growth and storage kinetics variation under unsteady feast conditions for heterotrophic biomass. *Bioprocess and Biosystems Engineering*, submitted.

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