ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

ACUTE AND CHRONIC EFFECTS OF SULFAMETHOXAZOLE ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT LOW SLUDGE AGE

M.Sc. THESIS

Gökçe KOR

Department of Environmental Engineering

Environmental Biotechnology Programme

JUNE 2012

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

ACUTE AND CHRONIC EFFECTS OF SULFAMETHOXAZOLE ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT LOW SLUDGE AGE

M.Sc. THESIS

Gökçe KOR
(501091820)

Department of Environmental Engineering

Environmental Biotechnology Programme

Thesis Advisor: Prof. Dr. Emine UBAY ÇOKGÖR
Co-advisor: Prof. Dr. Derin ORHON

JUNE 2012

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SÜLFAMETOKSAZOL'ÜN AEROBIK ORTAMDA DÜŞÜK ÇAMUR YAŞINDA ASETATIN BİYOLOJİK AYRIŞABİLİRLİĞİ ÜZERİNE AKUT VE KRONİK ETKİLERİ

YÜKSEK LİSANS TEZİ

Gökçe KOR
(501091820)

Çevre Mühendisliği Anabilim Dalı

ÇevreBiyoteknolojisi Programı

Tez Danışmanı: Prof. Dr. Emine UBAY ÇOKGÖR
Eş Danışmanı: Prof. Dr. Derin ORHON

HAZİRAN 2012

Gökçe KOR, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID 501091820, successfully defended the **thesis** entitled "**ACUTE AND CHRONIC EFFECTS OF SULFAMETHOXAZOLE ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT LOW SLUDGE AGE**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Emine UBAY ÇOKGÖR** İstanbul Technical University	.....
Co-advisor :	**Prof. Dr. Derin ORHON** İstanbul Technical University	.....
Jury Members :	**Prof. Dr. İzzet ÖZTÜRK** İstanbul Technical University	.....
	Prof. Dr. Zeynep Petek ÇAKAR İstanbul Technical University	.....
	Assist. Prof. Dr. Bilge ALPASLAN KOCAMEMİ Marmara University	.....

Date of Submission : 04 May 2012
Date of Defense : 06 June 2012

To my family,

FOREWORD

It was great pleasure for me to have two promoters: Prof. Dr. Derin ORHON and Prof. Dr. Emine UBAY ÇOKGÖR. I would like to express my sincere thanks for their support, patience, their inspiring comments and recommendations during each phase of my personal and academic life during the whole journey of my M.Sc. study. I am very lucky that I have a possibility to work with two senior professors in my field. I am grateful to Prof. Dr. Derin ORHON for his endless energy, creative and brilliant suggestions. I will always admire his intelligence and will always be grateful to him for teaching me how to think scientifically. I am indebted to Prof. Emine UBAY ÇOKGÖR for her valuable supervision, understanding and scientific guidance since the beginning of my graduate studies. She has been continually supportive and has provided encouragement at the times when it was most needed. I have learned a lot from her and there are still a lot to be learned.

I am deeply thankful to Assoc. Prof. Dr. Tuğba ÖLMEZ HANCI and Assoc. Prof. Dr. Nevin YAĞCI for their contributions and providing the resources naively for the inconveniences, I have encountered during my laboratory studies. I would like to acknowledge the contribution of Assoc.Prof. Dr. Güçlü İNSEL for providing clarifications during my studies.

I am genuinely thankful to Aslıhan URAL for ensuring that I am never alone in life and my studies. I would like to sincere thanks to Research Assisstant İlke PALA ÖZKÖK and Research Assisstant Tuğçe KATİPOĞLU YAZAN for their assistance in the laboratory and also for sharing lots of information with me.Many thanks to Dr. Aslı Seyhan ÇIĞGIN for her contributions, support, patience and for her time spent to read the reports.

Special thanks to my friends and laboratory colleagues. I would like to thank all my professors and researchers which are the staff of Istanbul Technical University, Environmental Engineering Department. I would like to sincere thanks to Senem TEKSOY BAŞARAN and Merve AYSEL for their encouragements and support.

Last, but not the least, I want to thank my mother Şükran KOR, my father Hayri KOR, my sister İrem KOR and my family for always being on my side, supporting me and trusting in me during my life.

Finally, I could not end without expressing my deepest love and gratitude to my intending husband, Osman A. BIÇAKCI for his love, endless patience and help during my life.

I would like to dedicate this thesis to my family.

April 2012 Gökçe KOR
(Environmental Engineer)

TABLE OF CONTENTS

Page

FOREWORD ..... **ix**
TABLE OF CONTENTS ..... **xi**
ABBREVIATIONS ..... **xiii**
LIST OF TABLES ..... **xv**
LIST OF FIGURES ..... **xvii**
SUMMARY ..... **xix**
ÖZET ..... **xxi**
1. INTRODUCTION **1**
1.1 Aim of the Thesis 2
1.2 Scope of the Thesis 2
2. LITERATURE REVIEW **5**
2.1 Pharmaceutical Industry 5
2.2 Antibiotics 9
2.2.1 Classification of antibiotics 10
2.2.1.1 Beta-Lactams 11
2.2.1.2 Macrolides 11
2.2.1.3 Fluroquinolones 11
2.2.1.4 Sulphonamides 12
2.2.1.5 Tetracyclines 14
2.2.1.6 Aminoglycosides 14
2.2.2 Significance of antibiotics in the environment 15
2.2.3 Resistance of antibiotics in the environment 16
2.3 Removal of Antibiotics 20
2.3.1 Sorption 21
2.3.2 Photolysis 22
2.3.3 Hydrolysis and thermolysis 22
2.3.4 Technical oxidation processes 22
2.3.5 Biodegradation 23
2.4 Enzyme Reactivity and Inhibition 24
2.4.1 Conceptual approach of enzymes in biological processes 24
2.4.2 Conceptual approach on enzyme inhibition 26
2.4.2.1 Competitive inhibition 27
2.4.2.2 Noncompetitive inhibition 29
2.5 Researches on the Biodegradation of Sulfamethoxazole in Activated Sludge Process 31
2.6 Modelling of Activated Sludge Systems 35
2.6.1 Activated Sludge Model No. 1 (ASM1) 36
2.6.2 Activated Sludge Model No. 3 (ASM3) 40
2.6.3 Substrate Storage Phenomena 44
2.6.3.1 Polyhydroxyalkanoates (PHAs) 45

2.6.3.2 Polyhydroxybutyrate (PHB) 47
2.6.4 Metabolic models 49
2.6.5 Effects of SRT on storage kinetics 59
3. MATERIALS AND METHODS 63
3.1 Reactor Setup 63
3.2 Experimental procedure 64
3.3 Analytical procedures 69
3.3.1 Standard methods 69
3.3.2 Antibiotic measurements 70
4. RESULTS AND DISCUSSION 71
4.1 Acclimation Studies of Control Reactor 71
4.2 Experimental Results 73
4.2.1 Evaluation of control experiment 73
4.2.2 Evaluation of acute experiments 75
4.2.3 Evaluation of chronic experiments 82
4.3 Modelling of Experimental Results 95
4.3.1 Modelling of control and acute experiments 95
4.3.2 Modelling of chronic experiments 102
5. CONCLUSION 107
REFERENCES 109
CURRICULUM VITAE 123

ABBREVIATIONS

Acetyl-CoA : Acetyl-Coenzyme A
ASM1 : Activated Sludge Model No. 1
ASM3 : Activated Sludge Model No. 3
ASMG : Activated Sludge Model for Growth and Storage
ATP : Adenosine Triphosphate
$\mathbf{b_H}$: Endogenous Respiration Rate of Heterotrophs [T^{-1}]
BOD : Biochemical Oxygen Demand
$\mathbf{b_{STO}}$: Endogenous Respiration Rate of Storage Products [T^{-1}]
CoA : Free Coenzyme A
COD : Chemical Oxygen Demand
Cs : BiodegradableCOD Concentration [M COD.L^{-3}]
CSTR : Continuously Stirred Reactor
$\mathbf{C_T}$: Initial Total COD Concentration [M COD.L^{-3}]
DNA : Deoxyribonucleic Acid
DO : Dissolved Oxygen
$\mathbf{f_{ES}}$: Fraction of Soluble Metabolic Products
$\mathbf{f_{EX}}$: Fraction of Particulate Metabolic Products
$\mathbf{f_P}$: Fraction of Particulate Inert COD Generated in Biomass Decay
HAs : Hydroxyalkanoic Acids
HPLC : High-phase Liquid Chromatography
$\mathbf{k_{STO}}$: Maximum Storage Rate [T^{-1}]
$\mathbf{k_h}$: Maximum Specific Hydrolysis Rate [T^{-1}]
$\mathbf{K_I}$: Competitive Inhibition Coefficient
$\mathbf{K_M}$: Michaelis-Menten Coefficient
$\mathbf{K_S}$: Half Saturation Constantfor Growth of X_H[M.L^{-3}]
$\mathbf{K_{S1}}$: Half Saturation Constant for Direct Growth [M.L-3]
$\mathbf{K_{S2}}$: Half Saturation Constant for Growth on Stored Polymers [M.L-3]
$\mathbf{K_{STO}}$: Half Saturation Constant for Storage [M COD.(M COD)$^{-1}$]
$\mathbf{K_X}$: Half Saturation Constant for the Hydrolysis[M.L^{-3}]
MLSS : Mixed Liquor Suspended Solid [M.L^{-3}]
$\mathbf{m_P}$: Maintenance Coefficient for Growth on PHB
NADH : Nicotinamide Adenine Dinucleotide
OUR : Oxygen Utilization Rate [M.L^{-3}.T^{-1}]
PHA : Polyhydroxyalkanoate
PHB : Poly-β-hydroxybutyrate
PHV : Poly-β-hydroxyvalerate
PPCPs : Pharmaceuticals and Personal Care Products
$\mathbf{q_C}$: Specific Carbon dioxide Production Rate [M.(M COD.T)$^{-1}$]
$\mathbf{q_O}$: The Specific Oxygen Consumption Rate [M.(M COD.T)$^{-1}$]
$\mathbf{q_{p(g)}}$: Specific Storage Product Formation Rate[M.(M COD.T)$^{-1}$]
$\mathbf{-q_{p(g)}}$: Specific Storage Product Consumption Rate [M.(M COD.T)$^{-1}$]
$\mathbf{-q_S}$: Specific Substrate Removal Rate [M.(M COD.T)$^{-1}$]

RNA : Ribonucleic Acid
S_{AC} : Acetate Concentration [M COD.L^{-3}]
SBR : Sequencing Batch Reactor
S_H : ReadilyHydrolysable COD Concentration[M COD.L^{-3}]
S_I : Soluble Inert COD Concentration [M COD.L^{-3}]
STP : Sewage Treatment Plant
S_O : Dissolved Oxygen Concentration [M O_2.L^{-3}]
SRT : Sludge Age[T]
S_S : Readily Biodegradable COD Concentration [M COD.L^{-3}]
SMX : Sulfamethoxazole
TCA : Tricarboxylic Acid Cycle
ThOD : Theoretical Oxygen Demand
VFA : Volatile Fatty Acid
VSS : Volatile Suspended Solid
WWTP : Wastewater Treatment Plant
X_H : Heterotrophic Biomass Concentration [M COD.L^{-3}]
X_I : Particulate Inert COD Concentration [M COD.L^{-3}]
X_P : Inert Microbial Product Concentration [M COD.L^{-3}]
X_S : Slowly Biodegradable COD Concentration [M COD.L^{-3}]
X_{STO} : Storage Polymer Concentration [M COD.L^{-3}]
Y_H : Heterotrophic Growth Yield [M COD.(M COD)$^{-1}$]
Y_{H1} : Yield Coefficient for Direct Growth [M COD.(M COD)-1]
Y_{H2} : Yield Coefficient for Growth on Stored Polymers [M COD.(M COD)-1]
Y_{obs} : Heterotrophic Observed Yields [M COD.(M COD)$^{-1}$]
Y_{PX} : Yield of Biomass on PHB [M COD.(M COD)$^{-1}$]
Y_{SP} : Yield of PHB on acetate [M COD.(M COD)$^{-1}$]
Y_{STO} : Storage Yield [M COD.(M COD)$^{-1}$]
Y_{SX} : Yield of Biomass on Acetate [M COD.(M COD)$^{-1}$]
δ : Electron Acceptor
μ_{Hmax} : Maximum Spesific Growth Rate for X_H [T^{-1}]
μ_{H1} : Maximum Spesific Growth Rate for Direct Growth [T^{-1}]
μ_{H2} : Maximum Spesific Growth Rate for Secondary Growth [T^{-1}]
μ_{STO} : Maximum Spesific Growth Rate for Growth on PHA [T^{-1}]
υ : Enzymatic Transformation Rate

LIST OF TABLES

Page

Table 2.1 : Classification of antibiotics.....14
Table 2.2: The fate of different antibiotics in WWTPs and in hospital effluents (Avella et al, 2010).....34
Table 2.3 : Processes for carbon oxidation described in ASM1 (Henze et al, 1987)...37
Table 2.4 : Typical parameter values at neutral pH. 39
Table 2.5 : The simplified ASM3 for organic carbon removal under aerobic conditions. 41
Table 2.6 : Typical stoichiometric parameters of ASM3. 42
Table 2.7 : Typical values for kinetic parameters of ASM3. 42
Table 2.8 : Matrix representation of simplified ASM3 (Krishna and van Loosdrecht 1999).....55
Table 2.9 : Matrix representation of the model structure for simultaneous growth and storage (Orhon et al, 2009b).....57
Table 2.10 : Kinetic and stoichiometric parameters of ASM3 and modified ASM3...58
Table 2.11 : Experimentally found storage yield values for different SRT. 59
Table 3.1 : Composition of solution acetate, A and B. 65
Table 3.2 : Monitored data for experimental runs.....66
Table 3.3 : Outline of control and acute experiments at a sludge age of 2 days.....67
Table 3.4 : Outline of chronic experiments at a sludge age of 2 days. 68
Table 4.1 : Steady state characteristics of the control reactor.....71
Table 4.2 : Control and acute experiments at a sludge age of 2 days. 75
Table 4.3 : Control and chronic experiments at a sludge age of 2 days.....84
Table 4.4 : Matrix representation of modified ASM3 model structure in control and acute experiments.....96
Table 4.5 : Calibration results of the control and acute experiments.....101
Table 4.6 : Matrix representation of modified ASM3 model structure in chronic experiments. 103
Table 4.7 : Calibration results of the chronic experiments. 105
Table 4.7 : Calibration results of the chronic experiments (cont.).....106

LIST OF FIGURES

Page

Figure 2.1: Origins and fate of PPCPs in the environment (Url-1, 2012). 8
Figure 2.2: Sulfamethoxazole molecular configuration (Url-2, 2011). 13
Figure 2.3: Distribution of antibiotics in the environment (Kümmerer, 2003). 17
Figure 2.4: Enzyme in a lock-and-key model (Url-3, 2011).....25
Figure 2.5: Effect of substrate concentration (S) on enzymatic transformation rate (υ) based upon Michaelis-Menten kinetics. When S equals K_m, υ is one-half of the maximum rate υ_m. 25
Figure 2.6 : Competitive inhibition (Url-4, 2011). 28
Figure 2.7 : Typical oxygenase enzyme reactions for growth-substrate and co-metabolic (non-growth) substrate (Chang and AlvarezCohen,1995b). ...29
Figure 2.8 : Noncompetitive inhibition (Url-4, 2011). 30
Figure 2.9 : Comparison of competitive and noncompetitive inhibitors. 30
Figure 2.10 : Death regeneration process in ASM1 (Orhon and Artan, 1994). 39
Figure 2.11 : Differences between ASM1 and ASM3. 43
Figure 2.12 : General structure of PHA (R: methyl: PHB and R: ethyl: PHV). 48
Figure 2.13 : PHB production pathways in feast/famine conditions (adapted from Salehizadeh and Van Loosdrecht, 2004). 49
Figure 2.14 : Schematic representation of the metabolism of an organism capable of forming and consuming PHB (van Aalst-van Leeuwen et al, 1997a). ...50
Figure 2.15 : Metabolic route of biomass (a) in ASM3 and (b) metabolic model proposed Krishna and Van Loosdrecht (1999). 56
Figure 3.1 : A laboratory-scale control reactor. 64
Figure 3.2 : Applitek RA-Combo-1000 continuous respirometer. 66
Figure 3.3 : Chronic experiment reactor with SMX feeding. 68
Figure 3.4 : SMX calibration curve for obtained for HPLC measurements. 70
Figure 4.1 : SS and VSS results of the control reactor. 72
Figure 4.2 : VSS/SS ratio of the control reactor. 72
Figure 4.3 : pH measurements of the control reactor.....72
Figure 4.4 : Influent and effluent soluble COD concentrations of the control reactor. ..73
Figure 4.5 : OUR profile of the control experiment versus time (Run 1).....74
Figure 4.6 : Soluble COD profile of the control experiment versus time (Run 1).....74
Figure 4.7 : OUR profile versus time (Run 2). 76
Figure 4.8 : Removal efficiencies of Run 2 (a) soluble COD and acetate profiles (b) SMX profiles.....77
Figure 4.9 : OUR profile versus time (Run 3). 77
Figure 4.10 : Removal efficiencies of Run 3 (a) soluble COD and acetate profiles (b) SMX profiles.....78
Figure 4.11 : OUR profile versus time (Run 4). 79
Figure 4.12 : Removal efficiencies of Run 4 (a) soluble COD and acetate profiles (b) SMX profiles.....79
Figure 4.13 : Soluble COD & SMX profiles of Run 5 (SMX-200 mg/L). 80

Figure 4.14 : Removal efficiencies of Run 5 (a) soluble COD and acetate profiles (b) SMX profiles. 81
Figure 4.15 : Comparison of OUR profiles of all acute experiments with the control... 81
Figure 4.16 : SS,VSS and VSS/SS ratio results of the 12 L chronic reactor..... 82
Figure 4.17 : SMX effluent concentrations of the 12 L chronic reactor. 83
Figure 4.18 : OUR profile versus time (Run 6)..... 85
Figure 4.19 : Removal efficiencies of Run 6 (a) soluble COD and acetate profiles (b) SMX profiles. 86
Figure 4.20 : OUR profiles versus time (Run 7). 86
Figure 4.21 : Removal efficiencies of Run 7 (a) soluble COD and acetate profiles (b) SMX profiles. 87
Figure 4.22 : OUR profile versus time (Run 8)..... 88
Figure 4.23 : Removal efficiencies of Run 8 (a) soluble COD and acetate profiles (b) SMX profiles. 88
Figure 4.24 : OUR profiles versus time (Run 9.1, Run 9.2 and Run 9.3). 89
Figure 4.25 : Removal efficiencies of Run 9.1 (a) soluble COD and acetate profiles (b) SMX profiles..... 90
Figure 4.26 : Removal efficiencies of Run 9.2 (a) soluble COD and acetate profiles (b) SMX profiles. 90
Figure 4.27 : Removal efficiencies of Run 9.3 (a) soluble COD and acetate profiles (b) SMX profiles..... 91
Figure 4.28 : OUR data versus time (Run 10.1 and Run 10.2). 92
Figure 4.29 : Soluble COD & SMX profiles of Run 10.1. 92
Figure 4.30 : OUR data versus time (Run 11 and Run 11.1). 93
Figure 4.31 : COD removal efficiencies of Run 11.1 and Run 11.2. 93
Figure 4.32 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate)..... 94
Figure 4.33 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate)..... 94
Figure 4.34 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate)..... 95
Figure 4.35 : Model simulation of OUR profile for Run 1. 97
Figure 4.36 : Model simulation of PHB data for Run 1. 97
Figure 4.37 : Model simulation of OUR profile for Run 2. 98
Figure 4.38 : Model simulation of PHB data for Run 2. 98
Figure 4.39 : Model simulation of OUR profile for Run 3. 99
Figure 4.40 : Model simulation of PHB data for Run 3. 99
Figure 4.41 : Model simulation of OUR profile for Run 4. 100
Figure 4.42 : Model simulation of PHB data for Run 4. 100
Figure 4.43 : Model simulation of OUR profile for Run 8. 104
Figure 4.44 : Model simulation of OUR profile for Run 9.1. 104
Figure 4.45 : Model simulation of OUR profile for Run 11.1. 105

ACUTE AND CHRONIC EFFECTS OF SULFAMETHOXAZOLE ON THE BIODEGRADATION OF ACETATE UNDER AEROBIC CONDITIONS AT LOW SLUDGE AGE

SUMMARY

The global distribution of pharmaceuticals in terrestrial or aquatic environment has become one of the main environmental problems in the last decade. Among all other pharmaceutical drugs and substances, antibiotics are one of the major groups of pharmaceuticals. Parallel to the increase in the extensive usage of antibiotics, it is unavoidable that these chemicals are released into the environment. Therefore, the adverse effect on the environment and health brought from the processing and disposal of these products become important issue in the field of environmental engineering.

Very large numbers of antibiotics are used in human and veterinary medicine and also used to promote the growth of animals to improve the quality of the product. Antibiotics are industrially produced and commercially used, but they are not required to undergo the extensive level of testing for possible environmental impact. Antibiotics generally remain in the effluent of wastewater treatment plants due to difficult treatability with conventional treatment systems such as aerobic activated sludge system. The presence of these organic contaminants and their primary degradation products in the influents of wastewater treatment plants(WWTPs) may inhibit biological processes. For this reason, these substances are becoming an increasingly large problem in WWTPs, they also spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems.

In this context, the objective of this study was to investigate acute and chronic inhibition effects of antibiotic substances on the biodegradation of carbon source. Thus, the sulfamethoxazole was chosen as model antibiotic due to abundantly used antibiotic compound in the world and in Turkey. The sulfamethoxazole represents sulfonamide group of antibiotics and it blocks bacterial cell metabolism by inhibiting enzymes.

Acetate used as a readily biodegradable carbon source in this study. The system operated at steady state conditions as namely “control reactor”. In other words, the inhibitory impact of sulfamethoxazole on the biodegradation of acetate by an acclimated microbial culture was investigated under aerobic conditions at a sludge age of 2 d. Substrate was added once a day by pulse feeding and biomass was acclimated to pulse feeding pattern in the aerobic batch reactor.

After steady state conditions were established in the system, the acclimated biomass culture was taken to perform respirometric tests. Acetate was added on the biomass to have the same S_0/X_0 ratio with the control reactor and oxygen uptake rate was monitored for the assessment of substrate biodegradation behaviour.

Additional respirometric experiments were conducted on the activated sludge to determine acute and chronic inhibition effects of antibiotics. The antibiotic was

givento system with different pulse feedings of 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of SMX concentrations to evaluate the acute effects on the carbon removal performance, however the chronic experiments were done with continuous feeding of 50 mg/L of SMX concentration.

Moreover, it is also aimed to determine the responses of the activated sludge culture to antibiotic substances by means of modeling tools which enables to determine the kinetic and stoichiometric constants of the mixed culture. Therefore, relevant kinetic and stoichiometric coefficients were achieved using modified ASM3 pattern in the study.According to model simulations results of acute experiments, maximum specific growth rate (μ_{Hmax}) decreased whereas the half saturation coefficient (K_S) increased with the addition of high concentration of SMX to the system. In addition, the maximum specific growth rate for storage (μ_{STO}) also decreased in these system. On the other hand, model simulations results of chronic experiments indicated that, only a portion of acetate was utilized by the microorganisms in the growth process. The other portion of acetate made a complex with antibiotic that was consumed as readily hydrolysable COD fraction in the hydrolysis process. Approximately 40 mg COD/L antibiotic was removed throughout the chronic experiments with a specific hydrolysis rate.

As a consequence, all acute and chronic results under aerobic conditions at a sludge age of 2 d demonstrated that the metabolism of the activated sludge was shifting from storage to growth. The biomass prefers to direct growth process rather than storage process.

SÜLFAMETOKSAZOL'ÜN AEROBİK ORTAMDA DÜŞÜK ÇAMUR YAŞINDA ASETATIN BİYOLOJİK AYRIŞABİLİRLİĞİ ÜZERİNE AKUT VE KRONİK ETKİLERİ

ÖZET

İlaçların yeryüzünde genel dağılımı, son on yıllık süre içinde en önemli çevre problemlerinden biri olarak karşımıza çıkmaktadır. Tıbbi ilaç, insanlarda ve hayvanlarda hastalıklardan korunma, tanı, tedavi veya bir fonksiyonun düzeltilmesi ya da insan (hayvan) yararına değiştirilmesi için kullanılan, genelde bir veya daha fazla yardımcı maddeler ile formüle edilmiş etkin madde(ler) içeren bitmiş dozaj şekli olarak tanımlanabilir. Son yıllarda yapılan çalışmalarda sucul ve diğer ortamlarda (çevresel ortam) tıbbi ilaçlardan kaynaklanan mikrokirleticilerin görülmesi ve bunların bu ortamlarda yaşayan canlılara olan olumsuz etkilerinin ortaya konması, endişeleri arttırmıştır. Yapılan çalışmalarda; cinsiyet oranlarının değişmesi, cinsiyet tersinimleri, popülasyonların azalması, yumurtlama ve canlı kalma oranlarının azalması gibi sonuçlar organizmalar üzerinde ilaçlardan kaynaklanan ve tespit edilen bazı etkiler arasındadır.

Günümüzde ilaçların kullanım sonucu çevresel ortamlardaki taşınımı, bozunumu, dönüşümü, miktarları ve nihai akıbetleri konularında sınırlı bilgi mevcuttur. Son on yıllık süre içinde mevcut ilaçların sucul ortamlarda tespit edilmeleri, bu ilaçların daha önceleri sucul ortamlarda bulunmadığı anlamına gelmemektedir. Daha hassas ve daha düşük konsantrasyonları ölçülebilen yeni bilimsel metotların ve analitik ölçüm yöntemlerinin geliştirilmesi ile bu ilaçların oluşturabilecekleri olumsuz etkiler yavaş yavaş ortaya konmaya başlanmıştır.

Gelişmiş ülkelerde tıbbi kimyasal için uluslararası düzeyde detaylı mevzuatlar mevcut değildir. Ancak ürkütücü olabilecek potansiyel etkilerinden dolayı gittikçe popüler hale gelen bu konuda özellikle gelişmiş ülkelerde toplum baskısı artmaktadır. Dolayısıyla, yakın gelecekte tıbbi kimyasal olmasından şüphe edilen birçok sentetik ilaç için, yeni ve çok sıkı mevzuatların getirilmesi ve buna bağlı olarak da atıksu arıtma tesisi deşarj standartlarının da çok daha sıkı olması beklenmektedir.

Dünyada ve ülkemizde en çok tüketilen ilaç gruplarının başında antibiyotikler gelmektedir. Yunanca anti (karşı) ve bios (yaşam) sözcüklerinden türetilen antibiyotik sözcüğü; küf mantarlarında bulunan ya da yapay olarak üretilen, bakteri ve diğer mikroorganizmaların gelişimini durduran ya da onları yok eden maddelerin ortak adı olarak tanımlanmaktadır. 19. yüzyılın ortalarında Louis Pasteur bir mikroorganizmanın diğer bir mikroorganizmanın büyümesini engellediğini keşfetmiştir. Bu keşfi ise uygulama alanına ilk koyan kişi Sir Alexander Fleming olmuştur. 1928 yılında Fleming belirli bir küf (Penicillium notatum) tarafından enfekte olan kaplarda bakterilerin gelişmesinin durduğunu raslantı sonucu keşfederek, küf tarafından salınan bir madde ile bakterilerin öldüğü sonucunu çıkarmıştır. Üreyen küf mantarlarının Penisillinin türünden oluşundan esinlenerek, etkili maddeye 'penisilin' adını vermiştir. 1940 yılında penisilinin yüksek etkinliğinin deneysel olarak

kanıtlanmasından sonra, değişik gruplardan da birçok antibiyotik geliştirilmiştir. Bulaşıcı bakteri ve mantarlarla savaşmak için yararlanılan antibiyotikler 1940'dan bu yana kullanılmaktadır. Antibiyotikleri çeşitli kriterlere göre sınıflandırmak mümkündür. Antibiyotikler, mikroorganizmalar üzerindeki etki derecelerine, etki mekanizmalarına, kimyasal yapılarına ve farmakokinetik özelliklerine göre çeşitli şekillerde sınıflandırılabilir.

Antibiyotiklerin birçoğu veterinerlik ve tıp alanında kullanılmakta, ayrıca ürün kalitesini geliştirmek için hayvanların büyümesinde besin olarak da verilmektedir. Avrupa birliği ülkelerinde insan ve veteriner ilaç yapımında yaklaşık 4.000 farklı etken madde kullanılmaktadır. Antibiyotikler endüstriyel olarak üretilip, ticari olarak kullanılsa da, onların çevreye olan olası etkileri önemli bir ölçüde değerlendirilmemiştir.

Gelişmiş ülkelerde ilaçların tüketim sıralamasında antibiyotikler 5. sırada yer alırken, ülkemizde (%16,3) birinci sırada bulunmaktadır. Ülkemizde 40 dolar olan yıllık kişi başına ilaç tüketiminin %21'ini antibiyotikler ve benzeri ilaçlar oluşturmaktadır. Bu da ülkemizde antibiyotiklerin genel ilaç giderleri arasındaki payının dünya standartlarından yaklaşık 2,5 kat fazla olduğunu göstermektedir. Bu fazla tüketime ek olarak antibiyotik uygulamalarının yaklaşık %50'sinin uygunsuz olduğu bilinmektedir. Türk Eczacılar Birliği'nin verilerine göre, son beş yılda yaklaşık 1,5 milyar kutuya yakın antibiyotik tüketilmiştir. Ayrıca bu verilere; yüzde 80'ni hayvanlara verilen ve dolayısıyla hayvanlardan insanlara geçen antibiyotikler ile doktorlara promosyon olarak verilen antibiyotikler dâhil değildir. Bu kimyasalların gereksiz ve fazla kullanımının artmasıyla, antibiyotikler kaçınılmaz bir şekilde çevreye yayılmaktadır. Bu nedenle, antibiyotiklerin işlenmesinin ve bertarafının çevreye ve sağlığa negatif etkileri çevre mühendisliği alanında önemli bir sorun haline gelmiştir.

Hastahaneler, ilaç endüstrileri ve evler antibiyotiklerin başlıca kirletici kaynakları arasında yer almaktadır. Özellikle bilinçsiz kullanım sonucu giderek kullanımı artan antibiyotikler biyolojik ayrışmaya karşı dirençli olup, insan vücudu tarafından dışkı ve üre ile atılarak, evsel nitelikli atıksu arıtma tesislerine taşınmaktadır. Dolayısıyla evsel nitelikli atıksu arıtma tesisleri ortama antibiyotik etken maddesi veren ciddi birer işletme haline gelmiştir. Şuanda mevcut olan atıksu arıtma tesisleri büyük oranda, tesise düzenli olarak gelen organik maddelerin (öncelikle karbon, azot, fosfor gibi nütrientlerin) giderimi üzerine projelendirilmiştir. Aerobik aktif çamur sistemi gibi geleneksel arıtma sistemleri bu tür maddeleri gidermek için tasarlanmadığından çeşitli sorunlar da beraberinde gelmektedir. Atıksu arıtma tesislerinde antibiyotiklerin farklı kimyasal yapılarından dolayı farklı oranlarda arıtıldığı veya hiç arıtılamadığı bilinmektedir. Ayrıca bu organik kimyasalların ve onların birincil yıkım ürünlerinin atıksu arıtma tesislerinin giriş sularında bulunması biyolojik prosesler açısından inhibisyon etkisi oluşturabilir. Bu nedenle bu maddeler atıksu arıtma tesislerinde büyük bir sorun haline gelmiştir.

Atıksu arıtma tesislerinde bulunan antibiyotiklerin giderim oranları üzerindeki çalışmalar çoğunlukla giriş ve çıkış atıksuyu konsantrasyonlarının ölçülmesi esasına dayanmaktadır. Giderim oranları; kullanılan arıtma teknolojisine, hidrolik bekletme süresine, mevsimsel koşullara ve arıtma tesisinin performansına bağlı olarak büyük değişiklikler göstermektedir. Bu nedenle antibiyotikler ekosistemde ve biyolojik arıtma sistemlerinde organizmalar için toksisite etki oluşturarak ekolojik dengeyi de bozmaktadır. Kısmen veya hiç arıtılamadan alıcı ortama verilen antibiyotikler nehirlerin, göllerin, denizlerin ve sıklıkla da yeraltı ve içmesularının kirlenmesine neden olmaktadır. Gelişen bilimsel metotlar ve analitik ölçüm yöntemleriyle birlikte;

daha önceden tespit edilemeyen birçok mikrokirletici tespit edilmeye başlanmıştır. Bununla birlikte mevcut analitik yöntemlerle tespit edilemeyen birçok kirleticinin olduğu ve mevzuatlarla da sınırlandırılamadığı için düşük konsantrasyonlarda alıcı ortamlara doğrudan deşarj edildiği düşünülmektedir.

Antibiyotiklerin diğer bir kirletici kaynağı ise; veteriner ilaçlarının kullanımı sonucu oluşan gübrelerin tarımda kullanılması ile meydana gelmektedir. Tarımda kullanılan veya araziye serilen bu gübreler ve arıtma çamurları toprağa sızarak yeraltı sularına, nihai olarak da içme sularına ulaşmakta ve kirliliğe neden olmaktadır. Bu maddeler mikrobiyal ekolojiyi etkileyerek, antibiyotiklere dayanıklı patojenlerin üremesinin artmasına neden olmakta ve böylelikle başta insanlar olmak üzere hayvanlar ve bitkiler üzerinde olumsuz etkiler yaratmaktadır. Günümüze kadar çeşitli Avrupa ülkelerinde yapılan çalışmalarda arıtma tesisi çıkış sularında, yüzeysel ve yeraltı sularında 100'ün üzerinde ilaç bileşimine rastlandığı bildirilmiştir. Bu tip alıcı ortamdaki canlılara toksik etki yapabilecek bileşiklerin potansiyel toksik etkileri üzerine pek fazla çalışma yapılmamış olduğu da dikkat çekicidir. Yapılan araştırmalarda sistemde ya sadece kollektif parametrelerin ölçüldüğü ya da sadece antibiyotik konsantrasyonlarının izlendiği görülmektedir.

Antibiyotiklerin çevresel ortamda canlılar üzerindeki etkileri akut ve kronik etkiler olmak üzere iki farklı alanda değerlendirilebilir. Akut etki, antibiyotiklerin tek bir doz uygulaması sonucunda ve genellikle kısa bir süre içerisinde meydana getirdiği zarar olarak ifade edilir. Kronik etki ise, uzun süre boyunca canlıların bu kimyasal maddelere maruz bırakılması ile belirlenir. Zamanın belirli periyodlarında tekrar tekrar kullanılan küçük dozlardan ortaya çıkan her türlü zarar kronik etki olarak ifade edilir. Bazı antibiyotiklerin akut toksisite etkileri hakkında yapılan çalışmalarda çeşitli tedavi gruplarından farklı ilaçların toksisite verileri elde edilmiştir. Yapılan akut toksisite deneylerinde, sucul organizmalara akut etki yapan konsantrasyonların, ilaç kalıntılarından dolayı çevresel ortamlarda bulunan konsantrasyonlardan 100-1.000 kat daha yüksek olduğu görülmüştür. Bu nedenle ilaçların doğrudan sucul ortamlara dökülmesi durumunda, sucul canlılara akut toksisite tehditi oluşturacağı ifade edilmektedir. Bunun yanı sıra, birçok sucul ortamda yaşayan canlı türleri uzun süreli veya tüm yaşamı boyunca antibiyotik gibi mikrokirleticilerin etkisinde kalmış da olabilir. Bu nedenle bu mikrokirleticilerin kronik potansiyel etkilerinin değerlendirilmesi önem arz etmektedir. Ancak antibiyotiklerin kronik etkileri hakkında çok az veri bulunmakta olup, bunların kronik toksisite etkileri genellikle bilinmemektedir. Bu konuda çok daha fazla spesifik çalışmaların yapılması gerekmektedir.

Bu çerçevede, bu çalışmanın amacı; aktif çamur sistemlerinde antibiyotik mekanizmasının ortadan kaldırılmasının ve bu kimyasalların karbon kaynağı biyodegradasyonu üzerindeki akut ve kronik inhibisyon etkilerinin araştırılmasıdır. Bu nedenle, dünyada ve Türkiye'de yaygın olarak kullanılan sülfametoksazol model antibiyotik olarak seçilmiştir. Antibiyotik sınıflandırılmasında sülfonamid grubunu temsil eden sülfametoksazol, bakterinin bünyesinde olan enzimleri inhibe ederek metabolizmalarını bloke eder. Literatürde yapılan çalışmalarda sülfametoksazolün giderimi ile ilgili olarak kesin bir bilgi bulunmamaktadır.

Substrat aerobik kesikli reaktörde ani besleme ile günde bir defa sisteme eklenmiş ve biyokütle ani beslemeye aklime edilmiştir. Sülfametoksazolün, karbon kaynağına aklime olan bir aerobik mikrobiyal kültürde subsrat ayrışması üzerine olan etkilerinin araştırılması hedeflenmiştir. Bu kapsamda, asetat biyolojik olarak kolay ayrışabilir bir karbon kaynağı olarak kullanılmıştır. Çalışmanın başında iki çamur yaşında bir adet

doldur-boşalt tipi reaktör kurulmuş ve reaktör asetat karışımı ile beslenerek kararlı denge haline ulaştırılmıştır. Kontrol reaktörü adı verilen biyokütle kaynağı tüm deneysel çalışma boyunca çalıştırılmıştır. Daha sonra, sülfametoksazolün aerobik ortamda iki gün çamur yaşında asetatın biyolojik ayrışabilirliği üzerine akut ve kronik inhibisyon etkileri araştırılmıştır.

Sistemde kararlı denge koşulları sağlandıktan sonra, aklime olan biyokütle respirometrik deneyleri gerçekleştirmek için reaktörden alınmıştır. Asetat ve biyokütle karışımı, kontrol reaktörüyle aynı besin ve biyokütle oranıyla respirometreye eklenmiştir. Oksijen tüketim hızı, substrat biyodegradasyonu değerlendirmek amacıyla deney boyunca takip edilmiştir.

Respirometre deneyleriyle ayrıca antibiyotiğin aktif çamur sistemine akut ve kronik etkileri değerlendirilmiştir. Antibiyotiğin karbon giderimi üzerindeki akut inhibisyon etkilerini araştırmak için respirometreye 25 mg/L, 50 mg/L, 100 mg/L ve 200 mg/L'lik dört farklı antibiyotik konsantrasyonu anlık besleme olarak eklenmiştir. Akut inhibisyon etkileri 5 set deneysel çalışma ile gerçekleştirilmiştir.

Ayrıca aktif çamur sisteminin antibiyotiklere kronik maruz kalmalarının etkilerinin incelenmesi amacıyla, bir adet daha doldur-boşalt tipi reaktör işletilmiş ve farklı günlerde yapılan deneylerle toplam 10 set deneysel çalışma gerçekleştirilmiştir. Kurulan kronik reaktör 50 mg/L'lik sülfametoksazol konsantrasyonu ile sürekli olarak beslenmiştir. Deneysel çalışmalarda ise karışık substrat (antibiyotik ile asetat), yalnız asetat ve yalnız antibiyotik olmak üzere üç farklı karbon kaynağı uygulanmıştır.

Akut ve kronik çalışmalardaki substrat tüketimi, respirometre deneyleriyle ulaşılan oksijen tüketim profillerinin gözlemlenmesi ile incelenmiş ve antibiyotik etkisi altında olmayan kontrol reaktörünün oksijen tüketim profili ile model simülasyonlarından yararlanılarak karşılaştırılmıştır. Model simülasyon sonuçları ile gerekli kinetik ve stokiyometrik katsayılar modifiye edilmiş ASM3 modeli kullanılarak belirlenmiştir.

Akut inhibisyon deneylerinin sonuçları karşılaştırıldığında, antibiyotik konsantrasyonunun arttırılmasıyla; maksimum heterotrofik çoğalma hızı (μ_{Hmax}) azalırken, heterotrofik yarı doygunluk sabiti (K_S) artmaktadır. Ayrıca, maksimum depolama hızı (μ_{STO}) da deneyler sırasında azalmıştır. Diğer taraftan kronik inhibisyon deneylerinin sonuçları değerlendirildiğinde, asetatın belli bir kısmı çoğalma prosesinde tüketilmiş, kalan kısmı ise antibiyotik ile kompleks oluşturarak hızlı hidroliz olabilen yavaş ayrışabilir çözünmüş KOİ olarak tüketilmiştir. Tüm kronik deney boyunca yaklaşık olarak 40 mg KOİ/L antibiyotik belirli bir hidroliz hızıyla giderilmiştir. Tüm akut ve kronik inhibisyon sonuçları değerlendirildiğinde; aktif çamur metabolizmasının depolama prosesinden çoğalma prosesine doğru kaydığı saptanmıştır.

1. INTRODUCTION

Life on this planet is based on the continuous cycling of elements. In recent years the massive mobilization of natural resources and the industrial synthesis of chemicals have generated a number of environmental problems as a consequence of the limited incorporation of natural and synthesized molecules into ongoing biological cycles. This is particularly true for pharmaceutical compounds, which are accumulated or induce adverse effects in terrestrial or aquatic organisms (Kümmerer, 2009a).

The global distribution of pharmaceuticals in the aquatic environment has become one of the main environmental problems in the last decade (Zuccato et al, 2005). Among all pharmaceutical drugs and substances, antibiotics are one of the major groups of pharmaceuticals due to its serious irreversible increase in the release to the environment. 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 (Gotvajn et al, 2007).Antibiotics are also used to promote the growth of animals where they are used at low doses in animal feeds and are considered to improve the quality of the product.

Antibiotics generally remain in the effluent of wastewater treatment plants due to difficult treatability with conventional treatment systems such as aerobic active sludge systems. For that reason, they spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems.The biodegradability and adverse effects of antibiotic compounds in engineered systems was investigated in a number of studies which focused on their adverse effects on treatment systems. Recent works have reported the presence of a large variety of antibiotics in WWTP effluents and surface waters, with concentrations up to several μg/L. According to literature, antibiotic concentrations in pharmaceutical wastewaters are reported to increase up to 200 mg/L (Ozkok et al, 2011; Drillia et al, 2005). 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. The sulfamethoxazole was chosen as model antibiotic to represent sulfonamide group of antibiotics. This substance, which is among the abundantly used antibiotic compounds in the world and in Turkey (Kümmerer and Henninger, 2003).

1.1 Aim of the Thesis

The aim of this study is to investigate acute and chronic effects of sulfamethoxazole on the biodegradation of acetate under aerobic conditions at low sludge agein the activated sludge system. After steady state conditions were established in the system, activated sludge culture was taken to perform respirometric tests for acetate removal mechanisms and acute and chronic effects of antibiotic substance. In literature, the majority of the studies examined effects of lower concentrations of antibiotics characterizing wastewater generated after usage of these compounds. However, control of antibiotics at source, in the effluent of the pharmaceutical industry is equally important. Therefore, in this study, higher antibiotic concentrations were chosen in order to represent antibiotic production wastewaters.

Finally, the biochemical processes occurring under inhibiting and non-inhibiting conditions were modelled with the aid of the results obtained from experimental studies. It is also aimed to determine the responses of the activated sludge culture to antibiotic substances by means of modelling tools which enables to determine the kinetic and stoichiometric constants of the mixed culture.

1.2 Scope of the Thesis

This study was started up with the set up a laboratory-scale fill and draw reactor, namely “control reactor”. The control reactor was operated at a sludge age of 2 days with the initial carbon source concentration of approximately 400 mg COD/L. Acetate representing readily biodegradable substrate was chosen as sole carbon source. The reactor was inoculated with activated sludge taken from the acetate acclimated biomass used in another study. Substrate was added once a day by pulse feeding and biomass was acclimated to pulse feeding pattern in the aerobic batch reactor. Reactor was operated until it reached steady state conditions, which was

monitored by conventional parameter analysis such as suspended solids, volatile suspended solids and chemical oxygen demand measurements.

After the control reactor reached at steady state conditions; the acclimated biomass was used for respirometric experiments. Acetate mixture was added on the biomass to have the same S_0/X_0 ratio with the control reactor and oxygen uptake rate was monitored for the assessment of substrate biodegradation behaviour. Additional respirometric experiments were conducted on the activated sludge to determine acute and chronic inhibition effects of antibiotics. The antibiotic was given to system with different pulse feedings of 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of SMX concentrations to evaluate the acute effects on the carbon removal performance, however the chronic experiments were done with continuous feeding of 50 mg/L of SMX concentration.

2. LITERATURE REVIEW

2.1 Pharmaceutical Industry

Xenobiotics are chemicals which are not generated by living organisms and alien to natural systems such as poisons, drugs, and environmental pollutants. The evaluation of the metabolism processes of xenobiotics gain interest in recent years. Metabolism of the xenobiotics, which refers the various metabolic pathways depending on the structure of the chemicals, is important for life, as it allows an organism to neutralize and eliminate foreign toxins that would otherwise interfere with the chemical processes that keep it alive. These chemicals are continuously produced in greater numbers, mostly as raw materials or auxiliaries for industrial activities and they end up in waste streams. By their nature, they are mostly resistant to biodegradation (Orhon et al, 2010).

As xenobiotics are not normally found in an organism as part of its natural biochemistry, they are not easily incorporated into biogenic element cycles and consequently they are permanently or transitory accumulated in the environment. These chemicals have multiple applications in the synthesis of polyurethane foams, herbicides, insecticides, pharmaceuticals, and explosives, all of which are more recalcitrant than the raw material from which they are synthesized (Ramos et al, 2001).

The products of xenobiotics that are indispensable part of our daily life help to pursue the modern way of living and contribute to our high standards in living and safety, mobility, communication technologies, food, health, textiles and drinking water treatment, among many others. These products are generally labelled under the categories such as: pharmaceuticals, medicines, disinfectants, pesticides, detergents, fertilizers, dyes, paints, food additives and personal care products (Kümmerer, 2009a).

Pharmaceutical substances often have similar physico-chemical behaviour as other harmful xenobiotics that are accumulated or induce adverse effects in terrestrial or

aquatic organisms. Pharmaceuticals (antibiotics, antidepressants, and may other chemicals) are examples of such xenobiotics which have the potential to accumulate in the food chain and threaten human health (van der Meer et al, 1992). The global distribution of pharmaceuticals in the aquatic environment has become one of the main environmental problems in the last decade (Zuccato et al, 2005). Very large numbers of substances 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 (Gotvajn et al, 2007).

Parallel to the increase in the extensive usage of pharmaceuticals, it is unavoidable that these chemicals are released into the environment. Therefore, the adverse effect on the environment and health brought from the processing and disposal of these products become important issue in the field of environmental engineering (Hirsch et al, 1999). Because of its continuous introduction in the environment, are considered as "pseudo persistent compounds" in the environment. Pharmaceutical residues and/or their metabolites that have been detected in different environmental compartments including antibiotics, analgesic and anti-inflammatory, lipids regulator agents, antiepileptics, contraceptives, steroids and related hormones. Although, the environmental concentrations of pharmaceuticals are generally at trace levels (ng/L to low µg/L) in the environment, can be sufficient to induce toxic effects (Hernando et al, 2006).

The literature is sparsely documented with reported cases of pharmaceuticals from the early 1950s; however it wasn't until the mid 1990s that focused research into the fate and consequences of such compounds commenced (Hirsch et al, 1999). At the end of the last century, it was realised that the products of chemical and pharmaceutical industries are presenting a new type of environmental pollution that may also pose a health risk to the consumer. Pharmaceuticals and their metabolites have been subject to many years of unrestricted emission to the environment, directly or via sewage treatment plant effluents or sludge. Persistence toward biological degradation and their biological activity are key properties of these pollutants, since they have been designed to cause DNA damage to bacteria or eukaryotic cells. They retain their chemical structure long enough to do their therapeutic work and because of their continuous input they could remain in the environment for a long time (Alexy et al, 2004). The topic of pharmaceuticals and personal care products

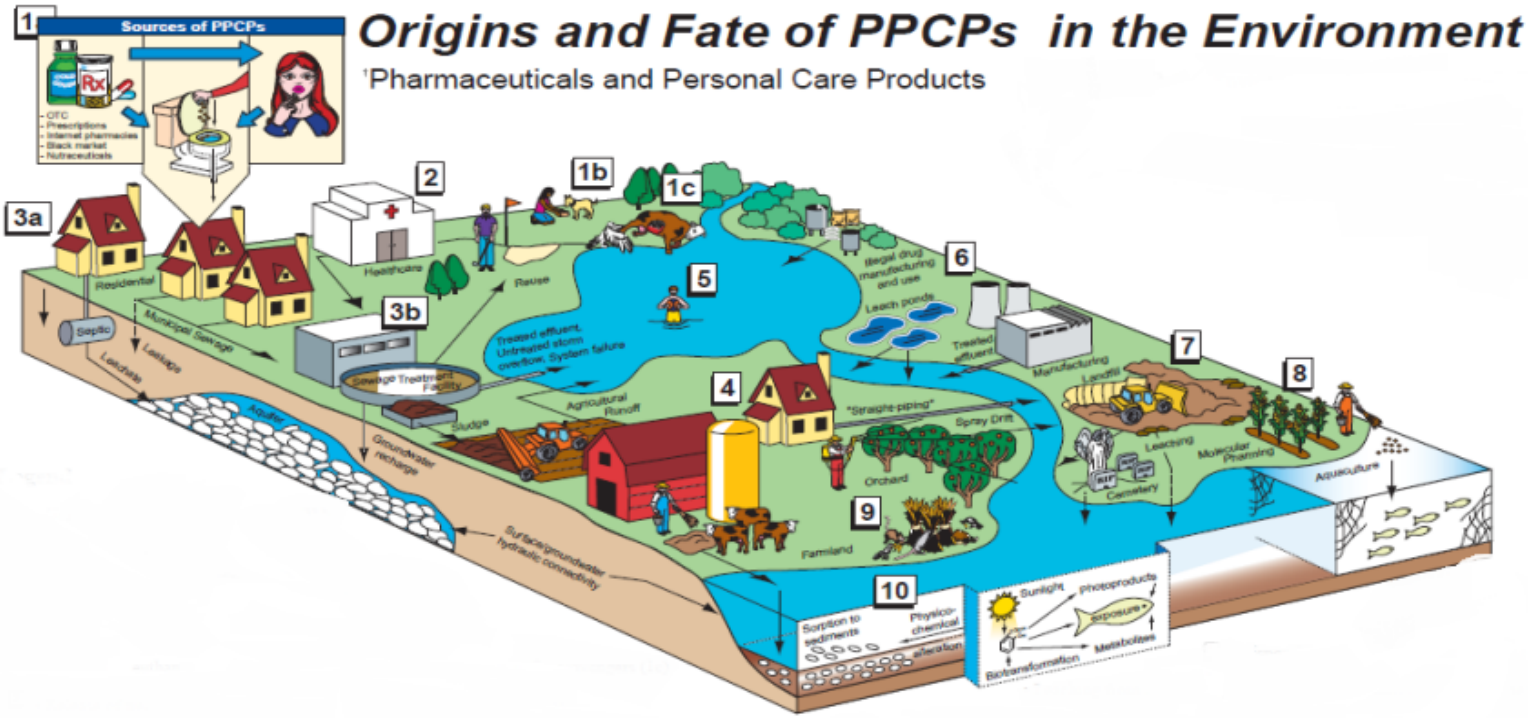
(PPCPs) as pollutants is intimately tied to a bewildering array of phenomena that transport and transform these chemicals from one place to another via a multitude of distinct "routes" by which the chemical is emitted, dispersed, or otherwise introduced to the next "compartment" or ultimately to a biological receptor. The fate and transport complexity of PPCPs can be seen in Figure 2.1. (Petrović and Barceló, 2007).

Reduction of pharmaceutical compounds in municipal sewage, through conventional biological treatment systems is a significant but insufficient means in mitigating their potential environmental impact. Pharmaceutical compounds in discharges derived from hospitals and pharmaceutical manufacturing sites can further increase the risk placed on the environment by municipal wastewater treatment plant (WWTP) effluents. Aga et al. (2005) stated that many of the pharmaceutical pollutants are resistant to biodegradation in WWTPs are also eliminated in the effluent by sorption on sludge.

Typical pharmaceutical wastewater has the COD, sulphate and total suspended solids (TSS) concentrations of 12500 mg/L, 9000 mg/L and 36000 mg/L, respectively. Moreover the antibiotic concentrations in the pharmaceutical wastewaters have been reported to increase until 200 mg/L (Drilla et al, 2005; Kim et al, 2005). Coagulation, chemical precipitation and biological treatment by activated sludge systems can be given among the classical treatment methods of pharmaceutical wastewaters.

Elimination of pharmaceuticals in wastewater treatment plants is depended on many parameters like the sludge age, hydraulic retention time, temperature, pH, biomass concentration, polarity and biodegradability of the substance (Press and Kristensen, 2007).

Wastewater treatment plants (WWTPs) are usual receptors of pharmaceuticals in which these compounds co-treated with municipal wastewaters before being discharged to the environment. The presence of these organic contaminants and their primary degradation products in the influents of WWTPs may inhibit biological processes (Kornaros et al, 2006).Therefore, these substances are becoming an increasingly large problem in WWTPs, since they are relatively new substances and are very difficult to categorize.



LEGEND

1.Usage by individuals (1a) and pets (1b):
Disposal of euthanized/medicated animal carcasses serving as food for scavengers (1c)
2.Release of treated/untreated hospital wastes to domestic sewage systems ;
also disposal by pharmacies, physicians, humanitarian drug surplus
3.Release to private septic/leach fields (3a)
Treated effluent from domestic sewage treatment plants discharged to surface waters (3b)
Overflow of untreated sewage from storm events and system failures directly to surface waters (3b)
4.Transfer of sewage solids ("biosolids") to land (e.g., soil amendment/fertilization)
Release from agriculture: spray drift from tree crops (e.g., antibiotics)
5.Direct release to open waters via washing/bathing/swimming
6.Discharge of regulated/controlled industrial manufacturing waste streams
7.Disposal to landfills via domestic refuse, Leaching from landfills, medical wastes, and other hazardous wastes
8.Release to open waters from aquaculture (medicated feed and resulting excreta)
Future potential for release from molecular pharming (production of therapeutics in crops)
9.Release of drugs that serve double duty as pest control agents:
10. Ultimate environmental transport/fate

Figure 2.1 : Origins and fate of PPCPs in the environment (Url-1, 2012).

In addition, some of them are not completely degraded in WWTPs and remain in the water effluent. Others may be release in a modified form, either hydrolysed or conjugated (e.g. ibuprofen is removed in more than 90%, and transformed in hydroxyl and carboxy transformation products) (Hernando et al, 2006). For instance, antibiotics are excreted as the parent compound, in conjugated form, or as oxidation or hydrolysis products (Plosz et al, 2010).

Most of pharmaceuticals were still detected in biologically treated gray water (kitchen sinks, laundry, hand basins, showers, and baths) in the low µg/L range. Until now, gray water treatment technologies focused on parameters such as chemical and biochemical oxygen demand (COD and BOD, respectively), nutrients, and pathogens. Eriksson et al. (2003) identified most ofpharmaceuticalsin gray water, and this may have a negative impact on the environment if they persist in recycled water or if discharged to water bodies.

2.2 Antibiotics

Antibiotics are one of the major groups of pharmaceuticals. Among all the other pharmaceutical drugs and substances, antibiotics are important compounds due to its serious irreversible increase in the release to the environment. Antibiotics are generally remain in the effluent of treatment plants and reach to receiver media due to difficult treatability with conventional treatment systems such as aerobic active sludge system and also their hydrophilic property. For that reason, they spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems (Demirden, 2005). The word "antibiotics" comes from the Greek anti ("against") and bios ("life"). An antibiotic is a drug that kills or slows the growth of bacteria. Antibiotics are one class of antimicrobials, a larger group which also includes anti-viral, anti-fungal, and anti-parasitic drugs. The classical definition of an antibiotic is a compound produced by a microorganism which inhibits the growth of another microorganism (Beers et al, 2003).

The first antibiotic was discovered by Alexander Fleming in 1928 in a significant breakthrough for medical science. Over the last 50 years, antibiotics have transformed the patterns of disease and death. Each different type of antibiotic affects different bacteria in different ways. Some antibiotics can be used to treat a wide

range of infections and are known as 'broad-spectrum' antibiotics. Others are only effective against a few types of bacteria and are called 'narrow-spectrum' antibiotics. Over the years, this definition has been expanded to include synthetic and semi-synthetic products (Kümmerer, 2009a).

Antibiotics are suspicious environmental contaminants as they are biologically active, which obviously is a part of their nature. Antibacterial agents are usually very soluble. In order to be as effective as possible, they often have a low biodegradability. This property poses a potential for bioaccumulation and persistence in the environment. Drugs, which have specific mode of actions, may probably exert effects on terrestrial and aquatic ecosystems, when released into the environment (Wollenbergera et al, 2000).

An antibiotic in a broader sense is a chemotherapeutic agent that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoa because antibiotics are designed to affect microorganisms. Although, the term antibiotic originally referred to any agent with biological activity against living organisms, ‘‘antibiotic” now refers to substances with their antibacterial, anti-fungal, or anti-parasitical activity. There are currently about 250 different chemical entities registered for use in human and veterinary medicine (Kümmerer and Henninger, 2003). Currently, antibiotics are obtained by chemical synthesis, such as the sulpha drugs (e.g. sulfamethoxazole), or by chemical modification in natural origin of compounds. Many antibiotics are relatively small molecules with a molecular weight of less than 1000 Da (Kümmerer, 2009a).

2.2.1 Classification of antibiotics

Antibiotics can be grouped by either their chemical structure or mechanism of action. The most common method classifies them according to their chemical structure as antibiotics sharing the same or similar chemical structure will generally show similar patterns of antibacterial activity, effectiveness, toxicity and allergic potential (Ternes and Joss, 2007; Beers et al, 2003).

The main classes of antibiotics are:

- Beta-Lactams
- Macrolides
- Fluoroquinolones

- Sulphonamides
- Tetracyclines
- Aminoglycosides

Most commonly used types of antibiotics are *Aminoglycosides*, *Penicillins*, *Fluoroquinolones, Cephalosporins, Macrolides,* and *Tetracyclines.* They are often complex molecules, which may possess different functionalities within the same molecule. Therefore, under different pH conditions, antibiotics can be neutral, cationic, anionic, or zwitterionic. Because of the different functionalities within a single molecule, their physico-chemical and biological properties (Cunningham, 2008), sorption behaviour, photo reactivity and antibiotic activity and toxicity may change with pH (Kümmerer, 2009a).

2.2.1.1 Beta-Lactams

Penicillins were the first and oldest class of antibiotics produced by fungi in the genus Penicillium, or streptomycin from bacteria of the genus Streptomyces. Penicillins are generally bactericidal, inhibiting formation of the cell wall (Url-5 and Url-6, 2012).

2.2.1.2 Macrolides

The macrolide antibiotics are derived from *Streptomyces* bacteria, and got their name because they all have a macro cyclic lactones chemical structure. The macrolides are bacteriostatic, binding with bacterial ribosomes to inhibit protein synthesis. Erythromycin, the prototype of this class, has a spectrum and is used similar to penicillin (Url-5 and Url-6, 2012).

2.2.1.3 Fluroquinolones

Fluoroquinolones (fluoridated quinolones) are the newest class of antibiotics. Their generic name often contains the root "floxacin". They are synthetic antibiotics, and not derived from bacteria. Fluoroquinolones belong to the family of antibiotics called quinolones.Fluoroquinolones inhibit bacteria by interfering with their ability to make DNA. This activity makes it difficult for bacteria to multiply (Ternes, and Joss, 2007; Beers et al, 2003).

2.2.1.4 Sulphonamides

Sulphonamides (SA) are synthetic antimicrobial with a wide spectrum against most gram-positive and many gram-negative organisms. The term “sulphonamides” is a monomer as it includes the amides of all organic sulfonic acids, but only the amides of sulfanilic acid (para-aminobenzoesulfonic acid) exhibit antibacterial activity. Sulfonamides are bacteriostatic agents, i.e. they block bacterial growth, but cooperation of the host immune defense is required to eradicate an infection. Therefore, molecular structure of sulfonamide is similar to p-aminobenzoic acid (PABA) which is needed in bacteria organisms as a substrate of the enzyme dihydropteroate synthetase for the synthesis of tetrahydrofolic acid (THF). Sulfonamides, derived chiefly from sulfanilamide, are capable of interfering with the metabolic processes in bacteria that require PABA. They act as antimicrobial agents by inhibiting bacterial growth and activity and called sulfa drugs. They are used in the prevention and treatment of bacterial infections, diabetes mellitus, edema, hypertension, and gout.

Sulfonamides inhibit the multiplication of bacteria, because they are competitive inhibitors of p-amino benzoic acid in the folic acid metabolism cycle (O’Neil et al, 2001), thus causes a decline in folate concentration. This decline is detrimental to the bacterium because folic acid is the precursor of purines and pyrimidines, the bases used in the construction ofDNA, RNA and other important cell constituents. The resulting inhibition of purine and pyrimidine synthesis leads to cessation of protein synthesis and DNA replication, thus the pathogen dies.

Resistance against sulfonamides has been increasing because many formerly susceptible pathogens have developed an ability to take up folic acid from their environment while bacteria are initially are not able to synthesize their own folic acid, whereas most animals obtain folic acid from their own diet (Madison et al, 2006). Nowadays sulfonamides are often used in fixed combinations with a diaminopyrimidine, either trimethoprim (TMP) (Ternes and Joss, 2007). Antimicrobial therapy with sulfamethoxazole plus trimethoprim, a related folic acid synthesis competitor, is still effective in many instances because the drug combination produces sequential blocking of the folic acid synthesis pathway.

Even though the sulfonamides are amphoteric, they generally function as weak acids at physiologic pH range. Because of this property, they are usually seen as sodium salts that have increased solubility as pH increases (Halling-Sorensen, 2000).

Sulfamethoxazole:

Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) is one of the most commonly administered sulfonamides. Sulfamethoxazole (sulfa drugs) are synthetic antimicrobials that are widely used in both veterinary and human medicine (Figure 2.2). Sulfamethoxazole has widespread used in the treatment of human infections. In human medicine, the sulfa drugs are used in the treatment of infectious diseases such as bronchitis and urinary tract infections (Perez et al, 2005). In livestock production, they are used in the prevention and treatment of infections as well as improving feed efficiency when used at subtherapeutic doses.

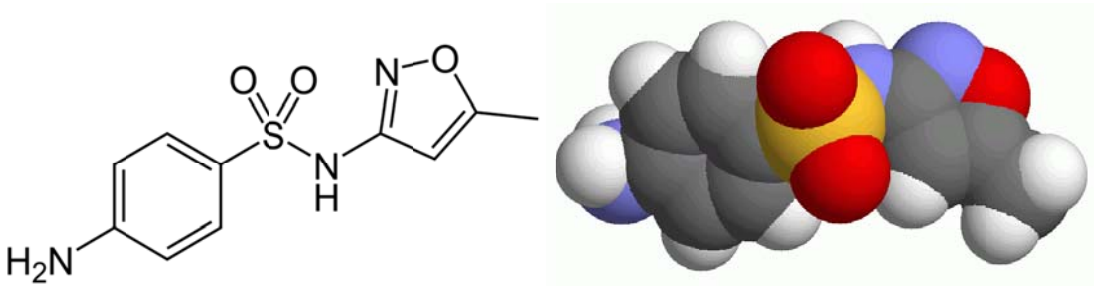


Figure2.2 : Sulfamethoxazole molecular configuration (Url-2, 2011).

Sulfamethoxazole prevents the formation of dihydrofolic acid, a compound that bacteria must be able to produce in order to survive. Although it was once a very useful antibiotic, it is almost obsolete as a single agent today due to the development of bacterial resistance to its effects. Sulfamethoxazole is now used primarily in combination with trimethoprim a combination product known as Bactrim or Septra. This antibiotic has proven to be very persistent to biodegradation according to recent researches (Drillia et al, 2005).

Antimicrobials are generally adsorbed in tissues and may be subject to metabolic reactions resulting in biotransformation products that are excreted via urine and feces along with the unmetabolized fraction of the parent compounds. For instance, approximately 15% of sulfamethoxazole is excreted from the human body in its original form and drug residues are eventually discharged into the sewage(Perez et al, 2005).Another study showed that when metabolism of SMX was considered,

approximately 45 % of SMX is excreted via urine (McEvoy et al, 2004). Monitoring data on raw and treated sewage from municipal wastewater treatment plants (WWTPs) indicated that the removal of sulfonamides during sewage treatment is incomplete, resulting in the discharge of antimicrobial residues into the receiving water bodies (Perez et al, 2005).

2.2.1.5 Tetracyclines

Tetracycline antibiotics are derived from a species of *Streptomyces* bacteria. They are broad-spectrum bacteriostatic agents, that inhibit bacterial protein synthesis. Tetracyclines may be effective against a wide variety of microorganisms, including rickettsia and amebic parasites (Url-5 and Url-6, 2012).

2.2.1.6 Aminoglycosides

Aminoglycosides are derived from various species of *Streptomyces* genus. Aminoglycoside antibiotics are used to treat infections caused by gram-negative bacteria. Aminoglycosides may be used along with penicillins or cephalosporins to give a two-pronged attack on the bacteria. (Beers et al, 2003).In summary, most commonly used types of antibiotics are shown in Table 2.1.

Table 2.1 : Classification of antibiotics.

Class (chemical structure)	Action mechanism	Examples
B-lactam antibiotics	Inhibit bacterial cell wall synthesis	• Penicillins • Cephalosporins • Carbapenem
Macrolides	Inhibit bacterial protein synthesis	• Erythromycin • Azithromycin • Clarithromycin
Fluoroquinolones	Inhibit bacterial DNA synthesis	• Norfloxacin • Ciprofloxacin • Enoxacin • Ofloxacin
Sulphonamides	Blocks bacterial cell metabolism by inhibiting enzymes	• Co-trimoxazole • Trimethoprim/Sulfamethoxazole
Tetracyclines	Inhibit bacterial protein synthesis	• Tetracycline • Minocycline • Doxycycline
Aminoglycosides	Inhibit bacterial protein synthesis	• Gentamicin • Amikacin

2.2.2 Significance of antibiotics in the environment

Antibiotics have been detected in various compartments of the environment such as in sewage, surface, ground and drinking waters or in soil (Kümmerer 2003). According to data supplied by the European Federation of Animal Health (FEDESA) in 1999, 13 288 tons of antibiotics were used in the European Union (EU) and Switzerland, of which 65% was used in human medicine; 29% was used in the veterinary field and 6% as growth promoters.

In 1996, about 10 200 tons of antibiotics were used in the EU, of which approximately 50% was applied in veterinary medicine and as growth promoters. Several hundred different antibiotic substances are used in human and veterinary medicine, e.g. more than 250 in Germany (Kümmerer and Henninger, 2003). In the USA, approximately 16 200 tons were produced in 2000 of which 70% was used in livestock farming. This is eight times of the amount used in human medicine. Wise estimated total world-wide antibiotic consumption is lie between 100 000 and 200 000 tons (Kümmerer, 2003).

Consumption for humans in total, per capita and the individual share of each compound varies from country to country. In total, 412 ton of antibiotics were used in Germany 1998. Taking the compound specific metabolization rates into account, 305 ton are emitted into wastewater, of which 92 ton were due to hospitals. The data demonstrate that there are no point sources but diffuse input by the general public/wastewater treatment plants(Kümmerer and Henninger, 2003).

Consumption by animals for the purposes of prevention or therapy is largely determined by modern animal breeding and fattening methods and conditions. Antibiotics are also used to promote the growth of animals (Gaskins et al, 2002) in some countries where they are used at low doses in animal feeds and are considered to improve the quality of the product, with a lower percentage of fat and higher protein content in the meat (Cromwell, 2002).

Antibiotics have been used since the 1950s to control certain bacterial diseases of high-value fruit, vegetable, and ornamental plants. Today, the antibiotic, which is most commonly used on plants, is streptomycin with oxytetracycline to a minor extent (Kümmerer, 2009a). In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. Antibiotics authorized for use in

aquaculture are oxytetracycline, florfenicol, premix, sarafloxacin, erythromycin, sulphonamides potentiated with trimethoprim or ormethoprim (Serrano, 2005).

Antibiotics are also used in aquaculture, for the purpose of preventing or treating microbial infections (Kümmerer and Henninger, 2003). High loads of antibiotics in sediments at concentrations potent enough to inhibit the growth of bacteria have been reported for aquaculture. Resistant bacteria may be present in sediments because of the application of antibiotics in fish farming or because of selection through the antibiotics present in the sediments 1990s (Kümmerer, 2009b). Some investigations have demonstrated the presence and persistence of antibiotics applied extensively in fish farming in sediments beneath fish farms (Kümmerer et al, 2004). Fluoroquinolones, sulphonamides and tetracyclines are strongly adsorbed; therefore they can readily accumulate in sediments.

2.2.3 Resistance of antibiotics in the environment

Antibiotics have been reported in hospital wastewaters, wastewater treatment plant (WWTP) effluents, WWTP biosolids, soil, surface waters, groundwaters, sediments, biota and drinking water (Watkinson et al, 2009). The development and proliferation of antibiotic resistance in pathogenic, commensal, and environmental microorganisms is a major public health concern (McMahon et al, 2007). Resistant and even multi-resistant pathogenic bacteria have been detected in wastewater, as well as in other environmental compartments because of lots of natural processes. The municipal wastewater may be polluted by antibiotics due to the human excretions. Most antibiotics are not completely metabolized or eliminated in the body and between 30 and 90 % are discharged unchanged into the sewage system (Hirsch et al, 1999). Another study has shown that the non-metabolized fraction of antibiotics is excreted approximately 70% of the totals in Germany as a still-active compound (Kümmerer and Henninger, 2003). If the drugs are not degraded or eliminated during sewage treatment, in soil or in other environmental compartments, they will reach surface water and ground water and, potentially, drinking water. For instance, sulfamethoxazole concentration in the surface waters was measured between 30 and 85 ng/Lin Germany. Unmetabolized antibiotic substances are often passed into the aquatic environment. Therefore, this allows resistant bacteria to enter the food chain directly (Figure 2.3) (Kümmerer, 2003). It might reach humans again via drinking water. Furthermore, direct discharge, especially from poultry processing, meat

processing, and aquaculture, as well as from pets (e.g. aquariums) is also possible and can contribute towards an increase in the total concentration of antibiotics in sewage and surface water (Kümmerer, 2009a).

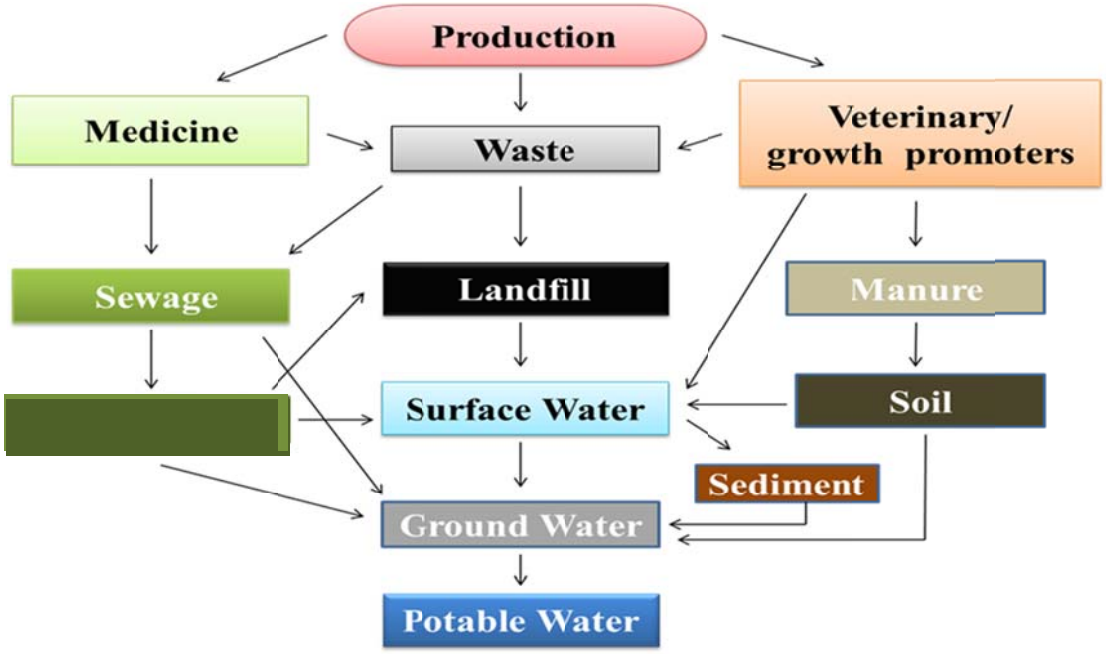


Figure 2.3 : Distribution of antibiotics in the environment (Kümmerer, 2003).

Antibiotics are among the most widely used for animal health and management. Their major use in livestock farming is not only to treat diseases but also to enhance growth and feed efficiency in livestock. As a general rule, drugs administered by oral route are slowly absorbed and excreted with faeces. Thus, animal wastes from intensive farming contain antibiotics in active form (Halling-Sorensen et al, 1998). These compounds may also find their way into the environment through several indirect pathways.

Veterinary antibiotics may release to the soil environment by manure spreading on the agricultural fields (Isidori et al., 2005), which would eventually be directed to the water through wash-off during rainfall events. For example, tetracyclines have been detected in concentrations of up to 0.2 µg per kg in soil whereas others have been found in the sediment under fish farms (Kümmerer, 2003).Very often wastes from terrestrial animals are used for field manuring, while in marine intensive aquaculture fish dejects go directly into the sea. In intensive fish farming, the antibacterials are mostly given as medicated feed pellets and calculations have indicated, that 70 % of these compounds ends up in the environment (Hektoen et al, 1995).

The widely detection of antibiotic containing compounds in water resources has became increasing concern because of their contribution to the presence and persistence of resistance in populations of both pathogenic and non-pathogenic microbes (Kolpin et al, 2002). Bacteria resistant to antibiotics have been found in the aquatic environment (Kümmerer et al, 2004; Kim and Aga, 2007; Watkinson et al, 2007) and in soil (Schmidt and Römbke, 2008). In addition, antibiotic-resistant bacteria were detected in drinking water as early as the 1980s and later in the 1990s (Kümmerer, 2009b).

Resistance of antibiotics is one of the major challenges for human medicine and veterinary medicine (Kümmerer, 2009b). The excretion from medicated animals and the application of the contaminated manure on agricultural land are among the major routes by which veterinary antibiotics enter the environment. Pharmaceutically active ingredients are complex molecules with different functionalities and physico-chemical and biological properties. They can have undesired effects on non-target organisms present in the environment (Kümmerer, 2009a), since they have been designed to cause damage in DNA of bacteria or to eukaryotic cells.

To understand the ecology of antibiotic resistance, it is necessary to characterize the occurrence, fate, and transport of antibiotic-resistant bacteria. A first step in this endeavour is the identification of major sources of antibiotics and antibiotic resistance to the environment. Resistant bacteria may be detected by antibiotic substances in hospital effluent, municipal sewage, aeration tanks or the anaerobic digestion process of WWTPs. The dilution of hospital effluents by municipal sewage will lower the concentration of antibiotics only moderately, because municipal wastewater also contains antibiotic substances and disinfectants from households, veterinary sources and to a minor extent from livestock. Antibiotics have been detected in the μg/L range in municipal sewage, in the effluent of wastewater treatment plants (WWTPs), in surface water and in groundwater. These included quinolones such as ciprofloxacin, sulphonamides, roxythromycin, dehydrated erythromycin and others. As the hospital effluents contribute to less than 1% of the total amount of municipal sewage (Kümmerer, 2008), it is plausible that hospitals are not the main source for resistant bacteria in municipal sewage. Resistant bacteria are also present in municipal sewage not receiving hospital effluent. Because of the use of antibiotics in the home, the conclusion is that it is probably the general community

that is responsible for the main input of resistant bacteria into WWTPs.Resistant and multi-resistant bacteria such as *E. coli, P. aeruginosa, Acinetobacter, Pseudomonads, Enterobacteriaceae*, and phylogenetically distant bacteria, such as members of *α-* and *β-Proteobacteria,* are present in municipal sewage as well as in the aeration tanks and the anaerobic digestion process of WWTPs. Resistance against b-lactams, quinolones, tetracycline and sulfamathoxazole/trimethoprim and other sulphonamides has been found in wastewater and sewage sludge all over the world using classical means such as cultivation and resistance testing as well as the detection of resistance encoding genes (Kümmerer et al, 2004).

Another study also determined that between 16% and 35% of *Escherichia coli* isolates cultured from treated effluent were resistant to tetracycline (Reinthaler et al, 2003). Additionally, the incidence of antibiotic resistance among culturable *Escherichia coli*in WWTPs and a large fraction (57%) of that were resistant to tetracyclines were reported (Reinthaler et al, 2003).

A multi-resistant *Acinetobacter* strain which is known to be able to survive in sewage was introduced to a laboratory-scale sewage treatment plant (LSSTP) containing a mix of antibiotics at a concentration up to 100-fold above that expected in the aquatic environment in Germany and reflecting nation-wide antibiotic use. Furthermore, 2 weeks after introduction of the bacterium into the treatment plant, the genetic material responsible for the multi-resistance could no longer be detected. These results suggest that the continuous input of resistant bacteria due to the application of antibiotics is by far much more important than the input of antibiotics (Kümmerer, 2009b).

Moreover, antibiotics were recently classified as a priority risk group due to their high toxicity to algae and bacteria at low concentrations and their potential to cause resistance amongst natural bacterial populations. The sensitivity of algae towards antibiotics varies widely. As algae are the basis of the food chain, even slight decreases in the algal population may affect the balance in an aquatic system. Blue-green algae (*Cyanobacteria*) seems to be sensitive to many antibiotics, for example amoxicillin, benzyl penicillin, sarafloxacin, spiramycin, tetracycline and tiamulin (Boxall et al, 2003). The presence in surface waters of antibiotics is often reported in lower concentration level (low ng/L) and several publications about sulfamethoxazole, erytromicyn evidencing the frequency of both drugs in the aquatic

compartment (Hernando et al, 2006). Similarly, antibiotics were detected quite frequently in the low ng/L range, up to 2 μg/L in the surface waters of six investigated rivers including freshwater, estuarine and marine samples (Watkinson et al, 2009).Hence, antibiotics in surface waters have the potential to disrupt key bacterial cycles/processes critical to aquatic ecology (nitrification/denitrification) or agriculture (soil fertility) and animal production (rudimentary processes) (Watkinson et al, 2009).

2.3 Removal of Antibiotics

Antibiotics are considered highly toxic to the environment and to human life and therefore have to be removed from contaminated waters and wastewaters to the highest possible extent. Wastewater usually contains antibiotics either as excretion products resulting from metabolism or as a consequence of the inaccurate disposal of unused or out-of-date drugs. In wastewater treatment plants (WWTPs), a pharmaceutical compound and its metabolites may undergo a partial or complete mineralization, a slow biodegradation after binding on solid sludge, or may pass unchanged through the wastewater treatment plants. A number of drugs have been detected both in sewage and in WWTP effluents from various sources, with levels ranging from below detection limits up to mg/L and with removal efficiencies ranging widely depending on their physical chemical characteristics (Carucci et al, 2006). These substances are usually removed from wastewaters by biological processes, among which the activated sludge process is applied as the most common treatment method. In principle, they can be removed in the activated sludge process through different mechanisms: biodegradation, volatilisation, air stripping or adsorption on primary or secondary sludge (Dionisi et al, 2006).

Antibiotics may be removed in WWTPs by transformation processes such as hydrolysis, chlorine or ozone oxidation, and aerobic or anaerobic biodegradation. Varying degrees of transformation depend upon the nature of the compound, and the specific conditions and processes existing within the treatment plant. A key issue associated with any transformation process is that the parent compound is rarely mineralized (e.g., converted to inorganic carbon, nitrogen, phosphorus, etc.) Numerous studies have suggested that, in general, sorption of PPCPs to biosolids, aerobic biodegradation, membrane processes, and disinfection with chlorine or

ozone, may each provide for significant removals of many PPCPs under certain common treatment conditions (Adams, 2008).

Elimination of antibiotic compounds in the environment is the result of different processes. These processes can be biotic ones, i.e. biodegradation by bacteria and fungi. Non-biotic elimination processes are sorption, hydrolysis, photolysis, oxidation and reduction. It has to be noted that the results of bio-or photodegradation studies depend on conditions such as temperature, composition of matrix, latitude etc. (Kümmerer, 2009a).

Different studies on fate of antibiotics after treatment highlighted three different ways for antibiotics disappearance. The most favourable case is biodegradation, another way is accumulation on biomass defined as biosorption, which leads to the release of molecules after biomass death, and a third way is hydrolysis (Amrane et al, 2009). The main removal mechanisms of antibiotics are given below in detailed.

2.3.1 Sorption

Sorption was found to be the main mechanism of antibiotics removal by the activated sludge, this mechanism has been studied in more detail with the aim of determining the sorption equilibrium and kinetics, in order to provide useful data for the modelling of the fate of them in biological wastewater treatment. Sorption was found to be a very fast mechanism of organic substance removal from the liquid phase, and only a few minutes were needed to reach 90% of the final sorption for most of the substances (Dionisi et al, 2006).

Contaminants present in wastewater can partition between the liquid and solids phase, with sorption onto dissolved and colloidal organic matter (DCOM). This can subsequently influence the availability of contaminants for biodegradation. Mechanisms other than hydrophobic partitioning, such as cation exchange, cation bridging, surface complexation, and hydrogen bonding can have a significant impact on sorption (Plosz et al, 2010).

Binding to particles or the formation of complexes may cause a loss in detectability, as well as a loss in antibacterial activity. The loss of antibacterial activity, for example, was demonstrated for an aquaculture antimicrobial in seawater driven by the formation of complexes with the magnesium and calcium naturally present in

marine water. For instance, tetracyclines are able to form complexes with double cations, such as calcium or magnesium (Christian et al, 2003).

2.3.2 Photolysis

If a substance is light sensitive, photo-decomposition may be of major significance in the elimination process. Photo-decomposition takes place mainly in clear surface water. Photochemical decomposition can play an important role in surface water as an additional elimination pathway or for effluent treatment (Kümmerer, 2009a). Some antibiotics are light sensitive (e.g. quinolones, tetracyclines, sulphonamides, tylosin, nitrofuran antibiotics). However, not all compounds are photo-degradable.

Antibiotics may be degraded abiotically via photodegradation and/or hydrolysis or biotically by aerobic or anaerobic organisms. The quinolones and tetracyclines are rapidly photodegraded with half-lives ranging from <1 h to 22 d. In contrast, trimethoprim and sulfonamides are not readily photodegradable (Kümmerer, 2009a).

2.3.3 Hydrolysis and thermolysis

Another important pathway for the non-biotic elimination of organic substances in the environment is hydrolysis. In general, the hydrolysis rates for oxytetracycline increase as the pH deviates from pH 7 and as temperature increases. However sulphonamides and quinolones are resistant to hydrolysis. In laboratory biodegradability testing with sewage sludge, it has been found that β-lactams are rapidly hydrolysed. This leads to the deactivation of antibiotic activity (Längin et al, 2009).

Hydrolysis of particulate and colloidal organic matter along with biodegradation of the soluble mostly readily biodegradable substrates (S_S) can therefore influence the biodegradation of compounds such as antibiotics in WWTPs. In activated sludge, it is also possible that certain metabolites of pharmaceuticals can be transformed back to the parent compound as a result of enzyme-catalyzed reactions (Plosz et al, 2010).

2.3.4 Technical oxidation processes

Antibiotic formulation effluents are well known for the difficulty of their elimination by traditional biological treatment methods and their important contribution to environmental pollution is due to their fluctuating and recalcitrant nature. For

advanced effluent treatment oxidation processes are usually applied (Kümmerer, 2009a). The effect of ozonation on the degradation of oxytetracycline in aqueous solution at different pH values (3, 7 and 11) was investigated by Li et al. (2008). It has been shown that COD (chemical oxygen demand) removal rates increase with increasing pH as a consequence of enhanced ozone decomposition rates at elevated pH values. The results of bioluminescence data indicate that the initial by-products after partial ozonation (5–30 min) of oxytetracycline were more toxic than the parent compound.

On the other hand, sulfamethoxazole was efficiently degraded by ozonation (Dantas et al, 2007). A biodegradability enhancement expressed as the increment of BOD_5/COD (biological oxygen demand after 5 d/chemical oxygen demand) ratio from 0 to 0.28 was observed by the authors after 60 min of ozonation. The complete sulfamethoxazole removal was achieved for an in photo-Fenton process (González et al, 2007).

2.3.5 Biodegradation

Most antibiotics tested to date have not been biodegradable under aerobic conditions (Richardson and Bowron, 1985; Al-Ahmad et al, 1999; Wiethan et al, 2000; Kümmerer et al, 2000; Ingerslev and Halling-Sørensen, 2001; Gartiser et al, 2007; Li et al, 2008). The biodegradability of some clinically important antibiotic drugs (ciprofloxacin, ofloxacin, metronidazole) and hereby the elimination of their genotoxicity was investigated as the first step of an environmental risk assessment using the Closed Bottle Test (CBT) (OECD 301 D). The CBT is simple test for the assessment of the biodegradability of organic compounds and the standard test period of the CBT is 28 days (Kümmerer et al, 2000). According to this test, it has been determined that sulfamethoxazole was stable during the test period of 28 days and seen to be resistant to biodegradation (Gartiser et al, 2007).

Biodegradability tests were conducted on SMX as Zahn WellensTest (OECD 302B) and CO_2 Evoluation Test (OECD 301B). Zahn Wellens Test (DOC elimination based) and CO_2 Evoluation Test indicated inherent biodegradability and readily biodegradability, respectively whereas their combination results were used to assess inherent biodegradability and DOC elimination by sorption. The results showed that

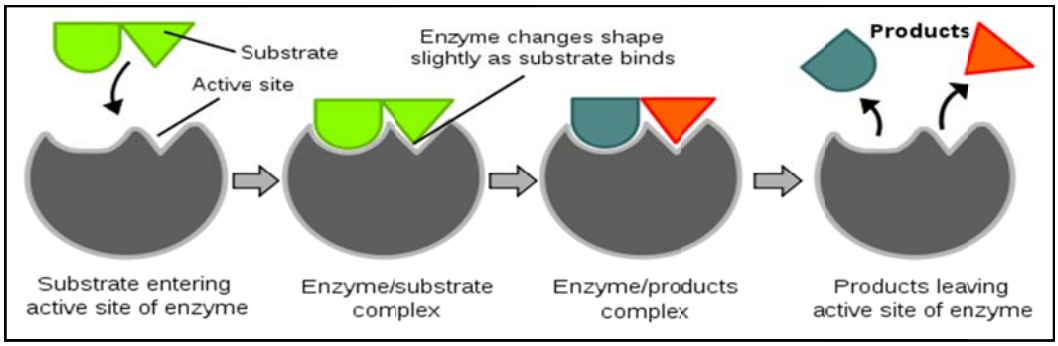
SMX had no ultimate biodegradation (< 20 % removal) and only minor DOC elimination (< 20 % removal) in inherent tests (Gartiser et al, 2007).

Biodegradability has been poor for most of the compounds investigated in laboratory tests such as the OECD test series (301– 303, 308) even for some of the ß-lactams (Alexy et al, 2004). Out of 16 antibiotics tested, only benzyl penicillin (penicillin G) was completely mineralized in a combination test (combination of the OECD 302 B and OECD 301 B tests; Gartiser et al, 2007). No evidence of biodegradation for tetracycline was observed during a biodegradability test (sequence batch reactor), and sorption was found to be the principal removal mechanism for tetracycline in activated sludge (Kim et al, 2005). Some antibiotics occurring in soil and sediment proved to be quite persistent in laboratory testing as well as in field studies. Some do not biodegrade well under anaerobic conditions (Gartiser et al, 2007).

2.4 Enzyme Reactivity and Inhibition

2.4.1 Conceptual approach of enzymes in biological processes

All organisms control its activity through enzymes, which are protein molecules produced by microorganisms that catalyze chemical reactions. Enzymes comprise the largest and most specialized group of protein molecules within the cell (Rittmann and McCarty, 2001).The basic function of an enzyme is to increase the rate of a reaction rate by lowering the activation energy of the biochemical reaction (Orhon and Artan, 1994). Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Most enzymes act specifically with only one reactant (called a substrate) to produce products. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell (Orhon and Artan, 1994).The reactivity of an enzyme toward a substrate involves specificity and kinetics. The conventional view is that a substrate molecule or a portion of it fits into the active site of the enzyme in a lock-and-key model. Figure 2.4 shows the induced fit hypothesis of enzyme action. (Rittmann and McCarty, 2001).



The rate (or kinetics) of an enzyme-catalyzed reactions is, in general, governed by the same principles that govern other chemical reactions. In many cases, the rates of growth and substrate utilization by microorganisms can be described quite well by kinetics developed to describe the rate of transformation of a single substrate by a single molecule. Hence, the kinetics of enzyme catalysis are of interest for describing the individual reactions and, often, complex biochemical processes (Rittmann and McCarty, 2001).

A charateristic of enzyme reactions is substrate saturation. Figure 2.5 illustrates the observed effect of substrate concentration on the rate of an enzyme-catalyzed reaction. At very low substrate concentrations, the rate increases in direct proportion to substrate concentration; thus, the rate here is first-order with respect to substrate concentration. As substrate concentration increases, however, the rate of increase begins to decline, giving a mixed-order reaction. At higher substrate concentrations, the enzyme becomes saturated with substrate, and the rate increases no further. This rate is the maximum rate for the reaction. Here, the rate becomes zero order with respect to substrate concentration. This saturation effect is typical of all enzyme-catalyzed reactions (Rittmann and McCarty, 2001).

0 K_m S

Figure 2.5 : Effect of substrate concentration (S) on enzymatic transformation rate (υ) based upon Michaelis-Menten kinetics. When S equals K_m, υ is one-half of the maximum rate υ_m.

L. Michaelis and M. L. Menten recognized this saturation phenomenon and, in 1913, developed a general theory of enzyme action and kinetics, which was later extended by G.E. Briggs and J.B.S. Haldane (Rittmann and McCarty, 2001). This theory suggest that enzyme *E* first reacts with substrate *S* to form an enzyme-substrate complex *ES*, which then breaks down to form free enzyme and products *P*. As a result ofreactions totake place, the coefficient K_M, which represents a composite of the various rate coefficients, is called the *Michaelis-Menten coefficient*. Equation **(2.1)** is the Michaelis-Menten equation, which defines the quantitative relationship between the substrate concentration and the reaction rate in relation to the maximum possible rate. The coefficient K_M equals the substrate concentration at which the velocity of the reaction is one-half of the maximum velocity. K_M represents the affinity between the substrate and the enzyme. A low value of K_M indicates a very strong affinity, such that the maximum rate is reached at a relatively low substrate concentration. A large value of K_M, on the other hand, shows a poor affinity (Rittmann and McCarty, 2001).

$$\upsilon = \upsilon_m \frac{S}{K_M + S} \qquad \textbf{(2.1)}$$

The Michaelis-Menten mechanism for the catalysis of biological chemical reactions is one of the most important chemical reaction mechanisms in biochemistry. It provides a practical tool to mathematically explain the behavior of microbial cultures under inhibitory effects (Rittmann and McCarty, 2001).

2.4.2 Conceptual approach on enzyme inhibition

Since biological processes deal with living organisms, they are subject to upsets by inhibitory or toxic agents. Inhibition is caused by chemical or physical agents, such as pH, temperature, oxidation-reduction potential of the medium, etc. So far two concepts have emerged, namely inhibition and toxicity. 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. When the inhibited biochemical reactions are vital to the cell then the agent is identified as toxic. The effect of toxicity is manifested in microorganisms' increased difficulty to take up nutrients and a decrease in growth rate or in the ultimate death of the cell. Translating this to the treatment system, a

lower degradation rate is observed and biomass activity is usually also altered (Orozco, 2008).

The kinetic expressions in biological transformations can be based on the mechanisms of enzyme kinetics that led to the Michaelis-Menten expression. These enzymatic mechanisms can be extended to cover special circumstances such as the intrusion of inhibitory compounds, which interfere with the active enzymes rendering them unfit for product formation (Orozco, 2008).

Inhibitory agents reduce enzyme reactivity, which is one way in which toxic chemicals can adversely affect a biological treatment process. For example, the chemical agent does not destroy the enzyme, and the reactivity can be reversed if the agent is removed. Two common types of reversible inhibition are competitive and noncompetitive inhibition (Rittmann and McCarty, 2001).

2.4.2.1Competitive inhibition

In competitive inhibition, a chemical that is similar in structure to the normal enzyme substrate competes with the substrate for the active site on the enzyme. 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 (Orhon and Artan, 1994). In competitive inhibition the maximal rate of the reaction is not changed, but higher substrate concentrations are required to reach a given maximum rate. A competitive inhibition model, developed from fundamental principles is;

$$\upsilon = \upsilon_m \frac{S}{K_M(1+\frac{1}{K_I})+S} \qquad \textbf{(2.2)}$$

Where I is the concentration of the competitive inhibitor, and K_I is a competitive inhibition coefficient having the same concentration units as I. As I increases, the rate decreases in a manner similar to that as if K_M were increased in Equation **(2.1)**. If S is large enough, the inhibitor is removed from the active site, and the maximum rate of substrate transformation is achieved (Rittmann and McCarty, 2001) (Figure 2.6).

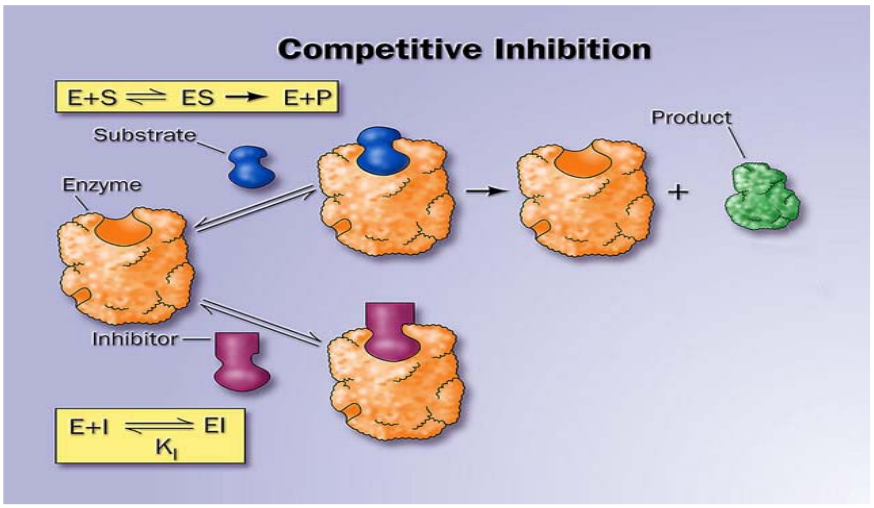


Figure 2.6: Competitive inhibition (Url-4, 2011).

In addition, competitive inhibition has often been observed between the growth and co-metabolic substrates. Co-metabolism is the biological transformation of a non-growth (co-metabolic) substrate by bacteria through enzymes which can only be induced in the presence of a growth-substrate providing energy for cell growth and maintenance. In recent years, specific organic chemicals have become of concern and the goal is often to reduce concentrations to the low microgram per litre level, or even lower. Lower limits can also be achieved by proper design of treatment systems that base on the principle of co-metabolism (McCarty, 2000).

In generally, the aerobic co-metabolism is catalyzed by nonspecific oxygenase enzymes that use molecular oxygen as the electron acceptor and NAD(P)H as the reducing energy to oxidize both growth- and non-growth substrates (Chang and Alvarez-Cohen, 1995 a, b). Non-growth substrates have a similar structure to growth-substrates and can bind to enzymes and be transformed due to non-specific activity. However, some factors such as the inhibitory effects of the initial growth-substrate or co-metabolic substrate on the enzyme as well as the inhibitory effects of products and limitations in NAD(P)H generation may adversely affect the degradation. As shown in Figure 2.7, short-lived toxic intermediate products may cause damage on oxygenase enzyme or cell. In this case, co-metabolic oxidation of the non-growth substrate consumes the reductant NAD(P)H which is then not regenerated (Figure 2.7). Thus, NAD(P)H, which is a potentially limiting reactant during co-metabolic reactions, must be provided by external or endogenous regenerants.

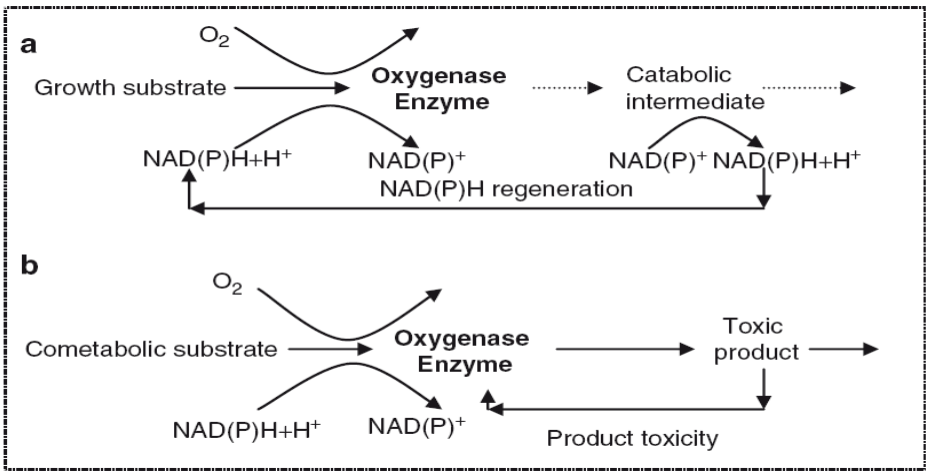


Figure 2.7 : Typical oxygenase enzyme reactions for growth-substrate and co metabolic (non-growth) substrate (Chang and AlvarezCohen,1995b).

The products of growth-substrate oxidation may undergo further degradation that regenerates NAD(P)H for additional substrate oxidations. Therefore, it is essential that a growth-substrate is provided at least periodically for growth of new cells and induction of enzyme production. Co-metabolic degradation occurring in the absence of external regenerants is referred to as the resting cells condition. In this case, the reducing energy can only be provided by endogenous energy sources or general biomass that often limits the co-metabolic degradation rate and leads to product toxicity (Chang and Alvarez-Cohen, 1995b) (Çeçen et al, 2010).

2.4.2.2 Noncompetitive inhibition

In noncompetitive inhibition, the chemical agent acts by complexing a metallic activator or by binding at a place on the enzyme other than the active site. The enzyme is then less reactive toward its substrate. 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_I, and it will not be infuenced by S or ES (Figure 2.8) (Orhon and Artan, 1994). A noncompetitive inhibition, developed from fundamental principles is;

$$\upsilon = \upsilon_m \frac{S}{K_M + S} \cdot \left(\frac{1}{1 + \frac{1}{K_I}}\right) \qquad \textbf{(2.3)}$$

An increase in I here effectively reduces the value of υ_m, if Equation **(2.1)** were used to describe the enzyme reactivity.

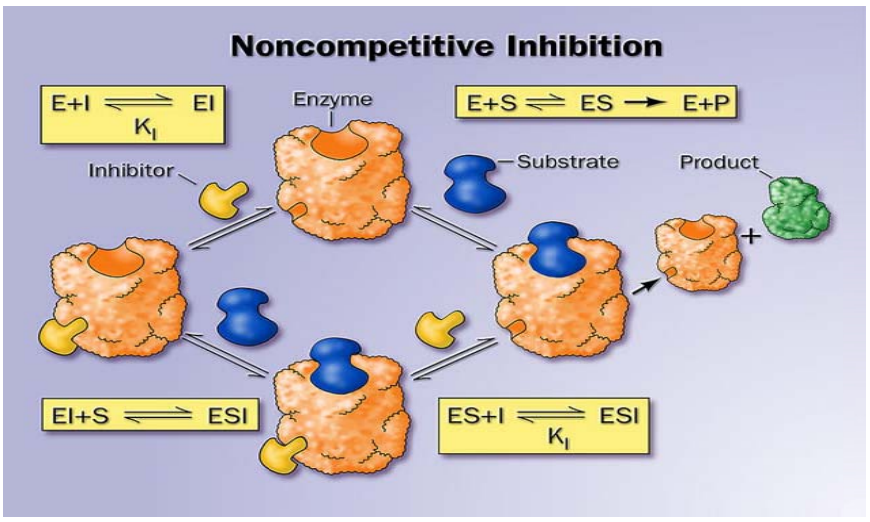


Figure 2.8 : Noncompetitive inhibition (Url-4, 2011).

As a summary; when a drug is a competitive inhibitor, the drug competes with the normal substrate for the active site and the concentration of competitive inhibitor must be kept high at all times. If the concentration of the competitive inhibitor drops, the velocity of the reaction will increase. However; when a drug is a noncompetitive inhibitor, the drug binds strongly to the enzyme and does not compete with substrate. Only the initial concentration of the drug is important. The reaction will not increase as the concentration of noncompetitive inhibitor drops (Figure 2.9).

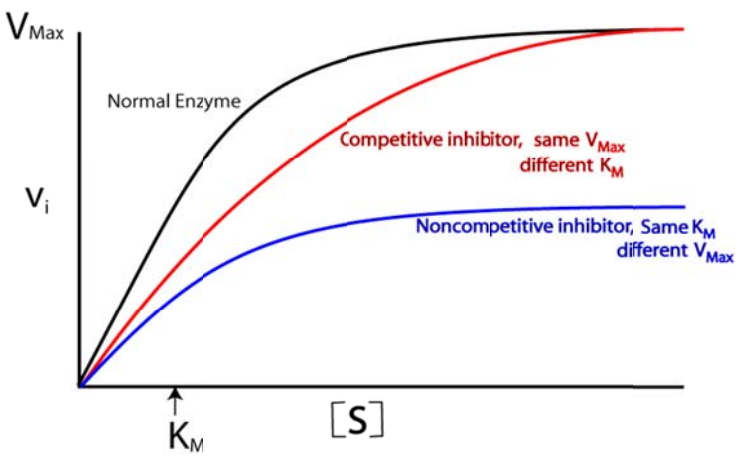


Figure 2.9 : Comparison of competitive and noncompetitive inhibitors.

2.5 Researches on the Biodegradation of Sulfamethoxazole in Activated Sludge Process

The activated sludge process is a biological method of wastewater treatment that is performed by mixed microbial cultures in an aerobic aquatic environment. These microorganisms derive energy from carbonaceous organic matter in aerated wastewater for the production of new cells in a process known as synthesis, while simultaneously releasing energy through the conversion of this organic matter into compounds that contain lower energy, such as carbon dioxide and water, in a process called endogenous respiration. This consortium of microorganisms, the biological component of the process, is known collectively as activated sludge (Metcalf and Eddy, 2004).

The higher removal efficiency of antibiotics has been reported in the activated sludge process under aerobic conditions in compared to anaerobic conditions. Although, the removal of antibiotics from wastewater was the highest in the aerobic system and in the combined anaerobic and aerobic system, antibiotic concentrations after anaerobic treatment indicated their overall poor removal (Leal et al, 2010).

Most of antibiotics come either from domestic sewage or from hospital or industrial discharges and enter WWTPs. 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. Their removal efficiencies are influenced, apart by the chemical properties of specific compounds, by microbial activity and environmental conditions (Omil et al, 2004). A major factor influencing the efficiency of these pollutants removal from water is their ability to interact with solid particles, both natural (clay, sediments, microorganisms) or added to the medium (active carbon, coagulants), because this facilitates their removal by physical - chemical (settling, flotation) or biological processes (biodegradation). However, some compounds with low adsorption coefficients tend to remain in the aqueous phase, which favors their mobility through the sewage treatment plant (STP) and the receiving environment (Omil et al, 2004). This leads to inhibition of the microbial processes. Therefore, for determination of most efficient treatment strategies, it is important to understand the role of enzymes and inhibition mechanism as well as the

treatment behavior of antibiotics with other carbon sources. Omil et al.(2004) has investigated that the treatment of sulfamethoxazole has been surveyed along the different units of a municipal STP with a population of approximately 100 000 inhabitants in Spain. The plant includes three main sections: pre-treatment, primary treatment and secondary treatment. Sulfamethoxazole was quantified with concentrations of around 0.6 μg/L in these wastewaters, which is removed around 67%, during the biological step.

Another study by Perez et al. (2005) has showed that the synthetic antimicrobials sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim have the potential to be microbially degraded during sewage treatment. The biodegradability tests were conducted in aerated batch reactors by spiking the sewage with 20 mg/L of each of the test substance. Concentration profiles of the assayed compounds were monitored during a 54 days period using liquid chromatography/electrospray ionization/mass spectrometry. The highest degradation rates were obtained in the sewage from the activated sludge treatment, where no adaptation phase was observed. On the other hand, the onset of biodegradation in the sewage from the primary treatment was preceded by a lag phase ranging from 10 to 15 days.

The occurrence of antibiotics in WWTP effluents has been also carried out in several studies. Sulfamethoxazole was also found in raw influent and final effluent, indicating that it could withstand different treatment processes in the STPs (Xu et al, 2007). Sulfamethoxazoleand trimethoprim or erythromycin are often founded at medium - high ng/L level (128 and 886 ng/L) (Hernando et al, 2006). Investigated WWTPs were highly effective in removing antibiotics from the water phase, with an average removal rate of greater than 80% for all targeted antibiotic. However, antibiotics were still detected in WWTP effluents in the low ng/L range up to a maximum of 3.4 μg/L, with the macrolide, quinolone and sulphonamide antibiotics most prevalent.

The sulphonamides all demonstrated high removal in WWTP effluent (roughly 80%) with the tetracyclines only demonstrating little removal (roughly 30%) from the water phase. The highest recorded antibiotic concentration in WWTP effluents was tylosin (3.4 μg/L); however, sulfamethoxazole had the greatest median value (0.05 μg/L) (Watkinson et al, 2009).

In addition, the fate of sulfamethoxazole in the activated sludge process was studied using a sequencing batch reactor. According to study, SMX serves as a carbon and/or a nitrogen source for the enhanced culture of microorganisms that are able to degrade it (Drillia et al, 2005). Drillia et al. also investigated the removal of SMX in presence of excess ammonium and readily biodegradable carbon source (acetate), which is a common situation at wastewater treatment plants. It has been found out that under these conditions, the enzymes responsible for the removal of the antibiotic were inactivate, and that if enough time was given for the synthesis of these enzymes, degradation of SMX was also possible under ammonium rich conditions. According to the results obtained from the study, it has been concluded that SMX can be removed in systems like extended aeration systems, where there is an absence of a readily biodegradable substrate.

The example treatment strategies and efficiencies of different antibiotics in WWTPs are summarized in Table 2.2 (Avella et al, 2010).

Table 2.2: The fate of different antibiotics in WWTPs and in hospital effluents (Avella et al, 2010).

Antibiotic	WWTP Antibiotic Influent (ng/L)	Bioreactor (ng/L)	Secondary Effluent (ng/L)	Hospital Effluents (ng/L)	Comments
Erythromycin					**Erythromycin:** macrolide antibiotic. Macrolides were the second antibiotic class mostly used in human care in european countries between 1998–2005 (ESAC). Erythromycin is usually applied for respiratory tract infections, infections of soft tissues and when infections are persistent to penicillin antibiotics. Its principal metabolite Erythromycin-H_2O is the mostly frequently detected macrolide antibiotic in WWTP effluents (Watkinson et al, 2009). This indicates its potential to pass through conventional treatment (Hirsch et al 1999). The elimination of Erythromycin in conventional WWTP is incomplete.
Gulkowska et al. (2008)	470-810				
Göbel et al. (2005)	70 ±120		80±60		
Roxithromycin					**Roxithromycin:** macrolide antibiotic. It is better tolerated by human than Erythromycin. Göbel et al. (2005) observed that Roxithromycin was persistent in the effluent from WWTP with conventional activated sludge treatment. They reported that no significant sorption of this antibiotic was detected on biological sludge
Watkinson et al. (2007)	0-18	0-60	0-100		
Watkinson et al. (2009)	~ 8		~ 20	~ 50	
Göbel et al. (2005)	20±20		20±10		
Sulfamethoxazole					**Sulfamethoxazole:** In conventional WWTP, the elimination of Sulfamethoxazole was found to be incomplete. Therefore, it is frequently detected in rivers. In sequencing batch reactor, Drillia et al. (2005) suggested that bacteria used Sulfamethoxazole as a nitrogen source when more readily biodegradable source of nitrogen was absent. Göbel et al. (2005) reported the sorption of Sulfamethoxazole on biological sludge.
Watkinson et al. (2007)	360-500	185-200	270-320	1300-2100	
Brown et al. (2006)				400-2100	
Watkinson et al. (2009)	250±2750		50±150	100±200	
Göbel et al. (2005)	430±200		280±560		
Amoxicillin					**Amoxicillin:** penicillin antibiotic class. The penicillin was the most consumed antibiotic in EU (ESAC, 1998–2005). Amoxicillin belongs to b-lactams (cyclic amide) which are not generally considered as important environmental pollutants because they undergo thermal degradation until ultimate mineralisation to CO_2 and water (Hirsch et al, 1999).
Watkinson et al. (2007)	190-280		0-30		
Watkinson et al. (2009)	~ 1400		<50	90	
Tetracycline					**Tetracycline** undergoes photochemical degradation and forms a number of degradates products. Many of them are highly soluble and stable in receiving waters. Tetracycline has also a tendency to form relatively stable complexes with double cations such as calcium and magnesium and with particulates matter (Halling-Sorensen et al, 2002). Tetracycline removal of water phase is not complete (Watkinson et al, 2009). However, Tetracycline is eliminated by more than 50% due to sorption on sewage sludge (Watkinson et al, 2007).
Watkinson et al. (2007)	0-35	0-20	0-30		
Watkinson et al. (2009)	<10		<20	<40	

2.6 Modelling of Activated Sludge Systems

Different 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 in activated sludge plants is very essential for modelling purposes. Thus, modelling of aerobic biodegradation of substrates has been continuously evolving that enables improved interpretation of the process kinetics of substrate removal mechanisms in activated sludge processes. The trend of improving the effluent quality from wastewater treatment plants leads to an increasing complexity in the design and operation of the plants. To design the plants and optimize and control the operation, it is necessary to use dynamic models (Henze, 2005).

Deterministic models are the most used in wastewater treatment plant design. The deterministic models, also known as black-box type models that are used for controlling purposes, aim to give a realistic description of the main processes of the plant. However, models are never true in the process sense and always simplify the complicated processes occurring in a biological treatment plant. A major gap in deterministic modelling is lack of knowledge about the development of the microbiology in treatment plants (Henze, 2005). For this reason, it is commonly agreed that the only way to provide a reliable basis for design and operation of treatment plants is to identify a rational mechanistic description of the process in terms of microbial kinetics and material balance (Orhon and Artan, 1994).

Mechanistic models would provide a reasonably acceptable description of major biochemical processes in terms of microbial kinetics and stoichiometric relationship among significant parameters. Models developed can only provide a simplified approximation of complex biochemical reactions taking place in the activated sludge systems (Orhon et al, 2009a).

A new conceptual approach of wastewater characterization relies on the existence and identification of a number of substrate fractions with different biodegradation characteristics. This approach also differentiates active biomass from other particulate biomass components (Orhon et al, 2009a). Compared with traditional models (Pearson, 1968; Jenkins and Garrison, 1968; Lawrence and McCarty, 1980)

having only two components (substrate/biomass) and two processes (growth/decay), the number of model components is increased to account for all significant substrate and biomass fractions (Orhon et al, 2009a).

A mechanistic model should include a minimum eight components for the modelling of organic carbon removal. They can be divided into two groups: substrate components and biomass components. The readily biodegradable COD, S_S, the slowly biodegradable COD, X_S, the soluble inert COD, S_I and the particulate inert COD, X_I define the biodegradation characteristics of the wastewater. The rapidly hydrolysable COD, S_H is often considered as an additional model component for most wastewaters. The major biomass components obviously include active heterotrophic biomass, X_H, together with soluble and particulate inert microbial products, S_P and X_P. Dissolved oxygen concentration, S_O is the indispensable parameter for the model (Orhon et al, 2009a).

Most models for activated sludge processes are based on the IAWQ Activated Sludge Model No. 1, called ASM1 (Henze et al, 1987). This model is used as a platform for further modeling approachs and is today the international standard for advanced activated sludge modeling, although this model has been extended considering some important processes such as biological phosphorus removal (ASM2, Henze et al, 1994) and storage phenomena (ASM3, Gujer et al, 1999).

2.6.1 Activated Sludge Model No. 1 (ASM1)

The International Association on Water Quality (IAWQ, formerly IAWPRC) was established in 1983 for the development and application of practical models in activated sludge systems. After extensive studies, Activated Sludge Model No.1 (ASM1) was developed by IAWQ Task Group with mathematical modelling for design and operation of biological wastewater treatment systems (Henze et al, 1987). ASM1 have still provided basis for the design and modelling of activated sludge systems. It is essential to have comprehensive knowledge on this first conceptual model ASM1, to understand all basic processes of activated sludge. ASM1 generally takes into consideration these three components: biomass, substrate and oxygen. The basic components and process of ASM1 for carbon oxidation is shown in Table 2.3.

Table 2.3: Processes for carbon oxidation described in ASM1 (Henze et al, 1987).

Component → **Process ↓**	S_O	S_S	X_S	X_P	X_H	**Process rate**
Growth	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$			1	$\hat{\mu}_H \cdot \left(\frac{S_o}{K_o+S_o}\right) \cdot \left(\frac{S_S}{K_S+S_S}\right) X_H$
Hydrolysis		1	-1			$k_h \cdot \left(\frac{S_o}{K_o+S_o}\right) \cdot \left(\frac{X_S/X_H}{K_X+X_S/X_H}\right) X_H$
Decay			$1-f_P$	f_P	-1	$b_H . X_H$
Parameter	O_2 COD		COD	COD	Cell COD	

As seen from the simplified representation of ASM1, this model integrates two stoichiometric and six kinetic parameters to describe the utilization of wastewater by heterotrophic microorganisms. The biodegradable substrate in influent wastewater consists of two fractions: readily and slowly biodegradable substrate. Although, existing readily biodegradable substrate (S_S) is assumed to consist of simple molecules which are able to pass through the cell wall and immediately be used for microbial growth, The slowly biodegradable substrate (X_S), which consists of larger complex molecules, is adsorbed by activated sludge, and then broken down by extracellular enzymes (hydrolysis) before being transferred through the cell wall and used for metabolism. The processes of simplified ASM1 can be described as:

- *Growth*: A fraction of the readily biodegradable substrate is used for growth of heterotrophic biomass and oxidized for energy requirement of the cell. The growth is modelled by using Monod kinetics. Both the concentration of S_S and dissolved oxygen (S_O) may be rate limiting for growth process. This process is generally the main contributor to the production of new biomass and removal of substrate.

- *Hydrolysis*: Slowly biodegradable substrate (X_S) is broken down to readily biodegradable substrate by extracellular enzymes, and then the latter one is available to the organisms for growth. This process is modelled on the basis of surface reaction kinetics. Therefore, the rate is expressed by first-order kinetic with respect to the present heterotrophic biomass.

- *Decay*: This process is modelled according to the death-regeneration hypothesis. Inert microbial product (X_P) is generated during the lysis of heterotrophic microorganism (X_H) with fraction f_P. The remaining fraction of ($1-f_P$) is transformed into X_S. It is assumed that carbon is not removed during this transformation and as a consequence no electron acceptor is utilized.

The main stoichiometric parameters represented in simplified ASM1 are the heterotrophic yield coefficient (Y_H) and fraction of biomass, leading to particulate product (f_P). The coefficient f_P, which is consumed as inert particulate product after decay process, is affected by the death-regeneration concept. If the decay process is modelled as endogenous decay, this value is usually assumed to be approximately 0.2. However, the recycling of biomass by death-regeneration, results in significantly lower f_P value in order to reach the same amount of particulate inert mass. Y_H is a stoichiometric constant known as observed growth yield. It is simply defined as weight of new biomass produced from the growth-limiting substrate. The yield term indicates the amount of substrate, which is used both for new biomass production and also for energy generation to carry out biosynthetic reactions (Ciggin, A.S, 2011a). In addition, the Y_H is an important parameter, as it effects estimation of sludge production and oxygen demand; it also has an impact on kinetic parameters. As the Y_H highly depends on the biomass, it is assumed to be rather constant in different municipal wastewater treatment plants (WWTPs). The typical value of Y_H is given as 0.67 for municipal wastewaters in ASM1.

Kinetic parameters are highly dependent on the nature of the substrate. Therefore, it is necessary to determine these parameters by case specific analyses. The interaction of different processes makes the determination of decay coefficient (b_H) difficult related to the death regeneration concept. The maximum specific growth rate (μ_{Hmax}) depends on the sludge ageand therefore depends on both characteristics of wastewater and operation of the WWTPs. The half saturation coefficient for heterotrophic biomass (K_S) also depends on the operation of the WWTPs, due to the dependency of the floc size and structure on diffusion of substrate to the cell and thereby on the apparent value of saturation coefficients. The hydrolysis process in ASM1 is used to describe the degradation of slowly biodegradable organic substance originating from the influent carbon source and from internal turnover of substrate in the death regeneration cycle. In ASM1, the kinetic parameters of hydrolysis process,

which are the maximum specific hydrolysis rate (k_h) and half saturation coefficient for hydrolysis (K_X) were suggested as model parameters that have to be adjusted during model calibration procedure (Ciggin, 2011a). The stoichiometric and kinetic parameter values used by the task group in the modelling studies reported are listed in Table 2.4 for 10 °C and 20 °C.

Table 2.4 : Typical parameter values at neutral pH.

Symbol	Characterization	Temperature		Units
		10 °C	20 °C	
Y_H	Heterotrophic yield coefficient	0.67	0.67	g cell COD.(g COD)$^{-1}$
f_p	Fraction of biomass for particulate product	0.08	0.08	-
μ_H	Maximum specific growth rate for heterotrophicbiomass	6.0	3.0	d^{-1}
K_S	Half-saturation coefficient for heterotrophicbiomass	20.0	20.0	g COD m^{-3}
b_H	Decay coefficient for heterotrophicbiomass	0.62	0.20	d^{-1}
k_h	Hydrolysis rate constant	3	3	d^{-1}
K_X	Hydrolysis saturation constant	0.03	0.03	g.COD.(gCOD)$^{-1}$

ASM1 is basically structured on the assumption that active biomass is converted to a combination of particulate residual products and slowly biodegradable substrate, through death and lysis as schematically plotted in Figure 2.10. No loss of COD is involved and no direct electron acceptor is utilized. The slowly biodegradable substrate released is hydrolysed, generating an equivalent amount of readily biodegradable COD. Under aerobic conditions, new cells are produced at the expense of this COD fraction together with oxygen uptake (Orhon et al, 2009a).

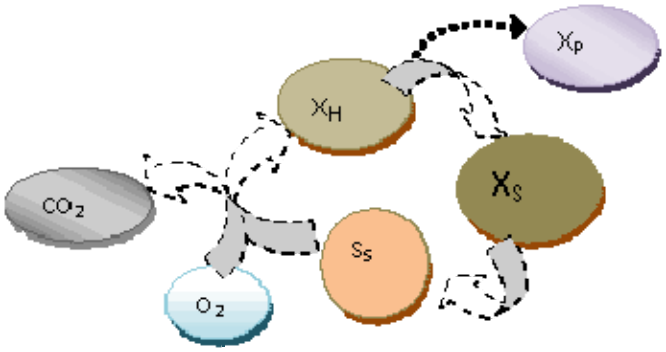


Figure 2.10 : Death regeneration process in ASM1 (Orhon and Artan, 1994).

2.6.2 Activated Sludge Model No.3 (ASM3)

ASM1 was first introduced by Henze et al. (1987) to interpret the carbon oxidation based on the assumption that the external substrate is consumed only for biomass growth. For this reason, models generally presume that microbial growth is the only biochemical mechanism for growth; substrate is directly converted into biomass which is subsequently used for endogenous respiration. However, in the last decade several researchers observed the phenomenon of storage of carbon sources as intracellular polymers that plays a significant role in carbon removal process (van Loosdrecht et al, 1997b; Majone et al, 1999). It has been recognized that bacterial populations adopt a survival strategy when exposed to feast (presence of external substrate) and famine (absence of external substrate) conditions, because of transient conditions. For this reason, the Activated Sludge Model No.3 (ASM3) model was built on the basis of storage concept of readily biodegradable substrate by IWA Task Group (Gujer et al, 1999; Gujer et al, 2000).

In ASM3, it is hypothesized that all readily biodegradable substrate (S_S) is first stored as an internal storage product (X_{STO}), either directly or through preliminary hydrolysis, and then growth occurs only on internally storage polymers during the famine (unavailability) phase. ASM3 is one of the most referenced models for the quantitative analysis of activated sludge processes. The storage yield (Y_{STO}), is one of the most important parameters of the simultaneous storage and growth models, since it represents the stoichiometric amount of the substrate converted into storage products, which are then utilized for growth. The assessment of Y_{STO} is therefore crucial for the accurate estimation of the overall electron acceptor utilization and sludge production. As the Y_{STO} mainly depends on the carbon source, it is essential to evaluate the storage in relation to the type of the produced storage polymers. Consequently, the substrate uptake rate and consequently Y_{STO} rely on the external carbon source, which determines the type of storage polymers. It was also suggested that the storage rate (k_{STO}) is changing with environmental conditions (Hanada et al, 2001).As these stoichiometric and kinetic values highly depend on the environmental conditions, it is better to evaluate these parameters based on the operating parameters of activated sludge systems. The growth environment, namely operating conditions, controls the expression of the genetic capabilities of the organisms such as the levels of the enzymes, which vary with the growth environment. The type of storage

polymer and operating conditions also determine the species of microorganisms present in activated sludge systems.

The ASM3 model represents an alternative process description for heterotrophic bacteria. Under transient loading conditions, heterotrophic bacteria can store organic matter as polymeric compounds like polyhydroxyalkanoate (PHA) or glycogen (GLY). This storage can influence the overall process of activated sludge by supplying organic material under starvation conditions. The reaction kinetics and stoichiometry of ASM3 simplified for organic carbon removal is given in Table 2.5. The mechanisms participating in oxygen utilization are storage, growth, endogenous decay and respiration of storage products.

The net (true) yield of heterotrophic biomass (X_H) produced per unit of readily biodegradable substrate (S_S) removed in ASM3 are found from following equation.

$$Y_{net,O2} = Y_{STO,O2}.Y_{H,O2} \qquad \textbf{(2.4)}$$

Table 2.5 : The simplified ASM3 for organic carbon removal under aerobic conditions.

Component → Process ↓	**S_O**	**S_S**	**S_I**	**X_S**	**X_I**	**X_H**	**X_{STO}**	**Process rate**
Hydrolysis		$-(1-f_{SI})$	$-f_{SI}$	-1				$k_h\left(\frac{X_S/X_H}{K_X+X_S/X_H}\right)X_H$
Storage of S_S	$-(1-Y_{STO})$	-1					Y_{STO}	$k_{STO}\cdot\left(\frac{S_o}{K_o+S_o}\right)\cdot\left(\frac{S_S}{K_{STO}+S_S}\right)X_H$
Growth on X_{STO}	$-\frac{1-Y_H}{Y_H}$					1	$-\frac{1}{Y_H}$	$\hat{\mu}_H\cdot\left(\frac{S_o}{K_o+S_o}\right)\left(\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}\right)X_H$
Endogenous respiration	$-(1-f_I)$				$-f_I$	-1		$b_H\cdot\frac{S_o}{K_o+S_o}.X_H$
Respiration of X_{STO}	-1						-1	$b_{STO}\cdot\frac{S_o}{K_o+S_o}.X_{STO}$
Parameter	O_2	COD		COD		Cell COD	COD	

Stoichiometric parameters of ASM3 under aerobic conditions are identified together with their units and a typical value in Table 2.6. In relation to removal of all soluble compounds, the kinetic expressions of ASM3 rely on switching functions, which are

hyperbolic or saturation terms, Monod equations, S/(K+S). Similarly, the switching functions are affected for particulate compounds by the ratio of X_{STO}/X_H and X_S/X_H.

Table 2.6 : Typical stoichiometric parameters of ASM3.

Symbol	Characterization	Value	Units
Y_{STO}	Aerobic yield of stored product per S_S	0.85	g COD_{XSTO}.(g COD_{SS}) $^{-1}$
Y_H	Aerobic yield of heterotrophic biomass	0.63	g COD_{XH}.(g COD_{XSTO}) $^{-1}$
f_{XI}	Production of X_I in endogenous respiration	0.20	g COD_{XI}.(g COD_{XBM}) $^{-1}$
$I_{SS,XI}$	SS to COD ratio for X_I	0.75	g SS.(g COD_{XI}) $^{-1}$
$I_{SS,XS}$	SS to COD ratio for X_S	0.75	g SS.(g COD_{XS}) $^{-1}$
$I_{SS,BM}$	SS to COD ratio for biomass, X_H	0.90	g SS.(g COD_{XBM}) $^{-1}$

The units and typical values for kinetic parameters of ASM3 under aerobic conditions at 10 ˚C and 20 ˚C are identified in Table 2.7.

Table 2.7 : Typical values for kinetic parameters of ASM3.

Symbol	Characterization	Temperature		Units
		10 °C	20 °C	
k_h	Hydrolysis rate constant	2	3	g COD_{XS}.(g.COD_{XH})$^{-1}$.d^{-1}
K_X	Hydrolysis saturation constant	1	1	g.COD_{XS}.(g.COD_{XH})$^{-1}$
k_{STO}	Storage rate constant	2.5	5	g COD_{SS}.(g.COD_{XH})$^{-1}$.d^{-1}
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})$^{-1}$
μ_H	Heterotrophic maximum growth rate of X_H	1	2	d^{-1}
b_H	Aerobic endogenous respiration rate of X_H	0.1	0.2	d^{-1}
b_{STO}	Aerobic respiration rate for X_{STO}	0.1	0.2	d^{-1}

ASM1 adopted substrate utilization by direct growth, whereas ASM3 suggested storage of substrate in the form of internal storage polymers followed by its utilization for other biochemical processes (Katipoğlu et al, 2010). The main difference between ASM1 and ASM3 is the recognition of the importance of storage

polymers in activated sludge processes. At the same time the decay process from ASM1 is replaced by an endogenous respiration process. The endogenous respiration concept originated from the observation that internal storage materials are used for maintenance purposes when the external substrate is depleted (Van Loosdrecht and Henze, 1999).

A major difference for the wastewater characterization between ASM1 and ASM3 is that soluble (S_S) and particulate (X_S) biodegradable components in ASM3 are supposed to be differentiated with filtration over 0.45 µm membrane filters, whereas a significant fraction of the slowly biodegradable organic substrates (X_S) in ASM1 would be contained in the filtrate of the influent wastewater (Gujer et al, 1999).

The observation from OUR tests with activated sludge which revealed the fact that bacteria will rapidly take-up readily biodegradable COD and store it as internal substrate which will further be converted slowly (conversion ofreadily biodegradable COD into slowly biodegradable COD). When the acetate (defined substrate) is added to the activated sludge the observed OUR suggests the presence of two substrates; the OUR associated rapid and slow degradation of substrate can be observed in Figure 2.11.

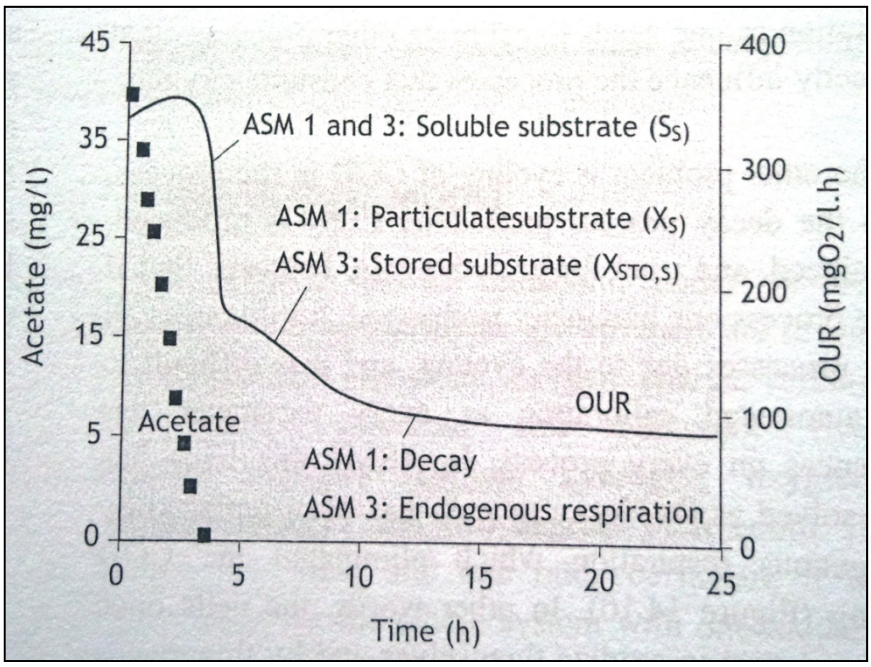


Figure 2.11 : Differences between ASM1 and ASM3.

In ASM1 it is reflected as if two substrate are present (S_S and X_S) while in reality only acetate (S_S) is dosed. In order to describe the observed OUR by ASM1 in this

case, it is necessary to define that the acetate is partly soluble and partly particulate, which is not recommended. This deficiency is solved by the introduction of a storage compound, $X_{STO,S}$ in ASM3. It means that substrate is taken up rapidly and stored, while growth occurs with the stored substrate. Both models will describe the observed OUR, but only ASM3 will describe well the acetate uptake (Henze et al, 2008).

2.6.3 Substrate Storage Phenomena

Storage has been explained as the survival mechanism when microorganism is prevented from growing at its maximum rate (Van Looshdrect et al, 1997b). Although the substrate is transported into the cell, the biosynthetic growth limitations may prevent the consumption of all energy. This leads to an increase in energy charge which, in turn, leads to storage of the substrate. In this way, favourable growth conditions are established to face against this highly dynamic environment in activated sludge processes (Ciggin, 2011a).

As a result of highly dynamic feeding conditions in activated sludge systems, microorganisms are subjected to feast-famine regime. This process is also currently known as‘‘aerobic dynamic feeding(ADF)”.These periods were explained as: when external carbon source is available only short periods of time, microorganisms consume substrate and produce storage polymers in feast period whereas no external substrate is found and consumption of stored polymer occurs for growth in famine period (Van Loosdrecht et al, 1997b). If there was no external substrate, bacteria would undergo long starvation periods. Starvation by substrate for a certain period of time can cause a decrease in the amount of intracellular components (RNA and enzymes) needed for growth. Following this period of substrate limitation, cells faced with an excess of substrate can take it up rapidly. However, the growth rate does not increase at a rate that corresponds with the substrate uptake rate. This difference is related to the fraction of substrate stored or used for cell maintenance. After starvation, storage occurs preferentially instead of cell growth because the amount of enzymes required for storage are lower than the enzymes needed for growth at maximum rate. During the famine period, the stored substrate is used for cell growth and cell maintenance. Operation of a reactor under feast and famine conditions can selectively enrich a mixed population with a high storage capacity (Reis et al, 2006). Owing to storage ability, microorganisms have a competitive

advantage over the other bacteria without the capacity of substrate storage. If bacteria can not store the substrate; extra energy is needed for rapid growth in feast period (Van Loosdrecht et al, 1997b). Thus, storage process requires significant amount of energy for removal of soluble substrate.

2.6.3.1Polyhydroxyalkanoates (PHAs)

It is reported that when activated sludge culture is fed with volatile fatty acids (VFAs), the storage polymers are Polyhydroxyalkanoates (PHAs) which are intracellular energy and carbon storage materials synthesized by numerous microorganisms (Lee, 1996). Microbial production of PHA, both under aerobic and anaerobic conditions, has gained considerable interest because of the potential applications of these polyesters as biodegradable plastics (Brock and Madigan, 1991). PHAs can be thermoplastically moulded and can be used as new plastics which have the advantage over polypropylene and polyethylene of being biodegradable. They can be transformed into sheets, fibres and hollow bodies like bottles and mugs. Besides being the only linear biodegradable polyester, they are also biocompatible with numerous applications in medicine, pharmacy and packaging (Steinbüchel and Wiese, 1992). While PHA-based plastic substitutes are less flexible than traditional plastics, they are completely biodegradable and leave behind no residue. More than 40 different hydroxyalkanoic acids (HAs) have been detected as constituents of PHA, but only few homopolyesters besides poly(3-hydroxybutyrate) (PHB) are available from bacteria (Steinbüchel and Wiese, 1992).

More recently, recombinant strains for cost-effective PHA production (properties include: rapid growth, high cell density, ability to use several inexpensive substrates, and simple polymer purification) have been developed by cloning the PHA synthase genes from many microorganisms, including *Ralstonia eutropha* (recently renamed as *Cupriavidus necator*).

Industrial processes for the production of PHAs as a bioplastic employ generally pure cultures such as *Ralstonia eutropha, Alcaligenes latus*, and *Burkholderia sacchari* (Reis et al., 2006) of natural PHA producers or recombinant bacteria. For example, the production of PHA by recombinant *Escherichia coli* harboring *R. Eutropha* can reach 80–90% of the cell dry weight (Reis et al, 2006). However, the production costs are still too high for PHAs to become a competitive commodity plastic (Dias et al, 2006). Production costs could potentially be reduced by using

open undefined mixed culture processes for the production of PHA and waste streams rather than pure chemicals as substrates (Kleerebezem et al, 2009). With the aim of commercializing PHA, a great deal of effort has been devoted for reducing the production cost by using activated sludge as a mixed culture and a renewable resource (Satoh et al, 1999; Kumar et al, 2004; Yan et al, 2006; Cai et al, 2009).

In general, mixed cultures are microbial populations of unknown composition, which are able to perform specific intracellular and extracellular reactions, and are selected by the operational conditions imposed on the biological system. Mixed cultures selected for PHA production can have a high intracellular storage capacity due to operational conditions that limit their primary metabolism. PHA synthesis assume that accumulation occurs when growth is limited by external factors such as a lack of nutrients (for example, phosphorus or nitrogen) or internal factors such as an insufficient amount of RNA or enzymes required for growth (Reis et al, 2006). Activated sludge is known to accumulate up to 65% PHA per cell dry weight. There are currently many possibilities of industrial and agricultural wastes that could be directed to the production of PHA on the basis of open mixed microbial systems (Lemos et al., 2008).

A promising approach for producing PHAs with mixed cultures is the use of a two-step process. In the first step a PHA producing culture is enriched using alternating presence and absence of the carbon source (feast and famine periods) as a selective pressure, hereby making use of the ecological role of PHAs as a storage polymer. This feast - famine regime can be implemented using a sequencing batch reactor (SBR). In the second step the culture enriched in the first step is subjected to continuous presence of the carbon source, usually under growth limiting conditions, in order to maximize the cellular PHA content (fed-batch reactor) (Kleerebezem et al, 2009).

In other words, the strategy for the enrichment of heterotrophic organisms in the sludge is a critical factor for the process productivity. The feast and famine feeding regimen was shown to be an effective strategy for selecting the heterotrophic organisms with high PHA storage capacity. The results of many studies demonstrate undeniably that mixed cultures submitted to the feast and famine regimen have high PHA storage capacity, high PHA yields, and high specific PHA productivity (Reis et al, 2006).

The type of PHA components produced depends on the composition of substrate. Well known monomeric units of PHA in activated sludge are poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV). In addition to these two main polymers, the production of other PHA components such as 3-hydroxy-2-methylbutyrate (3H2MB) and 3-hydroxy-2-methylvalerate (3H2MV) may also be significant (Satoh et al, 1992). More than 40 different hydroxyalkanoic acids (HAs) have been detected as constituents of PHA, but only few homopolyesters besides poly (3-hydroxybutyrate) (PHB) are available from bacteria (Steinbüchel and Wiese, 1992).

2.6.3.2 Polyhydroxybutyrate (PHB)

The most common forms of PHA accumulated by microorganisms are polyhydroxybutyrate (PHB) which is most well-known storage polymer associated with simple external substrates such as volatile fatty acids and especially acetate (Satoh et al, 1992; Dionisi et al, 2001; Karahan-Gül et al, 2002a).

In 1926, Lemoigne was the first to describe PHB as cell internal component. It would take until 1960 before a renewed interest was created because of the presumed metabolic significance of PHBs. The storage polymer was particularly encountered under certain growth conditions such as limiting nutrient conditions (low N/COD ratio) (Braunegg et al, 1978; Baetens, 2001). PHB is an energy and carbon source derived from the fermentation processes of several kinds of bacteria, such as *Bacillus megaterium* and *Ralstonia eutrophus.* In addition, PHB is water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable plastics, which are either water soluble or moisture sensitive. This compound is also good oxygen permeability and soluble in chloroform and other chlorinated hydrocarbons (Baetens, 2001). PHB is present as granules enclosed by a membrane in the cytoplasm of the cells. The granules of PHB have typical diameters of 0.2 to 0.5 μm. Figure 2.12 depicts a general structure of PHAs.

The regulation of PHB synthesis is controlled at enzymatic level and mainly depends on the intracellular concentration of acetyl-CoA and free coenzyme A (CoA). The production of storage polymers is thought as serving a NADH overflow mechanism to control the redox state of heterotrophic cells during unbalanced growth conditions (Van Niel et al, 1995).

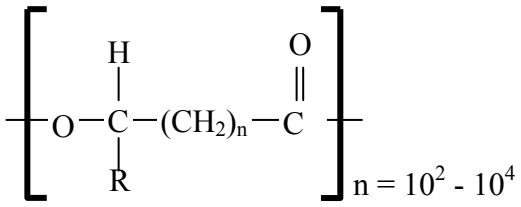


Figure 2.12 : General structure of PHA (R: methyl: PHB and R: ethyl: PHV).

As illustrated in Figure 2.13, after bacteria take up acetate into cell, it is converted to acetyl-CoA and the produced acetyl-CoA is primarily used in the tricarboxylic acid (TCA) cycle to produce ATP, $NADH_2$ and to form biomass. When growth conditions are unbalanced, acetyl-CoA cannot enter TCA cycle to obtain energy for cells due to high concentrations of NADH. High concentrations of NADH inhibit citrate synthase, one of the key enzymes of TCA cycle, leading to an increase in the level of acetyl-CoA. For PHB synthesis, two molecules of acetyl-CoA are condensed to form acetoacetyl-CoA and release a CoA. The enzyme 3-ketothiolase catalyzes this condensation reaction. The increase in the concentration of acetyl-CoA drives the equilibrium reaction of 3- ketothiolase into the direction of PHB synthesis. The acetoacetyl-CoA is reduced to (R)-3-hydroxybutyryl-CoA. This reaction is catalyzed by NADPH-dependent acetoacetyl-CoA reductase and (R)-3-hydroxybutyryl-CoA molecules synthesize PHB with the release of free CoA (Ciggin, 2011a).

Subsequently, PHB is accumulated in order to keep the concentration of intermediates at a balanced level in cells. It is likely that when the ratio acetyl-CoA/CoA is high, the cells will respond with the production of more anabolic enzymes, i.e. increase their growth rate. If the period of excess external substrate availability is long enough, the specific growth rate of the biomass will increase to its maximum and the PHB synthesis rate will slow down (Ciggin,2011a). Depolymerization of PHB appears to be inhibited in the presence of a soluble substrate favouring rapid growth. However, certain observations seem to indicate that degradation of PHB occurs at the same time as its synthesis. When PHB is used by biomass while no external substrate is available, PHB is converted to D(-)-3-hydroxybutyrate by PHB depolymerise and then produced D(-)-3-hydroxybutyrate is converted to acetoacetate by D(-)-3-hydroxybutyrate dehydrogenase. Finally, acetoacetyl-CoA is formed by acetoacetyl-CoA synthetase. Metabolic pathway involved in the synthesis and degradation of PHB require energy and reducing

power. Although, the knowledge of the PHB degradation pathway is very limited, it is accepted that one of the steps in the biochemical pathway of PHB degradation must be the rate limiting (Ciggin, 2011a).

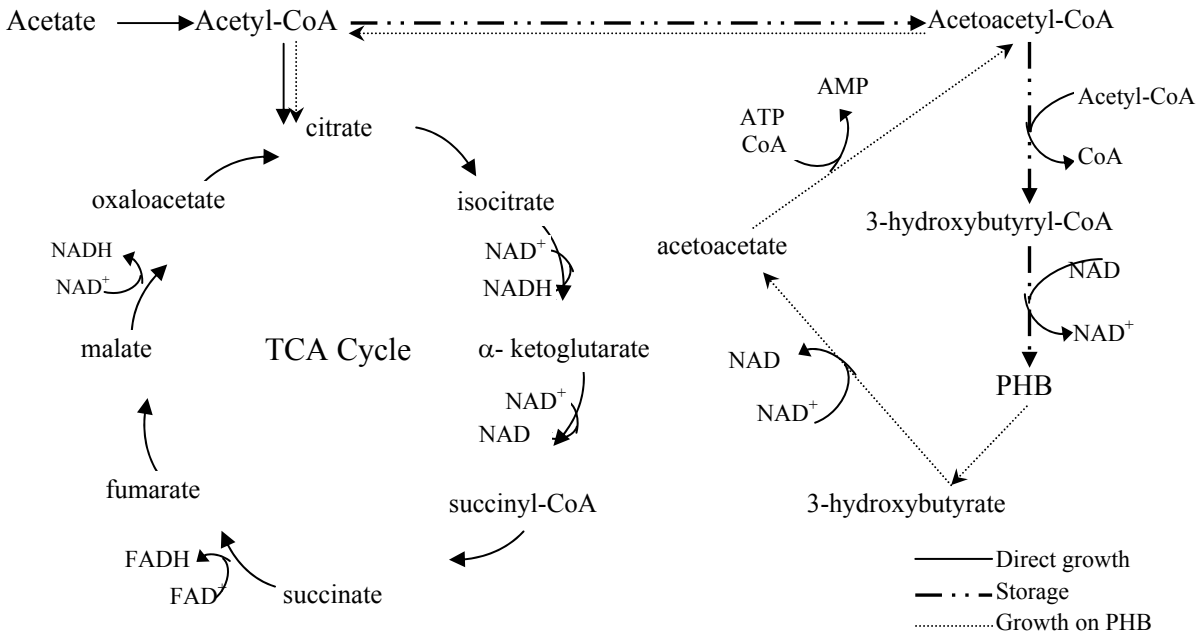


Figure 2.13 : PHB production pathways in feast/famine conditions (adapted from Salehizadeh and Van Loosdrecht, 2004).

2.6.4 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 (Roels, 1983). 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.

A metabolic model was proposed that acetate is aerobically stored as PHB under dynamic conditions by van Aalst-van Leeuwen et al. (1997a). This metabolic model reduces the number of unknown parameters and describes the observed kinetics of PHB formation and consumption by selected microorganism.

The substrate uptake rate will be larger than required for growth when carbon source is given periodically. It was 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 needed for growth is limited, ATP will accumulate

which is lead to accumulation of NADH2. 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 $NADH_2$ formation).

Van Aalst-van Leeuwen et al. (1997a) used acetate limited continuous culture of Paracoccus pantotrophus sp. in order to observe the metabolism of a microorganisms capable of producing and consuming which was described by seven internal reaction (Figure 2.14). Bacterial biomass is assumed to consist of two compartments: (i) an active biomass compartment and (ii) a poly(β)-hydroxybutyrate compartment. The active biomass is capable of reproduction and growth; the PHB is used as storage for carbon and energy. The fraction of PHB in the total biomass is called f_{PHB}. The fraction of active biomass in the total biomass is $(1-f_{PHB})$.

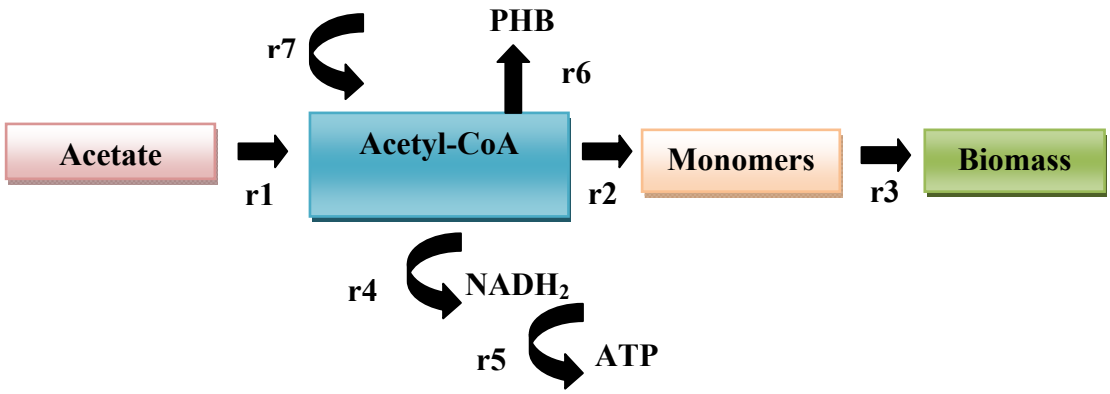


Figure 2.14 : Schematic representation of the metabolism of an organism capable of forming and consuming PHB (van Aalst-van Leeuwen et al, 1997a).

- **Synthesisof Acetyl-CoA from Acetate, (r_1):** Acetate is taken up by the cell by means of active transport and activated to form Acetyl-Pi, then this is further converted into acetyl-CoA.
- **Synthesis of biomass monomers from Acetyl-CoA, (r_2):** Anabolism, the synthesis of active biomass, involves two distinct types. First the biomass monomers are synthesized.
- **Polymerization of biomass precursors and maintenance, (r_3):** the biomass precursors are polymerized into active biomass.
- **Carbon source catabolism, (r_4)**
- **Oxidative phosphorylation, (r_5):** In oxidative phosphorylation, ATP is produced from $NADH_2$.

- **Synthesis of the storage product PHB from Acetyl-CoA, (r_6):** The substrate is used not only for synthesis of biomass and for catabolism but also for the synthesis of 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.

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 below Equation **(2.5)**.

$$(-r_S) = \frac{1}{Y_{SX}^{\max}} r_X + \frac{1}{Y_{SP}^{\max}} r_P + m_S C_X \qquad \textbf{(2.5)}$$

As similar, PHB consumption, biomass growth, and maintenance are expressed with the following equation for the famine period Equation **(2.6)**:

$$(-r_P) = \frac{1}{Y_{PX}^{\max}} r_X + m_P C_X \qquad \textbf{(2.6)}$$

where,

$$Y_{SX}^{\max} = \frac{4\delta - 2}{4.13\delta + 4.32} \qquad \textbf{(2.7)}$$

$$Y_{SP}^{\max} = \frac{4\delta - 2}{4.5\delta} \qquad \textbf{(2.8)}$$

$$Y_{PX}^{\max} = \frac{4.5\delta - 0.5}{4.13\delta + 4.32} \qquad \textbf{(2.9)}$$

$$m_S = \frac{m_{ATP}}{2\delta - 1} \qquad \textbf{(2.10)}$$

$$m_P = \frac{m_{ATP}}{4.5\delta - 0.5} \qquad \textbf{(2.11)}$$

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 $r_X=\mu C_X$, $r_S=q_S.C_X$, $r_P=q_P.C_X$ followed by division by biomass concentration, C_X gives the following Equation **(2.12)**:

$$(-q_S) = \frac{1}{Y_{SX}^{\max}}\mu + \frac{1}{Y_{SP}^{\max}} q_P + m_S \qquad \textbf{(2.12)}$$

From earlier experiments (Pot, 1996), 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]. The P/O ratio, δ= 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 the related equations and above values, also these parameters are considered to be constant within the experimental range. Maximum yield of PHB on acetate, Y_{SP}^{max} = 0.648 [C-mol/C-mol] was found by using the equation 3.4. Substituting these yield coefficients in Equation **(2.12)** gives Equation **(2.13)**:

$$-qs = 2.22\mu. + 1.544q_P + 0.038 \qquad \textbf{(2.13)}$$

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_PEquation **(2.14)** and **(2.15)**:

$$q_C = (-q_S) - \mu - q_P = 1.22\mu + 0.544q_P + 0.038 \qquad \textbf{(2.14)}$$

$$(-q_O) = 1.188\mu + 0.419q_P + 0.038 \qquad \textbf{(2.15)}$$

Stoichiometry of the kinetic model for the PHB production can be based upon Equations **(2.13- 2.15)**. 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. Below equations were derived by using these values:

$$(-q_P) = \frac{1}{Y_{PX}^{\max}} \mu + m_P \quad \textbf{(2.16)}$$

$$-q_P = 1.532\mu + 0.0131 \quad \textbf{(2.17)}$$

$$q_C = 0.532\mu + 0.0131 \quad \textbf{(2.18)}$$

$$(-q_O) = 0.693\mu + 0.0147 \quad \textbf{(2.19)}$$

Stoichiometry of the kinetic model for the PHB consumption can be based upon Equations **(2.16 -2.19)**. It was observed from experimental and modeling results that, the substrate uptake rate reaches its maximum value immediately after the pulse addition of carbon source. However, the growth rate is only influenced by maximal growth rate, and slowly decreases during growth on PHB presumably being related to the reduction in PHB amount (van Aalst- van Leeuwen et al, 1997a). According to the standard theory, the substrate uptake rate in a chemostat is found by the multiplication of the maximum uptake rate and Monod factor for the substrate relationship. This is an evidence for that the organisms will induce a maximal level of substrate uptake enzymes, while the enzyme system for cell growth will not be completely induced. So, even though the cells are cultivated close to growth rate of zero, the maximum substrate uptake activity will be maintained. Since the conditions are firmly different from a chemostat in wastewater treatment processes under dynamic conditions, it can be discussed that under such conditions microorganisms that have a fully induced substrate uptake system, accumulate more substrate and out compete organisms that do have lower substrate uptake rates.

The maximum substrate uptake rate of an organism is generally independent of the, real growth rate of that organism (Roels, 1983). In the model of van Aalst-van Leeuwen et al. (1997a), it was proposed that PHB is generally used as a buffer for the substrate taken up but not directly used for growth. The storage polymers can play a significant role in microbial growth under unbalanced conditions (van Loosdrecht et al, 1997a).

To evaluate storage mechanism, Krishna and Van Loosdrecht (1999) have observed that ASM3 failed to model two significant experimental observations: (i) the

discontinuity in the growth rate of biomass observed experimentally in feast and famine phases and (ii) it required prediction of higher levels of internal storage polymers than measured to fit the oxygen consumption during feast and famine phases. Guisasola et al. (2004), moreover, showed that for batch OUR data, ASM3 approach also causes severe practical identification problems that resulted in unrealistic and nonmechanistic parameter estimates which is the traditional way in model calibration. The major reason of this failure was the experimentally observed fact that storage and growth occur simultaneously during the feast phase as opposed to the assumption of ASM3 that only storage occurs during the feast phase (Krishna and Van Loosdrecht, 1999).

The experiments carried out in activated sludge systems, operating under dynamic conditions indicate that although the storage of internal polymers is usually the main mechanism for the removal of readily biodegradable carbon sources, bacteria can grow on both external and internal substrate under dynamic conditions (Ciggin, 2011a). It has been reported that bacteria use external substrate preferentially for cell growth (i.e. to optimize the growth rate) or for storage processes (i.e. to optimize the substrate uptake rate). This fact led to the formulation of the first simultaneous storage and growth model in order to interpret the experimental data better by Krishna and Van Loosdrecht (1999). From a mechanistic modelling point of view, it becomes clear that ASM3 should be extended to account for simultaneous storage and growth.

Activated Sludge Model for Growth and Storage (ASMG) had six components, namely, readily biodegradable substrate (in this study HAc was used as substrate), S_S, organics stored by heterotrophs (in this study storage is equivalent to PHB), X_{TSO}, heterotrophic biomass, X_H, inert particulate organics, X_I, total suspended solids, X_{TS} and finally, dissolved oxygen, S_O. Accordingly, it incorporated five biochemical processes: aerobic storage of COD; aerobic growth on PHB; aerobic endogenous respiration; aerobic respiration of PHB and aerobic growth on acetate (Krishna and Van Loosdrecht, 1999). A matrix representation of the metabolic model for aerobic conditions, including rate expressions and model coefficients of all relevant processes is given in Table 2.8.

Table 2.8 : Matrix representation of simplified ASM3 (Krishna and van Loosdrecht 1999).

Component → **Process** ↓	S_O	S_S	X_I	X_H	X_{STO}	X_{TS}	**Process rate**
Aerobic storage of COD (PHB storage)	$-(1-Y_{STO})$	-1			Y_{STO}		$k_{STO}.\frac{S_S}{K_S+S_S}.X_H$
Aerobic growth on PHB	$-\frac{1-Y_{H2}}{Y_{H2}}$			1	$-\frac{1}{Y_{H2}}$		$\mu_{H2}.\frac{S_S}{K_S+S_S}.\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}.X_H$
Aerobic endogenous respiration	$-(1-f_I)$		f_I	-1			$b_H.X_H$
Aerobic respiration of PHB	-1						$b_{STO}.X_H$
Aerobic growth on acetate	$-\frac{1-Y_{H1}}{Y_{H1}}$	$-\frac{1}{Y_{H1}}$		1			$\mu_{H1}.\frac{S_S}{K_S+S_S}.X_H$

For simultaneous storage and growth, three yield factors have been introduced 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. The schematic comparison of the ASM3 and metabolic model involving simultaneous growth and storage is illustrated in Figure 2.15.

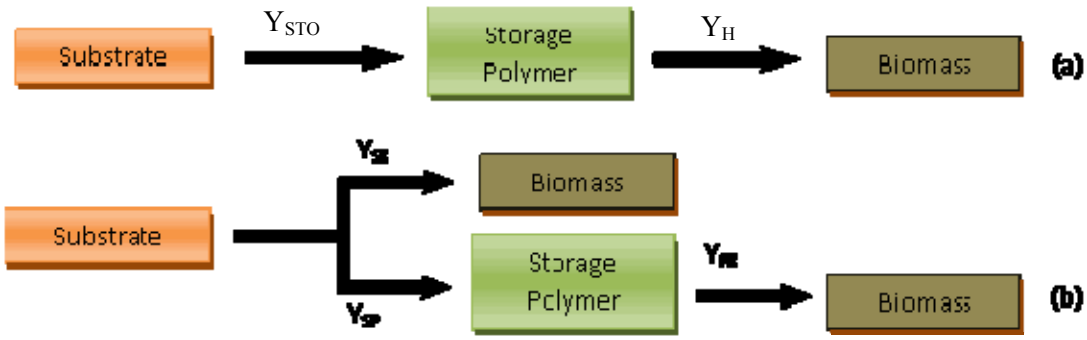


Figure 2.15 : Metabolic route of biomass (a) in ASM3 and (b) metabolic model proposed Krishna and Van Loosdrecht (1999).

In addition to Krishna and Van Loosdrecht (1999), several models have been proposed to improve the mechanistic modelling of simultaneous storage and growth processes in activated sludge systems. Orhon et al. (2009b), also evaluated that modeling of peptone biodegradation with simultaneous PHA storage was basically constructed on the same modified ASM1 template utilized in the earlier part of the experimental evaluation. The adopted model structure (growth-storage model) included a new model component reflecting the concentration of the storage products, X_{PHA} and two new processes, namely storage and growth on stored PHA, as given in a matrix format in Table 2.9. The storage process was conventionally defined in terms of a similar Monod-type of an expression where k_{STO} denotes the maximum storage rate. In this study, peptone was selected as carbon source because it is very similar to domestic sewage in terms of biodegradation characteristics. Also, it contains a similar balance between readily biodegradable COD and slowly biodegradable COD fraction. The kinetic and stoichiometric parameters of other related studies are given in Table 2.10.

Table 2.9 : Matrix representation of the model structure for simultaneous growth and storage (Orhon et al, 2009b).

Component → **Process** ↓	S_{O2}	S_S	S_{H1}	S_{H2}	X_H	X_{STO}	X_P	**Process rate**
Hydrolysis of S_{H1}		1	-1					$k_h.\frac{S_{H1}/X_H}{K_X+S_{H1}/X_H}.X_H$
Hydrolysis of S_{H2}		1		-1				$k_{hx}.\frac{S_{H2}/X_H}{K_{XX}+S_{H2}/X_H}.X_H$
Storage of PHA	$-(1-Y_{STO})$	-1				Y_{STO}		$k_{STO}.\frac{S_S}{K_S+S_S}.X_H$
Growth of X_H	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$			1			$\mu_H.\frac{S_S}{K_S+S_S}.X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$				1	$-\frac{1}{Y_H}$		$\mu_{STO}.X_{STO}$
Decay of X_H	-(1-*fp*)				-1		*fp*	$b_H.X_H$

Table 2.10 : Kinetic and stoichiometric parameters of ASM3 and modified ASM3.

Parameters	Insel et al, 2012		Cokgor et. al, 2011	Ural, A., 2011	Ciggin, A.S., 2011a		Orhon et al, 2009b	Cokgor et. al, 2008	Karahan et al, 2008	Insel et al, 2007	Karahan et al, 2006	Karahan-Gül et al, 2003		Krishna and van Loosdrecht, 1999	
Model Type	Modified ASM3		Adopted model	Modified ASM3	Modified ASM3		ASM3	ASM3	Modified ASM3	Proposed model	Modified ASM3	ASM3	Modified ASM3	ASM3	Modified ASM3
SRT(day)	10	2	10	10	8	2	10	10	-	5	5	20		2.5	
Carbon Source	Acetate		Peptone	Acetate	Acetate		Peptone	Synthetic wastewater	Acetic acid	Glucose	Soluble starch	Acetate		Acetate	
μ_{Hmax}(1/day)	3.9	6.8	4.1	3.5	1.5	2.5	6.1	3.2	3	15.1	3	-	4	-	2
μ_{STO}(1/day)	4.7	2.2	1.25	3.5	1.5	2.0	1.10	-	5	-	4	3 & 1.8	3	-	3.5
K_S (mg COD/L)	6	25	4.0	6.0	5	10	2.3	40	5	16	20	4	3	0.1	0.1
K_{STO}(mg COD/L)	1	5	0.41	0.3	0.45	0.5	-	0.002	0.5		0.4	1	0.4	1	1
b_H (1/day)	0.22	0.22	0.20	0.2	0.20	0.20	0.20	0.20	0.10	0.15	0.10	0.24	0.24	0.2	0.2
b_{STO} (1/day)	-	-	-	-	0.10	0.10	-	0.20	0.05		0.05	0.24	0.24	0.2	0.4
k_{STO} (1/day)	5.7	6.3	0.90	5.7	8.0	6.5	0.45	-	15	1.4	25	16	14	10	10
Y_H (mg COD/ mg COD)	0.66	0.66	0.60	0.66	0.68	0.68	0.60	0.67	-	0.47	0.79	-	0.65	-	-
Y_{STO} (mg COD/ mg COD)	0.80	0.80	0.80	0.80	0.85	0.85	0.80	0.78	0.76	0.90	0.91	0.80	0.80	-	-
k_h (1/day)	-	-	4.34	-	-	-	5.95	-	-	-	30	-	-	-	-
K_X (mg COD/ mg COD)	-	-	0.03	-	-	-	0.05	-	-	-	0.15	-	-	-	-
f_{ES}	0.05	0.05	0.05	0.05	-	-	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	-	0.15	0.10	0.15	0.15	-	-
SBR cycles	1	1	1	1	6	6	1	1	6	1	6	1	1	6	6

2.6.5 Effects of SRT on storage kinetics

As the average growth rate of the biomass is mainly controlled by the sludge age (SRT), SRT has been used for the determination of the mean microbial life-time, and hence microbial population in activated sludge systems (Frigon, 2006). Although, ASM3 suggested the default value of storage yield as 0.85 g COD/g COD, extensive experimental studies showed that storage yield ranges from 0.38 to 0.79 gCOD/gCOD according to different SRT. Storage yield values for different substrates and related sludge ages found in several studies are given in Table 2.11.As seen from the Table 2.11, different yield values were reported by different authors for the same substrates.

Table 2.11 : Experimentally found storage yield values for different SRT.

Reference	Growth conditions of activated sludge		Carbon source	Y_{STO} (g COD/ g COD)
	SRT (days)	Feed composition (Ratio, COD based)		
Beun et al.(2000)	3.8	Acetate	Acetate	0.69
Beun et al. (2002)	2-20	Acetate	Acetate	0.68
Carta et al.(2001)	6.1	Acetate+glucose (1:1)	Acetate+glucose	0.6-07
Dionisi et al.(2004)	1	Acetate + Lactate + propionate (2:2:1)	Acetate Propionate Lactate	0.49 0.38 0.32
Goel et al. (1998)	10	Acetate + Glucose + Peptone + Yeast Extract (2:1:1:1)	Glucose Acetate Starch	0.68 0.45 0.36
Krishna and Van Loosdrecht (1999)	2.5	Acetate	Acetate	0.73
Martins et al. (2003)	7-10	Acetate	Acetate	0.47-0.59

The storage yields directly calculated from the fraction of removal substrate to produced PHB $(q_P/-q_S)$, which is recovered as storage compound. The faster substrate uptake rate ($-q_S$) has been usually interpreted as indirect evidence of more relevant presence of a storage response in activated sludge process (Van Loosdrecht et al, 1997a). Briefly, experiments showed that while substrate uptake rate ($-q_S$) decreased linearly, storage compound production rate (q_P) linearly increased in the

feast period when extra cellular substrate is available. The fraction of substrate removed to storage polymer produced ($q_P/-q_S$) is used as an indication of which fraction of the substrate is stored. The substrate uptake rate is limited by the substrate concentration. Although a linear relation exists between substrate uptake and biomass growth in the systems where only biomass growth is observed, more complicated relations are present between the substrate uptake rate, biomass growth and PHB production.

Studies on the storage phenomenon with pure cultures at low SRT (i.e. high growth rate) showed that storage is dependent on the growth rate of the culture (Van Aalst-van Leeuwen et al, 1997a). In other words, the accumulation rate of storage products was linearly correlated to the difference between the maximum substrate uptake rate and the substrate uptake rate required for growth. When the culture is operated at a growth rate close to its maximum substrate uptake rate, storage is observed to be negligible. In this range, storage rate changes slightly with SRT while growth rate is strongly affected by variation of SRT (Van Loosdrecht and Heijnen, 2002).

The slower substrate uptake rate and PHA production rate have been observed in the SBR fed with acetate at SRT of 1 day then the SBR operated at SRT of 10 days although high organic loading was applied at low SRT (Lemos et al, 2008). The authors have concluded that the biomass was dominated by species which have high specific growth rates at low SRTs, as the growth yield was higher despite of lower uptake rates in low SRT. According to Beun et al. (2002); the ratio $q_P/-q_S$ has a constant value for dynamically fed systems operated at a sludge ageSRT>2d. This value is 0.6 Cmol/Cmol under aerobic conditions.

Beun et al. (2000) showed that using the PHB metabolism and adjusting the substrate uptake rate enable the bacteria to balance their growth rate under dynamic substrate supply and effectively compete for substrate. If acetate is present in excess (feast period), it is taken up with the maximal specific acetate uptake rate, which is dependent on the SRT. At the same time, PHB is produced with a constant rate. 66% to almost 100% of the acetate consumed in steady state systems growing under non-growth-limiting conditions is used for PHB synthesis processes. This percentage increases with increasing SRT. The remainder of the acetate consumed is used for growth and maintenance processes. The difference in specific growth rate between the feast and famine period decreases with increasing SRT.

Dircks et al. (2001) presented that PHB formation is found to be the dominant process in heterotrophic conversion of acetate in activated sludge under aerobic conditions and yield of PHB on acetate was found around 0.67 COD/COD. According to this study, the PHB degradation pattern found in this study is dependent on the amount of PHB present in the cells at the end of the feast period. The significant part of the total potential growth (90%) is found to occur in the famine period on stored PHB. The sludge with SRT of 4 d is found to degrade PHB faster than sludge of higher SRTs at high fractions of PHB.

Van Aalst-van Leeuwen et al. (1997b) observed that faster growing organisms accumulated less PHB. In addition to this observation, Majone et al. (2007) showed that the overall transient response (i.e. maximum specific substrate removal rate) increased as SRT decreased in their pure culture study. Same statement has been made by Van Loosdrecht and Heijnen (2002) that the shorter the SRT, the higher the growth rate and the less substrate converted into storage polymers. Ciggin et al. (2011c) also determined that low sludge age did not alter simultaneous storage and utilization for direct growth but it slightly favoured direct utilization due to faster growing biomass.

Alternatively, it was found that activated sludge with an SRT of 3 days had better PHA production capability than sludge with an SRT of 10 days (Chua et al, 2003). This observation was explained with the selection of microbial community having higher PHA production capacity at shorter SRTs than longer SRTs.

In addition, in the mixed culture, specific PHB production rate in the feast period was determined to be almost constant for different SRTs. Higher substrate uptake rate was observed in the culture acclimated at the SRT of 3.8 days, than the culture acclimated at the SRT of 9.5 and 19.1 days, which were essentially the same (Beun et al, 2000).

Storage yields for glycogen storage from glucose has been founded as 0.96 g COD/g COD (Chudoba et al, 1985) and accepted as 0.9 g COD/g COD (Goel et al, 1999). This difference in the storage yield of two type of substrate is explained that the formation of glycogen from glucose requires less energy than the PHB formation from acetate. While storage products are producing at a constant rate in the beginning, the rate of storage decreases due to reaching maximum storage capacity in time. This causes the overall and observed storage yields to vary over time on the

F/M ratio of the experiment. On the other hand, it was observed that F/M ratios did not affected the storage yields that were reported as 0.78 g COD/g COD for acetate and 0.87 g COD/g COD for glucose (Karahan-Gül et al, 2002a).

3. MATERIALS AND METHODS

Main aim of this study is to investigate acute and chronic inhibition effects of sulfamethoxazole on the biodegradation of acetate under aerobic conditions at low sludge age. Sulfamethoxazole (SMX) was chosen as model antibiotic to determine the effects of antibiotics on the aerobic activated sludge systems and the removal mechanism of the selected substrate at low sludge age. For this purpose, a control reactor operated in fill and draw mode was used for the acclimation of activated sludge to aimed conditions. Carbon source was added to system once a day and biomass was acclimated to pulse feeding pattern in the aerobic batch reactor. The control reactor was fed with acetate as a sole carbon source and operated at steady state conditions. Aftersteady state conditions were established in control reactor, activated sludge culture was taken to perform respirometric tests for acetate removal mechanism and acute and chronic effects of antibiotic substance.

3.1 Reactor Setup

Experimental study was started up with the set-up of the control reactor. A laboratory-scale fill and draw reactor, namely "control reactor" with a net volume of 4 L was inoculated with activated sludge taken from the acetate acclimated biomass used in another study. The control reactor was operated at a sludge ageof 2 days with the initial carbon source concentration of approximately 400 mg COD/L (Figure 3.1). Acetate representing readily biodegradable substrate was chosen as sole carbon source, pH was kept in the range of 6.0 to 8.0, suitable for biological activity and the temperature was maintained at 20 ± 1°C during the operation of reactor. During each daily feeding period, reactor was settled for 1 h (t_s) and decanted until 2 L (V_0). Aeration was continuously provided and the oxygen concentration in the reactor was kept above 2 mg/L to maintain aerobic conditions. An magnetic stirrer was also provided with the reactor in order to mix the contents uniformly. Reactor was operated until it reached steady state conditions, which was monitored by suspended solids (SS), volatile suspended solids (VSS) and COD measurements. After the

control reactor reached at steady state conditions; the acclimated biomass was used for respirometric experiments. At steady state, biomass concentration of the reactor was stabilized approximately 360 mg VSS/L.

Figure 3.1 : A laboratory-scale control reactor.

The substrate feed was prepared by diluting a stock solution of sodium acetate trihydrate (CH_3COONa) in distilled water. The nutrient requirements of the activated sludge were supplied with the addition of macro and micro nutrient solutions, namely Solution A and Solution B (O'Connor, 1972). The nutrients were supplied for the nutritional needs of the cells and to provide enough buffer capacity to the reactor so that pH is kept around 7.0. In the reactor, amount of Solution A and B were adjusted to supply 10 mL of solution for 1000 mgCOD/L of carbon source (O'Connor, 1972). The composition of these solutions is given in Table 3.1.

3.2 Experimental procedure

The main idea of respirometric analysis is to measure the change in the respiration capacities of activated sludge. Oxygen uptake rate (OUR) profiles should be comprehended for the evaluation of substrate removal mechanisms. OUR isalso now prescribed as a standard procedure for inhibition assessment (ISO, 2007). It is successfully used for the evaluation of the inhibitory impact induced by various organic compounds including antibiotics (Karahan et al, 2010).

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

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

Therefore, the OUR experiments were conducted in a respirometer during typical batch tests (Orhon et al., 2002).Acetate was added on the biomass to have the same S_0/X_0 ratio with the control reactor and oxygen uptake rate was monitored for the assessment of substrate biodegradation behaviour. In the respirometric tests, the initial acetate concentration was kept approximately200 mg COD/L, because of this both VSS and substrate concentrations were diluted by 1/2 ratio. The obtained OUR profile was considered as control in order to evaluate the effects of antibiotic substance. In other words, control analysis without antibiotic addition was conducted before inhibition analysis for each experiment. The OUR profiles obtained in this part of study was used for the experimental assessment of individual stoichiometric and kinetics coefficients.

In this study, OUR measurements were performed with an Applitek RA-Combo-1000 (Applitek Co., Nazareth, Belgium) continuous respirometer with PC connection (Figure 3.2). During each analysis, 1.04 g Allyl Thio Urea (ATU) (Formula 2533TM, Hach Company) was added to the OUR reactors as a nitrification inhibitor to prevent any possible interference induced by nitrification. The respirometer used in the experiments involved a continuous flow-through measurement using the DO concentration in the liquid phase to calculate the respiration rate of the activated sludge (Spanjers et al, 1996). In principle, the activated sludge was continuously transferred to the respirometer with a peristaltic pump. After passing through the respiration vessel (0.75 L), where the dissolved oxygen at the inlet and outlet were measured by a single DO probe, the sample returned to the OUR reactor. Hereby the activated sludge was continuously recirculated. Due to the use of a single DO-

electrode the measuring frequency was limited by the response rate of the DO-electrode (Spanjers et al, 1996). The OUR reactor was continuously aerated and samples were taken for COD, PHA and antibiotic analyses until the endogenous decay was observed after the depletion of carbon source. At steady state, biomass concentration of all respirometric experiments was stabilized same ratio control reactor at the steady state conditions. In all respirometric experiments, nine 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.

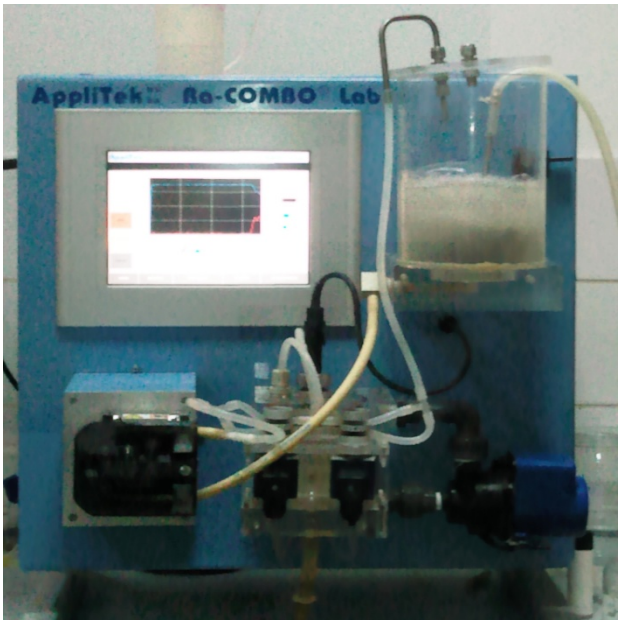


Figure 3.2 : Applitek RA-Combo-1000 continuous respirometer.

Table 3.2 : Monitored data for experimental runs.

Time	pH	SS/VSS	COD filtered	SMX filtered	PHA
-30 min	x	x	x	x	x
20 min			x	x	x
40 min			x	x	x
60 min			x	x	x
80 min			x	x	x
100 min			x	x	x
140 min			x	x	x
200 min			x	x	x
24 h			x	x	x

Additional respirometric experiments were conducted on the activated sludge to determine acute and chronic effects of antibiotics on the activated sludge system. These respirometric tests were started with the approximately same loading ratio (S_0/X_0) with control reactor. After observation of the endogenous decay level in terms of straight line, the antibiotic was added to OUR reactor. The antibiotic was given to system with different pulse feedings of 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of SMX concentrations to evaluate the acute effects on the carbon removal performance. All experiments with antibiotic were conducted in duplicate. The summary of control and acute experiments were shown in Table 3.3.

Although acute effects were characterized by sudden exposure to the substance, chronic effects are characterized by prolonged exposures over many times. To understand chronic effects of antibiotic substance to activated sludge systems; 12 L chronic reactor was set using the seed sludge taken from the control reactor (Figure 3.3). For understanding the chronic effects, the experimental observation of the study involved a period of 18 days after reaching steady state conditions. All chronic experiments were done with the same antibiotic concentration (50 mg/L SMX). The chronic experiments were given detailed in Table 3.4. Using respirometric studies, the chronic effects of SMX on the activated sludge culture was determined by the change of the OUR profile in time. In addition, the OUR reactor wrapped in aluminum foil to prevent possible photodegradation of sulfamethoxazole in the all acute and chronic experiments.

Table 3.3 : Outline of control and acute experiments at a sludge age of 2 days.

SUBSTRATE			**ANTIBIOTIC Acute Experiments**		
Runs	**Type**	**Concentration** (mg COD/L)	**Type**	**Concentration** (mgSMX/L)	**Concentration** (mg COD/L)
Run 1	Acetate	195	Control	-	-
Run 2	Acetate	230	SMX	25	34.5
Run 3	Acetate	250	SMX	50	69
Run 4	Acetate	240	SMX	100	138
Run 5	Acetate	217	SMX	200	276

Figure 3.3 : Chronic experiment reactor with SMX feeding.

Table 3.4 : Outline of chronic experiments at a sludge age of 2 days.

Runs	**Acclimation period**	**Carbon Source**	**Acetate Concentration** (mg COD/L)	**Antibiotic Concentration** (mg SMX/L)	**AntibioticCon centration** (mg COD/L)
Run 1	0. day – control	Acetate	195	-	-
Run 5	1. day	Acetate + SMX	250	50	69
Run 6	2. day	Acetate + SMX	230	50	69
Run 7	4. day	Acetate	230	-	-
Run 8	6. day	Acetate + SMX	230	50	69
Run 9.1	8. day	Acetate + SMX	230	50	72
Run 9.2	8. day	Acetate	230	-	-
Run 9.3	8. day	SMX	-	50	72
Run 10.1	10. day	Acetate + SMX	230	50	72
Run 10.2	10. day	SMX	-	50	72
Run 11.1	18. day	Acetate + SMX	230	50	70
Run 11.2	18. day	Acetate	230	-	-

3.3 Analytical procedures

3.3.1 Standard methods

Temperature of all experiments was maintained at 20 ± 1◦C. pH was kept around 7.0 suitable for biological activity and minimum dissolved oxygen concentration of 2.0 mg/L was supplied in the system, respectively. During the experiments an Orion 520 pH meter was used for pH measurements and before each usage of the device the pH meter was calibrated.

Samples, which were taken for soluble COD and antibiotic measurements, were filtered through 0.45 μm polyvinylidene fluoride (PVDF) syringe filters and COD samples were preserved with H_2SO_4. COD measurements were performed as described in the ISO 6060 method (ISO 6060, 1986). To understand the definite SMX concentration in terms of COD, macro COD measurements were also performed for stock antibiotic solution. The biomass concentration was monitored by total suspended solids (TSS) and volatile suspended solids (VSS) analysis as described in Standard Methods (APHA, 2005). TSS and VSS measurements were carried out after filtering the sample from Milipore AP40 glassfiber filters with an effective pore size of approximately 1.2 μm.

The gas chromatographic method as described by Braunegg et al. (1978) for the determination of poly-β-hydroxybutyric acid (PHB) consists of a mild acid or alkaline methanolysis of poly-β-hydroxybutyric acid directly without previous extraction of PHB from the cells; this is followed by gas chromatography of the 3-hydroxybutyric acid methylester. The method was characterized by high accuracy and excellent reproducibility, permitting determinations as low as 10^{-5} g/L. Only 4 h is required from sampling from the fermenter till completion of the PHB determination.

The only pretreatment of cell samples is simply centrifugation, the time for which can be considerably shortened by the addition of methanol to the suspension; this is particularly so when the cells contain large amounts of PHB. After decanting the liquid phase, the cells can be directly subjected to the acidic methanol treatment prior to GC analysis. Centrifuged cells are suspended in a mixture of 2 mL methanol (3% H2SO4, v/v) and 2 mL chloroform, then heated at 100 °C for 4 hours. After cooling to room temperature, 1 mL H_2O is added and the sample shaken vigorously for 10

min. The two phases are allowed to separate, during which cell detritus gathers at the interphase. The organic phase which is to be analyzed is placed in vials and can be stored unchanged at 4 °C for several weeks. Within the linear range of the detector used on the gas chromatograph (GC) apparatus, the results of this method for PHB determination depend neither on the volume of the cell suspension nor on the PHB content of the cells; the maximum standard deviation was ± 0.5% (Braunegg et al., 1978).

3.3.2 Antibiotic measurements

SMX samples were measured by high-performance liquid chromatography (HPLC, Agilent 1100 Series, USA) equipped with a Diode-Array Detector (G1315A, Agilent Series) and Novapack reverse phase C18 column. Detector was adjusted at a wavelength of 280 nm. The SMX samples filtered through 0.45 µm Milipore membrane filters were transferred to HPLC vials. Methanol–water solution (30:70, v/v) was used as mobile phase at a flow rate of 1.1 mL/min as proposed by Beltran et al. (2008). Mobile phase was acidified with phosphoric acid (0.1 %) at pH 2.5. Injection volume was selected as 40 µL. A calibration curve was plotted for SMX measurements with Diode Array Detector at 280 nm (Figure 3.4).

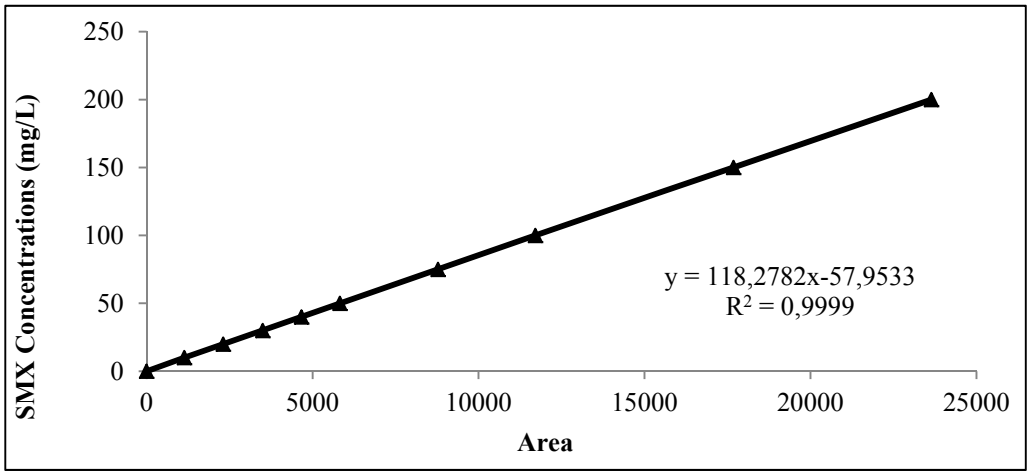


Figure 3.4 : SMX calibration curve forobtained for HPLC measurements.

4. RESULTS AND DISCUSSION

4.1 Acclimation Studies of Control Reactor

A laboratory-scale fill and draw reactor, namely "control reactor" a net volume of 4 L was operated at a sludge ageof 2 days with the initial carbon source concentration of approximately 400 mg COD/L. Acetate representing readily biodegradable substrate was chosen as sole carbon source. pH was kept in the range of 7.0 to 8.0, suitable for biological activity and the temperature was maintained at 20 ± 1˚C during the operation of reactor. At steady state conditions, biomass concentration of reactor was stabilized approximately 360 mg VSS/L, yielding the S_0/X_0 ratio of 1.1 mg COD/mg VSS, respectively.

In summary, the control reactor characteristics at steady state conditions were given in Table 4.1. The SS, VSS and VSS/SS ratio of the control reactor were monitored in a period of 5 months in detailed. Reactor monitoring data can be seen in Figure 4.1 and Figure 4.2.The pH measurements and influent and effluent COD concentrations of the control reactor in a period of 5 months were also represented in Figure 4.3 and Figure 4.4.

Table 4.1 : Steady state characteristics of the control reactor.

Carbon Source	SS	VSS	VSS/SS	S_0/X_0	CODinf.	CODeff.	Removal efficiency
	mg SS/L	mg VSS/L	-	mgCOD/ mgVSS	mg/L	mg/L	%
Acetate	505	360	0.71	1.1	400	45	89

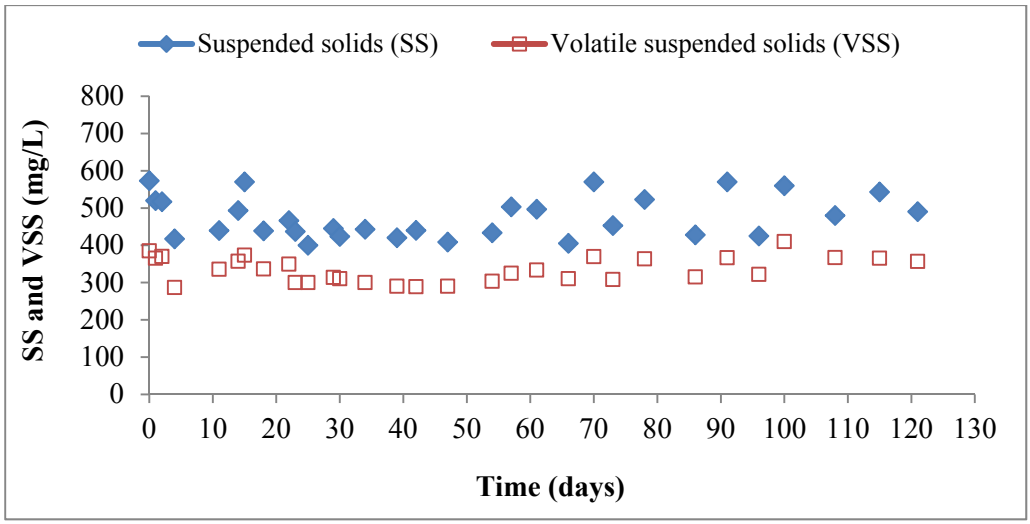


Figure 4.1 : SS and VSS results of the control reactor.

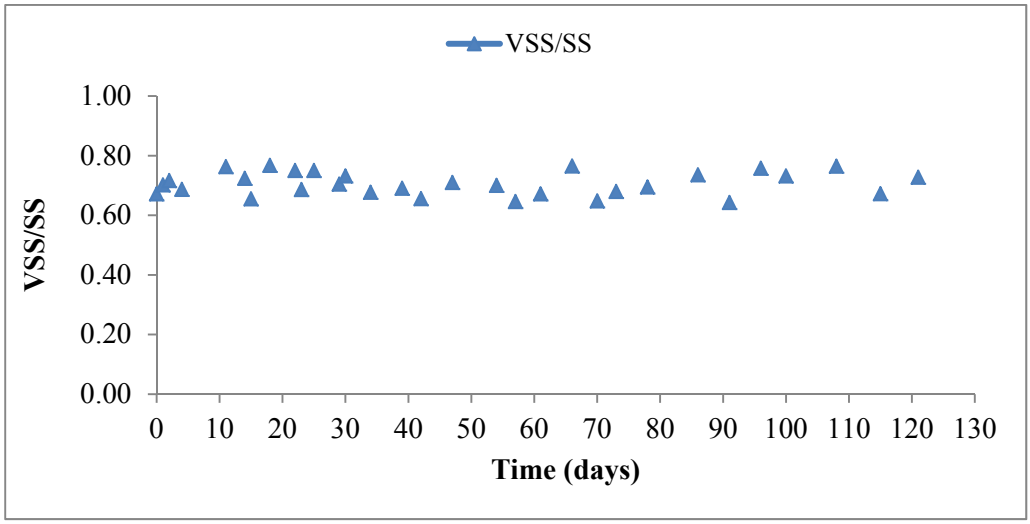


Figure 4.2 : VSS/SS ratio of the control reactor.

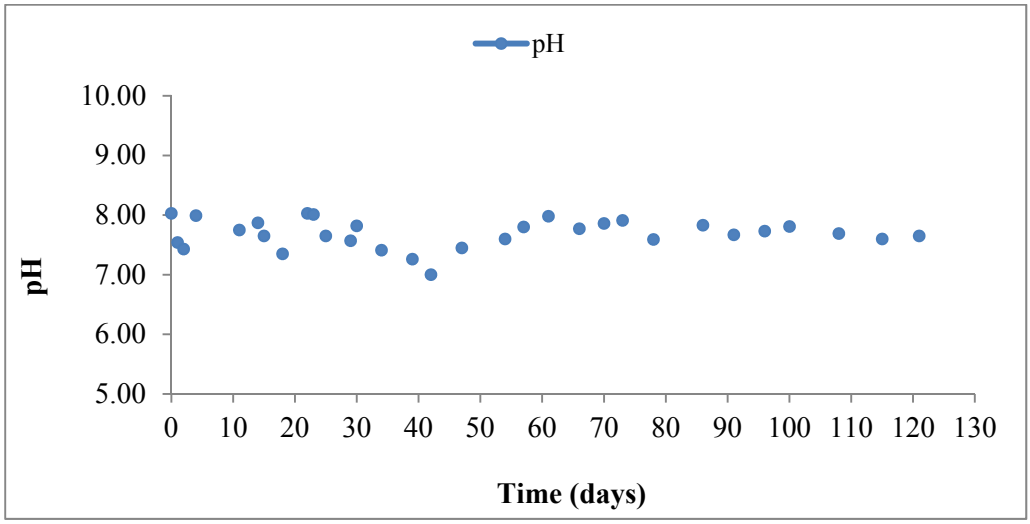


Figure 4.3 : pH measurements of the control reactor.

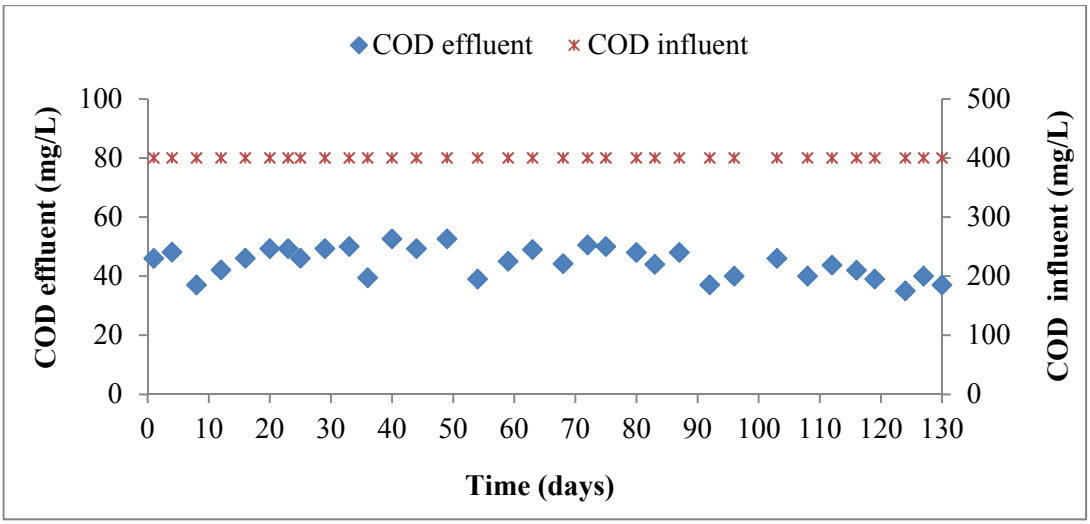


Figure 4.4 : Influent and effluent soluble COD concentrations of the control reactor.

4.2 Experimental Results

4.2.1 Evaluation of control experiment

First run of respirometric experiments was performed with using the acetate as sole carbon source and biomass taken from the control reactor in order to investigate the biomass behaviour under steady state conditions (Run 1). Respirometric test was started with the approximately same loading ratio (S_0/X_0) with control reactor. For this reason VSS and substrate concentrations were diluted by 1/2 ratio. After observation of the endogenous decay level in terms of straight line, the acetate was added to OUR reactor of respirometry as carbon source.

The oxygen utilization rate (OUR) curve obtained from biodegradation of acetate was used as control for evaluating the acute and chronic effects of antibiotic on biomass (Figure 4.5). In other words, the impact was interpreted by changes inflicted by antibiotics on the shape of the initial OUR profile obtained in the control test conducted without antibiotic addition. The OUR, defined as the rate of oxygen utilization in biochemical processes in the activated sludge models, is the change observed in the dissolved oxygen concentration (S_O) in time due to biochemical transformations. Thus, OUR is an overall process rate reflecting the cumulative impact of all oxygen/energy consuming reactions. The OUR curve obtained from the biodegradation of the acetate is shown in Figure 4.5. The maximum oxygen uptake rate of the biomass gave the first peak around 80 mg/L.h in 58 minutes, which is due to readily biodegradable COD component in the system. The area under the OUR

curve has shown that the total oxygen consumption for defined period of time (i.e. one minute).

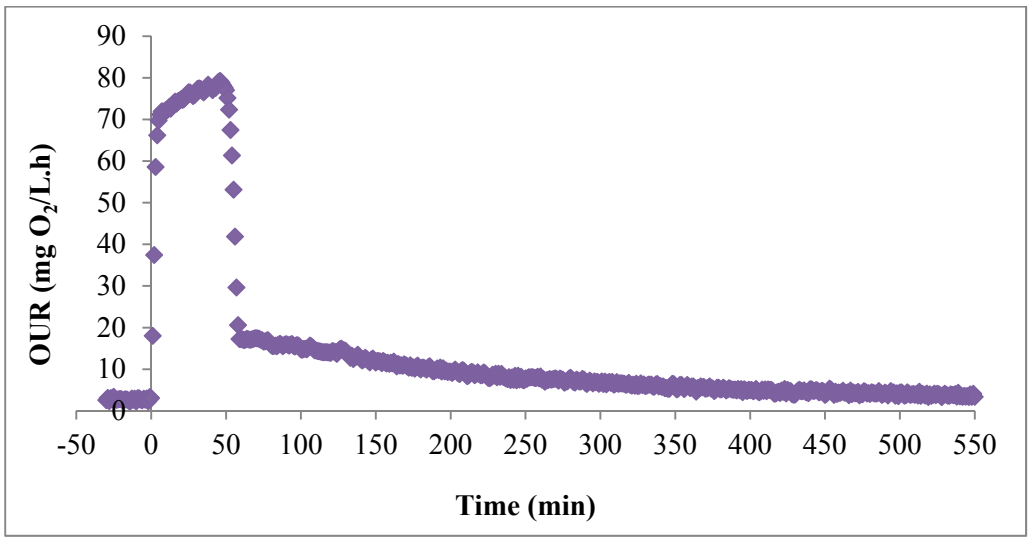


Figure 4.5 : OUR profile of the control experiment versus time (Run 1).

The COD removal efficiency of acetate in Run 1 was given in the Figure 4.6 which indicates removal of all influent acetate in the control reactor during a period of time. The organic substrate (acetate) used in the experiments is by nature totally biodegradable; this is one of the main reasons for its selection and recommendation as the standard substrate for biodegradation experiments. Because the biodegradable COD in the control reactor was completely depleted after the OUR profile dropped to the initial endogenous respiration level.

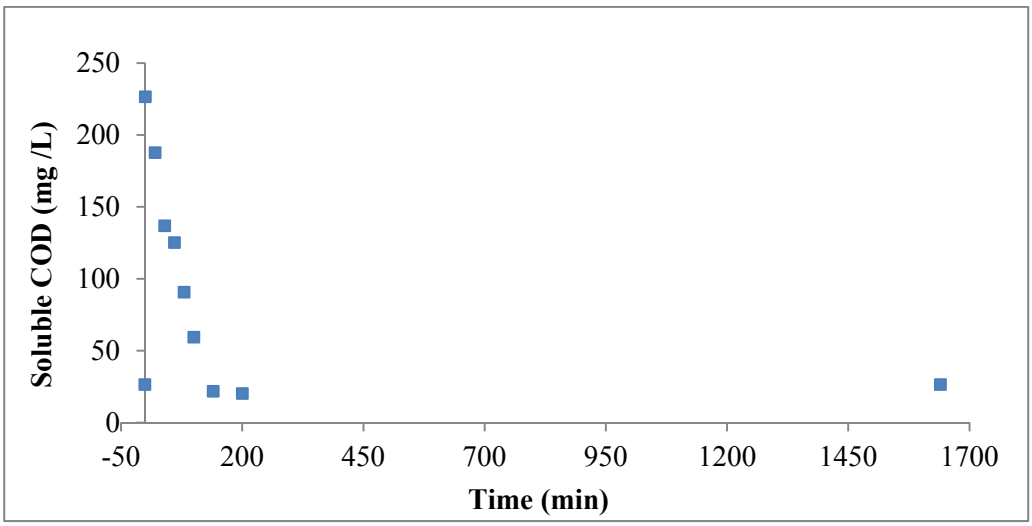


Figure 4.6 : Soluble COD profile of the control experiment versus time (Run 1).

4.2.2 Evaluation of acute experiments

Acute experiments were conducted on the activated sludge to determine acute effects of SMX on the activated sludge and carbon source removal performance. Acute effects were characterized by sudden exposure to the substance and microbial system is exposed to the antibiotic for the first time. According to literature review, antibiotics remain nonbiodegradable for the short term tests as acute inhibition effect.

Acute respirometric tests were also started with the approximately same loading ratio (S_0/X_0) with control reactor. After observation of the endogenous decay level in terms of straight line, the SMX was added to OUR reactor with carbon source. While approximately200 mg/L acetate was added to system at OUR experiments, the antibiotic was supplied in different concentration in order to evaluate the acute effects of SMX on the carbon source removal performance.

As mentioned before, the OUR tests were conducted with four different antibiotic concentrations from 25 mg/L to 200 mg/L, mainly because they are representative of the range commonly encountered in the individual effluent streams at related pharmaceutical plants (Ozkok et al, 2011). The results are expected to be meaningful because removal at the source is always advocated as the best means of controlling these chemicals. All experiments with antibiotic were conducted in duplicate. The summary of control and acute experiments were shown in Table 4.2.

Table 4.2 : Control and acute experiments at a sludge age of 2 days.

SUBSTRATE			**ANTIBIOTIC Acute Experiments**			
Runs	**Type**	**Acetate Conc.** (mg COD/L)	**Type**	**SMX Conc.** (mg SMX/L)	**SMX Conc.** (mg COD/L)	**VSS** (mg VSS/L)
Run 1	Acetate	195	Control	-	-	240
Run 2	Acetate	230	SMX	25	34.5	274
Run 3	Acetate	250	SMX	50	69	239
Run 4	Acetate	240	SMX	100	138	273
Run 5	Acetate	217	SMX	200	276	246

Acute effect of 25 mg/L antibiotic addition to acetate acclimated biomass was investigated in Figure 4.7. Run 2 was started with an initial VSS concentration of 274 mg/L. The OUR curve reaches a maximum level of 75 mg/L.h in 82 minutes.

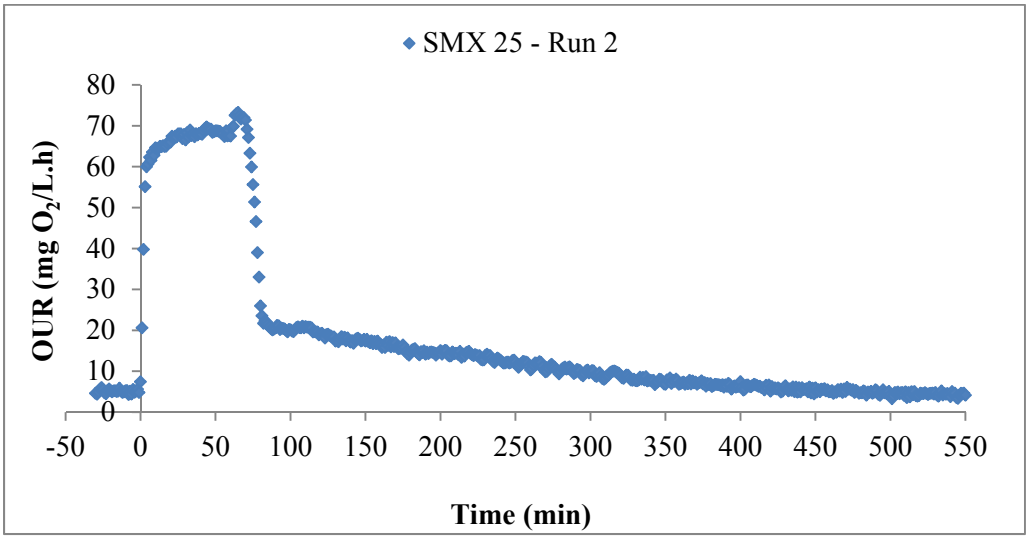
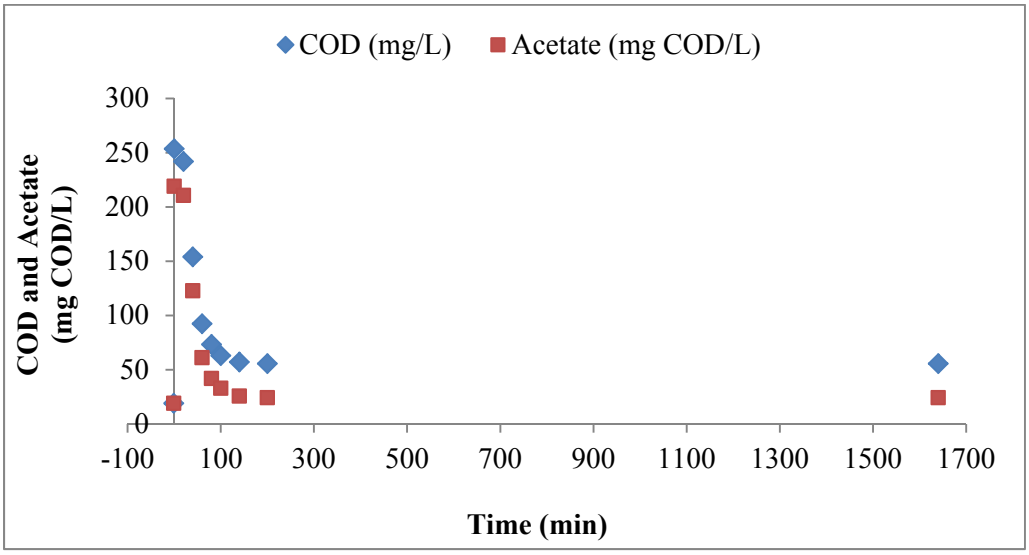
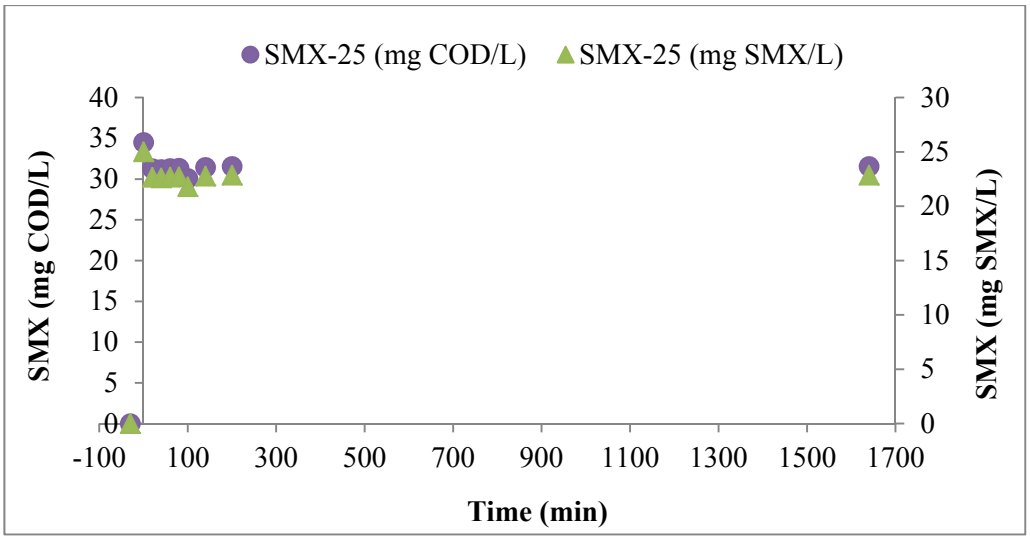


Figure 4.7 : OUR profile versus time (Run 2).

Addition of different concentrations of antibiotic did not change the removal efficiency of acetate in the activated sludge system. In addition, SMX removal was not observed during a period of time with the different concentration of antibiotic addition. Figure 4.8 shows soluble COD profile and 25 mg SMX/L antibiotic measurement. Antibiotic was not removed with respect to time as shown in figure and the removal of acetate was also calculated by substracting the COD of SMX from total soluble COD.



(a)



(b)

Figure 4.8 : Removal efficiencies of Run 2 (a) soluble COD and acetate profiles (b) SMX profiles.

OUR profile of 50 mg/L SMX addition was displayed in Figure 4.9.Run 3 was started with an initial VSS concentration of 239 mg/L. The OUR curve reaches a maximum level of 66 mg/L.h in 87 minutes. Figure 4.10 also shows acute effect of 50 mg/L SMX concentration and soluble COD profile.

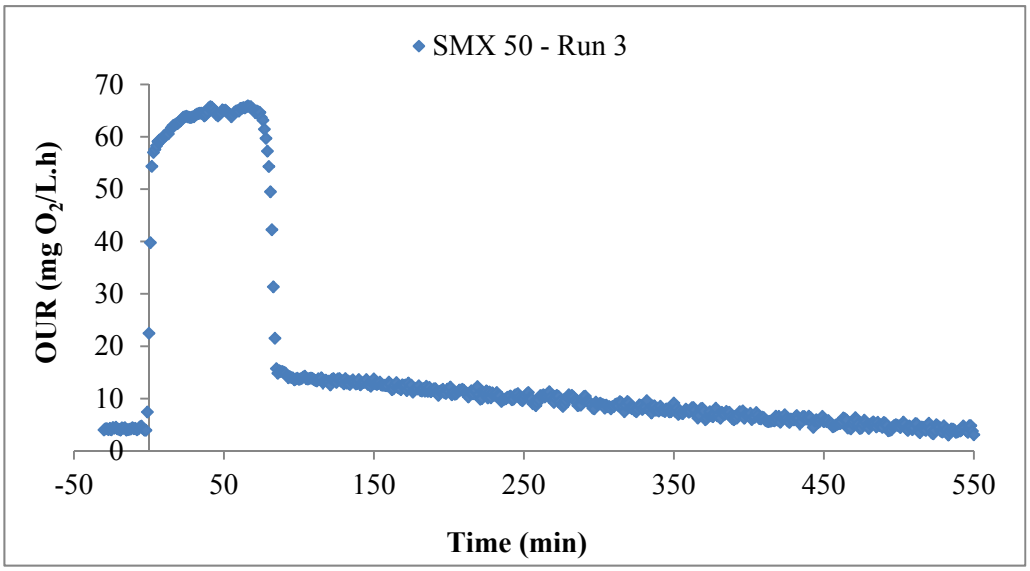
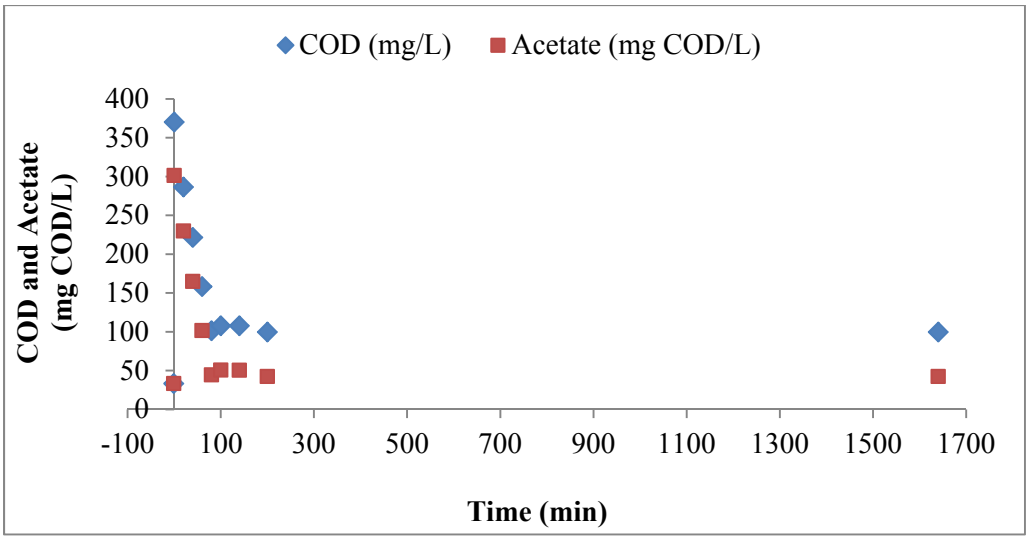
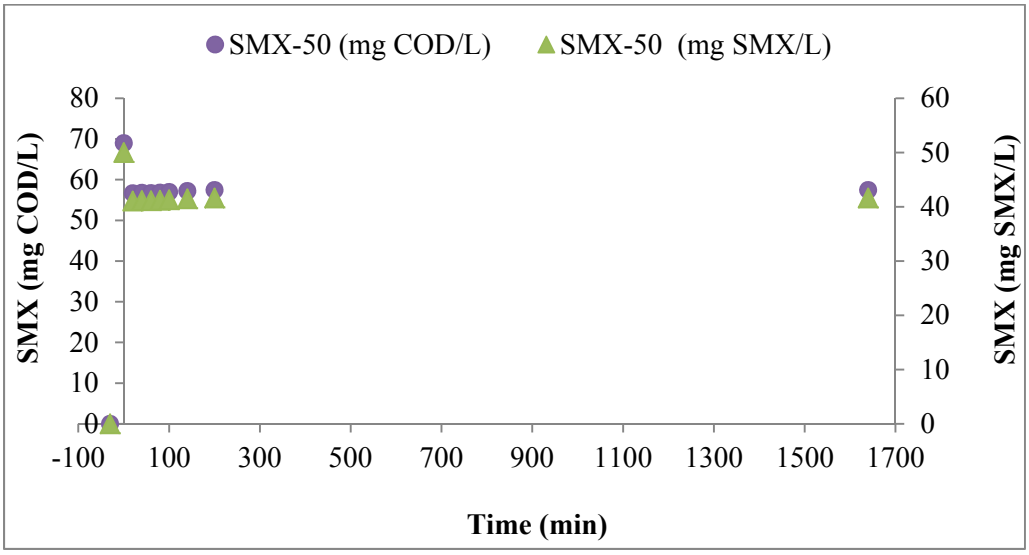


Figure 4.9 : OUR profile versus time (Run 3).



(a)



(b)

Figure 4.10 : Removal efficiencies of Run 3 (a) soluble COD and acetate profiles (b) SMX profiles.

100 mg/L antibiotic addition to acetate acclimated biomass was determined in Figure 4.11. Run 4 was started with an initial VSS concentration of 273 mg/L. The maximum oxygen uptake rate of the biomass gave the first peak around 62 mg/L.h in 82 minutes. Figure 4.12 gives COD profile of 100 mg/L SMX concentration.

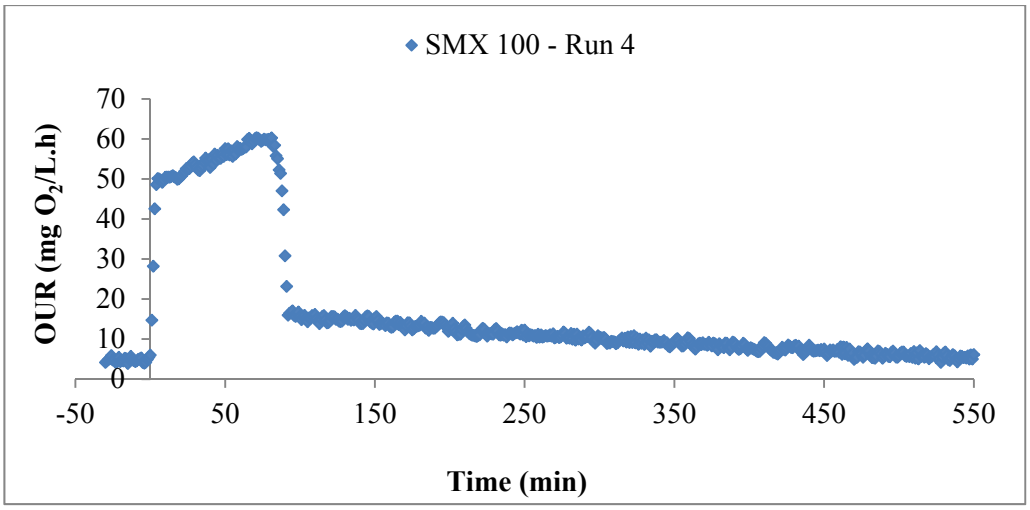
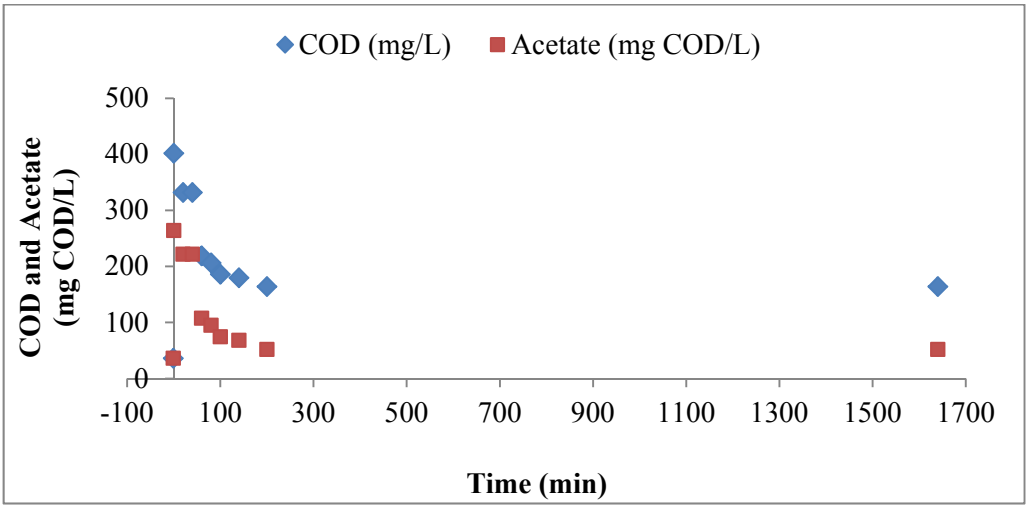
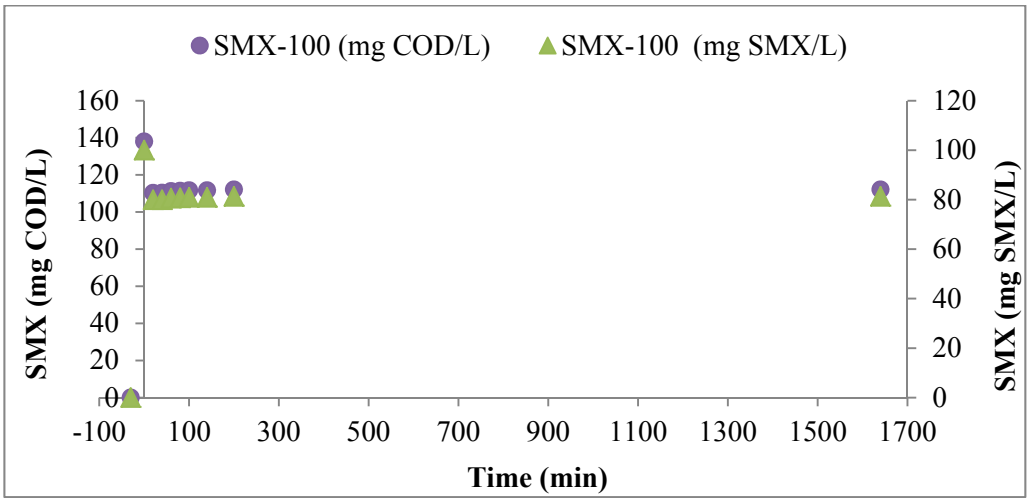


Figure 4.11 : OUR profile versus time (Run 4).



(a)



(b)

Figure 4.12 : Removal efficiencies of Run 4 (a) soluble COD and acetate profiles (b) SMX profiles.

Finally, 200 mg/L SMX concentration was tested for acute inhibition effect to activated sludge system (Figure 4.13). Run 5 was started with an initial VSS concentration of 246 mg/L.The OUR curve reaches a maximum level of 53 mg/L.h in 100 minutes.

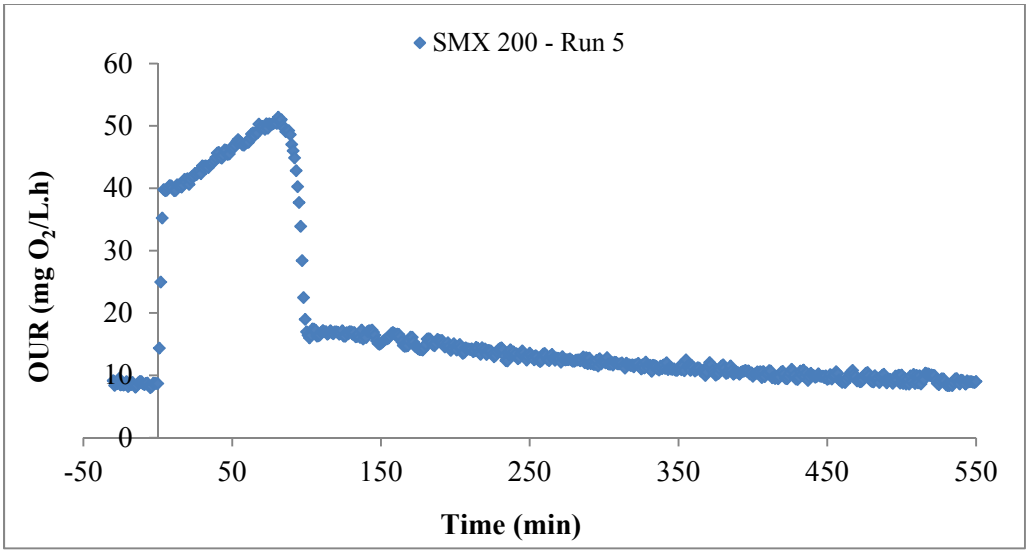
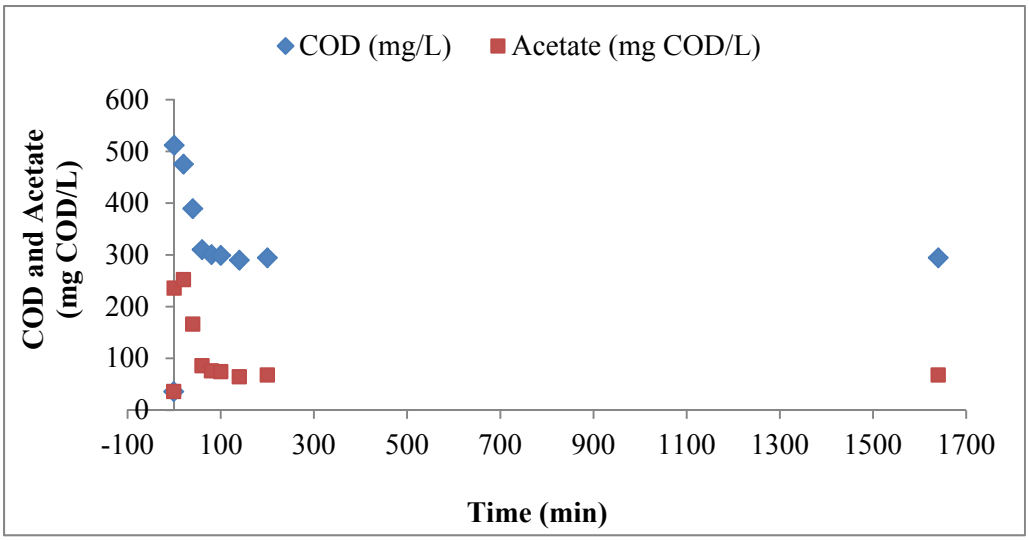
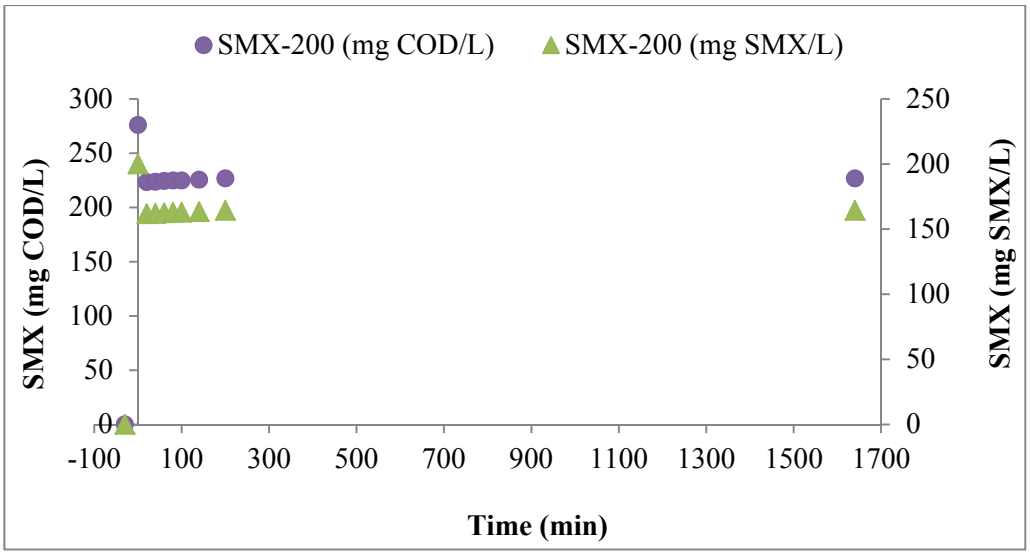


Figure 4.13 : OUR profile versus time (Run 5).

Figure 4.14 also displays acute effect of 200 mg/L SMX concentration and soluble COD profile. In summary, the COD profile results have indicated that antibiotic addition not to be effective for acetate removal in the activated sludge system as acute inhibition.



(a)



(b)

Figure 4.14 : Removal efficiencies of Run 5 (a) soluble COD and acetate profiles (b) SMX profiles.

The Figure 4.15 shows comparison of OUR profiles of control and acute experiments with respect to time. The antibiotic addition affected the shape of the OUR profile and the maximum OUR level. As it is seen from the Figure 4.15, addition of different SMX concentration to the activated sludge system decrease the maximum oxygen uptake rate of biomass in the acute tests.

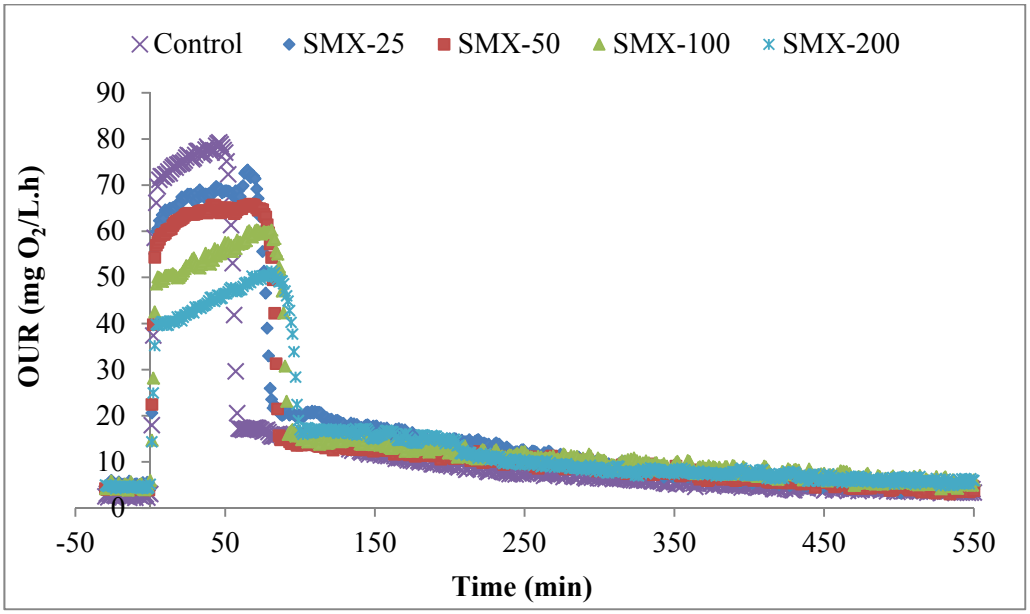


Figure 4.15 : Comparison of OUR profiles of all acute experiments with the control.

In control experiment, the OUR curve reaches a maximum level of 80 mg/L.h in 58 minutes, then the curve decreases due to the consuming of readily biodegradable substrate as acetate. However, acute tests represent that acetate is consumed for a long period of time and also the maximum OUR peak level decreases slowly with increasing SMX concentration according to control experiment. In other words, SMX addition as acute impact only retard substrate utilization so that it would take more time to utilize the available substrate.

4.2.3 Evaluation of chronic experiments

Chronic effect is a response that is developed in time by constant exposure. Chronic tests to observe the long-term effects of antibiotic in the aerobic system. For understanding the chronic effect of SMX, a second fill and draw reactor was set up with the same conditions and fed by the antibiotic in addition to acetate. All experiments were done with the same antibiotic concentration as 50 mg SMX/L. This experimental observation of the study involved a period of 18 days after reaching steady state conditions. In this context, the SS, VSS and VSS/SS ratio of the 12 L chronic reactor were given in Figure 4.16. Effluent SMX concentrations were also monitored in a period of 18 days (Figure 4.17). If there is no antibiotic removal was assumed in chronic reactor, the antibiotic accumulation in system would be as given in Figure 4.17.

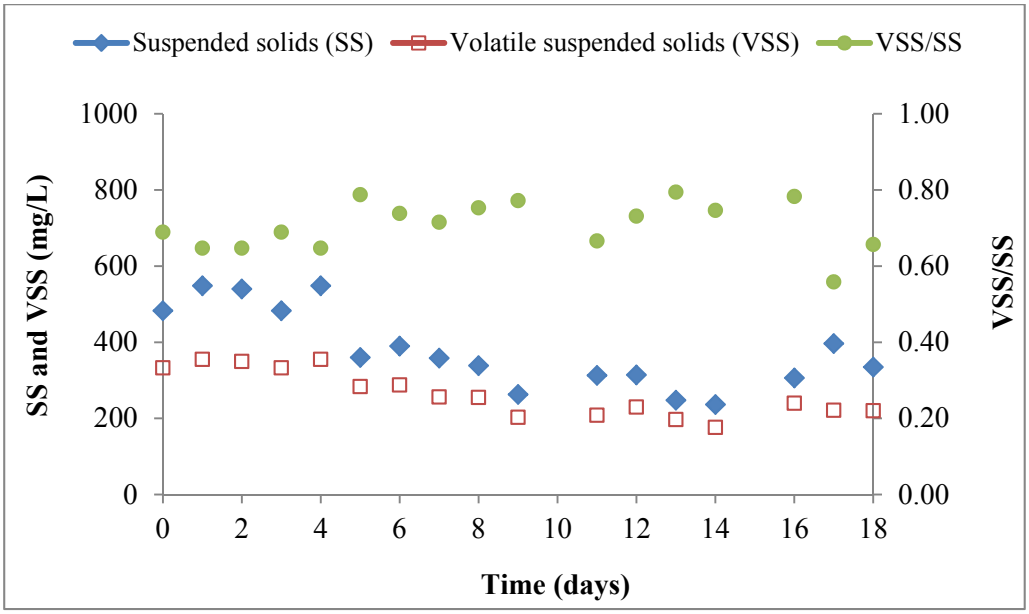


Figure 4.16 : SS,VSS and VSS/SS ratio results of the 12 L chronic reactor.

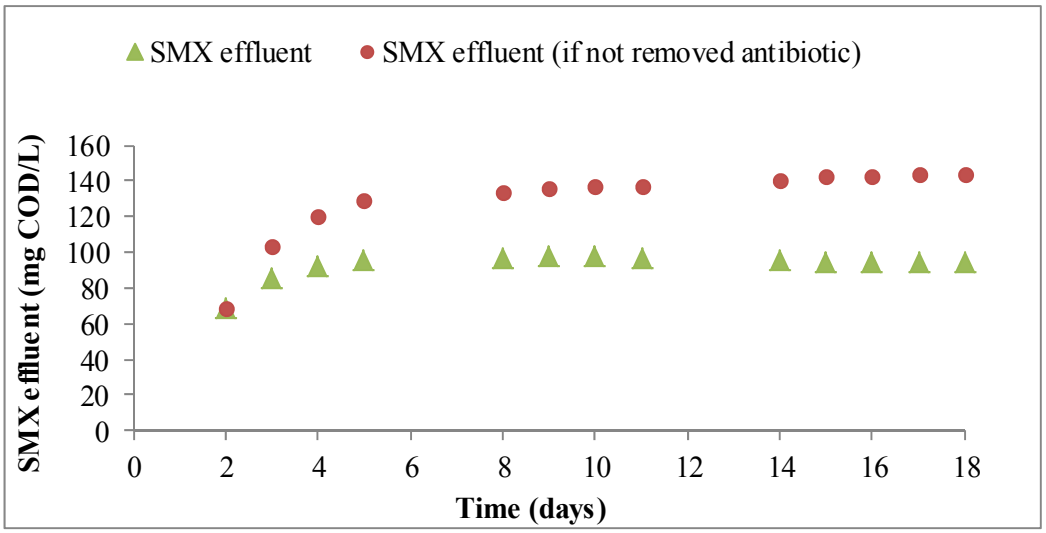


Figure 4.17 : SMX effluent concentrations of the 12 L chronic reactor.

10 runs of experiments were conducted; detailed information related to the batch experiments is given in Table 4.3. Experiments were conducted by using both acetate and antibiotic (called as mix), only acetate and only antibiotic as substrate under aerobic conditions. The chronic experiments were applied as;

- on the second day of antibiotic feeding →one experiment with mixed substrate
- on the fourth day of antibiotic feeding →one experiments with only acetate
- on the sixth day of antibiotic feeding→one experiment with mixed substrate
- on the eighth day of antibiotic feeding →three experiments with mixed substrate, only acetate and only SMX
- on the tenth day of antibiotic feeding →two experiments with mixed substrate and only SMX
- on the eighteenth day of antibiotic feeding → two experiments with mixed substrate and only acetate

In the all chronic experiments, the chronic reactor and the respiration vessel of respirometer wrapped in aluminum foil to prevent possible photodegradation of sulfamethoxazole.

Table 4.3 : Control and chronic experiments at a sludge age of 2 days.

Runs	**Acclimation period**	**Carbon Source**	**Acetate Concentration** (mg COD/L)	**Antibiotic Concentration** (mg SMX/L)	**Antibiotic Concentration** (mg COD/L)	**VSS** (mg VSS/L)
Run 1	0. day – control	Acetate	195	Control	-	240
Run 5	1. day	Acetate + SMX	250	50	69	239
Run 6	2. day	Acetate + SMX	230	50	69	144
Run 7	4. day	Acetate	230	-	-	128
Run 8	6. day	Acetate + SMX	230	50	69	150
Run 9.1	8. day	Acetate + SMX	230	50	72	165
Run 9.2	8. day	Acetate	230	-	-	165
Run 9.3	8. day	SMX	-	50	72	165
Run 10.1	10. day	Acetate + SMX	230	50	72	110
Run 10.2	10. day	SMX	-	50	72	110
Run 11.1	18. day	Acetate + SMX	230	50	70	120
Run 11.2	18. day	Acetate	230	-	-	120

.

Run 6 was conducted on the second day chronic impact of SMX to acclimated biomass. The OUR profile was given in Figure 4.18. The OUR curve reaches a maximum level of 75 mg/L.h in 60 minutes. The Figure 4.19 represents antibiotic measurements of 50 mg SMX/L and the trends of removal soluble COD in 2nd day chronic experiments (Run 6). The figure shows that 114 mg COD/L antibiotic concentration decreased to around 78 mg COD/L during time period.

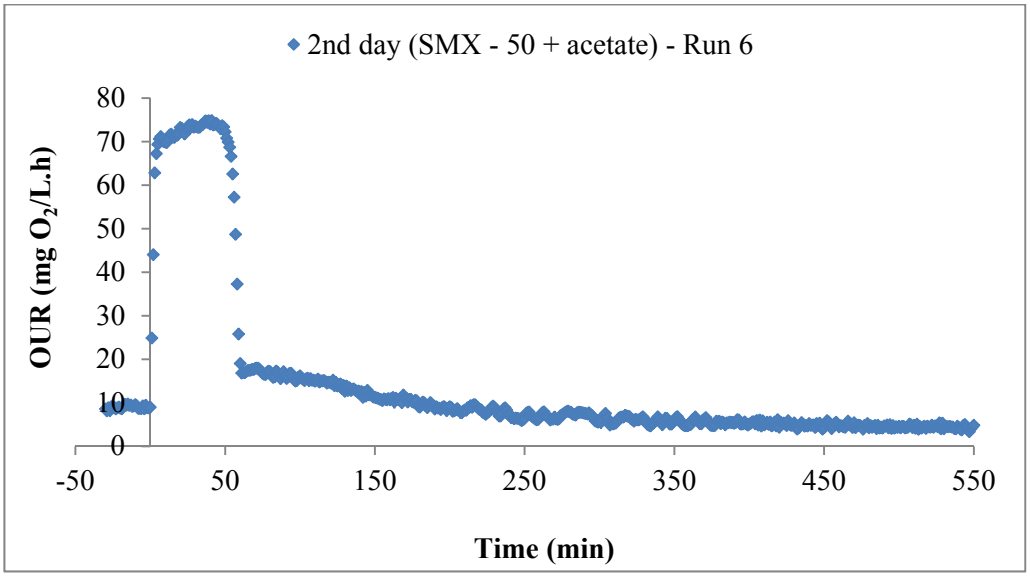
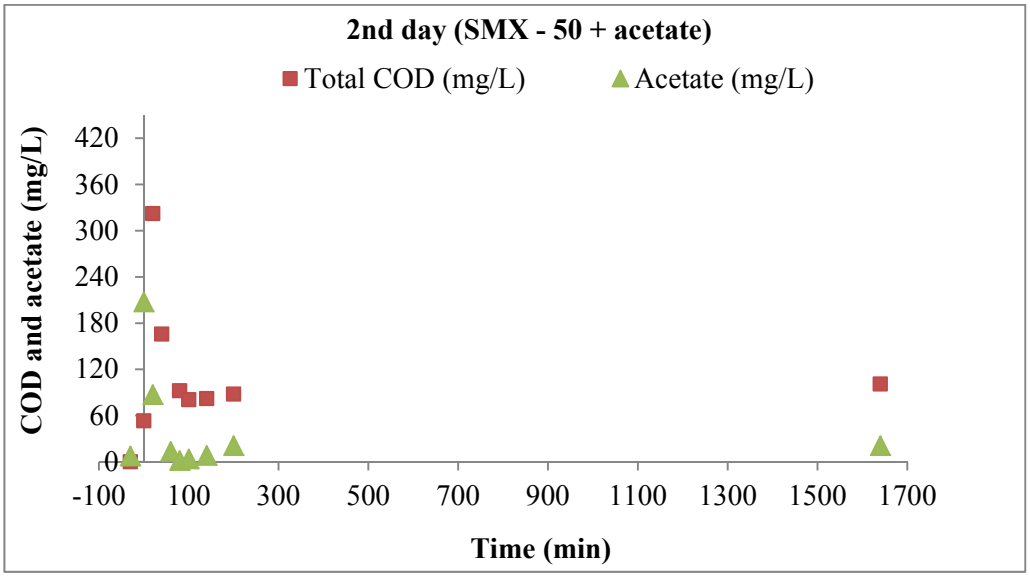
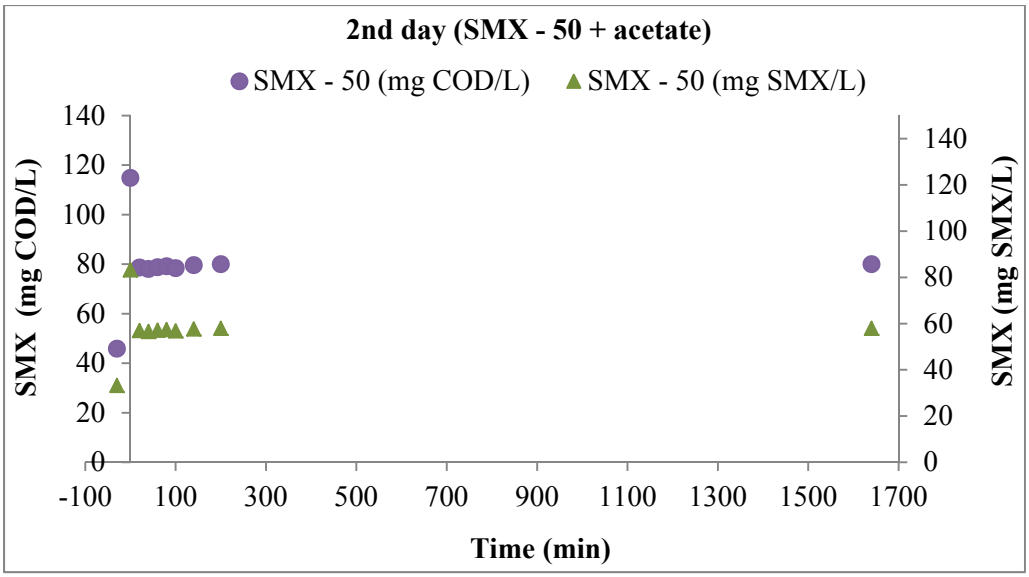


Figure 4.18 : OUR profile versus time (Run 6).



(a)



(b)

Figure 4.19 : Removal efficiencies of Run 6 (a) soluble COD and acetate profiles (b) SMX profiles.

Chronic effect of 50 mg SMX/L to activated sludge system was determined in 4th day as given in Figure 4.20. Run 7 shows that the respirometric evaluation of only acetate addition to system. The maximum oxygen uptake rate of the biomass gave the first peak around 32 mg/L.h in 122 minutes in Run 7. The Figure 4.21 shows antibiotic measurements and the removal efficiency of soluble COD in 4th day chronic experiments (Run 7).

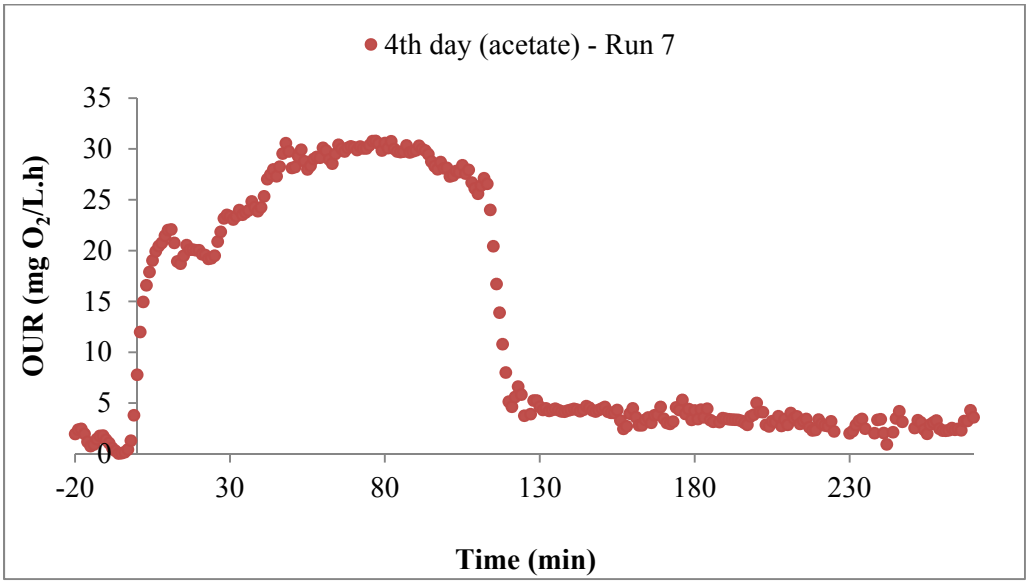
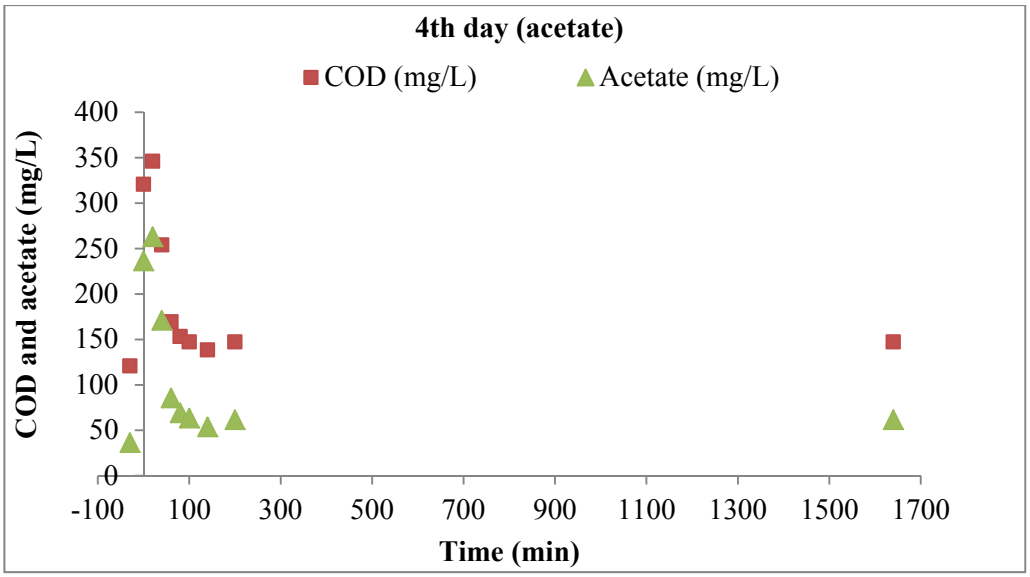
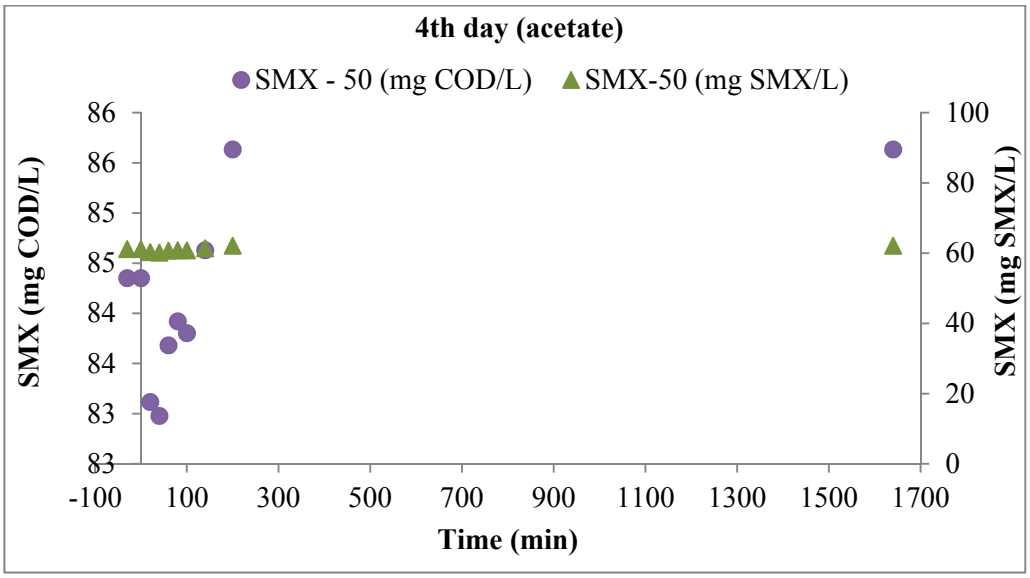


Figure 4.20 : OUR profiles versus time (Run 7).



(a)



(b)

Figure 4.21 : Removal efficiencies of Run 7 (a) soluble COD and acetate profiles (b) SMX profiles.

6th day chronic effect of 50 mg SMX/L was pointed in Figure 4.22 (Run 8). The OUR curve reaches a maximum level of 32 mg/L.h in 163 minutes. Soluble COD removal efficiencies and antibiotic measurements of 6th day were investigated in Figure 4.23 (Run 8). 40 mg COD/L antibiotic was removed with respect to time as shown in figure.

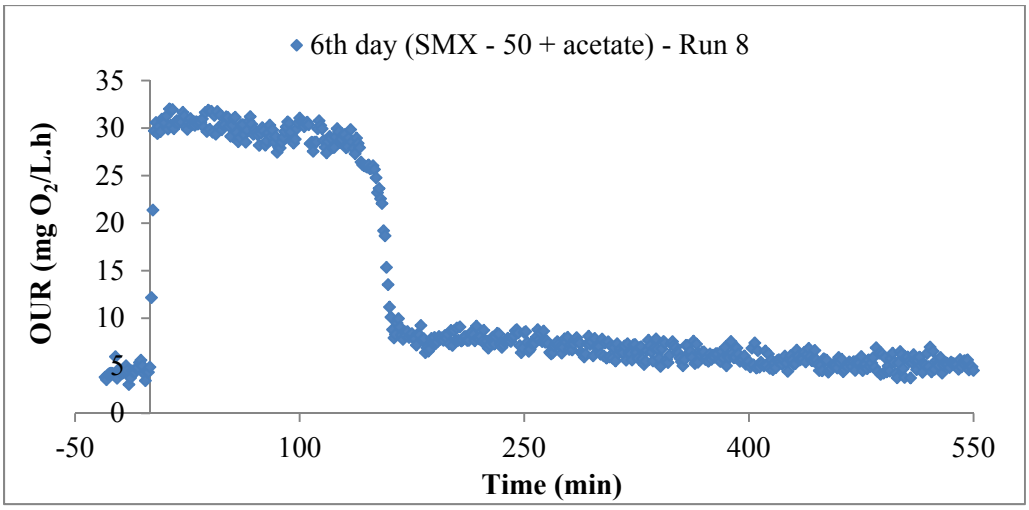
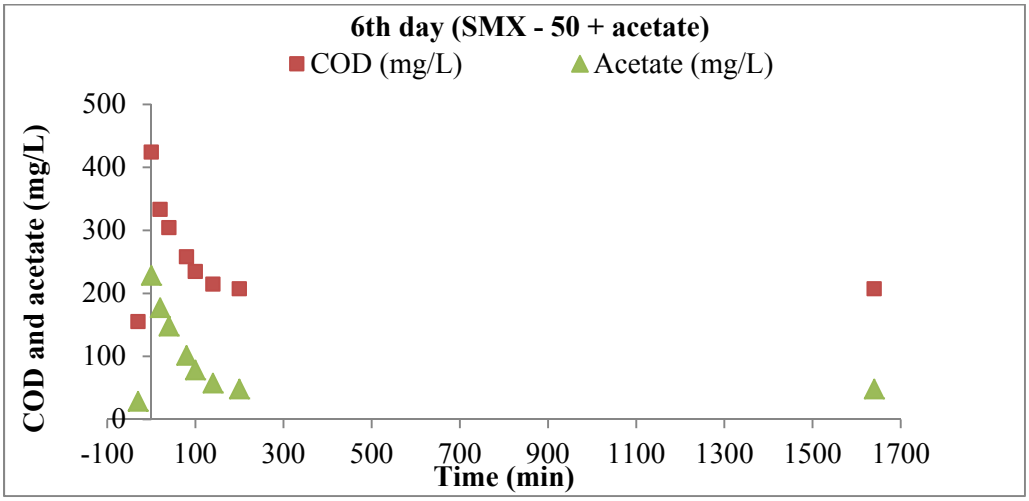
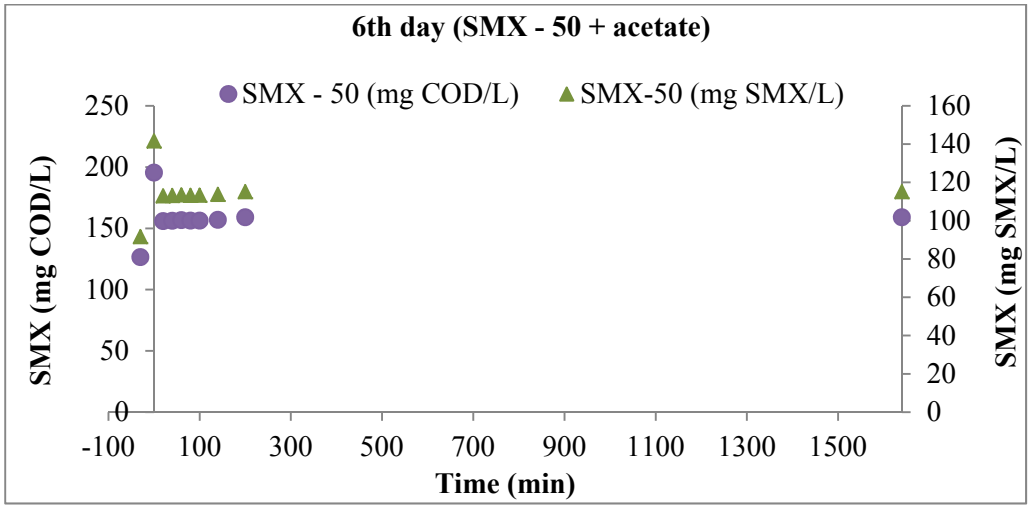


Figure 4.22 : OUR profile versus time (Run 8).



(a)



(b)

Figure 4.23 : Removal efficiencies of Run 8 (a) soluble COD and acetate profiles (b) SMX profiles.

8th day chronic effects of 50 mg SMX/L to system were shown in below Figure 4.24. In 8th day, three different OUR respirometric measurements were done at the same time. Run 9.1, Run 9.2 and Run 9.3 assigned respirometric determination of mixed substrate, only acetate addition and only SMX addition to the activated sludge system (Figure 4.24). The maximum oxygen uptake rate of the biomass gave the first peak around 42 mg/L.h in 124 minutes in Run 9.1 and 38 mg/L.h in 115 minutes in Run 9.2. In addition, as shown from Figure 4.24, the system progressed the endogenous decay level when only SMX added to the reactor (Run 9.3).

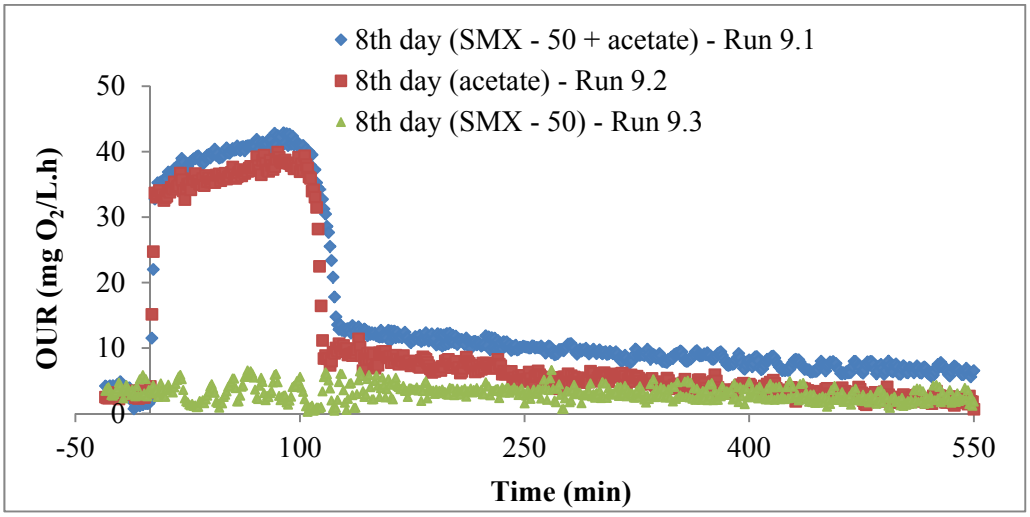
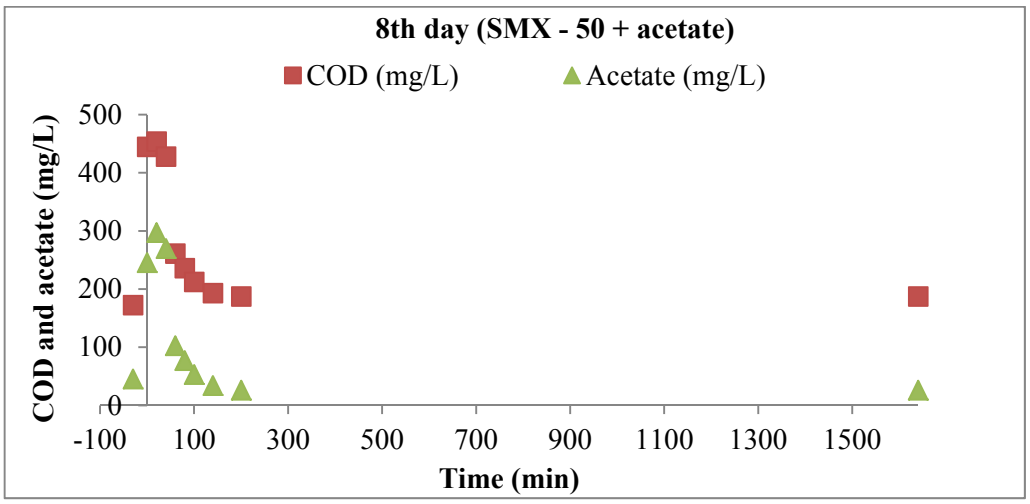
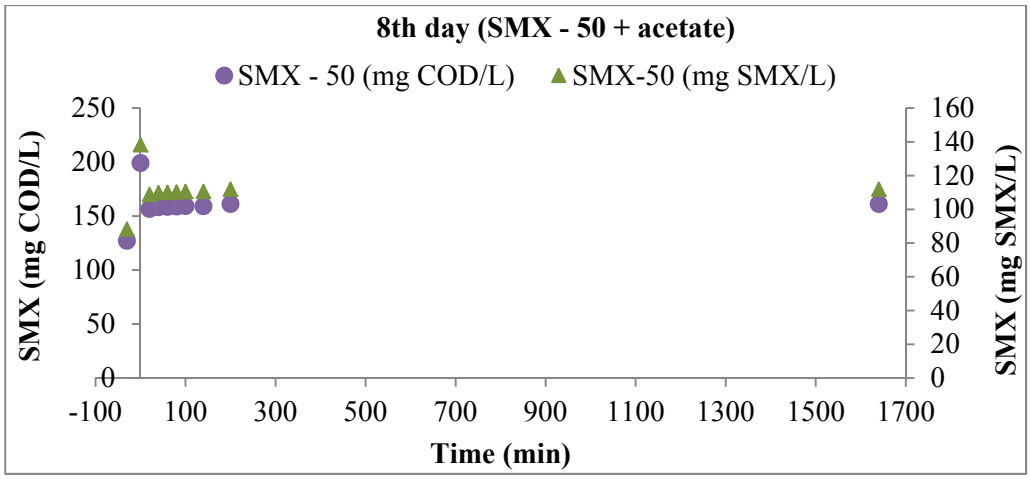


Figure 4.24 : OUR profiles versus time (Run 9.1, Run 9.2 and Run 9.3).

Soluble COD removal efficiencies and antibiotic measurements of 8th day of 50 mg SMX/L to chronic reactor were determined in Figure 4.25, Figure 4.26 and Figure 4.27 (Run 9.1, Run 9.2 and Run 9.3), respectively.42 mg COD/L antibiotic was removed with respect to time as shown in figures.

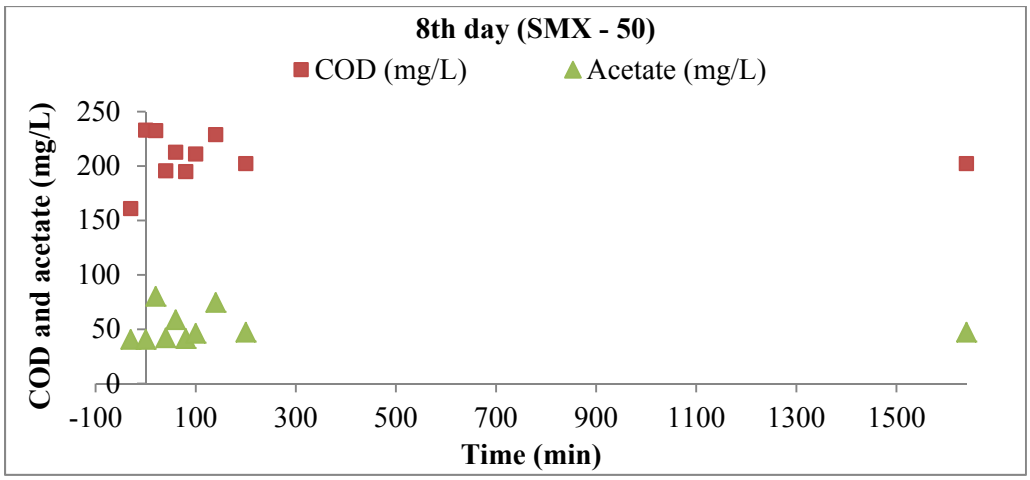


(a)

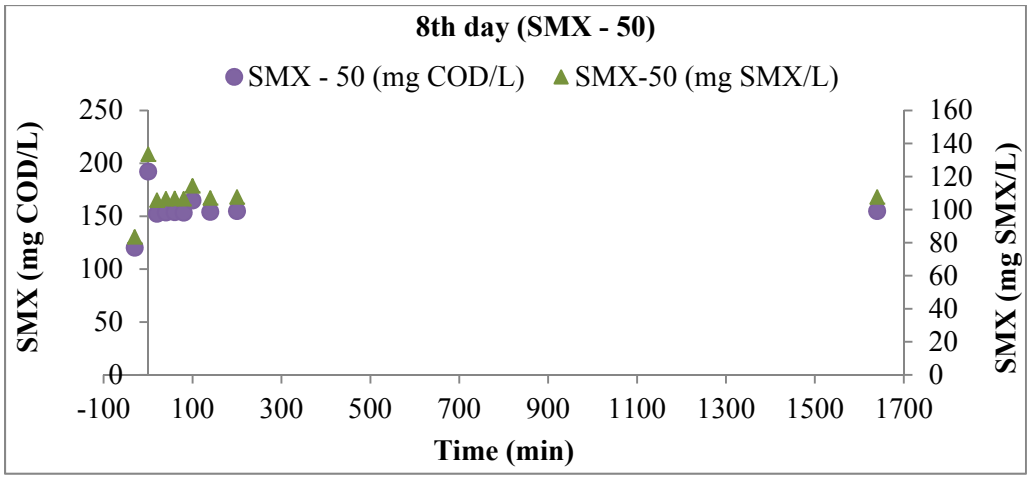


(b)

Figure 4.25 : Removal efficiencies of Run 9.1 (a) soluble COD and acetate profiles (b) SMX profiles.

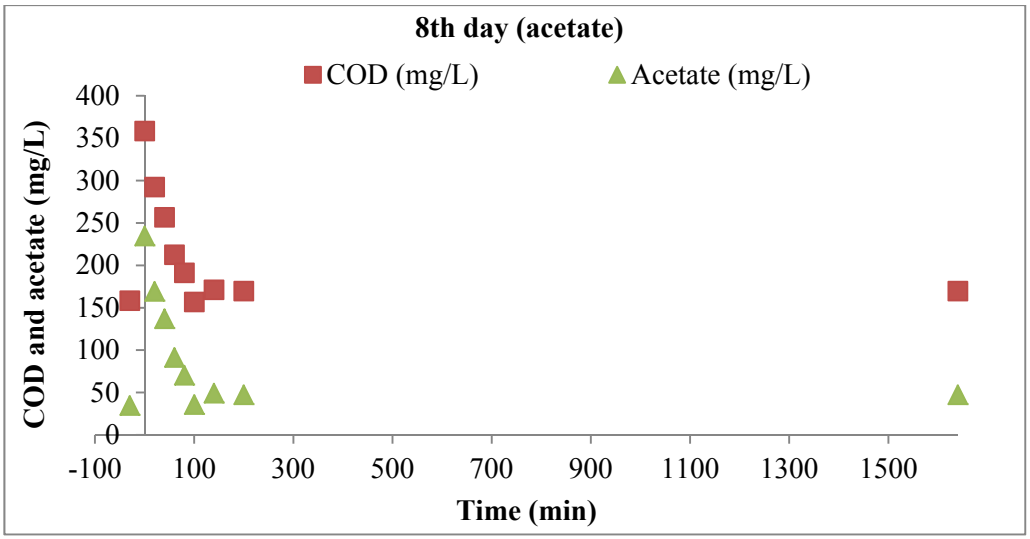


(a)

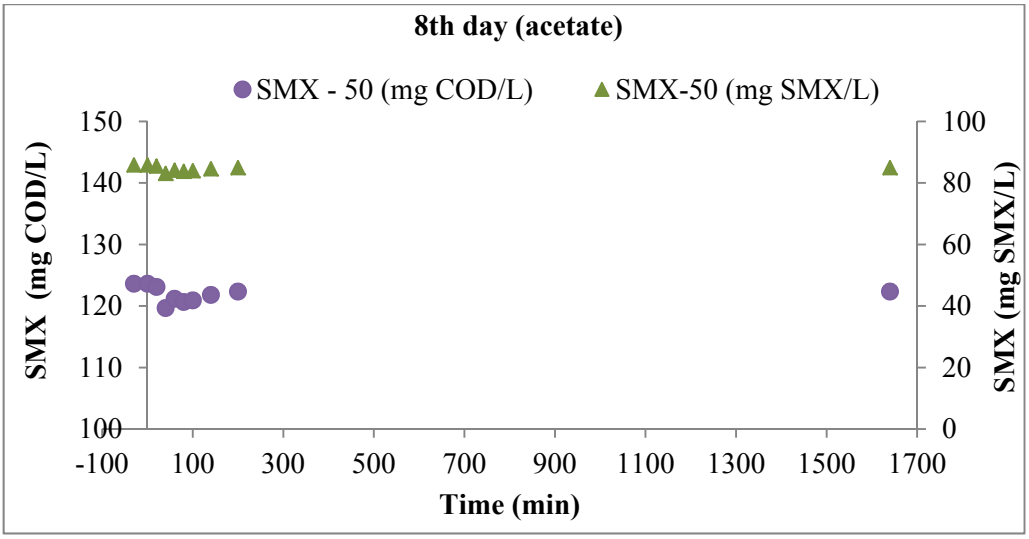


(b)

Figure 4.26 : Removal efficiencies of Run 9.2 (a) soluble COD and acetate profiles (b) SMX profiles.



(a)



(b)

Figure 4.27 : Removal efficiencies of Run 9.3 (a) soluble COD and acetate profiles (b) SMX profiles.

In the below Figure 4.28, it was observed that 10th day chronic effects were investigated (Run 10.1 and Run 10.2). OUR curve reaches a maximum level of 58 mg/L.h in 193 minutes. Again, the system progressed the endogenous decay level when only SMX added to the reactor as shown from Figure 4.28(Run 10.2). The Figure 4.29 indicates measurements of 50 mg SMX/L and the removal efficiencies of total soluble COD and acetate in 10th day chronic experiments (Run 10.1). 44 mg COD/L antibiotic was removed with respect to time as shown in figure.

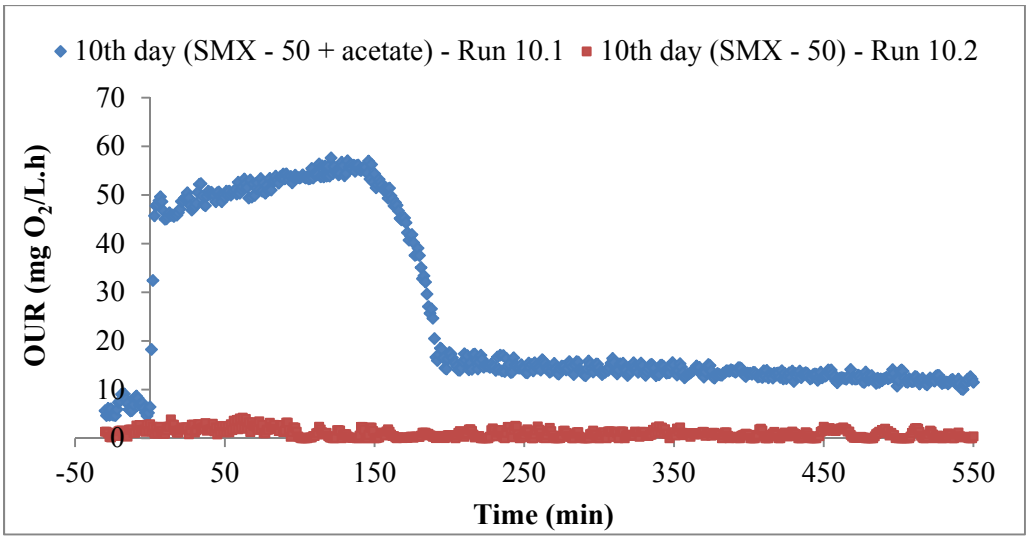
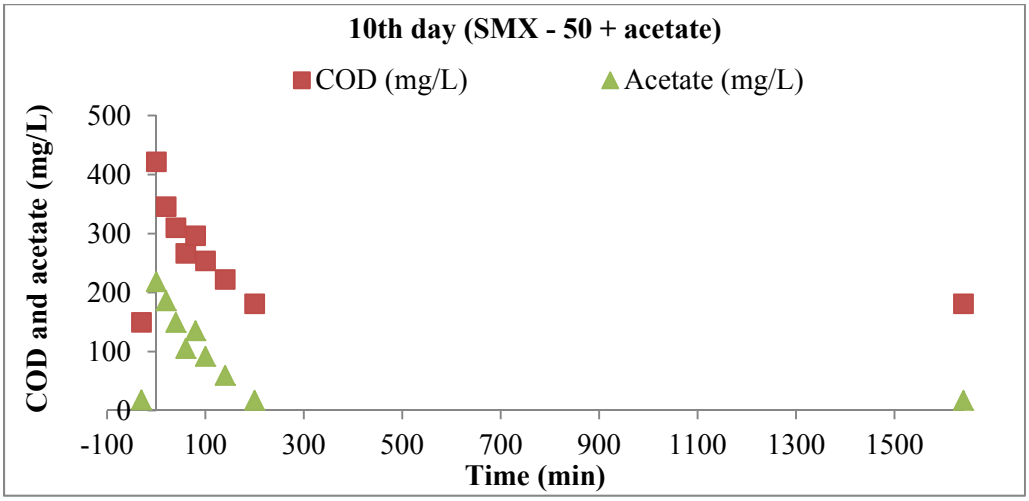
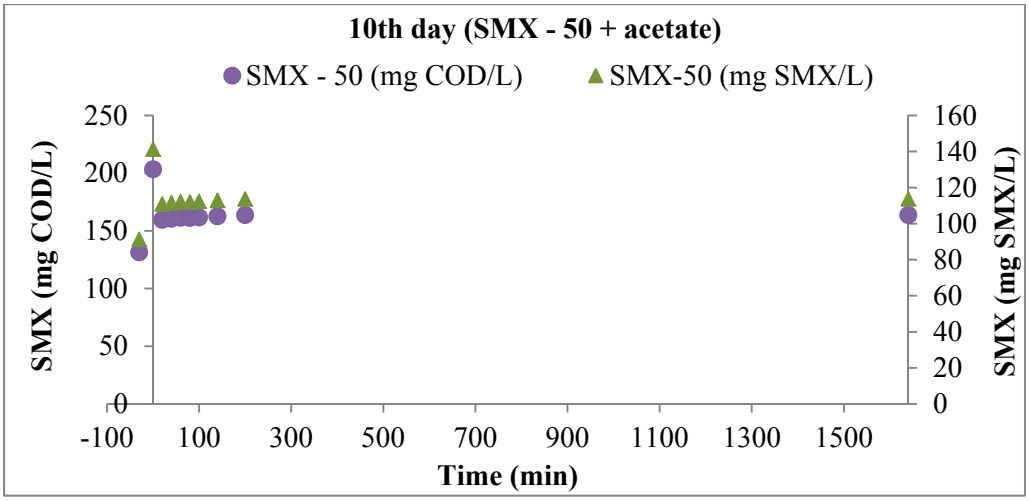


Figure 4.28 : OUR data versus time (Run 10.1 and Run 10.2).



(a)



(b)

Figure 4.29 : Removal efficiencies of Run 10.1 (a) soluble COD and acetate profiles (b) SMX profiles.

Finally, 18th day chronic effects of 50 mg SMX/L to system were shown in Figure 4.30. In 18th day, two different OUR respirometric measurements were done at the same time. Run 11.1 and Run 11.2 show investigation of mixed substrateand only acetate addition to the activated sludge, respectively (Figure 4.30). OUR curve reaches a maximum level of 35 mg/L.h in 177 minutes in Run 11.1 and 25 mg/L.h in 169 minutes in Run 11.2.

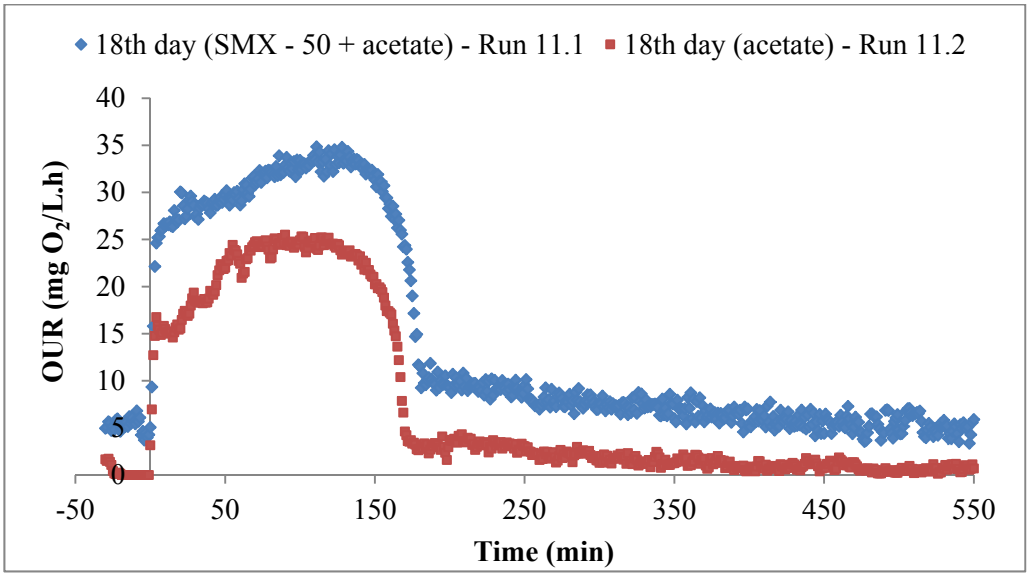


Figure 4.30 : OUR data versus time (Run 11 and Run 11.1).

Antibiotic measurement of 18th day of 50 mg SMX/L on chronic reactor of 12 L were not measured in HPLC. 18th day chronic experiment included only COD removal efficiencies of SMX 50 and acetate mixture and only acetate in Figure 4.31.

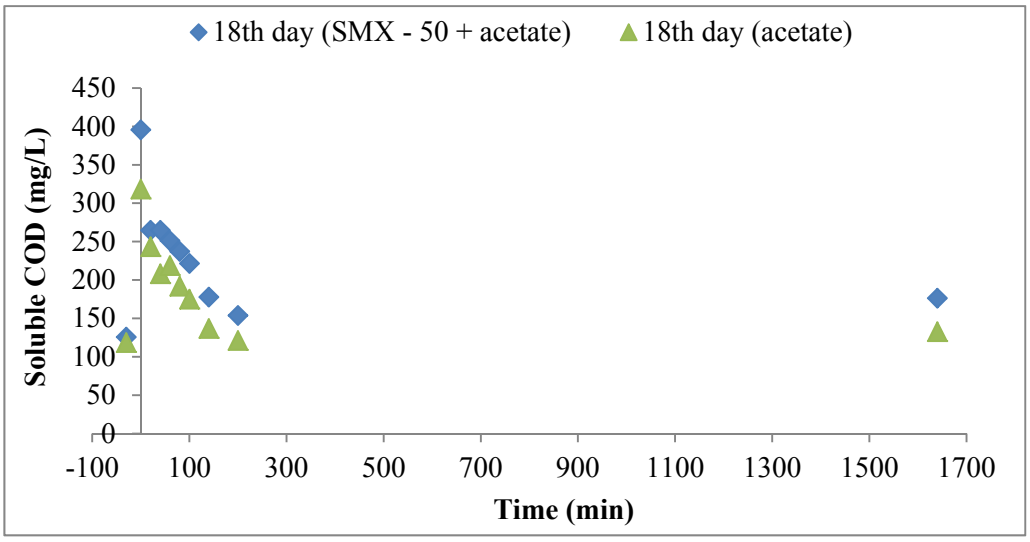


Figure 4.31 : COD removal efficiencies of Run 11.1 and Run 11.2.

Comparison of the OUR profiles of all chronic experiments (with mixed substrate, with only acetate and with only antibiotic) were indicated in Figure 4.32, Figure 4.33 and Figure 4.34, respectively.

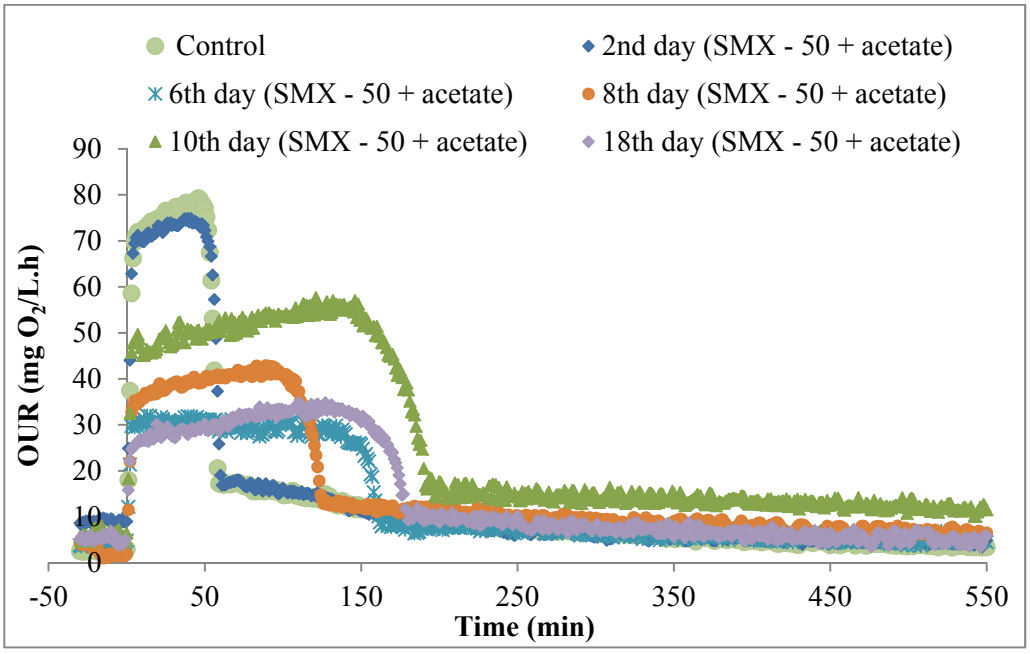


Figure 4.32 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate).

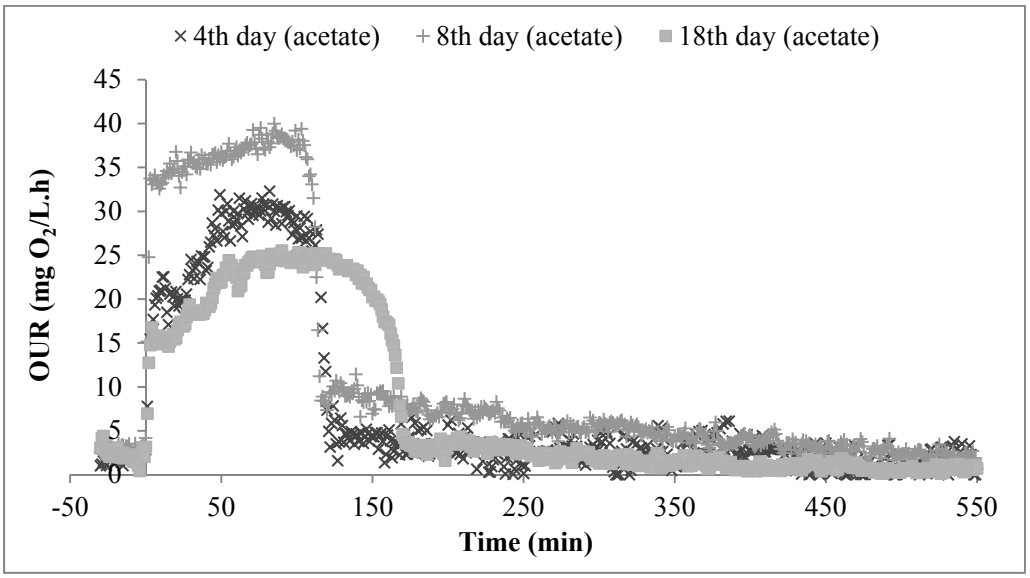


Figure 4.33 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate).

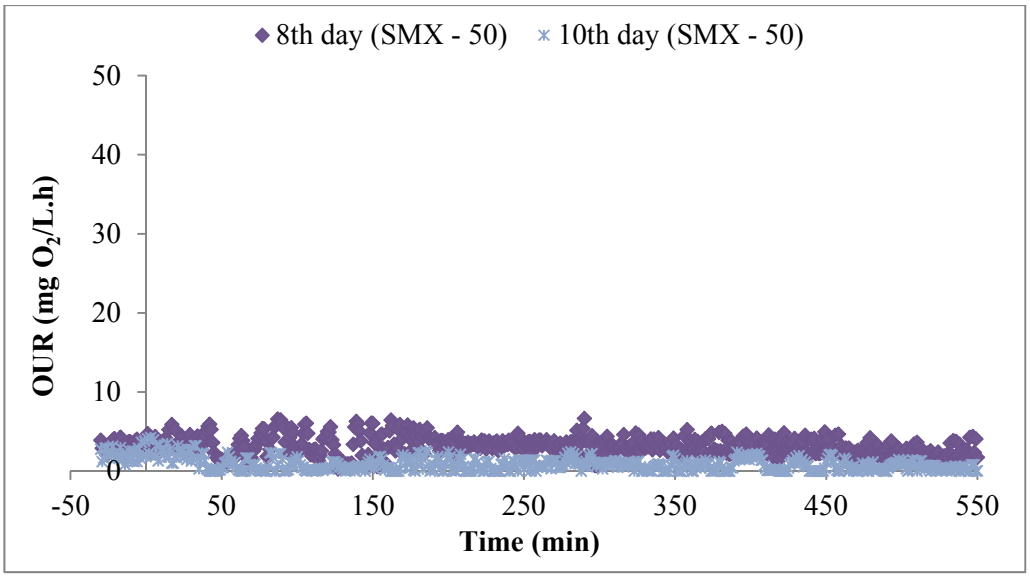


Figure 4.34 : Comparison of the OUR profiles of all chronic experiments (with mixed substrate).

4.3 Modeling of Experimental Results

Modeling is now regarded as a useful instrument for understanding and interpreting biodegradation and related mechanisms of substrate utilization. Recent models structured for this purpose operate with two major conceptual developments: (i) they use chemical oxygen demand (COD) and the organic carbon parameter and include COD fractions with different biodegradation characteristics based on the pioneering works of Dold et al. (1980) and Ekama et al. (1986); (ii) they incorporate dissolved oxygen concentration (S_0) as a significant model component which allow to calibrate and evaluate oxygen uptake rate profiles (Cokgor et al, 2011).

4.3.1 Modeling of control and acute experiments

Modeling consisted of analyzing and calibrating the OUR and PHA profiles. In this context, a mechanistic model involving model components and kinetic&stoichiometric parameters was developed for both acetate and SMX. Modified ASM3 model was used in this study. The model structure were composed of four model components, namely the readily biodegradable COD, S_S; active heterotrophic biomass, X_H; storage products, X_{STO} and dissolved oxygen, S_O. Generation of residual products (X_P) as a decay associated processes is implicitly involved in the model by means of the coefficient f_P which acts as a correcting factor for oxygen consumption due to endogenous respiration. The model structure

involved four microbial processes: growth on S_S; storage and growth on stored PHA and decay of X_H by means of endogenous respiration (Orhon et al, 2009b).The growth process associated with X_H was defined as conventionally adopted Monod-type equations. Both endogenous decay mechanisms were described as first-degree reactions with respect to biomass concentrations. The storage process was defined in terms of a similar Monod-type of an expression where k_{STO} denotes the maximum storage rate (Karahan et al, 2006). The matrix representation of modified ASM3 structure was indicated in Table 4.4.

Table 4.4 : Matrix representation of modified ASM3 model structure in control and acute experiments.

Component → **Process** ↓	S_{O2}	S_S	X_H	X_{STO}	X_P	**Process rate**
Storage of PHA	$-(1-Y_{STO})$	-1		Y_{STO}		$k_{STO}.\frac{S_S}{K_S+S_S}.X_H$
Growth of X_H	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$	1			$\mu_H.\frac{S_S}{K_S+S_S}.X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$		1	$-\frac{1}{Y_H}$		$\mu_{STO}.X_{STO}$
Decay of X_H	-(1-*fp*)		-1		*fp*	$b_H.X_H$

Kinetic and stoichiometric parameters of the modified ASM3 model were estimated using the AQUASIM simulation program(Reichert et al, 1998). In addition, modeling study was also performed for parameter estimation. The parameter estimation study provided a basis for observing the change in the kinetic parameters of activated sludge while adopting to different conditions (Insel et al, 2012).

Model simulation results of OUR profile and PHA data of control experiment (Run1) were shown in Figure 4.35 and Figure 4.36, respectively. According to simulation results, the model was calibrated with a maximum specific growth rate, μ_{Hmax} of 8.5/day for the portion of the biomass. The initial X_H concentration was determined as 300 mg cell COD/L. The calibration of the initial endogenous respiration part of the OUR curve also yielded an endogenous decay coefficient, b_H of 0.24/day. The model calibration was performed with the assumption of a yield coefficient Y_H of 0.66 mg cell COD/mg COD and a storage yield coefficient Y_{STO} of 0.80 mg cell COD/mg COD (Yavaşbay, 2010). The coefficient for residual products generation, f_P was taken as 0.20 in the all experimental runs (Orhon et al, 2009b).

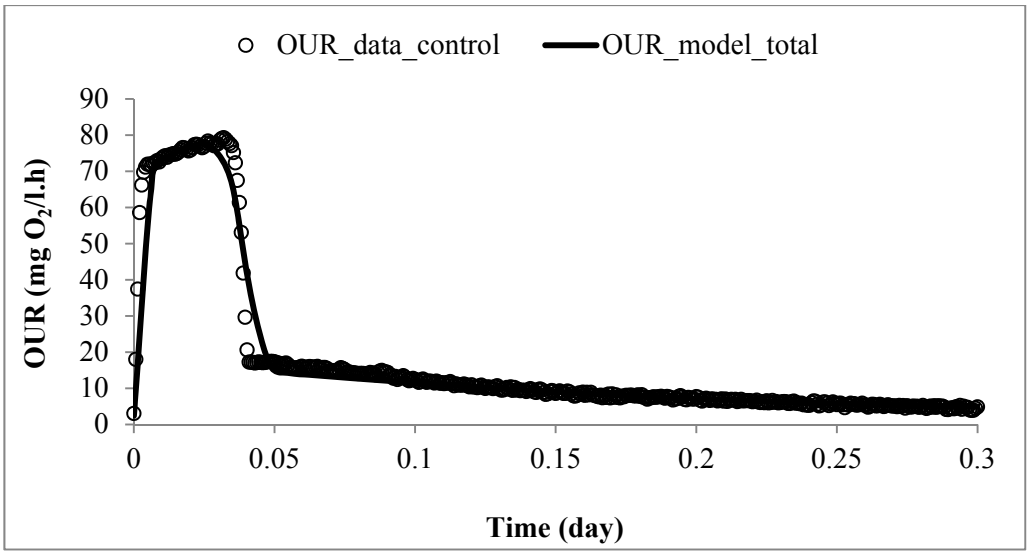


Figure 4.35: Model simulation of OUR profile for Run 1.

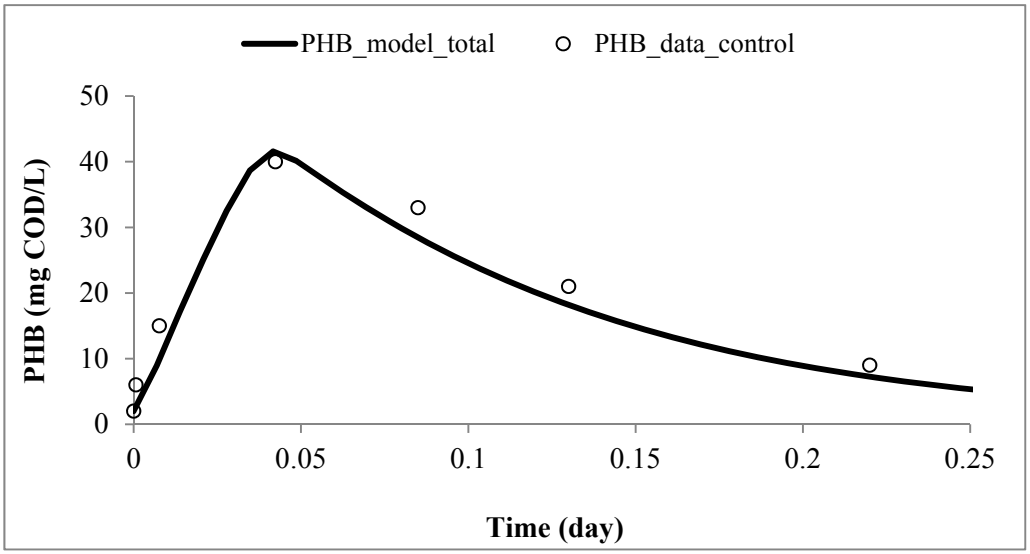


Figure 4.36: Model simulation of PHB data for Run 1.

Simulation results showed that the half-saturation constant (K_S) was found as 15mg COD/L with 2 days of sludge age. In the other study about the similar research, this value was found 6 mg COD/L with the same operational conditions only difference in 10 days of sludge age (Ural, 2012). Another study also showed that reducing the sludge age (SRT) from 10 to 2 days elevated the half-saturation constant for growth (K_S) from 5 to 25 mg COD/L (Insel et al, 2012).

Modeling results reflected the PHA storage, yielding with a k_{STO} value of 5.9/day. The maximum storage rate was nearly same as the default value of ASM3. For all experimental runs, the half saturation constant for storage K_{STO} was obtained as 0.3 mg COD/L (Ural, 2012).

The additional batch experiments were carried out to provide a model evaluation for acute inhibition effects of SMX on the biodegradation of acetate. Model simulation was applied to SMX-25,SMX-50and SMX-100 acute experimental results. Model simulation results of OUR profile and PHB data for Run 2 was shown in Figure 4.37 and Figure 4.38, respectively. According to the Run 2 (SMX-25), half saturation constant (K_S) was found 33 mg COD/L, whereas it was 15 mg COD/L for the control experiment. In the Run 2, endogenous decay rate (b_H) increased from 0.24/day to 0.35/day and maximum heterotrophic growth rate (μ_{Hmax}) decreased from 8.5/day to 8/day. In addition, maximum specific growth rate on the stored products (μ_{STO}), the maximum storage rate (k_{STO}) and half saturation constant for storage (K_{STO}) were found the control experiment.

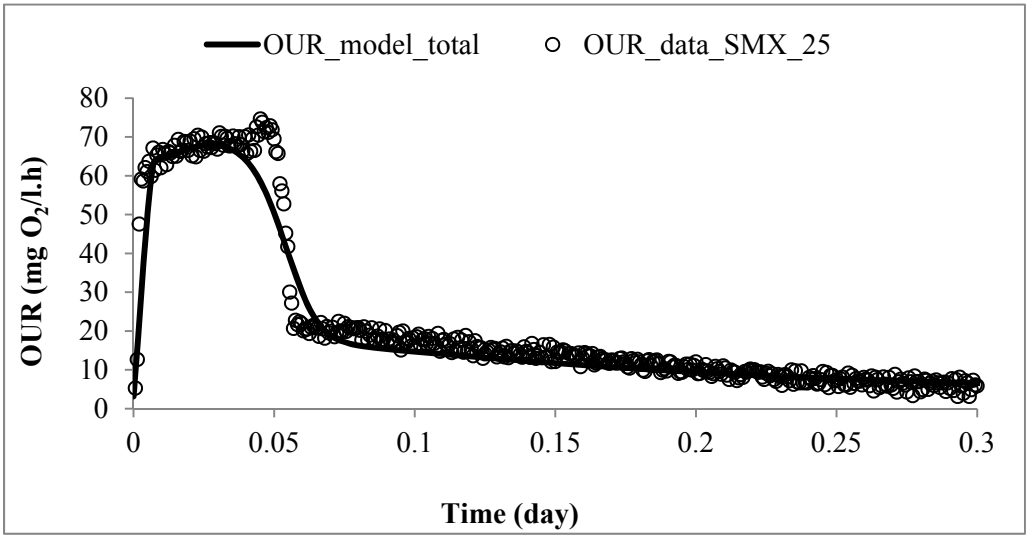


Figure 4.37 : Model simulation of OUR profile for Run 2.

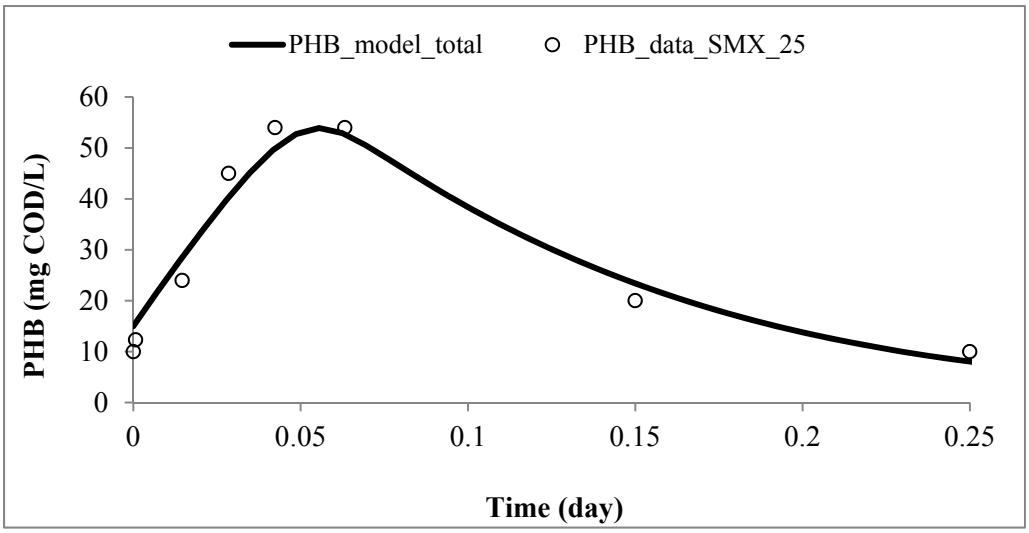


Figure 4.38 : Model simulation of PHB data for Run 2.

Model simulation results of OUR profile and PHB data for Run 3 (SMX-50) were indicated in Figure 4.39 and Figure 4.40, respectively. Run 3 (SMX-50) modelling results of modified ASM3 indicated that maximum heterotrophic growth rate (μ_{Hmax}), half saturation constant for readily biodegradable substrate (K_S), endogenous decay rate (b_H), maximum specific growth rate on the stored products (μ_{STO}),the maximum storage rate (k_{STO}) and half saturation constant for storage (K_{STO}) were the same as the Run 2.

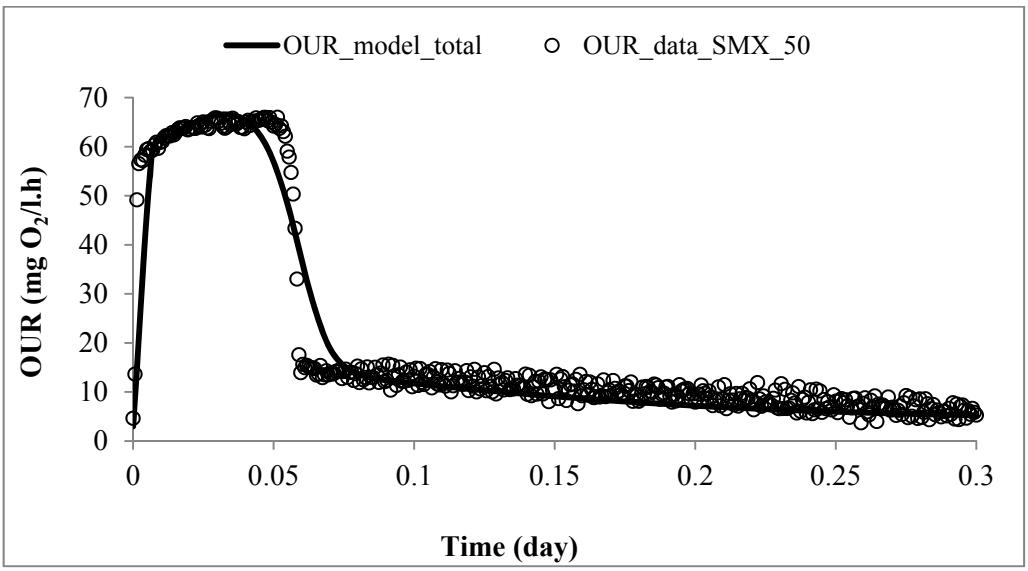


Figure 4.39 : Model simulation of OUR profile for Run 3.

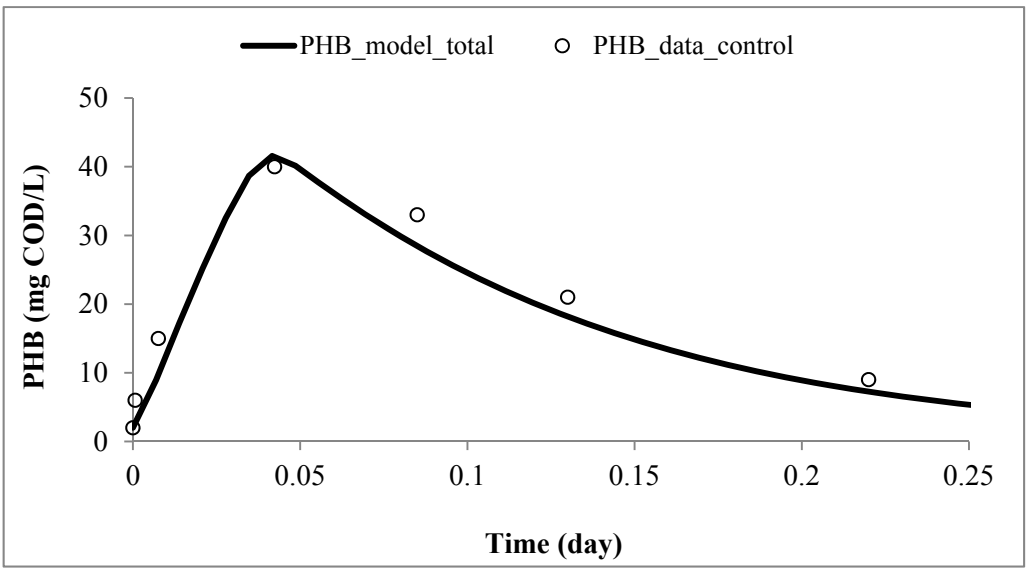


Figure 4.40 : Model simulation of PHB data for Run 3.

Model simulation results of OUR profile and PHB data for Run 4 were illustrated in Figure 4.41 and Figure 4.42 respectively. In the Run 4 (SMX-100), maximum heterotrophic growth rate (μ_{Hmax}) decreased from 8/day to 6/day whereas half saturation constant (K_S) was the same value with the Run 3. As it can be seen other parameters in the Run 4, the maximum specific growth rate for storage (μ_{STO}) also decreased according to other acute experiments. The model simulation results based on modified ASM3 of Run 1, Run 2, Run 3 and Run 4 were given in Table 4.5 in details.

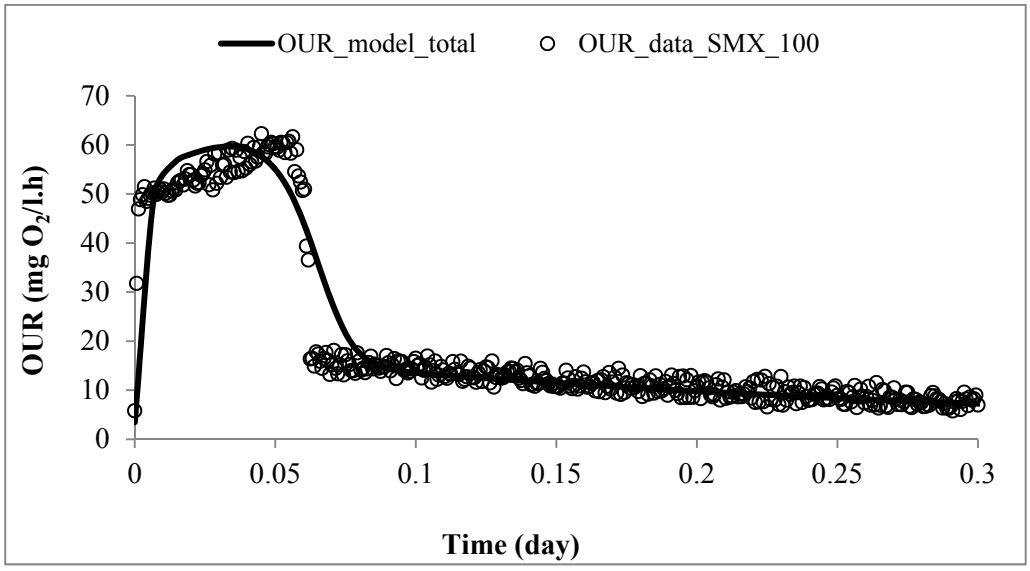


Figure 4.41 : Model simulation of OUR profile for Run 4.

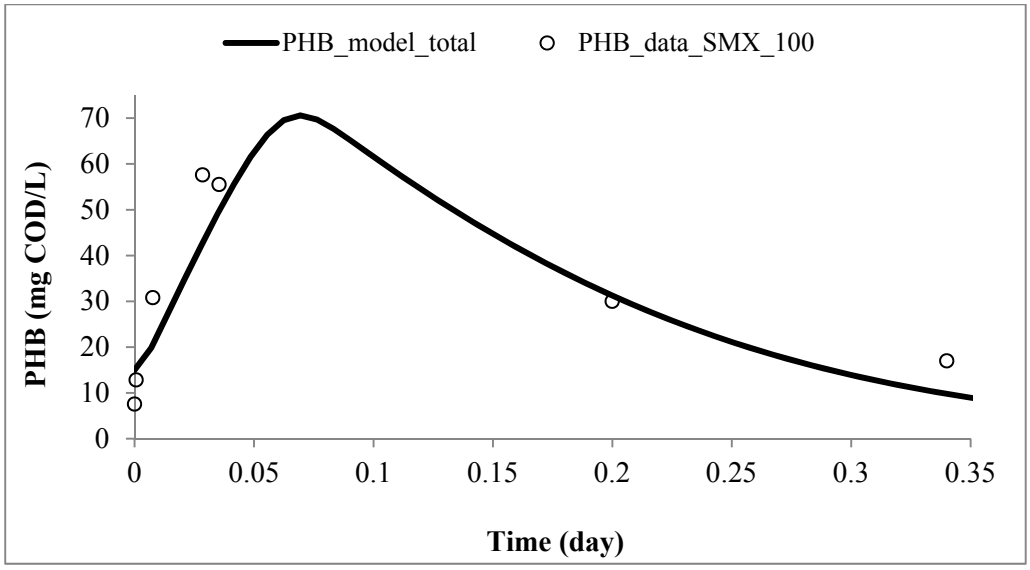


Figure 4.42 : Model simulation of PHB data for Run 4.

Table 4.5 : Calibration results of the control and acute experiments.

Model Parameters	**Unit**	**Control** (Run 1)	**Acute SMX-25** (Run 2)	**Acute SMX-50**(Run 3)	**Acute SMX-100** (Run 4)
μ_{Hmax}	1/day	8.5	8	8	6
K_S	mg COD/L	15	33	33	33
b_H	1/day	0.24	0.35	0.35	0.35
k_{STO}	1/day	5.9	5.9	5.9	5.9
μ_{STO}	1/day	3	3	3	2.1
K_{STO}	mg COD/L	0.3	0.3	0.3	0.3
f_{ES}	-	0.05	0.05	0.05	0.05
f_{EX}	-	0.15	0.15	0.15	0.15
State variables	**Unit**				
Total biomass	mg VSS/L	240	274	239	273
X_H	mg COD/L	300	280	265	310
Activity	%	88	72	78	80
$S_{ACini.}$	mg COD/L	195	230	250	240

Assumptions: Y_H = 0.66 g COD/g COD and Y_{STO} = 0.80 g COD/g COD (Yavaşbay, 2010).

Evaluated of the maximum specific growth rate(μ_{Hmax}) and the half saturation coefficient (K_S) in the acute experiments, μ_{Hmax} decreased from 8.5/day to 6/day while K_S increased from 15 mg COD/L to 33 mg COD/L. As related to this subject, Novak et al. (1994) argued that batch reactors would favor the fast growers group in the biomass and generate a higher μ_{Hmax} value for the same substrate with respect to biomass growing in continuous systems. According to Grady et al. (1996), the maximum specific growth rate (μ_{Hmax}), is closely related to the r-RNA level of the cell as it controls the protein synthesis mechanism. Then, culture history (sludge age, dilution rate, etc.) which regulates the biochemical mechanisms within the cell is likely to affect the growth kinetics (Orhon et al, 2009b). Furthermore, a recent study has shown that substrate utilization was highly influenced by the r-RNA level and the activity of substrate transforming enzymes (Lavallée et al, 2005). The latter basically reflects substrate affinity of microorganisms. This affinity increases with higher activity of transport enzymes inducing lower values for the half saturation coefficient(K_S) (Daigger and Grady, 1982; Ferenci, 1999). As further support to this situation, Kovarova-Kovar and Egli (1998) stated that the microbial cells could adjust their properties within a window of μ_H-K_S plane depending upon the type of

the bacteria having oligitrophic and copiotrophic properties. The oligitrophic properties correspond to lower maximum μ_H-K_s domain, however, the copiotrophic properties indicates higher μ_H-K_S levels. For instance, microorganisms are able to increase their affinity ($<<K_S$) during long-term adaptation from high to low substance concentration in such way that glucose is transported via high affinity galactose binding protein/maltose system rather than the glucose phosphotransferase system (Yavaşbay, 2010).

The model simulation results also showed that the OUR segments governing the direct growth and storage processes reveal that the biomass prefers to channel substrate (acetate) more to direct growth process rather than producing internal storage material (PHB) (Insel et al, 2012). Because, the maximum specific growth rate for storage (μ_{STO}) decreased from 3/d to 2.1/d.

4.3.2 Modeling of chronic experiments

In chronic experiments, the model structure were composed of five model components and hydrolysis process added to the system so microorganisms consumed SMX as readily hydrolysable COD with a specific hydrolysis rate. The slowly biodegradable COD fraction was composed of S_{AC} and SMX. Modified ASM3 model was used and the matrix representation of modified ASM3 structure was indicated in Table 4.6.

In the chronic experiments, only the 1st day mix (Run 2); 6th day mix (Run 8), 8th day mix (Run 9.1), and 18th day mix (Run 11.1) were simulated by the modified ASM3 model. Model simulation results of Run 2 were represented in Figure 4.39 and Figure 4.40, respectively. Model simulation result of 6th day mix (Run 8) was shown in Figure 4.43. According to model simulation results, the maximum specific growth rate (μ_H) decreased from 8.5/day to 8/day in the 6th day chronic experiment due to constant addition of antibiotics to the system. Therefore, only a portion of acetate (167 mg/L) was utilized by the microorganisms in the growth process. The other portion of acetate (63 mg/L) made a complex with antibiotic (40 mg/L) that was consumed as readily hydrolysable COD fraction in the hydrolysis process. As from 6th day chronic experiment, processes of storage and growth on stored PHA were disappeared. As a result of that, the metabolism of the activated sludge was shifting from storage to growth (Yavaşbay, 2010).

Table 4.6 : Matrix representation of modified ASM3 model structure in chronic experiments.

Component → **Process** ↓	S_{O2}	S_S	$(S_S+SMX)_{complex}$	X_H	X_{STO}	X_P	**Process rate**
Hydrolysis of $(S_S+SMX)_{complex}$		1	-1				$k_h.\frac{(S_S+SMX)_{complex}/X_H}{K_X+\left((S_S+SMX)_{complex}/X_H\right)}.X_H$
Storage of PHA	$-(1-Y_{STO})$	-1			Y_{STO}		$k_{STO}.\frac{S_S}{K_S+S_S}.X_H$
Growth of X_H	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$		1			$\mu_H.\frac{S_S}{K_S+S_S}.X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$			1	$-\frac{1}{Y_H}$		$\mu_{STO}.X_{STO}$
Decay of X_H	-(1-*fp*)			-1		*fp*	$b_H.X_H$

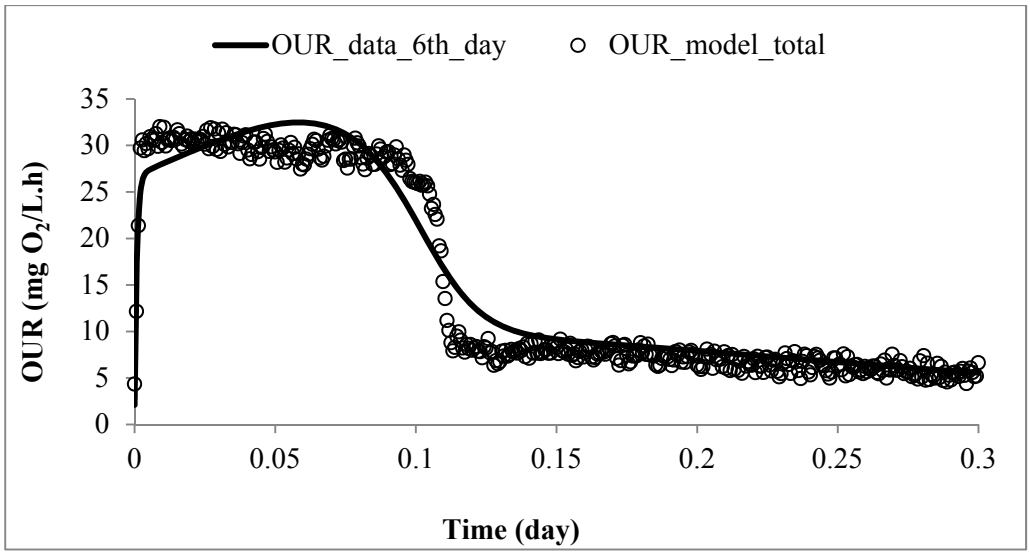


Figure 4.43 : Model simulation of OUR profile for Run 8.

Model simulation result of Run 9.1 was illustrated in Figure 4.44. In the 8th day chronic experiment, the maximum specific growth rate (μ_{Hmax}) again increased from 8/day to 8.5/day. 175 mg/L acetate was utilized by the microorganisms in the growth process. 55 mg/L acetate made a complex with 42 mg/L antibiotic in the hydrolysis process.

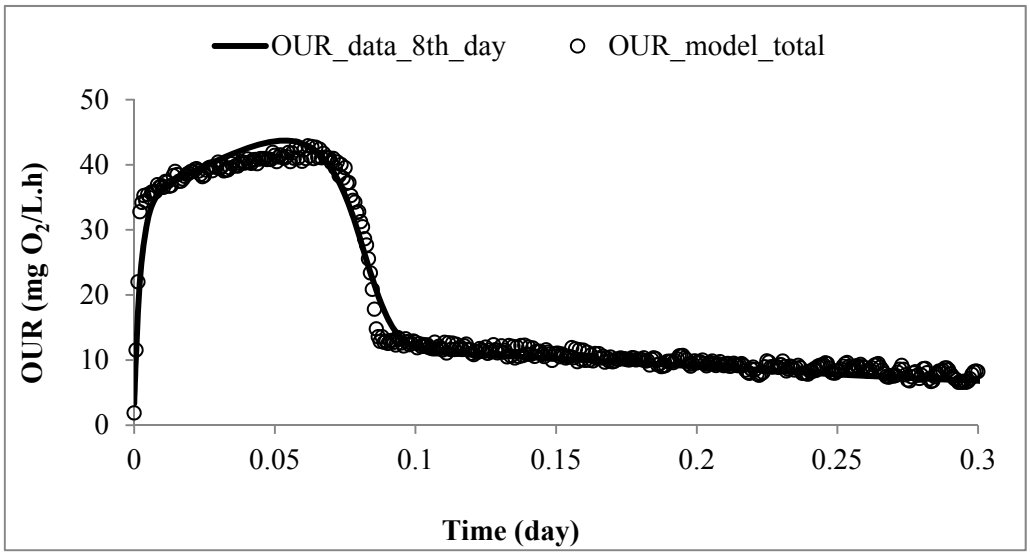


Figure 4.44 : Model simulation of OUR profile for Run 9.1.

Model simulation result of 18th day mix (Run 11.1) was shown in Figure 4.45. These model parameter results were found the with same 8th day experiment. 200 mg/L acetate was utilized by the microorganisms in the growth process.30 mg/L acetate made a complex with 44 mg/L antibiotic in the hydrolysis process.

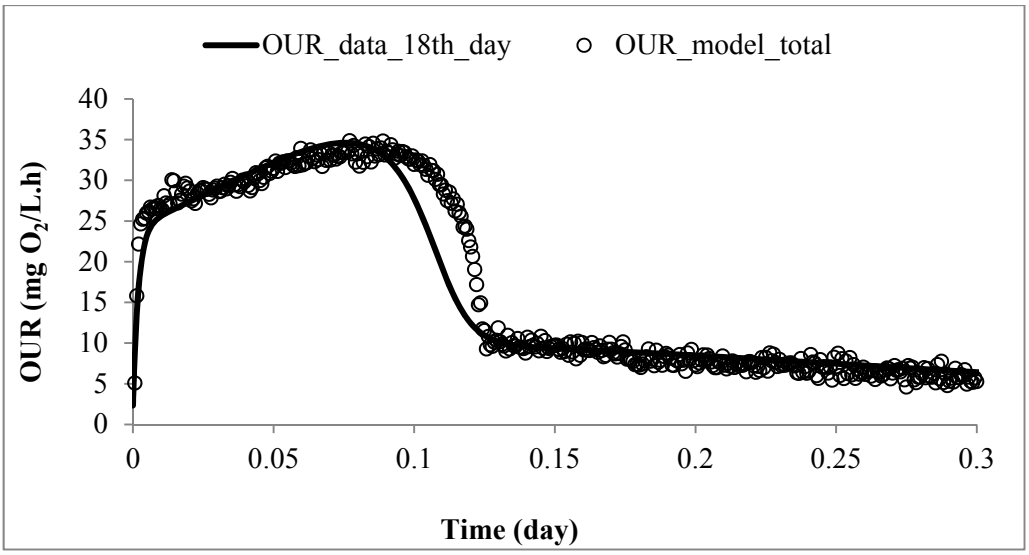


Figure 4.45 : Model simulation of OUR profile for Run 11.1.

According to modeling of chronic experiments, the biomass is able to increase its primary growth metabolism and minimize storage capability. Because, 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 chronic experiments. The model simulation results based on modified ASM3 of Run 1, Run 8, Run 9.1 and Run 11.1 were given in Table 4.7 in details.

Table 4.7 : Calibration results of the chronic experiments.

Model Parameters	**Unit**	**Control** (Run 1)	**1st day** (Run 3)	**6th day** (Run 8)	**8th day** (Run 9.1)	**18th day** (Run 11.1)
μ_{Hmax}	1/day	8.5	8	8	8.5	8
K_S	mg COD/L	15	33	33	15	15
b_H	1/day	0.24	0.35	0.35	0.35	0.35
k_{STO}	1/day	5.9	5.9	-	-	-
μ_{STO}	1/day	3	3	-	-	-
K_{STO}	mg COD/L	0.3	0.3	-	-	-
k_h	1/day	-	-	2.4	2.4	2.4
K_X	mg COD/ mg COD	-	-	0.15	0.15	0.15
f_{ES}	-	0.05	0.05	0.05	0.05	0.05
f_{EX}	-	0.15	0.15	0.15	0.15	0.15

Assumptions: Y_H = 0.66 g COD/g COD (Yavaşbay, 2010).

Table 4.8 : Calibration results of the chronic experiments (cont.).

State variables	**Unit**	**Control** (Run 1)	**1st day** (Run 3)	**6th day** (Run 8)	**8th day** (Run 9.1)	**18th day** (Run 11.1)
Total biomass	mg VSS/L	182	239	141	165	120
X_H	mg COD/L	300	265	170	200	140
Activity	%	88	72	85	85	82
$S_{ACini.}$	mg COD/L	195	250	230	230	230
$SMX_{ini.}$	mg COD/L	-	69	69	69	69
$S_{ACuti.}$	mg COD/L	215	255	167	175	200
$(S_{AC}+SMX)_{comp.}$	mg COD/L	-	12	63+40	55+42	30+44

5. CONCLUSION

The objective of this study was to investigate acute and chronic inhibition effects of antibiotics on the biodegradation of acetate. Therefore, this research focused on the fate and effect of sulfamethoxazole (SMX)which represents sulfonamide group of antibiotics. The inhibitory impact of SMX on the biodegradation of acetate by an acclimated microbial culture was investigated under aerobic conditions at a sludge age of 2 d.

Respirometric tests were performed for the assessment of substrate biodegradation behaviour with addition of SMX. The acclimated biomass was used for respirometric experiments which displayed a significant information about the inhibitory impact of SMX. To evaluate the acute effects of SMX on the acetate removal performance, the antibiotic was given to system with different pulse feedings of 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L of SMX concentrations. Antibiotic removal was not observed with the different concentration of antibiotic addition during a period of time in the acute experiments.

According to model simulations results of acute experiments, maximum specific growth rate (μ_{Hmax}) decreased from 8.5/day to 6/day whereas the half saturation coefficient (K_S) increased from 15 mg COD/L to 33 mg COD/L with the addition of SMX to the system. In addition, the maximum specific growth rate for storage (μ_{STO}) decreased from 3/d to 2.1/d. The results indicated that the metabolism of the activated sludge was shifting from storage to growth. The biomass prefers to channel substrate (acetate) more to direct growth process rather than producing internal storage material (PHB).

Additional respirometric experiments were conducted on the activated sludge to determine chronic inhibition effects of SMX. In the chronic experiments were done with continuous feeding of 50 mg/L of SMX concentration. According to model simulations results, a portion of acetate was utilized by the microorganisms in the growth process. The other portion of acetate made a complex with antibiotic that was consumed as readily hydrolysable COD fraction in the hydrolysis process.

Approximately 40 mg COD/L antibiotic was removed throughout the chronic experiments with a specific hydrolysis rate. Moreover, the biomass is able to increase its primary growth metabolism and minimize storage capability. Because, the storage process disappeared with the acclimation of activated sludge to SMX in the chronic experiments.

As a consequence, all acute and chronic results under aerobic conditions at a sludge age of 2 d demonstrated that the metabolism of the activated sludge was shifting from storage to growth. The biomass prefers to direct growth process rather than storage process.

Finally, it is recommended that future studies on antibiotics should include biodegradation of antibiotics and their toxic/inhibition effects on microbial community which must be defined in these systems. Because, there are studies present in the literature considering the detection and the biodegradation of these substances under different environmental conditions has been tried to determine by only conventional methods. Studies on the microbial groups and species that are responsible for degradation have not been done yet.

REFERENCES

Adams, C.D. 2008. Modeling the fate of pharmaceuticals and personal care products in sewage treatment plants.*Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, 10.1061/ASCE,1090-025X(2008)12:1(2).

Aga, D.S., Sungpyo, K., Eichhorn, P., Jensen, N.J., and Weber, S.A. 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science and Technology*, 2005, 39, 5816-5823.

Al-Ahmad, A., Daschner, F.D., and Kümmerer, K. 1999. Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G and sulfamethoxazole and inhibition of waste water bacteria. *Archives of Environmental Contamination and Toxicology*, 37, 158–163.

Alaton A.I., Dogruel, S., Baykal, E., and Gerone G. 2004.Combined chemical and biological oxidation of penicillin formulation effluent.*Journal of Environmental Management,***73**(2004), 155-163.

Alexy, R., Kümpel, T., and Kümmerer, K. 2004.Assessment of degradation of 18 antibiotics in the closed bottle test. Chemosphere, 57, 505–512.

Amrane, A.,Prado, N., and Ochoa, J. 2009. Biodegradation and biosorption of tetracycline and tylosin antibiotics in activated sludge system. *Process Biochemistry,***44**(2009), 1302-1306.

APHA, AWWA, WPCP, 2005: Standard methods for the examination of water and wastewater. Edited by Clesceri L.S., Greenberg A.E., Eaton, A.D. 21th edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Arslan-Alaton, I., and Caglayan,E.A. 2006.Toxicity and biodegradability assessment of raw and ozonated procaine penicillin G formulation effluent.*Ecotoxicology and Environmental Safety,***63**(2006), 131-140.

Avella, A. C., Essendoubi, M., Louvet, J. N., Görner, T., Sockalingum, G. D., Pons, M. N., Manfait, M., and Donato, Ph. de. 2010. Activated sludge behaviour in a batch reactor in the presence of antibiotics: study of extracellular polymeric substances. *Water Science and Technology*, **61**(12), 3147-3155.

Baetens, I.D. 2001. Enhanced biological phosphorus removal: modelling and experimental design, PhD thesis.

Beers M.H. 2003. The Merck manual of medical information, second edition: The world's most widely used medical reference-Now in everyday language. Whitehouse Station, NJ: Merck; 2003.

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

Benes, O., Spanjers, H., and Holba, M. 2002. Respirometry techniques and activated sludge models. *Water Science and Technology,***46**(4–5), 1-6.

Beun, J.J., Paletta, F., van Loosdrecht, M.C.M., and Heijnen, J.J. 2000. Stoichiometry and kinetics of poly- β -hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures. *Biotechnology and Bioengineering*, **67**(4), 379-389.

Boxall, A.B.A., Kolpin,D.W., Sorensen, B.H., and Tolls, J. 2003. Are veterinary medicines causing environmental risks?.*Environmental Science and Technology,***37**(2003), 286A-294A.

Braunegg, G., Sonnleitner, B. and Lafferty, R.M. 1978. A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass. European Journal of *Applied Microbiology and Biotechnology*, **6**(1), 29-37.

Brock, T.D., and Madigan,M.T. Biology of Microorganisms, 6th Ed., Prentice-Hall, Englewood Cliffs, US (1991)

Cai M., Chua H., Zhao Q., Sin N.S., and Ren J. 2009. Optimal production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation. Bioresource Technology, 100:1399–1405.

Carucci, A., Cappai, G., and Piredda, M. 2006. Biodegradability and toxicity of pharmaceuticals in biological wastewater treatment plants.*Journal of Environmental Science and Health Part A,***41(**2006), 1831–1842.

Çeçen, F., Kocamemi, A.B., and Aktaş, Ö. 2010. Xenobiotics in the Urban Water Cycle; Mass Flows, Environmental Process, Mitigation and Treatment Strategies, Environmental Pollution, Vol 16, p. 161-178, Eds. Fatta-Kassinos, D., Bester, K., Kümmerer K., Springer Dordrecht Heidelberg London, New York.

Chang, H., and Alvarez-Cohen, L. 1995a. Transformation capacities of chlorinated organics by mixed cultures enriched on methane, propane, toluene, or phenol. Biotechnology and Bioengineering, 45, 440-449.

Chang, H., and Alvarez-Cohen, L. 1995b.Model for the cometabolic biodegradation of chlorinated organics. Environmental Science and Technology, 29, 2357-2367.

Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T., and Goldbach, H.E. 2003.Determination of antibiotic residues in manure, soil, and surface waters.*Acta Hydrochimica Et Hydrobiologica*, 31, 36-44.

Chua, A.S.M., Takabatake, H., Satoh, H., and Mino, T. 2003. Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastetwater: effect of pH, sludge retention time (SRT), and acetate concentration in the influent. *Water Research*, 37, 3602-3611.

Ciggin, A.S.2011a. Growth and storage kinetics of activated sludge systems operated with various carbon sources for different sludge ages. Ph.D. Thesis, Istanbul Technical University, Turkey, April 2011.

Ciggin, A.S., Insel, G., Majone, M., and Orhon, D. 2011b. Respirometric evaluation and modelling of acetate utilization in sequencing batch reactor under pulse and continuous feeding. *Bioresource Technology,* **107**(2012), 61–69.

Ciggin, A.S., Rossetti, S., Majone, M., and Orhon, D.2011c.Effect of feeding and sludge age on acclimated bacterial community and fateof slowly biodegradable substrate.*Bioresource Technology,***102**(2011), 7794-7801.

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

Cokgor, E.U., Sözen, S., Insel, G., and Orhon, D. 2009. Respirometric evaluation of biodegradation characteristics of dairy wastewater for organic carbon removal.*Environmental Technology*, **30**(11), 1169-1176.

Cokgor, E.U., Arslan Alaton, I., Karahan, O., Dogruel, S., and Orhon, D. 2008.Biological treatability of raw and ozonated penicillin formulation effluent.Journal of Hazardous Materials, B116(2004), 159–166.

Cokgor, E.U., Karahan, O., Arslan-Alaton I., Meric, S., Saruhan, H., and Orhon D. 2006.Effect of perozonation on biodegradability and toxicity of a penicillin formulation effluent.*Journal of Environmental Science and Health Part A,* 41, 1887-1897.

Cokgor, E.U., Sozen, S., Orhon, D., and Henze, M.1998. Respirometric analysis of activated sludge behaviour--I.Assessment of the readily biodegradable substrate.*Water Research,***32**(2), 461-475.

Cromwell, G. 2002. Why and how antibiotics are used in swine production. *Animal Biotechnology,* 13, 7-27.

Dantas, R.F., Contreras, S., Sans, C., Esplugas, S. 2007. Sulfamethoxazole abatement by means of ozonation.*Journal of Hazardous Materials*, 150, 790–794.

Demirden, P., 2005. Treatability of pharmaceutical industry wastewaters containing antibiotic in anaerobic/aerobic sequential processes.M.Sc.Thesis, Dokuz Eylül University,Turkey, September 2005.

Dias, J.M., Lemos, P.C., Serafim, L.S., Oliveira, C., Eiroa, M., Albuquerque, M.G., Ramos, A.M., Oliveira, R., and Reis, M.A. 2006. Recent advances in polyhydroxyalkanoate production by mixed aerobic cultures: From the substrate to the final product. *Macromolecular Bioscience,***6**(11), 885–906.

Dionisi, D., Majone, M., Ramadori, R., and Beccari, M.2001.The storage of acetate under anoxic conditions.*Water Research,***35**(11), 2661–2668.

Dircks, K., Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., and Henze, M. 2000. Glycogen metabolism in aerobic mixed cultures.*Biotechnology and Bioengineering,***73**(2), 85-94.

Dircks, K., Pind, P.F., Mosbæk, H., and Henze,M.2001. Yield determination by respirometry - The possible influence of storage under aerobic conditions in activated sludge. *Water Research,***25**(1), 69-74.

Dizdaroglu-Risvanoglu, G., Karahan, O., Cokgor, E.U., Orhon, D., and van Loosdrecht, M.C.M. 2007. Substrate storage concepts in modeling activated sludge systems for tannery wastewaters. *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering,***42**(14), 2159-2166.

Drillia, P., Dokianakis S.N., Fountoulakis, M.S., Kornaros, M., Stamatelatou, K., and Lyberatos, G. 2005. On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. *Journal of Hazardous Materials,***122**(2005), 259-265.

Eriksson, E., Auffarth, K., Eilersen, A.M., Henze, M., and Ledin, A. 2003.Household chemicals and personal care products as sources for xenobiotic organic compounds in grey wastewater.*Water SA*, **29**(2), 135-146.

Frigon, D., Muyzer, G., van Loosdrecht, M.C.M., and Raskin, L. 2006. rRNA and poly-β-hydroxybutyrate dynamics in bioreactors subjected to feast and famine cycles. *Applied Microbiology and Biotechnology,***72**(4), 2322–2330.

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

Gaskins, H.R., Collier, C.T., and Anderson B.D. 2002. Antibiotics as growth promotants: Mode of action. *Animal Biotechnology*. 13, 29-42.

Goel, R., Mino, T., Satoh, H., and Matsuo, T.1999. Modeling hydrolysis processes considering intracellular storage. *Water Science and Technology,* **39**(1), 97-105.

Gonzalez, O., Sans, C., Esplugas, S., 2007.Sulfamethoxazole abatement by photo-Fenton toxicity, inhibition and biodegradability assessment of intermediates.*Journal of Hazardous Materials*, 146, 456–459.

Gotvajn, A.Z., Zagorc-Koncan, J., and Tisler, T. 2007.Pretreatment of highly polluted pharmaceutical waste broth by wet air oxidation.*Journal of Environmental Engineering,***133**(2007), 89-94.

Guisasola, A., Sin, G., Baeza, J.A., Carrera, J.,and Vanrolleghem, P. 2004. Limitations of ASM1 and ASM3: A comparison based on batch OUR profiles from different full-scale WWTPs. In Proceedings 2nd IWA congress, Marrakech, Morocco, 19–24 September.

Gujer, W., Henze, M., Mino, T., and van Loosdrecht, M.C.M. 1999. Activated sludge model no.3. *Water Science and Technolog,***39**(1), 183-193.

Gujer, W., Henze, M., Mino, T., and van Loosdrecht, M.C.M. 2000. Activated sludge model no.3. In: Activated sludge models ASM1, ASM2, ASM2D and ASM3, IWA Scientific and Technical Report No.9. IWA London.

Hanada, S., Hiroyasu, S. and Mino, T., 2001. Measurement of microorganisms with PHA production capability in activated sludge and its implication in activated sludge model no. 3. *Water Science and Technology*, **45**(6), 107-113.

Halling-Sorensen, B. 2000. Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere,***40**(2000), 731-739.

Halling-Sorensen, B., Nors-Nielsen, S., Lanzkey, P., Ingerslev, F., Holten, H., and Jorgensen, S. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment-a review.*Chemosphere,* 36, 357-393.

Hanada, S., Hiroyasu, S., and Mino, T. 2001. Measurement of microorganisms with PHA production capability in activated sludge and its implication in activated sludge model no. 3. *Water Science and Technology,***45**(6), 107-113.

Hektoen, H., Bergea, J.A., Hormazabal, V., and Yndestad, M. 1995.Persistence of antibacterial agents in marine sediments.*Aquaculture*, **133**(3–4), 175–184.

Henze M., Gujer W., Mino T., Matsuo T., Wentzel M.C., and Marais G.v.R.1994. Activated sludge model no.2. IAWQ Scientific and Technical Reports.IWAQ London.

Henze, M. 2005. Environmental biotechnology: Concepts and applications. Edited by Jördening, H.J. and Winter, J., pp. 121-135. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, ISBN: 3-527-30585-8.

Henze, M., Grady, C.P.L., Gujer, W., Marais, G.v.R., and Matsuo, T. 1987. Activated sludge model no.1, IAWPRC Scientific and Technical Reports, No. 1. London.

Henze, M., van Loosdrecht, M.C.M. Ekama, G.A., and Brdjanovic D. 2008. Biological wastewater treatment, principles, modelling and design.Pages 1-528, ISBN: 9781843391883, IWA publishing London, UK.

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

Hirsch, R., Ternes, T., Haberer, K., and Kratz K.-L. 1999. Occurrence of antibiotics in the aquatic environment. *The Science of the Total Environment,***225**(1999), 109-118.

Hoque, M. A., Aravinthan, V., and Pradhan, N.M. 2009. Assessment on activated sludge models for acetate biodegradation under aerobic conditions. *Water Science and Technology,***60**(4), 983-991.

Insel, G., Yavasbay, A., Ozcan, O., and Ubay Cokgor, E. 2012.Modeling of simultaneous growth and storage kinetics variation under unsteady feast conditions for aerobic heterotrophic biomass.*Bioprocess and Biosystems Engineering,* DOI 10.1007/s00449-012-0733-1.

Insel, G., Celikyilmaz, G., Ucisik-Akkaya, E., Yesiladali, K., Cakar, Z.P., Tamerler, C., and Orhon, D. 2007.Respirometric evaluation and modeling of glucose utilization by Escherichia coli under aerobic and mesophilic cultivation conditions.*Biotechnology and Bioengineering,***96**(1): 94-105.

Ingerslev, F., and Halling-Sørensen, B. 2001.Biodegradability of metronidazole, olaqiondox, and tylosin, and formation of tylosin degradation products in aerobic soil/manure slurries. Ecotoxicology and Environmental Safety, 48, 311–320.

International Standards Organization (ISO), 2007.Water quality-test for inhibition of oxygen consumption by activated sludge-ISO 8192; ISO, Switzerland.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., and Parrella, A. 2005. Science of the Total and Environment, 346, 87-98.

ISO 6060, 1986.Water quality-determination of the chemical oxygen demand. International Standards Organization, Switzerland.

Karahan, O. 2010. Inhibition effect of linear alkylbenzene sulphonates on the biodegradation mechanisms of activated sludge. *Bioresource Technology,***101**(1), 92–97.

Karahan, O., Van Loosdrecht, M.C.M., and Orhon, D.2006. Modeling the utilization of starch by activated sludge for simultaneous substrate storage and microbial growth. *Biotechnology and Bioengineering*, **94**(1), 43-53.

Karahan-Gül, O., Van Loosdrecht, M.C.M., and Orhon, D. 2003.Modification of activated sludge model no. 3 considering direct growth on primary substrate.*Water Science and Technology*, **47**(11), 219-225.

Karahan-Gül, O., Artan, N., Orhon, D., Henze, M., and van Loosdrecht, M.C.M. 2002a.Experimental assessment of bacterial storage yield.*Journal of Environmental Engineering-ASCE*, **128**(11), 1030–1035.

Karahan-Gül, O., Artan, N., Orhon, D., Henze, M., and van Loosdrecht, M.C.M. 2002b.Respirometric assessment of storage yield for different substrates.*Water Science and Technology,***46**(1-2), 345-352.

Katipoğlu, T., Cokgor, U E., Insel, G., and Orhon, D. 2010. Response of mixed microbial culture to 2,6-dihydroxybenzoic acid and peptone mixture at low sludge age-effect of culture history. *Journal of Environmental Science and Health Part A,* 45(2010), 875-882.

Katipoğlu, T. 2007. Evaluation of acclimation and inhibitory impact of 2,6-dihydroxybenzoic acid on the biodegradation of peptone under aerobic conditions. M.Sc. Thesis.Istanbul Technical University, Turkey, April 2011.

Kim, S., Jensen, J.N., Aga, D.S., and Weber, A.S. 2007. Fate of tetracycline resistant bacteria as a function of activated sludge process organic loading and growth rate. *Water Science and Technology*, **55**(1–2), 291–297.

Kim, S., and Aga, D.S. 2007. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. *Journal of Toxicology and Environmental Health. Part B,* 10, 559–573.

Kim, S., Horn, P., Jensen, J.N., Weber, A.S., and Aga, D.S. 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science and Technology*,**39**(2005), 5816-5823.

Kleerebezem, R., Johnson K., and van Loosdrecht M.C.M. 2009. Model-based data evaluation of polyhydroxybutyrate producing mixed microbial cultures in aerobic sequencing batch and fed-batch reactors.*Biotechnology and Bioengineering*, **104**(1), 50-67.

Koch, G., Kühni, M., Gujer, W., and Siegrist, H. 2000.Calibration and validation of Activated Sludge Model No. 3 for Swiss Municipal Wastewater.*Water Research*, **34**(14): 3580-3590.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., and Buxton, HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environmental Science and Technology*, **36**(6), 1202-1211.

Kornaros, M., Dokianakis,S N., and Lyberatos G. 2006. Impact of five selected xenobiotics on ısolated ammonium oxidizers and on nitrifying activated sludge. *Environmental Toxicology*, DOI 10.1002/tox.20199.

Krishna, C., and van Loosdrecht, M.C.M. 1999. Substrate flux into storage and growth in relation to activated sludge modeling.*Water Research*, **33**(14), 3149-3161.

Kumar M.S., Mudliar S.N., Reddy K.M.K., and Chakrabarti, T.2004. Production of biodegradable plastics from activated sludge generated from a food processing industrial wastewater treatment plant. *Bioresource Technology*, 95:327–330.

Kümmerer K.,Fatta-Kassinos, D., and Bester, K. 2010.Xenobiotics in the Urban Water Cycle; Mass Flows, Environmental Process, Mitigation and Treatment Strategies, Environmental Pollution, Vol 16, DOI 10.1007/978-90-481-3509-7_9, Springer Dordrecht Heidelberg London, New York.

Kümmerer, K. 2009a. Antibiotics in the aquatic environment – A review – Part I. *Chemosphere,***75**(2009), 417-434.

Kümmerer, K. 2009b. Antibiotics in the aquatic environment – A review – Part II.*Chemosphere,***75**(2009), 435-441.

Kümmerer, K. 2009c.The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges.*Journal of Environmental Management,***90**(2009), 2354-2366.

Kümmerer, K., 2008. Antibiotics in the Environment.Pharmaceuticals in the Environment, pp. 3-35, Eds. Kummerer, K., 3rd Ed. Springer, Verlag.

Kümmerer, K. 2003.Significance of antibiotics in the environment.*Journal of Antimicrobial Chemotherapy*, 52, 5-7.

Kümmerer, K., and Henninger, A. 2003.Promoting resistance by the emission of antibiotics from hospitals and households into effluents.*Clinical Microbiology and Infection,* 9, 1203-1214.

Kümmerer, K., Al-Ahmad, A., and Mersch-Sundermann, V. 2000.Biodegradability of some antibiotics, elimination of the genotoxicity and a ection of wastewater bacteria in a simple test.*Chemosphere,* **40**(2000), 701-710.

Kümmerer, K., Alexy, R., Hüttig, J., and Schöll A. 2004. Standardized tests fail to assess the effects of antibiotics on environmental bacteria. *Water Research*, 38(2004), 2111-2116.

Längin, A., Alexy, R., König, A., and Kümmerer, K., 2009. Deactivation and transformation products in biodegradability testing of ß-lactams piperacillin and amoxicillin.*Chemosphere*, **75**(3), 347-354.

Leal, L.H., Vieno, N., Temmink, H., Zeeman, G., and Buisman, C.J.N. 2010.Occurrence of xenobiotics in gray water and removal in three biological treatment systems.*Environmental Science and Technology*, 44, 6835-6842.

Lee, S.Y. 1996. Bacterial polydroxyalkanoates.*Biotechnology and Bioengineering*, 49:1–14.

Lemos, P.C., Dias, J.M.L., Oehmen, A., Serafim L.S, Reis, M.A.M., and Oliveira, R. 2008. Metabolic modelling of polyhydroxyalkanoate copolymers production by mixed microbial cultures. *BMC Systems Biology*, **2**(59), 1-21.

Li, K., Yediler, A., Yang, M., Schulte-Hostede, S., and Wong H.M. 2008.Ozonation of oxytetracycline and toxicological assessment of its oxidation by-products.*Chemosphere,***72**(2008), 473-478.

Majone, M., Beccari, M., Dionisi, D., Levantesi, C., Ramadori, R., and Tandoi, V. 2007.Effect of periodic feeding on substrate uptake and storage rates by a pure culture of Thiothrix (CT3 strain).*Water Research*, 41, 177-187.

Majone, M., Dircks, K., and Beun, J.J. 1999. Aerobic storage under dynamic conditions in activated sludge processes. The state of the art.*Water Science and Technology,***39**(1), 61-73.

Martins, A.M., Heijnen, J.J., and van Loosdrecht, M.C.M. 2003.Effect of feeding pattern and storage on the sludge settleability under aerobic conditions.*Water Research*, 37, 2555-2570.

McCarty, P. L. (2000). Novel biological removal of hazarodous chemicals at trace levels. Water Science and Technology, 42, 49–60.

McEvoy, G.K. 2004. AHFS Drug information; American Society of Health System Pharmacists: Bethesda, MD.

McMahon M.A.S., Xu, J., Moore E.J., Blair, S.I., and McDowell, A.D. 2007. Environmental Stress and Antibiotic Resistance in Food-Related Pathogens.*Applied and Environmental Microbiology*, **73**(1), 211-217.

Metcalf and Eddy, Inc. 2004. Wastewater Engineering: Treatment, Disposal, and Reuse. 4th Edition, McGraw-Hill Companies, Inc., New York, NY.

O'Connor, J.T. 1972. Environmental engineering unit operations and unit processes, Laboratory manual, Association of Environmental Engineering Professors, U.S.A.

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

O'Neil, M.J., Smith, A., Heckelman, P.E.,2001. Eds. The Merck Index (13th ed.), Merck, Whitehouse Station, NJ. (2001).

Orhon, D., and Artan, N. 1994.Modelling of activated sludge systems. Lancaster PA, USA: Technomic Press.

Orhon, D., Germirli Babuna, F., and Karahan, O. 2009a.Industrial wastewater treatment by activated sludge. ISBN: 9781843391449, IWA publishing London, UK,

Orhon, D., Cokgor, E.U., Insel, G., Karahan, O., and Katipoglu, T. 2009b. Validity of monod kinetics at different sludge ages - peptone biodegradation under aerobic conditions, *Bioresource Technology*, **100**(23): 5678-5686.

Orhon, D., Karahan, O., and Sozen, S. 1999. The effect of residual microbial products on the experimental assessment of the particulate COD in wastewaters.*Water Researc*h, **33**(14), 3191-3203.

Orhon, D., Okutman, D., and Insel, G. 2002. "Characterization and biodegradation of settleable organic matter for domestic wastewater", *Water SA*, No. 28, 299-305.

Orhon, D., Ubay Cokgor, E., Katipoglu, T., Insel, G., and Karahan O. 2010. Fate of 2,6-dihydroxybenzoic acid and its inhibitory impact on the biodegradation of peptone under aerobic conditions. *Bioresource Technology*, **101**(2010), 2665-2671.

Orozco, J.H.G. 2008. Toxicity, pp. 73-290, Eds. Henze, M., van Loosdrecht, M.C.M. Ekama, G.A., and Brdjanovic D., Biological wastewater treatment, principles, modelling and design. IWA publishing London, UK.

Ozkok Pala, I., Yazan Katipoglu, T., Cokgor Ubay, E., Insel, G., Talinli, I., and Orhon, D. 2011.Respirometric assessment of substrate binding by antibiotics in peptone biodegradation.*Journal of Environmental Science and Health, Part A*, **46**(2011), 1–10.

Park S., and Choi K. 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems.*Ecotoxicology.*

Perez, S., Eichhorn, P., and Aga, S.D. 2005.Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment.*Environmental Toxicology and Chemistry,***24**(6), 1361-1367.

Petrović, M., and Barceló, D. 2007. Analysis, fate and removal of pharmaceuticals in the water cycle, Volume 50, Pages 1-564, ISBN: 978-0-444-53052-3, Elsevier.

Physicians' Desk Reference, 2005.59th ed. Montvale, N.J.: Thomson PDR.

Plosz, B.G.Y., Leknes, H., and Thomas, K.V. 2010.Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment.*Environmental Science and Technology,***44**(2010), 734-742.

Pot, M., van Leeuwen, M.A., van Loosdrecht M.C.M., and Heijnen, J.J. 1996. Kinetic modelling of polyhydroxybutyrate production and consumption by Thiosphsera pantotropha under dynamic substrate supply.*Trends in Biotechnology*,**14**(11):431-438

Prado, N., Montéléon C., Ochoa, J., and Amrane, A. 2009. Evaluation of the toxicity of veterinary antibiotics on activated sludge using modified Sturm tests—application to tetracycline and tylosine antibiotics. *Journal of Chemical Technology and Biotechnology*, **85**(4), 471–477.

Ramos, J.L., Nunez, A., and Caballero, A. 2001. Biological Degradation of 2,4,6 Trinitrotoluene. *Microbiology and Molecular Biology Reviews*, **65**(3), 335-352.

Reichert, P., Ruchti, J., and Simon, W.1998.AQUASIM 2.0.Swiss Federal Institutefor Environmental Science and Technology (EAWAG), CH-8600 Duebendorf, Switzerland.

Reinthaler, F.F., Posch, J., Feierl, G., Wust, G., Haas, D., Ruckenbauer, G., Mascher, F., and Marth, E.2003.Antibiotic resistance of E. coli in sewage and sludge.*Water Research*, **37**(8), 1685-1690.

Reis, M.A.M.,Serafim, L.S., Lemos, P.C., and Oliveira, R. 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. *Biotechnology and Bioengineering,* Volume 87, No. 2.

Richardson, M.L., and Bowron, J.M. 1985. The fate of pharmaceutical chemicals in the environment.*Journal of Pharmacy and Pharmacology*, 37, 1–12.

Rieger, P.G., Meier, H.M., Gerle,M., Vogt, U., Groth, T., and Knackmuss, H.J. 2002. Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence. *Journal of Biotechnology*, **94**(2002), 101-123.

Rittmann, B. E. and P. L. McCarty (2001). Environmental Biotechnology: Principles and Applications. McGraw-Hill Book Co., New York.

Roels, J.A. 1983. Energetics and kinetics in biotechnology.Elsevier Biomedical Press, Amsterdam.

Salehizadeh, H., and Van Loosdrecht, M.C.M. 2004. Production of polyhydroxyalkanoates by mixed cultures: Recent trends and biotechnological importance. *Biotechnology Advances*, 22, 261-279.

Sapadin, A.N., ve Fleischmajer, R. 2006. Tetrasiklinler: Antibiyotik dısı özellikleri ve klinik uygulamaları. Jaad Turkish Edition, 3(2).

Satoh, H., Mino, T., and Matsuo, T. 1999.PHA production by activated sludge. International Journal of Biological Macromolecules, 25:105–109.

Schmidt, H., and Römbke, J., 2008. The ecotoxicological effects of pharmaceuticals (antibiotics and antiparasiticides) in the terrestrial environment – a review. In: Kümmerer, K. (Eds.), Pharmaceuticals in the Environment, 3rd Ed., Sources, Fate Effects and Risk Springer, Berlin, Heidelberg, pp. 285–303.

Serrano, P.H.2005.Responsible use of antibiotics in aquaculture.FAO Fisheries Technical Paper.No. 469. FAO, Rome, 97 p.

Sozen, S., Ubay Cokgör, E., Orhon, D., and Henze, M. 1998.Respirometric analysis of activated sludge behaviour--II.Heterotrophic growth under aerobic and anoxic conditions.*Water Research*, **32**(2), 476-488.

Spanjers, H., Vanrolleghem, P.A., Gustaf, O., and Dold, P. 1996. Respirometry in control of the activated sludge process.*Water Science and Technology,***34**(2), 117-126.

Steinbüchel, A., and Wiese, S.A. 1992.A Pseudomonas strain accumulating polyesters of 3-polyhydroxybutyric acid and medium-chain-length 3-hydroxyalkanoic acids.*Applied Microbiology and Biotechnology*, 37, 691-697.

Ternes, T., and Joss, A. 2007.Human pharmaceuticals, hormones and fragrances, the challenge of micropollutants in urban water management. ISBN: 9781843390930, IWA Publishing.

Ural, A.2012.Fate of sulfamethoxazole and its inhibitory impact on the biodegradation of acetate under aerobic conditions at high sludge age. M.Sc. Thesis, Istanbul Technical University, Turkey, January 2012.

Url-1<*http://epa.gov/nerlesd1/chemistry/pharma/images/drawing.pdf*>, accessed at 07.01.2012.

Url-2<*http://www.chemicalbook.com/CAS/GIF/723-46-6.gif*>,accessed at 21.12.2011.

Url-3<*http:// chemistrypictures.org/v/enzymes*>, accessed at 25.07.2011.

Url-4<*http:// en.wikipedia.org/wiki/Enzyme*>, accessed at 25.07.2011.

Url-5<http://www.emedexpert.com/classes/antibiotics.shtml>, accessed at 10.03.2011.

Url-6<http://www.dermnet.org.nz/treatments/antibiotics.htm>, accessed at 15.03.2011.

Url-7<*http://faculty.une.edu/com/courses/bionut/distbio/obj-512/Chap9*>, accessed at 03.05.2011.

Url-8<*http://en.wikipedia.org/wiki/Polyhydroxybutyrate*>, accessed at 18.10.2011.

Url-9<*http://curiosity.discovery.com/question/what-polyhydroxybutyrate-pbh-eco-friendly*>, accessed at 18.10.2011.

Van Aalst-van Leeuwen, M.A., Pot, M.A., Van Loosdrecht, M.C.M., and Heijnen, J.J.1997a. Kinetic modeling of poly (β-hydroxybutyrate) production and consumption by Paracoccus pantotrophus under dynamic substrate supply.*Biotechnology and Bioengineering,***55**(5), 773-782.

Van Loosdrecht, M.C.M., and Heijnen, J.J. 2002. Modeling of activated sludge processes with structured biomass. *Water Science and Technology*, 45, 13–23.

Van Loosdrecht, M.C.M., and Henze, M. 1999. Maintenance, endogeneous respiration, lysis, decay and predation.*Water Science and Technolog*, **39**(1), 107– 117.

Van Loosdrecht, M.C.M., Pot, M.A., and Heijnen, J.J. 1997b. Importance of bacterial storage polymers in bioprocesses.*Water Science and Technology,***35**(1), 41-47.

Van Niel, E.W.J., Robertson, L.A., and Kuenen, J.G. 1995. Rapid short term poly-β-hydroxybutyrate production by Thiosphaera pantothropha in the presence of excess acetate.*Enzyme and Microbial Technology,* 17(11), 977-982.

Watkinson, A.J., Murby, E.J., and Costanzo S.D. 2007. Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Research*, **41**(2007), 4164-4176.

Watkinson, A.J., Murby, E.J., Kolpin, D.W., and Costanzo, S.D. 2009. The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. *Science of the Total Environment*, **407**(2009), 2711-2723.

Watkinson, J.A., Micalizzi, B.G., Graham, M.G., Bates, B.J., and Costanzo D.S. 2007. Antibiotic-resistant Escherichia Coli in wastewaters, surface waters, and oysters from an urban riverine system. *Applied and Environmental Microbiology,***73**(17), 5667-5670.

Wiethan, J., Al-Ahmad, A., Henninger, A., and Kümmerer, K. 2000.Simulation des selektionsdrucks der antibiotika ciprofloxacin und ceftazidim in oberflächengewässern mittels klassischer methoden (Simulation of the selection pressure of the antibiotics ciprofloxacin and ceftazidim in surface water with classical methods).*Vom Wasser*, 95, 107–118.

Wollenbergera, L., Halling-Sùrensenb, B., and Kuska, K.O.2000.Acute and chronic toxicity of veterinary antibiotics to Daphnia magna.*Chemosphere,***40**(2000), 723-730.

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

Yan S., Tyagi R.D., and Surampalli R.Y. 2006.Polyhydroxyalkanoates (PHA) production using wastewater as carbon source and activated sludge as microorganisms.*Water Science and Technology*, **53**(6): 175–180.

Yavaşbay, A. 2010.Effects on intermittent feeding conditions on microbial growth kinetics. M.Sc. Thesis, Istanbul Technical University, Turkey, May 2010.

CURRICULUM VITAE

Name Surname: **Gökçe KOR**

Place and Date of Birth: Manisa / 21.10.1985

Address: Istanbul Technical University,
Civil Engineering Faculty,
Environmental Engineering Department
34469 Maslak, Istanbul / TURKEY

E-Mail: gkor@itu.edu.tr

B.Sc.: Marmara University, Engineering Faculty,
Environmental Engineering Department
(2004-2009)

Professional Experience and Rewards:

- Research Assistant at Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department (January 2012-)
- Environmental Engineer at ENVIS Energy and Environmental SystemsResearch Development Ltd. (April 2010 – August 2011)
- Marmara University, Environmental Engineering Department Graduated with Honor Degree (2nd in among all department graduates) (July 2009)
- Sertificate of Minor Education on Marmara University, Mechanical EngineeringDepartment (July 2009)