ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CHARACTERIZATION AND MODELING OF ACTIVATED SLUDGE PROCESS FOR PHARMACEUTICAL INDUSTRY WASTEWATERS

M.Sc. THESIS

DORA OLCAY

Department of Environmental Engineering

Environmental Biotechnology Programme

Thesis Advisor: Asst. Prof.Serdar Doğruel

JANUARY 2014

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CHARACTERIZATION AND MODELING OF ACTIVATED SLUDGE PROCESS FOR PHARMACEUTICAL INDUSTRY WASTEWATERS

M.Sc. THESIS

DORA OLCAY
501091828

Thesis Advisor: Asst.Prof.Dr. Serdar Doğruel

Department of Environmental Engineering

Environmental Biotechnology Programme

JANUARY 2014

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

İLAÇ ENDÜSTRİSİ ATIKSULARI İÇİN KARAKTERİZASYON VE AKTİF ÇAMUR PROSESİNİN MODELLENMESİ

YÜKSEK LİSANS TEZİ

DORA OLCAY
501091828

Çevre Mühendisliği Anabilim Dalı

Çevre Biyoteknolojisi Programı

Tez Danışmanı: Yar.Doç. Serdar Doğruel

OCAK 2014

Dora Olcay, a **M.Sc./a Ph.D.** student of ITU **Graduate School of Environmental Biotechnology** student ID 501091828, successfully defended the **thesis** entitled "**CHARACTERIZATION AND MODELING OF ACTIVATED SLUDGE PROCESS FOR PHARMACEUTICAL INDUSTRY WASTEWATERS**"

which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Asst.Prof.Serdar Doğruel** İstanbul Technical University	.....
Jury Members :	**Prof. Dr. Fatma Gülen İskender** Istanbul Technical University	.....
	Asst.Prof.Hatice Eser Ökten Bahçeşehir University	.....

Date of Submission : 16 December 2013
Date of Defense : 24 January 2014

To Tan and Mehmet ,

FOREWORD

I would like to express my sincere gratitude to my thesis supervisor Asst. Prof. Serdar Doğruel for his generous support, guidance, encouragement and tolerance throughout the study.
I am also grateful to Prof. Emine Ubay Çokgör and Asst.Prof.Dr. H.Güçlü İnsel for their valuable support and expertise during my study.
I would like to extend my special thanks and sincere appreciation to Hüseyin Orhan who gave very valuable support throughout my study.
I would also like to thank my managers at Eczacıbaşı-Baxter ; Ayşın Turan and Mihriye Çelik for their understanding and support.
I would also like to extend my special thanks to Ahmet Köseoğlu for his valuable support to begin this study.
Finally , I would like to extend my special thanks to my husband Mehmet Olcay and my son Tan Olcay.I'm very blessed to have had such loving, supportive and understanding family.I wish to dedicate this thesis to my dear family and thank them for everything.

December 2013

Dora OLCAY
(Molecular Biologist
and Genetic Engineer)

TABLE OF CONTENTS

Page

ABBREVIATIONS

ASM1 :Activated Sludge Model No.1
b_H :Endogenous Respiration Rate of Heterotrophs (1/T)
BOD :Biological Oxygen Demand mg/L
COD :Chemical Oxygen Demand mg/L
Cs :Biodegradable COD concentration
f_{ES} :Fraction of Soluble Metabolic Products
f_{EX} :Fraction of Particulate Metabolic Products
f_P :Fraction of Particulate Inert COD Generated in Biomass Decay
k_{La} : Volumetric Oxygen Tranfer Coefficient (T^{-1})
Ks :Half Saturation Constant for Growth of XH ($M.L^{-3}$)
μ_H :Specific Growth Rate for X_H (1/T)
OUR :Oxygen Utilization Rate ($M.L^{-3}.T^{-1}$)
SBR :Sequencing Batch Reactor
SS :Suspended Solids
VSS :VolatileSuspended Solids
WWTP:Wastewater Treatment Plant
WPCR :Water Pollution Control Regulation
X_H :Heterotrophic Biomass Concentration ($M.COD.L^{-3}$)

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHARACTERIZATION AND MODELING OF ACTIVATED SLUDGE PROCESS FOR PHARMACEUTICAL INDUSTRY WASTEWATERS

SUMMARY

Since the 1990s water contamination by pharmaceuticals has been an environmental issue of concern. Most pharmaceuticals are deposited in the environment through human consumption and excretion, and are often filtered ineffectively by wastewater treatment plants which are not designed to manage them. During the past decade, concern has grown about the adverse effects the use and disposal of pharmaceuticals might potentially have on human and ecological health. Research has shown that after passing through wastewater treatment, pharmaceuticals, amongst other compounds, are released directly into the environment.Among all other pharmaceutical drugs and substances, antibiotics are one of the major groups of pharmaceuticals. Little is known about the extent of environmental occurrence, transport, and ultimate fate and effects of pharmaceuticals in general, as well as of antibiotics in particular. Therefore, the adverse effect of the environment and health brought from the processing and disposal of these products become important issue in the field of environmental engineering.

Antibiotics are industrially produced and commercially used, but they are not required to undergo the extensive level of testing for possible environmental impact. For this reason, these substances are becoming an increasingly large problem in WWTP's, they also spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems.

Pharmaceutical industry formulation subcategory includes tablet, capsulle, liquid or pomad production and produced in requested dosage forms. It's important to determine wastewater sources, characterizations and volumes generated from this subcategory. In this thesis, It is 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 stochiometric constants of the mixed culture.

By comparing real and calculated values, it's been aimed to propose better operational modes for more efficient treatment.

For this purpose, the pharmaceutical plant in Turkey was selected which produces drugs in many forms and antibiotics. With its 763 personnel and 8 different production line, the plant generates 95.000 m^3 wastewater in one year.

The plant has biological treatment and chemical treatment systems. Chemical treatment is only used when SS value decreases below 1500 ppm. Wastewater COD , SS, pH values are measured daily for influent and effluent of treatment system. It is aimed to meet the Water Pollution Control Regulation criteria for pharmaceutical

industry. System has COD treatment efficiency around %90 which was shown also by conventional characterization experiments

Conventional characterization ,respiromeric analysis were conducted on the sampled wastewaters. Also Particle Size Distribution Analysis (PSD) was done via Sequential Filtration/Ultrafiltration analysis.Modelling study was also conducted using Aquasim program , ASM1 model.Stochiometric and kinetic coefficients were found and compared to literatural values.Ks and Mh values were found typical for pharmaceutical industry wastewaters.

Results of these tests show that COD removal efficiency of treatment meets the criteria of Water Pollution Control Regulation for dicharge limits of COD and pH. It is concluded that aerobic biological treatment is effective and sufficient for the wastewater generation of the plant.

The results of the model calibration related to the assessment of kinetic and stoichiometric coefficients reveals the biodegradable nature of the pharmaceutical wastewater.

From PSD analysis results it is seen that biological treatment was in removing all size ranges except soluble inert COD fraction.

İLAÇ ENDÜSTRİSİ ATIKSULARINDA AKTİF ÇAMUR PROSESİNİN KARAKTERİZASYON VE MODELLEMESİ

ÖZET

İlaçların dünya üzerindeki genel dağılımları ,günümüzün en önemli çevre problemlerinden biri olarak görülmektedir.Tıbbi olarak ilaç, insan ve hayvanlarda hastalıklardan korunma ve tedavi etme amacı güden, yardımcı maddeler ile zenginleştirilerek kullanıma sunulan dozaj şekli olarak tanımlanmaktadır. İlaçlardan kalan atıkların idrar, dışkı yoluyla veya duş sırasında suya karışması , canlı organizmalarla etkileşimde bulunmak üzere tasarlanan bu maddelerin, sudaki canlıları kısa sürede etkilemesine, bunun da ekosistem ve insanlar için potansiyel tehlike oluşturmasına yol açmaktadır.

Düşük düzeyde bile olsa bu tür bileşikler, su canlıları üzerinde teşhis edilemeyen, yavaş biriken etkilere neden olabilir hatta yeni su canlılarının ortaya çıkmasına yol açabilir. Diğer taraftan bu tür kirleticiler bakterilerde direnç gelişimine neden olabilmektedir. Yüzey ve yer altı sularının artan şekilde kimyasal maddelerle kirlenmesinin, sudaki yaşam ve insan üzerinde uzun vadeli ve tehlikeli sonuçlar doğurması beklenmektedir.Her yıl yaklaşık 300 milyon ton endüstriyel ve tüketiciler tarafından kullanılan yapay bileşiklerin atıkları, tarımsal olarak kullanılan 140 milyon ton gübre ve bir kaç milyon ton tarımsal ilaç ve kazayla 0,4 milyon ton petrol ve petrol ürünlerinin doğal sulara karışmakta olduğu düşünüldüğünde öngörülen zararlar daha da ön plana çıkmaktadır. Ürkütücü olabilecek potansiyel etkilerinden dolayı gittikçe popüler hale gelen bu konuda dünya çapında toplum baskısı artmaktadır.Bu nedenle yakın gelecekte birçok ilaç için mevzuatların sıkılaştırılması ve buna bağlı olarak arıtma tesislerinin uymak zorunda olduğu 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.Antibiyotikler, etki derecelerine, etki mekanizmalarına, kimyasal yapılarına ve farmakinetik özelliklerine göre çeşitli şekillerde sınıflandırılabilir.Avrupa birliği ülkelerinde insan ve veteriner ilaç yapımında yaklaşık 4000 farklı etken madde kullanılmaktadır.Bu maddelerin çevreye karşı olan olası etkileri yeterince değerlendirilmemiştir.

Antibiyotiklerin çevresel ortamda canlılar üzerindeki etkileri akut ve kronik etkiler olarak iki farklı alanda değerlendirilebilir.Akut etki, antibiyotiklerin tek bir doz uygulanması sonucunda ve genellikle kısa bir süre içinde meydana getirdiği zarar olarak ifade edilir.Kronik etki ise, uzun süre boyunca canlıların bu kimyasal

maddelere maruz bırakılması ile belirlenir.Zamanın belirli periyotlarında tekrar tekrar kullanılan küçük dozlardan ortaya çıkan küçük dozlardan ortaya çıkan her türlü zararlı 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 mikroorganizmalara akut etki yapan konsantrasyonların , ilaç kalıntılarından dolayı çevresel ortamlarda bulunan konsantrayonlardan 100-1000 kat daha yüksek olduğu görülmüştür.Bu nedenle ilaçların doğrudan sucul ortamlara dökülmesi durumunda, sucul canlılara akut toksisite tehtidi oluşturacğı ifade edilmektedir.Bunun yanı sıra, birçok sucul ortamda yaşayan canlı türleri uzun süreli veya tüm yaşamı boyunca antibiyotik gibi mikrokirleticilerin ekişinde kalmış da olabilir. Bu nedenle bu mikrokirleticilerin kronik potasiyel etkilerinin değerlendirilmesi önem taşımaktadır.Ancak antibiyotiklerin kronik etkileri hakkında çok az veri bulunmakta, kronik etkileri de genellikle bilinmemektedir. Bu konuda çok daha fazla spesifik çalışmaların yapılması gerekmektedir.

İlaç endüstrisi formulasyon altkategorisi atıksuları, farmasötik aktif bileşikler üretildikten sonra kullanıcıya sunulmak üzere uygun dozajlarda tablet, kapsül, sıvı veya merhem şeklinde formüle edilmesi işlemlerinden meydana gelmektedir.Bu nedenle formulasyon altkategorisinden oluşan atıksuların ürüne bağlı olarak kaynaklarının, miktarlarının ve karakterinin belirlenmesi önem taşımaktadır.Bu çalışmada ilaç endüstrisi formulasyon altkategorisinde kaynak bazında , Lak Tablet, Tablet, Likit ve Antibiyotik üretimlerinden meydana gelen atıksuların ürün esas alınarak miktarlarının belirlenmesi, karaterizasyon çalışmasının yapılması ve bu atıksuların arıtılabilirliğinin incelenmesi hedeflenmiştir.

Bu çerçevede Türkiye'de formulasyon kategorisinde üretim yapan bir ilaç üretim tesisi çalışma konusu olarak seçilmiştir. Türkiye'nin pazar payı ve üretim adedi olarak önde gelen firmalarından olan bu firmada katı formda tablet, kapsül, toz ve granül olarak, sıvı formda ampul ve flakonlar, şurup, süspansiyon ve spreyler, pomat formunda merhemler, suporizituvar ve jeller , antibiyotik çeşidi olarak da beta laktam antibiyotiklerden penisilin ve sefalosporinler çeşitli formlarda üretilmektedir.

Tesisin üretim prosesleri incelenmiştir.Yıl boyunca oluşan atıksular kaynakları ve miktarları ile ölçümlenmektedir. Tesiste 763 kişi üretimve bakım bölümleri 3 vardiyalı düzende olmak üzere çalışmaktadır. Oluşan atıksular evsel ve endüstriyel kaynaklı olmak üzere iki farklı karakterde olmakla birlikte arıtma tesisine beraber veilmektedir. Tesiste biyolojik arıtma sistemi mevcuttur. Ön arıtma olarak kimyasal arıtma sistemi mevcut olmakla birlikte askıda katı madde oranı 1500 ppm'nin altına düştüğünde devreye alınmaktadır.Biyolojik arıtma için 2 adet silindirik 675 m^3 hacimli reaktör kullanılmaktadır.Biyolojik arıtma; dolum, reaksiyon, çökme ve çıkış akımının çekilmesi evrelerinden oluşmaktadır.

Atıksu sistemine giren ve çıkan suyun hacim, KOI, BOI, pH değerleri günlük olarak ölçülmekte ve sistemin atıksu deşarj limitlerini (SKKY baz alınarak) sağladığı kontrol edilmektedir. Sistemin KOI bazında arıtım verimi ölçümleri %90'ın üzerinde seyretmektedir.

Çalışmada atıksu arıtma verimleri deneysel olarak ölçümlenmiştir. Atıksu arıtma sistemine giren ve çıkan sudan 2 farklı mevsimde alınan numunelerde yapılan

respirometrik ölçümlerde sağlanan değerlerin tesis ölçümleri ile uyumlu olduğu görülmüştür.

Repirometrik deneyler sonucunda elde edilen oksijen tüketim hızı verileri, Aquasim olarak adlandırılan bir bilgisayar programı aracılığı ile ASM1 modeline uygun olarak modellenmiştir.

Model kullanılmasının amacı,ilgili stokiyometrik ve kinetik katsayılarınn belirlenmesidir. Belirlenen katsayılar daha sonra literatürde benzer proseslere ait verilerle kıyaslanmıştır. Bu kıyaslama neticesinde sonuçalrın literatür verileri ile uyumlu olduğu görülmüştür.

Son zamanlarda partikül boyut dağılımı bazlı KOİ bileşen analizi endüstriyel atıksuların alternatif karakterizasyon çalışması olarak başarı ile kullanılmaktadır.Bu analiz KOİ bileşenlerinin partiküler anlamda boyutlarının biyolojik bozunma karakterlerine bağlantılı olduğunu göstermektedir. Partikül Boyut Dağılım analizi Sıralı filtrasyon/Ultrafiltrasyon metodu ile gerçekleştirilmiştir. PBD bazlı COD dağılımları giriş ve çıkış akımları için yapılmış, bu şekilde arıtma metodunun hangi boyutlarda ne ölçüde gerçekleştiği görülmüştür. İncelenen tesiste partiküler, kolloidal, suprakolloidal boyutlarda etkili bir arıtma verimi gözlemlenmekle birlikte çözünmüş fraksiyonda aynı verim görülmemiştir.Respirometrik ölçümlerde bulunan inert KOI değerleri ile de görülen bu sonuç biyolojik arıtmanın verimi ile bağlantılı olmamakla birlikte inert fraksiyonun sistemden bozunmadan geçmesi ile ilişkili olup ayrıca ölen mikroorganizma kalıntıları ile de çoğalmaktadır.

Respirometrik analiz ve Ardışık Filtrasyon / Ultrafiltrasyon analizleri ile de ölçümlendiği üzere MH ve Ks değerleri literatür verileri ile uyum göstermektedir. Biyoloji arıtma verimi sonuçları firmanın ölçümleri ile uyum göstermektedir. Aerobik biyolojik arıtma yöntemi tesisin atıksu karakteri için uygundur.Farklı proseslerden gelen atıksuların birleşerek arıtma sistemine verilmesinin antibiyotik türlerinin inhibisyon özelliğini dilüsyon yoluyla azalttığı, bu sayede ön arıtma sistemi olarak kullanılan kimyasal arıtmaya askıda katı madde oranı belli seviyenin altında olduğu için gerek görülmediği görülmüştür. Benzer sonuçlara deneysel olarak da ulaşılmıştır.

1. INTRODUCTION

Pharmaceutical Sector wastewaters have become a problem from the environmental point of view.The reason of it is non or limited biodegradability of some of their ingredients. The presence of human pharmaceutical compounds in surface waters is an emerging issue in environmental science .While water purification techniques could potentially remove these pollutants from wastewater streams , the high cost involved suggests that more attention should be given to the potential for the optimization of current treatment processes, and reduction at source in order to reduce environmental contamination.

Wastewater usually contain pharmaceuticals 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 (WWTP); 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 plant.Toxic effects must also being investigated. Many drugs are not fully metabolized in the body and so may be excreted to the sewer system. Numerous pharmaceutical compounds have been shown to pass through sewage treament plants (STPs) and contaminate the aquatic environment.

1.1 Purpose of Thesis

In this study, the wastewaters of the pharmaceutical plant in Turkey which has a biological treatment system had been investigated. It is 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 stochiometric constants of the mixed culture.Therefore, relevant kinetic and stochiometric coefficients were achieved using ASM1 pattern in the study.

By comparing real and calculated values, it’s been aimed to propose better operational modes for more efficient treatment.

1.2.Scope of Thesis

In the thesis, the following steps will be conducted:

- Conventional characterization of the investigated firm wastewaters.
- Respirometric analysis on the the investigated firm wastewaters.
- Particle Size Distribution analysis of the investigated plant wastewater by Ultrafiltration Method
- Modelling of the results
- Overall evaluation

2. LITERATURE REVIEW

2.1.Pharmaceutical Industry

The term ‘pharmaceutical’ covers a wide-ranging class of compounds with substantial variability of structures, function, behaviour and the activity. Developed to elicit a biological effect , they are used in both humans and animals to cure disease, fight infection, and/or reduce symptoms. Approximately 3000 different pharmaceutical compounds including painkillers , antibiotics, antidiabetics, beta blockers, contraceptives, lipid regulators, antidepressants, impotence drugs and cytostatic agents are used in Europe (Ternes et al, 2006). Globally production and consumption of these compounds are considered around 100.000 ton/year (Kummerer 2004).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 became important issue in the field of environmental engineering.Antibiotics generally remain in the effluent of wastewater treatment plants due to difficult treatability with conventional treatment systems.They also spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems.

2.1.1.Types of Pharmaceutical Processes and Products

The activities of the pharmaceutical industry can be classified into three main categories:

2.1.1.1. Research and development

2.1.1.2. Primary manufacturing to produce the bulk drugs

- Chemical Synthesis
- Fermentation

- Biological and Natural Extraction

2.1.1.3. Secondary manufacturing (mixing, compounding or formulating)

2.1.1.1. Research and Development

This subcategory contains the microbiological and pharmalogical research studies that are done for production of a new drug (Duman, 2006;Sert 2006).Common wastes are halogenated solvents, non-halogenated solvents, organic chemicals, organic chemicals, natural products, biomass, radionuclides,oxidizers, acids, bases, and myriad of reagents.The quantities are significant in comparison to those from manufacturing operations.

2.1.1.2.Primary manufacturing to produce the bulk drugs

- Chemical synthesis

Mostly drugs are produced by chemical synthesis.The process involves sequencing batch reactors.These reactors are used for solvent extraction and crystallization, mixing of solvents, boiling and cooling.Chemical synthesis wastewaters have complex structures and hard to treat.

Primary sources of wastewater from chemical synthesis operations are:

a. Process wastes such as spent solvents, filtrates, and concentrates;

b. Floor and equipment wash water;

c. Pump seal water;

d. Wet scrubber wastewater;

f. Spills.

Wastewater from chemical synthesis plants can be characterized as having high BOD, COD, and TSS concentrations; large flows; and extremely variable pH values, ranging from 1.0 to 11.0.Wastes can show inhibitory affect on biological treatment systems. (Duman, 2006;Sert, 2006)

-Fermentation

Most antibiotics and steroids are produced by the fermentation process, which involves three basic steps: inoculum and seed preparation, fermentation, and product recovery. Wastewater mostly come from fermentation and product recovery. For

fermentation process; acetone, methanol, isopropyl alcohol, etil alcohol and amil alcohol is used. Primery pollutants are methlene chloride, toluen and phenol. These wastewaters have high BOD, COD and SS.

The wastewater sources for fermentation process are given below

a.Surface and equipment cleaning waters

b.Used solvents coming from extraction process

c.Cooling waters

Sterilization of equipments used in fermentation process are mostly done by steam and phenol sometimes.Chemical disinfectants seriously increases pollution level. (Duman, 2006;Sert, 2006).

-Biological and natural extraction

Extraction is an expensive manufacturing process which requires collecting and processing large volumes of specialized plant or animal matter to produce small quantities of products.

The main sources of wastewater from biological/natural extraction operations are:

a. Spent raw materials (plan tor animal tissue)

b. Floor and equipment wash water;

c. Chemical wastes coming from purification and extraction processes (methilen chloride, toluen, chloroform, methanol, acetone, isopropanol)

d. Cleanup of spills.

Wastewater from extraction plants is generally characterized by low BOD , COD, and TSS concentrations; small flows; and pH values of approximately 6.0 to 8.0. (Duman, 2006;Sert, 2006)

2.1.1.3.Secondary manufacturing (mixing, compounding or formulating)

Pharmaceutically active ingredients are generally produced by batch processes in bulk form and must be converted to dosage form for consumer use. Common dosage forms for the consumer market are tablets, capsules, liquids, and ointments. In addition, active ingredients can also be incorporated into patches and time release capsules.

The primary objective of mixing, compounding, or formulating operations is to convert the manufactured products into a final, usable form. The necessary production steps typically have small wastewater flows because very few of the unit operations generate wastewater. The primary use of water is in the actual formulating process, where it is used for cooling and for equipment and floor washing.

Wastewater sources from mixing, compounding, or formulating operations are:

a. Floor and equipment wash water,

b. Wet scrubbers,

c. Spills.

An analysis of the pollutant information in the pharmaceutical manufacturing database shows that wastewater from mixing, compounding, or formulating plants normally has low BOD , COD, and TSS concentrations; relatively small flows; and pH values of 6.0 to 8.0.

2.1.2. Pharmaceutical Manufacturing Process Variability

The wastewater effluent flow and composition from a typical pharmaceutical manufacturing facility can be highly variable. Factors contributing to such variability are:

- Campaigning
- Batch processing
- Wastewater commingling

Because many pharmaceutical products are manufactured in campaigns, most wastewater is generated during product changeover. The process equipment must be cleaned out to avoid product contamination. The composition of the wastewater will vary according to the products that were manufactured on that process line.

Pharmaceuticals are manufactured by batch and continuous manufacturing operations. Batch-type production is by far the most common manufacturing technique. Many pharmaceutical facilities conduct multiple batch operations, some in series and some concurrently. Often several of the required batch processes are performed at the same time in separate reactors, each with its own schedule. Each batch may have unique waste stream characteristics. In fermentation operations, it

can take a few days to several weeks to complete the ferment, during which little or no wastewater is generated. However, during product recovery operations, high-volume, high-strength wastewaters are generated. It is also common practice in the pharmaceutical manufacturing industry to commingle organic contaminated wastewaters. In many cases commingling is necessary to collect sufficient wastewater volume to properly operate an economically sized treatment unit such as a steam stripper. Commingled wastes may be added to the treatment unit feed tank on a variable schedule, thus altering the feed composition on a real-time basis. In other cases, segregating for purposes of recovery and treatment may be appropriate and cost effective.

A variety of solvents are used in the pharmaceutical manufacturing industry and end up in the industry's wastewater. Many solvents are process-specific and cannot be interchanged in other pharmaceutical processes.

2.1.3. Pharmaceutical Dosage Forms

A dosage form is a combination of drug substances and excipients to facilitate dosing, administration, and delivery of the medicine to the patient. The design and testing of all dosage forms target drug product quality.

2.1.3.1. Aerosols

Aerosols are preparations packaged under pressure and contain therapeutic agent(s) and a propellant that are released upon activation of an appropriate valve system.The aerosol dosage form refers only to those products packaged under pressure that release a fine mist of particles or droplets when activated.

Aerosol preparations may consist of either a two-phase (gas and liquid) or a three-phase (gas, liquid, and solid or liquid) formulation.

2.1.3.2. Capsules

Capsules are solid dosage forms in which the Active Pharmaceutical Ingredients (API) and excipients are enclosed within a soluble container or shell. The shells may be composed of two pieces, a body and a cap, or they may be composed of a single piece.Two-piece capsules are commonly referred to as hardshell capsules, and one-piece capsules are often referred to as soft-shell capsules.

2.1.3.3. Dry powder ınhalers

The dry powder inhaler (DPI) consists of a mixture of drug(s) and carrier, and all components exist in a finely divided solid state packaged as a unit dose. The dose is released from the packaging by an appropriate mechanism and is mobilized into a fine mist only upon oral inhalation by the patient.

2.1.3.4. Creams

Creams may be formulated from a variety of oils, both mineral and vegetable, and from fatty alcohols,fatty acids, and fatty esters. The solid excipients are melted at the time of preparation. Emulsifying agents include nonionic surfactants, detergents, and soaps. Soaps are usually formed from a fatty acid in the oil phase hydrolyzed by a base dissolved in the aqueous phase in situ during the preparation of creams.

2.1.3.5. Lotions

Lotions usually are prepared by dissolving or dispersing the API into the more appropriate phase (oil or water), adding the appropriate emulsifying or suspending agents, and mixing the oil and water phases to form a uniform fluid emulsion.

2.1.3.6. Foams

Medicated foams are emulsions containing a dispersed phase of gas bubbles in a liquid continuous phase containing the API. Medicated foams are packaged in pressurized containers or special dispensing devices and are intended for application to the skin or mucous membranes.

The medicated foam is formed at the time of application.

Medicated foams intended to treat severely injured skin or open wounds must be sterile.

2.1.3.7. Medical gases (ınhalation materials)

Medical gases are products that are administered directly as a gas. A medical gas has a direct pharmacological action or acts as a diluent for another medical gas.

2.1.3.8.Gels

Gels (sometimes called jellies) are semisolid systems consisting either of suspensions of small inorganic particles or of organic molecules interpenetrated by a liquid.

2.1.3.9. Granules

Granules are solid dosage forms that are composed of agglomerations of smaller particles. Granules often are the precursors used in tablet compression or capsule filling. Although this application represents a pharmaceutical intermediate and not a final dosage form, numerous commercial products are based on granules.

2.1.3.10. Medicated gums

Medicated gum is a semisolid confection that is designed to be chewed rather than swallowed. Medicated gums release the API(s) into the saliva.

2.1.3.11. Implants

Implants are long-acting dosage forms that provide continuous release of the API for periods of months to years. They are administered by the parenteral route.For systemic delivery they may be placed subcutaneously,or for local delivery they can be placed in a specific region in the body.

2.1.3.12. Inserts

Inserts are solid dosage forms that are inserted into a body cavity other than the rectum.The API is delivered in inserts for local or systemic action.

2.1.3.13. Liquids

As a dosage form a liquid consists of a pure chemical in its liquid state. Examples include mineral oil, isoflurane,and ether.

2.1.3.14. Lozenges

Lozenges are solid oral dosage forms that are designed to dissolve or disintegrate slowly in the mouth. They contain one or more APIs that are slowly liberated from the flavored and sweetened base.

2.1.3.15. Ointments

Ointments are semisolid preparations intended for external application to the skin or mucous membranes.APIs delivered in ointments are intended for local action or for systemic absorption.

2.1.3.16. Pastes

Pastes are semisolid preparations of stiff consistency and contain a high percentage of finely dispersed solids. Pastes are intended for application to the skin, oral cavity or mucous membranes.

2.1.3.17. Transdermal systems (patches)

Transdermal drug delivery systems (TDSs) are discrete dosage forms that are designed to deliver the API(s)through intact skin to the systemic circulation.

2.1.3.18. Pellets

Pellets are dosage forms composed of small, solid particles of uniformshape sometimes called beads.Pellets may be administered by the oral (gastrointestinal) or by the injection route

2.1.3.19. Pills

Pills are API-containing small round solid bodies intended for oral administration. At one time pills were the most extensively used oral dosage form, but they have been replaced by compressed tablets and capsules.Pills are distinguished from tablets because pills are manufactured by a wet massing and molding technique,while tablets are formed by compression.

2.1.3.20.Powders

Powders are defined as a solid or a mixture of solids in a finely divided state intended for internal or external use.Powders used as pharmaceutical dosage forms may contain one or more APIs and can be mixed with water for oral administration or injection.

2.1.3.21. Medicated soaps and shampoos

Medicated soaps and shampoos are solid or liquid preparations intended for topical application to the skin or scalp followed by subsequent rinsing with water.

2.1.3.22. Solutions

A solution is a liquid preparation that contains one or more dissolved chemical substances in a suitable solvent or mixture of mutually miscible solvents.

2.1.3.23. Sprays (nasal, pulmonary, or solutions for nebulization)

A spray is a preparation that contains a therapeutic agent(s) in either the liquid or solid state and is intended for administration as a fine mist of small aqueous droplets.

2.1.3.24. Suppositories

Suppositories are dosage forms adapted for application into the rectum. They usually melt, soften, or dissolve at body temperature.

2.1.3.25. Suspensions

A suspension is a biphasic preparation consisting of solid particles dispersed throughout a liquid phase.

2.1.3.26. Tablets

Tablets are solid dosage forms in which the API is blended with excipients and compressed into the final dosage. Tablet presses use steel punches and dies to prepare compacted tablets by the application of high pressures to powder blends or granulations.

2.1.3.27. Tapes

A tape is a dosage form suitable for delivering APIs to the skin. It consists of an API(s) impregnated into a durable yet flexible woven fabric or extruded synthetic material that is coated with an adhesive agent.

2.1.4. Turkish Pharmaceutical Sector

Turkey has a developed pharmaceutical industry in terms of production standards, technology and capacity. The production facilities have been inspected continuously

by Ministry of Health, and accredited internationally by International Accreditation Authorities (IEIS, 2008).

Today, there are approximately 300 pharmaceutical firms operating in Turkey, many of them just maintain a marketing organization. Among 42 manufacturing facilities in Turkey, 14 belong to multinational firms.

Approximately 25.000 people are employed in the sector due to the sector report of 2010, of which 50% have a university degree. (pharmacist 4, 5%, physicians 3%, chemical engineers 7, 5%, chemists 7%, biologists 9, 5%) (Petrol-İş Pharma.Sector Analysis)

In 2007, pharmaceutical imports increased by 16% and reached 3.52 billion USD whereas the export increased by 14% and reached 357 million USD. The export-import ratio in 2007 was 10.1% (after 10.3% in 2006).

2.2. ANTIBIOTICS

Compounds which are alien to existing enzyme systems are called xenobiotics.These compounds are generally synthetically produced and cover many groups of chemicals including persistant compounds (van der Meer et al, 1992). Pharmaceuticals are examples of such xenobiotic compounds which have the potential to accumulate in the food chain and threaten human health (van der Meer et al, 1992)The pharmaceuticals may contain antibiotics , antidepressants and many other chemicals.

2.2.1. Classification of Antibiotics

Antibiotics can be grouped by either their chemical structure or mechanisms 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; Beers et al, 2003)

Most commonly used types of antibiotics are Aminoglycosides, Penicillins, Fluoroquinolones, Cephalosporins, Macrolides and Tetracylines.Classification of antibiotics is shown on Figure 2.1.

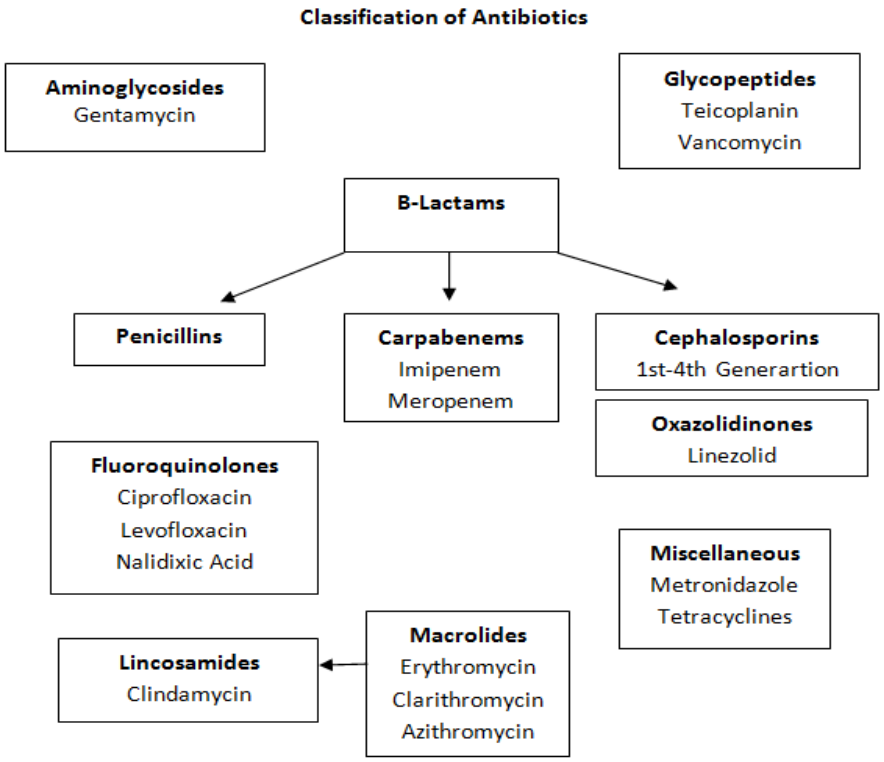

Figure 2.1. Classification of Antibiotics.

2.2.1.1.Penicillins

Penicillins are produced from some kinds of fungi and have bactericidal effect.They can be produced synthetically or semi-synthetically. The basic structure in all of them is 6-aminopencillicacid (6-APA).They show their effect by inhibiting cell wall production.

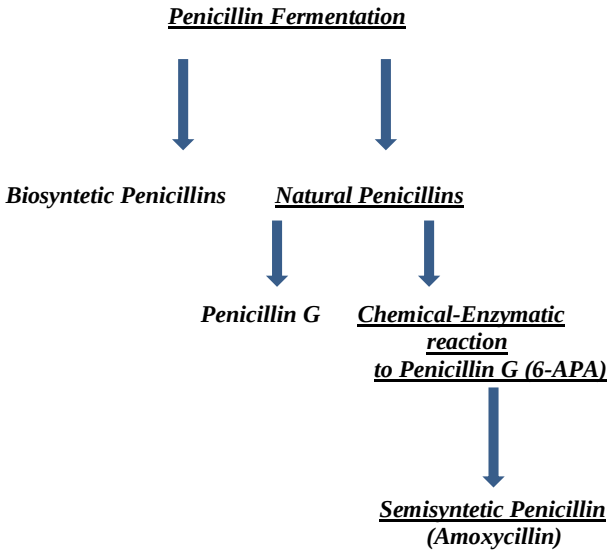

Figure 2.2. Classification of Penicillins.

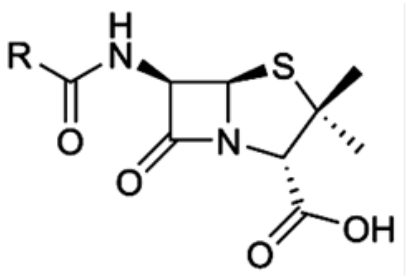

Figure 2.3. General Chemical Structure of Penicillins.

2.2.1.2. Cephalosporins

They've been produced first in 1940's from the fungi *Cephalosporicum acremonium.* They're wide spectrum beta lactam antibiotics. They have the same effect mechanism as other penicillins.They inhibit cell wall synthesis of bacteria.They are classified by their different properties and molecular structures.

1. Generation cephalosporins are active mostly on aerobic Gr (+) Coccies.
2. Generation cephalosporins are active mostly on selected Gr (-) microorganisms
3. Generation cephalosporins are active mostly on Gr (-) microorganisms
4. Generation cephalosporins have the widest range of effect.

(Şensoy, 2011)

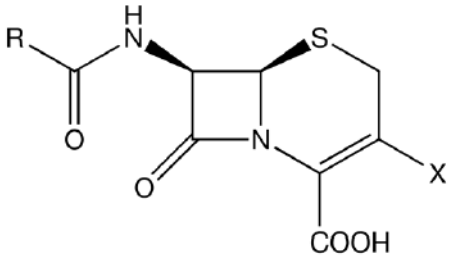

Figure 2.4. General Structure for Cephalosporins.

(Nemutlu and Kır, 2009)

2.2.2.Degradation of *b*-Lactam Antibiotics

The instability of *b*-lactam antibiotics in solution was observed to be a major hurdle in the development of penicillin and other useful *b*-lactam antibiotics. Therefore, degradation or stability study of *b*-lactam antibiotics has been of paramount importance not only for their market availability, but also to evaluate their

pharmacokinetic properties and adverse reactions. It is also interesting to note that stability or rate of degradation of different members of *b*-lactam antibiotics *in vivo* as well as *in vitro* has been quite different. However, the major pathways of their degradation have remained similar, leading to various breakdown products in a majority of the *b*-lactams.(A. D. Deshpande et al, 2004)

2.2.2.1.Degradation of Penicillin

Penicillin is a unique molecule containing unstable, highly strained and reactive *b*-lactam amide bond. The degradation of penicillin takes place in various conditions, viz.alkaline or acidic, in the presence of enzyme *b*-lactamase or treatment of weak nucleophiles like water and metal ions.

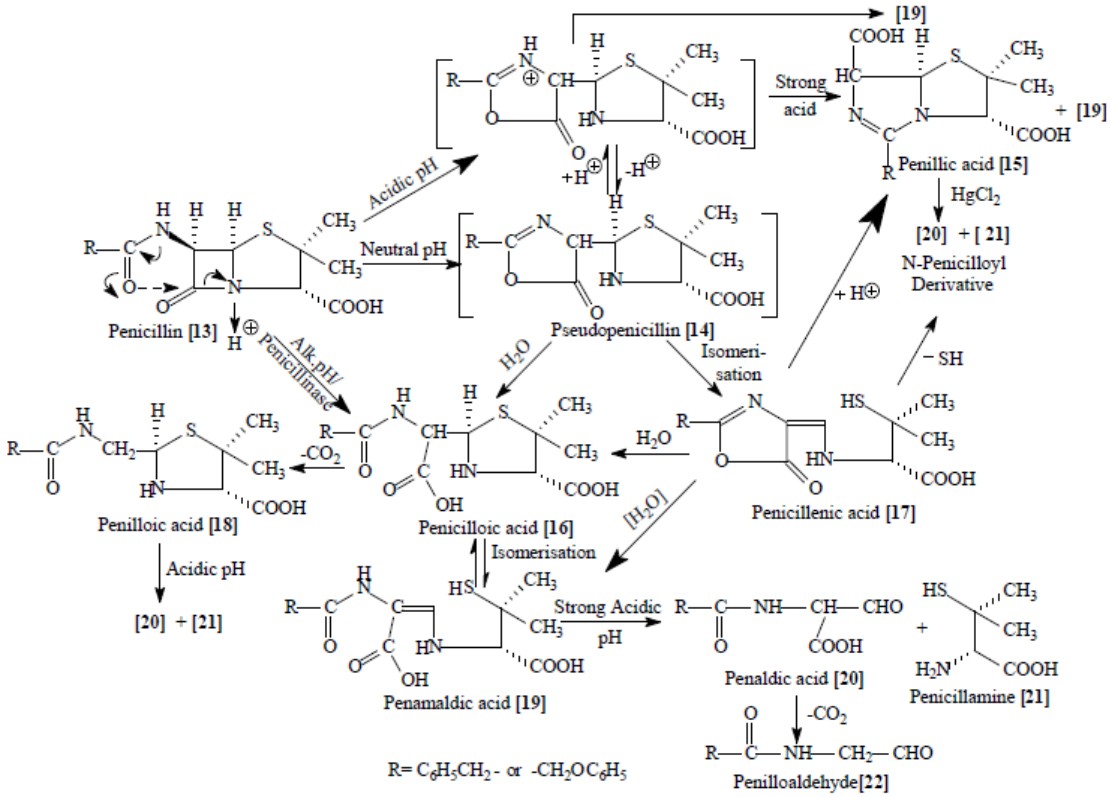

Figure 2.5. Pathways of degradation of penicillin in acidic and alkaline conditions.

2.2.2.2. Degradation of Cephalosporins

Although cephalosporins are more stable to hydrolytic degradation reactions than penicillins, they experience a variety of chemical and enzymatic transformations, whose specific nature depends on the side chain at C-7 and the substituenton C-3 atom.

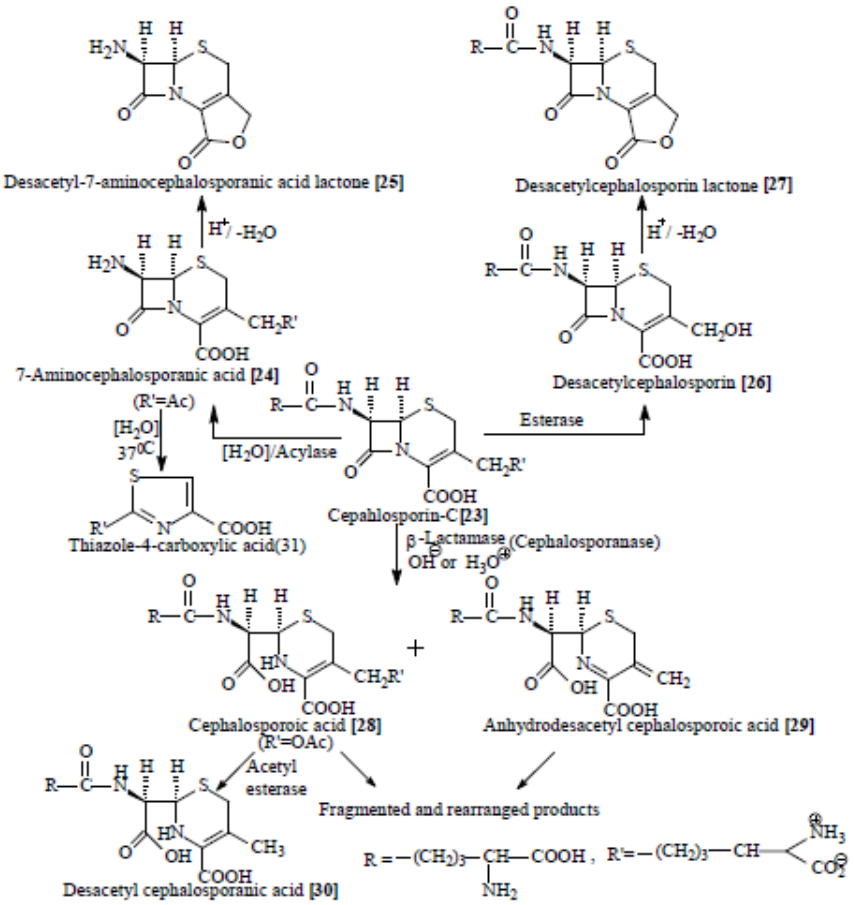

Figure2.6. Possible pathways of degradation of cephalosporin C.

2.2.3. Antibiotic Resistance Mechanisms

Resistance is decrease/loss of sensitivity to a certain antibiotic.Frequent and uncontrolled usage of antibiotics may cause this situation.Resistance to an antibiotic can be classified into 2 classes; natural resistance and achieved resistance (Yüce, A., 2001)

2.2.3.1. Natural resistance

The root cause is microorganisms are in inactive state metabolically or they don't have a target structure for the effect mechanism of that antibiotic.

2.2.3.2. Achieved resistance

- **Achieved resistance by mutation**

This effect is generally not the result of drug-microorganism contact, and there is no cause-effect relationship. Mutation occurs spontanously , there are lots of studies that

shows the mutation levels are almost the same in groups that are contact/not in contact with antibiotics.If resistance is chromosomal, this mutation may have been gained in one or more than one step.

- **One step mutation**

There is sudden occurance of resistance to the certain antibiotic after one or more contact. *Enterobacter, Serretia* , indol positive *Proteus, Pseudomonas aeroginosa* and in some other species may gain one step resitance. Mutation cause them to increase β-lactamase that break down Sephalosporins.

- **Mutual step mutation**

Resistance occur slowly and in increasing mode.This type of resistance is called also as penillin type resistance.For the occurance of this type of resistance there must be sequential mutations in different parts of DNA. This type of resistance may occur against Penicillins and Tetracycllins.

- **Achieved resistance due to transfer of resistance gene**

Plasmids are cirsular shaped double helix DNA molecules. They are extrachromozomal genetic elements.Their molecular weight changes from 1-200 billion daltons.More than one type of plasmids may take place in a cell. The plasmid is called a resistance (R) plasmid if they have resistance genes.

Transpozons are special kind of DNA parts that are smaller and mobile, they take place in transfer of resistance.They can recombine themselves both to chromosomal DNA and to plasmids.

The mechanisms of plasmids to transfer themselves from different environments into cells;

- **Transduction**

Bacteriophages (Bacterial viruses) transfer resistance plasmids.Upon entrance into bacterial cell, they take R plasmid into their viral protein cover. After division, bacteria with copies of plasmids are formed, after than bacterial cell burst and hundreds of plasmids are scattered to environment.They make other bacteria resistant too.

- **Conjugation:**

Resistant bacteria, together with sensitive bacteria form cytoplasmic bridge and one of R plasmids pass into sensitive cell , make it resistant too.

- **Transformation:**

R Plasmids and DNA parts in the environment due to the bacterial lysis, are taken by sensitive bacteria and make them resistant too.

2.2.4. Emissions of Antibiotics to the Environment

Antibiotics can enter the environment by a number of different pathways. Effluents of sewage treatment plant and pharmaceutical industry, waste from confined animal feeding operations and manure can be a source for antibiotic pollution (Kuımar et al.2005)

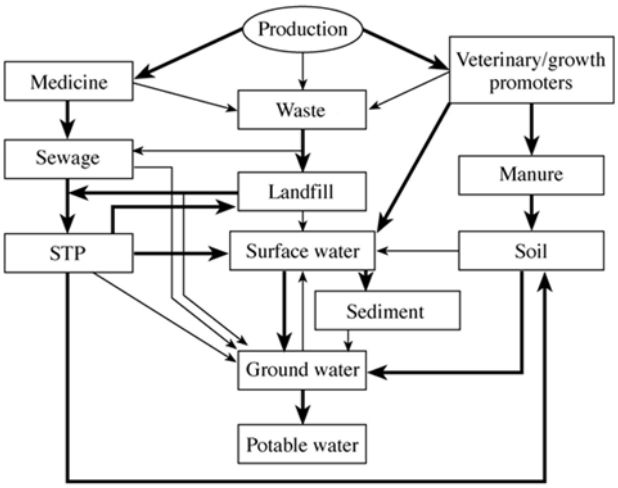

Figure 2.7. Principal routes of antibiotics in the environment.

From the late 1980s, occurrence of human derived antibiotics in different environmental compartments has been reported. Later, it was found that animal feeding operations and aquaculture facilities are the other sources of antibiotics. Until now,numerous studies have been documented to report occurrence of human used antibiotics in environment and measured concentration was generally less than 1 μg/L with few exception (Farre et al., 2001; Golet et al., 2002; Heberer, 2002; Miao and Koenig, 2002,Barreiro and Lores, 2003; McArdell et al., 2003; Stamatelatou et al., 2003; Vanderford et al., 2003; Cahill et al., 2004; Gobel et al.,

2004; Gross et al., 2004; Kolpin et al., 2004;Wiegel et al., 2004; Glassmeyer et al., 2005)

However, significantly higher concentration of veterinary antibiotics up to 46 mg/kg (Martinez-Carbollo et al., 2007) has been detected in manure and thus manure amended soil contains high amount of antibiotics such as 100 μg/g in soil (Accinelli et al., 2007). Antibiotics are also detected in other natural sources such as 246 μg/kg in sediment (Lalumera et al., 2004); 20 μg/L in fauna (Pathak and Gobal, 2005); and 1233 ng/g in plants (Migliore et al., 2003).

Antibiotics which are released to aquatic environment may contaminate raw , treated, recycled, irrigation and recretion water.Bacterial resistance to antibiotics may be gained by bacteria.On the other hand, negative effects such as toxicity and inhibition may be observed on ecosystem bacteria (Rang et al, 1999)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).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)Persistence toward biodegradation 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 therapotic work and because of their continious input they could remain in the environment for a long time (Alexy et al,2004) Antibiotics are biologically very active ingredients by their interference property in enzymatic reactions.From environmental point of view, they violate the ecological balance by effecting water species like Daphnia magna by their toxic effect.(Lanzky and Halling-Sorensen, 1997, Harrass et al, 1985, Macri et al, 1988). The inreasing usage of antibiotics in last 50 years have caused the genetical selection for many harmful bacteria.The genetic pool have changed dramatically.This irreversible effect have caused some type of bacteria to develop defense mechanism to this bactericidal effect.

They spoil ecological balance to form toxicity to organisms in ecosystem and biological treatment systems.

Toxic effects of common antibiotics on different organisms (bacteria, algae, Artemia saliva, Daphnia magna, etc.) have been found even at very low exposure doses

(Macri et al., 1988; Migliore et al., 1997;Wollenberger et al., 2000; Ferrari et al., 2003)

Abuse of antibiotics and the existence of residual antibiotics in the environment have been linked with the formation of antibiotic resistance (Boxall et al., 2003; Silver and Bostian, 1993).The occurrences of several kinds of antibiotics like macrolides and sulfonamides have been reported in many environmental samples such as municipal wastewater (McArdell et al., 2003), surface water, groundwater (Batt and Aga, 2005),sludge and sediments (Lindberg et al., 2005).

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)

2.3. The Treatability and the Toxicity of the Pharmaceutical Wastewater

The solvents and antibiotics, pain drugs and some raw materials used in the pharmaceutical industry have shown to cause toxicity in wastewaters. Since antibiotics are resistant to biological treatment, the discharge to environment without treatment, causes high toxic effect. The effect of toxic material to treatment system changes according to types of microorganisms, type and concentration of toxic material, sludge age, biomass concentration, time of exposure and temperature.

All microorganisms show different reaction to toxic materials.The effect of toxic material depends on the toxic material/biomass percentage in the treatment system.The smaller is this amount, the lower is the effect on the microorganisms.To lower the effect of toxic material, wastewater must be given to the sytem with dilution with domestic wastewater .The shortage of time of exposure of biomass results in smaller effect and biomass can return to its actual state but at the same time it may result with untreated toxic material. Therefore the care must be given on the below conditions; (Gülmez, 1997)

- Enough time must be given for the biomass adapt to toxic material
- To lower the toxic material amount with dilution with domestic wastewater to a level that biomass can compensate
- The wastewater containing toxic material must be given to treatment system gradually

- The loss of biomass during acclimation must be minimized.

The effect of antibiotics that are given to treatment systems after usage are not known widely. Kummerer et al, (2000) have investigated toxic effects of 3 different raw materials; Metronidazol, Ciprofloxacin and Ofloxacin. Metronidazol show low toxic effect to *algs* and *Daphnia* . Ciprofloxacin is effective on the aerobic Gr(-) and Gr(+) bacteria. Ofloxacin show lower effect than Ciprofloxacin to Gr(-) bacteria.It's been showed that the antibiotics cannot be treated biologically in Closed Bottle Test (CBT)

2.3.1. Pharmaceutical Industry Wastewater Treatment Options

Different treatment techniques are applied to pharmaceutical wastewaters according to process profiles since they generally occur in sequencing batch reactors and pollution concentration differ in time.The main treatment techniques applied these wastewaters are:

2.3.1.1.Biological treatment

Biological treatment methods are used for treatment of pharmaceutical wastewater (Suman Raj and Anjaneyulu, 2005). They may be subdivided into aerobic and anaerobic processes.

Aerobic applications include;

- activated sludge
- membrane batch reactors
- sequence batch reactors

(LaPara *et al*., 2002; Suman Raj and Anjaneyulu, 2005; Noble, 2006; Chang *et al*., 2008 and Chen *et al*., 2008).

Anaerobic methods include;

- anaerobic sludge reactors,
- anaerobic film reactors
- anaerobic filters

(Gangagni *et al*., 2005; Enright *et al*.2005; Chelliapan *et al*., 2006; Oktem *et al*., 2007;Sreekanth *et al*., 2009).

The wastewater characteristics play a key role in the selection of biological treatments.Solvents, APIs, intermediates and raw materials represent biologically recalcitrant substances which affect the efficiency of biological treatment systems (Oz *et al*., 2004; Helmig *et al.*, 2007). Activated sludge (AS) treatment is unsuitable for the treatment of wastewater where the COD levels are greater than 4000 mg/L (Suman Raj and Anjaneyulu, 2005).

Conventional activated sludge with a long hydraulic retention time (HRT) has historically been the method of choice for the treatment of pharmaceutical industry wastewater (El Gohary and Abou-Elea, 1995; Oz *et al*., 2004). It has a lower capital cost than more advanced treatment methods and a limited operational requirement; it is generally more environmentally friendly than chlorination. However,high energy consumption, the production of large amounts of sludge (Sreekanth *et al*., 2009) and operational problems including colour, foaming and bulking in secondary clarifiers are associated with activated sludge plants (Oz *et al*., 2004). Factors which affect the efficiency of activated sludge facilities for the treatment of pharmaceutical wastewater include HRT, temperature, pH, dissolved oxygen (DO), organic load, microbial community,presence of toxic or recalcitrant substances and the batch operation of pharmaceutical production facilities (LaPara *et al*., 2001a; LaPara *et al*., 2002; Suman Raj and Anjaneyulu, 2005). These variables require modification for adaptation to pharmaceutical industry wastewater.

The impact of pharmaceuticals on the AS process appears to be negligible under normal operating conditions (Stamatelatou *et al*., 2003). However at higher concentrations, which may be expected in the wastewater of pharmaceutical manufacturing facilities,they may become inhibitory. While there are a number of limited studies on the removal efficiency of APIs from pharmaceutical manufacturing facilities, it is known that removal efficiency of municipal facilities is dependent on the APIs present in the wastewater (Urase *et al*., 2005). AS is an efficient method for the removal of some APIs, but not all from municipal facilities (Zwiener and Frimmel, 2003; Castiglioni *et al.,* 2006; Watkinson *et al.*, 2007). β-Lactam and quinlone drugs in particular appear to be susceptible to aerobic oxidation. In a WWTP in Brisbane Australia,β-Lactam antibiotics showed high biodegradability due to hydrolic cleavage of the β-lactam ring. Lincomycin and sulphonamides were the least affected by AS treatment (Joss *et al*., 2005). Similar

studies have also found that the efficiency of the process is dependent on the compound under investigation (Joss *et al*., 2005). Ibuprofen, naproxen, bezafibrate and estrogens (estrone, estradiol and ethinylestradiol) showed a high degree of removal while sulfamethoxazole, carbamezapine and diclofenac showed limited removal (Clara *et al*., 2005; Joss *et al*.,2005; Xu *et al*., 2008). Removal efficiencies are likely to decrease due to the development of more resistant APIs (Khetan and Collins, 2007).

The advantages of anaerobic treatment over aerobic processes is its ability to deal with high strength wastewater, with lower energy inputs, sludge yield,nutrient requirements, operating cost, space requirement and improved biogas recovery. However, because a wide range of natural and xenobiotic organic chemicals in pharmaceutical wastewaters are recalcitrant and nonbiodegradable to the microbial mass within the conventional treatment system, anaerobic processes are not always effective in removing these substances.

2.3.1.2. Advanced treatment methods

Alaton & Dogruel (2004) investigated a variety of advanced oxidation processes (AOPs; O_3/OH^-, H_2O_2/UV, Fe^{2+}/H_2O_2, Fe^{3+}/H_2O2, Fe^{2+}/H_2O_2/UV andFe^{3+}/H_2O_2/UV) for the oxidative pre-treatment of real penicillin formulation effluent.The average COD_0, TOC_0, $BOD_{5,0}$ were 1395, 920 and 0 mg/l. The selected wastewater corresponded to approximately 24% of the total daily effluent (=150m^3/day) of which 47% was the process water. For the ozonation process the primary involvement of free radical species such as OH in the oxidative reaction could be demonstrated via inspection of ozone absorption rates. Alkaline ozonation and the

photo-Fenton's reagents both appeared to be the most promising AOPs in terms of COD (49–66%) and TOC (42–52%) removal rates. It was demonstrated that the penicillin active substance can be completely removed at the selected experimental conditions accompanied by effective oxidation (i.e. 51-58% TOC and 72-81% COD removal). In separate treatability experiments conducted with aqueous amoxicillin solution.The ozonation of amoxicillin was studied by Andreozzi, Canterino, Marotta &Paxeus (2005). As for other pharmaceuticals, ozonation was proposed as a process for its abatement from Sewage Treatment Plant effluents. Chemical investigations showed that the ozonation process was characterized by low degree of mineralization

even for long treatment times. The results indicate that ozone attack was partially directed towards a reaction center different from the phenol ring or the protonated amino group, possibly towards the sulfur atom. No direct evidences of attack on

sulfur atom with sulfoxide formation are found. Kinetic investigations allowed characterizing both the direct and radical mechanism of ozone attack to the molecule.It was found that the kinetic constant for the direct attack was strongly dependent upon the pH of the solutions. Hydrogen peroxide photolysis has been successfully adopted to evaluate the constant for OH radical attack to amoxicillin molecule at a pH 5.5.Boyd, Zhang & Grimm (2005) studied the chemical transformations of naproxen following chlorine oxidation, which was common in water and wastewater treatment systems. Synthetic waters containing elevated concentrations of naproxen were oxidized by free chlorine at naproxen:chlorine molar ratios of 0.02-3:1 and pH values of 5-9.The formation of naproxen products was dependent on pH, chlorine dosage and contact time. This study demonstrated that naproxen readily reacts with free chlorine and formed disinfection products. A bioreactor was fed with a naproxen solution and then fed with a solution at the same naproxen concentration following contact with free chlorine. Results indicated that naproxen was not degraded biologically. Dissimilarity, the naproxen solution containing products of chlorination could adversely affect the biofilm process, which potentially could impact the performance of biofilm.

A membrane system including reverse osmosis (RO) and ultrafiltration (UF) was performed for the treatment of an oxytetracycline (OTC) waste liquor by Lie S., Lie X. & Wang (2004). The liquid samples contained a residual OTC of around 1000 mg/l and a COD of about 10 000 mg/l. Using RO filtration with a volume reduction coefficient of 3.5, the organic content in its permeate was decreased from 10 000 mg COD/l to less than 200 mg COD/l, while OTC was reduced from more than 1000 mg/l to lower than 80 mg/l. Oxytetracycline was concentrated more than 3 times to 3000–4000 mg/l in the retentate. Due to likely the high concentration of large biopolymers interacting with OTC molecules, OTC could not be successfully recovered from the RO retentate by conventional crystallization. With additional

treatment of ultrafiltration by 3K membranes, OTC crystallization and recovery from the RO retentate were significantly improved. A recovery ratio of more than 60% and a purity of higher than 80% were achieved.

Ternes & et al. (2003) investigated about a pilot plant for ozonation and UVdisinfection received effluent from a German municipal sewage treatment plant (STP) to test the removal of pharmaceuticals in iodinated X-ray contrast media (ICM) and musk fragrances from municipal wastewater. In the original STP effluent, 5 antibiotics, 5 betablockers, 4 antiphlogistics, 2 lipid regulator metabolites, the antiepileptic drug carbamazepine, 4 ICM, the natural estrogen estrone and 2 musk fragrances were detected by LC-electrospray tandem MS and/or GC/MS/MS. ICM,derived from radiological examinations, were present with the highest concentrations. By applying 10–15 mg l^{-1} ozone (contact time: 18 min), all the

pharmaceuticals investigated as well as musk fragrances and estrone were no longer detected. However, ICM (diatrizoate, iopamidol, iopromide and iomeprol) were still detected in appreciable concentrations. Even with a 15 mg l^{-1} ozone dose, the ionic diatrizoate only exhibited removal efficiencies of not higher than 14%, while the non-ionic ICM were removed to a degree of higher than 80%. Advanced oxidation processes (O_3/UV-low pressure mercury arc, O_3/H_2O_2), which were non-optimized for wastewater treatment, did not lead significantly to a higher removal efficiency for the ICM than ozone alone.The performance of the application of advanced oxidation processes to different industrial wastewaters-ozonation of pharmaceutical effluent was investigated by Balcıoglu et al. (2003). Depending upon the type of antibiotic 60-72% COD and 30-96% aromatic amine (UV_{254}) removals were achieved with an 2592 mg/l h applied ozone dosage at a pH=7. The experiments were carried out in four different applied ozone doses in the range of 640-2960 mg/l h indicated that the fractional ozone utilization was more efficient at lower ozone concentrations. Increasing ozone döşe did not cause any change in BOD_5/COD ratio.Balcıoglu & Otker (2003) studied the ozonation of three different synthetic pharmaceutical formulation wastewater containing two human antibiotics and a veterinary antibiotic to enhance the their biodegradability. The effects of pH and initial chemical oxygen demand (COD) value as well as addition of hydrogen peroxide on ozonation process were investigated. Ozonation experiments were performed in a 1500 ml capacity. Total organic carbon (TOC), COD, biochemical oxygen demand (BOD), and aromatic content (UV_{254}) were the parameters followed to evaluate the performance of ozonation process. While BOD_5/COD ratio of veterinary antibiotic formulation wastewater was increased from 0.077 to 0.38 with an applied ozone dosage of 2.96

g/l, the BOD_5/ COD ratio for human antibiotic I and human antibiotic II was increased from 0 to 0.1 and 0.27 respectively. The results of this investigation showed that the ozonation process is capable of achieving high levels of COD and aromaticity removals at about their natural pH values.

2.3.1.3. Chemical treatment methods

A total of 23 coagulants/flocculants was tested, including salts, polyhidroxyaluminates,synthetic polymers as well as natural gums by Torres (1997). In a second stage, some mixing aspects were studied. The effects of the specific impeller,the agitation speed during the coagulation and flocculation stages, and the absence or presence of baffles was evaluated in a six-place agitation system. It was demonstrated that the appropriate coagulation-flocculation system was capable of diminishing the COD, the apparent color and the dissolved solids up to 40.6, 25.6 and 39.4%, respectively. The best results were observed when using BL-5086, guargum, Niad II-3, Niad II-4 and locus beam gum. The impeller performance was highly dependent on the agitation speed for each fixed system.

2.4. Respirometry and Activated Sludge Modeling

2.4.1. COD fractionation in wastewater characterization

COD fractination involves identification of inert and biodegradable COD together with readily biodegradable COD slowly biodegradable fractions.

The total organic matter,C_T which is measured as COD is first classified in 2 groups depending on its biodegradability.Non-biodegradable organic matter is inert since it is not changed during wastewater treatment.Total inert COD, C_I is the sum of the soluble, S_I and particulate,X_I forms of inert organic matter.S_I passes through activated sludge system whereas X_I retains and it can only be removed by sludge wastage.

The biodegradable fraction of COD is divided into readily biodegradable, Ss and slowly biodegradable COD, Xs as they have quite different biodegradation rates.Simple carbohydrates, volatile fatty acids, alcohols constitute readily biodegradable fraction.Xs fraction includes soluble, colloidal, larger complex organic matter which cannot pass through the cell wall of the cell. For utilization of Xs, hydrolysis and conversion to simpler organic matter is required.Hydrolysis is

assumed to proceed slower compared to the utilization of readily biodegradable COD. The complex nature of wastewater indicates the need for the seperation of hydrolysable COD in two fractions:slowly hydrolysable COD, S_H and readily hydrolysable COD, Xs.However, ASM1 model assumes single type of hydrolyzable COD.

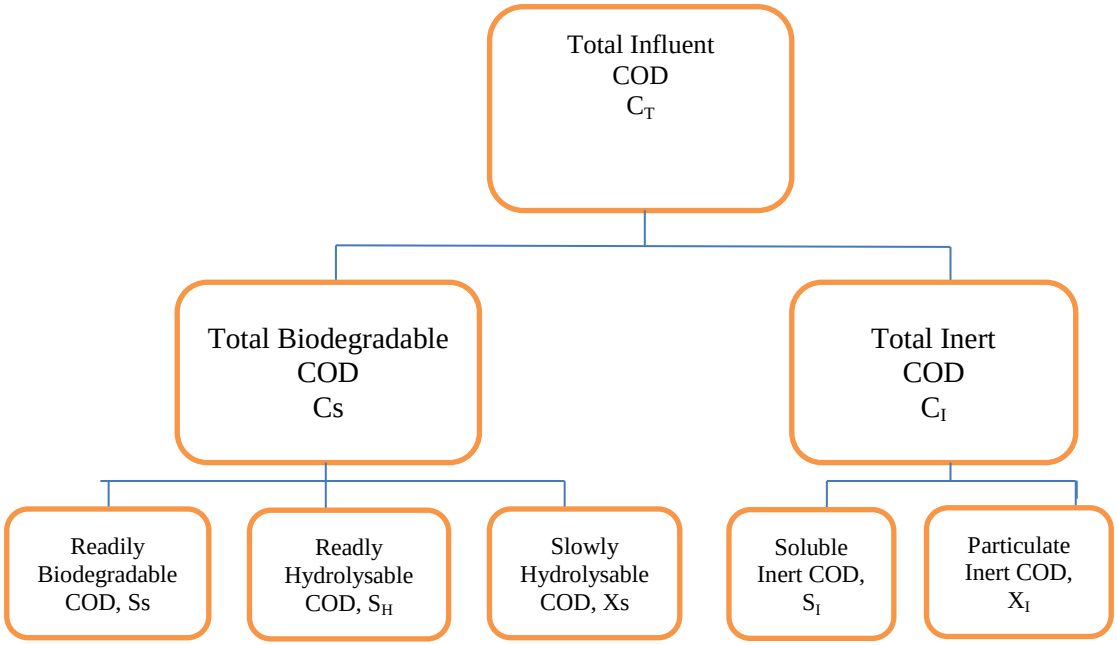

Figure2.8. Distribution of COD fractions in wastewaters (Artan and Orhon, 1994).

2.4.2. Respirometry

Dissolved oxygen and nitrate are known to be final electron acceptors in carbon and nutrient removing treatment systems.Dissolved oxygen is used in establishing electron balance in aerobic balance in aerobic processes such as biodegradable COD, biomass and electron acceptor. Activated sludge models were developed which involve final electron acceptors such as dissolved oxygen and nitrate (Orhon et al., 2009).It defines oxygen uptake rate (OUR) showing the rate of oxygen utilization.

Depletion of oxygen is monitored for a defined period of time. OUR is used for assessment of biodegradable COD fractions, wastewater characteristics, process kinetics and process control purposes in treatment systems (Ekama et al, 1986; Kappeler and Gujer,1992;Ubay Çokgör et al.,1998; Insel et al., 2003; Karahan et al.,2002; Sözen et al., 1998;Avcıoğlu et al.,1998;Sollfranf and Gujer, 1991)

2.4.3. Activated Sludge Modeling

The identification of wastewater characteristics with regard to the organic content is useful both from the standpoint of process kinetics and prediction of effluent quality. Collective analytical parameters such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD), routinely used to reflect the total organics in wastewaters, do not correspond to the real system variable, i.e. the growth limiting substrate.COD is estimated to be a more useful parameter than BOD as it enables one to make appropriate correlations among substrate,biomass and dissolved oxygen in terms of electron equivalence. (Orhon and Çokgör ,1996)

From a modelling standpoint, COD cannot differentiate between biodegradable and inert organic matter or between readily and slowly biodegradable fractions.The inert COD may be present in the influent or it may be generated during biological treatment as residual microbial products.This residual COD fraction is significant for industrial effluents and leads to misinterpretation of the biological treatability and kinetic analysis;the soluble inert COD fraction also becomes a challenging factor in meeting the effluent limitations expressed in terms of COD for a number of industrial categories. (Orhon and Çokgör ,1996).

Modelling is a valuable tool for design of activated sludge systems,because it enables the role and impact of significant parameters to be analysed and so helps to define and optimize the proposed process (Orhon and Çokgör ,1996)

In 1983, the International Association on Water Quality (IAWQ, formerly IAWPRC) formed a task group, which was to promote development, and facilitate the application of, practical models for design and operation of biological wastewater treatment systems. The first goal was to review existing models and the second goal was to reach a consensus concerning the simplest mathematical model having the capability of realistically predicting the performance of single-sludge systems carrying out carbon oxidation, nitrification and denitrification. The final result was presented in 1987 as the IAWQ Activated Sludge Model No. 1(A Gen. Desc. of the IAWQ Activated Sludge Model No. 1 by Ulf Jeppsson, MSc, PhDDept of Industrial Electrical Engineering and Automation Lund Institute of Technology Lund, Sweden)

Activated sludge models are generally based on IAWQ Activated Sludge Model No.1, ASM1 (Henze, 1987).Although it was designed for domestic or municipal

wastewater, for industrial applications the model should be carefully calibrated.It involve carbon removal, nitrification and denitrification processes.ASM1 generally takes into consideration 3 components:biomass, substrate and oxygen. The basic components and process of ASM1 for carbon oxidation is shown in Table 2.1.

Table.2.1. Processes for carbon oxidation described in ASM1 (Henze et al, 1987).

Table 1
The reduced-order ASM1 biological model in a matrix format

Compound → *i* / process ↓ *j* Process	S_S	S_{NO}	S_{NH}	P_j, *j* = process for the *n*-th tank
1. Aerobic growth of heterotrophs	$\frac{1}{Y_H}$	0	$-i_{XB}$	$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{S_O}{K_{OH}+S_O}\right)X_{BH}$
2. Anoxic growth of heterotrophs	$-\frac{1}{Y_H}$	$\frac{1-Y_H}{2.86Y_H}$	$-i_{XB}$	$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{K_{OH}}{K_{OH}+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\eta_g X_{BH}$
3. Aerobic growth of autotrophs	0	$\frac{1}{Y_A}$	$-i_{XB}-\frac{1}{Y_A}$	$\hat{\mu}_A\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_O}{K_{OA}+S_O}\right)X_{BA}$
4. Hydrolysis	1	0	0	$k_h X_{BH}\left(\frac{X_S/X_{BH}}{K_X+X_S/X_{BH}}\right)\left[\left(\frac{S_O}{K_{OH}+S_O}\right)+\eta_h\left(\frac{K_{OH}}{K_{OH}+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\right]$

2.4.3.1.Processes in ASM1

Basically there are four different main processes defined in ASM1 (Henze *et al.*, 1987):

- Growth of biomass
- Decay of biomass
- Ammonification of organic nitrogen
- Hydrolysis of particulate organic matter

The substrate flows in ASM1 are illustrated in Fig. 2.9.

2.4.4. COD components in ASM1

COD is selected as the most suitable parameter for defining the carbon substrates as it provides a link between electron equivalents in the organic substrate, the biomass and oxygen utilised. In ASM1 the COD is subdivided based on (1) solubility, (2) biodegradability (3) biodegradation rate and (4) viability (biomass):

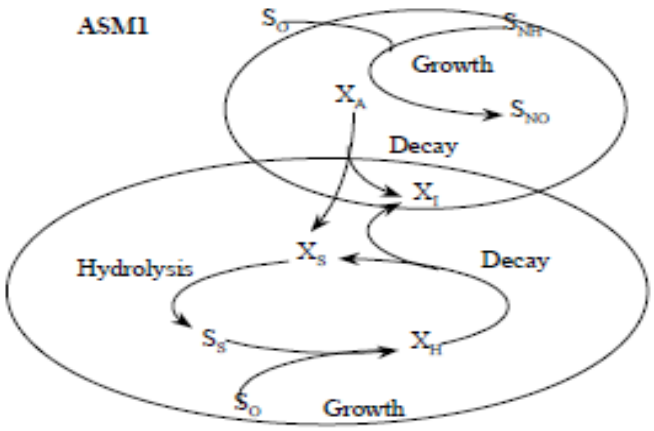

Figure 2.9. The substrate flows in ASM1.

• The total COD is divided into soluble (S) and particulate (X) components.

• The COD is further subdivided into non-biodegradable organic matter and biodegradable matter. The non-biodegradable matter is biologically inert and passes through an activated sludge system in unchanged form. The inert soluble organic matter (SI) leaves the system at the same concentration as it enters. Inert suspended organic matter in the wastewater influent (XI) or produced via decay (XP) becomes enmeshed in the activated sludge and is removed from the system via the sludge wastage.

• The biodegradable matter is divided into soluble readily biodegradable (SS) and slowly biodegradable (XS) substrate. Already here it should be stressed that some slowly biodegradable matter may actually be soluble. The readily biodegradable substrate is assumed to consist of relatively simple molecules that may be taken in directly by heterotrophic organisms and used for growth of new biomass. On the contrary, the slowly biodegradable substrate consists of relatively complex molecules that require enzymatic breakdown prior to utilisation.

• Finally, heterotrophic biomass (XBH) and autotrophic biomass (XBA) are generated by growth on the readily biodegradable substrate (SS) or by growth on ammonia nitrogen (SNH). The biomass is lost via the decay process where is it converted to XP and XS

Summarising, the total COD balance of ASM1 is defined by the following equation:

CODtot = SI + SS + XI + XS + XBH + XBA + XP

2.4.5. Ultrafiltration

Particles in wastewater have conveniently been grouped into operational size categories, namely dissolved (<1 nm), colloidal (1-10^3 nm) and settelable (>10^5 nm). Ultrafiltration, among other methods, is successfully used to identify and differentiate wastewater pollutants within much narrower ranges (Engstrom and Gytel, 2000; Sophonsiri and Morgenroth, 2004; Doğruel et al,2006). Particle Size Distribution (PSD)-based COD fractionation has been proposed and used successfully as an alternative approach for characterisation of municipal, industrial, agricultural waste streams (Sophonsiri and Morgenroth, 2004),domestic wastewaters (Dülekgurgen et al., 2006) with a suggestion of providing a more in-depth comprehension in terms of COD fractions and their biodegradability characteristics in relation to particle size categories.

3. MATERIALS AND METHODS

3.1.General Information About the Investigated Plant

The plant had been builded and started production on 1992. 763 personnel work, 447 of them (%59) work in 3 shift order. Total area is 338000 m².Total capacity is 425000 billions of packages in 3 shift and 6 days/week order.

3.1.1.Wastewater Produced per Drug Produced

Wastewater created in the investigated plant is given on the Table 3.1.

Table 3.1. Wastewater generation of the investigated plant for 2012.

Month	**Wastewater entering treatment system (m^3)**	**Total Discharged Wastewater from the System (m^3)**
January	6598	9237
February	6349	8824
March	6946	9242
April	6567	8593
May	6686	8338
June	6606	8529
July	1730	5009
August	5598	7400
September	5876	7314
October	5614	7268
November	6374	7843
December	6594	7902
Total	**71538**	**95499**

The investigated plant have produced 244000000 packages of drug in 2012 and created 95499 m³ wastewater discharged. 391 cm³ wastewater was produced per package of drug.

3.1.2.Drug Production in the Investigated Plant

The investigated plant have production activity in the formulation subcategory.There is high productive activity and many kind of different drug dosages and types are produced. The production units are given in Figure 3.1.

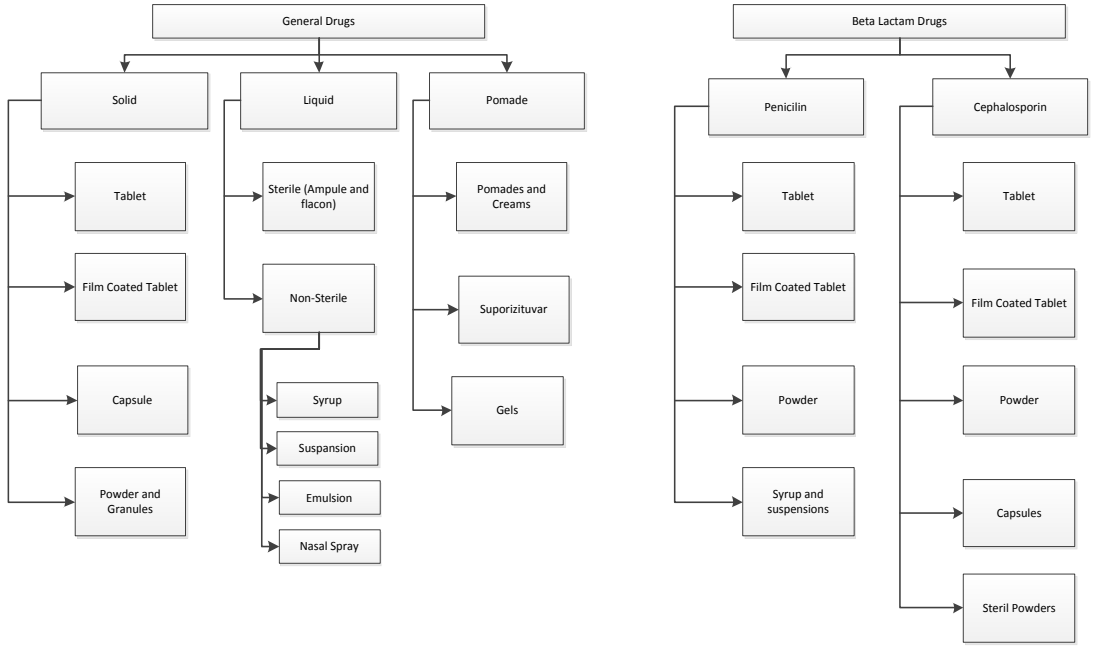

Figure 3.1. The production units of the investigated plant.

The percentage quantities of these forms are given on Table 3.2.

Table3.2. Production forms by percentage quantities 2011 and 2012.

	2011 %	2012 %
Solid	47.7	50.1
Liquid	25.0	23.0
Pomade	9.0	8.4
Beta Lactams	18.2	18.6

The investigated plant production capacity and the proposed production plans until 2016 is given at the Figure 3.2.

In 2011, 1560000000 packages of medicine was produced in Turkey. Investigated plant has produced 231000000 packages in 2011. With this values, The plant have produced %14.8 of all the medicines produced in Turkey. Plant production capacity and the proposed production plans 2010-2016 is shown on Figure 3.2.

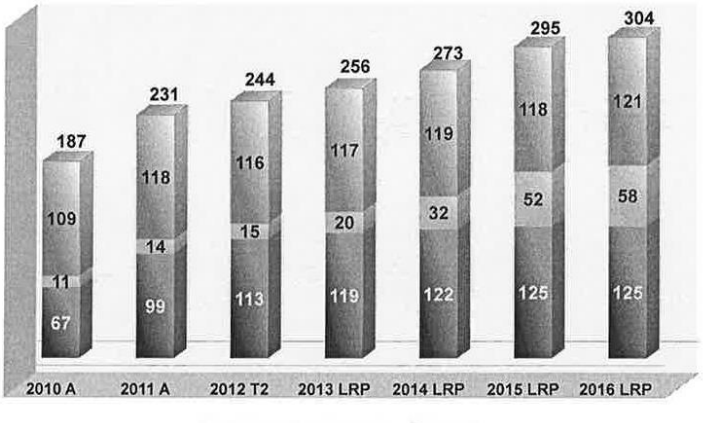

Figure 3.2. Plant production capacity and the proposed production plans 2010-2016.

3.1.3. Market Share of the Investigated Plant

The investigated firm is 18. biggest pharmaceutical firm with endorsement of 268927780 TL in Turkey (Türkiye İlaç Sanayi Meclisi Sektör Raporu-2011)

3.1.4. Process Schemes of the Investigated Plant

3.1.4.1. Solid production unit and process scheme

Solid production involves tablet preparation, printing and packaging.After raw material mixing, preparation is given into paste machine.Then granulation and drying steps are followed.If necessary cracking machines are used and the products are put into the printing machines.Finally packaging machines are used to package the drugs.

Solid Production Unit and Process Scheme is given on Figure 3.3.

3.1.4.2.Pomade production unit and process scheme

Pomade production involves melting and mixing of the raw materials in cauldron.Prepared pomade is taken to the filling machine.

Pomade Production Unit and Process Scheme is given on Figure 3.4.

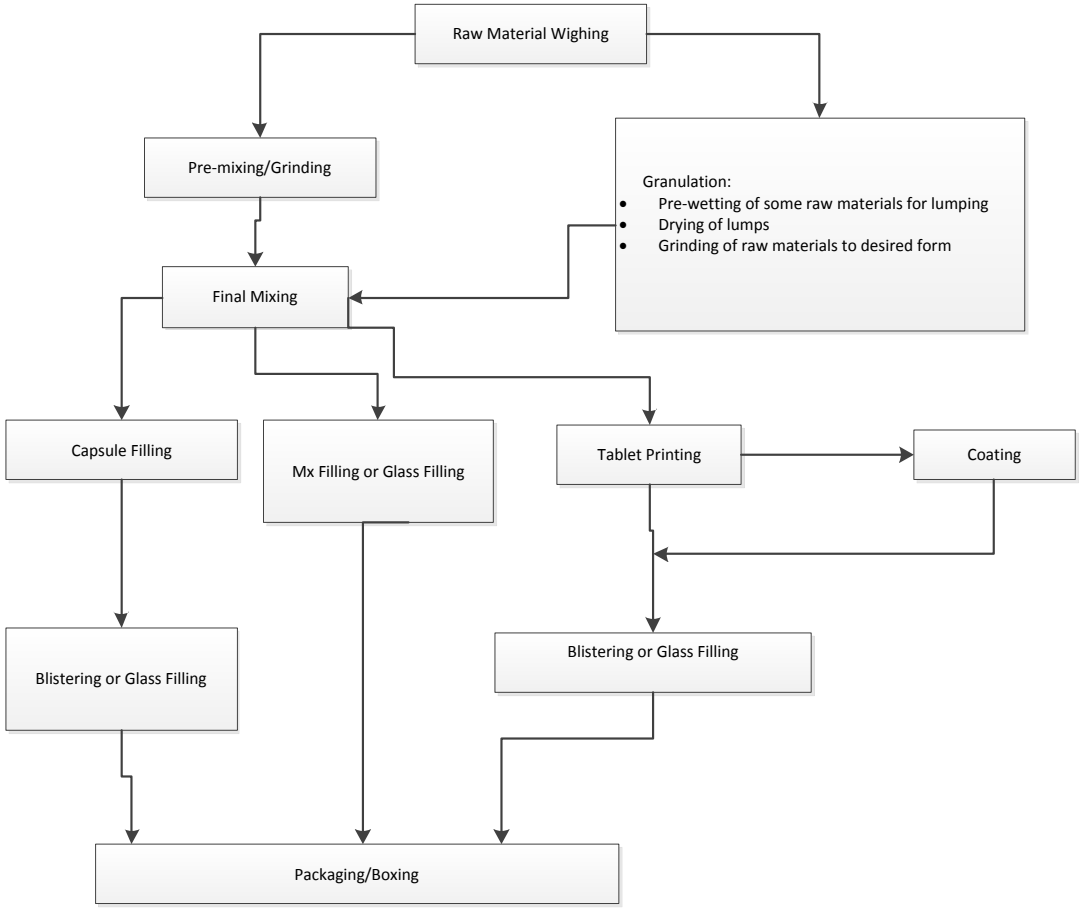

Figure 3.3. Solid Production Unit and Process Scheme.

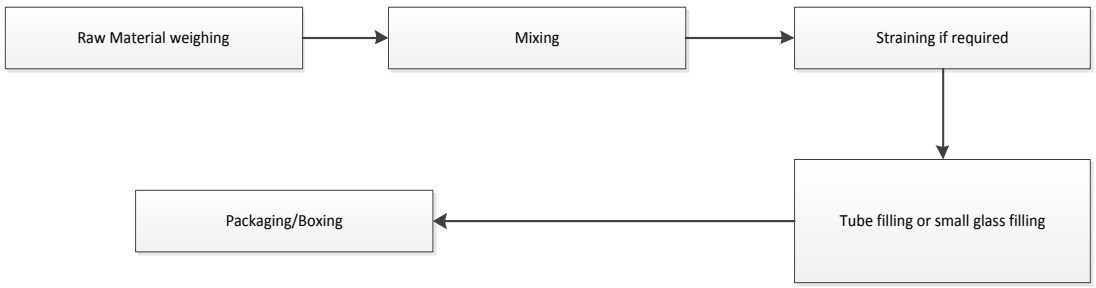

Figure 3.4. Pomade Production Unit and Process Scheme

3.1.4.3. Non-sterile production unit and process scheme

Non-sterile production involves raw material weighing, addition to cauldron, and filtering steps.Prepared solution is filled to glasses in liquid filling line and packaged. Non-Sterile Production Unit and Process Scheme is given on Figure 3.5.

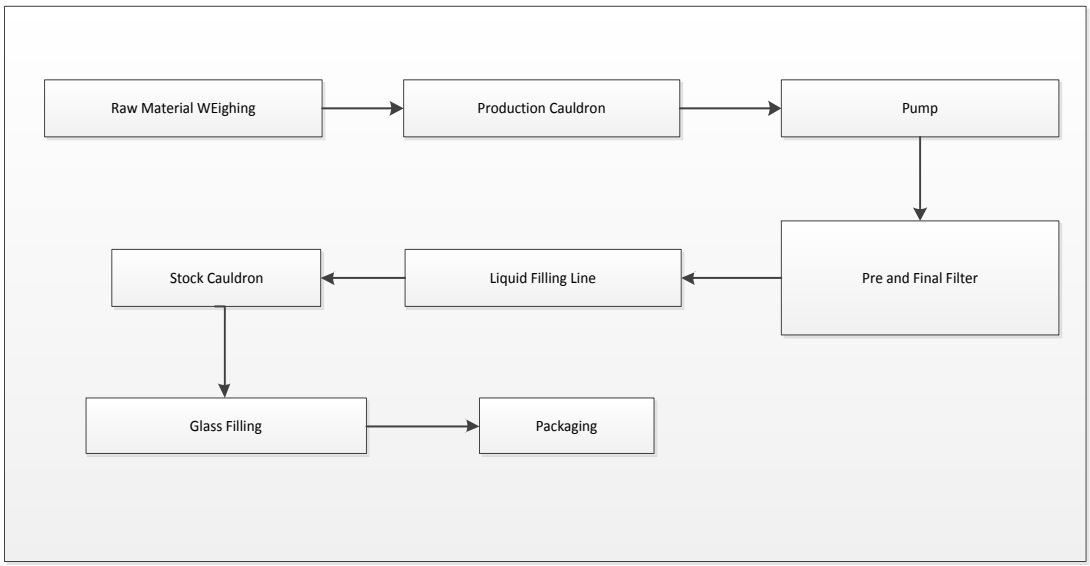

Figure 3.5. Non-Sterile Production Unit and Process Scheme.

3.1.4.4. Sterile liquid production unit and process scheme

Liquid production starts with suspension preparation.Raw materials and water is put to the cauldrons that have heating and mixing capacity.Prepared suspensions are taken to waiting cauldrons and than to the filling machines.Ampulles/Flacones are than autoclaved.After sterilization, all units are optically controlled for particles.After leak control, they're packaged. Sterile Production Unit and Process Scheme is given on Figure 3.6.

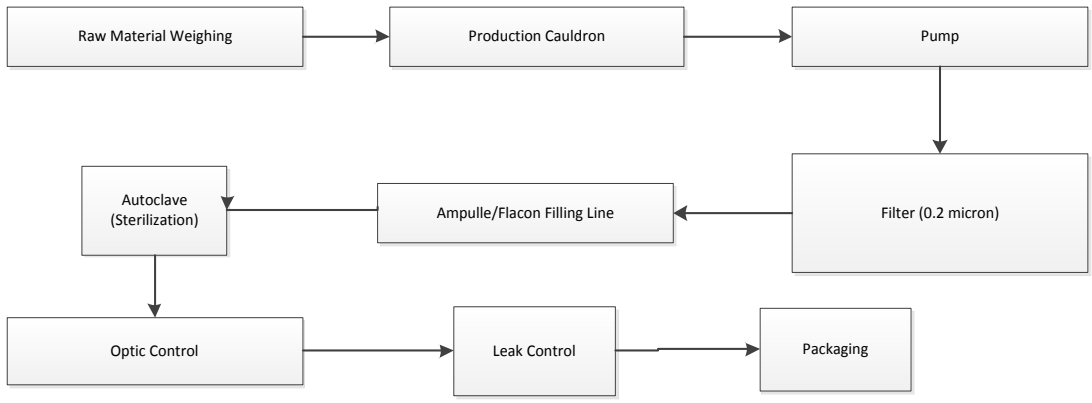

Figure 3.6. Sterile Liquid Production Unit and Process Scheme.

3.1.4.5. Cephalosporin non-sterile production unit and process scheme

Raw materials are weighed and after grinding/granulation steps they're mixed.According to final requested form, they're filled to capsules, mediflex / glass

containers or they're coated if final form will be tablette. Cephalosporin Non-Sterile Production Unit and Process Scheme is given on Figure 3.7.

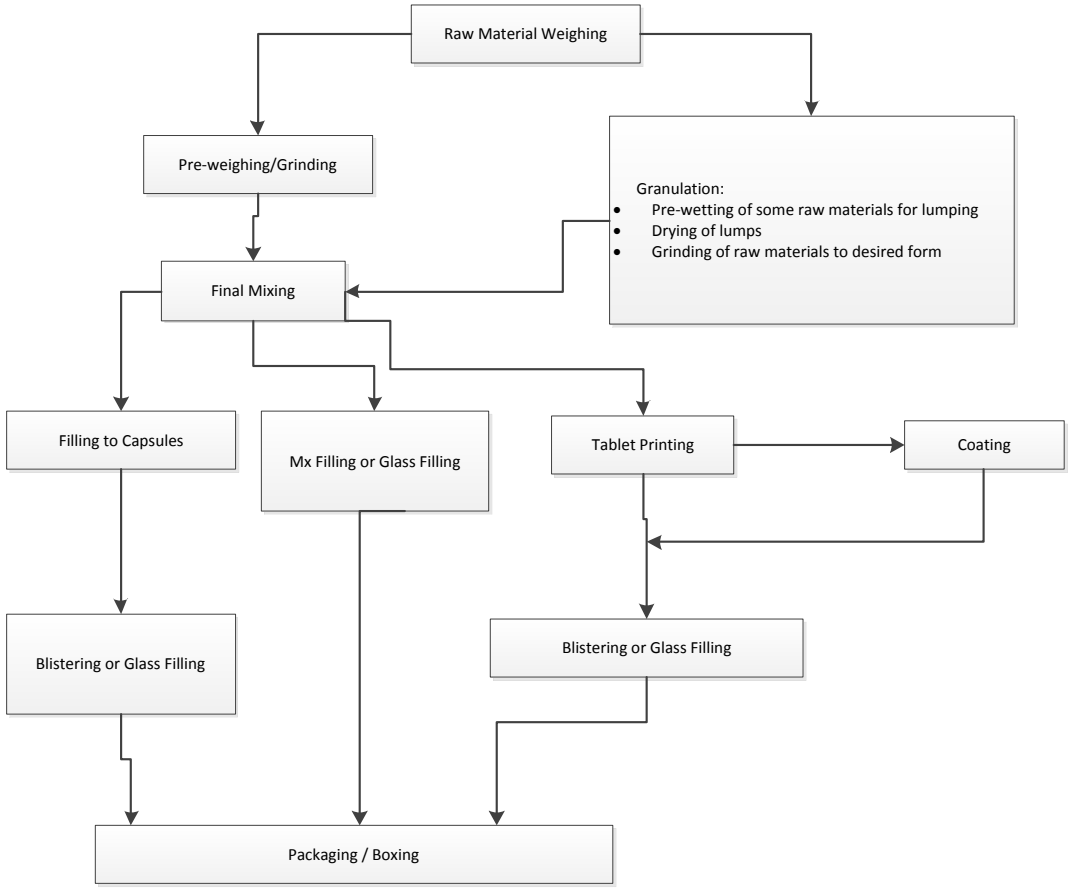

Figure 3.7. Cephalosporin Non-Sterile Production Unit and Process Scheme.

3.1.4.6. Cephalosporin sterile production unit and process scheme

For sterilization process, first flacons are washed and given to sterilization tunnel to be autoclaved.After sterilization, they're filled and capsized.After optical control, they're washed, coded, labeled, boxed, packaged and transported to warehouse.

Cephalosporin Sterile Production Unit and Process Scheme is shown on Figure 3.8.

3.1.4.7. Penicillin production unit and process scheme

Raw materials are weighed and after grinding/granulation steps they're mixed.According to final requested form, they're filled to capsules, mediflex / glass containers or they're coated if final form will be tablette.

Penicillin Production Unit and Process Scheme is shown on Figure 3.9.

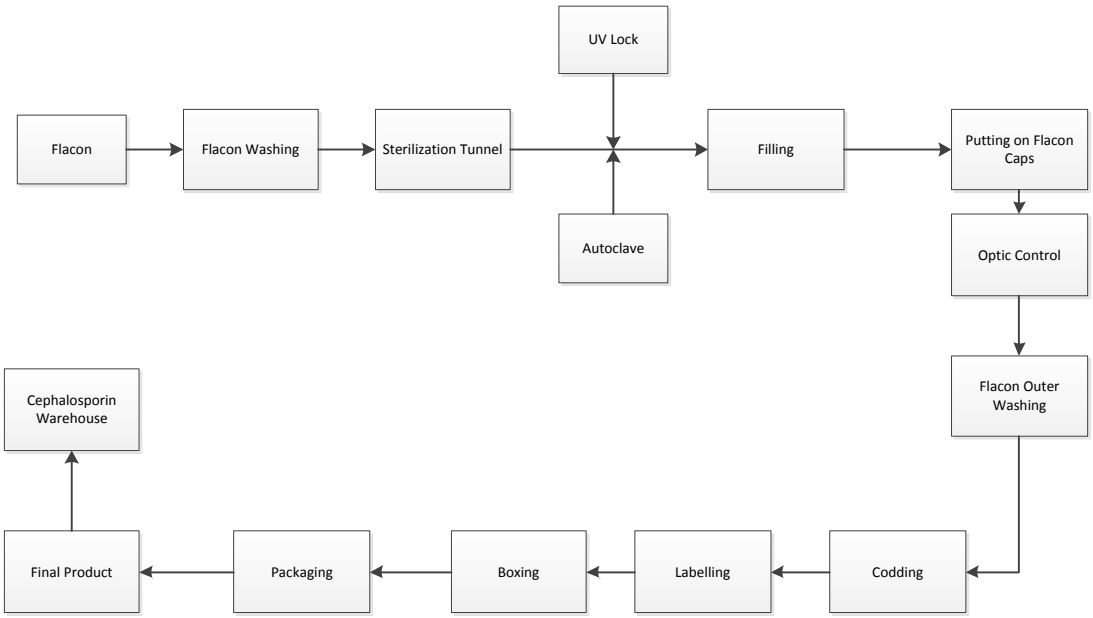

Figure 3.8. Cephalosporin Sterile Production Unit and Process Scheme.

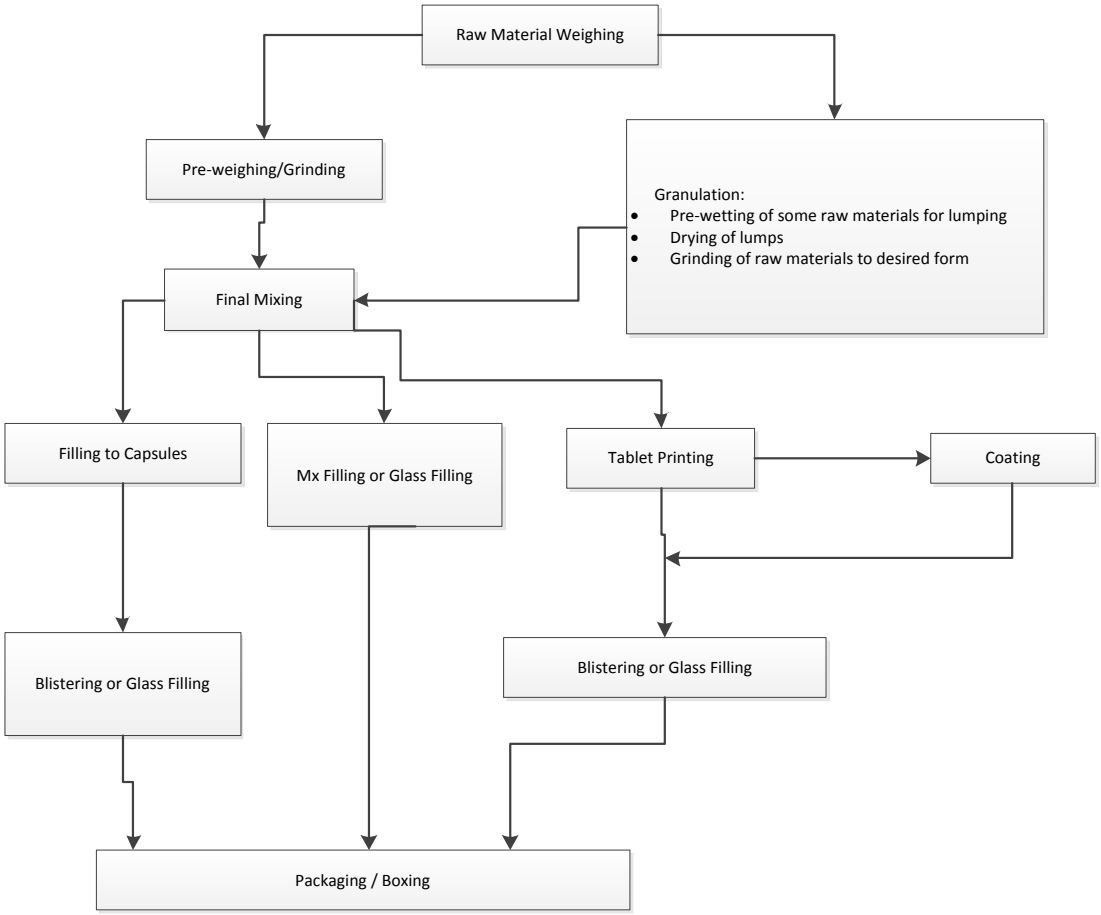

Figure 3.9. Penicillin Production Unit and Process Scheme.

3.1.5. Wastewater Characterization of the Investigated Plant

The investigated plant is in the Mixing, Compounding, Formulating subcategory.

Wastewater generation is from two different sources: Domestic and industrial wastewaters.

Wastewater characterization of the plant was examined on results section in detail.

3.1.6. Wastewater Treatment System in The Investigated Plant

Schematic representation of the Wastewater Treatment System is shown on Fig.3.10.

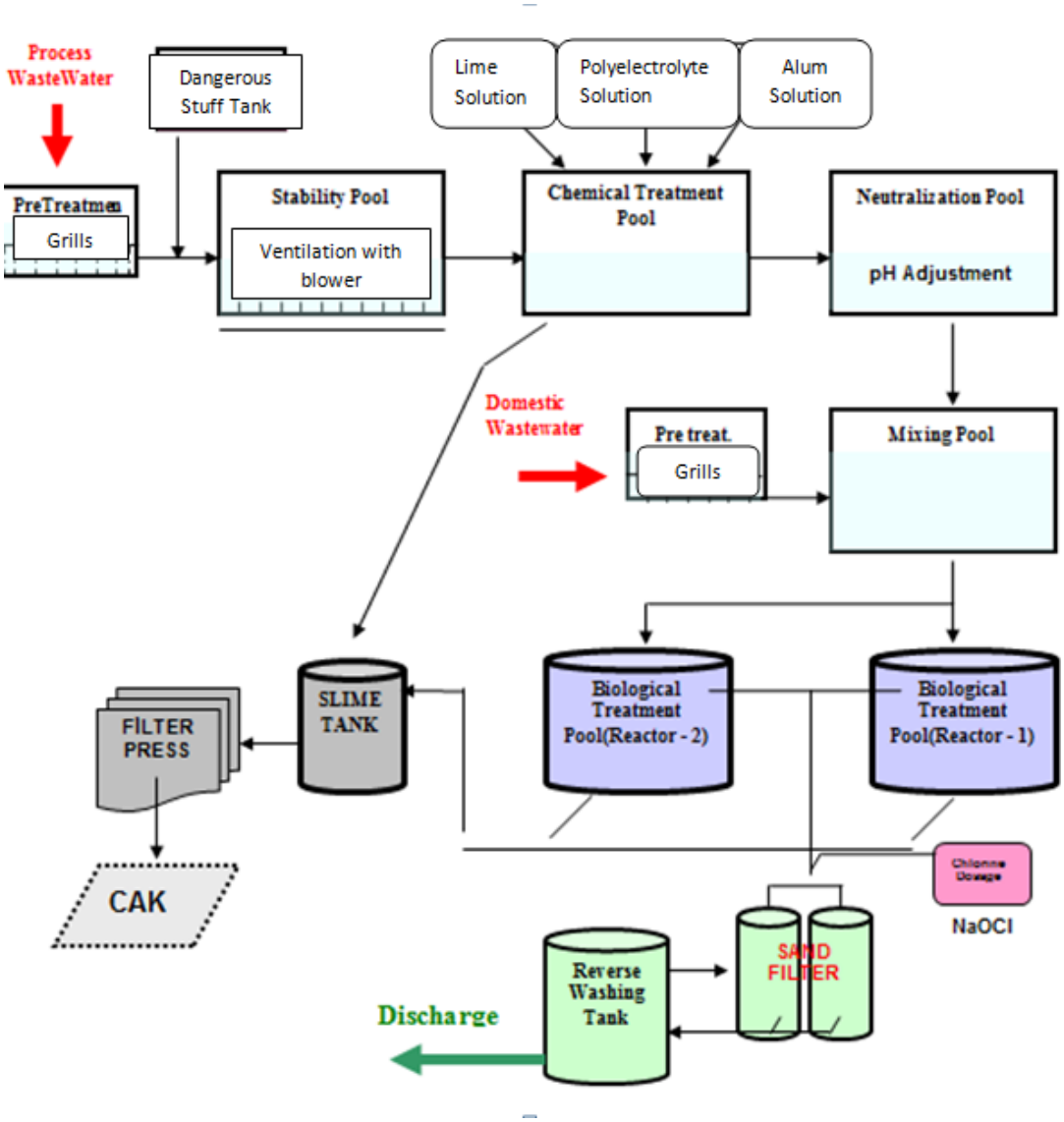

Figure 3.10. Schematic Representation of the Wastewater Treatment System.

Two types of wastewater is come out from the investigated plant. Each of them reaches to treatment system with seperate channel systems.

a. Process Wastewater :

Industrial wastewater : Wastewater produced after the cleaning of the cauldron with water before the next production process.Besides, laboratory and water system wastewater are belong to the same group.

Water system wastewater : Steam boiler, cooling system, water clearance (softener), de-ionizer and distilled water equipment and water from reverse washing.

b.Domestic wastewater :

Wastewater produced from human daily life needs and usage.Domestic wastewater and industrial wastewater enters and mixed in mixing pool, as shown in Figure 3.11.

Two types of treatment is applied to the wastewater reaches to treatment system:

- Chemical Treatment
- Biological Treatment.

Before these processes, pre-treatment is applied to wastewater.

Pre-treatment :

Process wastewater is passed from screens to stability pool.These screens are used to hold the big pieces before they reach to the tanks.

The chemical or chemical wastewater which can give harm to treatment system according to ÇGY-04.05 Regulation on the Control Hazardous Waste are taken to dangerous stuff tank.They are collected and aerated for decaying. Later, they're transferred to stability pool and the process continiues.

Wastewater taken to stability pool is aerated to be homogenized.Stabilized wastewater is pumped to chemical treatment pool with flow of 20 m^3/h. If the chemical treatment is not used than this step is skipped, wastewater is pumped out to the neutralization pool. Neutralization pool is shown on Figure 3.12.

Chemical treatment (Primary treatment) :

This process is not applied since suspended solid is always below 1500 ppm.

Biological treatment (Secondary treatment) :

With this process, required wastewater discharge values are obtained.

Figure 3.11. Domestic and Industrial Wastewater Entries to Mixing Pool.

Figure 3.12. Neutralization Pool.

Biological treatment reactors :

2 reactors are used for biological treatment. Figure 3.13.shows one of the biological treatment reactors. These are SBR (sequencing batch reactor), each of them have 675 m^3 volumes, cylindirical and reinforced concrete. Each of them has one aerator.

Aerator process :

There is one aerator in each aerator.They're used to mix the water in reactor to maintain homogenicity.Working of aerators in low or high motion provides dissolving of oxygen in air to a certain extent (2-4 ppm).At this instant, organotrophic bacteria which are in activated sludge decays organic material and turn them to water and carbondioxide.Figure 3.14. shows the working mode of aerators in biological reactor.

Application of biological treatment process :

Figure 3.13. Biological Treatment Reactor .

Water feeding to reactors for biological treatment is done from mixing tank.Process waste water is taken to neutralization pool and pH arrangement is done.After

neutralization process, it's transferred to mixing pool and mixed with domestic wastewater there.

- During transfer of wastewater to reactors, care is given for pH not to be out of efficient range for biological treatment.pH must be 6,5 - 8,5 in reactor.
- Working of reactors involves 4 steps, these steps are controlled and arranged from control panel.

  - Fill Time
  - React Time
  - Settle Time
  - Idle Time for Effluent Withdrawal

Total Cycle Time= Fill Time+React Time+Settle Time+ Idle Time for Effluent Withdrawal

Figure 3.14. Biological Treatment Reactor (aerators in working mode).

- **Fill time:** Each Reactor is fed with wastewater for 12 hours everyday.
- **React time:** During this step aerators are controlled. During reaction time 1 liter sample is taken and controlled for amount of sludge in İmhoff Cone.

(once a week from each reactor) (max. 200 ml/Lt). Dissolved oxygen in reactors is measured with portable oxygenmeter everyday and recorded.

- **Settle time:** During this step buoy is controlled.After water feeding is completed, aerator is stopped after 6 hours and rested for 2 hours to precipitation of bacteria as an active sludge.In rest of time, transparent upper water shell appears.
- **Idle time for effluent withdrawal:** After precipitation step (4 hours) tranparant water is transferred with floating sluice and pumped to sand filters.
- This transparent water is tested for pH, BOD, SS
- After precipitation step, electrical ventil is opened with signal coming from time relay.Then, filtration pumps start to work and treated water is sent to sand filters.
- When the level in SBR comes to low position,electrical ventil is closed with the signal and pumps are out of session and process is completed.
- Flow is recorded daily and treated water amount is calculated.

Withdrawn of sludge from reactors :

Wastes occured during biological treatment are accumulated as sludge at the bottom of the reactors. This sludge is emptied periodically according to Imhoff test made once a week.

Sludge treatment and filter press process :

Sludge taken from reactors are dehydrated by filter press (water max.%65).Excess sludge is pumped to sludge condensation tank. Accumulated biological and chemical sludges are pumped to filter press and stay as a sludge cake after an enough dehydration (%35-45).

Advance treatment (Tertiary treatment) :

Sand filters are used for an advanced treatment. Biologically treated outlet water are pumped to sand filters with outlet pumps.

Sand filter usage and back wash :

For filtration before discharge, mixed bed sand filters are used.Outlet water comes from sand filters are transferred to reverse wash tank. After accumulation in this tank, they're discharged to stream bed.

3.2. Wastewater Treatment Standards in Turkey

Wastewater Treatment Standards in Turkey according to Water Pollution Control Regulation is shown on Table 3.3.

Table 3.3. Wastewater Treatment Standards in Turkey ,Sector: Chemical Industry (Pharmaceutical Production and Similars).

PARAMETER	UNIT	REFERANCE MOMENTARY OR 2 HOURS COMPOSITE SAMPLE
Chemical Oxygen Demand (COD)	(mg/L)	150
Oil and Grease	(mg/L)	10
Total Organic Carbon (TOC)	(mg/L)	5
Phenols*	(mg/L)	0.3
Fish Bioexperiment (ZSF)	-	6
pH	-	6-9

* (4-Chloro-3-methylphenol, 2-Chlorophenol, 2,4-Dichlorophenol, 2,4-Dimethylphenol, 2-Methylphenol (o-Cresol), 3-Methylphenol (m-Cresol), 4-Methylphenol (p-Cresol), 2,4-Dinitrophenol, 2-Methyl-4,6-dinitrophenol, 2-Nitrophenol, 4-Nitrophenol , Pentachlorophenol , Phenol, 2,4,6-Trichlorophenol total amount is measured) (Draft SKKYEK)

3.3. Conventional Wastewater Characterization

COD was measured according to ISO6060 method [ISO6060, 1986]. For soluble COD determination, samples were subjected to vacuum filtration by means of Millipore membrane filters with a pore size of 0.45 μm. Other experiments were performed according to the Standard Methods [Standard Methods, 2005]. The Millipore AP40 glass fiber filters were used for total suspended solids (TSS) and volatile suspended solids (VSS) measurements.

3.4. Respirometric Biodegradation Test

The respirometric tests were conducted with acclimated biomass sampled from activated sludge unit of the wastewater treatment plant. Batch respirometric (OUR) experiment was conducted at desired initial Food/Microorganism (S_o/X_o) ratio. The OUR measurements were performed with Applitek Ra-Combo continuous respirometer with PC connection. A nitrification inhibitor (Formula 2533^{TM}, Hach

Company) was added to the reactors to prevent possible interference induced by nitrification. The biomass and wastewater mixture was continuously aerated with compressed air in order to keep the dissolved oxygen level above 5 mgO_2/L in the aeration vessel. The respirometric test was performed with approximately 2.3 times diluted wastewater sample. After observing the endogenous OUR level, a wastewater sample is added to mixed liquor having 6630 mgVSS/L was added in order to maintain initial substrate to biomass (S_o/X_o) ratio at 0.062 gCOD/gVSS.day. The model parameters were estimated in accordance with the method proposed by [Insel et al., 2003 and Dochain et al., 1997]. Model simulations and parameter estimation works were performed using AQUASIM program [Reichert et al., 1998]. Figure 3.15 shows Applitek Ra-Combo continuous respirometer

Figure 3.15 Applitek Ra-Combo continuous respirometer.

3.5. Particle Size Distribution Analysis

Filtration/Ultrafiltration experiments were conducted under positive pressure (0.6-1.2 atm;N_2 as the inert gas), in a continiously-stirred cellwith a volumetric capacity of

400 ml (Amicon,Model 8400).A final volume of 100 ml. permeate was determined to be sufficient to run COD experiments in duplicates.Samples were filtered sequentially through conventional filters with pore sizes of 1200-1600 nm(Millipore AP40, glass fiber),450 nm (Durapore HV, polyvinylidene flüoride (PVDF) and 220 nm(Durapore GV, PVDF)(Millipore Corp, Bedford, MA 01730).The working pressure was kept at 0.35 atm throughout the filtration experiments with disposable filters.during the ultrafiltration experiments , permeates from 220 nm membrane filter were filtered successively through ultrafiltration membranes with nominal molecular weigt cut-off (MWCO) values of 100,30,10,3 and 1 kDa (PL series , Millipore,MA).All experiments were conducted at room temperature and at a pH range of 7.2 -8.8.The pressure applied to the 100 kDa ultrafiltration membrane discs was 0.6 atm, and to the others was 1.2 atm (values all below the ones recommended by the manufacturer)

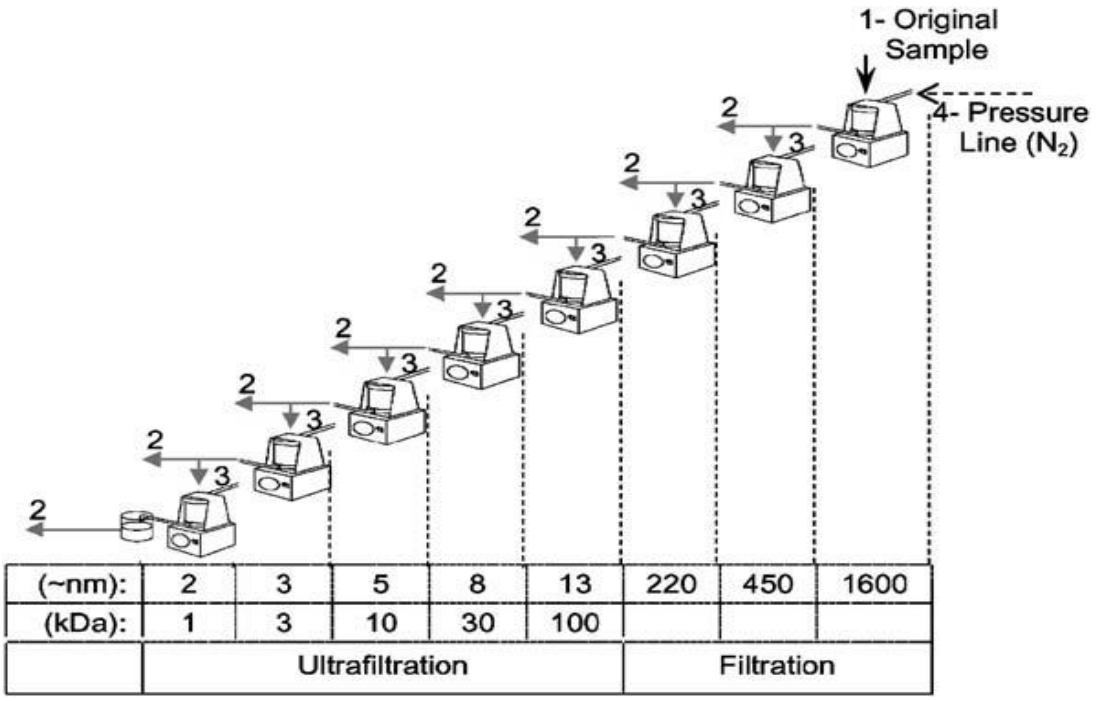

Fig.3.16. Schematic presentation of the sequential filtration/ultrafiltration procedure.

(1—Non-settled, nonfiltered,but mixed original sample, 2—aliquot of filtrate from the previous step, subjected to COD measurements, 3—aliquot of filtrate from the previous step, subjected to subsequent filtration/ultrafiltration procedure, 4—gas line providing positive pressure.

4. RESULTS

4.1.Wastewater Generation of the Plant:

The investigated plant had been observed throughout the year (2012) to reflect the relation between the drug produced and wastewater generated.The plant uses water for both production and domestic purposes. Table 4.1. shows Wastewater Generation of the Plant in 2012 and Figure 4.1. shows the volumes of industrial and discharged wastewater in 2012.As seen from the table , 23961 m³ water was used for domestic purposes by 763 personnel. 86 L of domestic wastewater was created per person each day.

Table 4.1. Wastewater Generation of the Plant for 2012.

DATE	TOTAL INDUSTRIAL WASTEWATER	TOTAL DISCHARGED WASTEWATER
	Wastewater (m³)	Discharged Wastewater (m³)
JANUARY	6598	9237
FEBRUARY	6349	8824
MARCH	6946	9242
APRIL	6567	8593
MAY	6686	8338
JUNE	6606	8529
JULY	1730	5009
AUGUST	5598	7400
SEPTEMBER	5876	7314
OCTOBER	5614	7268
NOVEMBER	6374	7843
DECEMBER	6594	7902
TOTAL	**71538**	**95499**

As seen on the table and graph, wastewater values had their lowest values on July.This sharp decrease of wastewater volume was because of production shut-down period which took 3 weeks and done each year for maintenance and repair purposes in production area.

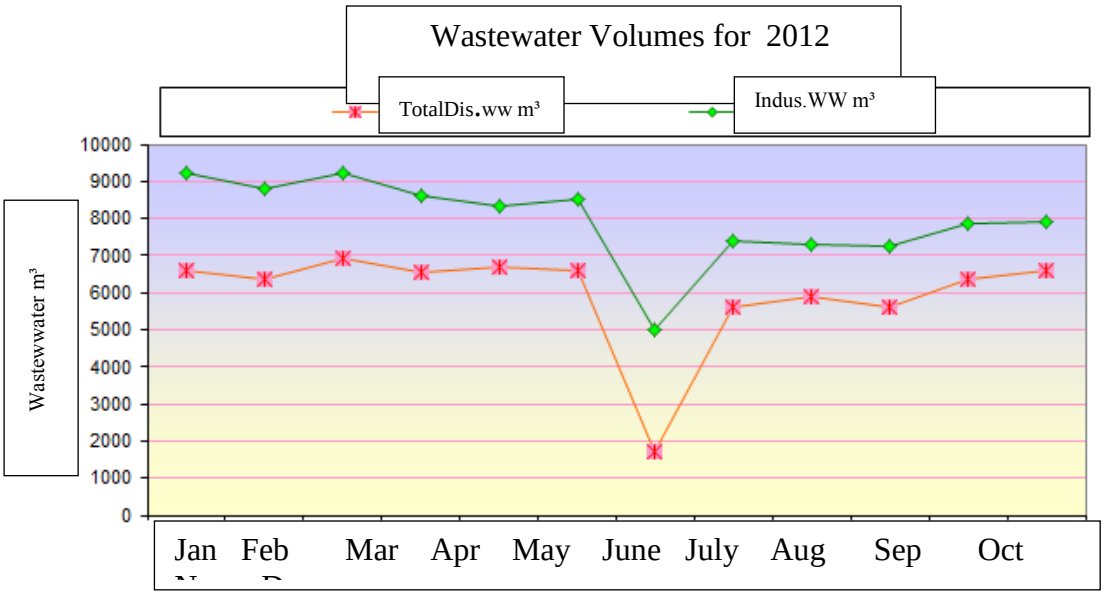

Figure 4.1. Schematic Representation of Industrial and Total Wastewater Produced.

4.1.1.Process Profile of the Plant

As shown in Table 4.1., wastewater generation of the plant for 2012 is 95500 m³/day.Total drug production is 244000000 packages/year. According to these values, 391 m³ wastewater is generated per package of drugs.

4.1.2.Pollution Profile of the Plant

Characterization of pollution profile is given on Table 4.2. Wastewater Treatment Results for the Investigated Plant - 2012

As represented on Table 4.2. , average COD level for 2012 is 902 mg/l.. The plant have generated 236 kg COD/day Considering the production of 244000000 packages of drug per year, 0.35 mg/L COD was generated per packages of drug.

4.1.3.Wastewater Treatment Results for the Investigated Plant – 2012

Wastewater treatment results for 2012 are given on the Table 4.2. on a monthly averaged basis. Wastewater generations, COD , BOD, pH, SS and Sludge production values are measured and followed regularly. From the data, treatment efficiencies can be followed, also control of meeting the standards of Water Pollution Control Regulation is possible.

Table 4.2. Wastewater Treatment Results for the Investigated Plant – 2012.

MONTHS	Working Day	Total Disharged Water		Industrial WW		Domestic WW		pH		Chemical Oxygen Demand (COD)			Biological Oxygen Demand(BOD)			Suspended Solids(SS)	Filtered Solids
		Monthly m³	Daily m³	Monthly m³	Daily m³	Monthly m³	Daily m³	Influent	Effluent	Influ. (mg/l)	Efflu. (mg/l)	Effic.	Influ. (mg/l)	Efflu. (mg/l)	Effic.	mg/l	Sludge Weight Kg
		-	-	-	-	-	-	(6-9)	(6.5-8.5)	-	<100	-	-	<30	-	<25	-
JANUARY	31	9237	298	6598	213	2639	85	7.42	8.04	684	34	95	360	6.15	98	5.65	3600
FEBRUARY	28	8824	315	6349	227	2475	88	7.28	7.86	798	57	93	320	6.67	98	4.25	4200
MARCH	31	9242	298	6946	224	2296	74	7.13	7.74	1208	95	92	440	2.07	100	8.48	5400
APRIL	30	8593	286	6567	219	2026	68	7.63	7.60	779	48	94	600	9.46	98	3.30	6000
MAY	31	8338	269	6686	216	1652	53	7.48	7.69	833	52	94	300	12.96	96	5.50	5400
JUNE	30	8529	284	6606	220	1923	64	7.21	7.74	1298	78	94	480	13.48	97	4.58	5400
JULY	31	5009	162	1730	56	3279	106	7.67	7.75	862	61	93	300	14.27	95	4.65	2200
AUGUST	31	7400	239	5598	181	1802	58	7.52	7.78	991	52	95	520	9.04	98	6.73	3000
SEPTEMBER	30	7314	244	5876	196	1438	48	7.42	7.71	786	25	97	520	6.15	99	3.58	4200
OCTOBER	31	7268	234	5614	181	1654	53	7.56	7.68	698	44	94	440	10.08	98	3.64	3600
NOVEMBER	30	7843	261	6374	212	1469	49	7.55	7.57	1310	63	95	360	9.48	97	6.40	5400
DECEMBER	31	7902	255	6594	213	1308	42	7.55	7.36	577	44	92	320	8.77	97	3.20	4800
AVERAGE	Total 365	7958 +/- 1161	262 +/- 41	5962 +/- 1400	196 +/- 47	1997 +/- 582	66 +/- 19	7.45 +/- 0,16	7.71 +/- 0,16	902 +/- 246	54 +/- 19	94 +/- 1,35	413 +/- 101	9.05 +/- 3,5	98 +/- 1,22	5.00 +/- 1,61	4433 +/- 1163

Considering the discharge standards based on Water Pollution Control Regulation (Table 3.3), effluent COD parameter must be below 150 mg/L. To meet this criteria, %85 treatment efficiency must be reached. As seen from the table, treatment system efficiency was %94 which meets the criteria. pH is also considered as one of the effluent parameters.Average pH of the effluent was 7.71. 6-9 range was expected to meet the criteria, which was acceptable according to regulations.

Figure 4.2. represents Suspended Solid Results of Biological Treatment System for 2012. As seen from the graph, SS values varies between 3-9 mg/L, on average 5 mg/L. These values are low as expected from the filtration process .

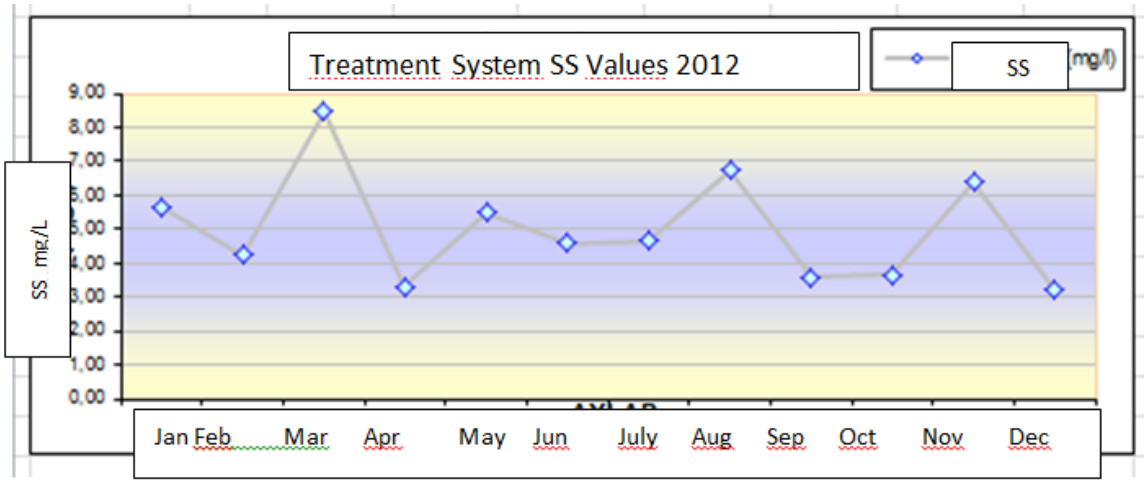

Fig. 4.2. Biological Treatment System SS values for 2012.

Figure 4.3. represents treatment system sludge amounts. The lowest value is on July as expected from the shut-down period. 3000-6000 kg of sludge produced on other months which differs according to type and amount of drug produced. Total sludge production is around 50.000 kg/year.

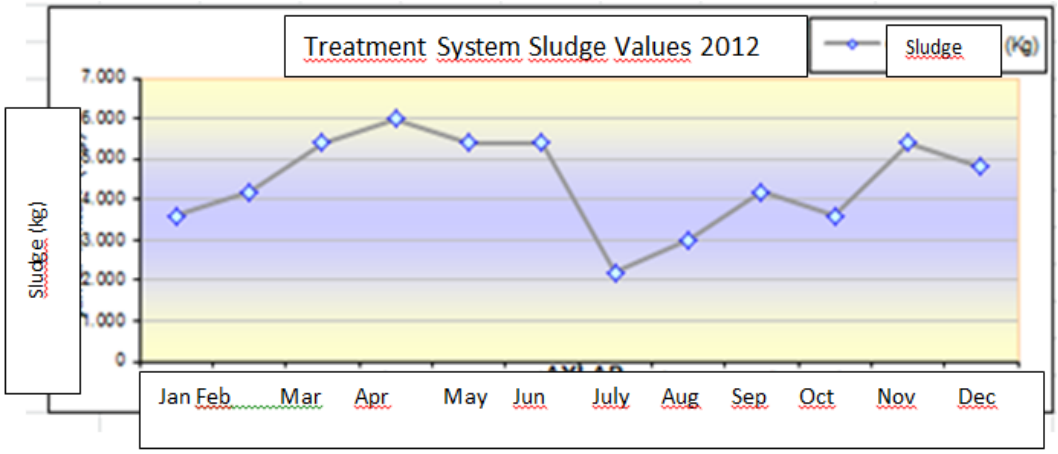

Fig.4.3. Biological Treatment System Sludge Values for 2012.

Figure 4.4. represents pH values of treatment system.pH values are around 7-8 for both influents and effluents. These results meet WPCR criteria.Conventional characterization experiments also gave similar results (6.96 and 7.04)

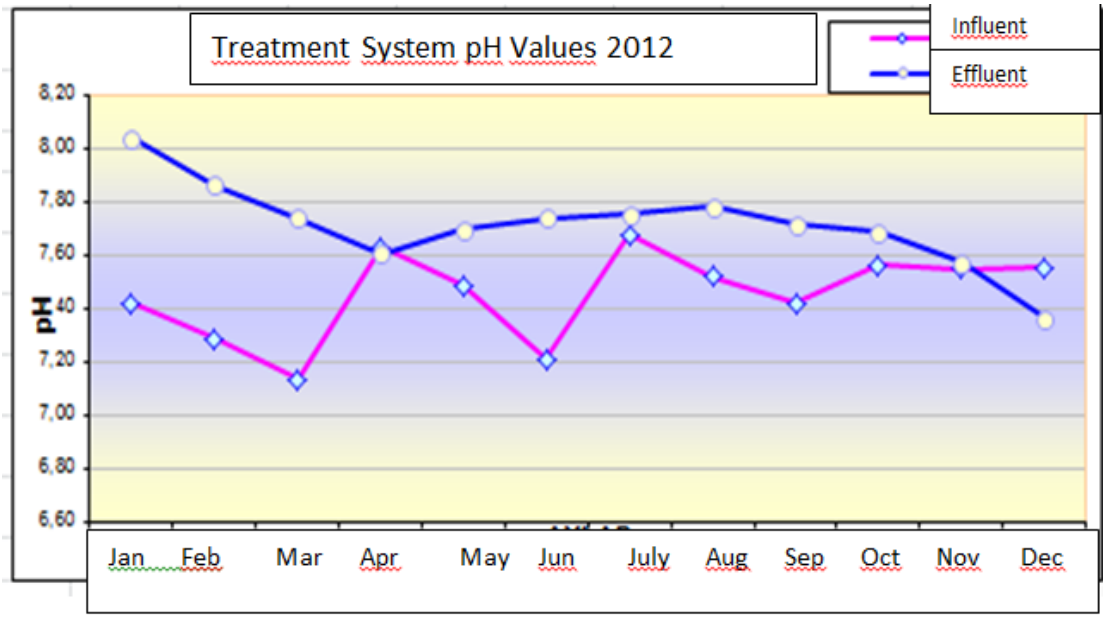

Figure 4.4. Biological Treatment System pH values for 2012.

4.2.Experimental Results

4.2.1.Conventional Characterization

An influent grab sample from the equalization tank and an effluent grab sample from the SBR of the biological WWTP of the pharmaceutical mill (expalined in detail on section 3.1.6), were collected during spring and summer seasons for conventional characterization.

Total COD (mg/L),Soluble COD (mg/L),TOC (mg/L),DOC (mg/L),TSS (mg/L),VSS (mg/L),TKN (mg/L),NH3-N (mg/L) ,TP (mg/L),OP (mg/L),pH,Alkalinity (mg CaCO3/L),Cl- (mg/L),SO42- (mg/L) analysis were performed on the samples.

Table 4.3. represents the values found in conventional characterization studies for the sample taken on April 2012.

Table 4.4. represents the values found in conventional characterization studies taken on June 2012.

Table.4.3. Conventional Characterization Experimental results for Sample1.

Parameter	April 2012	
	Influent	**Effluent**
Total COD (mg/L)	790	80
Soluble COD (mg/L)	485	65
TOC (mg/L)	225	25
DOC (mg/L)	140	20
TSS (mg/L)	200	20
VSS (mg/L)	190	20
TKN (mg/L)	55	25
NH_3-N (mg/L)	30	20
TP (mg/L)	7	2
OP (mg/L)	3	1
pH	6.75	6.96
Alkalinity mg$CaCO_3$/L)	65	75
Cl^- (mg/L)	120	100
SO_4^{2-} (mg/L)	100	85

As seen from the results of the two samples, average COD level is 900 mg/L. This level is concordant with the literature for pharmaceutical industry (Çokgör et al,2004)

Soluble COD level is 475 mg/L which is %55 of total COD. TSS are mainly volatile.

COD/TOC ratio is 3.67 . TKN, 60 mg/L and TP , 8 mg/L on average is similar to domestic wastewater (around 50 mg/L for TKN, 10 mg/L for TP, Orhon et al, 2009)Experimental data reveal an overall COD removal efficiency of %90 for the investigated pharmaceutical wastewater. This value meets acceptance criteria of Water Pollution Control Regulation. Biological treatment yielded satisfactory removal efficiencies for all parameters. COD removal efficiency results are also concordant with the plant's measurements.(%94 COD removal efficiency)

Table 4.4. Conventional Characterization Experimental results for Sample 2.

Parameter	June 2012	
	Influent	Effluent
Total COD (mg/L)	945	120
Soluble COD (mg/L)	465	95
TOC (mg/L)	265	35
DOC (mg/L)	135	30
TSS (mg/L)	350	20
VSS (mg/L)	330	20
TKN (mg/L)	65	30
NH_3-N (mg/L)	40	30
TP (mg/L)	9	3
OP (mg/L)	4	1
pH	6.94	7.05
Alkalinity (mg$CaCO_3$/L)	85	90
Cl^- (mg/L)	145	120
SO_4^{2-} (mg/L)	120	100

4.2.2.Respirometric Test Results - Date 21.06.2012

The respirometric tests were conducted with acclimated biomass sampled from activated sludge unit of the wastewater treatment plant. The biomass and wastewater mixture was continuously aerated with compressed air in order to keep the dissolved oxygen level above 5 mgO_2/L in the aeration vessel. The respirometric test was performed with approximately 2.3 times diluted wastewater sample. After observing the endogenous OUR level, a wastewater sample is added to mixed liquor having 6630 mgVSS/L was added in order to maintain initial substrate to biomass (S_o/X_o) ratio at 0.062 gCOD/gVSS.day. The results of the respirometric measurements of OUR and Soluble COD are given on Table 4.5.

Respirometric tests were performed with relevant acclimated biomass seeding alone to obtain the level of initial endogenous oxygen uptake rate (OUR).

Table 4.5. Respirometric Measurements of OUR and Soluble COD.

Parameter	Value	Unit
V_{Sludge}	1000	mL
$V_{Tap\ Water}$	300	mL
V_{WW}	1000	mL

Time (min)	Time	OUR (mg/L/h)	Soluble COD (mg/L)
0	09:12	119.0	210
29	09:41	28.6	76
59	10:11	23.6	66
119	11:11	16.7	54
150	11:42	end. resp. level	38

Determination of endogenous respiration rate of the biomass sample, was the first step of respirometric measurements.After the endogenous respiration rate was stabile for half an hour, main respirometry reactor was fed up with wastewater that was filtered from 0.45 μm membrane filter in a way that F/M ratio was 0.03 COD/g VSS.

At the beginning, very high OTH Profile was observed due to the consumption of readily biodegradable COD component. According to the method that was used to determine the readily biodegradable COD component by Ekama et al (1986), the concentration of readily biodegradable COD concentration was found from the area of time segment until clear and instant decrease was seen. OUR Profiles of respirometric measurements conducted on soluble wastewater samples was shown on Figure 4.11. COD measurements conducted on samples taken from respirometer on selected times were shown on Figure 4.12.

4.2.3.Ultrafiltration

In this study , Particle size distribution (PSD) via sequential filtration/ultrafiltration was used as the tool for COD fractionation of pharmaceutical company wastewaters before and after treatment.

Treatment efficiency were determined via comparing the profiles of the effluents from biological-treatment to those of the corresponding influents. Particle Size Distribution (PSD) analysis was used by means of sequential filtration and

ultrafiltration, to attain a better image of distribution of organic carbon content, thus the COD fingerprint, of the investigated pharmaceutical wastewater. For that purpose, influent and effluent samples were collected from the biological treatment system of the investigated plant , and sequential filtration/ ultrafiltration experiments were executed to obtain the PSD-based profiles for the samples. Profiles obtained for the influents and effluents have revealed COD characteristics for the wastewaters. Comparison of influent and effluent profiles described the specific impact of applied treatment processes on distribution of COD fractions.

The cumulative values for a given filter size indicate the COD of the permeate and thus, each one describes the total COD below the selected filter size. The differential COD between the two consecutive filter sizes can then be calculated as the difference between the two relevant absolute CODs. The first values seen in the table correspond to the total COD contents (sum of particulate, settleable and supracolloidal, colloidal, and soluble portions) of the samples. The profiles for the effluents, when compared with those of the influents, roughly imply the treatment efficiencies.

Tables and Figures below represents Filtration/Sequential Filtration results, PSD Analysis, Percent COD Distribution and Percentage contributions from each size interval for the 2 wastewater samples taken before and after the biological treatment.

Results of Sample 1:

Table 4.6 . Cumulative and differential COD values of pharmaceutical wastewater before and after biological treatment.

Separation Technique	**Particle size (nm)**	**Cumulative COD (mg/L)**		**Size Category (nm)**	**Differential COD (mg/L)**	
		Biological Treatment			**Biological Treatment**	
		Influent	**Effluent**		**Influent**	**Effluent**
Total		790	80			
Filtration						
AP40 filter	1600	605	70	> 1600	185	10
HV filter	450	485	65	450-1600	120	5
GV filter	220	465	65	220-450	20	0
Ultrafiltration						
100 kDa	13	355	65	13-220	110	0
30 kDa	8	340	65	8-13	15	0
10 kDa	5	330	60	5-8	10	5
3 kDa	3	305	60	3-5	25	0
1 kDa	2	285	55	2-3	20	5
				< 2	285	55

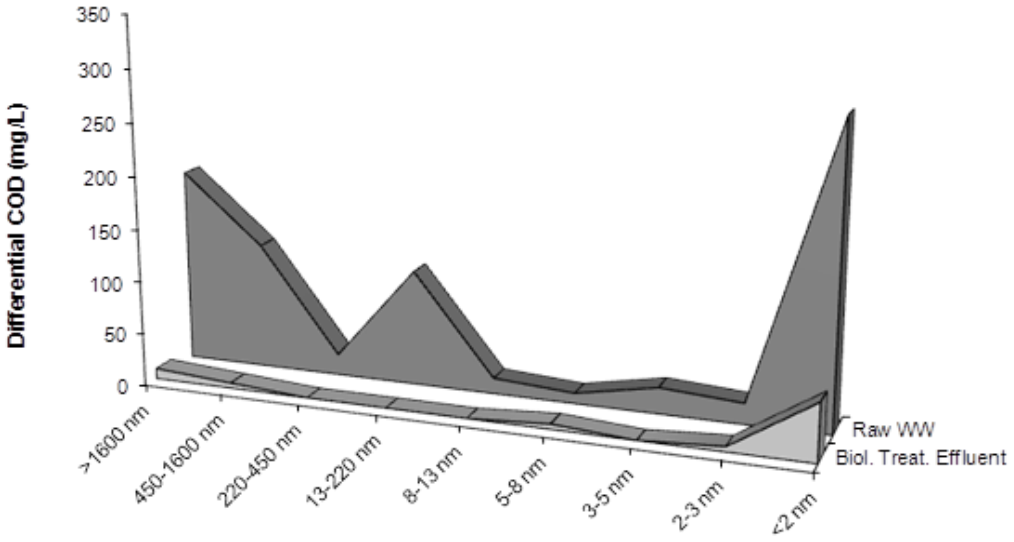

Figure 4.5. Particle size distribution of COD before and after biological treatment.

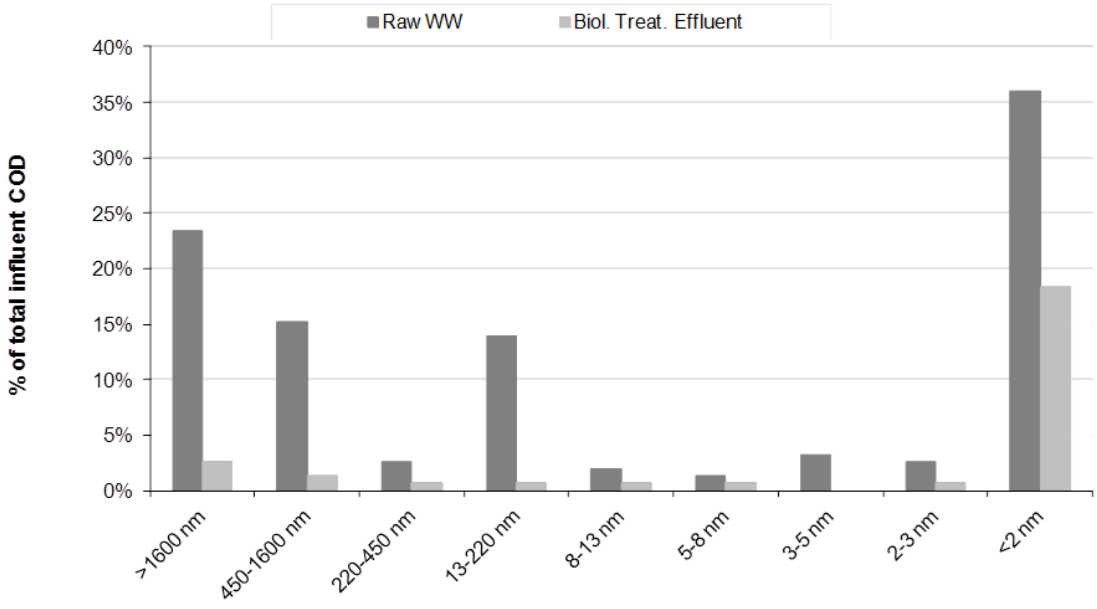

Figure 4.6. Percent COD distribution of the pharmaceutical wastewater before and after biological treatment.

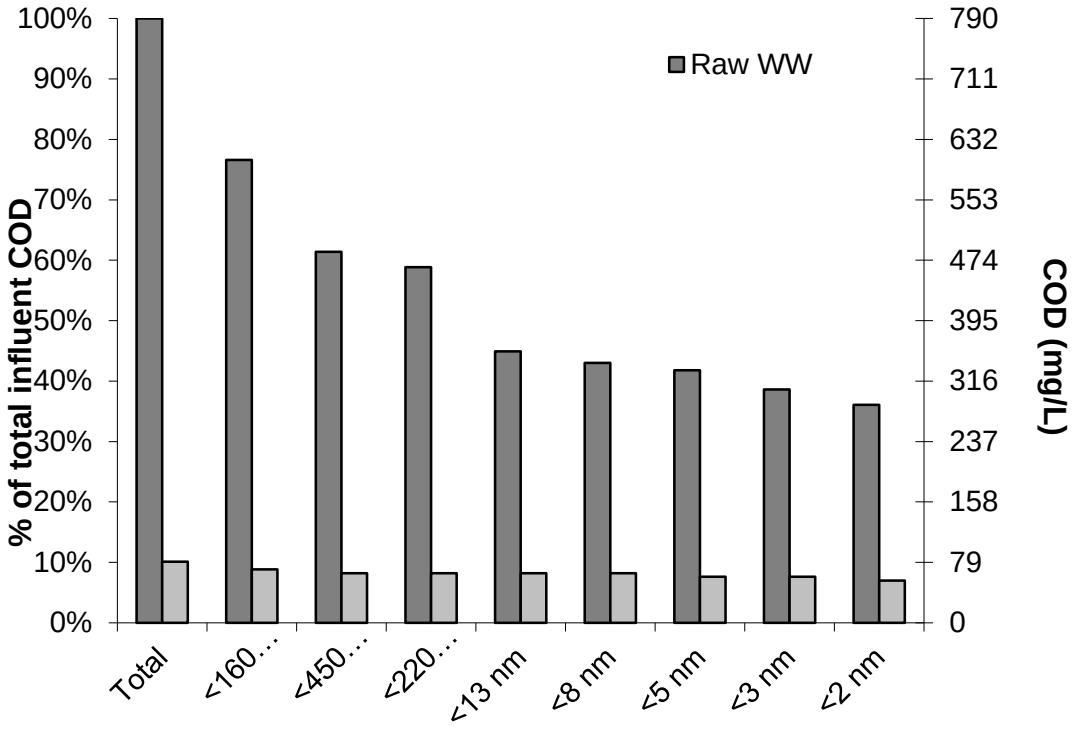

Figure 4.7. Percentage contributions from each size interval before and after biological treatment.

Results of Sample 2:

Table 4.7. Cumulative and differential COD values of pharmaceutical wastewater before and after biological treatment.

Separation Technique	Particle size (nm)	Cumulative COD (mg/L)		Size Category (nm)	Differential COD (mg/L)	
		Biological Treatment			Biological Treatment	
		Influent	Effluent		Influent	Effluent
Total		945	120			
Filtration						
AP40 filter	1600	590	105	> 1600	355	15
HV filter	450	465	95	450-1600	125	10
GV filter	220	445	95	220-450	20	0
Ultrafiltration						
100 kDa	13	330	95	13-220	115	0
30 kDa	8	320	95	8-13	10	0
10 kDa	5	315	85	5-8	5	10
3 kDa	3	280	85	3-5	35	0
1 kDa	2	260	80	2-3	20	5
				< 2	260	80

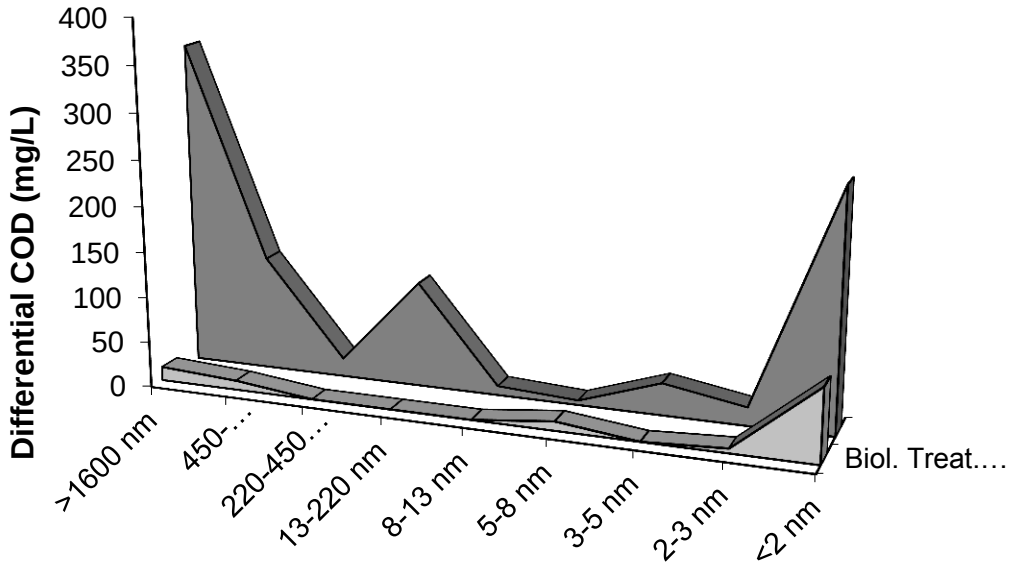

Figure 4.8. Particle size distribution of COD before and after biological treatment.

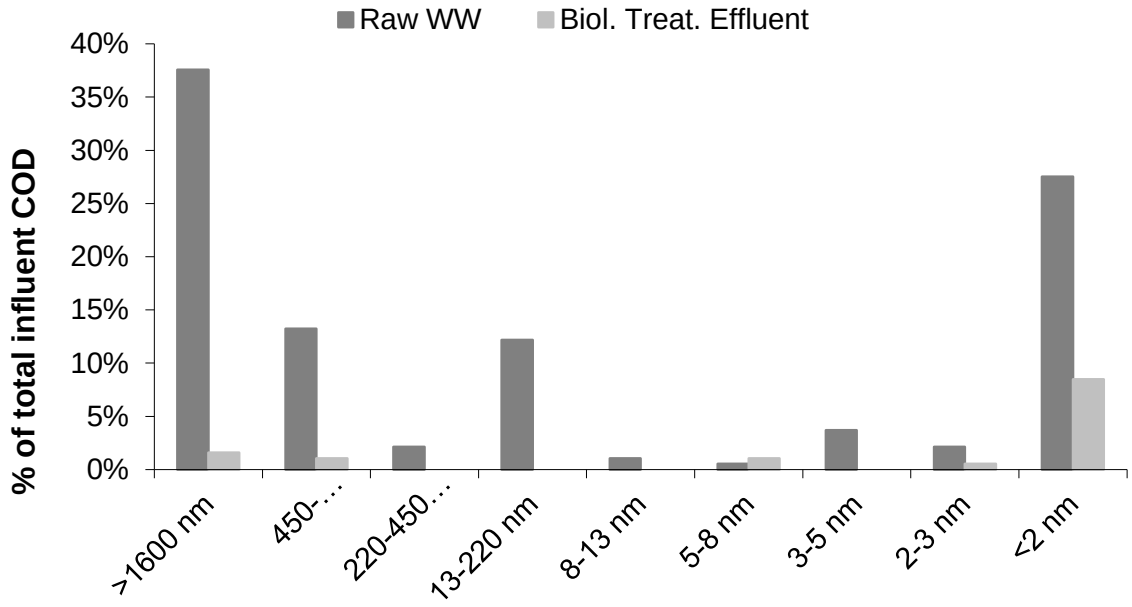

Figure 4.9. Percent COD distribution of the pharmaceutical wastewater before and after biological treatment.

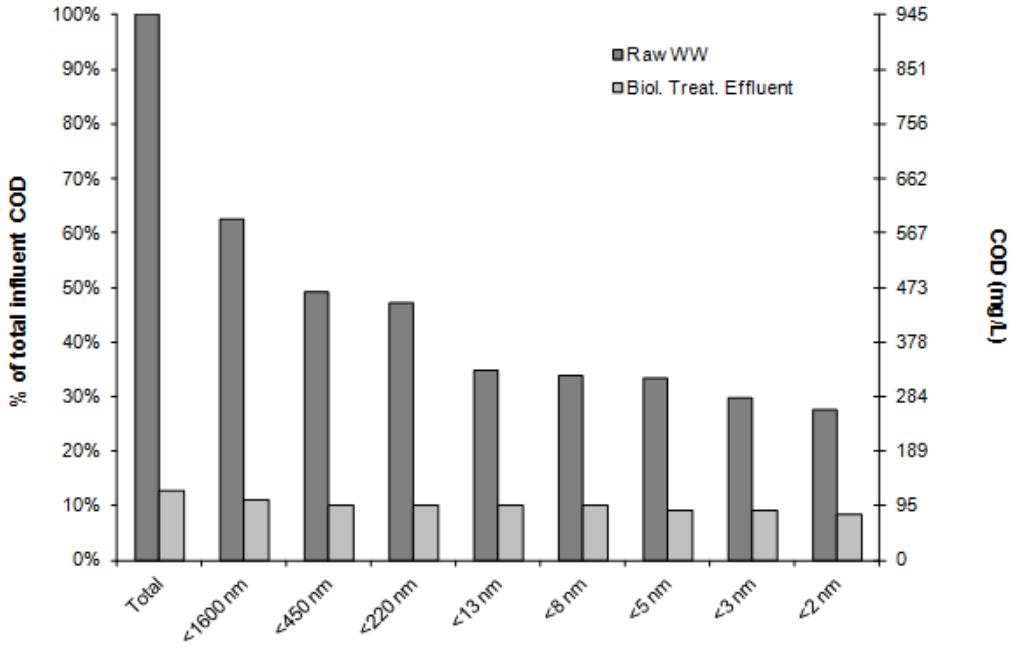

Figure 4.10. Percentage contributions from each size interval before and after biological treatment.

As seen from the results; COD fingerprints of 2 influent samples taken were quite similar at all the size ranges; with the settleable fraction (>nm) and soluble fraction (<2 nm) being the most apparent ones. Whereas for effluents only the soluble fractions are apparent. Biological treatment was effective at both ends of size distribution, with almost total removal at the particulate range and most of the soluble portion. Biological treatment was successful in removing all size ranges except soluble inert COD fraction.

It was concluded that the COD content of the effluents accumulating at the <2 nm size interval was mostly due to initial soluble inert COD by-passing treatment and soluble inert microbial products being generated in the course of biological treatment. This can also be seen from soluble inert COD concentration results (38 mg/L soluble COD concentration at endogenous respiration level) measured by respirometer. (Table 4.5)

4.2.4.Modeling of Experimental Results

In this study, the Aquasim Software was used to model the chronic experiment in order to determine related kinetic and stochiometric coefficients. A multicomponent model

referred to as Activated sludge Model (ASM1) was used in modelling studies. (Reichert et al, 1998).

Modeling consist of analyzing and calibrating the OUR profiles. The calibrated model provided a close fit with the experimental OUR profile displayed on Fig. 4.11., defining the (1) relative magnitude of biodegradable COD fractions and (2) appropriate kinetic and stoichiometric coefficients applicable for the biodegradation of the pharmaceutical wastewater. The model simulation of COD data is displayed on Figure 4.12. The OUR experiments were started with a diluted soluble COD level of 210 mg/L. The calibration exercise was initiated and confirmed with an endogenous decay coefficient, bH, of 0.20/day, fE of 0.20, and a heterotrophic yield, YH, of 0.67 mg cell COD per mg COD commonly determined for similar evaluations.

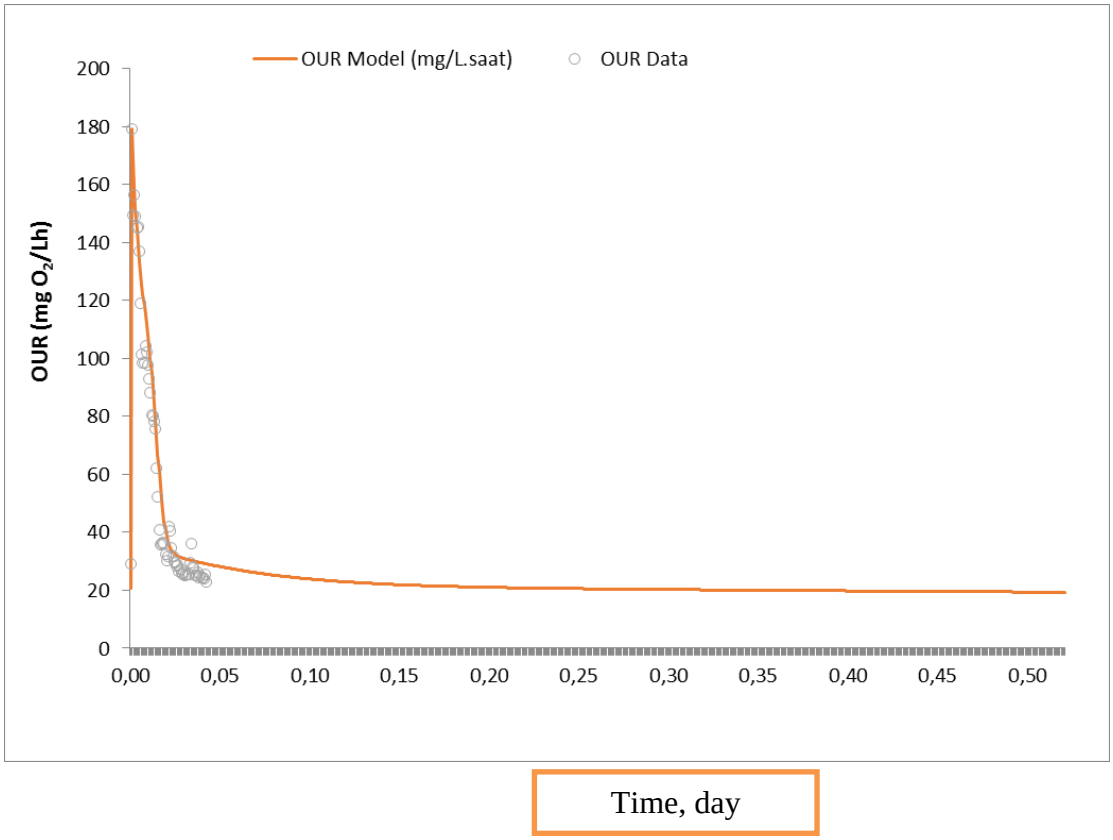

Figure 4.11. Model simulation of OUR data (ASM1).

The results of the model calibration related to the assessment of kinetic and stoichiometric coefficients are outlined in Table 4.8. Regarding the biodegradation kinetics, the maximum specific growth rate, μH, associated with readily biodegradable COD utilization for microbial growth was defined as 6.0/day, a value

within the range of 3.5–6.5/day reported for domestic sewage (Henze et al. 2000; Sözen et al. 1998). The corresponding half-saturation coefficient, KS, was 30 mg COD/L. In general, the model evaluation reveals the biodegradable nature of the pharmaceutical wastewater.

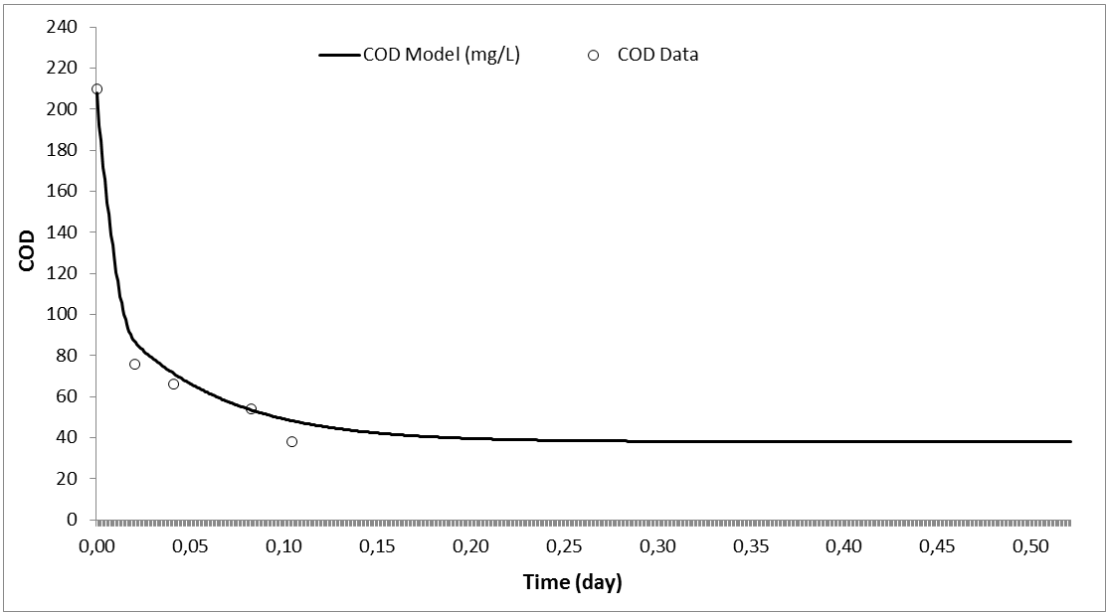

Figure 4.12. Model Simulation of COD data.

Table 4.8. Results of Model Calibration.

Model Parameters/State Variables	**Unit**	**Value**	**Model Parameters/State Variables**	**Unit**	**Value**
µHmax	1/day	6	KX*S2*		0.1
K*s*	mgCOD/l	30	Ks	mg COD/L	30
bH	1/day	0.2	µH	1/day	6
CSin	mg/COD/L	170	Ss*in*	mg COD /L	24
fE		0.2	SH2 *in*	mg COD/L	66
K h *S1*	1/day	2.7	SH1 *in*	mg COD/L	80
K X *S1*	mg COD/COD	0.006	YH		0.67
Kh*S2*		2	XH*in*	mg COD/L	3100

4.3. Optimization Studies of Modeling Data and Experimental Results

4.3.1. Estimation of $f_{XS} = 0.28$ $f_{XI} = 0.23$ rate constants

Table 4.9 shows the results of convantional characterization of the sample that was taken on June 2012.

Table 4.9. Results of Convantional Characterization of 2.sample.

Influent	Unit(mg/L)
Total COD	945
Sol. COD	465
Part. COD	480

With the help of respirometric measurements (OUR data), soluble COD fractions (S_I, S_S and S_H) were found as below;

$S_I = 75$ mg/L

$S_S = 53$ mg/L

$S_H = 330$ mg/L

$S_{Total} = \approx 460$ mg/L

From these results, stochiometric coefficients can be found as below:

$f_{SI} = \%8 = S_I$ / Total COD

$f_{SS} = \%5.6 = S_S$ / Total COD

$f_{SH} = \%35 = S_H$ / Total COD

From the knowledge that the sum of these values is %48.6 of the total COD, it can be predicted that the sum of f_{XS} (= X_S / Total COD) and f_{XI} (= X_I / Total COD) is % 51.4. Since it's impossible to find Xs ve X_I values seperately with experimental methods, trial and error method was used to find the concordant values for f_{XS} and f_{XI} with the model data.

Since total COD is around 945 mg/L and S_{Total} is around 460 mg/L ;

$X_S + X_I$ can be found as 480 mg/L

After several tests , concordant values were found as;

$f_{XS} = 0.28$ and $f_{XI} = 0.23$.

These values are applied to the model to make comparisons between measured and calculated values of S_{COD}. Table 4.10 shows measured and calculated values for selected months and the average values.

Table 4.10. Comparison of measured and model S_{COD} values.

Date	COD_{in} (mg/L)	S_{COD} (Effluent) (mg/L)	S_{COD} model (mg/L) (Effluent S_I and $S_I + S_P$)
February	798	57	40 and 52
March	1208	95	60 and 74
April	779	48	39 and 49
July	862	61	43 and 55
August	991	52	50 and 61
November	1310	63	65 and 80
December	577	44	29 and 39
Average	902	54	45 and 57

*Θx was taken 20 on average

As seen from the table, resulting S_I and S_P values (S_I and $S_I + S_P$) were close to the measurements taken by the plant itself. The harmony of model values and measured values are higher in the months that have average COD close to the yearly average COD. This concordancy was not observed in all months, because the comparison was made with one sample but COD varies throughout the year due to many contributing factors.

4.3.2.Calculation of k Value

$f_{XS} = 0.28$ and $f_{XI} = 0.23$ values was used to calculate X_S and X_I values;

$X_S = f_{XS} *$ Total COD = 265 mg/L

and $X_I = 215$ mg/L ($X_S + X_I = 480$ mg/L)

With these values , biodegradable total COD:

$COD_{biodeg} = S_S + S_H + X_S$ = around 650 mg/L (L_0 value that will be used in BOD ultimate equation)

Approximate BOD/COD value is found from the plant's measurements.

(for 945 mg /L COD, BOD_5 value is 435 mg/L)

$BOI_5 = L_o (1-e^{-k.5})$

$435 = 650 (1-e^{-k5})$ equation was used to find k value of 0.22 day^{-1}. This value is similar to domestic wastewater values.

4.3.3.Evaluation About the Aeration System

Mechanical aerators that are used in biological treatment reactors works in slow or fast modes to maintain required level of oxygen. They work with 18.5 kW/hr in slow mode, 25 kW/hr in fast mode.

V_T volume is 675 m^3

V_o volume is 545 m^3 (675-260/2)

Oxygen given to system is 1.5-2.0 kg O_2/kWh , on average 1.7 kg O_2/kWh

In slow mode;

18.5 * 1 h * 1.7 = 32 kg O_2/hr

When this value is divided to V_T ,

oxygen given per m^3 :47 mg O_2/L.h

In fast mode this value:

25 * 1 h * 1.7 / V_T = 62 mg O_2/L.h

$OTR / V = k_{La} (C_{So} – C_{O2})$

$47 = k_{La} (8-2)$

$k_{La} = 7.83 \times 24 = 188\ h^{-1}$ (for slow mode) and

$k_{La} = 248\ h^{-1}$ (for fast mode)

Daily oxygen transfer rate is always higher than 5 mg/L

When these results were compared to OUR data coming from the model, it is seen that aeration of the Biological Reactors are around 1.5 times higher than needed.

5. CONCLUSIONS

In this study, the wastewaters of the pharmaceutical plant in Turkey which has a biological treatment system had been investigated. It is 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 stochiometric constants of the mixed culture. Relevant kinetic and stochiometric coefficients were achieved using ASM1 pattern in the study. By comparing real and calculated values, it's been aimed to propose better operational modes for more efficient treatment.

The investigated plant produces %15 of all drugs in Turkey. With 8 different production lines, in 21 different dosage forms, around 250000000 packages of drugs are produced per year. Plant produces 95000 m^3 of wastewater each year for domestic and industrial purposes. 75000 m^3 of it is used for production processes. It has biological treatment system having two cylindirical reactors each having volumes of 675 m^3 with aerators. Overall COD level of the combined wastewaters of domestic and industrial based is around 900 mg/L, a level which is quite representative for the pharmaceutical industry.

Conventional characterization tests, particle size distribution analysis by sequential filtration / ultrafiltration were conducted on both samples taken from influent and effluent of the biological treatment system. Respirometric analysis and modelling studies using Aquasim ASM1 Model were conducted on the second influent.

Results of these tests show that COD removal efficiency of treatment is around %90.According to Water Pollution Control Regulation, dicharge limits for COD and pH levels are 150 mg/L and 6-9 respectively. Treatment system generates effluent at COD level of around 100 mg/L and pH of 7 as measured experimentaly.These values are concordant with the plant measurements and meets the Water Pollution Control Regulation criteria. It is concluded that aerobic biological treatment is effective and

sufficient for the wastewater generation of the plant.

The results of the model calibration related to the assessment of kinetic and stoichiometric coefficients reveals the biodegradable nature of the pharmaceutical wastewater.

From PSD analysis results it is seen that biological treatment was effective at both ends of size distribution, with almost total removal at the particulate range and most of the soluble portion. Biological treatment was successful in removing all size ranges except soluble inert COD fraction.

Results of optimization studies of modeled and calculated data shows the concordancy of the both results in most of the months. Results are more adaptable for the months that influent CODs are more close to average COD.

Calculated k value during optimization studies, 0.22 day^{-1}; is similar to domestic wastewater values as expected.

Evaluation of aeration system shows the excess aeration around 1.5 times than needed. This fact can be considered by the plant in the future feasibility studies.

Finally, performing PSD-based COD fractionation of biological treatment effluents of various wastewaters with different characteristics should be considered as an interesting issue for further research.

REFERENCES

Arslan-Alaton,I., &Doğruel,S (2004). Pre-treatment of penicillin formulation effluent by advanced oxidation processes.Journalof Hazardous Materials, 112, 105-113.

A. D. Deshpande, K. G. Baheti and N. R. Chatterjee*,Padmashree Dr D. Y. Patil Degradation of *b*-lactam antibiotics,Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411 018, India

Carucci Alessandrai ,Cappal Giovanna, Piredda Martina , 2006,Biodegradability andToxicity of Pharmaceuticals in Biological Wastewater Treatment Plants, Journal of Environmental Science and Health Part, 41:1831-1842

Çetecioğlu Zeynep, 2011Evaluation of Anaerobic Biodegradability Characteristics of Antibiotics and Toxic/Inhibitory Effect on Mixed Microbial Culture, Msc Thesis , İstanbul Teknik Üniversitesi Çevre Müh. İstanbul

Çokgör Emine Ubay; Alaton İdil Aslan; Karahan Özlem; Doğruel Serdar; Orhon, Derin ,2004 , Biological treatability of Raw and Ozonated Penicillin Formulation Effluent, Journal of Hazardous Materials 159-166

Dochain D., Vangolleghem P.A.,Daele Van M., 1995, Structural identifiability of biokinetic models of activated sludge respiration, Wat.Res.29 2571-2579

Duman,E., 2006 İlaç Endüstrisi Atıksularının Fenton Oksidasyonu İle Arıtılabilirliğinin Araştırılması, Yük. Lis. Tezi , Hacettepe Üniversitesi Çevre Müh. A.B.D.,Ankara

Dulekgürgen, Ebru; Doğruel, Serdar; Karahan, Özlem; Orhon, Derin, 2006,Size Distribution of Wastewater COD FRactions as an Index for Biodegradability, Water Research 40 -273-282

Gürses, Filiz. Treatability of Antibiotic Formulation Effluents by Fenton-Like and Photo-Fenton-Like Advanced Oxidation Processes.Msc Thesis

Henze M, Gujer W, Mino T, van Loosdrecht MCM (2000) Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA Scientific and Technical Report No. 9. IWA Publishing, London**.**

IEIS İlaç Endüstrisi İşverenler Sendikası, Sektör Raporu, 2008

Insel G, Orhon D, Vangolleghem P.A., 2003, Identification and Modelling of Aerobic Hydrolysis mechanism-application of optimal experimental design, J Chem.Tech. Biotech. 78(4)437-445

ISO 6060, Water Quality, Determination of the Chemical Oxygen Demand, 1986

Jones O.A.H., Voulvoulis N; Lester J.N. 2005,Human Pharmaceuticals in Wastewater Treatment Processes, Critical Reviews in Environmental Science and Technology, 35:401-427

Katipoğlu Tuğçe, 2012 Evaluation of Biodegradation Characteristics and Toxicity/Inhibiton Effects of Xenobiotics on Nitrification Systems, Msc Thesis , İstanbul Teknik Üniversitesi Çevre Müh. İstanbul

Kor, Gökçe 2012 Acute and Chronic Effects of Sulfamethoxazole On the Biodegradation of Acetate Under Aerobic Conditions At Low Sludge Age, İstanbul Teknik Üniversitesi Çevre Müh. İstanbul

Kummerer, K., 2004.Pharmaceuticals in the Environment, Ed Kummerer, K., 2nd Ed.Springer, Verlag

Mogens Henze, Modeling of Aerobic Wastewater Treatment Processes, Environmental Biotechnology. Concepts and Applications. Edited by H.-J. Jördening and J. 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Orhon Derin; Artan Nazik 1994,Modelling of Activated Sludge Systems, Technomic Publishing Company, USA

Orhon Derin; Ubay Çokgör Emine, 1997, COD Fractionation in Wastewater Characterization –The State of the Art, J.Chem. Tech.Biotechnology, 68, 283-293

Ötker, Havva Merih 2002, Oxidative Treatment of Antibiotics in Pharmaceutical Effluents,Msc.Thesis Boğaziçi University,İstanbul

Reichert P, Ruchti J,Simon W, 1998, Aquasim 2.0 Swiss Federal Institute for Environmental Science and Technology (EAWAG), CH-8600, Duebendorf, Switzerland

Samuk, Beyza 2002 İlaç Endüstrisi Formulasyon ve Antibiyotik Atıksularının Biyolojik Arıtılabilirliğinin Arttırılması, Yük. Lis. Tezi , İstanbul Teknik Üniversitesi Çevre Müh. İstanbul

Sert, D., 2006**,** İlaç Endüstrisi Atıksularında Fenton Prosesi İle Renk Ve KOİ Giderimi, Yük.Lis. Tezi,İstanbul Ünv. Fen Bilimler Ens. Çevre Müh. A.B.D., İstanbul

Sözen S, Çokgör EU, Orhon D, Henze M (1998) Respirometric analysis of activated sludge behaviour—II. Heterotrophic growth under aerobic and anoxic conditions. Water Res 32:476–488

Standard Methods, 2005.Standard methods for the Examination of Water and Wastewater, 21st ed,American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC

Ternes, T.A., Joss, A (Eds), 2006. Human Pharmaceuticals , Hormones and Fragnances.The Challenge of Micropollutants in Urban Water Management.IWA Publishing, London

USP Pharmacopeial Forum, 1260-1272 IN-PROCESS REVISION Vol. 35(5) [Sept.–Oct. 2009]

Yüce,A.2001. Antimikrobik ilaçlara direnç kazanma mekanizmaları, Cilt 14, Sayı:2 S:41-46

Van der Meer JR, de Vos WM, Harayama, S., and Zehnder AJB., 1992, Molecular Mechanisms of Genetic Adoptation to Xenobiotic Compounds.Microbiological Rewievs, 56 (4), 677-694

Zanowiak P 1982, pharmaceuticals Kirk-other encyclopedia of chemical technology, 17, 3.edition

http://water.epa.gov/scitech/wastetech/guide/pharm/upload/1998_08_27_guide_pharm_techdev_ch3.pdf

CURRICULUM VITAE

Name Surname: Dora Olcay

Place and Date of Birth: Istanbul, 1970

Address: Yeşilbahar Sok., Zeynep Hn. Apt, No:48; D:4 Göztepe/Kadıköy, İstanbul

E-Mail: dora_olcay@hotmail.com

B.Sc.: Boğaziçi University, Molecular Biology and Genetics

Professional Experience and Rewards: Working on Eczacıbaşı-Baxter Hospital Products Company as Microbiology Lab Senior Specialist