İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECTS OF OZONATION AND THE O_3/H_2O_2 PROCESS ON THE BIOLOGICAL TREATMENT OF SYNTHETIC PENICILLIN FORMULATION WASTEWATER

M.Sc. Thesis by

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Date of submission : 26 April 2004

Date of defence examination: 21 May 2004

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MAY 2004

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SENTETİK PENİSİLİN FORMÜLASYON ATIKSULARININ BİYOLOJİK ARITIMI ÜZERİNE OZONLAMA VE O_3/H_2O_2 PROSESLERİNİN ETKİLERİ

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Tezin Enstitüye Verildiği Tarih : 26 Nisan 2004
Tezin Savunulduğu Tarih : 21 Mayıs 2004

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MAY 2004

PREFERENCES

It is my pleasant duty to record my thanks to my valuable teacher Associate Professor Emine Ubay Cokgor for all her support and patience during the process and finalizing of my project

I shall also express my gratitude to Research Assistant M.Sc. Özlem Karahan and Associate Professor Idil Arslan Alaton who helped in many ways in the preparation and production of my thesis.

I am also thankful to M.Sc. Seda Oktay, Muazzez & Robert Antonios and all my family for their continuous support.

And finally I am particularly indebted to my biggest supporter, my dear mother Reha Saruhan with my deepest love and thanks.

MAY 2004 HANDE SARUHAN

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NOTATION LIST

b_H : Endogenous respiration rate of heterotrophs [T^{-1}]
b_{STO} : Endogenous respiration rate of storage products [T^{-1}]
BOD_5 : Biochemical oxygen demand [ML^{-1}]
COD : Chemical oxygen demand [ML^{-1}]
f_{SI} : Fraction of the soluble metabolic products
f_I : Fraction of the particulate metabolic products
F/M ratio : Food to microorganisms ratio
K_O : Half saturation constant of oxygen [ML^{-1}]
K_S : Half saturation constant of substrate [ML^{-1}]
k_{STO} : Maximum rate of storage [T^{-1}]
K_{STO} : Half saturation constant of storage [$MCODMCOD^{-1}$]
OUR : Oxygen uptake rate [$ML^{-1}T^{-1}$]
S_O : Oxygen concentration [ML^{-1}]
S_S : Readily biodegradable substrate concentration [$MCODL^{-1}$]
$S_{Tinitial}$: Initial soluble COD concentration [$MCODL^{-1}$]
$S_{Tutilized}$: Utilized soluble COD concentration [$MCODL^{-1}$]
X_H : Concentration of heterotrophic biomass [ML^{-1}]
X_{STO} : Concentration of storage products [ML^{-1}]
Y_H : Growth yield [$MCODMCOD^{-1}$]
Y_{STO} : Storage yield [$MCODMCOD^{-1}$]
μ_H : Maximum growth rate [T^{-1}]

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SENTETİK PENİSİLİN FORMÜLASYON ATIKSULARININ BİYOLOJİK ARITIMI ÜZERİNE OZONLAMA VE O_3/H_2O_2 PROSESLERİNİN ETKİLERİ

ÖZET

Bu çalışmada Penisilin Prokain G atıksularının (pH= 7 ve1800 mg /l. saat ozon akım oranı) O_3 ve O_3/H_2O_2 ileri oksidasyon prosesleri uygulaması incelenmiştir. Penisilin Prokain G atıksuyunun KOI ve BOI_5 değerleri yaklaşık olarak, sırasıyla, 1175 ve 50 mg/l değerlerindedir. Biyolojik arıtılabilirlik çalışmaları yalnız sentetik atıksu ve ham, ozonlanmış ve perozonlanmış penisilin formulasyon çıkış suları ilave edilmiş atıksularla yürütüldü. Ham ve 60 dakika ozonlanmış reaktörün performansı paralel sonuçlar verdi, toplamda % 80 KOI giderimi oldu. Biyolojik sistemde hidroliz mekanizmasının Penisilin Prokain G çıkış suları için çok önemli olduğu görüldü.Etkili Penisilin Prokain G giderimi için 24 saatten fazla bir bekletme süresi gerekmekte.5 ve 20 dakika ozonlanmış numunelerle yapılan deneylerde maximum spesifik büyüme oranında bir inhibisyon etkisi görülmektedir. Ancak toplam verime bakıldığında sistem deney sonunda kendini düzeltmektedir. 5, 20 ve 40 dakika ozonlanmış atık sularla yürütülen respirometrik analizlerde yaklaşık olarak %60 toplam verim elde edilmiştir. Bu verim ile karşılaştırma yapıldığında, 60 dakika ozonlanmış atıksu ile yürütülen deneyde, uzun süreli ozonlamanın inhibisyon etkisi yüzünden, toplam giderim veriminde yaklaşık %10 bir düşüş olduğu görülmüştür. Çalışmalar, 5, 20 ve 40 dakika perozonlanmış penisilin fraksiyonunda (toplam KOI nin %25 'i) bir arıtım olmadığını ve sentetik atık suyun tamamen ayrıştığını gösterdi. Respirometrik çalışmalar paralel ve güvenilir sonuçlar verdi. Bu sonuçlara gore 60 dakika perozonlanmış penisilin formulasyonu atık suyunun oksijen tüketiminde %20 bir artış olduğu gözlendi. 5, 20 ve 40 dakika perozonlanmış penisilin formulasyonu atık suyundaki sentetik atık su fraksiyonunda bir inhibisyon gözlenmedi, ancak 60 dakika perozonlanmışda kalıcı oksidasyon ürünleri yüzünden inhibisyon görüldü. Modelleme çalışmaları, respirometrik testlerin açıklanmasında, Aktif Çamur Modelindeki (ASM3) inhibisyon kinetikleri hakkında deneysel destek ve bilgi sağladı.

EFFECTS OF OZONATION AND THE O_3/H_2O_2 PROCESS ON THE BIOLOGICAL TREATMENT OF SYNTHETIC PENICILLIN FORMULATION WASTEWATER

SUMMARY

In the present study, oxidative pre-treatment of Procain Penicillin G effluent via ozonation and ozonation with H_2O_2 at pH of 7 and ozone feed rate of 1800 mg/l in 1 hour was investigated. COD and BOD_5 values of Procain Penicillin G were approximately 1175 and 50 mg/l, respectively. Biological treatability studies were performed with a synthetic wastewater alone and supplemented with raw, ozonated, and ozonated with H_2O_2 penicillin formulation effluents. Raw and 60 min ozonated reactor performance gave parallel result, with overall COD removal of 80%. It was seen that hydrolysis mechanism for penicillin procaine G effluent was very crucial for biological system. For effective biological penicillin procaine G removal, more than 24 hours retention time is necessary. Experiments on the biological reactor with 5 and 20 min. ozonated wastewater with synthetic addition were showed that there was an inhibition effect on maximum specific growth rate. The system was recovered at the end of the experiment, according to overall efficiency. Respirometric analyses performed with 5, 20 and 40 min ozonated wastewaters were shown that overall efficiency was approximately 60%. The removal efficiency obtained for 60 minutes ozonated with synthetic wastewater was decreased approximately 10 % according to 5, 20 and 40 min ozonation results because of long terms of ozonation effect on inhibition. The studies showed that no degradation of 5, 20 and 40 min ozonated with H_2O_2 penicillin fraction (25% of total COD) occurred, and degradation of the synthetic wastewater was completely treatable Respirometric studies conducted gave parallel and reliable results found as 20 % decrease in the total oxygen consumption rate established for 60 min ozonation with H_2O_2 penicillin formulation effluent compared to the results obtained from fill and draw reactor. No inhibition of the synthetic fraction was observed for the 5, 20 and 40 min- ozonated with H_2O_2 penicillin formulation effluent, whereas inhibition re-appeared for the 60 min-ozonated with H_2O_2 penicillin effluent as a consequence of recalcitrant oxidation product accumulation. The modeling study provided experimental support and information on inhibition kinetics in Activated Sludge Model No.3 (ASM3) by means of respirometric tests.

1 INTRODUCTION

1.1. Aim

Pharmaceutical industries have an important role for the pollution of the environment. The sources of antibiotics in natural water systems may be manufacturing operations in pharmaceutical industry and therapeutical use of them for human and animals. Pharmaceutical industry often generates high strength wastewater changing in character and quantity depending upon the used manufacturing processes and seasons. Among the wastewaters from different operations in this industry, formulation effluent that rises from washing of equipment is characterized by small effluent flow and low pollution load. Recent studies showed that antibiotics, which are produced to restrain bacteria in humans and animals, have been found in surface water and sewage treatment plant effluents. These results inferred that antibiotics cannot be completely eliminated during biological treatment and they are emitted into receiving water systems. In an environment aspect, the most important effect of antibiotics is the exerting toxic effects to aquatic organisms that would upset the ecological balance. Moreover, the presence of antibiotics in natural systems leads to the development of multi-resistant strains of bacteria. Hence, it is necessary to treat the effluents containing antibiotics before discharging into biological treatment process and receiving water systems. However the effluents originated from the formulation of antibiotics have low biodegradability and bioinhibitory compounds since they contain almost only active substance. Hence a chemical pretreatment is necessary for pharmaceutical effluents, like antibiotic formulation, containing high concentrations of bioinhibitory compounds. Most known practices and technologies have appeared to be inappropriate for pharmaceutical effluent treatment and hence more advanced and effective oxidative treatment processes such as the Fenton's reagent (Fe^{2+}/H_2O_2/acidic pH), ozonation at elevated pH, or H_2O_2 – assisted ozonation all being categorized and described as Advanced Oxidation Processes (AOPs).

Recently, respirometry has been introduced as a useful tool for the identification of organic fractions with different biodegradation characteristics in wastewater for the assessment of the appropriate values of coefficients defining process kinetics and stoichiometry and for model calibration of experimental data defining the response of activated sludge in wastewater treatment. However, respirometric applications have so far been quite limited in testing the toxicity effects of different pollutants on activated sludge processes. Consequently, no practical application of a toxicity control strategy for activated sludge systems is presently available the literature.

Aim of the study is to provide an evaluation of the biological treatability of raw, ozonated, and ozonated with H_2O_2 synthetic wastewaters.

1.2. Scope

In the thesis, the following steps will be conducted:

- Conventional characterization of raw, ozonated, and ozonated with H_2O_2 syntetic penicillin wastewaters.
- Acclimation to raw, ozonated, and ozonated with H_2O_2 syntetic penicillin wastewaters.
- Respirometric analysis on control, raw, ozonated, and ozonated with H_2O_2 syntetic penicillin wastewaters.
- Evaluation of the results
- Modelling of the results
- Overall evaluation

2 PHARMACEUTICAL INDUSTRY

2. 1 Introduction

Since the 1950's, Turkish pharmaceutical industries have shown considerable progress. With the development in pharmaceutical industry, nowadays, %60 of demand has been met by Turkey's own production. In 1984, Good Manufacturing Practices (G.M.P) became valid in Turkey. By this way, especially pharmaceutical industries made required investments; they sped up their technological improvements and reached the qualities of the technologies in Europe and U.S.A. This improvement has also contributed to Turkish economy. In order to compete with European countries and U.S.A., pharmaceutical industries in Turkey should give importance to the environment and human health.

2.2 Categories in Pharmaceutical Industries

The production of pharmaceutical products can be divided into five main stages;

1. Research and development
2. Chemical Synthesis
3. Biological and Natural Extraction
4. Fermentation
5. Formulation

2.2.1 Research and development

New drug research and development are complex, costly and time-consuming efforts. It is based on developing new drug substances, pharmaceutical applications and products. Research areas include chemical, microbiological and pharmaceutical researches.

New drug in development involves four phases; Pre-Clinical Research and Development, Clinical Research and Development, Review of New Drug Application and Post Marketing Surveillance.

Pre-Clinical Research and Development begins after a promising compound has been discovered. In this phase, laboratory and animal tests are made in order to determine whether the compound is biologically active and safe. In the Clinical Research and Development, attention to waste minimization considerations is most effective. After this phase, a New Drug Application is made and this should include the data that the drug is safe and effective for use.

The development of a new drug requires the cooperative efforts of a large number of trained personnel specializing in medical, organic and analytical chemistry; microbiology; biochemistry; physiology; pharmacology; toxicology; chemical engineering; and pathology.

2.2.2 Chemical Synthesis

Chemical synthesis is the process of manufacturing pharmaceuticals using organic and inorganic chemical reaction. Most of the pharmaceuticals are prepared by chemical synthesis, generally by a batch process. Vitamins, antibiotics and antihistamines are just a few examples of the bulk pharmaceutical substances made by this process.

In a typical manufacturing plant, one or more batch reactor vessels is used in a series of reaction, separation and purification steps to make the desired end product. Many types of chemical reactions, recovery processes and chemicals are employed in order to produce a wide variety of drug products each conforming to its own rigid product specification.

It is not possible to provide a single process flow diagram for this industry because each bulk pharmaceutical substance is different in its production and several intermediate materials may be produced within the manufacturing process before the production of the final product.

In chemical synthesis the waste process solutions and vessel wash water may contain residual organic solvents. Because of high toxicity, biological treatment is not appropriate for this wastewater. Therefore, chemical pretreatment may be necessary for some chemical synthesis wastewater.

A schematic illustration of chemical synthesis having five steps including reactors, separation, crystallization, purificacation and drying is shown in Figure 2.1.

Reactors: Raw materials or ingredients are used to produce the intermediate or bulk substances. The condensers may be attached to reactors for recover solvents or control the pollution in order to remove volatile organics.

Separation: Several separation ways in pharmaceutical industries are extraction, decanting, centrifugation and filtration. By these ways, intermediates or bulk substances are separated from the reaction solution and pollutants are removed.

Crystallization: This is the most commonly used separation technique. Within this method, a supersaturated solution is created which crystals of the desired compound are formed in it. Further separations of the crystals are done by centrifuging or filtration.

Purification: After the separation process, an intermediate or bulk substance has to be purified. Purification is again made through several separation processes or recrystallization.

Drying: Drying of the intermediate or bulk substances are the final step in the chemical synthesis. It is employed in order to dry up the solvents from the solids.

2.2.3 Biological and Natural Product Extraction

Biological and natural product extraction is the production of pharmaceuticals from natural material sources such as roots, leaves and animal glands. This process is employed for producing allergy relief medicines, insulin, morphine, anti-cancer drugs or other pharmaceuticals with unique properties. The amount of finished drug product is small compared to the amount of natural source material used. Since there are large volume reductions in this process, several operation stations are used. In each station inert material is removed from the active ingredient. These active

ingredients are recovered by precipitation, purification and solvent extraction methods. Simplified process flow diagram for natural/biological extraction is given in Figure 2.2.

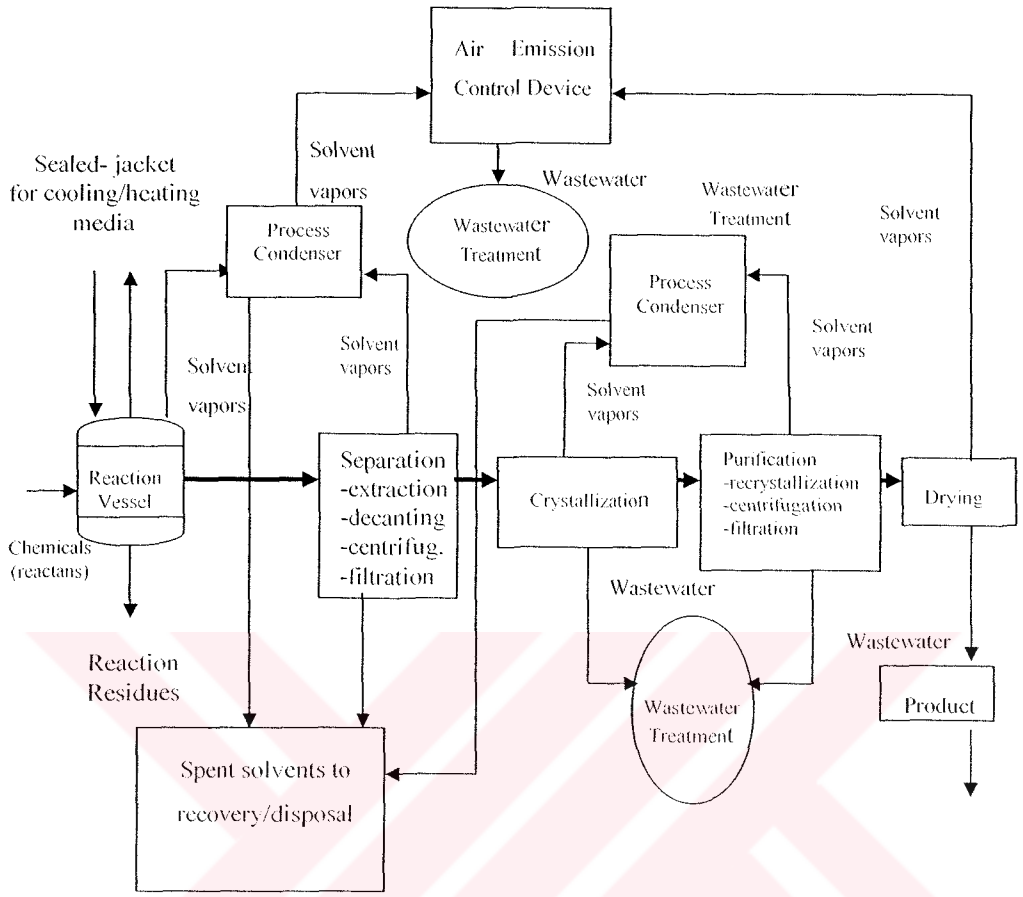

Figure 2.1. Simplified Process Flow Diagram for Chemical Synthesis

2.2.4 Fermentation

Steroids, antibiotics and certain food additives (such as vitamins) are typically produced using fermentation processes. Fermentation processes involve three main steps: inoculum and seed preparation, fermentation, and product recovery (Figure 2.3.). In this process, microorganisms (bacteria, yeast or fungi) are typically inoculated in a dense liquid. Microorganisms are acclimated to an environment (a constant temperature, pH, oxygen...etc.) for growing rapidly. These microorganisms produce the desired product such as antibiotic, steroid, and vitamin.

Seed Preparation: This step is the beginning of the fermentation. Within this step, microbial strain is introduced to primary seed fermentation. The cells are transferred

to a seed tank which operates like a full scale fermenter and is designed for maximum cell growth.

Fermentation: When the fermentorinoculum is ready, it is charged into a sterilized fermentor. Once these sterilized nutrient materials are added to the vessel, fermentation commences. Under this process, fermentor is agitated and aerated with sterile air via a sparger. Dissolved oxygen content, pH, temperature and several other paremeters are carefully monitored throughout the fermentation cycle. Final step, the fermentor "broth" is produced that is later filtered or centrifuged to remove the solid residues.

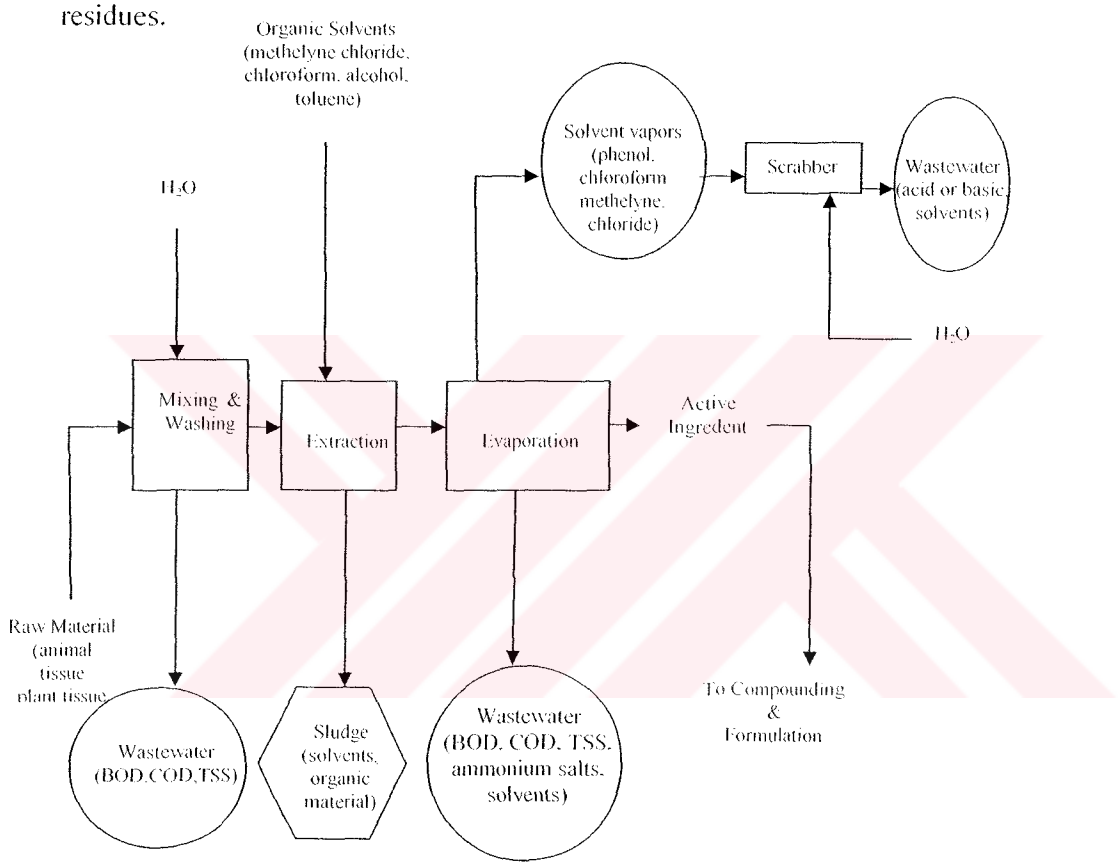

Figure 2.2 Simplified Process Flow Diagram for Natural/Biological Extraction

Product Recovery: The filtrate which obtained from the end of the fermentation is processed to recover the desired product in product recovery process. Product recovery has three different ways: solvent extraction, direct precipitation and ion exchange or adsorption. In addition, sometimes the active material may contain cells of the microorganisms that will be broken by heat or ultrasound. In all of the three steps, product is recovered by special methods.

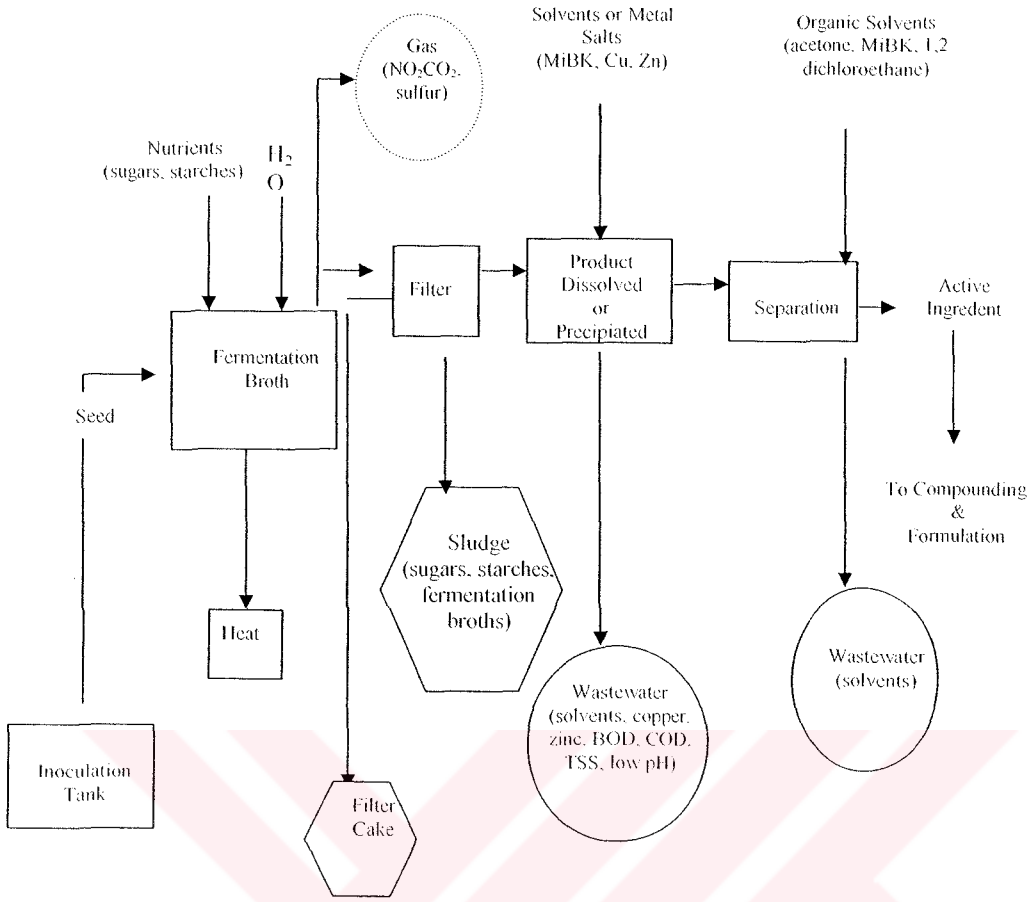

Figure 2.3 Simplified Process Flow Diagram for Fermentation Process

2.2.5 Formulation

Formulation is the last step in the production process. Dosage forms such as tablets, capsules, liquids, creams and ointments are prepared in pharmaceutical formulation process (Figure 2.4.). During this step, produced bulk substances are converted into their final and usable forms.

The good manufacturing practices (GMP) are conducted to make certain that the drug is safety, its quality and purity characteristics are as required.

Tablets: This is one of the most widely used dosage forms. Tablets account for over 90 percent of all medications taken orally. They produced by mixing the active pharmaceutical ingredient with appropriate filters such as sugar or starch; and

binders, such as corn syrup. When the active ingredient reaches to the proper concentration, a binder is used in order to stick the tablet particles together. If needed, a lubricant such as magnesium sterate or polyethylene glycol can be added in order to make the equipment operation easier.

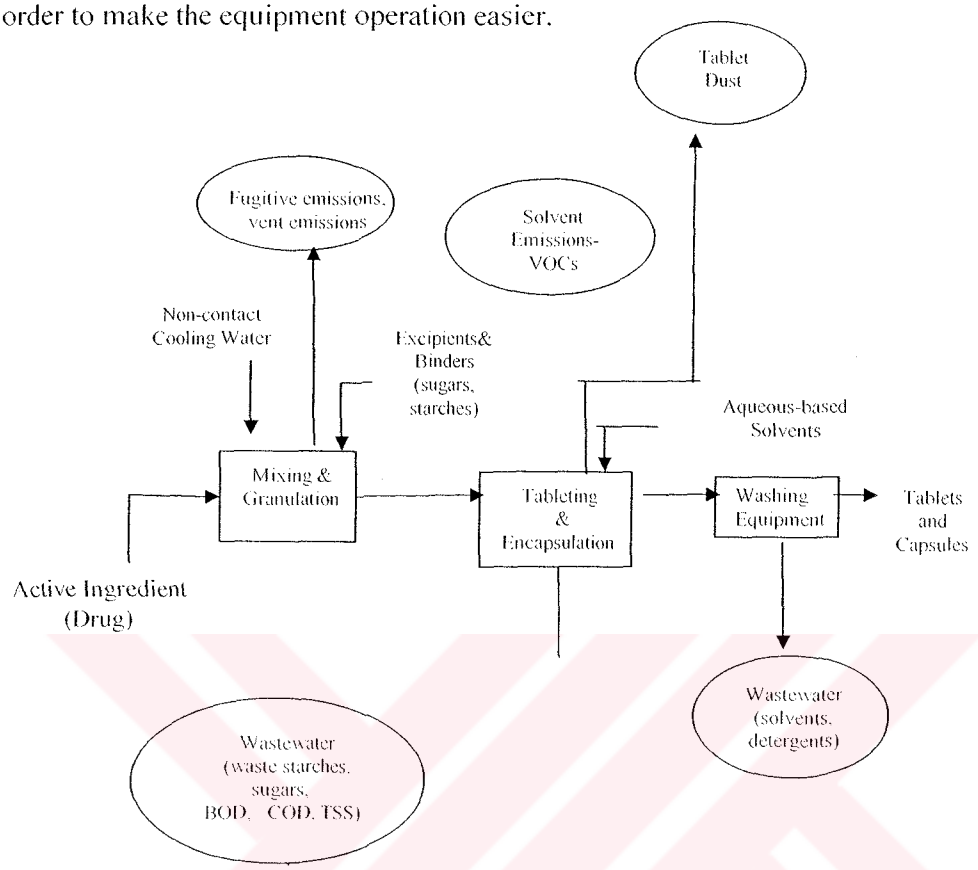

Figure 2.4 Simplified Process Flow Diagram for Compounding and Formulating

Capsules: Capsules are the next most widely used oral dosage form of solid drugs. They are prepared in soft or hard forms. Hard ones are formed by dipping metal pins into a solution of gelatin of a specific temperature. Soft ones are formed by placing two continuous gelatin films between rotary die plates later form two halves of the capsule. The drug, usually a nonaqueous solution or soft mass, is injected into the capsules.

Liquid dosage: Liquid dosage form is prepared for injection or oral use, which includes solutions, syrups...etc. During formulating the liquid product, the ingredients are first weighted and then dissolved in an appropriate liquid. Before final packaging, they are stored in tanks. Sometimes liquid dosage forms are

prepared with preservatives to prevent mold and bacterial growth, but they do not require sterilization if they are considered for oral or topical use.

Ointments and Creams: Ointments and creams are prepared for topical use. This dosage forms are made by mixing the bulk active material with a base such as a petroleum derivative or wax. This mixture is cooled, rolled out, and poured into tubes by machines and packaged.

Finally, the equipments are washed and cleaned in all dosage forms, based on the batch process requirements. However, because of the greasy nature of ointment and cream production, cleaning is often done with detergents.

Wastewater sources of formulation processes are:

- Floor and equipment wash water
- Wet scrubbers
- Spills

Generating wastewater has a low flow rate that between in a range of 80 and 200 m^3/day.

2.3 Pollution Profile

The wastes generated from the production of pharmaceutical materials. Pollution occurs because of cleaning, sterilizing equipment, chemical spill, rejected products and the processes themselves (Table 2.1.) In pollution profile, not only the role of raw materials and their toxicity is important, but also wastes and emissions generated are important.

2.3.1 Raw Materials

Lots of raw materials are required in pharmaceutical industry to produce various medicinal chemicals. Goods are produced in many different ways. Some of them need chemical synthesis; fermentation or some need different raw materials, bulk substances. In addition, there are so many kinds of amounts and methods.

Table 2.1 Pollution Sources in Pharmaceutical Industries

Waste Description	**Process Origin**	**Pollution Sources**
Process liquors	Organic synthesis	Polluted solvents
Spent fermentation broth	Fermentation processes	Polluted water
Spent natural product raw material	Natural product extraction processes	Tissues
Spent aqueous solutions	Solvent extraction processes	Polluted water
Leftover raw material containers	Unloading of materials into process equipment	Bags, drums, plastic bottles
Scrubber water from pollution control equipment	Dust or hazardous vapor generating	Polluted water
Volatile organic compounds	Chemical storage tanks,drums	Solvents
Out-dated products	Manufacturing operations	Various products
Spills	Manufacturing and labratory operations	Various chemicals
Wastewater	Equipment cleaning, extraction residues	Polluted water
Spent solvents	Solvent extraction or wash practices	Polluted solvents
Used production materials	manufacturing operations	Filters, tubing, diatomaceous earth
Used chemical reagents	R&D operations	Various chemicals
Natural gas combustion products	Steam boilers	Carbon compounds, oxides of nitrogen and sulfur

In fermentation operations, many chemical classifications such as carbohydrates, step liquors, nitrogen, phosphorus, anti-foam agents, various acids and bases are required. These are used as carbon and nutrient sources, as foam control additives and for pH adjustments.

For extraction and purification processes, different solvents, acids and bases are required. Besides, in a chemical synthesis process, organics and inorganic compounds are employed in gas, liquid and solids forms. Plant and animal tissues are also used in order to produce biological and natural extraction products.

Each manufacturing or formulation process is special and shows difference from the others. For example, bulk pharmaceutical reactions require organic solvents to dissolve chemical intermediates and reagents. These solvents must be non-reactive since many pharmaceutical intermediates and reagents have high reactivity. By this way, there will be optimum environment for allowing efficient heat transfer during endothermic and exothermic reactions and also for efficient electron transfer. The most commonly solvent used in pharmaceutical industry is methanol. Others are: ethanol, acetone and isopropanol.

2.3.2 Air Emissions

During pharmaceutical manufacturing and formulation gaseous organic, inorganic compounds and particulates may be emitted. Some of them are classified as hazardous air pollutants.

Since each pharmaceutical plant is special, the type and amount of the generated emissions depends on the operations, manufacturing and formulation processes.

Bulk Manufacturing: Most bulk pharmaceutical substances are produced by batch processes. Estimating typical or annual average emissions is difficult, because different reactants and solvents are used in manufacturing bulk substances. Therefore, generated emissions are predicated on what bulk substance or intermediate are produced and which equipment and raw materials are used. Dryers, reactors, distillation units, storage and transfer of materials are the major sources of emissions. Other sources are: filtration, extraction, centrifugation and crystallization.

Formulation: Most of the particulates and volatile organic compounds are generated during mixing, compounding, formulation and packaging steps of the production. They will be dangerous to the workers.

The quantity and type of the compound emitted depend on the operation employed. For example, sometimes formulation may not emit volatile organic compounds. Another method may be used for that.

2.3.3 Wastewater

Water is used both for process and non-process purposes in a pharmaceutical industry. Like air emissions, waste water may show differences. The use, discharge practices and the type of waste water may consider different.

Process water which used during production processes has either a direct contact with or originated from the use of any raw material or production of an intermediate, final product or waste. However, process waste may used or generated during the reaction or used to clean the equipment. Non-process waste water includes other sources from process wastewater.

2.3.4 Solid Wastes

Wastes which formed during the pharmaceutical manufacturing steps may be hazardous or non- hazardous. Both wastes include out-dated raw materials or products, spent solvents, reaction residues, used filter media, used chemical reagents, dusts from filtration, raw material packaging wastes etc.

Filter cakes and spent raw materials (plants, roots, animal tissues etc.) from fermentation and natural product extraction are two of the largest sources of residual wastes.

2.4 Pollution Prevention and Wastewater Treatment

2.4.1 Pollution Prevention

The minimization of waste in origin where pollution becomes is the best way to decrease pollution. Depending on the processes and materials involved, large amounts of extraction waste and fermentation media are generated which may contain hazardous components. Source reduction is always the most desirable option.

Effects of pollution on environment can be reduced by reducing material inputs, improving management practices and employing substitution of toxic chemicals.

Especially in fermentation and natural product extraction, within the bulk manufacturing process, large amounts of residual waste is formed in the pharmaceutical industry. Chemical synthesis processes generate wastes including hazardous spent solvents and reactants, combined with residual wastes such as reaction residues.

Source reduction is one method that can be used in the aim of reducing these wastes. It can be done by: material substitution, process modification, good operating practices and recovery and recycling.

2.4.1.1 Material Substitution

In order to reduce the volume or toxicity of waste generated , one or more raw materials used in production are changed in material substitution. However, product formulation is very difficult due to the testing required to make certain that the reformulation has the same therapeutic effect, stability and purity profile as the original drug. Material substitution has been used successfully in table coating operations to reduce hazardous waste generation.

2.4.1.2 Process Modification

This is another source reduction opportunity that can be achieved through modification or modernization of the existing process. For example, physical material losses may be reduced by redesigning the chemical transfer systems. Replacing gas pressurization with a pumped transfer eliminates the tank pressurizing step and its associated material losses.

2.4.1.3 Good Operating Practices

The good operating practices can help reduce hazardous and other waste generation and material losses. Management initiatives can support new ideas from experienced employees, which result in reduction or recycling of waste. All manufacturing employees must be trained with a waste management program which includes safe

operating procedures, equipment use, process control specifications and industrial hygiene. Another good operating practice is effective production and maintenance.

2.4.1.4 Recovery and recycle

Recovery and recycling include recovering used materials for another use, and removing pollutions from waste to obtain relatively pure substances. With this approach waste leaving the plant and costs are reduced.

2.4.2 Wastewater Treatment

The major wastewater treatment technologies used in the pharmaceutical manufacturing industry include (Environmental Protection Agency, 1998) ;

- Advanced biological treatment
- Multimedia filtration
- Polishing pond treatment
- Cyanide destruction
- Steam stripping and steam stripping with rectification
- Granular activated carbon adsorption
- pH adjustment/neutralization
- Equalization
- Air stripping
- Incineration

Advance Biological Treatment: Advance biological treatment is used in the pharmaceutical manufacturing industry to treat BOD_5, COD, TSS and to degrade various organic constituents. To provide reduction of ammonia in the waste water using advanced biological treatment, nitrification is necessary.

Multimedia Filtration: Multimedia filtration is used in the pharmaceutical manufacturing industry to reduce TSS in waste water. This technology may also serve to treat BOD in waste water by removing BOD associated with particulate matter.

Polishing Pond: Polishing ponds are used in the pharmaceutical manufacturing industry to remove TSS from waste water using gravity settling. Some BOD removal associated with the settling of suspended solids may also occur. This treatment is not currently common in the industry.

Cyanide Destruction: Several cyanide destruction treatment technologies are currently used in the pharmaceutical manufacturing industry, including alkaline chlorination, hydrogen peroxide oxidation and basic hydrolysis. Most of the facilities apply the cyanide destruction. Technologies in the process area that generates the cyanide-bearing wastewater and most of the treatment units are operated in batch mode.

Steam Stripping and Steam Stripping with Rectification: Steam stripping and steam stripping with rectification are used both in industrial chemical production and in industrial waste water to remove gasses and /or organic chemicals from waste water streams by providing steam to a tray or packed column. Under both Technologies, differences in relative volatility between the organic chemicals and water are used to achieve a separation. Pharmaceutical manufacturing industries which use steam stripping with rectification use them for solvent recovery operations.

Granular Activated Carbon Adsorption: Granular activated carbon adsorption is used in the pharmaceutical manufacturing industry to treat BOD_5, COD, or organic constituents in waste water.

pH Adjustment/Neutralization: Because many treatment technologies used in the pharmaceutical manufacturing industry are sensitive top H fluctuations, pH adjustment or neutralization, may be required as part of an effective treatment system.

Equalization: Because many of the treatment Technologies are performed continuously and some are sensitive to spikes of high flow or high contaminant

concentrations, it is necessary to include equalization as apart of most treatment systems.

Air Stripping: Air stripping is used in the pharmaceutical manufacturing industry to remove volatile organic constitutions from waste water. Air stripping can also be used to remove ammonia from wastewater. This technology is not common in the industry, and its use has decreased due to increasingly strict air emission regulations. Because the standard air stripper design simply transfers pollutants from water to air, the agency does not regard it as an acceptable treatment technology and is not including air stripping as part of the technology base of any of the regulatory options.

Incineration: Incineration is used in the pharmaceutical manufacturing industry to treat organic and inorganic constituents in waste water. Because incineration is costly and energy-intensive when used to treat high water content streams and does not allow for direct recycle or reuse of constituents contained in waste water, the agency is not including incineration as part of the technology basis for any of the regulatory options.

2.5 Fate of Pharmaceutical Drugs

After application, drugs are absorbed by humans or animals and are subject to metabolic reaction such as oxidation, reduction, hydrolysis...etc. (Hirsch et al., 1999) The degree of metabolites could be changed according to application time or health of patient and a range of 50-90 per cent of drug is excreted in unchanged form or metabolites might be reconverted to their parent compound after leaving the patient (Kümmerer et al., 2000; Wollenberger et al., 2000;Hirsch et al.,1999)

The drugs used by humans and its metabolites will be discharged to the sewer systems together with the urine and enter the sewage treatment plant (Figure 2.5. a-d) Outdated or residue pharmaceutical drugs also can be disposed of down household or hospital drains (Figure 2.5. b-c)

The fate of drugs and the metabolites of the parent drug in the sewage treatment plants may be divided into three groups (Hallig-Sorensen et al., 1998);

1. The drug or its metabolites might be readily biodegradable and mineralized by

microorganisms to carbon dioxide and water,

2. The drug or its metabolites could be more or less determination in the sewage treatment plant. A part of substance will be kept in the sludge and can cause the pollution of soil and ground water.(Figure e-f, e-h)

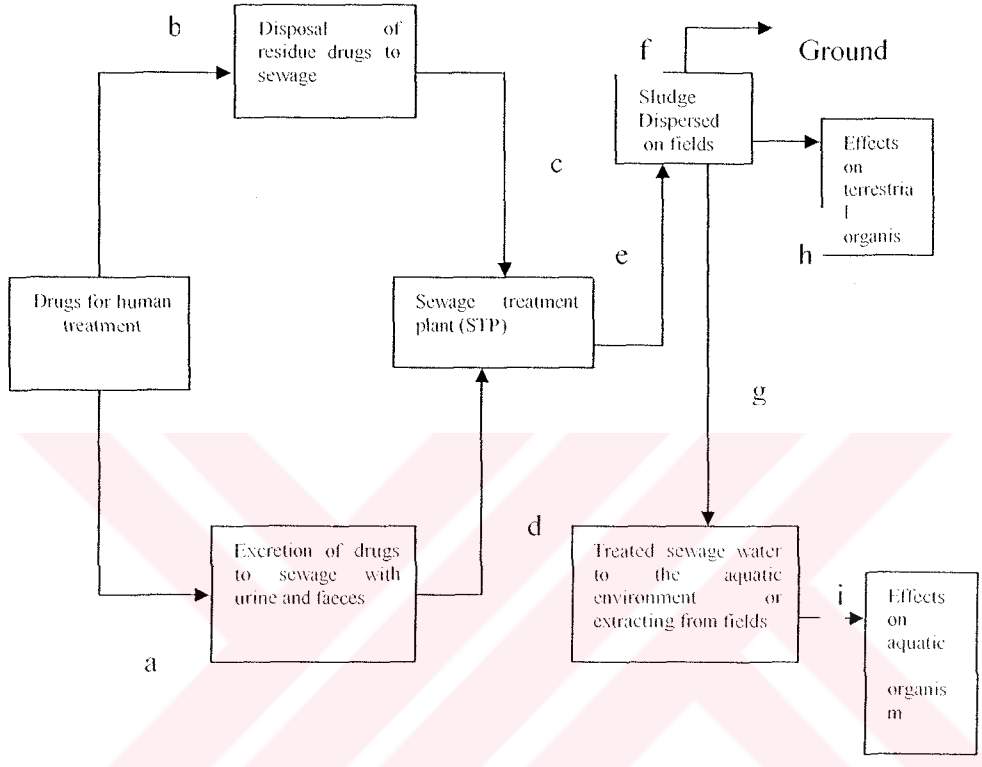

Figure 2.5 Fates of Pharmaceuticals Used for Human Treatment

3. Some pharmaceutical drugs that are uncontrollable to degradation and transformation have the potential to reach the aquatic environment.(Figure, i) Elimination of this kind of drugs in municipal sewage treatment plants are often uncompleted and elimination rates as ranging between 60 and 90 per cent (Ternes, 1998)

2.6 Antibiotics

Antibiotics are an important group of pharmaceutical substances in today's medicine. Biodegradability of the drug substances is an important characteristic in terms of removal of these substances from sewage water. Antibiotics are non-biodegradable and have the potential to survive sewage treatment.

Today antibiotics are an important group of pharmaceuticals. In addition to the treatment of human infections, they are also used in veterinary medicine. Recently, there has been a growing interest in the presence of pharmaceutical substances in the aquatic environment. A large variety of drug residues were found in sewage treatment plant effluents and surface waters.

The literature on antibiotics is much more developed among all other pharmaceutical drugs and substances, because of its serious final effects on aquatic and terrestrial environment. Effects of antibiotics on organisms (e.g. bacteria, algae, Daphnia magna) have been found not only in high concentrations, but also in low concentrations in chronic tests.

2.6.1 The Properties of Antibiotic Formulation Wastewaters

pH value for antibiotic formulation wastewaters is between a range of 6 to 8, it has a medium strength COD (chemical oxygen demands) values that between 220-1800 mg/l, a low BOD_5 (biological oxygen demands) values that between 0-143mg/l and a low VSS (volatile suspended solids) values that between 120-400 mg/l

3 ADVANCED OXIDATION PROCESSES

3.1 Introduction

During the formulation process of pharmaceutical substances, highly toxic liquid wastes containing recalcitrant organic compounds, which cannot be treated by the biological treatment systems are generated. Advanced oxidation processes are considered as an alternative for the treatment.

Previous studies indicate that antibiotics cannot be completely eliminated during biological treatment and they are emitted into receiving water systems. Most antibiotics are excreted by humans after administration and therefore reach the municipal sewage with the excretions. The various antibiotics were active against different groups of bacteria present in wastewater. (K. Kummerer, A.Al-Ahmad, V. Mersch- Sundermann, 2000)

Advanced oxidation processes are typically applied to wastewater containing non-biodegradable organic compounds, which are toxic or inhibitory to microbial growth. These processes are expensive to install and operate.

3.2 Advanced Oxidation Processes

Advanced oxidation processes have been defined as treatment processes that involve the generation of highly reactive radical intermediates, particularly the hydroxyl radicals (Glaze and Kang, 1989; Legrini *et al.*, 1993).

Some kind of wastewaters include difficult substrates for biological treatment due to their various content of a wide range of organic chemicals which may not be readily metabolized by the microbial steps in the bioreactors (Henry *et al.*, 1996)

Since the chemical oxidation is not economically feasible, its combination with cheaper biological processes is preferred instead of carrying chemical oxidation to the fullest extent of reaction (Eckenfelder, 2000).

Advanced oxidation processes are;

1. Non-photochemical Homogeneous AOPs

- Ozone/OH^-
- Ozone/Hydrogen peroxide
- The Fenton's Reaction (Fe^{+2}/ H_2O_2)

2. Photochemical Homogeneous AOPs

- Ozone/UV-C
- Hydrogen peroxide-UV-C
- The photo-Fenton's Reaction (Fe^{+2}/ H_2O_2/UV-C)

3. Photochemical Heterogeneous AOPs

- Photocatalytic oxidation (e.g.TiO_2/UV-A)

In this study, advanced oxidation processes including O_3 and O_3/H_2O_2 are investigated.

3.3 O_3 and O_3 /H_2O_2 Advanced Oxidation Processes

3.3.1 O_3 Process

Ozone has the ability to oxidize a great number of organic and inorganic materials. The U.S. Environmental Protection Agency has assumed hazardous materials which oxidation chemistries of ozone in aqueous media. Some of these materials are readily oxidized by ozone to produce CO_2 and water, others are easily oxidized to form biodegradable reaction products, while many others are not readily reactive with ozone at all. Although, most of the organic oxidation products of ozone are more

readily biodegradable than the original compounds. Therefore, less toxic than the starting materials.

Ozone may react with dissolved organic substances directly or it may decompose leading to more reactive species such as hydroxyl radicals, which determines the subsequent reactions.

The ozone reaction mechanism is uniquely dependent on the rate of mass transfer into an aqueous system. According top H of reaction medium, reactivity exist in different ways. At high pH, the major reaction mechanism with solutes entails hydroxyl radical, which has little selectively for oxidation of organic compounds. At neutral pH values both direct ozone and hydroxyl radical reactions are important, while at low pH, direct oxidation of solutes by ozone is the controlling reaction (Eckenfelder, 2000).

Decomposition of O_3 in water is given in Figure 3.1.

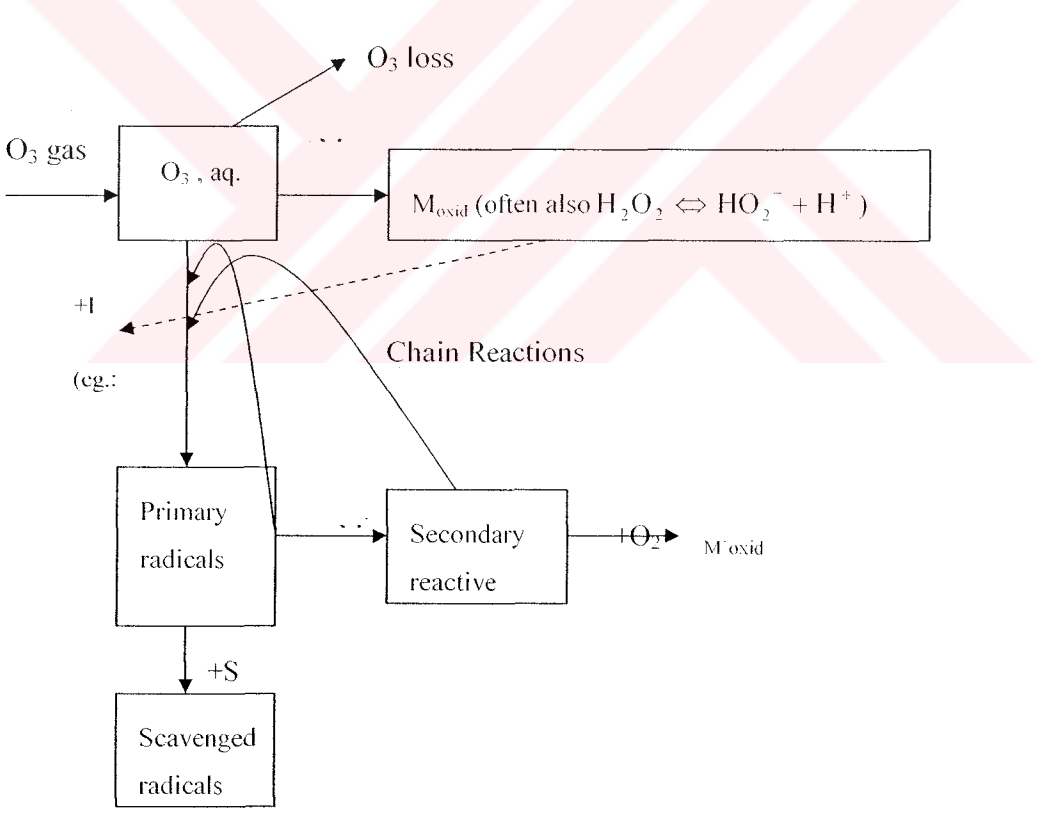

Figure 3.1. Schemes of Reactions of Aqueous Ozone

In the figure; M, solutes that consume ozone and become oxidized to M_{oxid} (and H_2O_2); I, solutes that indicate the decomposition of ozone to primary radicals; S, free radical scavenger; $M^{\cdot}$, solutes that react with $OH^{\cdot}$ and form secondary radicals $R^{\cdot}$ and become finally oxidized to $M^{\cdot}_{oxid}$.

Rey *et al.* (1999) studied the ozonation of the cytostatic drugs which are highly toxic and found ozonation as a suitable method.

The reactions of the decomposition of O_3 in water are;

$$O_3 + H_2O \rightarrow HO_3^+ + OH^- \quad (3.1)$$

$$HO_3^+ + OH^- \rightarrow 2HO_2\cdot \quad (3.2)$$

$$O_3 + HO_2\cdot \rightarrow OH\cdot + 2O_2 \quad (3.3)$$

$$OH\cdot + HO_2\cdot \rightarrow H_2O + O_2 \quad (3.4)$$

Decomposition of ozone may be initiated by hydroxyl ion at alkaline solutions. This kind of decomposition is pH dependent and is first order both in ozone and in hydroxyl ion concentration.

$$O_3 + OH^- \rightarrow O_2^{\cdot-} + HO_2\cdot \quad (3.5)$$

$$HO_2\cdot \Leftrightarrow H^+ + O_2^{\cdot-} \quad (3.6)$$

$$O_3 + O_2^{\cdot-} \rightarrow O_3^{\cdot-} + O_2 \quad (3.7)$$

$$O_3^{\cdot-} + H_2O \rightarrow OH^{\cdot} + OH^- + O_2 \quad (3.8)$$

$$3O_3 + H_2O \rightarrow 2OH^{\cdot} + 4O_2 \quad (3.9)$$

3.3.2 O_3/H_2O_2 Process

Many studies have confirmed that when H_2O_2 is added to a solution, ozone is rapidly degrade and $OH^{\bullet}$ radicals are formed, thus the process of degradation of pollutants takes place much faster (Stanislaw Ledakowicz, 1998).

Combining ozone with hydrogen peroxide to enhance oxidizing ability has been extensively researched and is considered to be a promising alternative for refractory organics removal from aqueous solutions hydrogen peroxide is a weak acid and when combined with water it partially dissociates into hydroperoxide ion.

The conjuge base of hydrogen peroxide (HO_2^-) can initiate the decomposition of ozone to form hydroxyl radicals (Glaze *et al.*, 1987; Masten and Davies, 1993).

The reaction between H_2O_2 and O_3 produces hydroxyl radical, however this reaction is very slow. A lower limit for the effectiveness of the O_3/H_2O_2 advanced oxidation process is in a range of 5 to 7 (Staehlin and Hoigne, 1982).

Isik Akmehmet Balcioglu and Merih Otker (2002) have studied on ozonation of three different synthetic pharmaceutical formulation wastewater to enhance the their biodegradability . They have studied on two human antibiotics and a veterinary antibiotic having initial COD value of 450 mg/l were subjected to ozonation at an applied rate of 2,96 g/l in buffered sollution at pH 7. They confirmed that the variation of biodegradability was associated with target compound. While BOD_5/COD ratio of veterinary antibiotic formulation wastewater was increased from 0,077 to 0,38, this ratio for human antibiotic I and human antibiotic II was increased from 0 to 0,1 and 0,27 respectively. Moreover the results indicate that the ozonation process is capable of achieving high levels of COD removals at about their natural pH values. The COD removal rates for different pH values obtained is showed in Table 3.1.

In the same study, the results obtained by O_3/H_2O_2 process for human I and veterinary antibiotic wastewaters indicate that during the 1 h reaction period the

Table 3.1. The COD removal rates for different pH values

COD Removal (%)	Human Antibiotic I	Human Antibiotic II	Veterinary Antibiotic
pH =3	53	24	65
pH=7	74	69	88
pH=11	82	71	79

COD results paralleled those without the added hydrogen peroxide. These similarities may be due to the high reactivity of compounds to ozonation and the presence of high ozone concentration. However for human antibiotic II , COD removals were enhanced from 69% and 29% to 95% and 90%, respectively, in the presence of the optimum dosage value of 20 mM hydrogen peroxide with an an applied ozone dosage of 2,96 g/l.h.

They concluded that ozonation could be successfully used as a pretreatment step to improve the biodegradability of wastewater containing antibiotics.

The decomposition rate of ozone by hydrogen peroxide increases with increasing pH (Langlais et al., 1991), but at very high pH values, the reaction of ozone with hydroxide ion predominates and the peroxide has little benefit. Therefore, the pH of the medium should be maintained at between 7 and 8.

Initiation steps;

$$H_2O_2 + H_2O \rightarrow HO_2^- + H_3O^+ \quad (3.10)$$

$$O_3 + HO_2^- \rightarrow O_3^- + HO_2^\bullet \quad (3.11)$$

$$HO_2^\bullet \leftrightarrow H^+ + O_2^- \quad (3.12)$$

Propagation Steps;

$$^{\bullet}O_2^- + O_3 \rightarrow {}^{\bullet}O_3^- + O_2 \quad (3.13)$$

$$^{\bullet}O_3^- + H^+ \rightarrow HO_3^{\bullet} \quad (3.14)$$

$$HO_3^{\bullet} \rightarrow OH^{\bullet} + O_2 \quad (3.15)$$

The overall reaction for the hydrogen peroxide initiated decomposition of ozone;

$$2O_3 + H_2O_2 \rightarrow 2OH^{\bullet} + 3O_2 \quad (3.16)$$

However hydrogen peroxide might act as a hydroxyl radical scavenger as well as an initiator when the concentration is so high (Glaze et al., 1987).

$$H_2O_2 + OH^{\bullet} \rightarrow O_2^- + H_2O + H^+ \quad (3.17)$$

$$HO_2^- + OH^{\bullet} \rightarrow OH^- + HO_2^{\bullet} \quad (3.18)$$

Zewiener and Frimmel (2000) carried out the oxidation experiments using ozone (O_3) and ozone/hydrogen peroxide (H_2O_2) to degrade the lipid lowering agent, clofibric acid and the analgesic agents, ibuprofen and diclofenac. The combined application of ozone and hydrogen peroxide improved the degradation efficiency of all investigated compounds. The application of increased oxidant concentration resulted in a better degradation of all compounds to more than 90 per cent at a concentration of 3,7 mg/L ozone and 1,4 mg/L hydrogen peroxide and more than 98 per cent at a concentration of 5 mg/L ozone and 1,8 mg/Lhydrogen peroxide.

Combining ozone with hydrogen peroxide to enhance oxidizing ability has been extensively researched recently and is considered to be a promising alternative for refractory organics removal from aquous solutions.(Glaze *et al.*, 1987; Masten and Davies, 1993)

Gulyas et al. (1995) studied the O_3 and O_3/H_2O_2 advanced oxidation processes for removal of preservative 1,1,1-trichloro-2-methyl-2-propanol (TCMP). However, the oxidation of the wastewater failed to be effective in TCMP degradation because of competitive ozonation of other organic solutes in the wastewater. Therefore, it was concluded that for the pharmaceutical wastewaters containing TCMP, biological

treatment prior to ozonation stage is necessary to remove biodegradable organic solutes, which will compete with difficult organics for oxidants.

3.4 Application Advanced Oxidation Processes Before Biological Treatment

Pre-treatment is necessary for Antibiotic Formulation wastewaters before biological treatment because of the reasons below;

- Converted the refracter components into the readily biodegradable components,
- Removing the toxic organic components,
- With pre-treatment, not only organic material removal is increased, but also inhibition effects are removed.

Advanced oxidation processes are important for toxic compounds elimination. However, total mineralization through this means is very expensive. On the other hand biological treatment is really cheap and reliable, but there are substances which are unable to deal with. A combination of both kinds of processes would mean a cheaper option for total organics degradation from a toxic wastewater.

Pre-treatment is necessary for Antibiotic Formulation wastewaters before biological treatment because;

Ozone or ozone -based technologies have been shown to effectively oxidize recalcitrant compounds in wastewater to more readily biodegradable oxidation products that can be treated using a conventional aerobic biological treatment. (Sontheimer et al., 1978; Marco et al., 1997; Ledakowicz and Gonera, 1999)

The interest in the development of sequential chemical and biological processes for the treatment of wastewater has considerably grown. (Scott and Ollis, 1995, 1997)

Advanced oxidation processes have been investigated looking for the best effectiveness to eliminate toxic substances. This is the general idea that makes some investigators think of a combination of chemical oxidation process followed by a biological one (Scott and Ollis, 1994; Esplugas and Ollis, 1993).

Combination of chemical and biological processes may result at the end in a cheaper option for the treatment of toxic wastewater. Each process is affected by its own parameters. Chemical oxidation will be affected by the global amount of oxidizable organics, reaction rate between oxidants and organic compounds and chemical conditions such as pH. Biological processes are affected by pH, occurence of toxic substances, redox potential, presence of oxygen...etc. (Antonio Marco, Santiago Esplugas, Gabriele Saum, 1997)

4 BIOLOGICAL TREATMENT AND MODELLING

4.1 Biological Treatability Studies of Antibiotics

Biological treatment is the most common approach to treating organic contaminants in water. It generally offers low unit costs but is inefficient or totally ineffective in treating a large number of organics that may be present in industrial wastewaters and also some in municipal wastewaters.

The concept of biological treatability has changed during the last decade. The determination of inert and biodegradable chemical oxygen demand (COD) fractions and the respirometric assessment of the coefficients of microbial growth kinetics in the context of multi-component activated sludge modeling gained an increasing importance.

Recent studies showed that antibiotics, which are produced to restrain bacteria in humans and animals, have been found in surface water (Stan et al., 1994; Meyer et al.,1999) and sewage treatment plant effluents (Richardson and Brown, 1985; Halling-Sorensen et al.,1998; Kümmerer et al., 2000).

Biodegradability of the drug substances is an important characteristic in terms of removal of these substances from sewage water. Because of the serious toxicity to different microbial species, poor removals for antibiotics are expected in sewage treatment plants (Daughton, 2001).

In an environment aspect, the most important effect of antibiotics is the exerting toxic effects to aquatic organisms that would upset the ecological balance (Lansky and Halling-Sorensen, 1997; Migliore et al., 1997).

The presence of antibiotics in natural systems leads to the development of multi-resistant strains of bacteria. Hence, it is necessary to treat the effluents containing

antibiotics before discharging into biological treatment process and receiving water systems.

The study conducted by Richardson and Bawron (1985) with some antibiotics, indicated that these antibiotics are nonbiodegradable and had the potential to survive sewage treatment. This property poses a determination of these compounds in the environment and potential for bioaccumulation (Wollenberger et al., 2000)

In recent studies concerning antibiotics in the aquatic environment have indicated that elimination in municipal sewage treatment plant is often incomplete Table 4.1.

Table 4.1. Occurrence of antibiotics in surface water and ground water

ANTIBIOTICS	Suface Water (µg/L)	Ground Water (µg/L)	Reference
Erythromycin	1,7	-	Hirsch et al.(1999)
Erythromycin	1	-	Watts et al.(1983)
Sulphamethoxazole		0,47	Hirsch et al.(1999)
Tetracycline	1	-	Watts et al.(1999)
Penicilloyl groups	0,025	0,010	Richardson and Bawron(1985)

The absence of penicillin in surface water samples is the existence of chemically unstable β-lactam rings which are readily susceptible to hydrolysis (Morrison and Boyd, 1987)

Increasing production and consumption of antibiotics resulted in changing in the genetic pool of microorganisms in nature (Jacobsen and Guildal, 2000; Rabolle and Spliid, 2000; Iwane et al., 2001)

Generally conventional biological processes are used for the treatment of wastewater at the pharmaceutical industries. However, new products may be synthesized during

the biological methods applied. For this reason, system should be monitored in order to control the biodegradability of new wastes (Rosen et al., 1998).

Aerobic treatment systems have been employed for the treatment of pharmaceutical wastewater including activated sludge systems (Andersen, 1980; Bernard and Gray, 2000; Gohary et al., 1995).

Gohary et al. (1995) performed the biological treatment of wastewater from pharmaceutical and chemical company. The wastewater was very acidic and contains high concentrations of organic compounds and total solids. The results obtained indicated that biological treatment using extended aeration activated sludge or biological filters followed by activated sludge process significantly removed the organic pollutants in wastewater (approximately 95 per cent of COD and BOD reduction).

Most of the pharmaceutical manufacturing industries use aerobic treatment technologies which are activated sludge systems, aerated lagoons, trickling filters and rotating biological contactors. These systems are initially designed to treat BOD and COD.

Rosen et al. (1998) also found that the biological treatment of chemical synthesis based pharmaceutical wastewater provided high removal of COD and toxicity.

Activated sludge processing has traditionally been the usual approach for the treatment of municipal sewage and wastewater from many industrial activities. However, it is not feasible to treat toxic or recalcitrant substances, since these reduce the biodegradation performance and eventually could inhibit the process.

Biological treatments have shown to be very economic and reliable systems for most cases (municipal wastewater, food and farm processing water...etc.)There are, however, cases in which effectiveness of these treatments drops, for example toxic substances. In this case, different chemical processes are being applied. Processes such as ozonation have been investigated looking for the best effectiveness to eliminate these toxic soluble substances. On the other hand, these kinds of treatment have proved to be expensive (at least compared with the biological treatment)

D.Orhon et al. (2002) have investigated the effect of partial ozonation of textile wastewater, both at the inlet (pre-ozonation) and the outlet (post-ozonation) of biological treatment, for the optimization of COD and color removals, both typical polluting parameters associated with the textile industry. Pre-ozonation provides at optimum contact time of 15 minutes 85% color removal, but only 19% COD reduction. Removal of the soluble inert COD fraction remains at 7%, indicating selective preference of ozone for the simpler compounds. Post-ozonation is much more effective on the breakdown of refractory organic compounds and on colour removal efficiency.

Ozonation and subsequent biodegradation in batch tests of the oxidation products were studied by Gilbert (1987) for 28 substituted aromatic compounds. Good biodegradability of the oxidation products, defined as a ratio of 5- day biochemical oxygen demand to chemical oxygen demand (BOD_5/COD) of 0,4 g/g was achieved along with approximately 100% removal of the target compounds.55 to 70% COD and 30 to 40% dissolved organic carbon (DOC) removal by ozonization.

Pharmaceutical industry often generates high strength wastewater changing in character and quantity depending upon the used manufacturing processes and seasons (Nemerow, 1978). Among the wastewaters from different operations in this industry, formulation effluent that rises from washing of equipment is characterized by small effluent flow and low pollution load.

However the effluents originated from the formulation of antibiotics have low biodegradability since they contain almost only active substance. Hence a chemical pretreatment is necessary for pharmaceutical effluents, like antibiotic formulation, containing high concentrations of bioinhibitory compounds.

A penicillin formulation effluent generated by a pharmaceutical company located in İstanbul had a total COD value of 710 mg/l with a soluble part of 97%, negligible BOD content The total daily effluent flow rate of the company was 150 m^3/day. It included wash water as 30 % and process water 48 % (E. Ubay Cokgor et al., 2004). In the mention study, ozonation at varying pH and ozone input concentrations and biological treatability of the penicillin Sultamycillin Tosylate Diydrate was investigated. Assessment of the efficiency of ozonation as a pre-treatment process

was evaluated with the collective parameters COD, TOC and BOD. The optimum COD and TOC removal obtained at pH 11.5, 34% and 24 % respectively, with an increase in the BOD_5 value from 16 mg/l to 128 mg/l after 40 minutes of ozonation. Biological treatability studies were conducted with synthetic wastewater, raw and ozonated penicillin formulation effluents. The degradation of the synthetic wastewater is being completely treatable without penicillin addition. But in addition to the non-biodegradable character of raw penicillin formulation effluent, synthetic wastewater degradation was inhibited by 7% due to the generation of non-biodegradable chemical complexes. The synthetic wastewater could be completely oxidized upon 40 min. ozonation and ozonated penicillin wastewater removal has provided as 35%. Prolonged ozonation of 60 minutes did not provide satisfactory pre-treatment results and inhibition has been observed when the ozonated effluent was subjected to biological treatment. The study showed that ozonation is a considerable process as a pre-treatment before biological treatment.

4.2 Microbial Growth

Microbial growth is commonly defined by means of of a differential equation:

$$\frac{dX}{dt} = \mu X \tag{4.1}$$

where

X=the biomass concentration(M(biomass)/L^3)

μ =specific growth rate (T^{-1})

The growth expression, combined with the equation defining the yield relationship between bacterial growth and substrate removal,

$$\frac{dX}{dt} = -Y\frac{dS}{dt} \tag{4.2}$$

where Y is the yield coefficient, describes the rate of substrate removal related to growth.

$$\frac{dS}{dt} = -\frac{\mu}{Y}X \tag{4.3}$$

The specific substrate removal rate, q is related to substrate removal with the following equation:

$$\frac{dS}{dt} = -qX \tag{4.4}$$

Then, q may be expressed in terms of the specific growth rate, μ, as:

$$q = \frac{\mu}{Y} \tag{4.5}$$

Monod expression is;

$$\mu = \hat{\mu}\frac{S}{K_S + S} \tag{4.6}$$

S=growth limiting substrate concentration (M(substrate)/L^3)

$\hat{\mu}$=maximum specific growth rate (T^{-1})

K_S=half -velocity constant, substrate concentration at one-half the maximum growth rate (M(substrate)/L^3)

If the value of μ from equation 6 is substituted in equation 1 and 5 the rates of microbial growth and substrate utilization can be expressed as follows:

$$\frac{dX}{dt} = \hat{\mu}\frac{SX}{K_S + S} \tag{4.7}$$

and

$$\frac{dS}{dt} = k_m\frac{SX}{K_S + S} \tag{4.8}$$

Equation 8 is expressed in terms of the maximum specific substrate removal rate k_m, defined as:

$$k_m = \frac{\hat{\mu}}{Y} \tag{4.9}$$

Combination of equations 5 and 9 yields an expression similar to the Monod equation for the specific substrate removal rate, q:

$$q = k_m \frac{S}{K_S + S} \tag{4.10}$$

4.3 Activated Sludge Model 3

The Activated Sludge Model No:3 (ASM3) can predict oxygen consumption, sludge production, nitrification and denitrification of activated sludge systems. It is based on the Activated Sludge Model No:1 (ASM1) and corrects for some defects of ASM1. In addition of ASM1, ASM3 includes storage of organic substrates as a new process. One important difference in ASM3, soluble and particulate components can be differentiated with filtration over 0.45 μm membrane filters whereas a significant fraction of slowly biodegradable organic substrates X_S and ASM1 would be contained in the filtrate of the inflowing wastewater. The lysis process is exchanged for an endogenous respiration process. ASM3 is provided as a reference in a form which can be implemented in a computer code without further adjustments. Biological phosphorous removal is contained in the Activated Sludge Models No.2 and 2d (Henze et al 1995 and 1998) and not considered in ASM3. ASM3 includes 13 components and 12 processes

Different points between ASM1 and ASM3 are ;

- ASM1 does not include kinetic expressions which can deal with nitrogen and alkalinity limitations of heterotrophic organisms; ASM3 includes this kinetic expressions.

- ASM1 includes biodegradable soluble and particulate organic nitrogen as model components; in ASM3, decomposed of organic nitrogen components are associated with hydrolysis, growth and decay processes.

- The kinetics of ammonification in ASM1 cannot really be quantified; in ASM3, ammonification process is inserted the other expressions.

- ASM1 differentiates inert particulate organic material depending on its orgin, influent or biomass decay, it is impossible however to differentiate these two fractions in reality; ASM3 has just one expression for inert particulate COD.

- The process of hydrolysis has a dominating effect upon the predictions of oxygen consumption and denitrification by heterotrophic organisms in ASM1;Hydrolysis process is less effective in ASM3, because of the addition of the storage mechanism.

- Lysis combined with growth is used to describe the collected effects of endogenous respiration of storage compounds, death...etc. of the biomass. This leads to difficulties in the evaluation of kinetic parameters.;in ASM3 endogenous respiration approach is used.

- Storage of poly-hydroxy-alkanoates is observed under aerobic and anoxic conditions in activated sludge plants, provided that elevated concentrations of readily biodegradable organic substrates (S_S) are available. This process is not included in ASM1; Storage expression is added to ASM3.

- ASM1 does not include the possibility to differentiate decay rates of nitrifiers under aerobic and anoxic conditions; Two different endogenous respiration expressions are described in ASM3.

- ASM1 does not allow for the prediction of directly observable mixed liquor suspended solids:Stochiometry equation is established for solid substances in ASM3.

The following components are used in ASM3 for carbon removal:

Definition of soluble components, S;

$S_O[M(O_2)L^{-3}]$:Dissolved Oxygen,O_2.

S_I[M(COD)L^{-3}]: Inert soluble organic material. These material is assumed to be part of the influent and maybe produced in the context of hydrolysis of particulate substrates,X_S

S_S[M(COD)L^{-3}]:Readily bio-degradable organic substrates. In ASM3 it is assumed that all these substrates are first taken up by heterotrophic organisms and stored in the form of X_{STO}.

Definition of particulate components, X.

X_I[M(COD)L^{-3}]: Inert particulate organic material. X_I may be a fraction of the influent or may be produced in the context of biomass decay.

X_H[M(COD)L^{-3}]:Heterotrophic organisms

X_{STO}[M(COD)L^{-3}]:A cell internal storage product of heterotrophic organisms. It occurs only associated with X_H; it is however not included in the mass of X_H.

ASM3 considers the following transformation processes for carbon removal:

1.Aerobic storage of readily biodegradable substrate: this process describes the storage of readily biodegradable substrate S_S in the form of cell internal storage products X_{STO}. This process required energy in the form of ATP which is obtained from aerobic respiration. It assumed that all substrates first become stored material and then are later assimilated to biomass.

2.Aerobic growth of heterotrophs: The substrate for the growth of heterotrophic organisms is assumed to consist entirely of stored organics X_{STO} this assumption simplifies ASM3 considerably.

3.Aerobic endogenous respiration: This process describes all forms of biomass loss and energy requirements not associated with growth by considering related respiration under aerobic conditions: Decay,(maintenance),endogenous respiration, lysis, predation motility, death,.... The model of this process is significantly different from the decay (lysis) process introduced in ASM1

4.Aerobic respiration of storage products: This process is analogous to endogenous respiration. It assures that storage products decay together with biomass.

The kinetic expressions of ASM3 are based on switching functions for all soluble components consumed. This form of kinetic expression is chosen because of experimental evidence and for mathematical convenience. These switching functions stop all biological activity as educts of a process approach zero concentrations. All kinetic rate expressions of ASM3 are defined with a typical value at 10 ^{0}C and 20 ^{0}C. It is recommended to interpolate kinetic parameters for different temperatures with the following temperature expression :

$$k(T) = k(20^{\circ}C) \cdot \exp(\theta_T \cdot (T - 20^{\circ}C)) \tag{4.11}$$

where ;

$$\theta_T = \frac{\ln(k(T_1)/k(T_2))}{T_1 - T_2} \tag{4.12}$$

A simplified ASM3 matrix presentation for carbon removal is illustrated in Table 4.2

Table 4.2. Matrix Representation of Activated Sludge Model No.3 (ASM3)

COMPONENT PROCESS	S_O O_2	S_S COD	S_I COD	X_I COD	X_H COD	X_{STO} COD	RATE
Storage of S_S	$-(1-Y_{STO})$	-1				Y_{STO}	$k_{STO} \frac{S_O}{K_O+S_O} \frac{S_S}{K_S+S_S} X_H$
Growth on X_{STO}	$-\frac{(1-Y_H)}{Y_H}$				1	$-1/Y_H$	$\mu_H \frac{S_O}{K_O+S_O} \frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H} X_H$
Endogenous Respiration	$-(1-f_I-f_{SI})$		f_{SI}	f_I	-1		$b_H \frac{S_O}{K_O+S_O} X_H$
Respiration of X_{STO}	-1					-1	$b_{STO} \frac{S_O}{K_O+S_O} X_{STO}$

4.4 Inhibition

Recently, interest in inhibition has increased. Inhibition and toxic effects on microorganisms in activated sludge can be caused by industrial wastewaters. Combining industrial and municipal wastewater for treatment plant can include

inhibitors. Organic removal efficiencies may be reduced by inhibitory effects on biological systems. Poor settling characteristics of activated sludge organisms or complete inhibition of the system with reduced biological activity can occurred.

Biologically resistant substances such as heavy metals and toxic organics are called inhibitors. Inhibition can effect on the treatment plant, the receiving water and sludge. There are two inhibition reactions; competitive and noncompetitive Inhibition.

4.4.1 The Analogy of Enzyme Kinetics

While proposed on the basis of experimental studies, the Monod expression is compatible with the Michaelis*Menten enzymatic reaction model. It provides a practical tool to mathematically explain the behavior of microbial cultures under inhibitory effects. The general equation for enzyme-substrate reactions as applicable to biological treatment systems;

$$E + S \Leftrightarrow ES \rightarrow E + P \tag{4.13}$$

where S, E, ES and P represent concentrations of substrate, free enzyme, enzyme substrate complex and product, respectively.From this equation;

$$\frac{dES}{dt} = k_1 E \cdot S - (k_2 + k_3)ES \tag{4.14}$$

If we assume that the reactions go on under equilibrium concentrations of the substrate-enzyme complex, the above equation is found;

$$E = \frac{(k_2 + k_3)}{k_1}\frac{ES}{S} \tag{4.15}$$

A mass balance on enzyme species yields;

$$E = E_0 - ES \tag{4.16}$$

where E_0 is the initial free-enzyme concentration. If the value of E in Equation (5.4) is substituted in Equation (5.3), the resulting expression is:

$$ES = E_0 \frac{S}{K_S + S} \tag{4.17}$$

$$K_S = \frac{(k_2 + k_3)}{k_1} \tag{4.18}$$

On the other hand, the rate of substrate utilization is defined as:

$$\frac{dS}{dt} = -k_1 S \cdot E + k_2 ES \tag{4.19}$$

or:

$$\frac{dS}{dt} = -k_3 ES \tag{4.20}$$

Substituting the value of ES in Equation (5.5) into the above equation yields:

$$\frac{dS}{dt} = -V_M \frac{S}{K_S + S} \tag{4.21}$$

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

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

$$V_M = k_3 E_0 = k_m X \tag{4.22}$$

4.4.2 Inhibition Types

There are four types of inhibition called competitive, non competitive uncompetitive, mixed.

4.4.2.1 Competitive Inhibition

Competitive inhibition results when inhibitor (I) and substrate (S) compete directly for the same enzyme site; the slopes of Lineweaver-Burk plots increase with rising inhibitor concentration in this case. (Figure 4.1)

The inhibitor can take the place of the substrate to form an enzyme-inhibitor complex (EI), which can not undergo further reactions with the substrate. Competitive inhibition can be defined that additional reaction;

$$E + I \Leftrightarrow EI \tag{4.23}$$

This concept defines a kinetic constant,K_I

$$K_I = \frac{E \cdot I}{EI} \tag{4.24}$$

The mass balance for enzyme components yields;

$$ES = E_0 - E - EI \tag{4.25}$$

ES can be defined in terms of the initial free –enzyme concentration,E_0;

$$ES = \frac{E_0 S}{K_S\left(1 + \frac{I}{K_I}\right) + S} \tag{4.26}$$

where I is the inhibitor concentration.

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}\frac{S \cdot X}{K_S\left(1 + \frac{I}{K_I}\right) + S} \tag{4.27}$$

$$K_S' = K_S\left(1 + \frac{I}{K_I}\right) \tag{4.28}$$

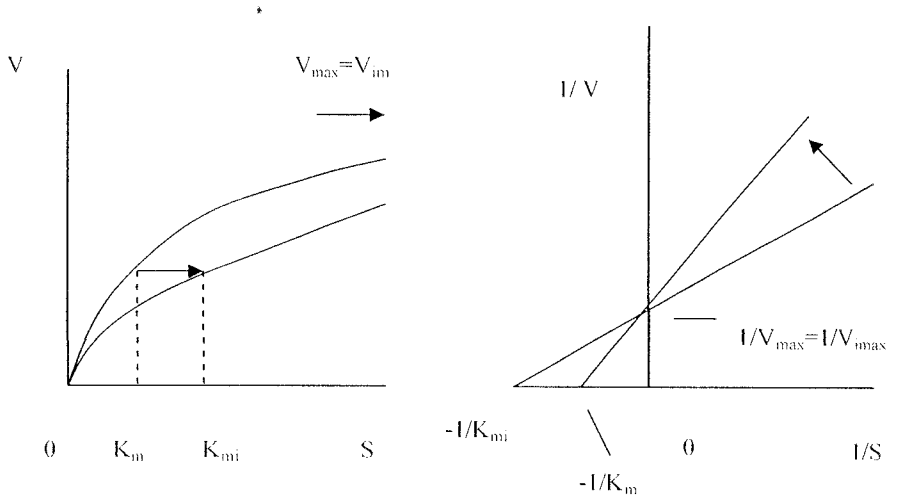

Figure 4.1. Competitive Inhibition

4.4.2.2 Uncompetitive Inhibition

Uncompetitive inhibition results when the inhibitor combines with an enzyme form (e.g. ES), the enzyme substrate complex different from with which the substrate combines. This type of inhibition results only in increased intercept with increased inhibitor concentration since there is no direct competition between inhibitor and substrate for the free enzyme. (Figure 4.2)

Uncompetitive inhibition can be formulated as:

$$\mu = -\frac{\hat{\mu}\left(\frac{K_i}{K_i + I}\right)S}{K_S\left(\frac{K_i}{K_i + I}\right) + S} \qquad (4.29)$$

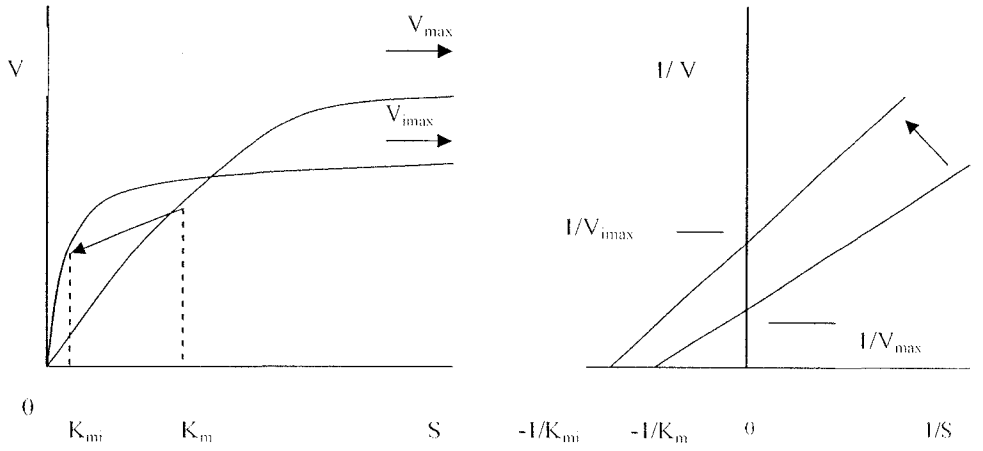

Figure 4.2. Uncompetitive Inhibition

4.4.2.3 Noncompetitive Inhibition

Noncompetitive Inhibition is a combination of competitive and uncompetitive inhibition and both slope and intercept vary with inhibitor concentration.

The inhibition reaction cannot be inverted by raising the substrate concentration in noncompetitive inhibition. The substrate is then unable to prevent the combination of the inhibitor with the enzyme. The magnitude of inhibition will accordingly depend on I and K_I, and it will not be influenced by S or ES. The mathematical interpretation of the preceding statement gives:

$$E'_0 = E_0 - EI \tag{4.30}$$

This equation indicates that the inhibitor blocks a portion of the initially present enzyme concentration ,E_0, so that only E_0 is practically available for substrate.

Then;

$$K_I = \frac{E'_0 \cdot I}{EI} \tag{4.31}$$

combining with this two equation ,EI is;

$$EI = \frac{E_0 \cdot I}{K_I + I} \tag{4.32}$$

For the substrate -enzyme interaction the following mass balance applies:

$$E'_0 = E_0 - EI = E + ES \tag{4.33}$$

ES may be arranged as;

$$ES = \frac{E_0\left(\frac{K_I}{I + K_I}\right) \cdot S}{K_S + S} \tag{4.34}$$

The corresponding rate for substrate then becomes;

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}\left(\frac{K_I}{I + K_I}\right)\frac{SX}{K_S + S} \tag{4.35}$$

If the system contained less enzyme in the presence of a non-competative inhibitor, maximum growth rate , $\hat{\mu}'$, will be reduced to a lower level;

$$\hat{\mu}' = \hat{\mu}\left(\frac{K_I}{I + K_I}\right) \tag{4.36}$$

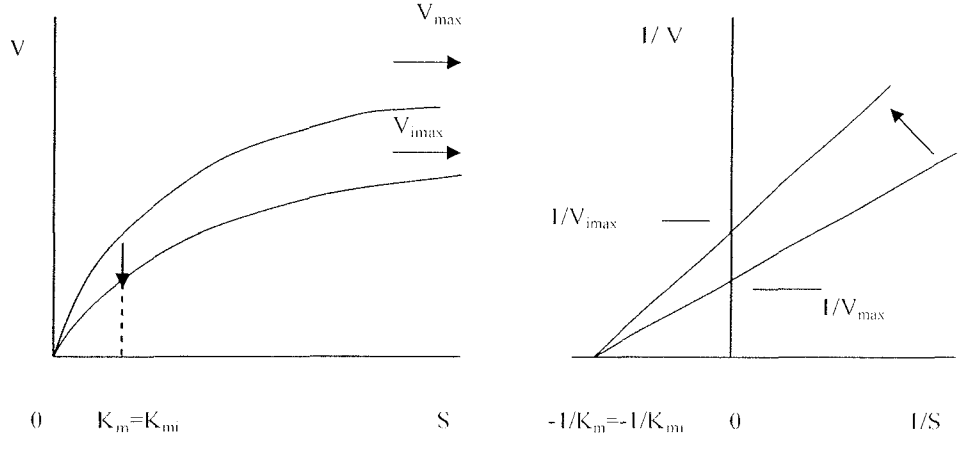

Figure 4.3. Noncompetitive Inhibition

4.4.2.4 Mixed inhibition

Mixed inhibition involves several enzymes or microbial species.

Mixed inhibition can be formulated as:

$$\mu = -\frac{\hat{\mu}\left(\frac{K_i}{K_i + I}\right)S}{K_S\left(1 + \frac{I}{K_i}\right) + S} \tag{4.37}$$

If $\hat{\mu}$ and K_S is shown as $\hat{\mu}^*$ and K_S^* in the presence of inhibitor, qualitative and quantitative effects on inhibition kinetics according to inhibitor types can be shown as in Table4.3 and Table 4.4

Table 4.3. Qualification of inhibitory effects (Volskay and Grady, 1988)

Inhibitor Type	Effect on $\hat{\mu}$	Effect on K_S
Competitive	None	Increase
Noncompetitive	Decrease	None
Uncompetitive	Decrease	Decrease
Mixed	Decrease	Increase

Table 4.4. Quantification of inhibitory effects (Volskay and Grady, 1988)

Inhibitor Type	Effect on $\hat{\mu}$	Effect on K_S
Competitive	$\hat{\mu}^*=\hat{\mu}$	$K_S^* = K_S/(1+I/K_i)$
Noncompetitive	$\hat{\mu}^*=\hat{\mu}/(1+I/K_i)$	$K_S^* = K_S$
Uncompetitive	$\hat{\mu}^*=\hat{\mu}/(1+I/K_i)$	$K_S^* = K_S/(1+I/K_i)$
Mixed	$\hat{\mu}^*=\hat{\mu}/(1+I/K_i)$	$K_S^* = K_S/(1+I/K_i)$

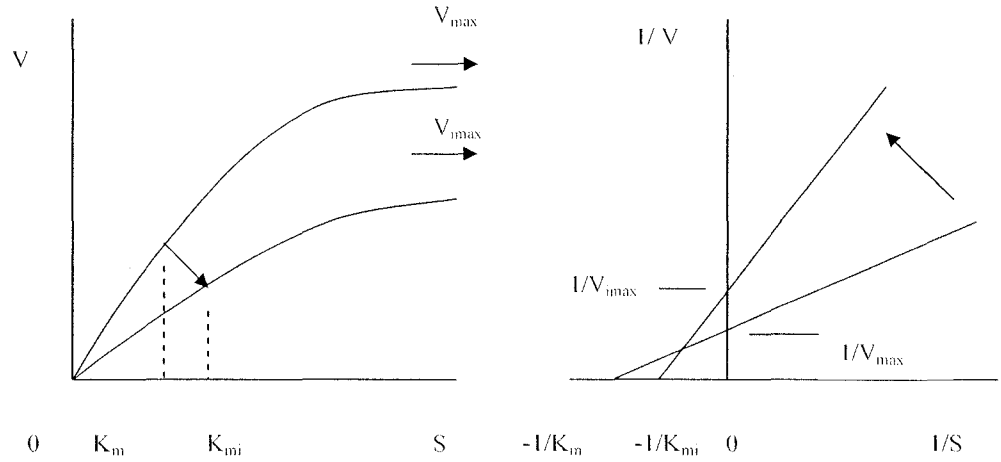

Figure 4.4. Mixed Inhibition

4.4.3 Substrate Inhibition

Substrate Inhibition is described on the basis of the assumption that some substrates have the ability to bind with the enzyme – substrate complex as well as with the free enzyme at high concentrations. This leads to the formation of a substrate-enzyme-substrate complex (SES) which cannot undergo further reaction.

$$E + S \Leftrightarrow ES \rightarrow E + P$$

$$E + S \Leftrightarrow SES \tag{4.38}$$

where

$$K_{IS} = \frac{ES \cdot S}{SES} \tag{4.39}$$

by using the approach previously outlined, the following rate expression can be developed;

$$\mu_{IS} = \hat{\mu}\frac{S}{\left(K_S + S + \frac{S^2}{K_{IS}}\right)} \tag{4.40}$$

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

Table4.5. Kinetic Models

Eq. No	Kinetic Model
1	$\mu_I = \hat{\mu}\frac{S}{(K_S + S)(1 + S/K_{IS})}$
2	$\mu_I = \hat{\mu}\frac{S(1+S/K_S)}{K_S + S + S^2/K_{IS}}$
3	$\mu_I = \hat{\mu}\frac{S}{K_S + S + (S^2/K_{IS})(1+S/K)}$
4	$\mu_I = \hat{\mu}\frac{S}{K_S + S}e^{-S/K_{IS}}$
5	$\mu_I = \hat{\mu}\left(e^{-S/K_{IS}} - e^{-S/K_S}\right)$
6	$\mu_I = \hat{\mu}\frac{S}{K_S + S}$ when $S<S^*$
7	$\mu_I = \hat{\mu}\frac{S}{K_S + S} - K_{IS}(S - S^*)$ when $S<S^*$
8	$\mu_I = \hat{\mu}\frac{S}{K_S + S}\left(1 - \frac{S}{S_m}\right)^{\infty 2}$

S^*=Threshold substrate concentration below which there is no subtrate inhibition.

S_m=Substrate concentration above which growth is completely inhibited.

This expression generally known as Haldane's equation. This expression is the only one of the similar models ,shown in Table 4.5.

Halane's equation is of little practical value for activated sludge modelling, which primarily uses substrate removal kinetics at low concentrations. The main problem in this case is the identification of inhibitory substrate components when using nonspecific parameters, such as BOD_5 or COD,to characterize sewage. Another modelling approach is presented for substrate inhibition , based upon the fact that sewage is not just a single substrate , but a mixture of substrate , and a minor fraction of this mixture may be expected to exhibit inhibiting properties. Generally, this can be reflected by

$$S=S_S+S_I \tag{4.41}$$

Where S is the total substrate concentration in sewage , and S_S and S_I its noninhibiting and inhibiting fractions respectively. This concepts is intoduced into the following model:

$$E+S \Leftrightarrow ES \rightarrow E+P \tag{4.42}$$

$$ES+S_I \Leftrightarrow SES_I \tag{4.43}$$

Model equations yields;

$$\mu_{IS}=\hat{\mu}\frac{S}{K_S+S+\frac{S\cdot S_I}{K_{SI}}} \tag{4.44}$$

This equation represents a totally different biochemical mechanisms for substrate inhibition in sewage treatment. It is also identical in format to the one derived for product inhibition on the basis of the following reaction ;

$$E+S \Leftrightarrow ES \rightarrow E+P \tag{4.45}$$

$$ES+P \Leftrightarrow ESP \tag{4.46}$$

We can obtain the expression for Inhibition at extreme substrate levels as it is shown below.

The rate expression for substrate utilization for low substrate concentrations;

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{YK_S}SX \tag{4.47}$$

We can modify equations, given for competitive and noncompetitive inhibition equations by using this expression;

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{YK_S}\frac{K_I}{K_I + I}SX \tag{4.48}$$

When $S>K_S$(high substrate concentrations), substrate utilization rate will be;

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}X \tag{4.49}$$

The impact of competitive inhibition may be ignored when $S>K_S$. Then noncompetitive inhibition may be modify as;

$$\frac{dS}{dt} = -\frac{\hat{\mu}}{Y}\frac{K_I}{K_I + I}X \tag{4.50}$$

5 MATERIAL AND METHOD

5.1 Experimental Plan

Biological treatability experiments were conducted in four laboratory-scale fill-and-draw reactors. The reactors were operated at a sludge age of 10 days and a hydraulic retention time (HRT) of one day for a period of 8 months. A synthetic wastewater consisting of an appropriate mixture of acetic acid, propionic acid, ethanol, glutamic acid and glucose has been prepared in accordance with Henze (1992) to simulate the readily biodegradable COD fraction in domestic sewage. This synthetic wastewater served as the main substrate in all biodegradation experiments. One of the reactors was fed with the synthetic wastewater alone, which was supplemented in the second reactor with raw penicillin formulation effluent, in the third reactor with the ozonated effluent and in the last reactor with the ozonated with H_2O_2 effluent. The penicillin effluent additions were adjusted to constitute approximately 30% of the total COD in the reactor.

For synthetic wastewater the components have been used as shown in (Table 5.1). Since wastewater used has limited nutrient, buffer solutions and other necessary materials added to the reactors.

5.2 Measurement Methods

All analysis on conventional parameters were performed as defined in Standart Methods (1998) except for COD. For COD determination , samples were filtered through 0,45 μm membrane filters. The analytical survey also used Whatman GF/C glass-fibre filtersfor suspended solids, (SS), and Volatile suspended solids (VSS) measurements. COD measurements were performed as described in the method proposed by ISO6060 (1986). OUR measurements were conducted with a Manoterm RA-1000 continuous respirometer with PC connection.

Table 5.1. Stock Synthetic Wastewater

1. STOCK SYNTHETIC WASTEWATER		
Feed Component	Feed Concentration	Compound
C	10,00 ml/l	Acetic Acid
C	3,10 ml/l	Propionic Acid
C	1,68 ml/l	Ethanol
C	3,62 g/l	Glutamic Acid
C	4,54 g/l	Glucose
2. STOCK N+P SOLUTIONS		
N	60 g/l	NH_4Cl
P	67,35 g/l	K_2HPO_4
P	52,645 g/l	KH_2PO_4
3. STOCK MICRO NUTRIENTS SOLUTION		
Mg	15 g/l	$MgSO_47H_2O$
Fe	0,5 g/l	$FeSO_47H_2O$
Ca	2 g/l	$CaCl_2$
Mn	0,3 g/l	$MnSO_4H_2O$
Zn	0,5 g/l	$ZnSO_47H_2O$

1 litre-penicillin wastewater samples were ozonated for 5, 20, 40 and 60 min in a borosilicate glass bubble column at semi-batch mode wherein the ozone + oxygen gas mixture was continuously sparged at a rate of 2 l min^{-1} through a fritted dispersion disc with a diameter of 5 cm. Ozone was produced by a corona discharge PCI GL-1 model pilot scale ozone generator with a maximum capacity of 20 g h^{-1} ozone. Teflon tubing was used for all connections from the ozone generator to the reaction vessel.

The respirometric tests were started with the biomass seeding alone to obtain the initial endogenous oxygen uptake rate (OUR) level. At the desired F/M ratios, samples was added on the biomass in the reactor, and the OUR data was monitored. Model simulations were performed with Aquasim (Reichert et al.,1998) for the estimation of kinetic and stoichiometric model parameters.

6 BIOLOGICAL TREATABILITY RESULTS AND EVALUATION

6. 1 Raw Synthetic Penicillin Procaine G

Biological treatability studies of raw penicillin formulation, the ozonated and the ozonated with H_2O_2 effluents are investigated in the thesis. The penicillin oriented effluent additions were adjusted to constitute approximately 30% of the total COD in the reactor. In the study penicillin formulation effluent is Penicillin Procaine G (PPG) as a sole source. The properties of Penicillin Procaine G are indicated in the Table 6.1. The basic structure of PPG is depicted in Figure 6.1

Table 6.1 Properties of Procaine Penicillin G

Source	Penicillin Culture
Chemical Group	β-lactam
Target Bacteria	Staphylococcus (gram positive)
Molecular Formulation	$C_{16}H_{18}N_2O_4S.C_{13}H_{20}N_2O_2.H_2O$
Molecular Weight	588.72 gr/mol
Solubility in water	100 gr/l
Procaine Penicillin G/COD	1.47 mg COD/ mg PPG

Biological treatability of Penicillin Procaine G has been investigated previous studies that have been shown it is not biodegradable. Therefore, its biological treatability had been investigated after advanced oxidation processes in this study.

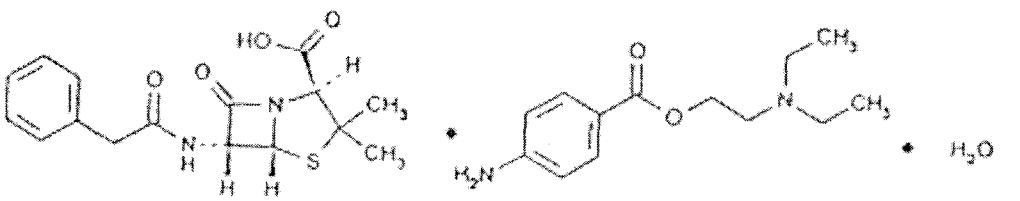

Figure 6.1 The Structure of Penicillin Procaine G

Biological treatability of Penicillin Procaine G has been investigated previous studies that have been shown it is not biodegradable. Therefore, its biological treatability had been investigated after advanced oxidation processes in this study.

6.2. Effects of O_3 and O_3/H_2O_2 Advanced Oxidation Processes on Penicillin Procaine G Effluent Removal

Raw PPG used through all experimental study performed with ozonation/ozonation with H_2O_2 with 1800 mg/L for 1 hour reaction time was arranged as 600 mgCOD/l as initial. The values are summarized in Table 6.2. and 6.3. COD removal efficiency was obtained between 6, 20, 27 and 37 % with ozonation time 5, 20, 40 and 60 min respectively. Ozonation with H_2O_2 experiments were given better results than only ozonation in all ozonation times.

Table 6.2. COD Results after Ozonation, pH=7, 1440 mg/h ozon flow rate (Influent BOD_5 = 48 mg/l)

OZONATION TIME (min)	INFLUENT COD (mg/l)	EFFLUENT COD (mg/l)	EFFLUENT BOD_5 (mg/l)	COD REMOVAL EFFICIENCY
5	600	560	-	6%
20	600	485	42	20%
40	600	440	34	27%
60	600	375	37	37%

Table 6.3. COD Results After H_2O_2/O_3, pH=7, 1440 mg/h ozon flow rate (Influent BOD_5 = 48 mg/l)

OZONATION TIME (min)	INFLUENT COD (mg/l)	EFFLUENT COD (mg/l)	EFFLUENT BOD_5 (mg/l)	COD REMOVAL EFFICIENCY
5	600	392	-	35%
20	600	390	46	35%
40	600	280	55	53%
60	600	165	43	73%

6.3. Biological Treatability Studies

Four laboratory-scale fill-and-draw reactors for a period of 8 months were operated for biological treatability experiments. All of the reactors were fed with synthetic wastewater; the first one with no synthetic penicillin effluent addition, raw, ozonated and ozonated with hydrogen peroxide synthetic penicillin formulation effluent were also added to the second, third and forth reactors respectively. Food and microorganism ratios (F/M) were between 0.27 - 0.35 mg COD/mg VSS .day for four reactors.

Control Reactor Results (Reactor 1): Control reactor was fed only synthetic wastewater given in Chapter 5. The obtained values from the measurements are evaluated as shown Table 6.4. Overall COD removal for Reactor 1 was 91% with a microbial product of 9%.

Penicillin Procaine G Reactor Result (Raw) (Reactor 2): 30 % of PPG and 70% synthetic wastewater added in this reactor for initial COD. The data obtained through the study for raw PPG was given in Figure 6. 2. All sets illustrations were shown in Appendix section (Figure A.1-A.8).

Ozonated Reactor Results (Reactor 3): Ozonated reactor performance was shown that COD removal was 80% as similar to Penicillin Procaine G Reactor (Raw) (Reactor 2).

Table 6.4. Reactor Performance

	F/M	Reactor Volume	Synthetic Substrate COD	Raw Penicillin COD	Total COD	Effluent COD	Overall Removal Efficiency	VSS	SS	VSS/SS
	(mgCOD/mgVSS.day)	(l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(%)	(mg/l)	(mg/l)	(-)
Synthetic Reactor	0.29	3L	785	-	785	67	91	2680	3100	0.86
Raw PPG Reactor	0.33	2L	441	189	630	123	80	1925	2315	0.83
O_3 PPG Reactor	0.35	3L	441	189	630	126	80	1825	2330	0.78
O_3/H_2O_2 PPG Reactor	0.27	1L	225	75	300	135	55	1110	1530	0.73

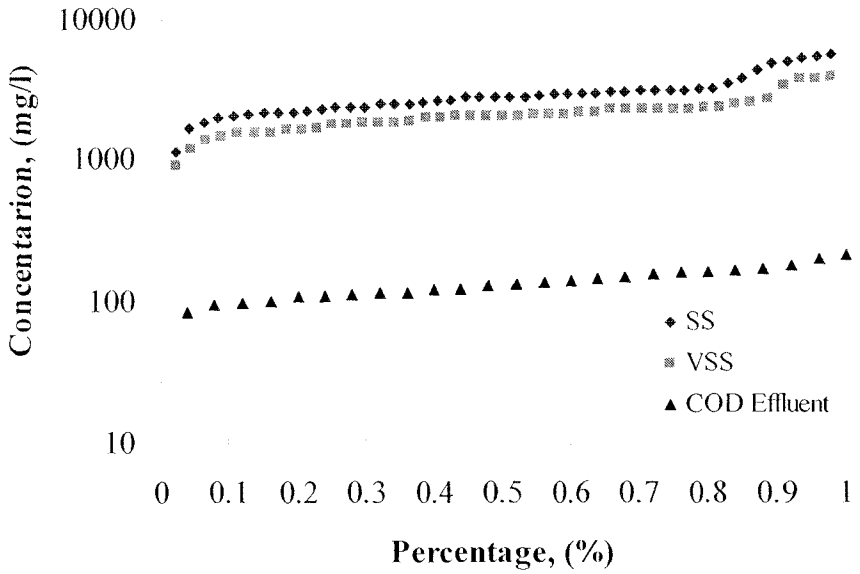

Figure 6.2. Data for a period of 8 months (Reactor 2)

Ozonated with H_2O_2 Reactor Results (Reactor 4): Ozonated with H_2O_2 performance was not given better results than Reactor 2 and Reactor 3 and synthetic substrate removal was also effected with a decrease of 18%.

6.4. Respirometric Analysis

Respirometric tests were performed with relevant acclimated biomass (to raw, ozonated and ozonated with hydrogen peroxide effluents), seeding alone to obtain the level of initial endogenous oxygen uptake rate (OUR). Several respirometric batch tests were conducted at desired F/M ratios, with only synthetic wastewater and raw, ozonated (5, 20, 40, 60 minutes) and ozonated with hydrogen peroxide (5, 20, 40, 60 minutes) synthetic penicillin formulation effluents added to the same amount of synthetic wastewater. In all respirometric tests, synthetic wastewater was arranged acetic acid as a sole carbon source.

6.4.1. Raw Reactor Analysis

Three sets of respirometric analysis were conducted using only synthetic, only raw penicillin formulation and synthetic with raw penicillin formulation wastewater addition with acclimated sludge to all mentioned wastewater types.

100 % removal efficiency is obtained from the experiment used only synthetic wastewater as 300 mg/l COD at the beginning (Figure A.9 and Table 6.5).

Table 6.5. General Evaluation for Synthetic Wastewater, Raw Penicillin Formulation Wastewaters

Model Coefficient	Synthetic	Raw	Raw with Synthetic
F/M (g COD/g VSS)	0.26	098	0.58
Y_{STO} (g COD/ g COD)	0.72	-	0.72
Y_{HWW} (g COD/ g COD)	0.65	0.65	0.65
$S_{Tinitial}$ (mg COD.l-1)	300	1200	900
$S_{Tutilized}$ (mg COD.l-1)	300	640	459
$S_{AAinitial}$ (mg COD.l-1)	300	-	300
$S_{AAutilized}$ (mg COD.l-1)	300	-	300
$S_{WWinitial}$ (mg COD.l-1)	-	1.200	600
$S_{WWutilized}$ (mg COD.l-1)	-	640	159
Removal Efficiency for S_T (%)	100	53	50
Removal Efficiency for S_{AA} (%)	100	-	100
Removal Efficiency for S_{WW} (%)	-	53	27

At the second experiment, the system was fed together with raw (600 mg COD/l) and synthetic wastewater. No effect was observed on μ_{max} in the first plateau, but hydrolysis of raw penicillin formulation wastewater begins with the end of the synthetic wastewater consumption. (Figure A.10) After the hydrolysis step of penicillin wastewater, the system went to the b_H. No penicillin consumption was occurred until completely synthetic wastewater consumption. In this experiment, the removal efficiency was obtained 27% for penicillin and 100 % for synthetic wastewater with a good agreement in the batch reactor system performance. Retention time is very important for biological treatment of penicillin formulation wastewater, because of slowly hydrolysis biodegradable fraction.

At the last experiment, the system was fed with only 1200 mg/l COD at the beginning. Hydrolysis of penicillin formulation wastewater occurred in the system by four steps (Figure 6.3). It is very obvious that the retention time is extremely important for this type wastewater. Removal efficiency is 53 % for approximately 15 hours.

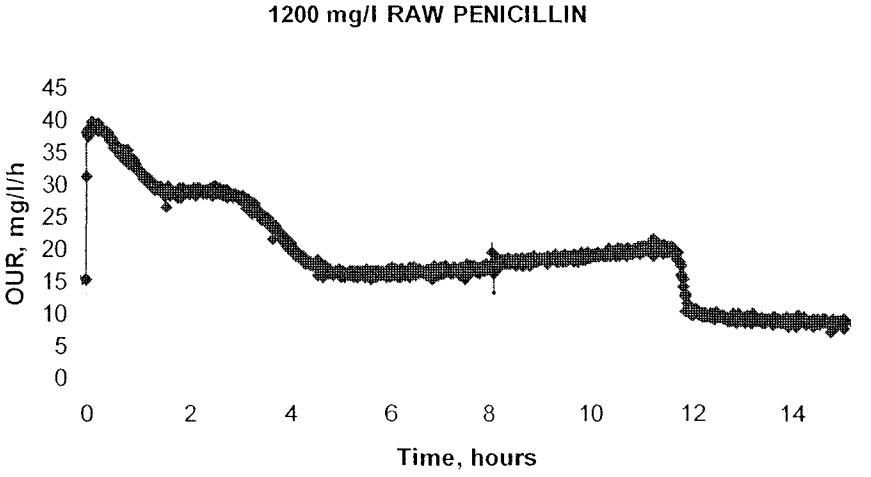

Figure 6.3. Respirometric Data for only Raw Penicilin Addition (1200 mgCOD/l)

In-cycle observation was conducted on raw reactor using only penicillin formulation wastewater of 600 and 1200 mg COD/l, respectively, (Figure 6.4). COD removal efficiencies for 8 hours were 34% and 43 % respectively for penicillin formulation wastewater of 600 and 1200 mg/l Retention time is important for the obtained COD values. More than 80% of COD removal efficiency for 24 hours evaluation was obtained for both systems. This shows that selecting of true retention time is extremely crucial for this type of wastewater.

6.4.2. O_3 Reactor Analysis

Six sets of respirometric measurements were conducted using ozonated reactor sludge. These experiments included just synthetic wastewater (Figure A.11), 5, 20, 40, 60 minutes ozonated penicillin formulation wastewater with synthetic wastewaters and finally just 20 minutes ozonated penicillin formulation wastewater.

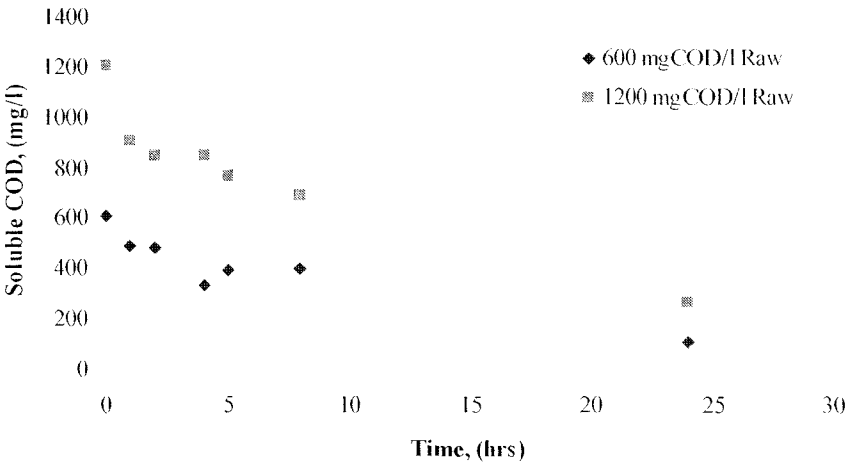

Figure 6.4. COD Concentration Changing with Time

It was observed that 5 and 20 min. ozonated wastewater with synthetic addition on the biological reactor gave an inhibition effect on maximum specific growth rate because of a decrease on the first plateau (Figure A.12-A.13). But after a while the system was recovered it selves with synthetic wastewater removal efficiency of 100 % and ozonated penicillin wastewater removal efficiency of more than 55 % for both (Table 6.6).

There is no inhibition effect on maximum growth rate for 40 minutes ozonation (Figure A.14). Approximately, the same efficiency was obtained with the 5 and 20 minutes ozonation.

There is an inhibition effect for 60 minutes ozonation (Figure A15). The removal efficiency was decreased approximately 10 %. 100 % synthetic removal occurred and 21 % ozonated wastewater removal has obtained. For conclusion, long terms of ozonation could cause of inhibition. But, after a while the system recovers it selves.

The same efficiencies (60%) were obtained for only 20 minutes ozonated (Figure A.16) and 20 minutes ozonated with synthetic wastewater.

Table 6.6 General Evaluation for Synthetic, Ozonated Penicillin Formulation Wastewaters

Model Coefficient	Synthetic	Ozonated with t20	Synthetic with t5	Synthetic with t20	Synthetic with t40	Synthetic with t60
F/M (g COD/g VSS)	0.21	0.08	0.23	0.42	0.12	0.17
Y_{STO} (g COD/ g COD)	0.72	-	0.72	0.72	0.72	0.72
Y_{HWW} (g COD/ g COD)	-	0.65	0.65	0.65	0.65	0.65
$S_{Tinitial}$ (mg COD.l-1)	435	87	631	481	251	285
$S_{Tutilized}$ (mg COD.l-1)	435	52	545	456	218	218
$S_{AAinitial}$ (mg COD.l-1)	435	-	435	435	200	200
$S_{AAutilized}$ (mg COD.l-1)	435	-	435	435	200	200
$S_{WWinitial}$ (mg COD.l-1)	-	87	196	46	51	85
$S_{WWutilized}$ (mg COD.l-1)	-	52	110	28	18	18
Removal Efficiency for S_T (%)	100	60	86	87	87	77
Removal Efficiency for S_{AA} (%)	100	-	100	100	100	100
Removal Efficiency for S_{WW} (%)	-	60	56	60	35	21

6.4.3. O_3/H_2O_2 Reactor Analysis

Five sets of respirometric measurements were performed ozonated with hydrogen peroxide reactor including 5,20,40,60 minutes ozonated with hydrogen peroxide Penicillin Procaine G and one for the controlling with synthetic wastewater. 20 min ozonation with H_2O_2 result was shown in Figure 6.5. All respirometric data illustrations were arranged in Appendix section (Figure A.17-A.21).

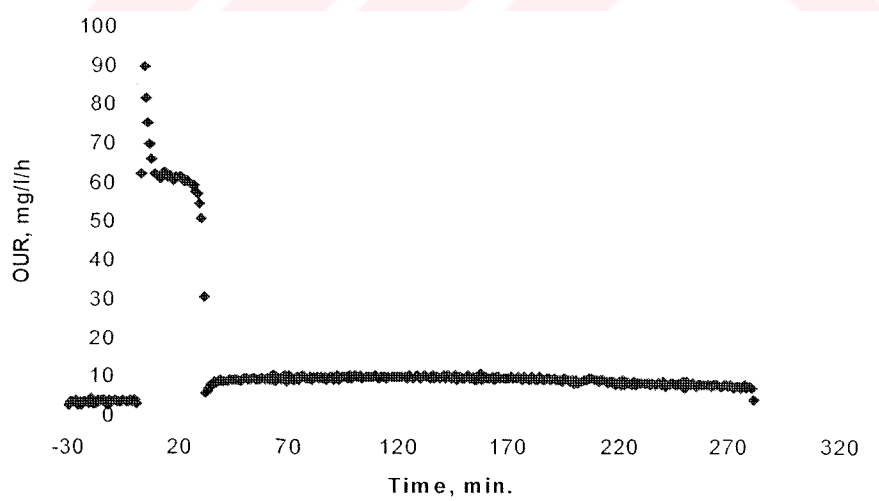

Figure 6.5. OUR Data for 20 min ozonation with H_2O_2

6.5. Modelling Results for O_3/H_2O_2 Reactor Analysis

Results of model simulations obtained for the biodegradation experiments are depicted in Figure 6.6. The model simulations present reliable predictions of the respirometric behaviour of the effluent samples during biodegradation. Table 6.7 elucidates the total amount of soluble COD ($S_{Tinitial}$) fed to the reactors during batch experiments, the amount of utilized COD ($S_{Tutilized}$) and the values of kinetic and stoichiometric parameters obtained for each wastewater mixture.

Table 6.7. ASM3 model coefficients obtained for synthetic wastewater, ozonated with H_2O_2 penicillin formulation wastewaters

Model Coefficient	Synthetic	Synthetic with t_5	Synthetic with t_{20}	Synthetic with t_{40}	Synthetic with t_{60}
$S_{Tinitial}$ (mg COD.l^{-1})	220	280	295	295	295
$S_{Tutilized}$ (mg COD.l^{-1})	220	200	220	220	***180***
b_H (d^{-1})	0.2	0.2	0.2	0.2	0.2
b_{STO} (d^{-1})	0.2	0.2	0.2	0.2	0.2
Y_{STO} (gCOD.(gCOD)$^{-1}$)	0.80	0.80	0.80	0.80	0.80
Y_H (gcellCOD.(gCOD)$^{-1}$)	0.54	0.54	0.54	0.54	0.54
k_{STO} (d^{-1})	10	10	10	10	10
K_S (mg COD.l^{-1})	3	3	3	3	3
μ_H (d^{-1})	3	3	3	3	3
K_{STO} (gCOD.(gcellCOD)$^{-1}$)	0.002	0.002	0.002	0.002	***0.4***

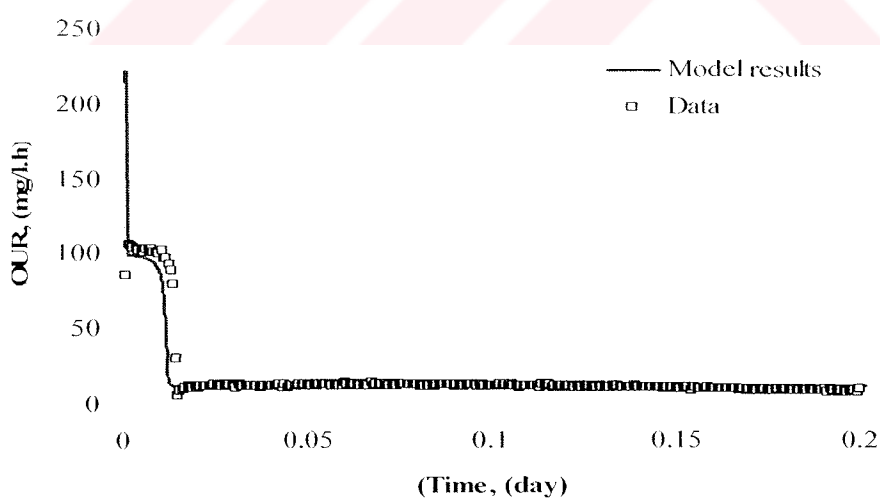

Figure 6.6. Experimentally observed and modeled OUR profiles obtained for 20 min–ozonated penicillin formulation and synthetic wastewater mixture.

Process stoichiometry of the applied model has been defined in accordance with ASM3 and the previously published literature. A storage yield, Y_{STO}, of 0.8 gCOD.(gCOD)$^{-1}$ and a heterotrophic growth yield, Y_H, of 0.54 g cellCOD.(gCOD)$^{-1}$ have been estimated. The stoichiometric coefficients, f_{SI}, fraction of the soluble metabolic products, and f_I, fraction of the particulate metabolic products, have been chosen as 0.10 gCOD.(gCOD)$^{-1}$.

The values obtained for substrate storage rate, k_{STO}, and for the maximum heterotrophic growth rate, μ_H, of synthetic wastewater bearing five different readily biodegradable COD components, were in complete accordance with those values reported for acetic acid. The substrate storage affinity constant, K_S, being found as 3 mg l^{-1}, appeared to be applicable for acetic acid in the scientific literature.

According to the OUR profiles presented in Appendix Section, the addition of 5, 20 and 40 min ozonated with H_2O_2 penicillin formulation wastewater to the synthetic wastewater at a volumetric ratio of 25 % resulted no removal in the penicillin formulation effluent. Results of the model simulation done for this batch test have indicated that the kinetic coefficients established for substrate storage and growth processes were identical to those obtained for synthetic wastewater. Conclusively, organic matter present in ozonated penicillin formulation wastewater was not biodegradable. This trend has also been observed on COD removal in the system.

The 60 min-ozonated penicillin formulation effluent exerted an inhibitory effect upon synthetic wastewater substrate degradation. Only 180 mg l^{-1} COD of the synthetic wastewater substrate having an initial COD of 295 mg l^{-1} could be utilized upon the addition of 60 min-ozonated penicillin formulation effluent, corresponding to a approximately 20 % decrease in substrate utilization. This effect is in complete accordance with the information reported in literature for the inhibitory effect of prolonged ozonation periods on successive biological treatment (Karahan et al., 2002). Although the kinetic parameters of substrate storage obtained for 60 min. ozonated wastewater were the same as that of synthetic wastewater, the inhibiting behaviour could be observed on heterotrophic growth with a high K_{STO} value of 0.4 g COD·(gcell COD)$^{-1}$.

7 CONCLUSION

The study described herein provides an evaluation of the biological treatability of raw and ozonated and ozonated with H_2O_2 penicillin formulation effluent as Procain Penicillin G.

Penicillin Procaine G and 60 min ozonated system performances were shown that overall COD removal was 80%. But, for penicillin procaine G effluent, hydrolysis mechanism was very crucial for biological system. For effective biological penicillin procaine G removal, more than 24 hours retention time is necessary.

Experiments on the biological reactor with 5 and 20 min. ozonated wastewater with synthetic addition were showed that there was an inhibition effect on maximum specific growth rate. The system was recovered at the end of the experiment, according to overall efficiency. Respirometric analyses performed with 5, 20 and 40 min ozonated wastewaters were shown that overall efficiency was approximately 60%. The removal efficiency obtained for 60 minutes ozonated with synthetic wastewater was decreased approximately 10 % according to 5, 20 and 40 min ozonation results because of long terms of ozonation effect on inhibition.

The addition of 5, 20 and 40 min ozonated with H_2O_2 penicillin formulation wastewater to the synthetic wastewater at a volumetric ratio of 25 % resulted no removal in the penicillin formulation effluent. Prolonged ozonation of the penicillin formulation effluent did not provide satisfactory results in terms of biocompatibility. 60 min-ozonation with H_2O_2 of the penicillin formulation effluent appeared to reduce the COD removal rate of the synthetic effluent fraction by 20 % during successive biodegradation. The heterotrophic growth was also inhibited by the oxidation products formed after ozone "overdosing". Ozonation with H_2O_2 advanced oxidation process effluent for Procain Penicillin G was not applicable for biological treatment.

The study presented herein also provided experimental support and information regarding activated sludge modelling using respirometric methods and inhibition kinetics. Further research should be conducted on the assessment of specific inhibition kinetics for pharmaceutical industry wastewaters originating from antibiotic formulation being most well known for their inhibitory nature. The description type and mechanism of inhibition still needs additional experimental studies including specific data on storage products.

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APPENDIX

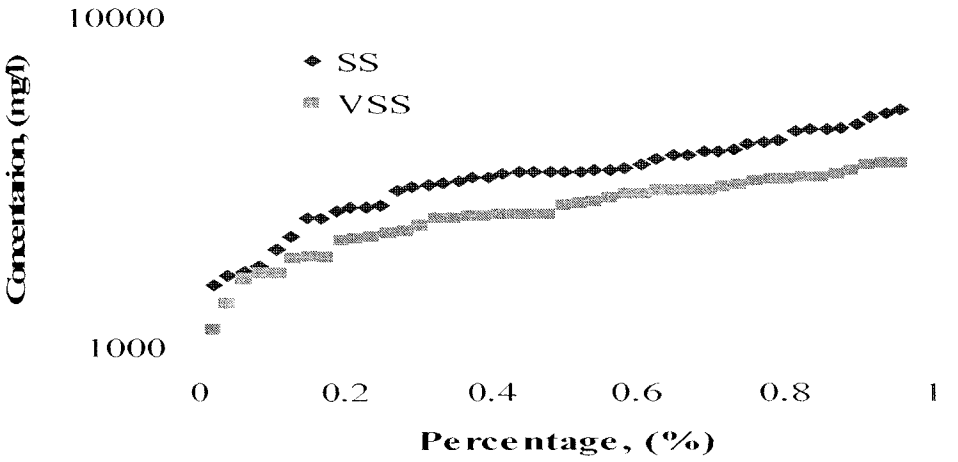


Figure A.1. Data for a period of 8 months for Synthetic Reactor (Reactor 1)

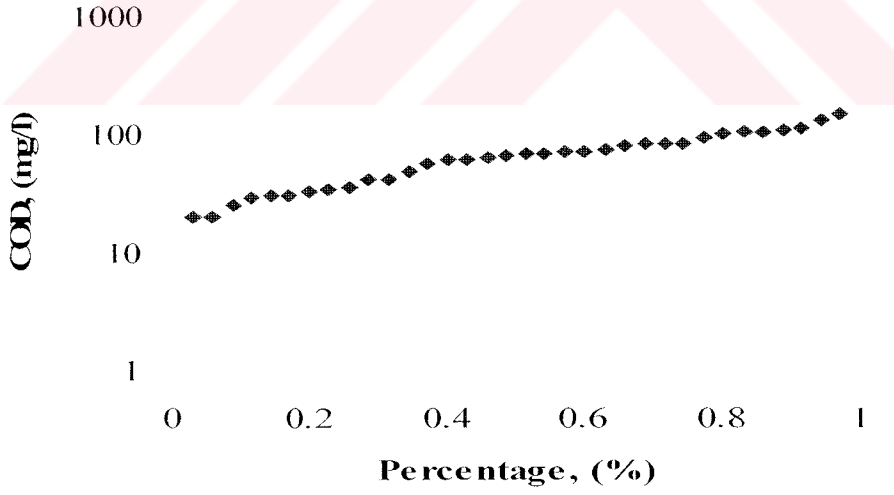


Figure A.2. Data for a period of 8 months for Synthetic Reactor (Reactor 1)

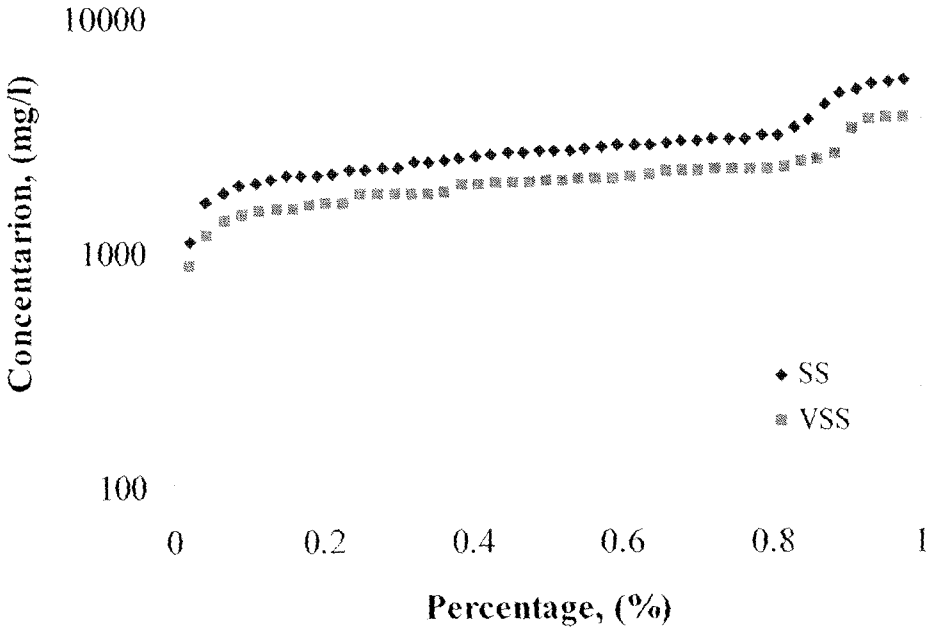
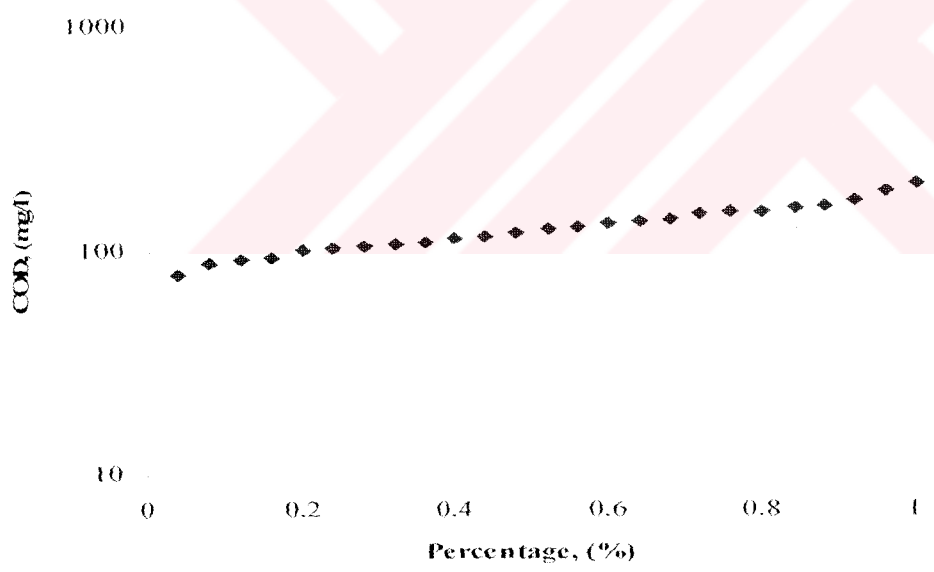


Figure A.3. Data for a period of 8 months for Raw Reactor (Reactor 2)



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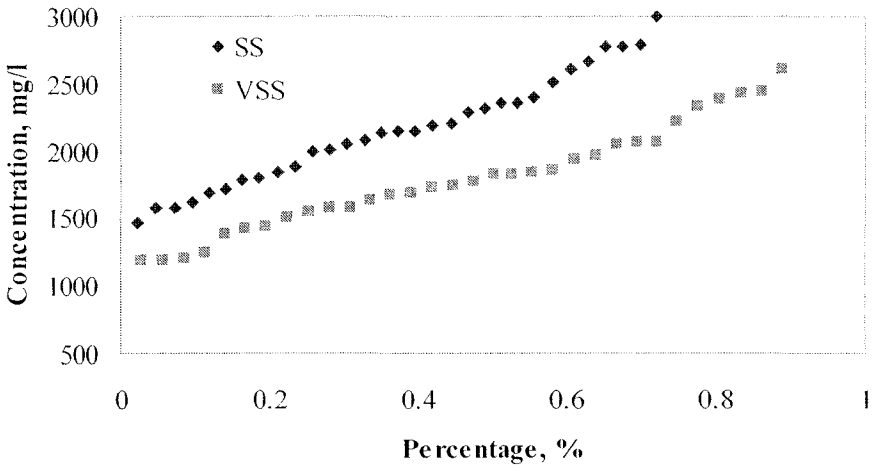
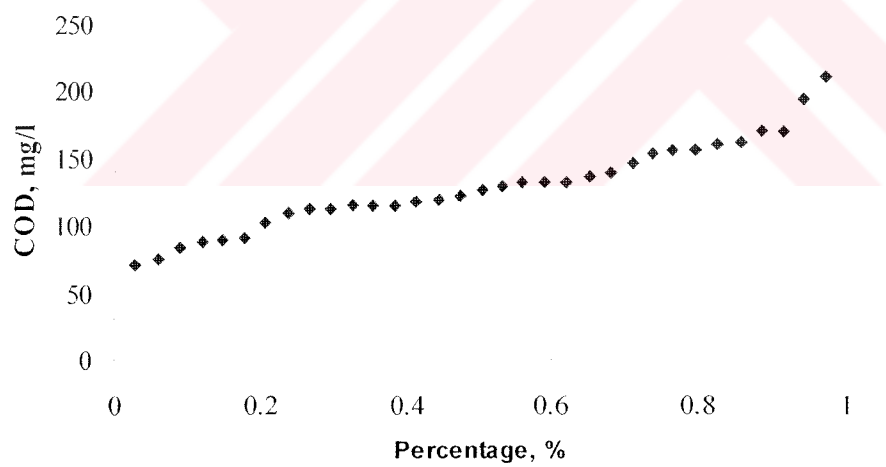


Figure A.5. Data for a period of 8 months for Ozonated Reactor (Reactor 3)



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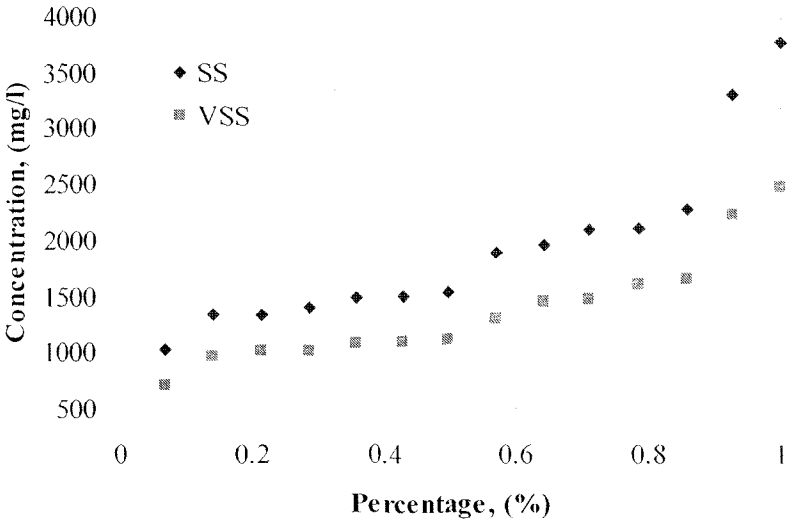


Figure A.7. Data for a period of 8 months for Ozonated with H_2O_2 Reactor (Reactor 4)

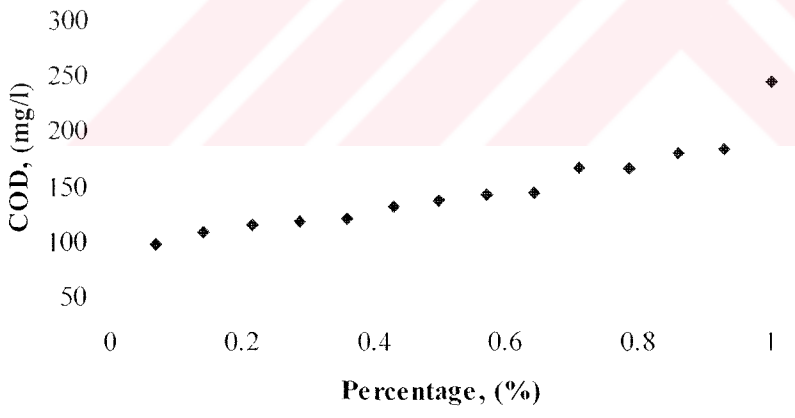


Figure A.8. Data for a period of 8 months for Ozonated with H_2O_2 Reactor (Reactor 4)

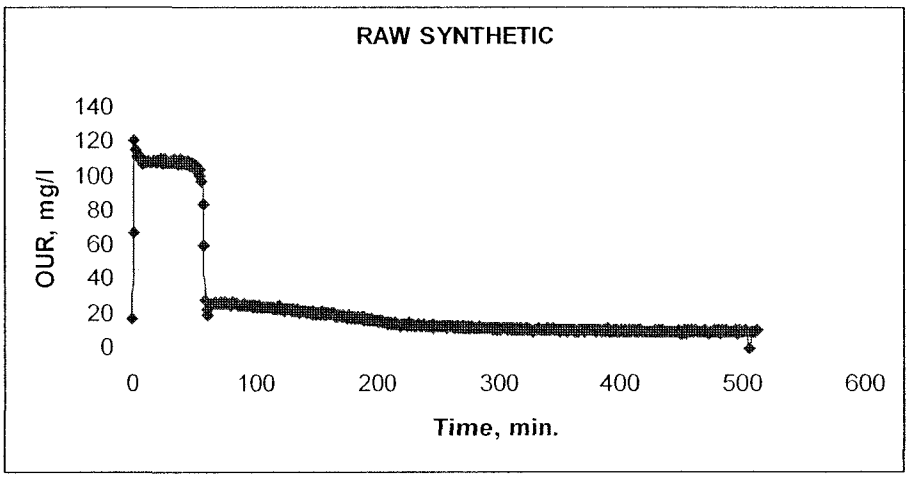


Figure A.9. OUR Data for Synthetic Wastewater (Raw Reactor)

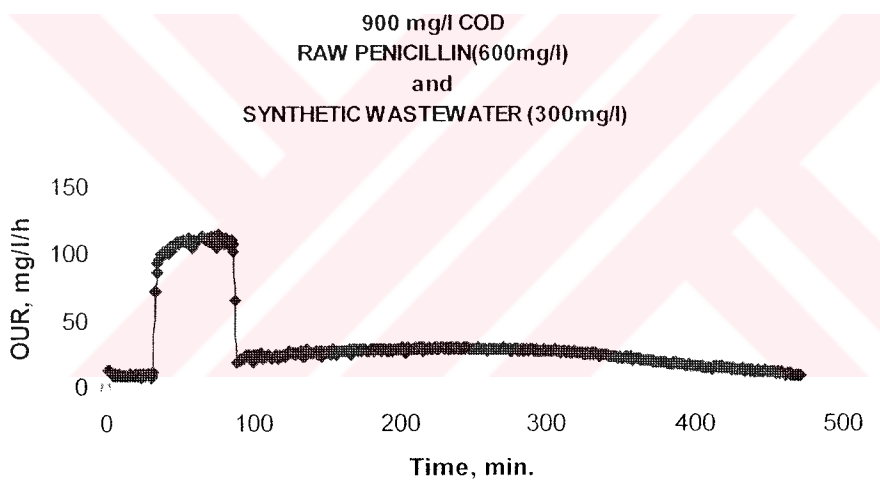
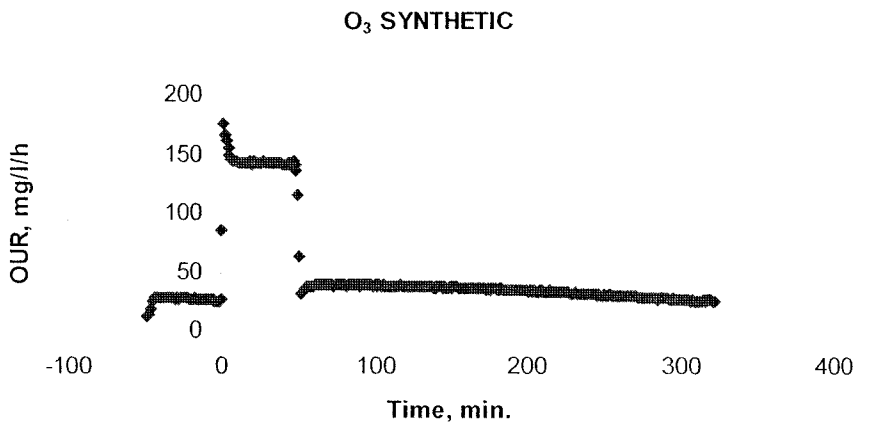


Figure A.10. OUR Data for Raw Penicillin and Synthetic Wastewater



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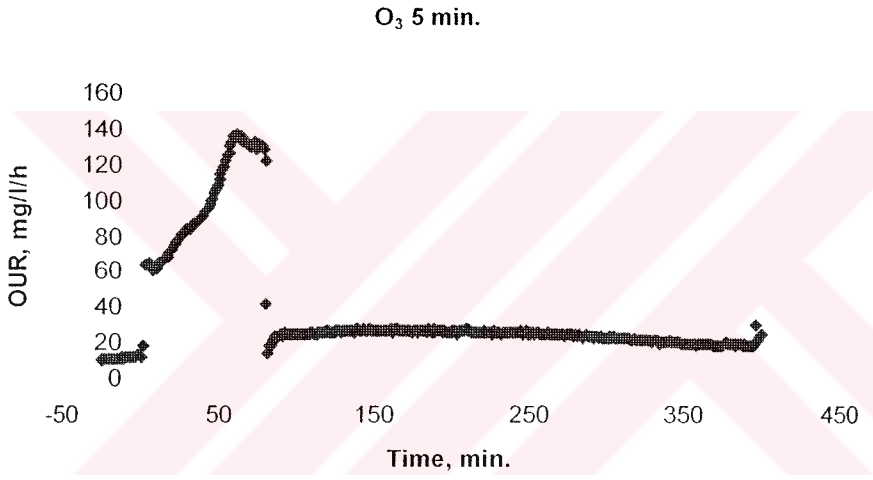


Figure A.12. OUR Data for 5 min Ozonation

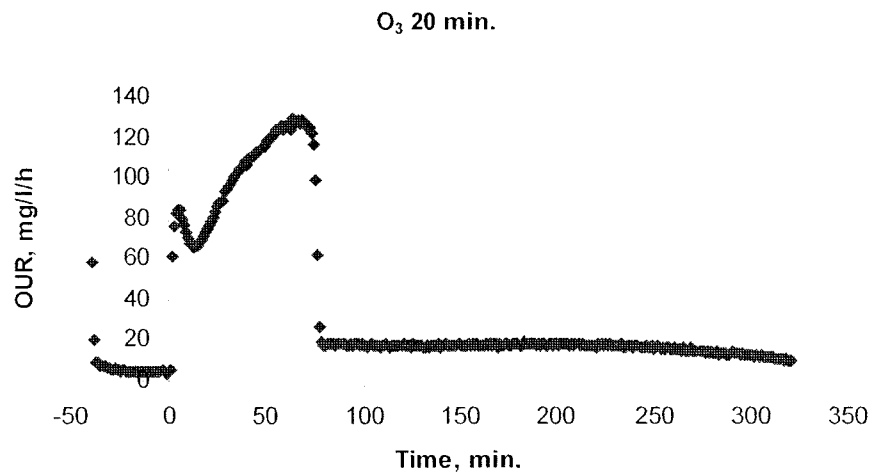


Figure A.13. OUR Data for 20 min Ozonation

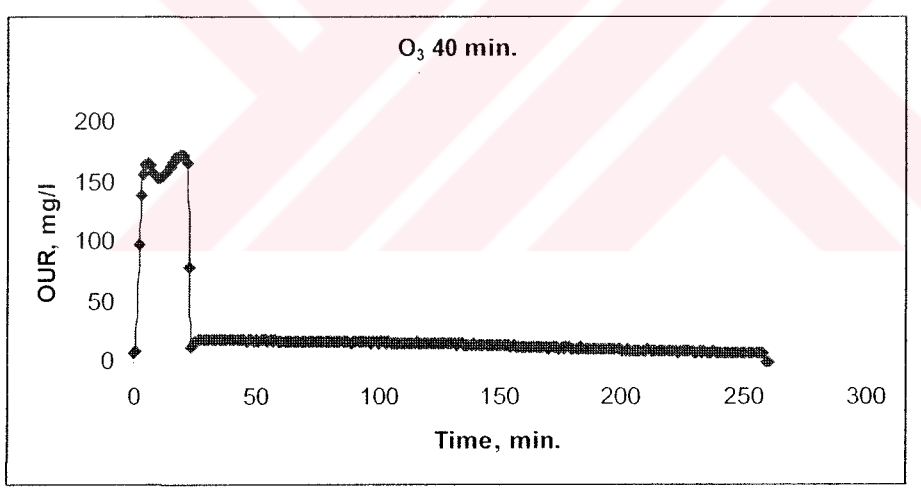


Figure A.14. OUR Data for 40 min Ozonation

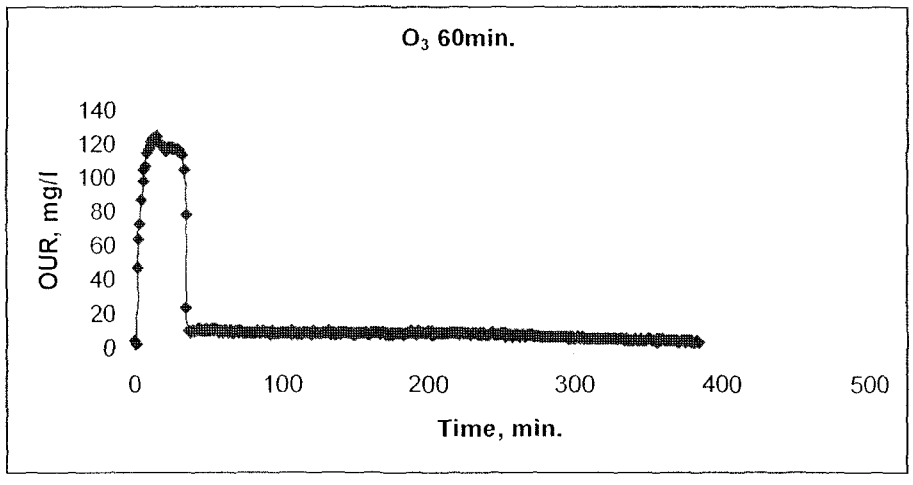


Figure A.15. OUR Data for 60 min Ozonation

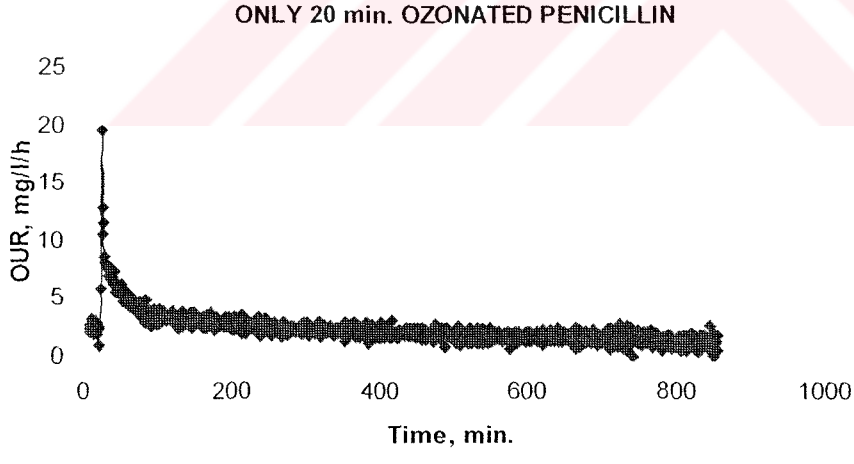


Figure A.16. OUR Data for only 20 min Ozonated Penicillin

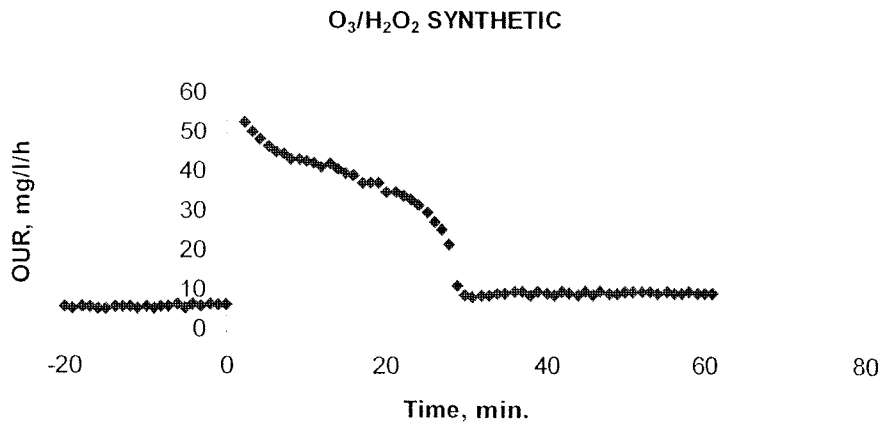


Figure A.17. OUR Data for Synthetic Wastewater (Ozonated with H_2O_2 Reactor)

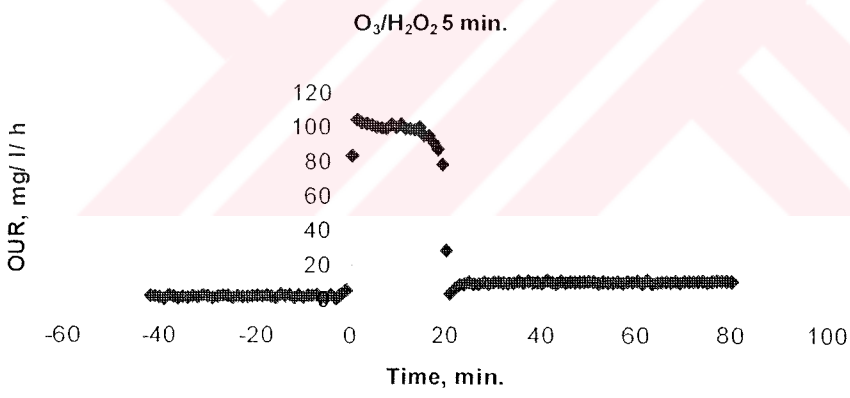


Figure A.18. OUR Data for 5 min ozonation with H_2O_2

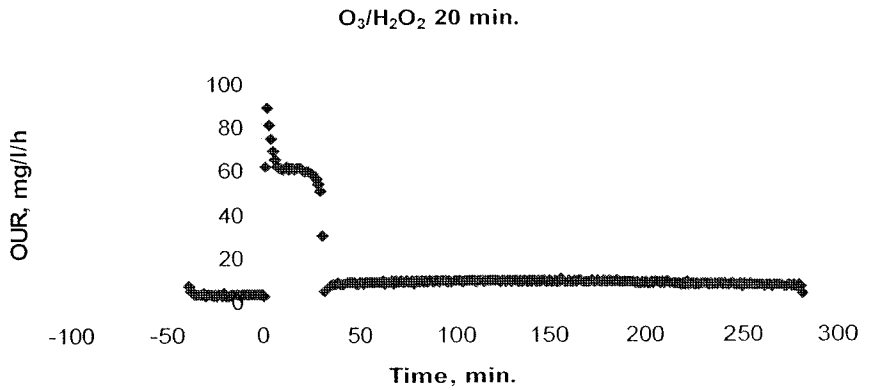


Figure A.19. OUR Data for 20 min ozonation with H_2O_2

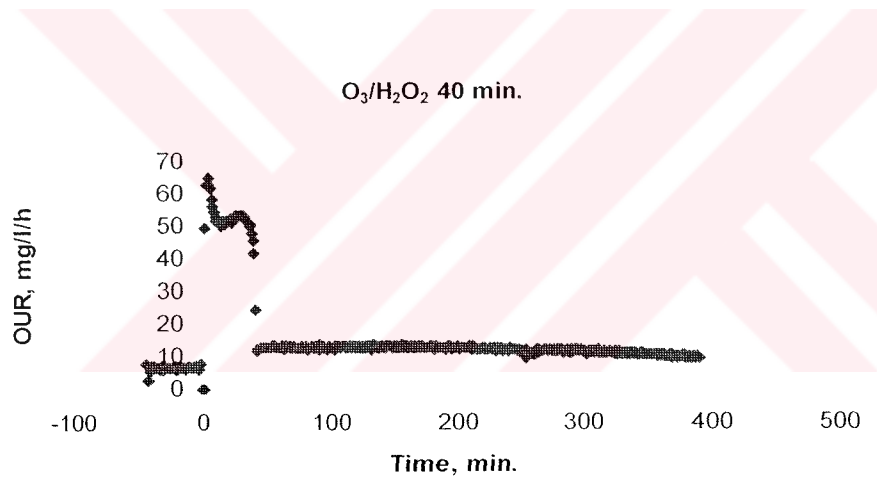


Figure A.20. OUR Data for 40 min ozonation with H_2O_2

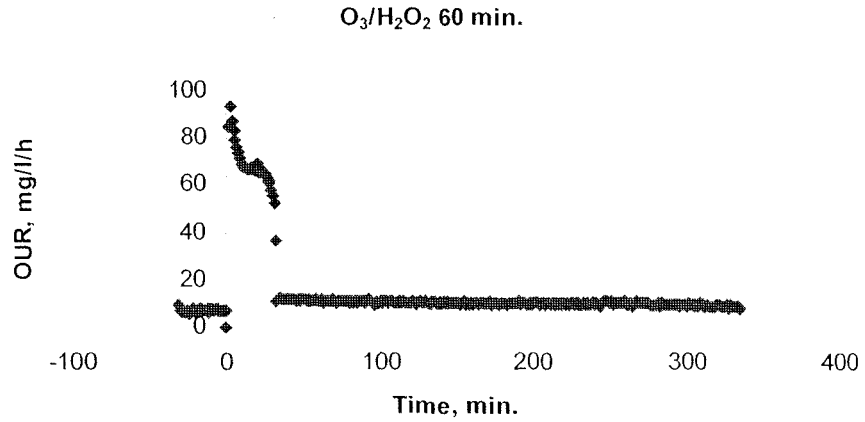
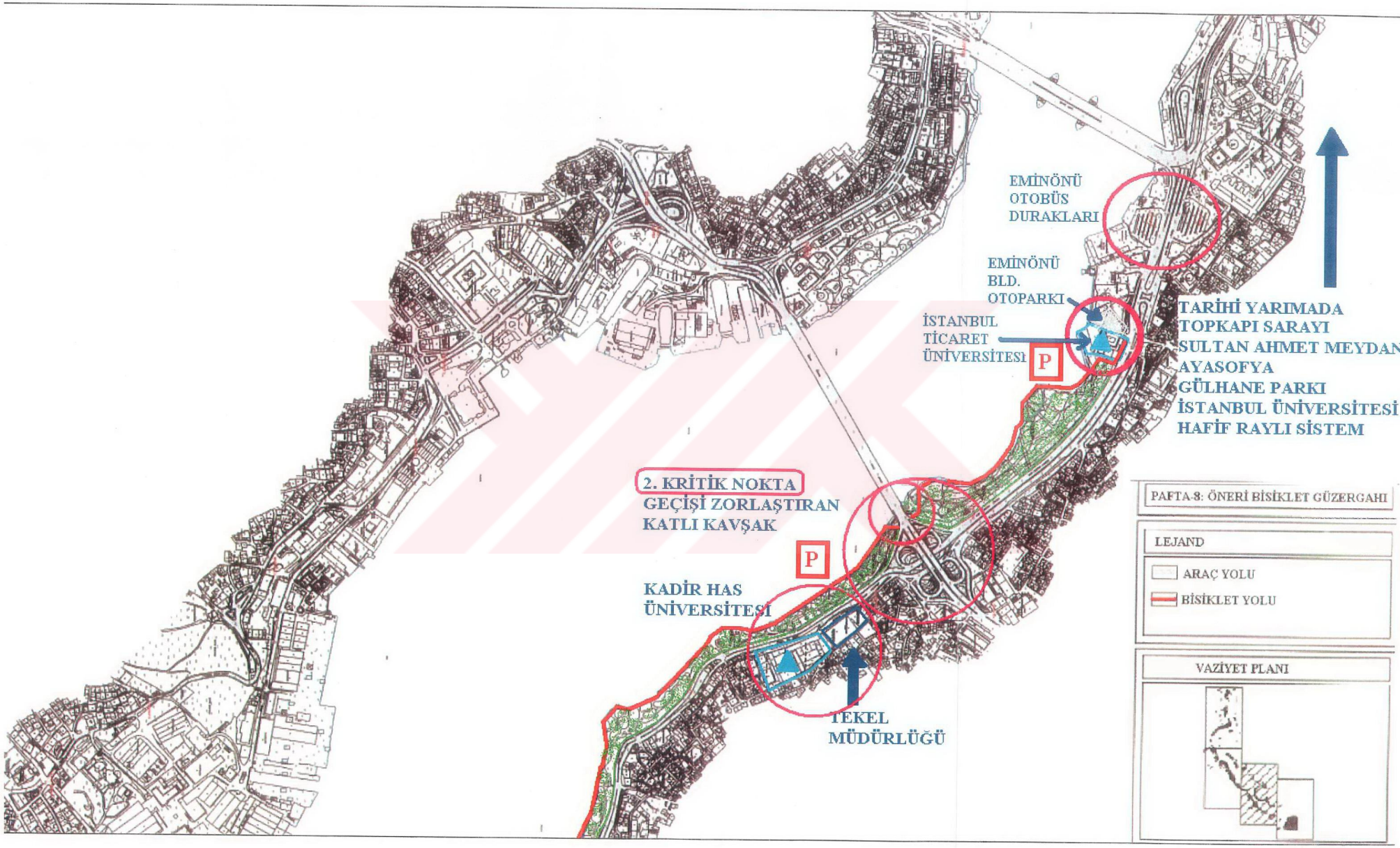


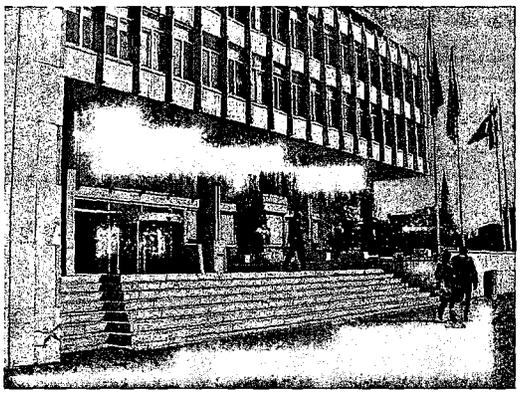
Figure A.21. OUR Data for 60 min ozonation with H_2O_2

CURRICULUM VITAE

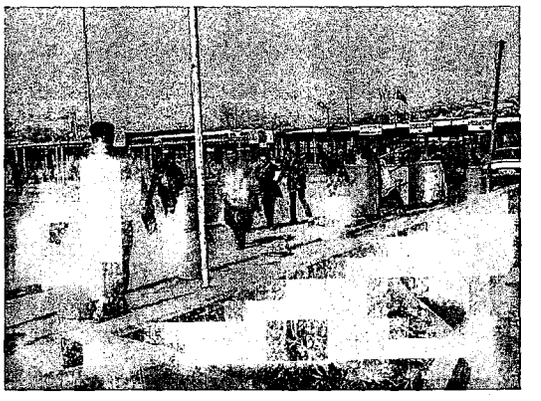
Hande Saruhan was born in Ankara in 1978. She graduated from Trabzon Affan Kitapcioglu High School in 1995. She received her B.Sc. degree in Environmental Engineering from Yildiz Technical Uviversity in 2001. She started studying for her master's degree in 2002 in Environmental Biotechnology Programme of İstanbul Technical University.


EMİNÖNÜ
OTOBÜS
DURAKLARI
EMİNÖNÜ
BLD.
OTOPARKI
İSTANBUL
TİCARET
ÜNİVERSİTESİ
P
TARİHİ YARIMADA
TOPKAPI SARAYI
SULTAN AHMET MEYDAN
AYASOFYA
GÜLHANE PARKI
İSTANBUL ÜNİVERSİTESİ
HAFİF RAYLI SİSTEM
2. KRİTİK NOKTA
GEÇİŞİ ZORLAŞTIRAN
KATLI KAVŞAK
P
KADİR HAS
ÜNİVERSİTESİ
TEKEL
MÜDÜRLÜĞÜ
PAFTA-8: ÖNERİ BİSİKLET GÜZERGAHI
LEJAND
ARAÇ YOLU
BİSİKLET YOLU
VAZİYET PLANI

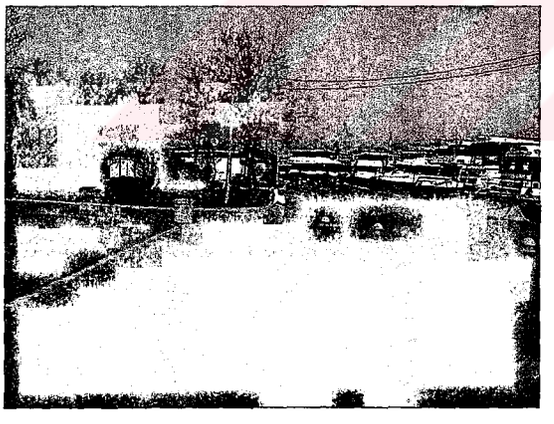
EK 2 . PROJE ALANINDAN FOTOĞRAFLAR



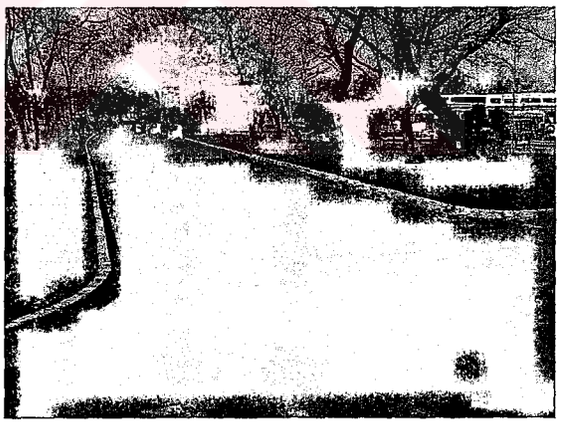
Fotoğraf-1: İstanbul Ticaret Üniversitesi (Eminönü). Bisiklet Yolu Projesinin Başlangıç Noktası



Fotoğraf-2: Eminönü İETT Otobüs Durakları



Fotoğraf-3: Park Alanı İçerisindeki Yaya Alanları



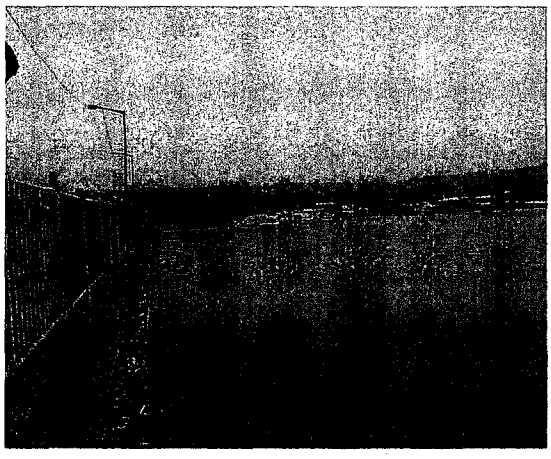
Fotoğraf-4: Park Alanı İçerisindeki Yaya Alanları

Fotoğraf-5: Park Alanı İçindeki Çamurlu Bozuk Yaya Yolları

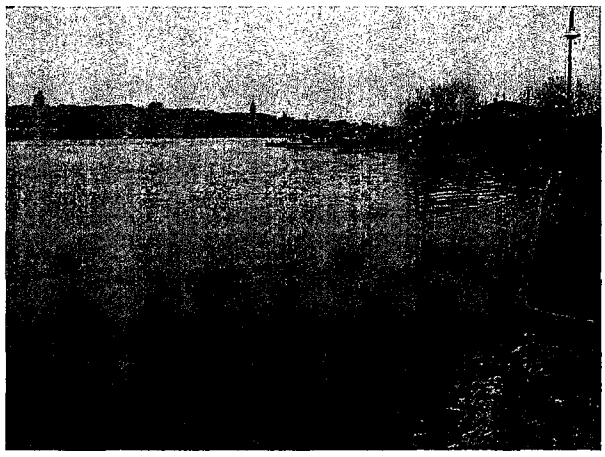
Fotoğraf-6: Park Alanı İçindeki Çamurlu Bozuk Yaya Yolları

Fotoğraf-7: Galata Kulesi'ne Bakan Haliç Kıyısı Ve Balıkçılar

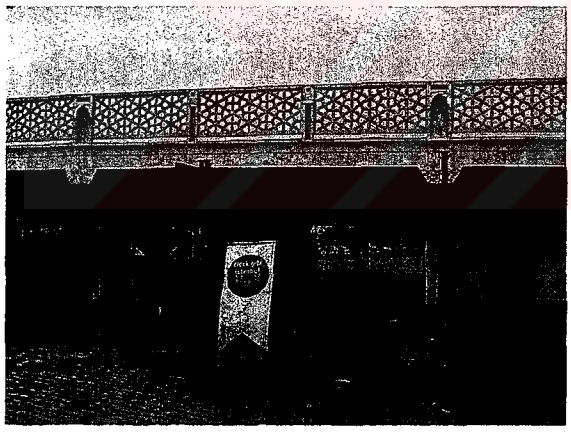
Fotoğraf-8: Haliç Kıyısındaki Bozuk Zemin



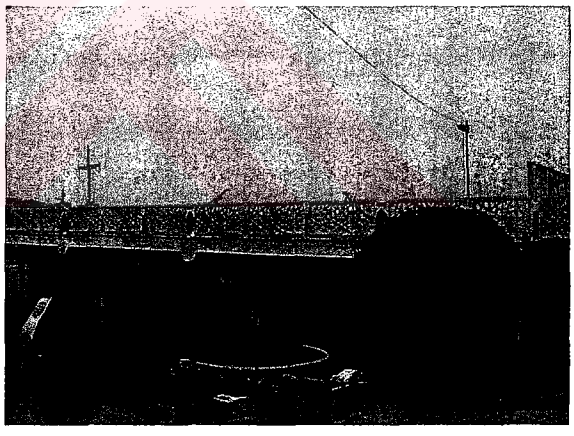
Fotoğraf-9: Çökerek Sular Altında Kalan Dolgu Alanı



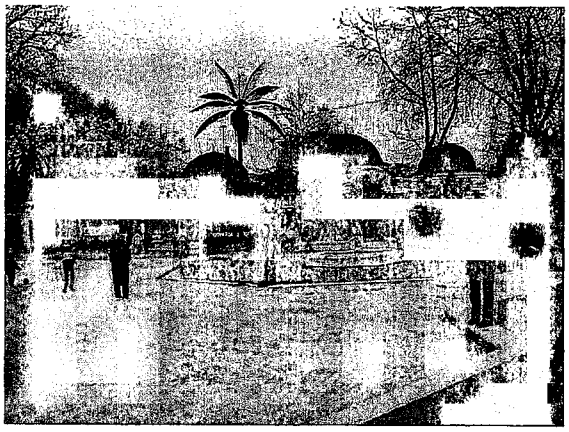
Fotoğraf-10: Çökerek Sular Altında Kalan Dolgu Alanı



Fotoğraf-11: Altından Geçişin Mümkün Olan, Fakat Şu Anda Depo Olarak Kilitli Kullanılan Galata Köprüsü



Fotoğraf-12: Altından Direk Geçilmesi ve Üstünden de Karşı Kıyıya Geçilmesi Tasarlanan Galata Köprüsü



Fotoğraf-13: Eyüp Meydanı

Fotoğraf-14: Eyüp Meydanı

Fotoğraf-15: Eyüp Meydanı'nda Yayalaştırılmış Taşıt Park Alanı Olarak Kullanılan Yollar

Fotoğraf-16: Eyüp Meydanı'nda Yayalaştırılmış Yollar

ÖZGEÇMİŞ

1978 yılında Ankara'da ailemin beşinci ve en küçük ferdi olarak dünyaya geldi. İlköğretim eğitimimi Cezayirli Gazi Hasana Paşa İlkokulu'nda, orta ve lise eğitimimi ise Kadıköy İ.H.L'inde tamamladı. 1996 yılında İstanbul Teknik Üniversitesi, Mimarlık Fakültesi, Şehir ve Bölge Planlama Bölümü'nde lisans eğitimine İngilizce hazırlık sınıfıyla başladı ve 2001 yılında mezun oldu. 2001 yılında yine İstanbul Teknik Üniversitesi, İnşaat Fakültesi, Ulaştırma Mühendisliği Yüksek Lisans Programı'nda yüksek lisans eğitimine başladı.