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AEROBIC BIOLOGICAL TREATMENT OF OPIUM ALKALOID WASTEWATER - EFFECT OF GAMMA RADIATION AND FENTON'S OXIDATION AS PRETREATMENT

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AUGUST 2008

METU 2008

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A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

CAVİT BURAK BURAL

IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

AUGUST 2008

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

AEROBIC BIOLOGICAL TREATMENT OF OPIUM ALKALOID WASTEWATER-EFFECT OF GAMMA RADIATION AND FENTONS OXIDATION AS PRETREATMENT

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August 2008, 143 pages

In this study, aerobic biological treatment of opium alkaloid wastewater and the effect of gamma preirradiation and Fenton's oxidation were investigated. First, the biodegradability of alkaloid wastewater was investigated by batch reactors and wastewater was found to be highly biodegradable providing 83 – 90 % COD degradation. In order to evaluate the effect of irradiation, original wastewater and irradiated wastewaters (40 & 140 kGy) were compared by means of BOD_5/COD values and through aerobic batch experiments. Results indicated that irradiation imparted no further enhancement in biodegradability.

Sequencing batch reactor (SBR) studies revealed that the treatment operation was not possible due to sludge settleability problem observed beyond an influent COD value of 2 g/L. Possible reasons for problem were investigated, and the high molecular weight, larger size and aromatic structure of the organic matters present in wastewater was thought to contribute to poor settleability characteristics. Some operational modifications including phosphate buffer addition cured the settleability problem. Influent COD was then increased to 5,000 mg/L. Significant COD removal efficiencies (> 70 %) were obtained in SBRs fed with

both original and irradiated wastewaters. Preirradiated wastewater provided a better settling sludge in comparison to original wastewater.

Degradation of the complex structure was followed by GC/MS analyses, particle size measurements and enhancement in filterability. Pre-irradiation enhanced the filterability of wastewater more than Fenton's treatment and degradation by irradiation was proved by GC/MS analyses.

Key words: Opium Alkaloid Wastewater, Biodegradability, SBR, Gamma Radiation, Fenton Oxidation.

ÖZ

AFYON ALKALOİD ATIKSULARININ AEROBİK BİYOLOJİK ARITIMI – ÖN ARITMA OLARAK GAMA RADYASYON VE FENTON OKSIDASYONUNUN ETKİSİ

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Ağustos 2008, 143 sayfa

Bu çalışmada, afyon alkaloid endüstrisi atıksularının aerobik biyolojik arıtımı ve ön ışınlama ve Fenton oksidasyonunun arıtılabilirliğe etkisi araştırılmıştır. Çalışmanın ilk aşamasında alkaloid atıksuyunun biyolojik parçalanabilirliği kesikli reaktörler kullanılarak araştırılmış, sonuç olarak atıksuyun yüksek oranda biyolojik parçalanabilir olduğu tespit edilmiştir (% 83 – 90 KOİ giderimi). Işınlamanın etkisini araştırmak için orjinal ve ışınlanmış atıksular (40 kGy ve 140 kGy dozlarında) $BOİ_5$/KOİ değerleri ve aerobik kesikli reaktörlerde yapılan arıtılabilirlik deneyleri ile kıyaslanmış ve ışınlamanın biyolojik arıtılabilirliğe önemli bir etkisinin olmadığı görülmüştür.

Ardışık kesikli reaktörlerde (AKR) yapılan çalışmada 2 g/L giriş konsantrasyonundan itibaren gözlemlenen çamur çökme probleminden ötürü arıtımın mümkün olmadığı görülmüştür. Problemin olası sebepleri araştırılmış ve atıksudaki organiklerin yüksek molekül ağırlıklı, büyük boyutlu ve aromatik yapısının çamur çökme probleminden sorumlu olduğu düşünülmüştür. Fosfat tampon ilavesini de içeren bazı operasyonel değişiklikler uygulanmış ve uygulanan değişiklikler sonucunda çamur çökme problemi aşılabilmiştir. Giriş

KOİ değeri 5,000 mg/L'ye kadar çıkarılmıştır. Orjinal ve ışınlanmış atıksuyla beslenen reaktörlerde yüksek KOİ giderim verimleri (>70 %) elde edilmiştir. Ön ışınlamaya tabi tutulan atıksuyla beslenen reaktörde daha iyi çamur çökmesi tespit edilmiştir.

Atıksuyun kompleks yapısındaki parçalanma GC/MS analizleri, tanecik boyut analizleri, ve süzülebilirlik testleri ile takip edilmiştir. Ön ışınlamanın, süzülebilirliği, Fenton arıtımına oranla daha fazla iyileştirdiği tespit edilmiş ve atıksu yapısındaki parçalanma GC/MS analizleri ile de gösterilmiştir.

Anahtar kelimeler: Afyon Alkaloid Atıksuyu, Biyolojik Arıtılabilirlik, Ardışık Kesikli Reaktör, Işınlama, Fenton Oksidasyonu.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor Prof. Dr. Filiz B. Dilek for her valuable guidance and encouragement throughout this research. I would like to thank my co-supervisor Prof. Dr. Göksel N. Demirer for his helpful recommendations and motivational support.

I would like to acknowledge TAEK (Turkish Atomic Energy Authority) for supplementing wastewater and conducting gamma irradiation for this study. I am thankful to Dr. Ömer Kantoğlu and Yüksel Mert for their assistance during this research.

I would like to thank Ramazan Demir, Kemal Demirtaş, Aynur Yıldırım, Gizem Uğurlu, Mehmet Yazıcıoğlu for their assistance in laboratory.

Sincere thanks to Fadime Kara and Mete Yüksel for their support. I would also like to thank my friends Recep Özdemir, Kadir Gedik, Murat Varol, Özge Yılmaz, Onur Güven Apul, Gülçin Özsoy, Emrah Alkaya, Hakan-Çiğdem Moral, Firdes Yenilmez, Aysun Vatansever and Umay Yücel.

I wish to express my special thanks to Dr. Erkan Şahinkaya who spent many hours with me answering countless laboratory questions.

Finally, I would like to dedicate this study to my family for their life-long support, encouragement and patience.

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BOD : Biochemical oxygen demand

COD : Chemical oxygen demand

COD:N:P : Chemical oxygen demand to Nitrogen to Phosphorus ratio

DO : Dissolved oxygen

HRT : Hydraulic retention time

MLSS : Mixed liquor suspended solids

MLVSS : Mixed liquor volatile suspended solids

METU : Middle East Technical University

N : Nitrogen

P : Phosphorus

SBR : Sequencing batch reactor

SRT : Solid retention time

SS : Suspended solids

SVI : Sludge volume index

TAEK : Turkish Atomic Energy Authority

TKN : Total kjeldahl nitrogen

VSS : Volatile suspended solids

X : Biomass concentration

Greek letters:

μ : Specific growth rate

μ_m : Maximum specific growth rate

θc : Sludge age

CHAPTER 1

INTRODUCTION

1.1. General

A variety of industrial processes generate highly polluted wastewater which constitute a major environmental concern and require adequate treatment prior to discharge into receiving waters. Meeting the stringent discharge limits set by environmental regulations is a challenge for many industrial facilities.

Biological treatment of wastewater is commonly used in industry. The most widely used process today is the activated sludge process in which microorganisms suspended in wastewater metabolize the soluble organic matter present under aerobic conditions. The microorganisms convert the carbonaceous organic matter into various gases and cell tissue. The biological treatment is completed by the removal of produced cell tissue from treated liquid by gravity settling (Metcalf and Eddy, 1991).

Many variations of the process have been developed since its inception. Sequencing Batch Reactor (SBR) is a variation of the activated sludge process which operates on a batch fill-and-draw principle. It combines all the unit processes of conventional activated sludge system into a single tank operating in time, rather than space (EPA, 1999). The concept has become accepted worldwide and employed successfully as an alternative to the continuous flow activated sludge process in treatment of both municipal and industrial wastewaters (Wilderer et al., 2001).

Several factors affect the performance of biological wastewater treatment systems such as temperature, pH, nutrient and trace element availability, hydraulic retention time and dissolved oxygen (DO) concentration. Although the biodegradability of a wastewater is high, its treatability may still be complicated due to some operational problems such as sludge bulking. Along with numerous other factors, the composition of wastewater itself is a significant factor on success of biological treatment systems.

In this present research, high strength effluents from an Opium Alkaloid Processing Plant located in Afyon Province of Turkey was investigated for its biological treatability, as its treatment has been standing problematic for years due to complex structural compounds contained in the wastewater.

In this plant, opium poppy harvested under the control and license of Turkish Government is processed to produce morphine and other important alkaloids for medicinal purposes. Alkaloid products are exported in substantial quantities after meeting the domestic demand and provide a considerable amount of financial income to our country (Sevimli et al., 1999).

The plant has a capacity to process 72 tons of opium per day and the specific wastewater production is about 6.7 m^3 per ton of opium capsule. Thus, the plant generates around 480 m^3/day wastewater with high organic matter concentration, and intense dark brown color (Sevimli et al., 1999). Discharging of this effluent to Eber Lake together with sugar, cement and other industry effluents imparts considerable pollution causing unacceptable environmental conditions which have detrimental effect on fish and bird species that use the lake as their habitat.

Current literature on the treatment of opium alkaloid factory effluents is very limited because the cultivation and processing of opium is not practiced in most of the developed countries (Sevimli et al., 1999). Several treatment options including

chemical, physicochemical and biological methods were investigated so far by other researchers (Section 3.2.4). However these studies could not present a solid solution to the problem. Therefore, the treatment of this industrial wastewater is still a challenging task.

Chemical pre-treatment prior to biological treatment can be used to improve the system performance for this kind of complex industrial wastewaters. Recently, chemical treatment methods, based on the generation of strong oxidizing hydroxyl radicals, known as advanced oxidation processes (AOPs), have been widely applied for pollutant degradation (Pérez et al., 2002). Fenton's oxidation is one of the most common AOPs and its association with an aerobic biological stage is accepted as a promising methodology in wastewater treatment (Souza et al., 2005).

Among the AOPs, application of radiation technology - including ^{60}Co-gamma irradiation and e-beam accelerator - in wastewater treatment is drawing considerable attention around the world (Bao et al., 2002; Diaz et al., 2003; Jo et al., 2006). Their application is based on interaction of reactive oxidizing and reducing species, that are formed by radiolytic ionization of water, with organic pollutants in both primary (direct effects) and secondary ionizations (indirect effects) (Drzewicz et al., 2003; Meeroff et al., 2004). For complex industrial wastewaters, radiation treatment alone may require high absorbed doses. Therefore, the combination of radiation technology with conventional processes such as chemical (Fang and Wu, 1999) and in particular biological treatment (Jo et al., 2006) is more promising.

1.2. Purpose and scope of the study

The aim of this study was to investigate the aerobic biological treatability of opium alkaloid industry wastewaters in conjunction with gamma irradiation,

anaerobic treatment and Fenton's oxidation as means of pre-treatment. A special emphasis was placed on radiation application as a relatively new research area.

The tasks carried out during this study are:

- Investigation of aerobic biological treatability of opium alkaloid wastewater in batch reactors,
- Determination of the effect of Fenton's oxidation on treatment of opium alkaloid wastewater,
- Determination of further treatability of anaerobically pre-treated opium alkaloid effluents in aerobic batch reactors,
- Comparative investigation of aerobic biological treatability of pre-irradiated and raw opium alkaloid wastewaters in batch and sequencing batch reactors (SBRs).

The following chapter (Chapter 2) presents theoretical background relevant to the treatment processes/methods (such as biological wastewater treatment, sequencing batch reactors, Fenton's oxidation and radiation technology and its environmental applications) used in this study. Information is provided on opium alkaloid industry, its wastewater and previous treatability studies conducted with alkaloid wastewater in Chapter 3. Chapter 4 outlines the experimental procedures and analytical methods employed throughout the study. The results of experiments are presented in Chapter 5. Chapter 6 presents the conclusions drawn from this study. Appendices contain sample calculations, experimental details, and other analysis reports.

CHAPTER 2

THEORETICAL BACKGROUND

2.1. Biological Wastewater Treatment

The general objectives of the biological treatment of wastewater are to remove the non-settleable colloidal solids and to stabilize the organic matter. Biological treatment can be applied almost all wastewaters with proper analysis and environmental control. For domestic wastewater, the main objective is to reduce the organic content and, in many cases the nutrients. The objective in industrial wastewater treatment is to remove or reduce the concentration of organic and inorganic compounds. In case these compounds are toxic to microorganisms, pretreatment may be required.

Biological processes can be divided into five major groups as aerobic, anoxic and anaerobic processes, combination of these, and pond processes. Further subdivision can be made depending on whether treatment is accomplished in suspended or attached growth systems. As the name implies, suspended growth processes are the ones in which the microorganisms accomplishing the biological treatment are maintained in suspension within the liquid. On the other hand, in attached growth or fixed-film processes microorganisms are attached to some inert medium such as rocks, slag, or specially designed plastic or ceramic materials.

The principal applications of these processes are for:

- the removal of the carbonaceous organic matter in wastewater
- nitrification / denitrification

- phosphorus removal and
- waste stabilization

The principal suspended-growth processes used for the removal of carbonaceous organic matter include the activated sludge process, aerated lagoons, aerobic digestion process and sequencing batch reactors. However, by far the most commonly used suspended growth biological treatment process today is the activated sludge process (Metcalf and Eddy, 1991).

2.1.1. The Activated Sludge Process

The activated sludge process is the most widely used technology in biological wastewater treatment in the world. It is a suspended growth process in which a mixed culture of microorganisms removes organic contaminants from the wastewater. The invention of the process is connected with the efforts of British and American engineers to intensify biological purification in fixed-film systems (Wanner, 1994).

In 1914, the activated sludge process was discovered in England by E. Ardern and W. T. Lockett who noted that aeration of sewage led to formation of flocculent suspended particles. They discovered that the time to remove organic contaminants was reduced from days to hours when these particles were held in the system. They referred to the suspended particles as being “activated” (Rittman and McCarty, 2001). Activated sludge was in fact an active biomass responsible for the improvement of the process intensity and treatment efficiency (Wanner, 1994).

Since its development, the activated sludge process has gained increasing importance in the treatment of both municipal and industrial wastewaters.

Furthermore, due to its flexibility and adaptability to almost any kind of biological waste treatment problem, many modifications have been developed up to now.
The conventional process consists of the aeration tank, a settling tank, solids recycle from settling tank to the aeration tank, and a sludge wasting line. A basic setup is illustrated in Figure 2.1. The aeration tank is a suspended growth reactor which contains *flocs* or microbial aggregates of microorganisms. Suspension in reactor is maintained through mixing by aeration or other mechanical means. The reactor contents are referred to as *mixed liquor*. When the mixed liquor pass to the settling tank, the flocs are removed from the treated wastewater by settling and some portion is returned to the aeration tank to maintain desired biomass concentration therein; and some other portion is wasted to control the solids retention time (SRT).

Through recycling, a high concentration of biomass can be kept in aeration tanks allowing effective oxidization of organic compounds in acceptable detention times, generally measured in hours (Rittman and McCarty, 2001; Wanner, 1994).

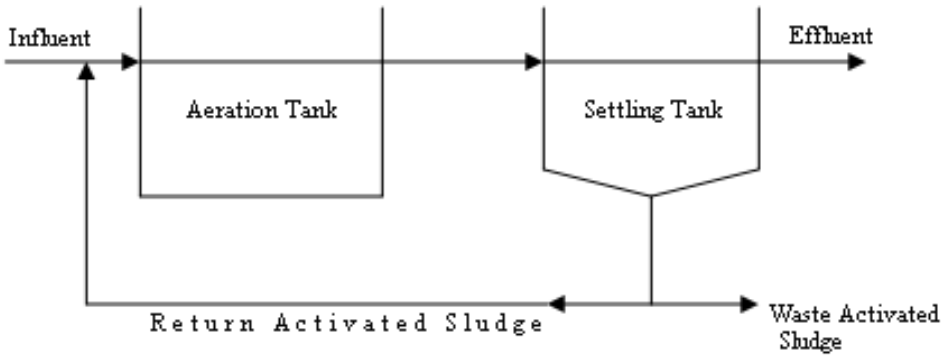


Figure 2.1. Simplified representation of the conventional activated sludge process

The level of biological mass to be kept in the reactor depends on the desired treatment efficiency and also on the other considerations related to growth kinetics (Metcalf and Eddy, 1991). Wasting the excess sludge from a separate line allows SRT to be separate from and much larger than the hydraulic detention time (Rittman and McCarty, 2001).

The most important function in the activated sludge process is the flocculent nature of the microbial biomass. The flocs have to be efficient in the adsorption and subsequent absorption of the organic fraction of the wastewater, and they also have to rapidly and effectively separate from the treated effluent in the settling tank. Any change in the reactor operation leads to changes in the nature of the flocs, and this may adversely affect the overall performance in a number of ways, most notably in poor sludge settlement resulting in biomass loss and turbid effluent (Gray, 2004).

As presented, capturing the flocs in the settler and recycling them back to the aeration tank are the two keys to the activated sludge process in order both to maintain a clear effluent and a sufficient biomass concentration in the aeration tank. These facts underline the importance of good settling properties of mixed cultures.

2.1.1.1. The Activated Sludge Microbiology

The activated sludge contains a wide variety of microorganisms. The main biological groups present include bacteria, protozoa, nematodes, rotifers and in certain conditions fungi. The limiting conditions in which the activated sludge is cultivated lead to strong competitions between individual groups of microorganisms, hence only the best-adapted ones can prevail. Predominance of certain microorganisms in community can be used to assess the process efficiency (Cheremisinoff, N.P. 1996). As the influencing factors such as the wastewater

characteristics, pH, temperature, DO, nutrient concentration, and sludge age change in wastewater treatment systems, the microbial composition of activated sludge and dominant species also change reflecting the effects that activated sludge system is exposed to (Wanner, 1994).

The primary consumers of the organic wastes in activated sludge are the heterotrophic bacteria, though with certain organic particles, protozoa may also be involved. Activated sludge flocs also harbor autotrophic bacteria such as nitrifiers Nitrosomonas and Nitrobacter (Bitton, 1994). By mass, the dominant members of this community are heterotrophic bacteria (Pike and Curds, 1971). They form the basis of flocs. The process itself selects the most efficient flocculating bacteria by returning back those flocs that rapidly settle in the sedimentation tank. Those species associated with flocs and other settleable material will also be returned to the aeration tank and thrive (Gray, 2004).

The group of bacteria involved in activated sludge systems belongs primarily to the Gram negative species, including carbon oxidizers and nitrogen oxidizers, floc-formers and nonfloc-formers, and aerobes and facultative anaerobes (Liu and Lipták, 1997). The major genera found in flocs include *Zoogloea*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Bacillus*, *Achromobacter*, *Corynebacterium*, *Comomonas*, *Brevibacterium*, and *Acinetobacter*, as well as a variety of filamentous microorganisms including *Sphaerotilus*, *Beggiatoa*, *Vitreoscilla* which are associated with bulking problems (Bitton, 1994).

Protozoa and other higher life forms may constitute approximately 5 % of activated sludge by weight and are represented by about 200 species (Curds, 1973; Curds, 1975). Total numbers can be on the order of 50,000 cells/mL (Pike and Curds, 1971). Protozoa are motile, single celled eukaryotes and the majority of protozoa are aerobic heterotrophs (Metcalf and Eddy, 1991). They are classified by means of locomotion as *flagellates, amoebae, and ciliates*. Ciliates

are further subdivided into free, creeping, and stalked ciliates. Creeping ciliates graze on bacteria on the surface of flocs (Bitton, 1994). Stalked protozoans that are attached to the flocs and the free swimming protozoans both feed on dispersed bacteria and organic particulate matter that is in suspension (Curds and Vandyke, 1966). So, they consume non or loosely flocculated bacteria, helping provide a clarified effluent (Curds et al., 1968).

Ciliates are accepted as the indicators of good floc formation and those ciliates that attach to flocs with a stalk, rather than the free swimming ones, are the best indicators of stable sludge. Furthermore, because protozoa are more sensitive to toxins than bacteria, their presence or absence can be used as an indicator of toxicity (Rittman and McCarty, 2001; Jenkins et al., 2004).

Other microorganisms in activated sludge include fungi, nematodes, and rotifers. Fungi are consumers of organic matter and predators for rotifers and nematodes. These eucaryotic organisms produce long filaments called hyphae, which forms a mass called mycelium. Activated sludge does not usually favor growth of fungi. They may grow abundantly under specific conditions of low pH (=5), toxicity, and nitrogen deficiency. A proliferation of fungi usually imparts poor settleability to the sludge (Bitton, 1994; Liu and Lipták, 1997).

Rotifers are aerobic heterotrophic, multicellular animals (metazoa) which are attached to activated sludge flocs. Since they need long time for development, they are more abundant in processes with long sludge ages (Doohan, 1975). They help in the removal of freely suspended (nonflocculated) bacteria and also contribute to floc formation by producing fecal pellets surrounded by mucus. Moreover, they break up big flocs into smaller ones which encourage new floc formation. Their presence in conventional plants indicates stable operating conditions (Gray, 2004; Bitton, 1994; Metcalf and Eddy, 1991; Cheremisinoff, 1996).

Little is known about the role of metazoa nematodes as they represent only a minor part of the biomass, if any. Their population doubling time is so long that normal sludge ages do not allow them to develop (Chaudhuri et al. 1965; Schiemer 1975; Gray, 2004).

2.1.1.2. The Activated Sludge Floc

Floc is the basic operational unit of activated sludge. Activated sludge flocs consist of biological and nonbiological components. Biological component includes a wide variety of bacteria, fungi, protozoa and metazoa, and non-biological component is made up of inorganic and organic particles. The basis of the floc is heterotrophic bacteria which include genera such as *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, and *Zooglea* (Jenkins et al., 2004). These bacteria like many other chemorganotrophic bacteria are able to convert organic substrates to specific extracellular polymeric substances (EPS), termed glycocalyx (Costerson et al., 1981). These biopolymers are composed mostly of proteins and carbohydrates (Jenkins et al., 2004).

Organic polymer glycocalyx increases water viscosity and enables the individual cells to stick together or to attach to solid surfaces or larger aggregates. Thus, bioflocculation can be described as an interaction of exopolymers of individual flocculating cells which form a three dimensional matrix. The bridging of cells is based on a decrease of surface electrostatic charge (Wanner, 1994). At approximately neutral pH values these polymers carry net negative charges. Divalent cations such as calcium and magnesium are thought to interact with negatively charged polymers to form bridges that allow cells to adhere to each other (Higgins and Novak, 1997).

Sezgin et al. (1978) described the filamentous backbone theory which assumes that the structure of activated sludge floc is formed in two levels. The first level,

termed the microstructure, is provided by processes of microbial adhesion, aggregation, and bioflocculation. It is the basis for floc formation because, without the ability of one microorganism to stick to another, large aggregates of microorganisms would not form. Microstructure flocs are small (up to 75 µm), spherical and compact, but mechanically rather weak. The second level, termed macrostructure, is provided by filamentous microorganisms. The filamentous microorganisms form a backbone for the flocs and the floc-formers firmly attaches to backbone by their extracellular polymers (EPS). Since the shape of macrostructure flocs follow the shape of the filamentous backbone, larger, irregularly-shaped flocs are built.

Although widely accepted, this theory does not explain the absence of filamentous backbones in well-flocculating activated sludges (Chudoba, 1989; Bitton, 1994). Because floc strength depends on the integrity of the biopolymer bridging, strong and weak flocs can be exist both with and without filaments (Jenkins et al., 2004).

2.1.1.3. The Activated Sludge Separation Problems

The separation of biomass from treated wastewaters is crucial to the quality of the final effluent from a wastewater treatment plant. A successful separation requires that the sludge floc settles and compact well in the settling tank. It must settle well so that the effluent has low amount of suspended solids; and must compact well so that the sludge can be returned to the aeration tank successfully. A well-compacted sludge also reduces the costs related with dewatering and disposal of the waste solids (Rittman and McCarty, 2001).

Unfortunately a successful separation is not always possible. The major problem in operation is the development of poor settling sludge. When this occurs, the desired SRT can not be maintained, effluent BOD often exceeds the limitations, and suspended solids pass to the effluent in concentrations that often exceed

regulatory standards. The suspension escaping from secondary settling tanks is formed by flocs, i.e. agglomerates of living microorganisms, and they consume the dissolved oxygen in receiving waters (Wanner, 1994; Rittman and McCarty, 2001).

Many sludge settling problems may occur but their causes are not all the same. The cause may be related to the particular configuration of the treatment plant, to the loading applied, to the environmental conditions, to the presence of particular wastewater constituents or by the absence of others (Rittman and McCarty, 2001). Jenkins et al. (2004) tabulated a summary of different activated sludge separation problems with their possible causes and effects as given in Table 2.1.

Dispersed Growth

Dispersed growth is a situation in which bacterial cells do not aggregate and do not form flocs. The microorganisms are dispersed freely as individual cells or very small clumps with a diameter of up to 10 – 20 µm (Wanner, 1994). The external polymer amount in such systems is too small to permit good flocculation (Rittman and McCarty, 2001). The sedimentation rate of individual cells or bacterial clumps is too low, resulting in a very turbid effluent. No zone settling occurs in settling tanks.

Dispersed growth is often observed during startup of activated sludge systems and where the SRT is too short (1 – 3 days) for the formation of the normal structure of good-settling sludge (Wanner, 1994; Rittman and McCarty, 2001). Excessive turbulence caused by aggressive aeration may be another reason hindering floc formation and resulting in dispersed growth.

Table 2.1. Sludge separation problems that can develop in Activated Sludge Systems (adapted from Jenkins et al., 2004)

Problem	Cause of Problem	Effect of Problem
Dispersed growth	Microorganisms are dispersed, they do not form flocs but only small clumps of single cells	Turbid effluent; no zone settling
Pinpoint floc	Flocs are small, compact, weak, roughly spherical. Larger ones settle rapidly; smaller ones slowly	Low SVI; cloudy turbid, high SS effluent
Blanket rising	Denitrification in the settler releases poorly soluble N_2 gas. Released gas attaches to flocs and floats them to the surface	Chunks of activated sludge float on surface of settler and anoxic zones of aeration tank.
Viscous (nonfilamentous) bulking	Microorganisms present in large amounts of exocellular slime. In severe cases, slime imparts jelly-like consistency to the activated sludge	Reduced settling and compaction rates; in severe cases virtually no solids separation; sludge blanket may overflow the secondary clarifier; formation of viscous foam.
Filamentous bulking	Large amount of filamentous organisms extend from flocs, or create open diffuse flocs, interfering with compaction, settling, and thickening	High SVI, very clear supernatant; in severe cases sludge blanket overflows the secondary clarifier; solid handling units become hydraulically overloaded
Foam/scum formation	Caused by nondegradable surfactants and by Nocardia sp. and sometimes *M. Parvicella*.	Foams can float large amounts of SS to surfaces of treatment units; microorganism-caused foams are persistent and difficult to break mechanically; foams can overflow tank freeboards

Pinpoint Flocs

Another phenomenon that results in the formation of small particles that do not settle well is pinpoint floc. The flocs have a pure microstructure without any filamentous microorganisms. Due to lack of macrostructure the flocs come to be small, compact, and spherical with a weak structure so that they are easily broken up into smaller flocs in the aeration tank (Gray, 2004). While the larger compact flocs settle rapidly; the smaller aggregates that sheared off from larger ones settle slowly creating a turbid supernatant (Jenkins et al., 2004). The unsettleable particles are of larger dimension (50 – 100 μm) than is the case with dispersed growth (Wanner, 1994).

In contrast to dispersed growth, pinpoint floc is mainly associated with systems with long SRT, such as extended aeration systems which operate at much lower F/M ratio than conventional systems. In these systems the old floc is subject to heavy predation by eukaryotic cells, resulting in the destruction of the floc characteristics and the production of biological debris, most of which is not active (Rittman and McCarty, 2001). Also, excessive turbulence caused by over aeration may hinder floc formation and result in pinpoint flocs.

Activated sludge plants treating industrial wastewaters, especially from chemical and the pharmaceutical sectors, produce small weak flocs deprived of filaments, resulting in typical pinpoint floc problem. The reason in this case is the action of toxic compounds that prevents filament development outside flocs (Gray, 2004).

Filamentous Bulking

The presence of filamentous bacteria is not always associated with poor settleability and moreover filamentous bacteria are suggested to provide a backbone for the overall floc structure (Sezgin et al., 1978). In an ideal floc, the

filamentous organisms are retained largely within the floc giving it a definite structure and strength, and the filaments protruding from the floc are of sufficiently low length not to interfere with settlement (Gray, 2004). It is the excessive growth of filamentous bacteria that leads to poor settleability.

There are major physiological differences between floc-forming and filamentous organisms. Filamentous bacteria have a higher surface-to-volume ratio than their floc-forming counterparts, which helps them survive under low oxygen and low nutrient conditions. They also have a low half-saturation constant and a high affinity for substrates, thus surviving well under starvation conditions. Therefore, filamentous bacteria are able to predominate under low DO, low F/M, low nutrient conditions, and also high sulfide levels (Bitton, 1994).

When they are abundant, the filaments interfere with the settling and compaction of the activated sludge by producing a *diffuse* floc structure or by extending outside the flocs into the bulk solution and *bridging* between them. The type of floc formed, the type of compaction and settling interference depends on the type of filamentous organisms present. Some filamentous organisms can cause both types of interference (Jenkins et al., 2004).

More than 25 types of filamentous organisms are known to be able to cause activated sludge bulking. *Sphaerotilus natans*, *Thiothrix sp*, types *021N*, *0961*, *0803*, *0041*, and *Haliscomenobacter hydrosis* have been identified to cause *bridging* type bulking whereas types *1701*, *0041*, *0675*, *Nostocoida limicola*, and *Microthrix parvicella* are associated with diffuse floc structure (Anon, 1979; Gray, 2004).

Although settles much more slowly than a normal sludge, when such sludge settles, it produces a very clear supernatant because the extended filament network filters out the small particles that otherwise cause turbidity (Jenkins et al., 2004; Gray, 2004).

Viscous (Non-filamentous) Bulking

This type of bulking occurs in absence of filaments. It is mainly associated with floc-forming bacteria and results when they produce an excess amount of extracellular polymer (glycocalyx). Normally, the biopolymer production is characteristic of most floc-forming microorganisms, and when produced in moderate amounts it serves good floc formation. However when it is produced in excess amounts, it may impart a slimy, jelly-like consistency to the sludge causing sludge settleability problem (Wanner, 1994; Rittman and McCarty, 2001).

Dispersed and flocculent microbial cells are surrounded by large amounts of exocellular biopolymers (Jenkins et al., 2004) and since the biopolymers are hydrophilic colloids the activated sludge becomes highly water retentive and exhibits low settling and compaction velocities. Since the biopolymers are also natural surface-active agents they cause foaming under intensive aeration (Wanner, 1994).

Overproduction of extracellular biopolymers can also lead to other kinds of separation problems. Polymers make the activated sludge sticky, and when the thickened layer of sludge is not periodically mechanically disturbed, the activated sludge loose its ability to flow. Such a decreased ability to flow can cause trouble with removing the thickened sludge layer from secondary settling tanks (Wanner, 1994).

When viscous activated sludge has been established, it is very difficult to ameliorate the problem. According to Jenkins et al. (2004), poor activated sludge settling caused by an excessive growth of biopolymers can not be satisfactorily controlled with the addition of polymers or hydrogen peroxide. Wanner (1994) also confirms this conclusion as the chlorination of the viscous activated sludge of a tannery wastewater plant was not successful.

Since the occurrence of non-filamentous bulking is not as common as filamentous bulking, there are few studies conducted on its causes and possible control methods. Novak et al. (1993) mention followings as the causes of non-filamentous bulking: wastewater containing high fatty and oleic compounds; high sludge loading or deficiency of nitrogen, phosphorus and/or trace nutrients; use of selectors to control filamentous bulking; and biological phosphorus removal systems which causes an excessive growth of poly-P bacteria e.g. *Acinetobacter spp* that produce excessive polymer.

In nutrient deficient conditions, the bacteria can not degrade some of the soluble COD and store it within the floc as an insoluble polysaccharide or slime to solubilize and degrade later when nutrients become available (Grau, 1991). Highly loaded plants with significant amounts of easily degraded carbohydrates, volatile fatty acids or in general readily biodegradable COD in the wastewater are prone to viscous bulking. Zoogleal bulking is commonly observed in those activated sludge systems designed to improve settling properties by using a concentration gradient, due to high initial F/M ratio obtained. Like in nutrient deficiency, the reason is the high amount of soluble COD that can not be degraded and is stored within the floc (Martinez, 2005).

Rising sludge

In the rising sludge phenomenon, two phases can be distinguished. First the activated sludge settles rapidly and a rather compact bottom layer of settled sludge and a clear supernatant are formed. After a certain period of time (at elevated temperatures even less than 30 minutes which cause difficulties in the SVI test), a whole or a part volume of the settled sludge starts to float and rise to the water surface. The floating material is full of gas bubbles. When the gas bubbles are partially released from the scum, some fraction of the floating sludge may settle again, but a substantial part of the floating material may escape from the

secondary settling tank deteriorating the quality of the final effluent (Wanner, 1994).

The nature of this phenomenon is endogenous denitrification which takes place in the settled and thickened activated sludge. When the dissolved oxygen from the previous aeration is quickly depleted by high amount of biomass concentration, anoxic conditions can be established, provided that nitrification occurs in the system. Bacteria respire using nitrate in place of free oxygen and release nitrogen gas as a by-product. The nitrogen bubbles freed during this endogenous denitrification act as sludge carrier (Wanner, 1994).

Unlike the others, this problem is not associated with sludge structure but rather with operational practice. However, the problem will be more serious if flocs have a macrostructure because their irregular shapes entrains gas bubbles more readily than smaller, spherical flocs. Also, as flocs with macrostructure are considerably larger, more sludge can be lost from the system (Gray, 1994).

The most effective cure of rising sludge problem is to stop nitrification in aeration tank so that no nitrate is formed by nitrification, then none can be denitrified to N_2 gas in the sedimentation tank. This may be accomplished by reducing the SRT and washing out the slow-growing nitrifiers (Rittman and McCarty, 2001) or by decreasing the aeration intensity or increasing the sludge loading to the aeration tank (Gray, 1994). Another way of preventing this problem is to promote denitrification as a part of activated sludge process, by using an anoxic zone prior to aeration. If the nitrate is removed before the mixed liquor enters the settler, then denitrification cannot occur in the settler.

Improved settler design may be a solution too. The idea here is to ensure that the sludge is not being held too long in the settler. Circular settlers with vacuum-type sludge removal devices are particularly effective and rectangular clarifiers with

sufficient speed sludge-removal scrapers may work well as long as accumulation of sludge in quiescent corners is prevented (Rittman and McCarty, 2001).

2.1.2. Acclimatization of Biological Sludges

There are several conditions which have to be fulfilled for a successful activated sludge treatment of industrial wastewaters. They may include: supplementation of the required mineral nutrients; proper pretreatment to make the wastewater more amenable to biological treatment; and adaptation of sludge to the specific substrates (Ganczarczyk, 1983).

It is necessary to acclimatize the biomass when treating industrial wastewaters. As defined in Environmental Engineering Dictionary and Directory (Pancratz, 2001) acclimatization is the "physiological and behavioral adjustments of an organism to changes in its environment". Acclimation of microorganisms to a specific industrial wastewater means the development of organisms capable of decomposing the compounds in question (Ganczarczyk, 1983). The source of the seed biomass, the operating temperature and the sludge age will determine the time required for acclimation. Acclimation period maybe from several days to five to six weeks, or acclimation may not occur at all. For a wastewater which is readily degradable and susceptible to filamentous bulking, acclimation of the biomass is best achieved on a fill-and-draw basis (Eckenfelder, 1995).

After the acclimation period, the operation can be converted to a continuous flow basis using either a plug-flow mixing regime or a biological selector to control bulking. As acclimation proceeds the concentration of the wastewater should be gradually increased. Acclimation is assumed to be complete when the system reaches a steady-state condition that can be followed by the residual organic concentration, the specific oxygen uptake rate or mixed liquor suspended solids concentration (Eckenfelder, 1995).

2.1.3. Batch Reactor

A batch reactor is a completely mixed reactor without any continuous in and out flow. The substrate and biomass are placed instantaneously in a tank and the reaction is allowed to proceed to completion.

2.1.4. Sequencing Batch Reactor (SBR)

SBR is a variation of activated sludge process which operates on a fill-and-draw basis. Fill-and-draw batch processes similar to SBR were in operation since 1920's. Originally the term SBR was introduced in 1971 by R. L. Irvine at the Purdue Industrial Waste Conference. The concept has become accepted worldwide and used as an alternative to the continuous flow activated sludge process (Wilderer et al., 2001). SBR systems have been successfully employed in the treatment of both municipal and industrial wastewaters.

SBRs and conventional A.S. systems have the same unit operations of wastewater treatment, such as equalization, biological treatment and secondary clarification. The important difference between the two technologies is that, while conventional A.S. systems require separate tanks, the SBR performs the unit operations in a single tank, using a time sequence.

All SBR systems have five sequential process phases as illustrated in Figure 2.2 and enumerated as follows:

1. Fill: Wastewater is added to the reactor.
2. React: Reaction that initiated during Fill is completed.
3. Settle: Biomass separation occurs.
4. Draw: The supernatant (treated wastewater) is removed.
5. Idle: The period between draw and fill.

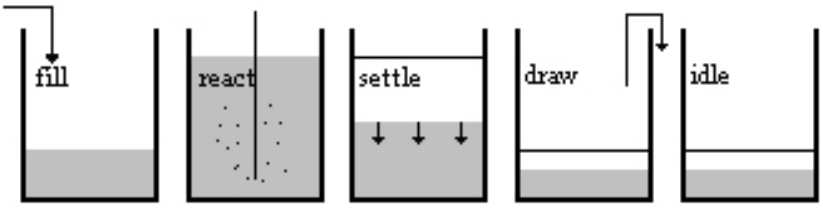


Figure 2.2. Operational phases during one cycle of Sequencing Batch Reactor

Three variations can be used in the Fill period, named as static, mixed and aerated fill. The selection of one strategy on other is based on the treatment objective. During static fill there is no mixing or aeration. Therefore, food to microorganism ratio (F/M) will be high in the system when mixing begins, and this will create a favorable environment for floc formers over filamentous organisms. Static fill may be compared to the selector compartments in conventional activated sludge systems which are used to control the F/M ratio.

In mixed fill, influent organics are mixed with the biomass, initiating biological degradation of organics. During mixed fill, bacteria use residual oxygen or alternative electron acceptors such as nitrate-nitrogen. Denitrification may occur under these anoxic conditions. Therefore, mixed fill may be compared to anoxic zone in a conventional biological nutrient removal A.S. system (BNR). In aerated fill, as the name implies, contents of the reactor are being aerated starting the aerobic reactions which will be completed in the React phase (EPA, 1999).

React period may be in mixed or aerated mode. With aeration, the aerobic reactions started in aerated fill are completed and nitrification can be achieved. In mixed mode, on the other hand, anoxic conditions for denitrification and also anaerobic conditions for phosphorus removal can be attained (EPA, 1999).

As mentioned above, when the reactions are complete, aeration and mixing is stopped, biomass is allowed to settle, and supernatant is removed from the system to make room for next batch of wastewater. There is no special designed period to waste the excess sludge in the cycle. Wasting can be done near the end of React period or during Settle period, and can take place weekly, daily or during each cycle (Wilderer et al., 2001).

Some of the advantages of the SBR systems are listed by EPA as following (EPA, 1999):

- Equalization, primary clarification, biological treatment and secondary clarification in single reactor
- Flexibility and control
- Small footprint
- Potential capital cost savings by eliminating clarifiers and other equipment

Franta and Wilderer (1997) studied biological treatment of papermill wastewater by SBR to reduce residual organics. The influence of process conditions was investigated varying the duration of Fill and React periods. Also, the influence of sludge age on residual organic concentration was investigated. It was found that longer Fill periods cause lower COD removals regardless of the sludge age and total cycle time. It was seen that high concentration gradients in SBR leads to an enhanced COD removal. Removal efficiencies achieved were over 90 %. As a whole, the degree of elimination was found to be dependent on the sludge age and optimized process strategy. The highest COD removal and the best sludge settling properties for the papermill wastewater were obtained at 20 days sludge age with 12 hours React period and 0.5 hours Fill period.

Martins et al. (2003) researched the effect of feeding pattern and storage on the sludge settleability characteristics. SBR systems were used to scale-down aerobic AS systems with aerobic selector. The experiments showed that the aerobic fill time ratio and the corresponding feast period, which is similar to contact time in an aerobic selector, had a strong effect on sludge settleability characteristics. SVI was strongly increasing with aerobic fill time ratio. Sludge settleability improved by changing the feeding strategy to pulse feed which resembles the substrate gradient as occurring in a plug flow process. An alternative hypothesis for filamentous bulking was also formulated in this study.

Farabegoli et al. (2004) investigated the feasibility of treating tannery wastewater containing an inhibiting compound, chromium, with SBR. It was observed that, at the same chromium concentration, nitrification and denitrification rates were higher in the lab-scale acclimated SBR than in batch experiments conducted with unacclimated biomass. This result confirms that, through acclimation, sequencing batch reactors are able to produce a biomass resistant to inhibiting conditions.

The performance of a sequencing batch reactor in treating complex chemical effluents (low BOD/COD) was investigated with a suspended biomass configuration (Mohan et al., 2005). A performance comparison with conventional AS process was also done. The conventional AS was operated at 1.1 kg COD/m^3/day with 5 days HRT and resulted in 55 % COD and 67 % BOD removal. In comparison, the SBR was operated at 0.8 kg COD/m^3/day and gave 66 % COD removal and 92 % BOD removal with only 24 hours HRT. Hence, the SBR showed relatively better performance than conventional suspended growth system. Enhanced performance with SBR was thought to be due to enforced short term unsteady state conditions and periodic exposure of the microorganisms to defined process conditions which can control the physiological state in SBR.

2.1.5. Bacterial Growth

An understanding of the basic principles governing the growth of microorganisms is important for an effective environmental control in biological waste treatment. The microorganisms of primary importance in biological treatment are bacteria. Bacteria generally reproduce by binary fission (i.e. by dividing). The time for each fission can vary from minutes to days. The various environmental limitations controlling bacterial dividing include system size, substrate concentration and nutrient concentration.

A typical growth pattern for bacteria based on the number of cells has four distinct phases, namely lag, log-growth, stationary and log-death. The growth pattern can also be discussed in terms of bacterial mass i.e. variation of mass of microorganisms with time. Phases of this growth pattern are as following (Metcalf and Eddy, 1991):

1. *The Lag Phase* – the time required for bacteria to acclimate to their nutritional environment.

2. *Log-Growth Phase* – the period when the rate of growth is only a function of the ability of the microorganism to process substrate.

3. *Declining Growth Phase* – The rate of increase of bacterial mass decreases because of limitations in the food supply.

4. *Endogenous Phase* – The microorganisms are forced to metabolize their own protoplasm because the concentration of food is minimum.

A typical growth curve for a single bacterial population grown in a batch reactor can be seen in Figure 2.3.

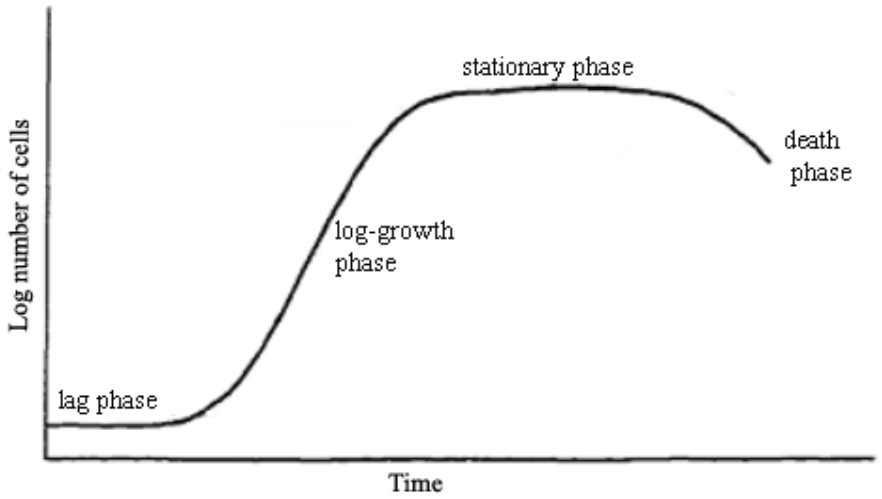


Figure 2.3. Typical bacterial growth curve

Specific growth rate

When all the requirements for growth are satisfied, the bacterial growth is proportional to the amount of biomass present. Exponential growth can be expressed as following;

$$\frac{dX}{dt} = \mu X \tag{2-1}$$

or with integration;

$$\mu = \frac{\ln X - \ln X_0}{t - t_0} \tag{2-2}$$

Where; X = Biomass concentration at time t, mg/L

X_o = Biomass concentration at the time 0, mg/L

μ = Specific growth rate, time^{-1}

Monod (1949) showed that the effect of a limiting substrate or nutrient on growth rate can be defined using the following expression;

$$\mu = \frac{\mu_m S}{K_s + S} \tag{2-3}$$

Where; μ = Specific growth rate, time^{-1}

μ_m = Maximum specific growth rate, time^{-1}

S = Concentration of growth-limiting substrate, mg/L

K_s = Half-velocity constant, substrate concentration value at one-half of the maximum growth rate, mg/L

The effect of substrate concentration on the specific growth rate is shown in Figure 2.4.

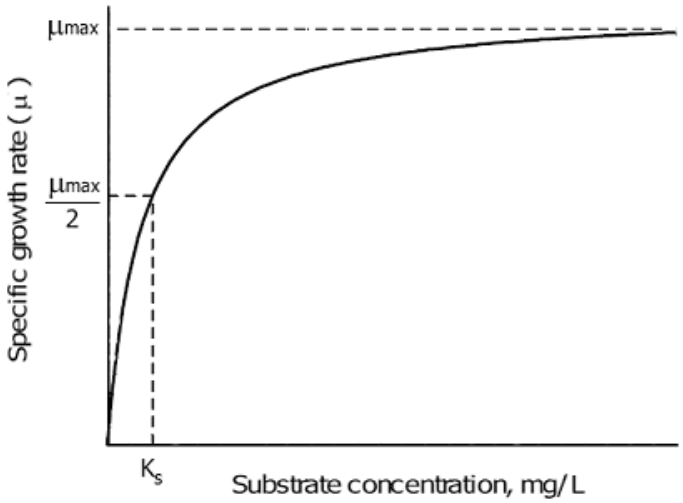


Figure 2.4. The effect of limiting substrate on the specific growth rate (Monod)

Growth Yield Coefficient

The relation between solids production and substrate removal rate is given as:

$$\frac{dX}{dt} = -Y\left(\frac{dS}{dt}\right) \tag{2-4}$$

Where; Y = Growth yield coefficient (mg/mg)

X = Biomass concentration (mg/L)

S = Substrate concentration (mg/L)

It is common to neglect minus sign in the literature. Y, *growth yield coefficient*, is also expressed as;

$$Y = \frac{\Delta X}{\Delta S} \qquad \text{or} \qquad Y = \frac{\mu}{q} \tag{2-5) (2-6}$$

Where q is the *specific substrate utilization rate* (time^{-1}).

Specific Substrate Utilization Rate

As it is seen in equation (2-6), the specific substrate utilization rate is directly proportional to the specific growth rate. Hence, the following equation holds:

$$\frac{dS}{dt} = qX \tag{2-7}$$

Monod model proposes the following equation for specific substrate utilization rate:

$$q = \frac{q_m S}{K_s + S} \tag{2-8}$$

Where; q = Specific substrate utilization rate, time^{-1}

q_m = Maximum specific substrate utilization rate, time^{-1}

S = Concentration of growth-limiting substrate, mg/L

K_s = Half-velocity constant, substrate concentration value at one-half of the maximum growth rate, mg/L

Solids retention time (SRT)

Sludge age or solids retention time (SRT) is basically the average length of time that biomass remains in the system. It can be expressed as the mass of organisms in the system divided by the mass of organisms removed from the system per day; mathematically as following:

$$\theta_c = \frac{V_r X}{Q_w X_w + Q_e X_e} \tag{2-9}$$

Where; θ_c = Solids retention time, d

V_r = Volume of the aeration tank, L

Q_w = Waste sludge flow rate, L/d

Q_e = Treated effluent flow rate, L/d

X_e = VSS concentration in the effluent, mg/L

X_w = VSS concentration in the waste sludge, mg/L

X = VSS concentration in the aeration tank, mg/L

When wasting is from the aeration tank ($X = X_w$) and the solids concentration in the effluent is neglected ($X_e \approx 0$), the equation reduces to following form:

$$\theta_c = \frac{V_r}{Q_w} \tag{2-10}$$

Therefore, SRT can be controlled by daily wasting of a quantity of flow determined by the following equation:

$$Q_w = \frac{V_r}{\theta_c} \tag{2-11}$$

2.2. Fenton's Oxidation

Fenton's reagent was discovered over hundred years ago. In 1894, H. J. H. Fenton reported that ferrous ion strongly promotes the oxidation of malic acid by hydrogen peroxide (Fenton, 1894). Subsequent work has shown that the combination of H_2O_2 and a ferrous salt was an effective oxidant of many organic substrates, then the combination of these two agents was called as *fenton's reagent*. Forty years later, Haber and Weiss (1934) proposed that the actual oxidant in Fenton reaction was the hydroxyl radical (Walling, 1975). In Fenton reaction, strong oxidative hydroxyl radicals are produced and the ferrous ions (Fe^{+2}) are oxidized to ferric ions (Fe^{+3}) as shown in chemical equation:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\cdot} \tag{2-12}$$

Upon generation, the hydroxyl radicals initiate non-selective oxidation processes on the surrounding organic and inorganic species. Particular attention must be paid to dosages of Fe^{+2} and H_2O_2 in order to avoid the following undesired hydroxyl radicals scavenging reactions that occur in the presence of an excess of each of the two reagents (Tang and Huang, 1997):

$$OH^{\cdot} + Fe^{2+} \rightarrow OH^- + Fe^{3+} \tag{2-13}$$

$$OH^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \tag{2-14}$$

Fenton process is a complicated system and it involves many reactions such as complexation, precipitation equilibrium, redox, etc. (Rivas et al., 2003). As a result, organic pollutant destruction, toxicity reduction, biodegradability enhancement, BOD, COD, odor and color removal is achieved in wastewater (Peres et al., 2004). Two important factors affecting the rate of the Fenton process are peroxide dose and iron concentration. While the peroxide dose is important for better degradation efficiency, the iron concentration is important for the reaction kinetics (Chamarro et al., 2001).

Generally, Fenton process comprises four steps (Bigda, 1995): pH adjustment, oxidation reaction, neutralization and coagulation, and precipitation. Some of the important features that makes Fenton process an attractive option for wastewater treatment can be summarized as following:

1. Harmful by-product generation is noticeably lower compared to some other AOPs (Liang et al., 1999).

2. Fe^{+2} is a non-toxic element, it is highly abundant and if needed it can be regenerated (Safarzadeh-Amiri et al., 1996, Tzedakis et al., 1989).

3. Hydrogen peroxide is easy to handle and environmentally friendly (Badawy and Ali., 2006).

4. Both Fe^{2+} and Fe^{3+} ions are coagulants, hence the Fenton process can have the dual function of oxidation and coagulation (Huh et al., 1996; Badawy and Ali., 2006).

Because of these features, Fenton process has been applied in the treatment of a wide variety of pollutants. Studies include but not limited to the treatment of trihalomethanes (Tang and Tassos, 1997), landfill leachates (Bae et al., 1997,

Lopez et al., 2004), nonionic surfactants (Kitiş et al., 1999), aromatic amines (Casero et al., 1997), pesticides (Chan and Chu, 2003), textile effluents (Kang and Chang, 1997; Kang et al., 2002), cork processing wastewaters (Peres et al., 2004), pigment wastewater (Kim et al., 2004), olive oil processing wastewater (Ahmadi et al., 2005), pharmaceutical wastewater (Tekin et al., 2006; Martinez et al., 2003).

2.3. Radiation Technology

The most widely used commercial ionizing radiation sources at the present time are cobalt-60 (^{60}Co) for gamma (γ) irradiation, and electron accelerators for electron-beam (EB) irradiation. The γ radiation sources are mainly used in applications such as medical product sterilization and food irradiation, where the greater penetration of γ radiation in comparison to EB radiation is an advantage. On the other hand, electron accelerators have the advantages in that penetration can be tailored to the application by controlling energy of the electrons, and the radiation source can be turned off by turning off the electric power (Woods, 1998).

The parameter which is of primary interest in radiation processing is the *absorbed dose*. It is the energy transferred from the incident radiation to the material being irradiated. Absorbed dose produces ions and excited species in the irradiated material, therefore it is responsible for radiation-induced chemical and biological changes observed. The SI unit for the absorbed dose is joule per kilogram (J/kg), which is given the special name gray (Gy). The absorbed dose can be calculated from dose rate (kGy/h) of the irradiation source (Woods, 1998).

The environmental applications of radiation technology include but not limited to treatment of industrial flue gases, disinfection of sewage sludge, deodorization, decolorization and disinfection of natural and polluted drinking waters, and

decomposition of toxic or recalcitrant pollutants in industrial wastewater (Woods and Pikaev, 1993). The utilization of radiation in wastewater treatment is drawing increasing attention around the world. Compare with other techniques, the ionizing radiation has its special properties and advantages: It can successfully and economically treat the organic systems which are biologically resistant. It does not cause secondary pollution. No trace of polluted materials are left in the wastewater after final degradation (Diaz et al., 2003; Bao et al., 2002). Furthermore, no radioactivity is produced during the treatment; the technology is safe for operating personal, general public and environment (Getoff, 1999).

Ionizing radiation generates hydroxyl radicals, the most powerful hydrolytic oxidative species, which therefore can degrade most pollutants. The composition of industrial waste is usually rather complex, resulting in very complex competitions for the radiolytically generated reactive species. Hydroxyl radicals present low selectivity in oxidation, which may lead to low efficiency in the full mineralization of pollutants; thus radiation treatment alone may require a relatively high absorbed dose and may be expensive. As a solution, the combination of radiation technology with other conventional methods such as chemical (e.g. ozonation) (Fang and Wu, 1999) and biological treatment is promising (Jo et al., 2006).

Bao et al. (2002) studied the effect of three treatment methods, namely: flocculation deposition, flocculation deposition combined with γ irradiation and direct irradiation on samples collected from a wastewater treatment plant which takes industrial and sanitary municipal wastewater. The original wastewater sample (COD = 145.0 mg/L) and the sample pretreated with 16 mg/L cationic starch-AAM flocculant (COD = 105.7 mg/L) were irradiated by ^{60}Co-gamma rays. The doses applied were 17.4, 25.0, 30.0 and 37.5 kGy. It was found that irradiation combined with flocculation was more effective in removing COD than

other two methods. The highest COD removal (65 %) was reached when the absorbed dose was about 17.4 kGy (Bao et al., 2002).

Diaz et al. (2003) studied treatment of a highly polluted industrial wastewater (COD = 3,400 mg/L) by three different methods namely: Electrocoagulation, Electrooxidation, and γ irradiation. Additionally, a combination of both electrochemical plus irradiation processes was performed. Irradiation was applied with doses varying from 5 to 35 kGy, using a ^{60}Co source. When applied directly, radiation reduced COD by 60 %, whereas removals by electro-oxidation and coagulation were 77 % and 68 %, respectively. On the other hand, when radiation was applied to pre-treated wastewater samples; 70 – 80 % further COD removals were reached at absorbed doses of 25 & 35 kGy respectively for electrocoagulation and electrooxidation treatment. The best conditions for treatment result when electrooxidation and γ irradiation were applied in sequence (95 % overall COD reduction). It is also noted in this study that there was no linear relationship between the radiation dose and the COD values. This agrees with above mentioned research (Diaz et al., 2003).

In order to evaluate the γ irradiation as a pretreatment to biological processes, Jo et al. (2006) investigated the effects of γ irradiation on biodegradability (BOD_5/COD) of textile and pulp wastewaters. For textile wastewaters, samples were gathered from desizing, weight-loss and final effluents; for pulp wastewater samples were gathered from cooking, bleaching E1 (first alkaline stage), C/D (acid stage including chlorine and chlorine dioxide), and final effluent. The irradiation was carried out with a high-activity ^{60}Co source. For all wastewater, the treatment efficiency based on TOC removal was insignificant even at 20 kGy dose. The improvement of biodegradability was largely dependent on chemical properties of effluents and the irradiation dose. For textile wastewaters, γ-ray treatment at 1 kGy slightly increased the biodegradability of desizing effluents due to degradation of polymeric sizing agents such as polyvinyl alcohol. At the

same dose, weigh-loss effluent gave large enhancement of biodegradability, however, COD of weigh-loss effluent were increased up to 80 % at higher doses. Biodegradability of the final effluent was reduced with an increase in COD at 1 kGy. For pulp wastewater, the γ-ray treatment did not improve the biodegradability of cooking and bleaching of C/D effluents. But, bleaching E1 and final effluents showed large improvement of biodegradability due to increase in BOD_5 concentration especially at a dose of 5 kGy. COD values of pulp wastewaters changed only little (Jo et al., 2006).

CHAPTER 3

OPIUM ALKALOID INDUSTRY AND WASTEWATER

3.1. Opium Alkaloid Industry

Opium is the air dried latex obtained from unripe seed capsule of the opium poppy, or by its botanical name *Papaver somniferum*. Opium contains an elaborate cocktail of sugars, proteins, ammonia, latex, gums, plant wax, fats, sulphuric acid, lactic acid and wide range of alkaloids which are partially chemically bonded to meconic acid. The significant parts are the alkaloids. Alkaloids are a group of highly complex nitrogenous organic bases which are found in certain species of plants (Booth, 1996). The alkaloid content of opium is approximately 10 - 20 % and over 40 individual alkaloids have been isolated in opium (Schiff, 2002).

Opium poppy is a medicinally important plant, because it is the only commercial source of the pharmaceutical alkaloids such as the pain reliever morphine, the cough suppressant codeine and the antitumor agent noscapine (narcotine) (Frick et al., 2007, Ye et al., 1998). Among this, morphine is by far the most prevalent alkaloid accounting for 8 – 17 % of total alkaloid content in opium (Schiff, 2002).

World Health Organization (WHO) recommends morphine and codein in its model list of essential medicines (WHO, 2007). Other opium alkaloids which are of great pharmaceutical value include the muscle relaxant papaverine, and the antimicrobial agent sanguinarine (Facchini and Park., 2003).

Opium poppy is cultivated on a licit basis in a number of countries among which Turkey and India are accepted as traditional producers. As India uses raw opium, Turkey uses poppy straw[1] for alkaloid extraction. As the global demand for alkaloids has been increasing over the past two decades, the demand has been mainly covered by poppy straw produced by Australia, France, Hungary, Spain and Turkey. According to International Narcotics Control Board (INCB) reports, Turkey was the leading producer of poppy straw (in morphine equivalent) accounting for 34 % of the world production in the year 2006 (INCB, 2008).

3.2. Opium Alkaloids Factory – Bolvadin/Afyon

In Turkey, harvested opium poppy is processed in Turkish Grain Board's (TMO) Opium Alkaloids Factory located in Bolvadin, Afyon. The factory has the capacity to process 72 tons of opium poppy capsule per day, and 3.3 kg of morphine is produced per ton of opium processed. The factory produces base morphine, base codein, base ethyl morphine, codeine phosphate and dionin (Sevimli et al, 2000). The plant exports 95 % of its products after meeting domestic demand. It is estimated that Turkey annually earns US$60 million from the export of poppy seeds and opium based medicines (TMO, 2005; Gecin and Hakbilen, 2005).

The opium alkaloids factory in Bolvadin consists of four main units which are grinding, liquid-solid extraction, liquid-liquid extraction and crystallization. The production flow scheme of the factory is given in Figure 3.1 and the tasks undertaken in these units are briefly described below (Aydın 2002, Sevimli et al. 1999):

[1] Poppy straw consists of all parts of the opium poppy plant after mowing except the seeds.

Grinding unit: Impurities in poppy capsules are removed, and capsules are grinded to the proper sizes to be used at the next unit.

Liquid-solid extraction unit: Capsules are treated with lime-water solution. The mixture is then pressed and morphine is extracted. This liquid extract is subjected to a pH adjustment with sulphuric acid and sodium bicarbonate. The extract with a final pH of 9 is sent to next unit after being cleaned.

Liquid-liquid extraction unit: The morphine in liquid phase is extracted with acetic acid and organic solvents such as toluene and butanol.

Crystallization unit: Morphine in acetate solution is sedimented by using ammonia solution and then the separated morphine is centrifuged and dried.

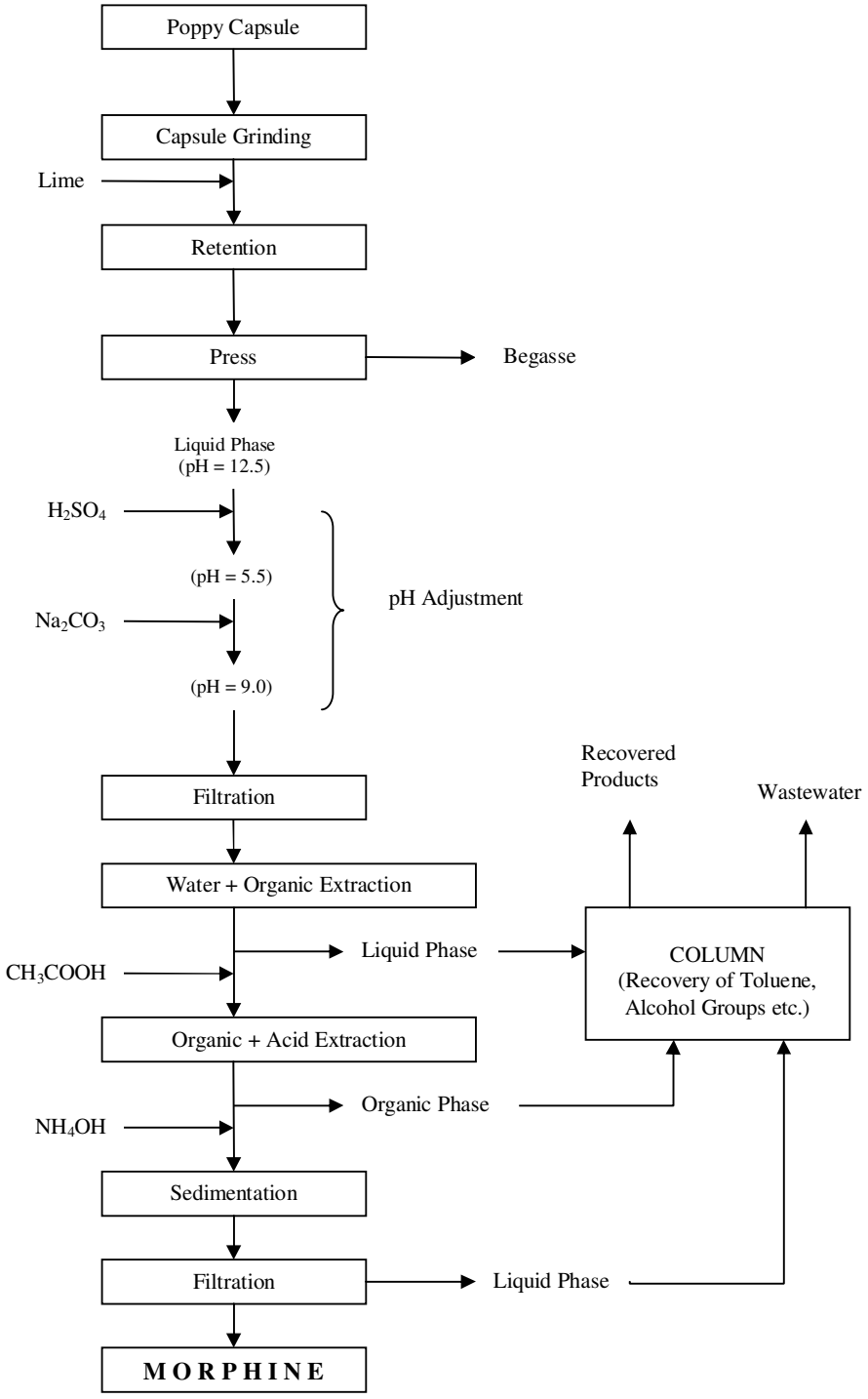


Figure 3.1. Production flow scheme of Opium Alkaloids Factory (Aydın, 2002)

3.2.1. Wastewater Generated

Effluent generated from alkaloid extraction and processing is on average 480 m^3/day. The average hourly flowrate of the wastewater is about 27.5 m^3/h (Sevimli et al., 1999). The effluent is discharged to Eber Lake through Akarçay River.

The wastewater generated by the opium alkaloid industry is heavily polluted with very high COD and BOD_5 content, and have intense dark brown color. Color causing substances in wastewater are almost totally dissolved and resistant to biodegradation (Koyuncu, 2003). The pollutant constituents in alkaloid wastewater mainly consist of phytin, morphine, codein, thebain, aniline, toluol, vax-like substances and cellulose (Kaçar et al., 2003; Aydın, 2002).

Sevimli et al. (1999) statistically analyzed COD and BOD_5 values of wastewater using the daily data gathered for three years. Median values of COD and BOD_5 were reported as 24,320 and 11,750 mg/L, and 95-percentile maximum values were found as 29,310 and 14,850 mg/L, respectively. These values were valid when the plant was working for 5 days in a week. At the weekend when the plant was closed, the plant effluent was being diluted by domestic wastewater coming to the treatment plant with a flowrate of 180 m^3/day.

Later on, Aydın (2002) observed that with the extension of process to 7 days, COD values of wastewater increased from 25,000-30,000 mg/L levels to 30,000-35,000 mg/L levels. The increase in the pollutant level of wastewater was attributed to lack of dilution as a result of extension of the process to whole week and also the effect of climate change on the level of pollutant originates from poppy capsule. The maximum COD and BOD_5 values observed during this study period were 42,500 and 22,215 mg/L, respectively.

Characteristics of wastewater used in previous studies are given in Table 3.1. The results reported by Aydın (2002) were obtained from analysis done by

TÜBİTAK-MAM, Bolvadin Opium Alkaloids Factory and ITU Environmental Engineering Laboratories.

Table 3.1. Opium alkaloid wastewater characteristics

Parameter	Unit	Çil et al., 1993	Kınlı, 1994	Sevimli et al., 1999	Aydın, 2002
TCOD	mg/L	21,200	23,251	36,500	18,300-42,500
SCOD	mg/L	-	-	32,620	17,050-39,470
TOC	mg/L	-	9,000	-	7,335-14,000
BOD_5	mg/L	14,700	14,450	-	4,250-22,215
pH	mg/L	5.10	4.89	-	4.9-6.3
Color	Pt-Co	-	-	-	2,150-2,550
TS	mg/L	-	-	-	27,235-29,750
TDS	mg/L	-	-	-	26,220-29,120
TSS	mg/L	1,214	1,450	1,400	565-2,295
VSS	mg/L	-	-	-	320-1,775
TKN	mg/L	404	203	1,030	550-841
NH_4-N	mg/L	147	62	140	73-141
TP	mg/L	15	29.2	65	3.1-15.0
PO_4-P	mg/L	-	-	-	20-30
SO_4	mg/L	-	-	-	8-912
Total alkalinity	mg $_{CaCO3}$/L	5,294	2,300	-	315-4,450
Conductivity	μ_{mhos}/cm	-	-	-	18,900-22,800
Na	mg/L	-	-	-	700-10,445
K	mg/L	-	-	-	315-457
Ca	mg/L	-	-	-	10-41
Mg	mg/L	-	-	-	7.3-36.0
Cu	mg/L	-	-	-	0.508-0.564
Zn	mg/L	-	-	-	2.057-2.226
Pb	mg/L	-	-	-	0.572-0.624
Cd	mg/L	-	-	-	<0.2
Cr	mg/L	-	-	-	<0.5
Co	mg/L	-	-	-	<0.2
Fe	mg/L	-	-	-	5.201-6.075
Ni	mg/L	-	-	-	0.404-0.482
Temperature	^oC	-	-	40	Summer: 35-38 Winter: 26-31

3.2.2. Existing Wastewater Treatment

The opium alkaloids factory formerly had a 2-stage activated sludge system to treat wastewater produced. In the early times, the treatment plant achieved over 95 % and 99 % COD and BOD_5 removal efficiencies, respectively. However, because of some operational problems, the treatment plant is not operating anymore. The major operating problems were claimed as uncontrolled temperature increase in the aeration basins which was due to the cover effect of thick scum layer and a long hydraulic detention time (Sevimli et al., 2000) and high aeration energy consumption (Koyuncu, 2003).

3.2.3. Discharge Standards for Alkaloid Industry

The discharge standards for alkaloid industry set by Turkish Water Pollution Control Regulation (WPCR) is as given in Table 3.2.

Table 3.2. Discharge standards for alkaloid production plants (WPCR, 2004)

Parameter	Unit	Composite Sample (24 h)
COD	mg/L	1,500
TKN	mg/L	15
TSS	mg/L	200
pH	-	6-9

3.2.4. Previous Treatability Studies on Opium Alkaloid Wastewater

Treatability of opium alkaloid factory wastewater was studied in TUBITAK Marmara Research Center (Kınlı, 1994). Physicochemical treatment, adsorption, chemical treatment and chemical oxidation techniques were applied to samples gathered from effluent of aerobic biological treatment plant. In physicochemical studies $Al_2SO_4.18H_2O$ (alum), $FeCl_3$, $Fe_2(SO_4)_3$ and $FeSO_4.7H_2O$; in adsorption

studies activated carbon, diatomite and perlite; in chemical treatment studies cement powder and eight different type carboxymethyl cellulose; in chemical oxidation studies potassium permanganate, hydrogen peroxide and potassium persulfate were used. As a result, the most significant COD removal efficiencies, around 40 – 45 %, were achieved by using potassium permanganate, alum, $Fe_2(SO_4)_3$ and $FeCl_3$ at dosages as high as 1,000 mg/L. Initial COD concentration was 1,010 mg/L.

Sevimli et al. (2000) studied anaerobic treatability of raw wastewater and also post treatment of the aerobic biological treatment plant effluent with lime and ozone. COD concentration for raw wastewater was 36,500 mg/L. Aerobically treated effluent COD and color values were 2,230 mg/L and 12,750 Pt-Co as given in elsewhere (Sarıkaya et al., 1998). In pilot-scale anaerobic treatability studies a 36 L UASB reactor was operated in mesophilic conditions for more than 5 months. 70 % & 45 % COD removal efficiencies were obtained for influent COD concentrations around 7,000 mg/L and 13,000 mg/L, respectively. In lab-scale anaerobic treatability studies COD removal efficiencies were descended from 90 % to 62 % for influent organic loads varying from 5,000 to 16,000 mg/L.

Chemical treatment studies with lime were carried out on plant effluent by standard jar-test experiments and resulted in 78 % color and 46 % COD removal efficiencies, at a high lime dosage of 25 g/L. Effluent was exposed to ozone oxidation at three different pH values, namely 2.5, 6.8 (original value) and 10. The effluent wastewater used in ozonation studies had a COD concentration of 2,090 mg/L, and color of 3,800 Pt-Co (Sarıkaya et al., 1998). The color removal efficiencies after 20 minutes of ozonation were 93, 83 and 76 %; and COD removal efficiencies after 30 minutes of ozonation were about 37, 30 and 25 % for pH values of 2.5, 6.8 and 10, respectively (Sevimli et al., 2000).

Aydın et al. (2002) applied Fenton's oxidation to effluents of a lab-scale two stage (UASB + SBR) biological treatment system. The COD and color values of SBR

effluent were 650 mg/L and 4,450 Pt-Co, respectively. Optimum pH and reaction time were found to be 4 and 30 minutes, respectively. 90 % COD and 95 % color removals were achieved by using 200 mg/L H_2O_2 and 600 mg/L $FeSO_4$, respectively.

Koyuncu (2003) applied membrane technology as an advanced treatment option. Effluent of full-scale aerobic treatment plant with COD 2,000 mg/L, color 1,750 Pt-Co; and also effluent of a lab-scale anaerobic/aerobic (UASB + SBR) treatment plant with COD 950 mg/L and color 1,100 Pt-Co were fed to membrane systems. For both wastewater, COD removal efficiencies greater than 95 %, and complete color removals were achieved with both nanofiltration (NF) and reverse osmosis (RO) membranes.

Özdemir (2006) investigated anaerobic treatability of opium alkaloid wastewater and also the effect of radiation pre-treatment on anaerobic treatability. Continuous reactor experiments were carried out in three UASB reactors (U1, U2, U3) for 138 days. U1 was fed with opium alkaloid wastewater only. U2 had 25 % (in terms of COD) calcium acetate co-substrate. U3 was fed with effluent of an acidification (SBR) reactor fed with alkaloid wastewater. Up to 78 % removal efficiency was obtained in U1 at initial COD concentration of 19 g/L (about 2/3 of original wastewater concentration). Highest overall efficiencies (above 80 %) were obtained in U2 for all initial COD concentrations studied (10, 15, 19 and 27.5 g/L).

Effect of radiation was sought in this study by using BMP assays with two initial COD concentrations of 14 and 25 g/L, and two radiation doses 40 and 140 kGy. At 14 g/L COD, there was no effect of radiation on gas production for both doses. However at initial COD of 25 g/L, reactors containing wastewater dosed with 140 and 40 kGy produced gas with higher rates above certain point with respect to raw wastewater. Reactor fed 140 kGy absorbed wastewater had the highest gas

production rate and original wastewater had the lowest. The net cumulative gas productions (NCGP) by the end of the experimental period were close. The highest NCGP (420.6 mL) was observed at reactor fed 40 kGy absorbed wastewater.

CHAPTER 4

MATERIALS AND METHODS

In this chapter, materials, experimental procedures and analytical methods used throughout this study are presented.

4.1. Raw Wastewater Characteristics

The wastewater used in the experiments was the raw wastewater generated from Opium Alkaloids Factory located in Afyon Province, Turkey. The characteristics of wastewater were variable throughout the study. The ranges for parameters are as given in Table 4.1. A photo taken of the opium alkaloid wastewater is presented in Figure 4.1. The influent COD values of wastewater used in each experiment are given under related sections.

Table 4.1. Characteristics of wastewater.

Parameter	Unit	Value
TCOD	mg/L	30,000 – 43,078
SCOD	mg/L	28,500 – 40,525
BOD_5	mg/L	16,625 – 23,670
pH	-	4.5 – 5.36
Conductivity	mS/cm	33.1 – 36.3
Alkalinity	mg $CaCO_3$/L	1,050 – 4,200
Color	Pt-Co	4,375 – 4,750
TSS	mg/L	555 – 2,193
VSS	mg/L	382 – 1,395
TKN	mg/L	396 – 1,001
NH_4-N	mg/L	61.6 – 259
Total P	mg/L	4 – 5.21
Protein	mg/L	5,330 – 6,630
Carbohydrate	mg/L	10,000

Figure 4.1. Appearance of Opium Alkaloid Wastewater

4.2. Chemicals

Chemicals used were obtained from Merck KgaA (Darmstadt, Germany). The list of the chemicals is as following:

- Ferrous sulfate heptahydrate, $FeSO_4.7H_2O$
- Hydrogen Peroxide, H_2O_2 (35%)
- Sulfuric Acid, H_2SO_4 (95-98%)
- Sodium Hydroxide, NaOH
- Potassium Dihydrogen Phosphate, KH_2PO_4
- Potassium Monohydrogen Phosphate, K_2HPO_4
- Ammonium Chloride, NH_4Cl
- Potassium Dichromate, $K_2Cr_2O_7$

- Mercury Sulfate, $HgSO_4$
- Silver Sulfate, Ag_2SO_4

4.3. Seed sludge (Inoculum)

The bacterial seed sludge used in the preliminary batch treatability studies was taken from an aerobic membrane bioreactor (MBR) treating domestic wastewater of ODTUKENT area at Middle East Technical University (METU) campus. Inoculum used in all the later studies, including batch and sequencing batch reactors, was obtained from the return line of the activated sludge unit of the Ankara Central Wastewater Treatment Plant. Prior to use, activated sludge was aerated one day and washed two times with tap water.

4.4. Nutrient addition

Although COD of the wastewater was quite high, nitrogen and phosphorus concentrations were not adequate for biological treatment (calculated COD:N:P = 100:2.3:0.01). Hence, nitrogen and phosphorus were supplied to sustain the microbial activity during the experimental periods. A mixture of di-potassium hydrogen phosphate (K_2HPO_4) and potassium di-hydrogen phosphate (KH_2PO_4) was added to the medium as the source of phosphorus to the microorganisms as well as to provide a buffer capacity, whereas ammonium chloride (NH_4Cl) was added as nitrogen source. Stock solutions of these nutrients were prepared and used in experiments. Distilled water was used for the preparation of all solutions. COD:N:P ratios supplied in each experiment were provided under related sections. A sample calculation for preparation of nutrient stock solutions was given in Appendix A.

4.5. Experiments

In order to investigate the effect of gamma radiation application, as pre-treatment, on the biological treatment of opium alkaloid wastewater, batch and sequencing batch reactor studies were conducted with irradiated wastewaters. Also, pre-treatment by Fenton's oxidation as an alternative to gamma irradiation was applied. As a base-line study, biological treatability of opium alkaloid raw wastewater was sought with batch and sequencing batch reactors. An operational problem (i.e. sludge settleability problem) was observed during SBR study and it was thought to originate from composition of wastewater itself. Therefore, gamma irradiation and Fenton's oxidation pre-treatments were also considered to help degradate this complex structure, and degradation was monitored by filterability tests, GC/MS analyses and particle size distribution analysis. Also aerobic treatment of anaerobically pre-treated effluents was studied in order to investigate the potential performance of a two-phase (anaerobic + aerobic) system. Details of the methods that were used in the course of these studies are described under this section.

4.5.1. Base-line Experiments: Aerobic biological treatment - Batch Reactors

4.5.1.1. Raw Wastewater

The batch studies were conducted to assess the biological treatability of the opium alkaloid wastewater. Preliminary experiments were performed in 500 mL erlenmeyer flasks with 500 mL working liquid. Five different initial COD concentrations (2,500, 4,500, 7,000, 18,000 and 26,000 mg/L), which were made up by appropriate dilutions, were studied. The reactors were initially inoculated with unacclimated culture taken from a MBR receiving domestic wastewater. Macronutrients were supplied from prepared stock solutions in order to provide COD:N:P of 100:5:2 not to limit biomass growth. The daily volume of water lost

via evaporation was compensated by tap water just before each sampling. 5 - 7 mL samples were taken at various time intervals and analyzed for COD concentration after filtering through 0.45-μm filters. All the analyses were done at least in duplicates. Aeration supplied via air pumps was enough to completely mix the reactor contents, hence no additional mixing was provided. These experiments were conducted under ambient air conditions.

In the first batch run, biodegradability of wastewater with initial COD concentrations 2,500, 4,500 and 7,000 mg/L was studied. Also a batch reactor which use peptone as carbon source (with 4,000 mg/L COD) was set in order to compare the degradability of opium alkaloid wastewater with a readily biodegradable substrate. All reactors were initially seeded with 70 mg/L biomass provided by adding 20 mL of sludge having 1,720 mg/L VSS into 500 mL-volume reactors. These experiments allowed to last for 11 days. The total COD of raw opium alkaloid wastewater used in this experiment was 42,000 mg/L.

In the second run, the higher initial COD concentrations, namely 18,000 and 26,000 mg/L were studied. Seed sludge VSS was around 55 mg/L (25 mL sludge having 1,100 mg/L VSS was added to 500-mL volume reactors). Experiments were allowed to continue 20 days. The total COD of raw opium alkaloid wastewater used in this experiment was around 30,000 mg/L

4.5.1.2. Anaerobically Pre-treated Effluents

In other set of experiment, in order to investigate the aerobic treatability of anaerobically pre-treated opium alkaloid wastewater, effluents produced by Özdemir (2006) from three lab-scale Upflow Anaerobic Sludge Blanket (UASB) reactors treating opium alkaloid wastewater were collected. The first reactor (U1), was being fed with opium alkaloid wastewater. The second reactor (U2) had feed composition of 25 % calcium acetate and 75 % alkaloid wastewater in terms of

COD. The third reactor, (U3), was the second stage of a two-phase system where the first phase (acidification) was a SBR reactor being fed with alkaloid wastewater. Initial COD concentrations of these anaerobic reactors were 10 g/L at the start-up and were increased gradually (Özdemir, 2006).

Anaerobic reactor effluents were collected at two different times for use in aerobic treatability studies and preserved at 4^{o}C until the experiments. First set of samples were collected when the influent COD concentration of anaerobic reactors was 10 g/L and the second set of samples was collected when the influent was 19 g/L. 80 - 85 % COD removal efficiencies had been obtained anaerobically for influent COD concentration of 10 g/L. Steady-state effluent total COD values were reported as 4155, 2470, and 4690 mg/L (corresponding to 78, 87, and 75 % efficiencies) for reactors U1, U2 and U3 respectively when influent COD concentration was 19 g/L (Özdemir, 2006).

Initial microbial seed concentrations for the first set of anaerobically pre-treated samples (UASB influent COD = 10 g/L) were adjusted to be 30 – 40 mg/L in reactors. COD:N:P ratio supplied was 100:5:2. For the second set of samples (UASB influent COD = 19 g/L) initial seed concentration and COD:N:P ratio supplied were around 2,800 mg/L and 100:5:5, respectively. P ratio was increased in order to supply some buffer capacity. Despite the effort, pH in reactors had a tendency to increase as high as to 9.6; hence pH was adjusted to 7 – 7.5 daily by using 5 N H_2SO_4 to minimize any potential inhibitory effect on the biomass. Other experimental conditions were the same as preliminary batch study.

4.5.2. Irradiation as a Pre-treatment

4.5.2.1. Radiation Application

Radiation applications were carried out by Turkish Atomic Energy Authority (TAEK) by using Issledovatel Px-γ-30 russian made ^{60}Co gamma rays irradiator

at a fixed dose rate of 2.23 kGy/h in air at ambient temperature (Figure 4.2). Mainly two absorbed doses of radiation, namely 40 kGy and 140 kGy were applied in the scope of this study. Various doses are used in literature for industrial wastewaters depending on chemical property of wastewaters. For example, Borrely et al. (2000) found that the complex mixture of industrial effluents required doses from 30 kGy to 100 kGy, especially when raw samples were extremely toxic. Jo et al. (2006) used doses from 1 to 20 kGy for pulp industry wastewaters having COD values 1,300 to 2,261 mg/L and textile industry wastewaters having COD from 1,614 to 9,497 mg/L. Diaz et al. (2003) studied treatment of a highly polluted industrial wastewater (COD = 3,400 mg/L) with irradiation doses from 5 to 35 kGy.

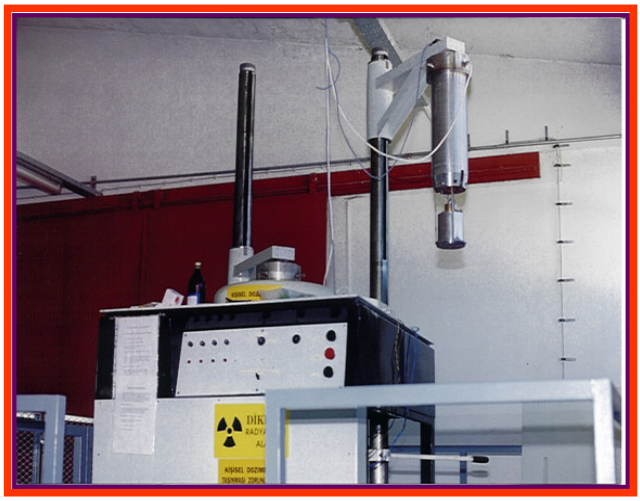


Figure 4.2. ^{60}Co γ-irradiator used during this study. Photograph courtesy of TAEK.

4.5.2.2. Biological treatability of Irradiated Wastewater - Batch Reactors

A set of batch reactor experiments was planned to be conducted using irradiated wastewater in order to investigate the effect of radiation application on biodegradability of opium alkaloid wastewater. Samples of wastewater were exposed to γ-radiation with 40 kGy and 140 kGy absorbed doses by TAEK. In order to observe the net effect of irradiation, a comparison was needed to be made with raw wastewater. Although a base-line batch treatability study was conducted earlier for this purpose (4.5.1.1), for a more accurate comparison parallel batch reactors fed by raw wastewater was set up again.

These experiments were carried out under constant temperature (25 ^{o}C) provided by a circulating water bath which pumps heated water. 500 mL erlenmeyer flasks with 500 mL working liquid were used as reactors. Raw and irradiated wastewater samples with various initial COD concentrations made up by appropriate dilutions were fed to reactors. Reactions were started by the addition of microbial seed. COD:N:P ratio was provided at 100:5:5 in order to supply nutrients and some buffer capacity. Since pH in reactors during reaction was observed to increase to levels 8.5 ± 0.2, pH was adjusted to 7 – 7.5 with 1 N and 5 N H_2SO_4 solutions daily. 5 - 10 mL samples were collected for analysis at various time intervals. Other conditions were the same as the preliminary study.

4.5.2.3. Biological treatability of Irradiated wastewater - Sequencing Batch Reactors (SBR)

The treatability of opium alkaloid wastewater was investigated in sequencing batch reactors. The details on reactor set-up, operations and experimental approach are presented below.

4.5.2.3.1. SBR Set-up and Operations

Experiments were conducted in 2.5 L glass vessels with total working volumes of 2 L, unless otherwise specified. At start-ups, reactors were inoculated at a volumetric ratio of 1/4 i.e. 25 % of working volume. Nutrients required by biomass were supplemented according to COD:N:P ratio of 100:5:2. Reactors were operated in fill-react-settle-draw modes following a 24 hour cycle. The cycle periods were controlled manually. Fill mode was applied as pulse feeding throughout the study. The react period allocated was at least 22 hours as the settling time was varied from minimum 20 minutes, up to maximum 2 hours according to operational needs. After the settling, the supernatant was discarded as effluent instantaneously and the same amount of feeding was done to start the next cycle. Various SRT values were studied throughout the experimental period, which are namely 20, 15, 10 and 6 days. Desired SRT values were maintained by wasting excess biomass daily from the mixed liquor at the end of each react period.

Dissolved oxygen concentration in reactors was kept above 2 mg/L by using air pumps. The intensity of supplied aeration was enough to allow complete mix condition to be attained in the reactors without any further need for mixing. Unless otherwise specified, all the experiments were conducted at 25°C, which was maintained by a temperature controlled water bath. The attainment of the steady state condition was followed by monitoring MLSS concentration and COD removal efficiencies. For MLSS analysis, completely mixed samples were taken from the reactors with a pipette at the end of react periods. COD analyses were performed at the beginning and the end of the react periods on samples filtered through 0.45-μm filters. All the analyses were done at least in duplicates. Settling characteristics were monitored based on the Sludge Volume Index (SVI). A schematic of the reactor set-up is given in Figure 4.3.

4.5.2.3.2. SBR - Experimental Approach

In total, 3 sets of sequencing batch reactor experiments were performed in the study. Due to complex nature and high strength of the opium alkaloid wastewater, gradual (stepwise) increments in the influent strength was attempted in order to allow the microbial biomass to acclimatize. Accordingly, the experiment was initiated with diluted raw wastewater having 500 mg/L COD, and influent strength was increased to 2,000 mg/L over time (Experiment 1). However, due to some operational problems (i.e sludge settling) experienced during the first experiment with the raw wastewater, some additional runs were conducted in an attempt to overcome these problems. For example, the effect of solids retention time and irradiation application (Experiment 2-1 and 2-2) were investigated.

For the investigation of the effect of irradation application on the biological treatment of alkaloid wastewater, two different irradiation doses, 40 kGy and 140 kGy were selected. One reactor was fed with original raw wastewater, second and third ones with wastewater pre-treated with 40 and 140 kGy irradiation, respectively (Experiment 2-1). SBRs were designed to operate under the same conditions and performance differences were evaluated. In the final experiment (Experiment 2-2) the effect of radiation application with 40 kGy dose was investigated in reactors run with increased buffer capacity (COD:N:P = 100:5:30). Influent strength was increased up to 5,000 mg/L. The set of experimental conditions studied are listed in Table 4.2. Further details are provided under related sections (see Results and Discussion).

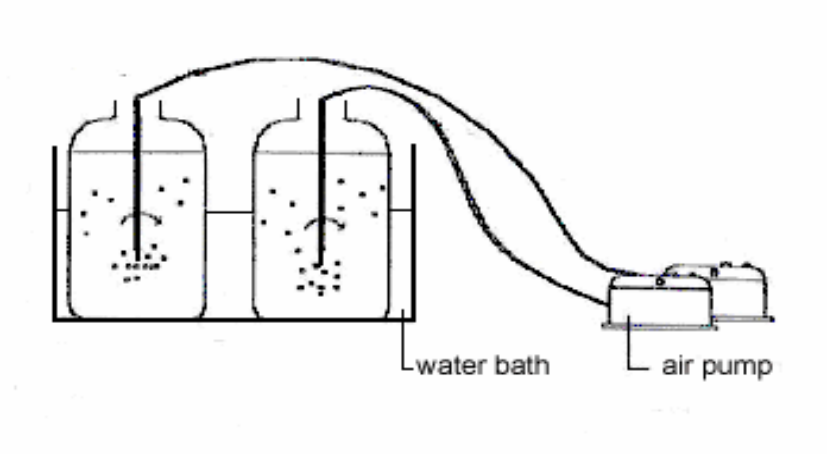


Figure 4.3. Experimental setup of Sequencing Batch Reactors

Table 4.2. Operational conditions of the SBR experiments

Experiment Number	Feed wastewater	Influent COD[(a)] (mg/L)	Filling time	SRT (days)	Working volume (L)	Temperature (^{o}C)
1	Raw	500 & 2000	Pulse	20	2	Ambient[(b)]
2-1	Raw	2000	Pulse	15	2	25
	40 kGy	2000	Pulse	15	2	25
	140 kGy	3000	Pulse	15	2	25
2-2	Raw	2000 & 5000	Pulse	6	1.6	25
	40 kGy	2000 & 5000	Pulse	6	1.6	25

a) Prepared by diluting the wastewater with tap water.

b) Summer time room temperature (20 – 23 ^{o}C).

4.5.3. Fenton's Oxidation as a Pre-treatment

To assess the pre-treatability of opium alkaloid wastewater, Fenton's oxidation, as an alternative advanced oxidation process, was applied. The COD and pH of raw wastewater used in this study was 33,000 mg/L, and 4.5, respectively. The experiments were carried out batch-wise using Aqualytic laboratory floc-tester (Dortmund, Germany). It has six agitator places; hence a set of six parallel experiments can be made. 1 L pyrex beakers were filled with 200 mL wastewater samples. pH was adjusted to fixed values (3) with concentrated sulphuric acid (95-98 %). Following pH adjustment step, solid ferrous sulfate ($FeSO_4.7H_2O$) was added to obtain scheduled Fe^{+2} dosages. Fenton's oxidation reaction was initiated by the addition of 35 % (w/w) hydrogen peroxide solution to give designated H_2O_2 concentration, and the samples were stirred at 100 rpm for 30 minutes. Then the floc tester was stopped and, pH was increased with 15 M sodium hydroxide solution to above 7 in order to eliminate H_2O_2 and stop oxidation. The samples were mixed for another 30 minutes at 30 rpm, and then left to settle under quiescent conditions for 2 hours in order to precipitate oxidized iron. Since no precipitation was observed in most of the studied dosages, all the samples were centrifuged 10 min at 3,500 rpm to remove formed ferric hydroxide and produce a clear supernatant for analyses. The centrifuged COD was used to assess the treatment efficiency.

It is known that the presence of residual hydrogen peroxide interferes with chemical oxygen demand analysis, resulting in a false high COD value. The reason of this interference is stated as the reaction of dichromate ions with H_2O_2 in an acidified solution (Kuo, 1992; Talınlı and Anderson, 1992; Kang et al., 1999; Tekin et al., 2006). Therefore, in order to overcome this interference, COD measurements in this study were performed after the coagulation stage where H_2O_2 loses its activity by decomposing (Kuo, 1992).

All the experiments were done in duplicates and results presented are the arithmetic averages of parallel measurements. Ferrous sulfate and hydrogen peroxide were added in single step. NaOH solution was prepared just before experiments to avoid any risk of contamination during storage.

4.5.4. Monitoring the Degradation of Complex Wastewater Structure

As mentioned, operational problems related to sludge settleability were observed in SBRs. Complex organic compounds in opium alkaloid wastewater was thought to contribute to this settleability problem. It was reasoned that if the chemical structure of wastewater change, then the microbial community in activated sludge would also change directly affecting the settleability characteristics. Therefore it was decided to alter this complex structure before biological treatment by means of irradiation and Fenton's oxidation treatments, respectively.

Degradation of the complex structure of the wastewater was followed by three different methods, namely filterability, GC/MS analysis and particle size distribution analysis. The details of these analyses are given under the following subsections.

4.5.4.1 Filterability tests

Filterability tests were conducted in an attempt to follow degradation of complex structure of the opium alkaloid wastewater by means of irradiation and Fenton's oxidation pre-treatments. The intention here was to observe any decrease in the resistance of wastewater to filtration which could be likely to occur with smaller molecules. To this purpose, two sets of filterability experiments were conducted, and time for filtration of wastewater was followed. In the first experiment, the filterability of raw wastewater was compared with wastewater pre-irradiated at

two doses namely 40 kGy, and 140 kGy, and also with wastewater chemically pre-treated by various Fenton's reagent dosages (up to 5 g/L).

In the second experiment, filterability of the wastewater samples irradiated at various doses including 5 – 10 – 20 – 25 – 30 – 35 – 40 kGy was tested and compared to that of raw wastewater. The aim was to investigate the possibility of decreasing the applied dose of irradiation by finding an optimum dose in terms of filterability, from economical and timely point of view.

The filterability test was performed filtering 50 mL of wastewater sample at an applied vacuum pressure of 74 kN/m^2 (71 kN/m^2 in experiment 2). The volume of filtrate was recorded as a function of time. Reported results are the averages of at least duplicate measurements.

In the first experiment, in order to eliminate the effect of sludge produced by Fenton reaction on filterability, and for the sake of comparability of samples; all samples including Fenton pre-treated, original and pre-irradiated were centrifuged (10 min at 3,500 rpm) prior to experiment. Then, filtration was done through 0.45 µm pore size membrane filters.

In the second experiment, which was done with wastewater samples that were pre-irradiated at various doses, and raw sample, samples were filtered through 20 – 25 µm pore size Whatman filter papers without applying centrifuging prior to the test (i.e. samples were used as they are).

4.5.4.2 Gas Chromatography Mass Spectrometry (GC/MS) Analysis

GC/MS analyses were performed using a Varian CP-3800 gas chromatograph (Varian Inc, Palo Alto, CA) equipped with a VF-5ms (30m x 0.25mm id x 0.25µm film thickness) capillary column and a Varian 1200L Quadrupole

MS/MS. Helium was used as carrier gas. The mass spectrometer was set to scan mass-to-charge ratios (m/z) in the range from 50 to 450 using the electron impact ionization mode. Instrument conditions that were used are given in Table 4.3.

Sample preparation procedure is as following: 20 mL sample is taken, and its pH is adjusted to 9 with 1 M NaOH. Then, aqueous solution is extracted three times with ethyl acetate. Organic phase is dried over Na_2SO_4. 95 % of the solvent is evaporated on a rotary evaporator. Residue is taken to a vial and solvent is evaporated to dryness with N_2 gas. Sample residue in vial is dissolved in methanol, filtered and analyzed.

Table 4.3. GC/MS Conditions

Parameter	**Condition**
Column type	VF-5ms, 30m x 0.25mm x 0.25µm
Carrier gas	Helium
Ionisation mode	Electron Impact (EI)
Temperature program	40 °C to 200 °C at 40 °C/min 200 °C to 280 °C at 5 °C/min (hold 20 min)

4.5.4.3 Particle Size Distribution Analysis

Particle size distribution analysis was conducted to observe how irradiation affects the structure of the particles in opium alkaloid wastewater. Analysis was carried out by TUBITAK Marmara Research Center (MAM) using a Malvern Mastersizer 2000® particle size analyzer instrument with Hydro 2000 MU sample dispersing unit. The instrument generates the results as the particle volume percent in discrete size ranges in between 0.02 µm and 2000 µm. Two consecutive measurements were made for each sample and the results were averaged to produce the overall distributions.

4.6. Analytical Methods and Other Analysis

Chemical oxygen demand (COD): COD analyses were performed according to U.S. EPA approved Reactor Digestion Method as described in Hach Water Analysis Handbook (HACH, 1997). High range (0-1500 mg/L) COD reagent vials were used. Vials were digested for 2 hours at 150 °C in Hach COD reactor model 45600-02 and the results were read with Hach DR/2000 direct reading spectrophotometer at 620 nm wavelength (Hach Company, Loveland, CO, USA).

Dissolved Oxygen (DO), pH, Temperature, Conductivity: pH measurements were performed with a portable pH meter (pH 310, Oakton Instruments, Vernon Hills, IL, USA)**.** DO, temperature and conductivity measurements were performed by using the same multi-parameter meter (HACH Sension. 378, USA).

Color: Color measurements were performed spectrophotometrically using Hach DR/2000 equipment according to platinum-cobalt standard method as described in Hach Water Analysis Handbook (HACH, 1997).

Mixed liquor suspended and volatile suspended solids (MLSS & MLVSS): MLSS and MLVSS were determined gravimetrically as described in Standard Methods 2540 D-E (APHA, 1998). Throughout the study MLSS, MLVSS measurements were done with 10 mL samples.

Ammonium and Total Kjeldahl nitrogen (TKN): TKN and ammonia nitrogen were determined according to Standard Methods 4500-Norg B, 4500-NH3 B-C (APHA, 1998).

Biochemical oxygen demand (BOD_5): BOD_5 assay was performed according to Standard Method 5210 B (APHA, 1998).

Total phosphorus: Total phosphorus was determined according to Standard Methods 4500-P B-E (APHA, 1998).

Carbohydrate: Carbohydrate concentration was measured using the Dubois method (Dubois *et al.*, 1956).

Protein: Protein concentration was measured using the Lowry method (Lowry *et al,* 1951).

Sludge volume index (SVI): Sludge settleability was monitored with the SVI parameter as defined in Standard Method 2710 D (APHA, 1998). Mixed liquor was allowed to settle for 30 minutes in an unstirred 1 L graduated glass cylinder. SVI value was then calculated by the following formula:

$$SVI = \frac{settled\ \ sludge\ \ volume\ \ (mL/L)\times 1000}{MLSS\ \ (mg/L)} \qquad (4.2)$$

Microscopical observations: Sludge samples obtained from completely mixed reactors were examined with Ernst Leitz Wetzlar (Germany) model microscope using 3.5 x 15 and 10 x 15 magnification.

CHAPTER 5

RESULTS AND DISCUSSION

In this chapter, the experimental results for the treatment of effluents from opium alkaloid factory located in Afyon Province are presented and discussed. The results of preliminary batch reactor experiments with raw wastewater and anaerobically pre-treated wastewater are presented in Section 5.1. The results of batch and sequencing batch reactor experiments with raw and irradiated wastewaters are presented and compared in Section 5.2. The results of Fenton oxidation experiments are provided in Section 5.3. Last section (Section 5.4) presents the results of analyses performed to monitor the degradation of complex wastewater structure.

5.1. Base-line Experiments: Aerobic Biological Treatment - Batch Reactors

Preliminary experiments were performed to assess the aerobic treatability of raw opium alkaloid wastewater and to investigate the further treatability of anaerobically pretreated wastewater. The results are presented in this section.

5.1.1. Raw Wastewater

Aerobic biological treatability of opium alkaloid wastewater was investigated in batch reactors. Various pre-designated initial concentrations of wastewater (COD between 2,500 and 26,000 mg/L) were added into reactors inoculated with unacclimated culture. Samples were taken from reactors at various time intervals to follow time course variation of COD concentration. The results obtained are presented in Figure 5.1. COD degradation profiles obtained in these experiments are given in Appendix B.

It is obvious from the results obtained that the wastewater is not inhibitory to bacteria even at the highest concentrations studied, i.e. there is no substrate inhibition. Results show also that almost all of the COD biodegradation occurs within the first 5 days for batch run 1, and occurs around 10 days for batch run 2 (Figures 5.1.A & 5.1.B). In experiments with high initial organic loads, namely 7,000, 18,000 and 26,000 mg/L, a slight increase in COD values is observed in the first day of experiment. This may be speculated that the solubilization rate of particulate COD may be higher than the rate of COD degradation at the start period of the reaction. The evolution of COD should be seen on Appendix B.

As it is seen in Figure 5.1.C., unacclimated biomass removed 83 – 90 % of the organic carbon, depending on the initial COD values. It should be recalled that the removal efficiency might be increased after a proper acclimation of seed biomass. Overall, these results suggest that the opium alkaloid wastewater has a good biodegradability and therefore it is suitable for aerobic biological treatment.

In Figure 5.2, the degradation of COD by time for reactor using peptone as carbon source is seen. A comparison of degradation behaviors between the reactors fed with peptone and fed with alkaloid wastewater (4,500 mg/L initial COD) showed a good fit, which also suggest that the wastewater is easily biodegradable.

The real figure of biomass evolution in reactors could not be followed during these studies. Since the opium alkaloid wastewater has a high inherent VSS content, the measured VSS values would not represent the actual microorganism concentration in reactors. For this reason, the specific substrate utilization rates, q, for each reactor were determined according to Equation 2-7 in terms of initially added seed VSS values rather than biomass concentrations at any time during exponential growth phases. The term dS/dt was gathered from the slopes of the trend lines drawn with Microsoft Excel to initial degradation phases (for 2,500 and 4,500 mg/L) or rapid decline phases (for 7,000, 18,000 and 26,000 mg/L,

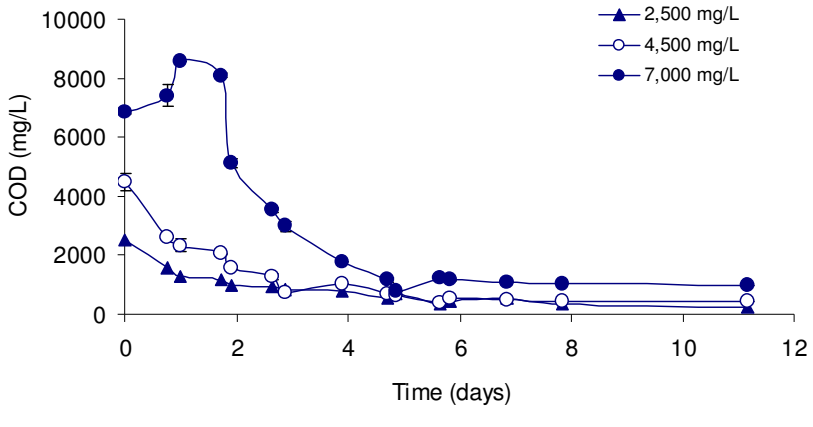
where COD increase was observed initially) of substrate degradation profiles provided in Figure 5.1 (Appendix C).

Following the calculation of q values, the Equation 2-8 was linearized in the form:

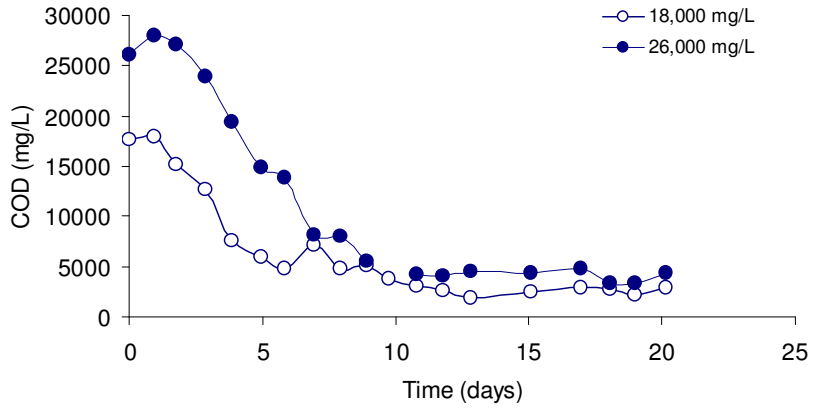
$$\frac{1}{q} = \frac{1}{q_{max}} + \frac{K_s}{q_{max}\ S} \tag{5-1}$$

In this form, a plot of 1/q versus 1/S gives a straight line whose intercept is $1/q_{max}$ and slope is K_s/q_{max}, respectively. This plot is named as double reciprocal or Lineweaver-Burk plot. Values of the Monod parameters K_s and q_{max} were determined by using Lineweaver-Burk plot and also through nonlinear regression analysis using SigmaPlot® software (Appendix C). R^2 values, which quantify the goodness of fit, were very high for both linear and non-linear regressions (0.97 and 0.99 respectively).

It is apparent also from visual observation of Figure 5.3 that Monod model fit well with experimentally found substrate concentration versus specific utilization rate data. This can be taken as further evidence to show that even at high concentrations, substrate utilization continues at high rate, consequently showing, there is no substrate related inhibition. However it should be again kept in mind that kinetic values were calculated in terms of initial seed biomasses and presented here because the data is consistent in itself. Yields, on the other hand, were not calculated because it could be misleading as the actual microorganism concentration could not be determined.



(A)



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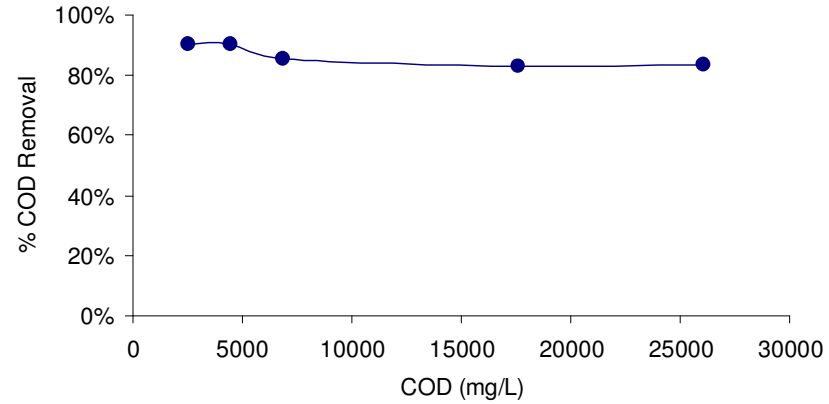


(C)

Figure 5.1. Soluble COD degradation performance in aerobic batch reactors. (A) Substrate degradation profiles for initial COD concentrations 2,500, 4,500 and 7,000 mg/L. (B) Substrate degradation profiles for initial COD concentrations 18,000 & 26,000 mg/L. (C) COD removal efficiencies at each initial concentration.

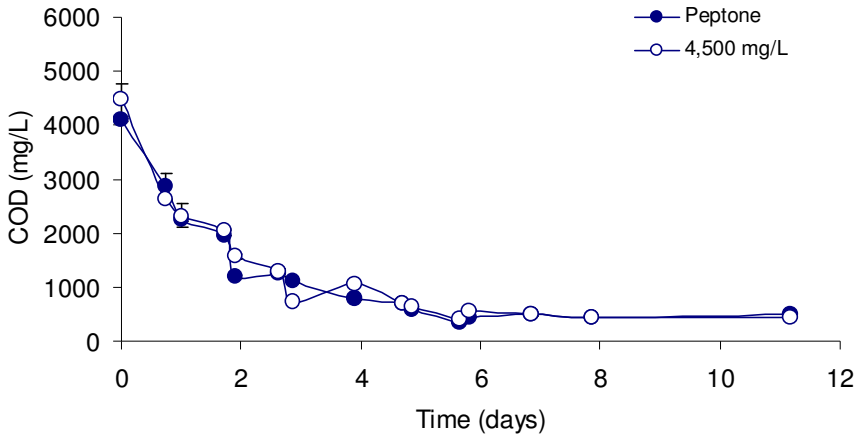


Figure 5.2. Comparison of soluble COD degradation profiles for reactor fed with peptone and reactor fed with alkaloid wastewater.

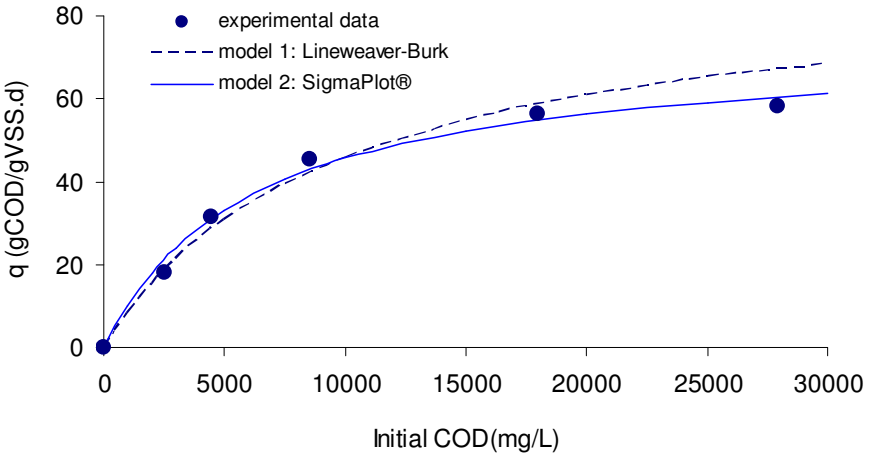


Figure 5.3. The fitness of the empirical monod model to experimental data.

5.1.2. Anaerobically Pre-treated Effluents

Treatability of anaerobically pre-treated opium alkaloid wastewater was investigated in aerobic batch reactors. As it is mentioned in Section 4.5.1.2, the effluents from three UASB reactors (U1, U2, U3) generated by Özdemir (2006) were fed to batch reactors with necessary nutrients and time course variation of COD values under aerobic conditions were followed. Two sets of anaerobically pre-treated samples were collected in different times, first when the UASB influent COD was 10 g/L and second when the UASB influent was 19 g/L. The results obtained for each set are depicted below.

UASB influent COD = 10 g/L:

Table 5.1 shows the total and soluble COD values for effluents obtained from three UASB reactors fed by 10 g/L COD. These anaerobic reactor effluents were fed to batch reactors together with necessary nutrients and reactions were started by seed addition.

As presented in Table 5.1, though the theoretical values of added seed concentrations in reactors were on the levels of 30 – 40 mg/L, measured initial MLVSS concentrations, which also include wastewater-originated VSS concentrations, were high above these. This was due to the escape of anaerobic suspended solids with UASB effluents.

Table 5.1. Characteristics of anaerobically treated effluents.

Parameter	Unit	Effluent from UASB1 (Raw Alkaloid)	Effluent from UASB2 (Co-Substrate)	Effluent from UASB3 (Preacidified)
Total COD	mg/L	2,080	1,400	2,260
Soluble COD	mg/L	1,250	1,120	1,308
MLVSS	mg/L	590 (as measured) 40 (added as seed)	430 (as measured) 30 (added as seed)	620(as measured) 40 (added as seed)

Soluble COD degradation profiles obtained in aerobic batch reactors are presented in Figure 5.4. It can be figured out that the escaped anaerobic biomass could not survive under aerobic conditions and contributed to the soluble COD increase that observed in the first day of experiment by solubilizing (Figure 5.4).

Effluent COD values (on day 6) were 868, 846, and 858 mg/L with corresponding removal efficiencies of 27.4, 22.7 and 32.1 % on the basis of soluble feed COD for aerobic batch reactors fed by effluents of U1, U2, and U3, respectively. These relatively low removal efficiencies indicate that most of the biodegradable part of the wastewater was already treated in anaerobic step. Evidently, while BOD_5/COD ratio of raw alkaloid wastewater fed to anaerobic reactors were 0.55, the measurements made on effluents from U1 and U3 (influents for aerobic batch reactors) gave the ratios of 0.33 and 0.27, respectively.

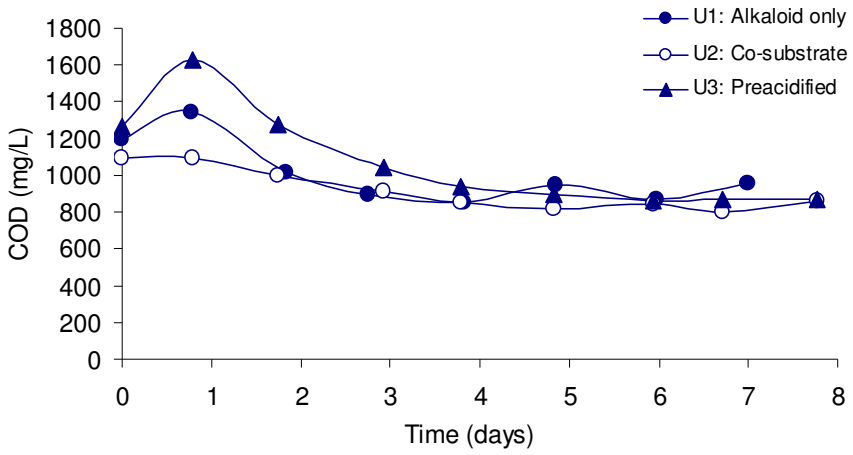


Figure 5.4. Soluble COD degradation profiles of anaerobically pre-treated opium alkaloid wastewater in aerobic batch reactors (UASB influent COD = 10 g/L).

As a whole, the COD removal performance of anaerobic + aerobic treatment was found to be approximately 92 %. This result is in accordance with a previous study carried out on opium alkaloid wastewaters by Aydın (2002) using anaerobic UASB + aerobic SBR system. Effluent CODs from two staged system in mentioned study was around 700 mg/L while influent COD concentrations were varied between 10 - 12 g/L in most of the study period (Aydın, 2002; Aydın et al., 2002).

UASB influent COD = 19 g/L

Effluents gathered from reactors U1 and U2 were centrifuged to separate anaerobic microorganisms before fed to aerobic batch reactors. In an attempt to simulate the conditions in a conventional activated sludge system, initial biomass concentration used in reactors was set around 2,800 mg/L. Time course variation of COD parameter is presented in Figure 5.5 for each reactor.

Effluent COD values were 1,320 and 1,300 mg/L with corresponding removal efficiencies of 66.1 % and 39.8 % for aerobic batch reactors fed with effluents from U1 and U2, respectively. In comparison to first experiment which was conducted by lower influent CODs (effluents from UASB reactors fed by 10 g/L COD), the removal efficiencies were higher in this experiment. This may show that biodegradability of anaerobically treated effluents used here was higher. Evidently, BOD_5/COD ratio was measured as 0.45 for effluent of U1 fed by 19 g/L COD, as it was 0.33 when the feed COD was 10 g/L.

As a whole, UASB + aerobic batch treatment system was able to decrease COD from 19 g/L to 1,300 mg/L which corresponds to a total efficiency of 93 %. The performance of aerobic treatment on anaerobically pre-treated effluents was only assessed with batch reactors in this study and some difference may be expected with a continuous system.

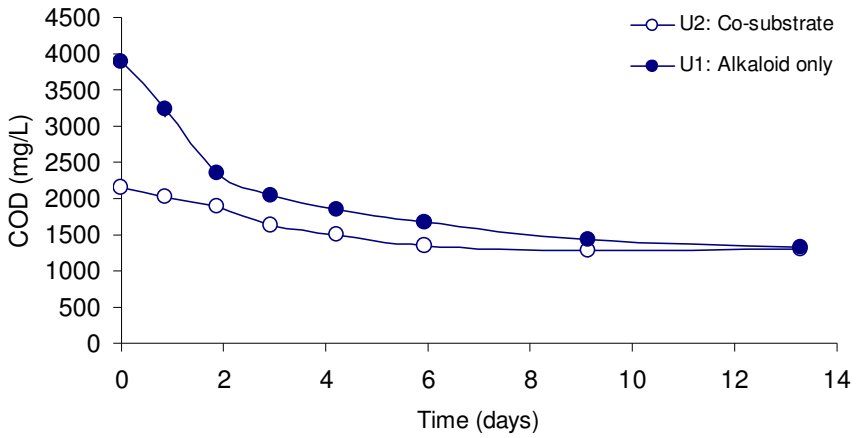


Figure 5.5. Soluble COD degradation profile of anaerobically pretreated opium alkaloid wastewater in aerobic batch reactors (UASB influent COD = 19 g/L).

5.2. Irradiation as a Pre-treatment

5.2.1. The Effect of Irradiation on Biodegradability

BOD_5/COD ratio can be used to evaluate the biodegradability of wastewaters. A wastewater is considered readily biodegradable if its BOD_5/COD ratio is between 0.4 and 0.8 (Al-Momani, 2002; Metcalf and Eddy, 1985). On this basis, the results of COD and BOD measurements conducted on opium alkaloid wastewater assert that the wastewater is readily biodegradable (Table 5.2). This suggestion is in agreement with the findings of the preliminary batch treatability study presented in the above section.

In order to observe the effect of irradiation on the biodegradability of opium alkaloid wastewater, BOD and COD measurements were performed on wastewater irradiated at doses 40 kGy and 140 kGy. As presented in Table 5.2

irradiation imparted no significant change in terms of COD, BOD_5 and accordingly biodegradability index (BOD_5/COD) at the studied doses.

Jo et al. (2006) investigated the effect of gamma irradiation on biodegradability (BOD_5/COD) of textile and pulp wastewaters. In general, the improvement of biodegradability was largely dependent on chemical properties of wastewaters and the absorbed radiation dose. For pulp wastewaters (cooking and bleaching C/D effluents having COD values 2,261 mg/L and 1,368 mg/L respectively) COD values changed only little after gamma-ray treatment at doses from 1 to 20 kGy and the biodegradability was not improved at all. It was stated that this might be caused by the formation of recalcitrant organic acids from aromatic compounds included in both effluents.

Diaz et al. (2003), who applied gamma irradiation on highly colored (3,750 Pt-Co) industrial wastewater, stated that the radiation effects can be limited due to the colloidal matter that contributes to the high color present in the wastewater.

Although the effect was not at significant levels, as can be seen from Table 5.2, radiation treatment was able to result in slight increase in COD concentration as well as a decrease. Melo et al. (2008) attributed this COD value increase after radiation treatment to the radiation-induced effect on the degradation of molecules that leads to the increase of the number of low-molecular weight substrates.

Furthermore, it was noted in several studies that there was no linear relationship between the applied doses of radiation and the resulted COD values as is the case with the current study (Jo et al., 2006; Diaz et al., 2003; Bao et al., 2002).

On the other hand, the opium alkaloid wastewater is highly polluted and therefore a large number of dilutions was needed for COD, BOD_5 measurements to reduce the concentrations to measurable levels. Since this might have caused some

uncertainty in results of the measurements, a series of batch biodegradability tests with original and irradiated samples were also conducted to assess the effect. The results obtained were presented in Section 5.2.2.

Table 5.2. COD, BOD_5 values for original and irradiated wastewater.

Sample Date	Wastewater	COD (mg/L)	BOD_5 (mg/L)	BOD_5/COD
October 2005	*Original*	43,078	23,666	0.55
	40 kGy	45,407	26,333	0.58
	140 kGy	46,290	27,083	0.59
Sample Date	**Wastewater**	**COD (mg/L)**	**BOD_5 (mg/L)**	**BOD_5/COD**
April 2006	*Original*	40,310	19,500	0.48
	40 kGy	42,013	17,750	0.42
	140 kGy	40,029	18,000	0.45
Sample Date	**Wastewater**	**COD (mg/L)**	**BOD_5 (mg/L)**	**BOD_5/COD**
April 2007	*Original*	41,640	22,375	0.54
	40 kGy	39,560	20,500	0.52

5.2.2. Biological Treatability of Irradiated Wastewater – Batch Reactors

A series of batch treatability experiments were carried out with April 2006 samples to support the results obtained in Section 4.2.1. Original and irradiated wastewater samples with various initial COD concentrations were fed to reactors. Substrate removal efficiencies that were achieved with irradiated wastewaters

were slightly lower than the case with original wastewater (Table 5.3). A sample substrate degradation curve obtained in this study is presented in Figure 5.6.

Apart from this, another set of batch experiments was conducted with full strength wastewaters, both raw and irradiated (COD around 35,000 mg/L). In order to simulate the conditions in conventional activated sludge systems, reactors were inoculated with higher amount of seed sludge, which was around 3,000 mg/L. COD removal efficiencies obtained were as high as 89, 88 and 84 % for original, 40 kGy and 140 kGy fed reactors, respectively. As it can be seen from Figure 5.7, the initial lag phase, which was observed in experiments conducted with lower initial biomass concentrations (Figure 5.6) did not occur when the seed biomass was increased.

As a whole, the results obtained in these batch experiments were in accordance with the findings presented in Section 5.2.1 indicating that no enhancement in biodegradability of alkaloid wastewater was able to be attained through gamma-ray treatment.

Table 5.3. Substrate removal efficiencies for original and irradiated wastewaters with various initial COD concentrations.

Initial COD	**Substrate Removal %**		
(mg/L)	***Original***	***40 kGy***	***140 kGy***
3,000	82	80	78
5,000	84	81	79
10,000	87	84	82
20,000	94	87	82
30,000	91	87	-

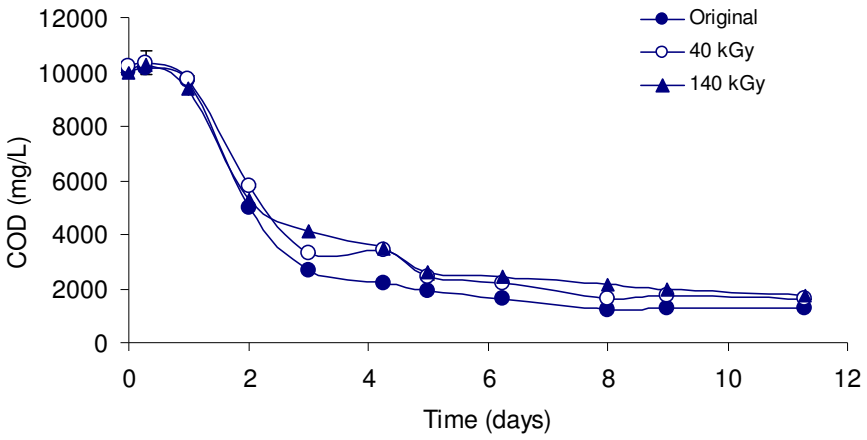


Figure 5.6. Soluble COD degradation for original and irradiated wastewaters (X_o = 350 mg/L)

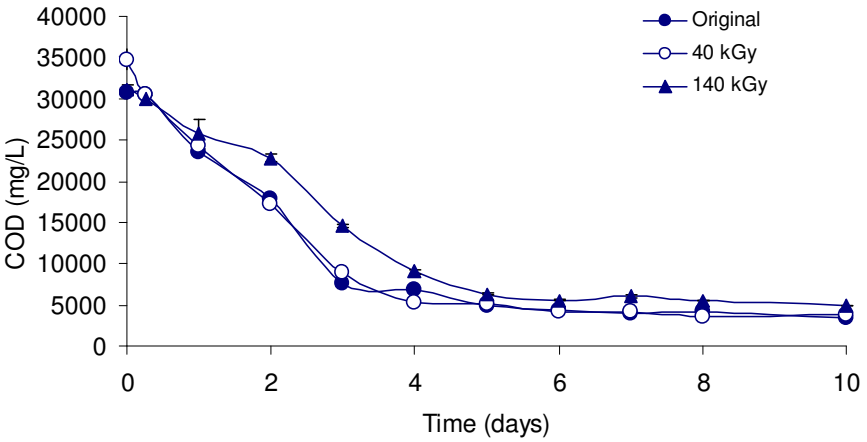


Figure 5.7. Soluble COD degradation for original and irradiated wastewaters (X_o = 3,000 mg/L).

5.2.3. Biological Treatability of Irradiated Wastewater - Sequencing Batch Reactors

In conducted batch treatability studies it was determined that the wastewater was amenable to aerobic biological treatment. In order to further investigate the treatability of wastewater in treatment plant operational conditions, further experiments were planned to be carried out. For this purpose the biological treatability of opium alkaloid wastewater was investigated in instantaneously fed sequencing batch reactors (SBR). COD, MLSS and MLVSS data were collected from the reactors. Performance differences among SBRs fed with raw wastewater and irradiated wastewaters were compared on the basis of COD removal efficiencies and sludge settling characteristics. Settling characteristics for the SBRs were assessed on the basis of sludge volume index (SVI). As sum, 3 sets of experiments were done and the results obtained are presented and discussed below.

5.2.3.1. Gradual Adaptation of Microorganisms to Increasing Influent Strength - Experiment 1

The aim was to feed the reactors with gradually increasing COD concentrations in order to acclimatize the biomass to opium alkaloid wastewater. For this purpose, the studies were started with a fairly low COD concentration, which was 500 mg/L. The raw wastewater had a COD concentration of 30,000 mg/L. About 35 mL of raw wastewater was fed to reactors and made up to 2 L by tap water instantaneously to reach the desired influent COD concentration at every cycle. Macronutrients were supplied at a COD:N:P ratio of 100:5:2. 100 mL excess sludge was wasted daily from the mixed liquor to keep the SRT at 20 days. Table 5.4 shows the results obtained after 32 days of operation, which was deemed enough for system to reach the steady state condition. Steady-state determination

was based on MLSS, MLVSS and COD measurements conducted during two consecutive days which provided almost constant results.

Table 5.4. Results obtained with SBR (COD = 500 mg/L; SRT = 20 days) - Experiment 1

Days	Influent COD (mg/L)	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)
32	510 ± 2	91 ± 1	1,865 ± 20	1,475 ± 4	107 ± 1

As it is seen in Table 5.4, over 90 % COD removal efficiency was achieved in the system and the sludge was settling well as indicated by low SVI value of 107 mL/g. An experiment was carried out on COD degradation profile before increasing the influent COD concentration. The obtained COD degradation profile shown in Figure 5.8 suggested that around 84 % COD removal (corresponding to 91 % of the total COD removal) was reached at the first 5 hours of operation. This may indicate that it might be convenient to study at a lower cycle time than 24 hours for this initial concentration.

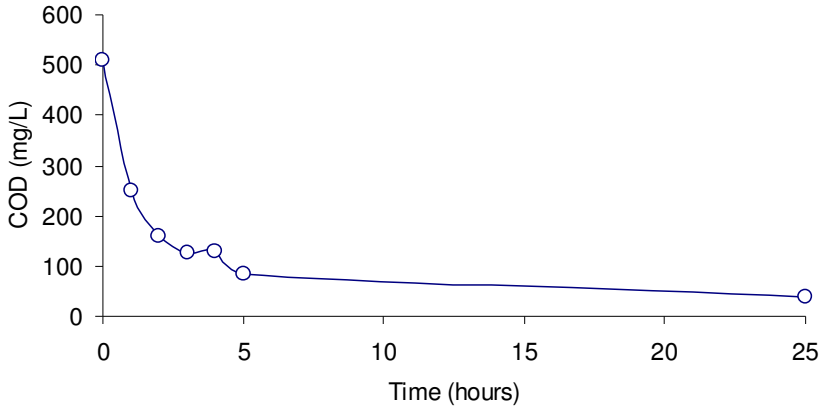


Figure 5.8. Degradation of COD over time in SBR reactor fed with 500 mg/L COD.

Following that, the influent COD concentration was increased to 2,000 mg/L while keeping the operational conditions the same as previous. This COD value was obtained by diluting the raw wastewater having COD of 33,000 mg/L. 125 mL of this raw wastewater was fed into 2 L reactors daily to give the desired influent COD concentration (2,000 mg/L) for the cycle.

Upon the increase of influent COD, sludge settlement problem was started to be observed, and therefore steady state conditions could not be achieved. After one SRT period (20 days), worsened settling was still a problem, hence remedial actions were taken. In one of the two reactors, SRT was decreased to 10 days (half) by doubling the daily sludge wastage. To other reactor, micronutrients (i.e. trace elements) were started to be added besides the macronutrients (nitrogen & phosphorus). Despite the efforts, problem could not be overcome. Experiment was halted as it was not possible to operate (fill & draw) the system anymore due to sludge settlement problem. The problem was the formation of small flocs which lack the filamentous backbones. Only few filaments existed.

5.2.3.2. Effect of Irradiation - Experiment 2-1

In this experiment, the effect of irradiation, with gamma-ray irradiated to 40 kGy and 140 kGy doses, on COD removal was investigated. Also, the effect of irradiation on settling properties of wastewater sludge was sought based on the results obtained through the filterability tests, GC/MS analyses and particle size distribution analyses (Section 5.4) in which irradiation was seen effective in changing the structure of complex molecules in opium alkaloid wastewater.

The COD of raw, 40 kGy dosed, and 140 kGy dosed wastewater were 40,310, 42,013, and 40,029 mg/L, respectively. To initialize the acclimation, influent CODs for the reactors fed with original and 40 kGy dosed wastewater were set as 2,000 mg/L by dilution. The influent COD for the reactor fed with 140 kGy dosed

wastewater was arranged as 3,000 mg/L. The selection of higher level COD was based on the filterability test (Section 5.4.1.1) in which 140 kGy dose provided better degradation, assumably making the substrate more available for bacteria. COD:N:P ratio was kept at 100:5:2 as in previous experiments. The reactors were operated at 15 days SRT in an attempt to reduce MLSS concentration. Steady-state was considered to be attained when MLSS, MLVSS and SVI values remained almost constant (varied less than 5 %) for 5 consecutive days. Because of the fact that the reactors became bulking by the end of first SRT period reactor operation was difficult. It was possible to operate the reactors fed with radiated wastewaters at this condition, however the reactor fed with original wastewater could not be operated (filled & drawn) longer due to higher volume of sludge which do not settle. Results obtained are summarized in Table 5.5.

Table 5.5. Results obtained with SBR (SRT = 15) - Experiment 2-1.

Feed ww	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)	SV_{30}* (mL/1L)
Raw	63	5,473	4,033	180	980
40 kGy	65	5,513	4,047	161	900
140 kGy	55	5,883	4.617	151	890

*The volume of sludge after 30 minutes in SVI test.

It is seen in Table 5.5 that the COD removal efficiency in reactor fed with 40 kGy dosed wastewater was only slightly better than the case with raw wastewater. Hourly COD degradation profile for the reactor fed with 40 kGy dosed wastewater is shown for one cycle in Figure 5.9. Profile suggests that almost all COD removal (corresponding to 99 % of the total COD removal) occurred in approximately 5 hours as similar to the case with raw wastewater in experiment 1 (Figure 5.8).

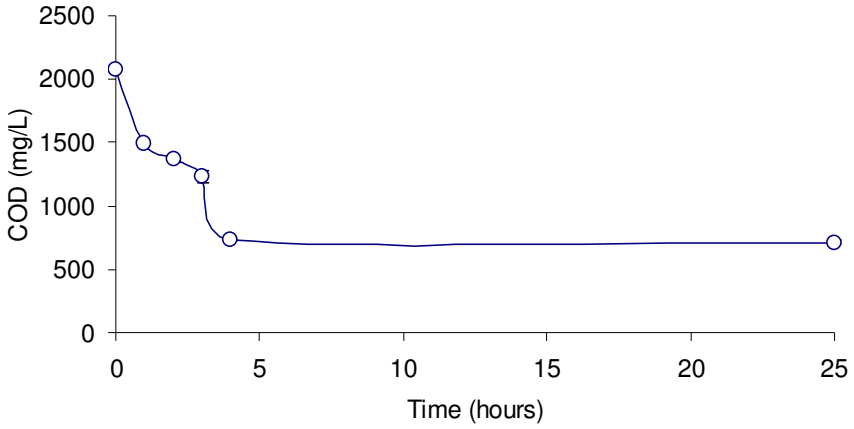


Figure 5.9. Degradation of COD over time in SBR reactor fed with 40 kGy dosed wastewater.

Furthermore, the application of higher radiation dose, namely 140 kGy, have resulted in lowered COD removal efficiency. However, such comparison may not be accurate because, as noted above, the influent COD concentration for 140 kGy fed reactor was higher than the ones fed with raw and 40 kGy, both. Here it should be also noted that the ultimate aim in these experiments was to observe the highest possible influent COD at which the reactors can be operated efficiently. For that purpose, stepwise increments in the influent strength was aimed to ensure acclimatization of microbial biomass. The starting concentrations, in this scope, were not needed or intended to be strictly the same.

Moreover, it was expected that the sludge settlement problem could be overcomed by application of pre-irradiation. However, although some improvement in SVI was observed in reactors fed with irradiated wastewater, reactor operation was still not possible in practice due to poor sludge settling condition evidenced by SV_{30} values presented in Table 5.5. The evolution of SVI value for reactor fed

with 140 kGy dosed wastewater was as presented in Figure 5.10. As it is seen, sludge turned to bulking condition by the end of the first SRT period.

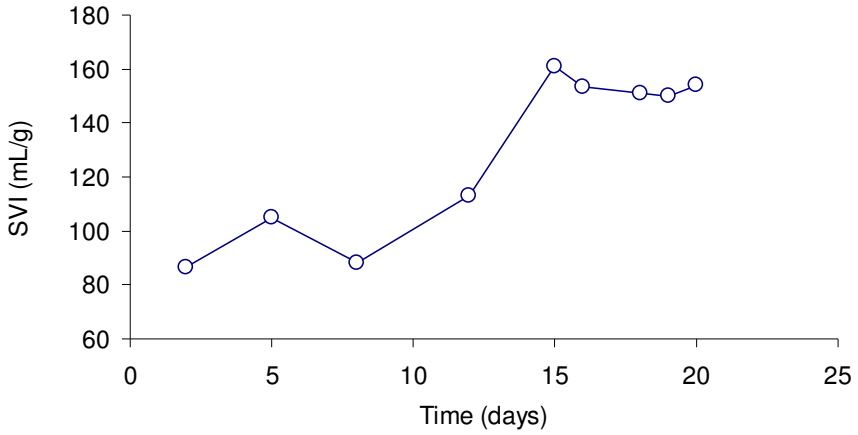


Figure 5.10. SVI course for SBR fed with 140 kGy irradiated wastewater.

Microscopical observation revealed that there were only few filamentous organisms and they do not act as backbone to strengthen the floc particles; the flocs were rather small and dispersed (Figure 5.11). Therefore, poor settling condition was not due to the filamentous bulking, but it might be due to poor floc properties i.e. small flocs. Moreover, foaming problem was observed during the experiment. Large quantities of foam bubbled out of the reactors causing loss of biomass, despite the effort made to collect and return lost solids to reactors.

Furthermore, the pH values measured at the end of the react periods were in the range of 8.6 ± 0.2. Microorganisms can cause an increase in pH by release of alkaline products. The most common cause of increased pH is the ammonia release through metabolism of proteins, peptides or aminoacids. Microorganisms decompose organic nitrogen by breakdown of organic matter, and convert it to

ammonia, a process called ammonification (Gaudy & Gaudy, 1980; Bitton, 1994). Alkaloids are highly complex nitrogenous organic bases which are mostly derived from aminoacids. Considering the high protein and TKN content of alkaloid wastewater (Table 4.1), pH increase may be speculated as a result of mentioned ammonification process.

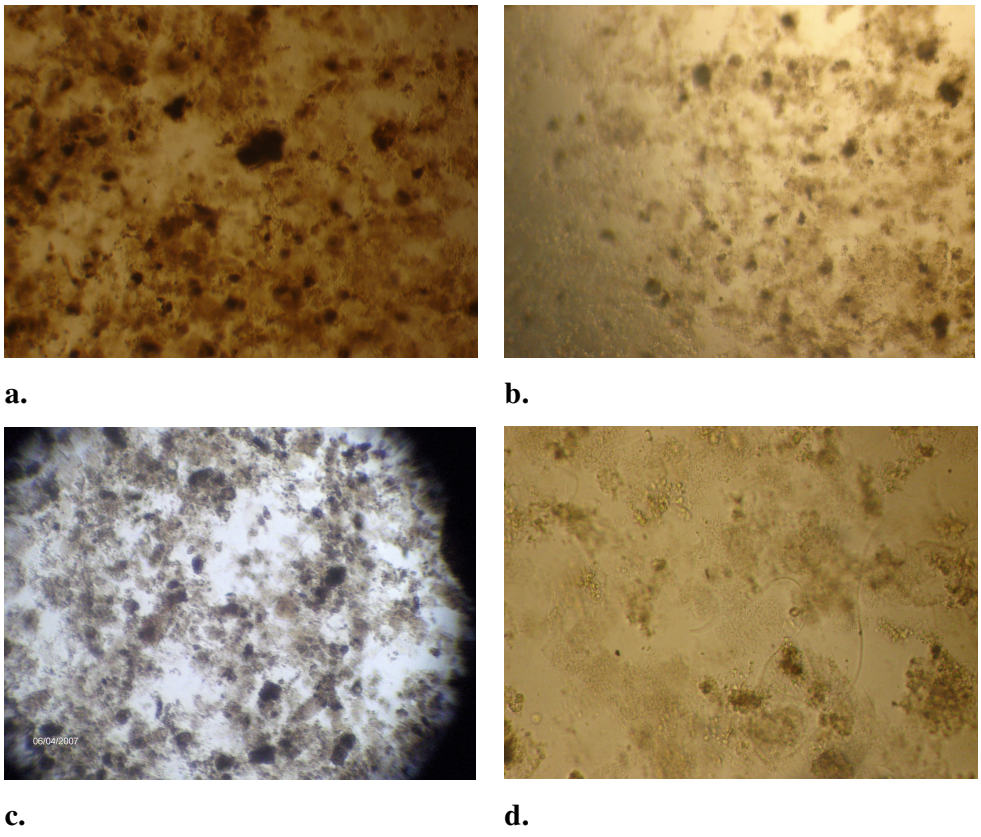


Figure 5.11. Sample microphotographs when influent COD is 2,000 mg/L for **a.** original 3.5x **b.** 40 kGy 3.5x **c.** 140 kGy 3.5x **d.** original 10x magnification

As a result, there was no significant improvement in COD removal efficiency and neither irradiation nor the use of different SRT value offered a solution to sludge settleability problem. Therefore, in these set of experiments, influent COD could not be further increased. Subsequently, it was decided to make some operational changes in order to overcome the existing problems and re-start the experiment. The planned changes were as following:

- Reactor volumes were reduced to 1.6 L in order to avoid overflows from reactors.

- SRT was reduced to 6 days in an attempt to reduce MLSS concentration in reactors.

- Fresh seed, taken from the return sludge line of Ankara Central Wastewater Treatment Plant, was mixed with biomass in reactors at a mass ratio of ½ to renew the culture and to allow a new microbial selection, if possible.

- Until this experiment the effluent pH values were high up to 9 at all times. In order to avoid this pH increase, phosphate addition was increased to serve both as nutrient and as buffering agent with the aim of supplying optimum conditions for the proper microorganisms to dominate.

- Moreover, few drops of paraffin oil were used to suppress foaming and therefore associated biosolids loss whenever needed.

One factor at a time method was applied in these operational changes and firstly SRT reduction was applied. It was seen that it has no positive effect on sludge settleability (data not shown). Following, biomass from these reactors were mixed with fresh seed and the experiment was re-started with cured operational conditions. Results were presented in the following section.

5.2.3.3. Effect of Irradiation -with cured operational conditions - Exp 2-2

Two SBRs were set up and seeded with this mixed sludge. One of the reactors, R1, was fed with original wastewater and R2 with 40 kGy dosed wastewater. Experiment was started with influent strength of 2,000 mg/L COD for both reactors. COD:N:P was supplied at a ratio of 100:5:30. By the increased buffer capacity, pH was kept at 7.2 ± 0.1 during the course of the experiment. SRT was maintained at 6 days. Obtaining almost constant results for SVI, MLSS (varied < 10 %) and COD removal (varied < 15 %) in 4 - 5 consecutive measurements conducted in at least 3 SRT periods indicated the attainment of steady-state.

Steady-state was achieved in a time period of 3 SRT in R1. SVI started to decrease after 6 days of operation and became constant at a level around 51 ± 3 mL/g by the attainment of steady-state condition (Table 5.6). Starting from day 12 sludge rising problem was observed in the settle period. Nitrogen bubbles in settled sludge were easily detectable. In order not to lose biomass, settling time was reduced from its generally used value of 120 minutes to 30 minutes which was enough for the biomass to settle well. Volume exchange ratio (VER) which is defined as volume of the effluent withdrawn to total working volume of the reactor was 75 % during steady-state period. Up to 80 % COD removal efficiency was achieved with a steady-state average of 76 ± 5 % for the influent overall average COD concentration about 2010 ± 255 mg/L.

R2 showed better settling characteristics in comparison to R1, indicated by lower SVI attained around 22 ± 2 mL/g (Table 5.7). Sludge was settling faster too. Over 45 minute settling time, sludge rising problem was observed as in the case with R1. Therefore, time allocated for the settle period was kept at 30 minutes which was enough to separate the settled sludge from the effluent. Because some biomass was lost at the starting period of this reactor due to sludge rising, R2 was allowed to run one SRT period longer than R1 and then steady-state condition was observed to be attained. VER was again 75 %. Similar to the results obtained in R1, up to 80 % COD removal was achieved with a steady-state average of 76 ± 4 % for the influent overall average COD concentration of 1930 ± 135 mg/L.

Table 5.6. Results obtained with SBR (R1) for raw wastewater (COD = 2,000 mg/L; SRT = 6) - Experiment 2-2 (shaded rows belong to steady-state data)

Days	COD influent (mg/L)	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)
0			3,470		
6	2,135	77	4,720	3,340	184
9	2,210	79	4,880	3,480	78
12	1,910	77	4,910	3,510	61
13	1,975	79	4,635	3,330	56
14	1,570	69	4,565	3,240	51
15	2,350	80	4,700	3,320	49
16	2,220	79	4,725	3,305	47
17	1,680	71	4,440	3,130	52

Table 5.7. Results obtained with SBR (R2) for irradiated (40kGy) wastewater (COD = 2,000 mg/L; SRT = 6) - Experiment 2-2 (shaded rows belong to steady-state data)

Days	COD influent (mg/L)	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)
0			3,280		
6	2,130	65	4,030	2,800	32
9	1,795	73	4,075	2,860	25
14	1,960	74	3,640	2,385	25
15	1,870	66	3,620	2,430	28
16	2,180	71	4,200	2,755	26
17	1,795	68	4,320	2,845	25
18	1,990	80	4,535	2,970	23
19	1,950	79	4,495	2,860	20
20	1,980	78	4,835	3,150	21
21	1,765	70	4,740	3,065	21
22	1,740	71	4,675	3,060	24

Following the collection of data, the feed COD concentration for both reactors were increased to 5,000 mg/L and the reactors were allowed to run until system performances re-stabilize to a new steady-state level. Results obtained are presented in Table 5.8 and 5.9 for R1 and R2 respectively.

After the increase of feed concentration to 5,000 mg/L in R1, sludge rising problem continued to prevail. One-hour anoxic cycle was implemented from day 6 to 12 in order to denitrify the wastewater before aerobic oxidation. However, no positive effect was observed, and especially between day 8 to day 14 settle period had to be kept around 20 – 30 minutes not to lose biomass due to rising. This settling time was enough for the sludge to settle well. However, SVI could not be calculated between these days as it requires the volume of sludge settled in 30 minutes. COD removal efficiency was as high as 77 % in that period (Table 5.8).

Sludge rising could not be observed visually at day 15, but then sludge settling relatively worsened with SVI values of 99 mL/g and sludge was bulking. Here it should be noted that, though SVI value (99 mL/g) may indicate acceptable settling condition, this low SVI value was due to high biomass concentration retained in the reactor in that period. The attainment of high MLSS concentration (9,500 mg/L) during that period might be due to accumulated COD in reactor caused by lower volume of effluent withdrawal due to worsened settling. Though COD:N:P ratio was kept at 100:5:30 again, it was observed that effluent pH values were at levels 8.5 ± 0.1. This high pH was though to be related with worsened settling, but no further increase in buffer amount was done. In order to continue safe reactor operation, time allocated for settle period was raised to its former duration (120 minutes) and kept at that value during rest of the experiment period. This bulking period lasted for 14 days from day 15 through day 28. VER decreased to 50 % in that period (i.e. around half of reactor volume was being withdrawn and fed daily). No operator intervention was made and reactor was allowed to reach a steady state.

From day 26 onward SVI showed a decreasing trend with an improvement in sludge settlement characteristic (Table 5.8). In sum, reactor was allowed to run around 9 SRT periods and steady-state data gathered between days 48 to 52. As seen in Table 5.8, SVI value leveled at 57 – 60 mL/g indicating good settling behavior and the average COD removal efficiency was 79 ± 1 % at steady-state. Microscopical observations of the reactor contents revealed that a moderate number of filamentous bacteria and a high amount of protozoa were present in the reactor. The presence of filamentous organisms in moderate amount is desirable as they provide backbone for flocs according to Sezgin et al. (1978). Protozoa (ciliates) are known to feed on the bacteria that are dispersed in the liquid phase and crawl over the surface of the flocs, helping to clarify the effluent and to flocculate the suspended matter and bacteria (Curds et al, 1968; Curds, 1973). Flocs formed were irregularly shaped (Figure 5.12 & 5.13).

Moreover, GC/MS analysis was also performed on both influent and effluent samples taken at steady-state period. The two chromatograms, presented in Appendix D Figure D.14, revealed that a remarkable alteration in wastewater composition was achieved in SBR. Signals of alkaloid compounds observable in raw influent sample were partly (thebain) or totally disappeared after biological treatment. This indicates that aerobic SBR was able to eliminate the main alkaloid pollutants that are present in wastewater, except thebain. Several unidentified peaks that are observed in chromatograms consist of nonalkaloid organic molecules such as residue from organic solvent, and some impurities originating from poppy seed.

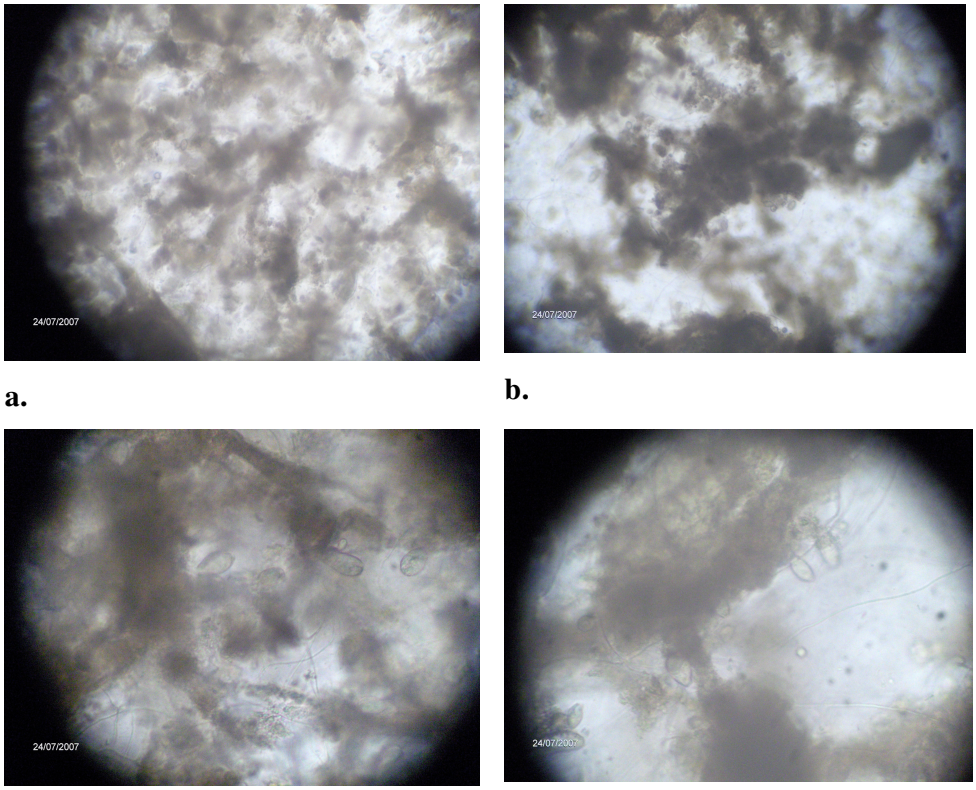
Table 5.8. Results obtained with SBR (R1) for raw wastewater (COD = 5,000 mg/L; SRT = 6) - Experiment 2-2. (shaded rows belong to steady-state data)

Days	COD influent (mg/L)	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)
6	4,625	76	7,980	5,695	46
9	5,000	77	8,775	6,250	Rising
10	4,765	74	8,320	5,930	Rising
18	6,300	-	9,520	6,770	99
25	-	-	9,300	6,150	99
26	4,705	67	9,500	6,250	92
29	4,960	73	7,950	5,870	73
30	4,535	78	7,440	5,360	58
48	5,080	76	8,090	5,610	57
49	4,910	79	7,890	5,580	57
50	4,820	79	8,220	5,709	58
51	4,450	80	8,005	5,670	60
52	4,813	79	7,960	5,635	60

In R2, sludge rising problem continued 6 more days (1 SRT period) after the increase of feed COD concentration to 5,000 mg/L (Table 5.9). Nitrogen bubbles were easily observable. No biomass was lost as 20 – 30 minutes settling time was enough for the sludge to settle well. In the next SRT period settleability of sludge worsened and bulking condition prevailed (day 8 to 12). During that period, the time allocated for settle period increased to 120 minutes; VER decreased to as low as 16 %. As a result MLSS concentration increased due to COD accumulation from former cycles, as in the case with R1. No operator intervention was made during that period and the reactor was allowed to reach a steady-state.

From day 13 onwards an improvement in sludge settlement was observed indicating that the reactor was reaching a steady state condition. In sum, reactor was allowed to run around 6 SRT periods and the steady state data were gathered between days 32 to 35. Average COD removal efficiency at steady-state was 73 ± 1 %. This value is only slightly lower than original-fed R1; as similar to the case with batch reactor studies (Section 5.2.2). One possible reason for this might be the difference in bacterial communities grew on original and irradiated wastewaters, respectively.

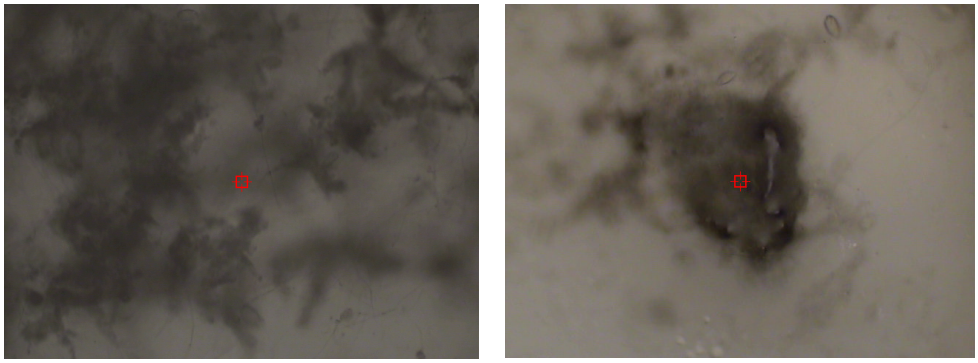
As can be seen in Table 5.9, SVI value leveled at 24 - 26 mL/g, which indicates very good settling behavior. Visual observation revealed a clear supernatant after SVI test. Microscopical observations of the reactor contents revealed a moderate number of filamentous organisms and a high density of protozoa. In comparison to R1, the density of protozoa (free swimming ciliates) was lower which could be due to the better floc formation i.e. lower concentration of bacteria dispersed in the liquid phase. More compact, larger and denser flocs were observed in this reactor (Figure 5.12 & 5.13).



a. **b.**

c. **d.**

Figure 5.12. Sample microphotographs when influent COD is 5,000 mg/L for **a.** original 3.5x **b.** 40 kGy 3.5x **c.** original 10x **d.** 40 kGy 10x magnification



a. **b.**

Figure 5.13. Sample microphotographs when influent COD is 5,000 mg/L for **a.** original **b.** 40 kGy – with nicolet almega xr confocal microscope at 10x magnification

Furthermore, GC/MS analysis performed on samples taken from influent and effluent of the reactor showed that the peaks corresponding to alkaloid compounds were fully disappeared upon SBR treatment. The resulted chromatograms are presented in Appendix D Figure D.15.

Table 5.9. Results obtained with SBR (R2) for irradiated (40kGy) wastewater (COD = 5,000 mg/L; SRT = 6) (shaded rows belong to steady-state data)

Days	COD influent (mg/L)	% COD Removal	MLSS (mg/L)	MLVSS (mg/L)	SVI (mL/g)
6	-	-	7,500	-	Rising
9	6,340	66	11,230	7,410	No settling
10	-	-	11,060	8,500	No settling
13	6,275	59	9,340	6,390	57
32	4,945	73	7,930	5,210	25
33	4,645	71	8,350	5,520	25
34	4,870	74	8,040	5,350	24
35	4,630	74	7,670	5,120	26

As a conclusion, after modifying the operational conditions it was possible to overcome sludge settlement problem, and influent COD concentration was raised up to 5,000 mg/L. Effective COD removal efficiencies were attained for both reactors and irradiation helped improve sludge settlement. The populations of protozoa and filamentous organisms required for proper flocculation was able to grow under cured conditions. As was expected, the average MLSS concentrations increased in both SBRs after the increase of influent COD to 5,000 mg/L. The SVI values for both reactors were very low partly because of the high concentrations of solids maintained in the SBRs. The SBR fed with irradiated wastewater consistently provided lower SVI values than the one fed with original.

5.3. Fenton's Oxidation as a Pre-treatment

In this part, results obtained from the Fenton's oxidation, which was applied as a pre-treatment alternative process, are presented. Firstly, a preliminary study was conducted in order to assess the required initial doses of Fenton's reagents to initiate the research. It was seen that at hydrogen peroxide concentrations higher than 40 g/L, high swelling and overflow occurs. Martinez et al. (2003) attributes this quick boiling and violent reaction observed over certain dosages to the high exothermic effects of quick COD oxidation. The concentrations where violent reaction occurred were excluded and the concentrations with moderate swelling were kept in experimental design. Because, it would be possible to reduce that swelling by various strategies such as two step addition of reagents, when needed. Also rapid mixing during 30 minutes were deemed sufficient for effective oxidation period, because there was no further COD removal up to two hours of oxidation (data not shown).

A set of experiment was conducted in order to examine the effect of hydrogen peroxide dose at different reagent ratios. First, hydrogen peroxide concentration was kept constant at 30 g/L and $FeSO_4.7H_2O$ dose was varied so as to provide H_2O_2 / Fe^{+2} mass ratios between 0.5 to 5. Experiment was repeated for 10 and 20 g/L hydrogen peroxide doses respectively providing the same H_2O_2 / Fe^{+2} ratios. The results obtained are shown in Figure 5.14.

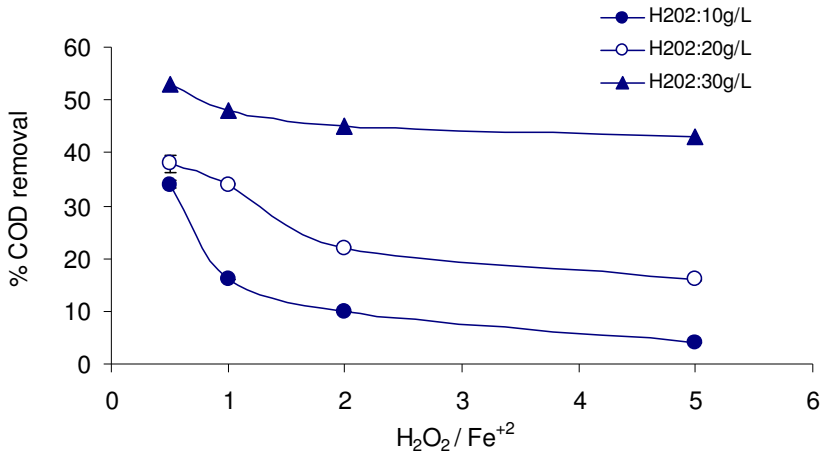


Figure 5.14. H_2O_2 / Fe^{+2} mass ratio versus COD removal efficiency. ($pH_{oxidation}$ = 3; 30 minutes rapid mix; $pH_{coagulation}$ = 10; 30 minutes slow mix).

As it is seen in Figure 5.14, higher hydrogen peroxide concentrations (corresponding to higher Fe loads as well) resulted in better COD removals due to generation of higher amount of ˙OH radicals. At all fixed dosages of peroxide (10, 20 and 30 g/L) COD removal performances increased with increasing iron dosages. For H_2O_2 dosage of 30 g/L such increase was not as significant in comparison to other oxidant doses. H_2O_2/Fe^{+2} mass ratio of 0.5 provided the best COD removal efficiencies at all H_2O_2 dosages studied. However, it should be noted that, the use of greater amounts of iron should be avoided. Because, the removal of settled iron hydroxide sludge would require an additional treatment step resulting in higher cost (Pérez, 2002; Schrank et al., 2005). Therefore, in the following experiments optimum dose of Fe^{+2} was sought keeping the hydrogen peroxide concentration constant at 30 g/L where better removal efficiencies were obtained especially at lower iron dosages (higher H_2O_2/Fe^{+2} ratios). The results are shown in Table 5.10 and Figure 5.15.

As it is seen in Figure 5.15, 6 g/L Fe^{+2} concentration (corresponding to 30 g/L $FeSO_4.7H_2O$) was the optimal dosage resulting in approximately 44 ± 1 % COD removal efficiency. An optimal Fe^{+2} : H_2O_2 mass ratio of 1:5 is in accordance with literature references Bigda (1995) and Guedes et al. (2003). However, for 480 m^3/day flow rate of Afyon alkaloid factory, this treatment would require very high amounts of both chemicals which would not be economical. Therefore biological treatment following Fenton's oxidation option was not studied further with these high concentrations.

Table 5.10. Results of Fenton Oxidation studies with constant H_2O_2 and various Fe^{+2} dosages.

H_2O_2 (g/L)	**Fe^{+2} (g/L)**	**H_2O_2 / Fe^{+2} (w/w)**	**% COD Removal**
30	60	0,5	53
30	30	1	48
30	15	2	45
30	10	3	43
30	6	5	44
30	5	6	35
30	3.75	8	37
30	3	10	13
30	1	30	3
30	0	-	0

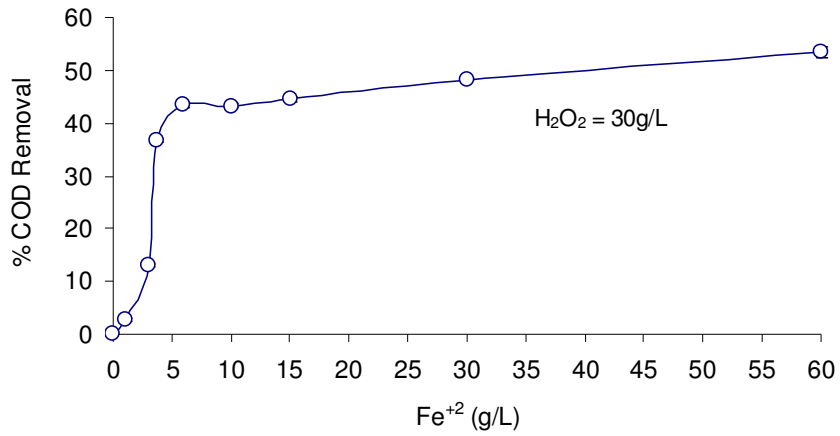


Figure 5.15. Effect of Fe^{+2} dosage on COD removal efficiency at constant H_2O_2 = 30 g/L. ($pH_{oxidation}$ = 3; 30 minutes rapid mix; $pH_{coagulation}$ = 10; 30 minutes slow mix)

5.4. Monitoring the Degradation of Complex Wastewater Structure

As it was mentioned in Section 5.2, some operational problems related to sludge settleability were observed in SBRs. Complex organic compounds in opium alkaloid wastewater was thought to contribute to this settleability problem. It was reasoned that if the chemical structure of wastewater change, then the microbial community in activated sludge would also change directly affecting the settleability characteristics. Therefore, it was decided to alter this complex structure prior to biological treatment by means of irradiation or Fenton's oxidation treatments. Degradation of the complex structure of the wastewater was followed by filterability tests, GC/MS analyses and particle size distribution analyses. The results of these analyses are presented under the following subsections.

5.4.1. Filterability Tests

As an evidence for degradation of complex structure and breakdown of bonds, enhancement in filterability of opium alkaloid wastewater was sought.

5.4.1.1. Filterability Tests for the effluent pre-treated with Fenton's Oxidation or Irradiation – Experiment 1

In this experiment, filterability tests were carried out with raw alkaloid effluent, effluents pre-treated with various Fenton's reagents dosages and also effluents pre-treated at two irradiation doses, namely 40 kGy and 140 kGy. Results were compared based on the filtration time.

As it is seen in Table 5.11, Fenton's treatment enhanced the filterability of wastewater, indicating the disintegration of complex structure. Enhancement in filterability was found to increase as applied hydrogen peroxide concentration increases. Fe^{+2}:H_2O_2 mass ratio of 1:2 (as in 1500 / 3000 and 2500 / 5000) was found to provide a better filterability than other studied ratios (1:1 and 1:5) when H_2O_2 was kept constant. This can be explained by the fact that H_2O_2 or Fe^{+2} can act as free-radical scavenger through reactions (2.13) and (2.14) provided in Section 2.2 unless an optimal ratio between reagents is fixed (Tang and Huang, 1997; Lopez et al., 2004).

In comparison to Fenton's treatment, gamma irradiation provided better enhancement in filterability of wastewater. Moreover, filtration time for 140 kGy irradiated wastewater to fill 50 mL volume was around half of the time required for 40 kGy irradiated wastewater to fill the same volume (28 seconds to 73 seconds), which evidenced that the filterability of the former was better.

It should be noted that, as mentioned in Section 4.5.2.1, Fenton pretreated samples were centrifuged prior to experiment in order to eliminate the effect of sludge produced by Fenton reaction on filterability, and then the same force and duration

of centrifugation was applied to raw and irradiated wastewater samples. Hence, the comparability of filterability test results acquired for all the samples was ensured.

Based on these results pre-irradiation, rather than Fenton's pre-treatment, was decided to be applied to ensure the alteration of complex structure prior to biological treatment in further treatability studies.

Table 5.11. Results of Filterability Tests for the effluent pre-treated with Fenton's Oxidation or Irradiation – Experiment 1.

Fe^{+2} / H_2O_2 (mg/L / mg/L)	**Time to Filter (sec)**
Original sample	943
500 / 1000	444
3000 / 3000	267
1500 / 3000	169
5000 / 5000	166
1000 / 5000	156
2500 / 5000	127
40 kGy	73
140 kGy	28

5.4.1.2. Filterability Test for the effluents pre-treated with various irradiation doses – Experiment 2

In this experiment, original and irradiated samples (at 5 to 40 kGy) were filtered through 20 – 25 µm Whatman filter papers. The aim was to assess the possibility of decreasing the applied dose of irradiation used prior to biological treatment,

from economical and timely point of view, by finding an optimum dose in terms of filterability. Evidenced from the sharp decrease observed in filtration time values, the filterability of wastewater had been appreciably enhanced upon the application of radiation (Table 5.12). It showed that, radiation application was effective in degradation of complex structure starting from the lowest dose applied. Moreover, the filterability of wastewater was further enhancing with increased doses of gamma radiation.

As a whole, it was concluded that a lower radiation dose could also be used towards the purpose of degradation judging by the easement in filterability. The current study was continued with 40 kGy dose.

Table 5.12. Results of Filterability Tests for the effluents pre-treated with various irradiation doses - Experiment 2.

Irradiation Dose (kGy)	**Time to Filter (sec)**
0	3632
5	465
10	323
20	164
25	151
30	155
35	120
40	110

Although the removal of COD was not the main aim of this experiment, COD concentrations were also decided to be measured and presented upon the observation that the lower irradiation doses were also quite effective in degradation. Because, it has been noted in several recent researches that there is

no linear relationship between the applied dose of radiation and the resulting COD value (Jo et al., 2006; Diaz et al., 2003; Bao et al., 2002).

COD removal efficiencies by irradiation and also by subsequent filtration are presented in Table 5.13. The percent COD removal at different doses of radiation is presented in Figure 5.16. It can be said that, in agreement with the above mentioned researches, there is no linear relationship between the dose applied and the removal percent acquired. However, it is also seen that, the best removal efficiencies were achieved for the highest doses, namely 30, 35 and 40 kGy. The COD of wastewater samples did not decrease further when the radiation dose was over 30 kGy. Although the COD removal efficiency achieved herein by irradiation of raw wastewater is the highest reported value throughout this study, the removal was still not at significant levels and stayed under 10 percent. The relative differences among the reported COD removal values (Table 5.2) may be due to the different characteristics of wastewater samples taken from factory at different times. Likewise, removals with filtration were found trivial.

Table 5.13. COD removal efficiencies by irradiation & microfiltration (20-25 μm)

Dose (kGy)	Total COD	Microfiltrate COD	% COD Removal by Radiation	% COD Removal by Microfiltration
Original ww	38,650	36,650	-	5.2
5	38,000	35,050	1.7	7.8
10	36,700	35,750	5.0	2.6
20	37,250	32,250	3.6	13.4
25	36,775	36,600	4.9	0.5
30	35,075	34,950	9.2	0.4
35	35,100	34,725	9.2	1.1
40	35,175	33,750	9.0	4.1

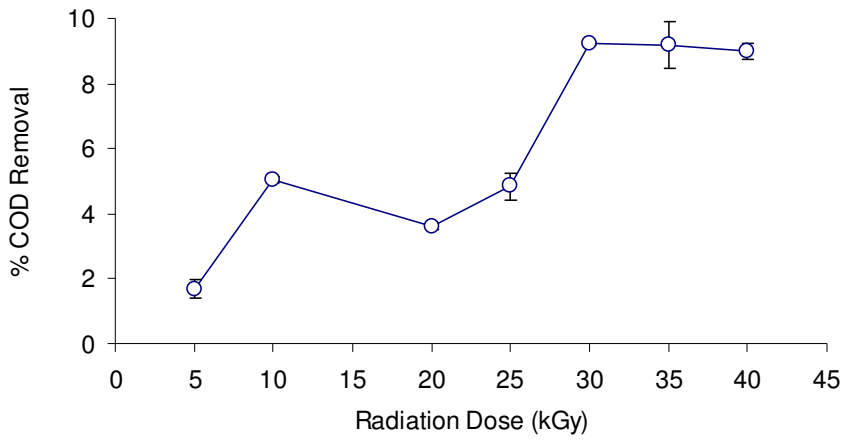


Figure 5.16. COD removal at different γ-irradiation doses

5.4.2. Gas Chromatography Mass Spectrometry Analysis

Gas Chromatography Mass Spectrometry (GC/MS) was used to investigate the presence of alkaloids in wastewater. Analysis was performed firstly on raw (untreated) wastewater. Some main alkaloids including morphine, codein, papaverine, thebain, protopine, laudanosine and noscapine were identified in wastewater as a result of this analysis. GC chromatogram which shows the peaks corresponding to detected compounds is presented in Figure 5.17. The validity of the identification of these GC peaks is verified by matching the measured mass spectra with standard mass spectral database stored in main library of GC/MS instrument. As a sample representation, the mass spectrum for the selected peak (labelled as 1A on chromatogram) is also presented in Figure 5.17. A library search of the mass spectral database identified this peak as Morphinan-6-ol,7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-,(5.alpha.,6.alpha.)- which is the systematic name for common alkaloid codeine. The molecular structure and standard mass spectra of codein provided by the instrument is presented in Figure 5.18 for comparison.

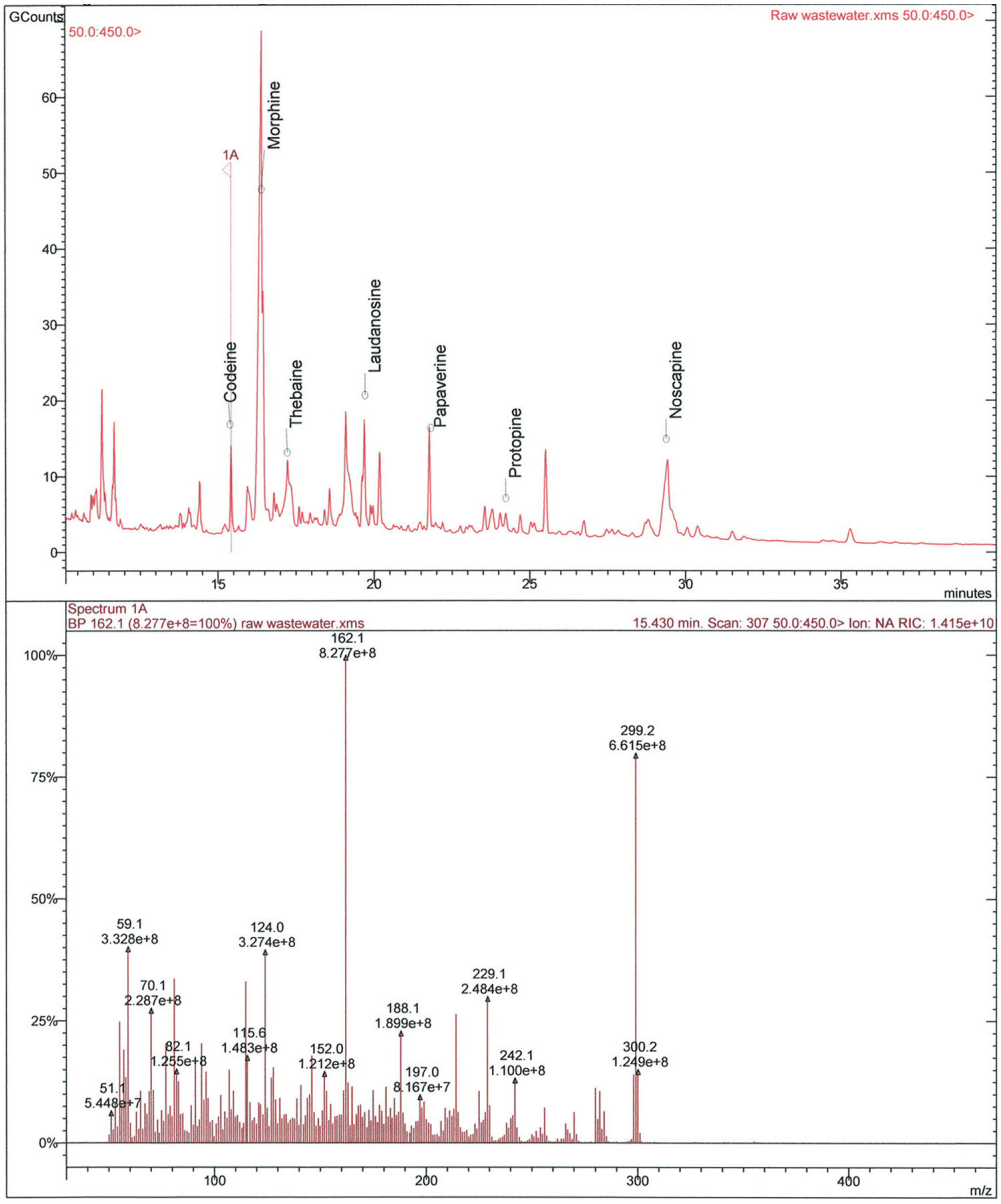


Figure 5.17. (*a*) GC Chromatogram of raw wastewater. Identified Peaks: 1-Codein; 2- Morphine; 3- Thebain; 4- Laudanosine; 5- Papaverine; 6- Protopine; 7-Noscapine. (*b*) EI Mass Spectrum of peak 1A obtained from GC/MS analysis.

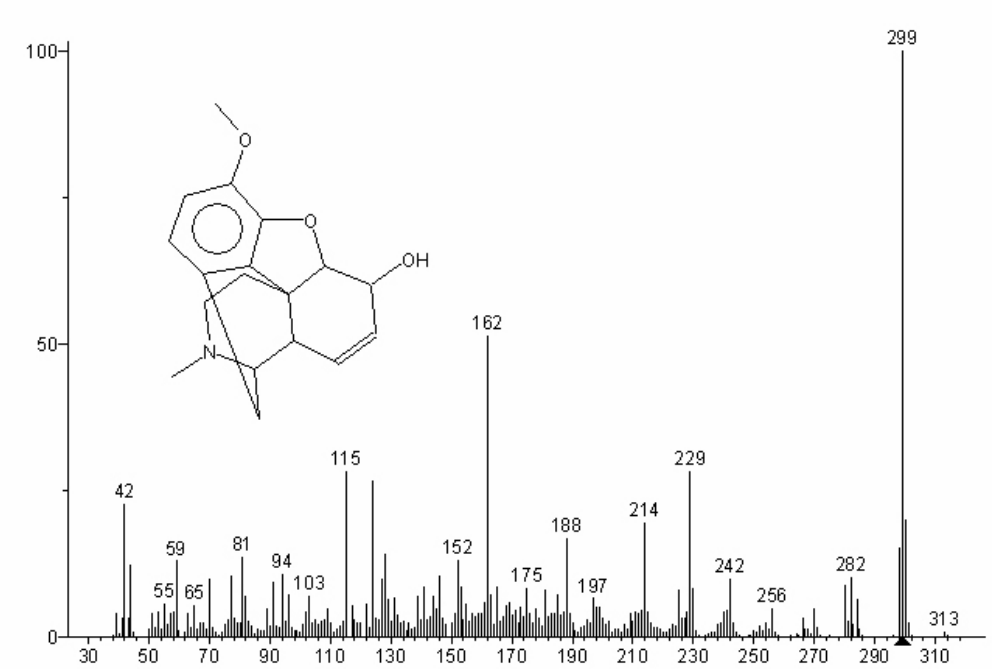


Figure 5.18. Molecular structure and mass spectrum of codein from instrument library. Chemical formula: $C_{18}H_{21}NO_3$; M.W. = 299.

Similarly, each alkaloid compound was identified by comparing with its standard mass spectrum. The mass spectra scanned for the selected chromatogram peaks and the matching internal standard spectrums are presented in Appendix D, Figure D.1 through D.12.

Following that GC/MS analysis was conducted with irradiated wastewater to assess whether these complex alkaloid compounds could be eliminated or degraded by radiation treatment. GC chromatogram for 40 kGy-irradiated wastewater was obtained and compared to that of raw wastewater by overlaying their chromatograms (Figure 5.19). The breakdown of compounds was qualitatively assessed by comparison of peak heights. Respective chromatogram for raw and irradiated wastewaters are presented in Appendix D, Figure D.13.

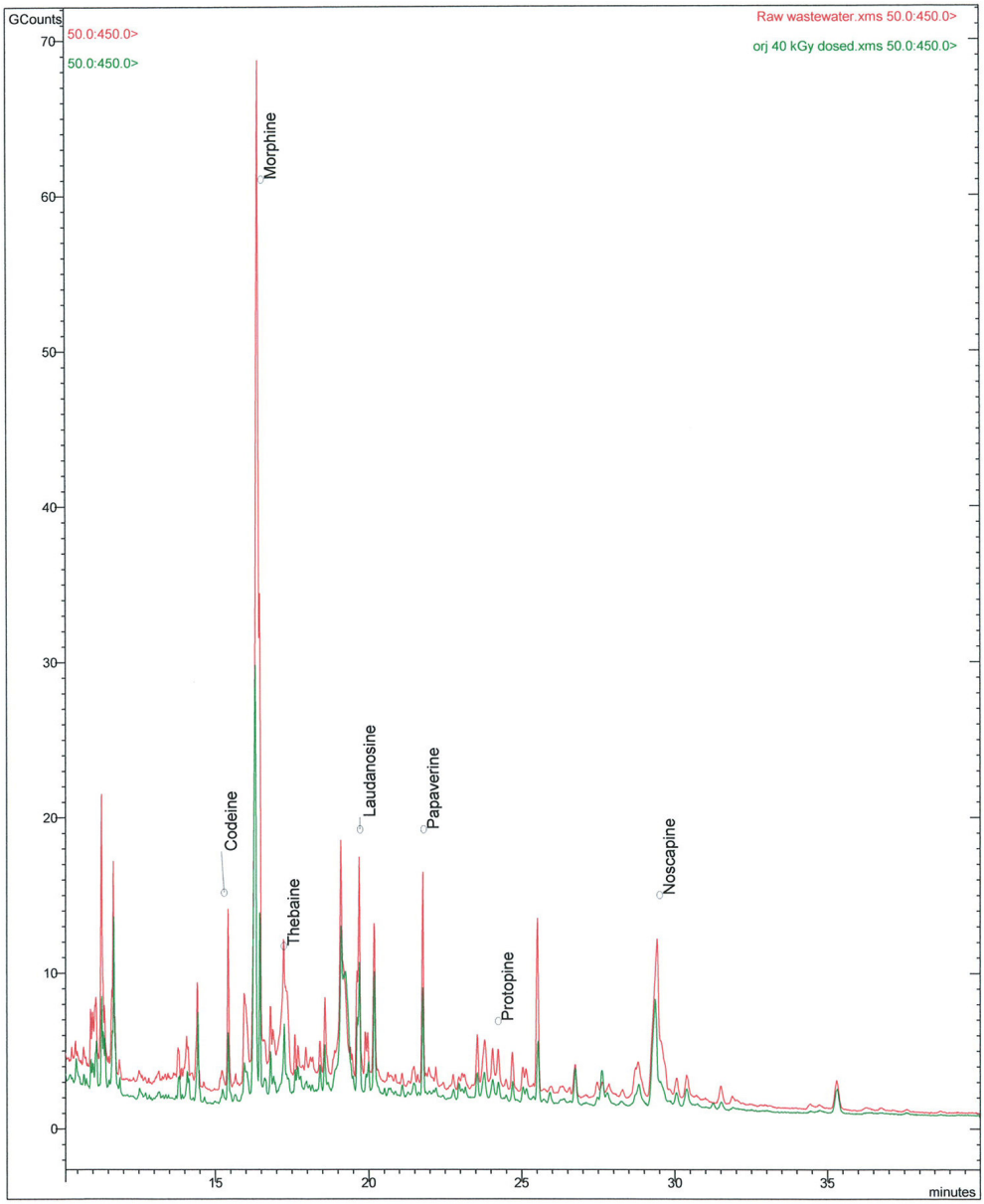


Figure 5.19. GC Chromatogram overlay of raw and irradiated samples.

Visual comparison of the overlaid chromatograms (Figure 5.19) indicates a significant decrease in GC peaks after radiation treatment proving that the compounds present in raw wastewater were degraded to a certain extent upon

irradiation. Signal-to-noise ratio (S/N) values provided for codein in Figure 5.20 quantitatively show this degradation on selected and expanded sector of overlaid chromatogram (Figure 5.20).

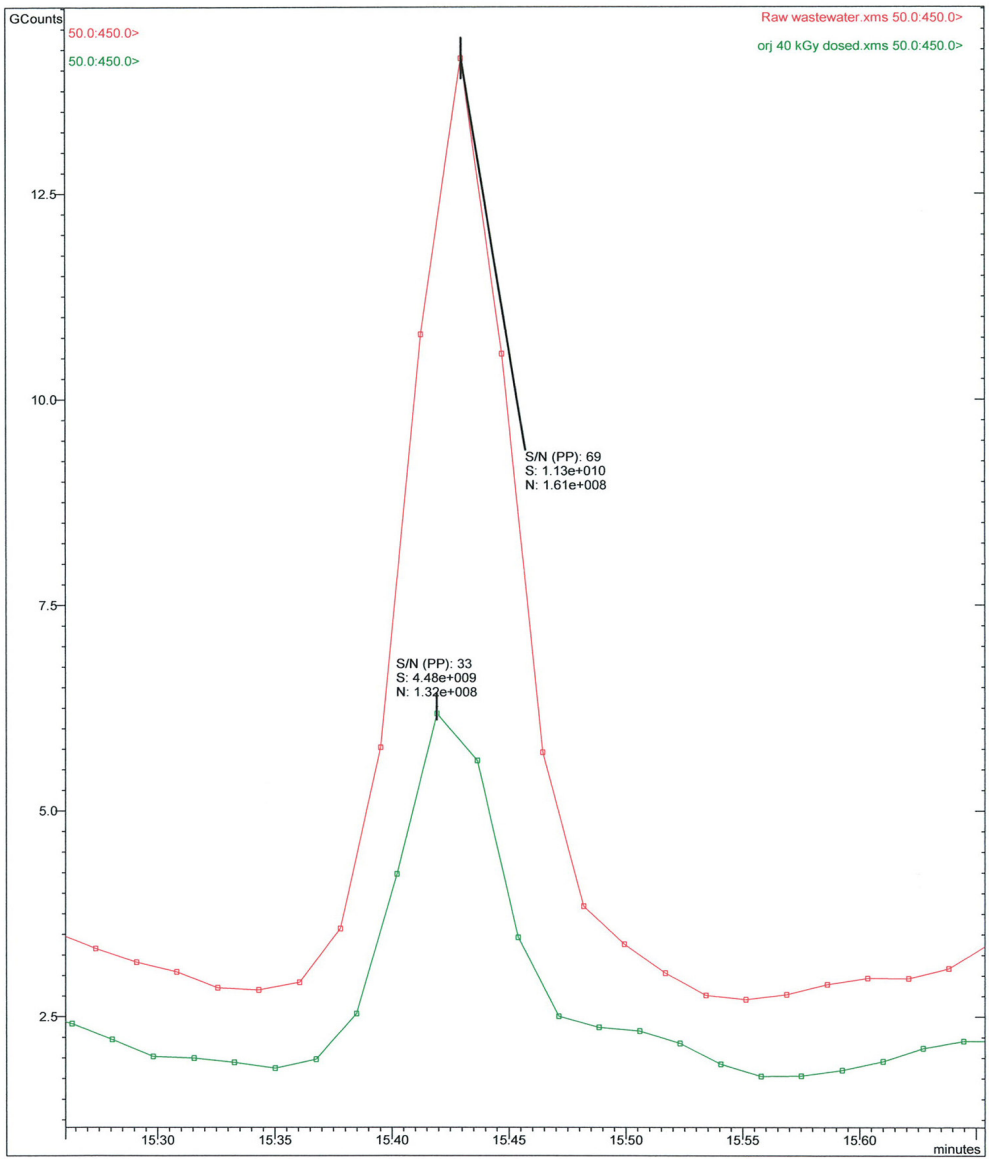


Figure 5.20. Overlaid Chromatogram showing degradation of Codein by irradiation.

5.4.3. Particle Size Distribution Analysis

Particle size analyses were done on both original and irradiated (40 kGy) wastewater samples. The particle size distribution of these samples which shows the percentage of particles by volume between 0.02 – 2000 μm is presented in Figure 5.21. The full result analysis reports may be seen in Appendix E.

As it is seen in the Figure 5.21 the original wastewater showed one main peak in the size range around 15 – 20 μm. Radiation application led shifts in the distribution. Although the main peak spanned a similar size range around 10 - 15 μm, its particle volume percent decreased nearly to half compared to original sample's. The small peaks on the larger and smaller size sides of the main peak became more pronounced by irradiation. In other words, the volume percent of both smaller and larger size particles increased upon radiation application confirming the alteration of uniformity and increase in polydispersity.

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As it is seen in Table 5.14, irradiation decreased the d (0.1) and d (0.5) values, while causing an increase in the d (0.9) value. The d (0.1), d (0.5) and d (0.9) values presented in the table indicate that 10 %, 50 % and 90 % of the particles measured were less than or equal to the size stated.

To further clarify this; the d(0.5) value for original sample is 15.77 microns, while the d(0.5) value for the irradiated sample is 10.33 microns. That means, 50 % of the particles in irradiated sample are lower than 10.33 microns in size compared to 50 % of particles in original sample being lower than 15.77 microns. The d(0.1) value have the same trend showing that particles got disintegrated into smaller ones upon irradiation. However, there is an increase in d(0.9) value after irradiation. It shows that 10 % of the particles are greater than 196.18 microns in irradiated sample compared to 43.73 microns in original.

The increase in specific surface area by radiation application proves the disintegration of relatively big size particles into smaller ones which results in that larger surface area. The increase in the ratio of the volume weighted mean to surface weighted mean diameters suggests an increase in the degree of polydispersity as mentioned earlier.

As a result, the findings prove that radiation application altered the structure of wastewater. As mentioned in Section 5.4, it was thought that if chemical structure of wastewater is changed, the microbial community fed by this wastewater would also change through microbial selection, directly affecting the sludge settleability characteristics. The result of particle size distribution analyses could be taken as evidence that the improvement in settleability characteristics observed in SBRs fed with irradiated wastewaters (Section 5.2.3.2 & 5.2.3.3) was due to the influence of that change in wastewater characteristics on microbial selection.

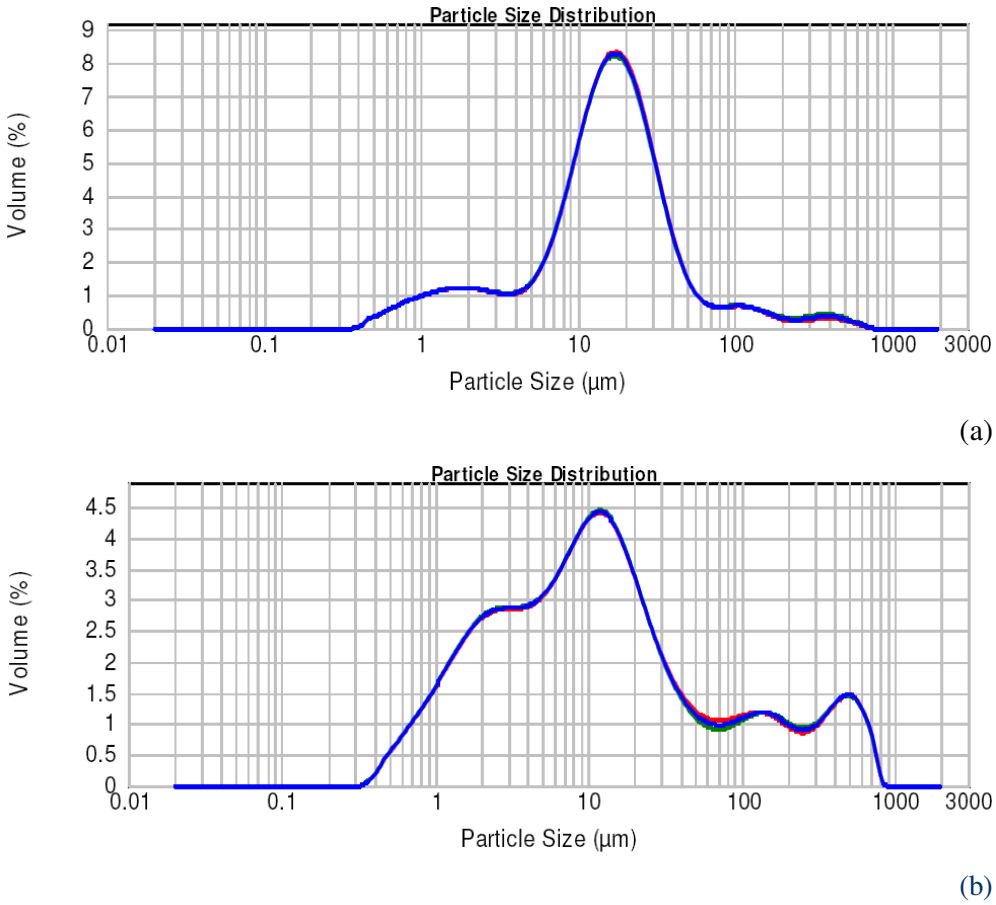


Figure 5.21. Particle size distributions of (a) Original and (b) 40 kGy irradiated wastewater.

Table 5.14. Results of particle size distribution analysis.

Wastewater	Particle size distribution (µm)			Specific surface area (m^2/g)	Surface weighted mean diameter (µm)	Volume weighted mean diameter (µm)
	d (0.1)	d (0.5)	d (0.9)			
Original	2.411	15.768	43.729	0.963	6.230	29.052
40 kGy	1.447	10.327	196.182	1.48	4.049	61.735

CHAPTER 6

CONCLUSIONS

In this study, treatability of high strength opium alkaloid industry wastewaters was investigated. The principal objective of the study was the investigation of the effect of gamma preirradiation on aerobic biological treatment of opium alkaloid wastewater. Two different types of bioreactors, namely, batch and SBR were utilized for this purpose. Moreover, aerobic biological treatment of anaerobically pre-treated wastewater was examined in batch reactors. The effect of Fenton's oxidation as a pre-treatment was also examined. The specific results obtained from this study can be summarized as follows:

- Biodegradability studies conducted in batch reactors revealed that opium alkaloid wastewater was not inhibitory to aerobic biomass and found to have a good biodegradability. Unacclimated biomass was able to remove 82 – 94 % of the organic carbon for studied influent COD concentrations from around 2,500 to 30,000 mg/L.

- Irradiation imparted no significant change in COD, BOD_5 and accordingly biodegradability of opium alkaloid wastewater.

- For anaerobically-pretreated effluents (UASB influent 10 g/L), aerobic batch treatment provided further 23 - 32 % COD removal. The additional COD removal provided by aerobic batch treatment was at higher level (40 – 66 %) for anaerobically-pretreated effluents when UASB influent was 19 g/L. The total COD removal efficiency of UASB + aerobic batch treatment was very high (>90 %).

- In SBR, at first, proper operation of reactor fed with raw wastewater was not possible due to sludge settleability problem experienced even with diluted wastewater samples. Remedial actions taken to disintegrate the complex structure of wastewater revealed that:

    - Better degradation of complex structure was attained by gamma radiation application in comparison to Fenton's oxidation.

    - Radiation application led shifts in the particle size distribution altering the uniformity and increasing the polydispersity.

    - Qualitative comparisons of GC chromatograms belong to raw and 40 kGy irradiated wastewaters showed that alkaloid compounds were able to be degraded to a certain extent by irradiation.

    - Furthermore, lower radiation doses could be used towards the purpose of disintegration of complex structure of wastewater judging by the easement in filterability.

- Preirradiation application imposed on wastewater provided relatively better sludge settleability characteristics in the following SBR treatment. However, reactor operation was still not possible due to poor settling condition. Operational changes, which include addition of fresh seed and phosphate buffer were effective in solving the sludge settlement problem.

- For 2,000 mg/L influent strength, a steady-state average of 76 ± 5 % COD removal efficiency was achieved in both reactors fed with original and 40 kGy irradiated wastewater. SVI value achieved in reactor fed with irradiated wastewater was lower in comparison to reactor fed with original wastewater (~20 to 50 mL/g).

- Influent COD strength was increased up to 5,000 mg/L COD. At steady-state condition significant average COD removal efficiencies were achieved for both reactors fed original and 40 kGy irradiated wastewater (79 and 73 %). The reactor fed with preirradiated wastewater produced sludge with enhanced settling properties which provides a lower SVI value in comparison to original wastewater (~25 to 60 mL/g).

- Significant floc structure change was evident after phosphate buffer addition; protozoa and filamentous organisms were observed in flocs indicating healthier floc formation.

- GC/MS analysis performed on samples taken from influent and effluent of SBRs fed with raw and 40 kGy irradiated wastewaters (COD: 5,000 mg/L) showed that alkaloid compounds were fully disappeared after biological treatment, with only that thebain was relatively degraded in the former one. Complete removal of thebain could be achieved only with radiated wastewater.

- Fenton oxidation method as a means of pretreatment resulted in 44 ± 1 % COD removal efficiency with the application of 30 g/L H_2O_2 and 6 g/L Fe^{2+} concentrations. This indicated that to provide significant level of COD removal the amounts of required reagent dosages are very high and this is not an economical choice.

It was shown that opium alkaloid wastewater with a COD around 5,000 mg/L, which could be attained by anaerobic pre-treatment, can be successfully treated by aerobic SBR. Radiation application improves the settling characteristics and provides more compact sludge which is considered advantageous with regard to sludge handling.

CHAPTER 7

RECOMMENDATIONS

This study can be a contribution to the recently developing research field on radiation treatment of industrial wastewaters. Following recommendations can be offered for future research based on the results obtained from this study and the study performed as a part of the overall intention by Ozdemir et al. (2006):

It was shown in current study that under aerobic conditions, significant COD removal efficiency (79 ± 1 %) could be accomplished for alkaloid wastewater with initial COD concentration around 5 g/L. Effluent limits set in Turkish Water Pollution Control Regulation (Table 3.2) was able to be achieved. It is recommended that COD concentration should be decreased to this level by anaerobic means prior to aerobic treatment.

There are several benefits to the use of anaerobic treatment such as decrease in electrical power requirements, reduced sludge production, production of methane etc. Anaerobic and aerobic treatments both have their limits, but when they are combined, sequential treatment could provide several economical and operational advantages.

The results from the study by Özdemir et al. showed that anaerobic (UASB) treatment may degrade alkaloid wastewater with a COD removal efficiency of 80 % for initial COD concentration up to 19 g/L, without any other process modifications. As presented in section 5.1.2, treatment of this anaerobically pre-treated wastewaters in aerobic batch reactors provided 66 % further COD removal, effluent satisfying the discharge limits, as claimed. Therefore, this anaerobic treatment with 80 % removal efficiency could be justified as the first

step in treatment process. Moreover, he suggested that opium alkaloid wastewater is inhibitory to the anaerobic microorganisms for concentrations above certain threshold level. Wastewater over 19 g/L COD could not be treated under anaerobic environment. However, conducted BMP studies showed that radiation pretreatment was able to decrease the inhibitory effect of alkaloid wastewater at high initial COD concentration of 25 g/L (Özdemir et al., 2006).

Therefore, it is recommended to study irradiation/anaerobic/aerobic treatment in sequence. The radiation could decrease inhibition effect of alkaloid wastewater, and higher initial COD concentrations could be fed to anaerobic/aerobic system. It is also recommended to make a further research on the effect of irradiation and also fenton's oxidation as post-treatment alternatives. This would lower the required doses and provide enhanced wastewater treatment.

REFERENCES

Ahmadi, M., Vahabzadeh, F., Bonakdarpour, B., Mofarrah, E., Mehranian, M. (2005). "Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation." *Journal of Hazardous Materials*, 123, 187–195.

Al-Momani, F., Touraud, E., Degorce-Dumas, J. R., Roussy, J., Thomas, O. (2002). "Biodegradability enhancement of textile dyes and textile wastewater by VUV photolysis" *Journal of Photochemistry and Photobiology*, 153 (1-3), 191-197.

APHA. (1998). *Standard Methods for the Examination of Water and Wastewater.* 20th ed. American Public Health Association, Washington, D.C.

Arceivala, S. J. (1998). *Wastewater Treatment for Pollution Control.* 2nd ed. McGraw-Hill Publishing, New Delhi, India.

Aydın, A. F., Altınbaş, M., Sevimli, M. F., Öztürk, I., and Sarıkaya, H. Z. (2002). "Advanced treatment of high strength opium alkaloid industry effluents." *Water Science and Technology*, 46(9), 323-330.

Aydın, A. F. (2002). "Afyon alkaloidleri endüstrisi atıksularının biyolojik prosesler ve Fenton oksidasyonu ile ileri arıtımı," *Doctoral Thesis*, İstanbul Technical University, İstanbul.

Badawy, M. I., Ali, M. E. M. (2006). "Fenton's peroxidation and coagulation processes for the treatment of combined industrial and domestic wastewater." *Journal of Hazardous Materials*, 136, 961-966.

Bae, J., Kin, S., Chang, H. (1997). "Treatment of landfill leachates: ammonia removal via nitrification and denitrification and further COD reduction via Fenton's treatment followed by activated sludge." *Water Science and Technology*, 36, 341–348.

Bao, H., Liu, Y. and Jia, H., (2002). "A study of irradiation in the treatment of wastewater." *Radiation Physics and Chemistry,* 63, 633–635.

Barrera-Diaz, C., Urena-Nunez, F., Campos, E., Palomar-Paradave, M., Romero-Romo, M. (2003). "A combined electrochemical-irradiation treatment of highly colored and polluted industrial wastewater" *Radiation Physics and Chemistry,* 67, 657-663.

Bigda, R. J. (1995). “Consider Fenton' s chemistry for waste water treatment.” *Chemical Engineering Progress*, 91, 62-66.

Bitton G. (1994). *Wastewater Microbiology*. John Wiley and Sons Inc., New York.

Booth, Martin. (1996). *Opium: A History*. St. Martin's Press, London: Simon & Schuster, Ltd., chapter 1.

Casero, I., Sicilia, D., Rubio, S., and Perez-Bendito, D. (1997). “Chemical degradation of aromatic amines by Fenton’s reagent.” *Water Research*, 31, 1985–1995.

Chaudhuri, N., Engelbrecth, R. S. and Austin, J. H. (1965). “Nematodes in an aerobic waste treatment plant.” *Journal of the American Water Works Association*, 57, 1561.

Chamarro, E., Marco, A., and Esplugas, S. (2001). “Use of fenton reagent to improve organic chemical biodegradability.” *Water Research*, 35, 1047-1051.

Chan K.H., Chu, W. (2003). “Modeling the reaction kinetics of Fenton_s process on the removal of atrazine.” *Chemosphere*, 51, 305–311.

Cheremisinoff, N.P. (1996). *Biotechnology for Waste and Wastewater Treatment*. Noyes publications, Westwood, New Jersey, USA.

Christensen, M. H., and Harremoes, P. (1978). “Nitrification and denitrification in wastewater treatment”, 391 – 414, in: *Water Pollution Microbiology*, Vol. 2, R. Mitchell, Ed. Wiley, New York.

Chudoba, J. (1989). Activated sludge - Bulking control, 171-202, in: *Encyclopedia of Environmental Control Technology*, Vol 3, Wastewater Treatment Technology, P.N. Cheremisinoff, Ed. Gulf Publishing Co., Houston, TX.

Costerson, J. W., Irvin, R. T. and Cheng K. J. (1981). “The bacterial glycoxalyx in nature and disease.” *Annual Review of Microbiology*, 35, 299-324.

Curds, C. R. (1973). “The role of protozoa in the activated sludge process.” *American. Zoologist*, 13, 161.

Curds, C. R. (1975), Protozoa, in ecological aspects of used water treatment: the organisms and their ecology, Curds, C.R., and Hawkes, H.A., Eds., Academic Press, New York, chap. 5.

Curds, C. R., Cockburn, A., and Vandyke, J.M. (1968), An experimental study of the role of the ciliated protozoa in the activated sludge process, *Water Pollution Control*, 67, 312.

Curds, C. R. and Vandyke, J. M. (1966). "The feeding habits and growth rates of some freshwater ciliates found in activated sludge plants." *Journal of Applied Ecology*, 3, 127-37.

Curds, C. R. and Fey, G.J. (1969), The effect of ciliated protozoa on the fate of escherichia coli in the activated sludge process, *Water Research*, 3, 853.

De Souza, D. R., Duarte, E. T. F. M., Girardi, G. D., Velani, V., Machado, A. E. D., Sattler, C., de Oliveira, L., de Miranda, J. A. (2006). "Study of kinetic parameters related to the degradation of an industrial effluent using Fenton-like reactions." *Journal of Photochemistry and Photobiology, A,* 179(3), 269-275.

Doohan, M. (1975). Rotifera. In Ecological Aspects of Used Water Treatment, Vol. 1. The Organisms and their Ecology (eds. C.R. Curds and H.A. Hawkes), pp 289-304. Academic Press, London.

Drzewicz, P., Bojanowska-Czajka, A. Trojanowicz, M., Nalecz-Jawecki, G., Sawicki, J., Wolkowicz, S. (2003). "Application of ionizing radiation for degradation of organic pollutants in water and wastes." *Polish Journal of Applied Chemistry*, 47(3), 127-136.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). "Colorimetric Method for the Determination of Sugar and Related Substances." *Analytical Chem*istry, 28, 350-356.

Eckenfelder, W. W., Musterman J. L. (1995). *Activated Sludge Treatment Of Industrial Wastewater*. Technomic Publishing Co, US.

EPA. (1999). "Wastewater Technology Fact Sheet: Sequencing Batch Reactors." *U.S. Environmental Protection Agency*, 832-F-99-073.

Facchini P.J., Park, S.-U. (2003). "Developmental and inducible accumulation of gene transcripts involved in alkaloid biosynthesis in opium poppy." *Phytochemistry*, 64, 177–186.

Fang, X., Wu, J. (1999). "Some remarks on applying radiation technology combined with other methods to the treatment of industrial wastes – Technical Note." *Radiation Physics and Chemistry*, 55, 465-468.

Farabegoli G., Carucci A., Majone M. and Rolle E. (2004). "Biological treatment of tannery wastewater in the presence of chromium." *Journal of Environmental Management*, 71, 345-349.

Fenton, H. J. H. (1894). "Oxidation of tartaric acid in presence of iron." *Journal of the Chemical Society*, 65, 899-910.

Franta, J. R., Wilderer, P. A. (1997). "Biological treatment of papermill wastewater by SBR technology to reduce residual organics." *Water Science and Technology*, 35, 129–136.

Frick, S., Kramell, R., and Kutchan, T.M. (2007). "Metabolic engineering with a morphine biosynthetic P450 in opium poppy surpasses breeding." *Metabolic Engineering*, 9, 169–176.

Gaudy, A. F. and Gaudy, E. T. (1980). *Microbiology for Environmental Scientists and Engineers*. McGraw-Hill Inc., USA.

Ganczarczyk, J. J. (1983). *Activated sludge process: theory and practice*. Marcel Dekker, New York, N.Y.

Gecin, G. and Hakbilen, S. (2005). "Opium Poppy's income to Turkey $60 million."Available online at: http://www.zaman.com.tr/haber.do?haberno=177842 Last accessed date: 24/07/2008.

Getoff, N. (1999). "Radiation chemistry and the environment." *Radiation Physics and Chemistry,* 54, 377-384.

Gray, N. F. (2004). *Biology of wastewater treatment*. 2nd Ed. Imperial College press, London, 1421p.

Guedes, A. M. F. M., Madeira, L. M. P., Boaventura, R. A. R., Costa, C. A. V. (2003). "Fenton oxidation of cork cooking wastewater-overall kinetic analysis." *Water Research*, 37, 3061-3069.

Haber, F. and Weiss, J. (1934). 'The catalytic decomposition hydrogen peroxide by iron salts.' *Proceedings of the Royal Society A*, 134, 332–351.

HACH. (1997). *Hach Water Analysis Handbook*. 3rd ed. Hach Company, Loveland, CO., USA.

Hawkes, H.A. (1983). Activated sludge. In: *Ecological Aspects of Used Water Treatment*, Vol. 1, C.R. Curds and H.A. Hawkes, Eds. Academic , London.

Higgins, M. J., Novak, J. T. (1997). "Characterization of exocellular protein and its role in bioflocculation." *Journal of Environmental Engineering*, 123(5), 479-485.

Huh, I. R., Kim, Y. K., and Lee, C. K. (1996). "Removals of refractory organic and color in improperly managed landfill leachate by Fenton oxidation." *Journal of Korean Society of Environmental Engineering*, 18, 43-54.

INCB (2008). *Narcotic Drugs Technical Reports: Estimated World Requirements for 2008 - Statistics for 2006*. United Nations, New York.

Jenkins, D., Richard, M. G., and Daigger, G. T. (2004). *Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems*. 3rd edt. Lewis Publishers Inc., Boca Raton, LA.

Jo, H. J., Lee, S. M., Kim, H. J., Kim, J. G., Choi, J. S., Park, Y. K., Jung, J. (2006). "Improvement of biodegradability of industrial wastewaters by radiation treatment." *Journal of Radioanalytical and Nuclear Chemistry*, 268, 145-150.

Kaçar, Y., Alpay, E., Ceylan, V. K. (2003). "Pretreatment of Afyon alcaloide factory's wastewater by wet air oxidation (WAO)." *Water Research*, 37, 1170-1176.

Kang, S. F., Chang, H. M. (1997). "Coagulation of textile secondary effluents with Fenton's reagent." *Water Science and Technology*, 36, 215–222.

Kang, Y. W., Cho, M. J., Hwang, K. Y. (1999). "Correction of hydrogen peroxide intereference on standard chemical oxygen demand test." *Water Research*, 33(5), 1247-1251.

Kang, S. F., Liao, C. H., Chen, M. C. (2002). "Pre-oxidation and coagulation of textile wastewater by the Fenton process." *Chemosphere*, 46, 923–928.

Kınlı, H. (1994). "Treatability studies of biological wastewater treatment plant effluent of TMO Opium Alkaloids Factory." Marmara Research Center, TUBITAK, Gebze-Kocaeli, Turkiye.

Kim, Y. O., Nam, H. U., Park, Y. R., Lee, J. H., Park, T. J., Lee, T. H. (2004). "Fenton oxidation process control using oxidation-reduction potential measurement for pigment wastewater treatment." *Korean Journal of Chemical Engineering*, 21, 801-805.

Kitiş, M., Adams, C. D., Daigger, G. (1999). "The effects of Fenton's reagent pretreatment on the biodegradability of nonionic surfactants." *Water Research*, 33, 2561-2568.

Koyuncu, I. (2003). "An advanced treatment of high-strength opium alkaloid processing industry wastewaters with membrane technology: pretreatment, fouling and retention characteristics of membranes." *Desalination*, 155, 265-275.

Kuo, W. K. (1992). "Decolorizing dye wastewater with Fenton's reagent." *Water Research*, 26, 881-886.

Liang, S., Palencia, L. S., Yates, R., Davis, M. K., Bruno, J. M., Wolfe, R. L. (1999). "Oxidation of MTBE by ozone and peroxone processes." *Journal AWWA*, 91, 104-114.

Liu, D. H. F. and Lipták, B. G. (1997). *Environmental Engineers' Handbook*. 2nd Ed., CRC Press Inc / Lewis Publishers, Boca Raton, Florida.

Lopez, A., Pagano, M., Volpe, A., Di Pinto, A. C. (2004). "Fenton's pre-treatment of mature landfill leachate." *Chemosphere*, 54, 1005-1010.

Martinez, N. S. S., Fernandez, J. F., Segura, X. F., Ferrer, A. S. (2003). "Pre-oxidation of an extremely polluted industrial wastewater by Fenton's reagent" *Journal of Hazardous Materials*, B101, 315-322.

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

Melo, R., Verde, S. C., Branco, J., Botelho, M. L. (2008). "Gamma radiation induced effects on slaughterhouse wastewater treatment." *Radiation Physics and Chemistry*, 77, 98-100.

Metcalf and Eddy. (1991). *Wastewater Engineering, Treatment, Disposal, Reuse*. 3rd Ed., Mc Graw Hill International Editions, New York.

Mohan, S. V., Rao, N. C., Prasad, K. K., Madhavi, B. T. V., Sharma, P. N. (2005), "Treatment of complex chemical wastewater in a sequencing batch reactor (SBR) with an aerobic suspended growth configuration." *Process Biochemistry*, 40, 1501-1508.

Novak, L., Larrea, L., Wanner, J., and Garcia-Heras, J. L. (1993). "Non-filmentous activated sludge bulking in a laboratory scale system." *Water Research* 27(8), pp. 1339-1346.

Özdemir, R. T. (2006). "Anaerobic treatment of opium alkaloid wastewater and effect of gamma-rays on anaerobic treatment." *Master Thesis*, Middle East Technical University, Ankara

Painter, H. A. (1970). “A review of literature on inorganic nitrogen metabolism in microorganisms.” *Water Research*, 4, 393-450.

Painter, H. A. and Loveless, J. E. (1983). Effect of temperature and pH value on the growth-rate constants of nitrifying bacteria in the activated sludge process. *Water Research*, 17, 237-248.

Pankratz, T. M. (2001). *Environmental Engineering Dictionary & Directory*. CRC Press Inc / Lewis Publishers, Boca Raton, Florida.

Peres, J. A., Beltran de Heredia, J., Dominguez, J. R. (2004). “Integrated Fenton’s reagent-coagulation/ flocculation process for the treatment of cork processing wastewaters.” *Journal of Hazardous Materials*, 107, 115–21.

Pérez, M., Torrades, F., Domènech, X. (2002). “Fenton and photo-Fenton oxidation of textile effluents.” *Water Research*, 36, 2703-2710.

Pike, E. B. and Curds, C. R. (1971). The microbial ecology of the activated sludge system. In G. Sykes and F.A. Skinner, Eds. Microbial Aspects of Pollution. London: Academic Press, pp. 123-147.

Rittman, B. E. and McCarty P. L. (2001). *Environmental Biotechnology: Principles and Applications*. USA: McGraw-Hill International Editions, Inc.

Rivas, F. J., Beltrán, F. J., Gimeno, O., and Alvarez, P. (2003). “Treatment of brines by combined Fenton’s reagent–aerobic biodegradation: II. Process modelling.” *Journal of Hazardous Materials*, 96, 259–276.

Safarzadeh-Amiri, A., Bolton, J.R., Cater, S.R. (1996). “The use of iron in advanced oxidation processes.” *Journal of Advanced Oxidation Technologies*, 1, 18–26.

Sarıkaya, H. Z., Özturk, I., Sevimli, M. F. and Aydın, A. F. (1998). “The report on rehabilitation of wastewater treatment plant of TMO Bolvadin Opium Alkaloids Plant and advanced treatment studies of treatment plant effluents.” Progress report No’s. 3-4, Department of Environmental Engineering, Istanbul Technical University, Turkiye.

Schiemer, F. (1975). Nematoda. In Ecological Aspects of Used Water Treatment, Vol. 1. (eds. C.R. Curds and H.A. Hawkes), pp 269-88. Academic Press, London.

Schiff, P. (2002). “Opium and its alkaloids.” *The American Journal of Pharmaceutical Education*, 66, 186–194.

Schrank, S. G., José, H. J., Moreira, R. F. P. M., Schröder, H. Fr. (2005). "Applicability of Fenton and H_2O_2/UV reactions in the treatment of tannery wastewaters." *Chemosphere*, 60, 644-655.

Sevimli, M. F., Aydın, A. F., Öztürk, I., and Sarıkaya, H. Z. (2000). "Evaluation of the alternative treatment processes to upgrade an opium alkaloid wastewater treatment plant." *Water Science and Technology*, 41, 223-230.

Sevimli, M. F., Aydın, A. F., Sarikaya, H. Z., and Öztürk, I. (1999). "Characterization and treatment of effluent from opium alkaloid processing wastewater." *Water Science and Technology*, 40(1), 23-30.

Sezgin, M., Jenkins D. and Parker D. S. (1978). "A Unified Theory Activated Sludge Bulking." *Journal of Water Pollution Control Federation,* 50, 362-381.

Shin, H.-S., Lee, S.-M., Seo, I.-S., Kim, G.-O., Song, J.-S. (1998). "Pilot-scale SBR and MF operation for the removal of organic and nitrogen compounds from greywater." *Water Science and Technology*, 38, 79-88.

Talınlı, I. and Anderson, G. K. (1992). "Interference of hydrogen peroxide on the standard COD test." *Water Research*, 26, 107-110.

Tang, W. Z., Huang, C. P. (1997). "Stoichiometry of Fenton's reagent in the oxidation of chlorinated aliphatic organic pollutants." *Environmental Technology*, 18, 13-23.

Tang, W. Z., Tassos, S. (1997), "Oxidation kinetics and mechanisms of trihalomethanes by Fenton's reagent." *Water Research,* 31, 1117-1125.

Tekin, H., Bilkay, O., Ataberk, S. S., Balta, T. H., Çeribaşı, I. H., Sanin, F. D., Dilek, F. B., Yetiş, Ü. (2006). "Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater." *Journal of Hazardous Materials*, 136, 258-265.

TMO. (2005). *2004 Yılı Haşhaş Raporu*. Ankara.

Tzedakis, T., Salvall, A. and Clifton M. J. J. (1989). "The electrochemical regeneration of Fenton's reagent in the hydroxylation of aromatic substrates: batch and continuous processes." *Journal of Applied Electrochemistry,* 19, 911-921.

U.S.EPA. (1975). *Process Design Manual for Nitrogen Control*. Office of Technology Transfer, Washington, D.C.

Walling, C. (1975). "Fenton's reagent revisited." *Accounts of Chemical Research*, 8, 125–131.

Wanner, J. (1994). *Activated sludge bulking and foaming control.* Technomic Publishing Company Inc., Lancaster, Pa.

WHO. (2007). "Model List of Essential Medicines." 15th Ed, Available online at: http://www.who.int/medicines/publications/EML15.pdf Last accessed date: 30/05/2007.

Wilderer, P. A., Irvine, R. L. and Goronszy, M. C. (2001). *Sequencing Batch Reactor Technology.* IWA Publishing, London.

Woods, R. J. (1998). "Radiation Chemistry and its application to Environmental Pollution." In *Environmental Applications of Ionizing radiation.* Ed. Cooper, W. J et al. John Wiley & Sons Inc.

Woods, R. J. and Pikaev, A. K. (1993). *Applied Radiation Chemistry: Radiation processing*. John Wiley & Sons Inc.

Ye, K., Ke, Y., Keshava, N., Shanks, J., Kapp, J. A., Tekmal, R. R., Petros, J., and Joshi, H. C. (1998). "Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells." *The Proceedings of the National Academy of Sciences U S A*, 95, 1601–1606.

APPENDIX A

SAMPLE CALCULATION FOR NUTRIENT STOCK SOLUTION PREPARATION

Alkaloid COD = 33,000 mg/L

Volume of reactor = 2,000 mL

Scheduled COD concentration in the reactor = 2,000 mg/L

Scheduled COD:N:P ratio = 100:5:2

Nitrogen Requirement = 100 mg/L

Phosphorus Requirement = 40 mg/L

Stock solutions are prepared so that 20 mL solution will be fed to 2,000 mL reactor

For Nitrogen: $C_1 * V_1 = C_2 * V_2$ (A-1)

where; C_1 = Desired nitrogen concentration in stock solution

V_1 = Volume of nitrogen stock solution to be fed to reactor

C_2 = Desired nitrogen concentration in reactor

V_2 = Volume of the reactor

$$C_1 * 20 \text{ mL} = 100 \text{ mg/L} * 2{,}000 \text{ mL}$$

$$C_1 = 10 \text{ g Nitrogen /L}$$

Ammonium chloride (NH_4Cl) was used as nitrogen source:

MW_{NH4Cl} = 53,5 g. MW_N = 14 g.

53,5 g NH_4Cl	14 g N
X	10 g N

$$\Rightarrow X_{N\text{-}STOCK} = 38{,}2 \text{ g } NH_4Cl / L$$

For Phosphorus:

Calculations were made so that concentration of K_2HPO_4 is two times of KH_2PO_4.

$X_{P\text{-}STOCK} = 13{,}7 \text{ g } K_2HPO_4 + 6{,}85 \text{ g } KH_2PO_4 \text{ / L}$

APPENDIX B

COD DEGRADATION PROFILE IN PRELIMINARY AEROBIC BATCH REACTOR EXPERIMENS

Table B.1. COD degradation profile for initial COD concentrations 2,500, 4,500, 7,000 mg/L.

Batch Run 1	**COD (mg/L) ***			
Time (days)	**Peptone**	**2500**	**4500**	**7000**
0	4104	2520	4473	6840
0.75	2860	1572	2625	7410
1	2250	1275	2325	8560
1.73	1970	1180	2058	8090
1.9	1200	973	1567	5130
2.64	1248	920	1279	3535
2.88	1110	825	739	3002
3.9	777	770	1048	1788
4.7	715	549	698	1178
4.85	577	685	644	778
5.65	350	353	410	1229
5.82	440	463	560	1159
6.85	512	567	488	1072
7.85	449	327	452	1011
11.17	487	246	429	1002

* Values given are the averages of duplicate measurements.

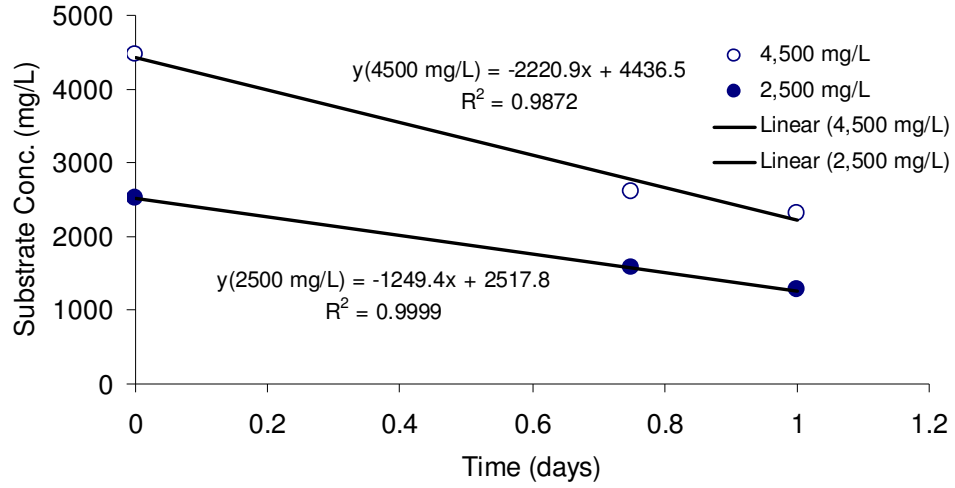
Table B.2. COD degradation profile for initial COD concentrations 18,000 and 26,000 mg/L.

Batch Run 2	**COD (mg/L) ***	
Time (days)	**18000**	**26000**
0	17580	26040
0.92	17950	27960
1.75	15190	27075
2.85	12620	23925
3.85	7565	19420
4.93	6015	14800
5.85	4860	13840
6.93	7160	8200
7.93	4785	7980
8.9	5090	5575
9.73	3762	-
10.8	3072	4200
11.78	2550	4150
12.83	1905	4470
15.1	2478	4350
16.94	2940	4845
18.07	2760	3400
19	2250	3300
20.13	2960	4300

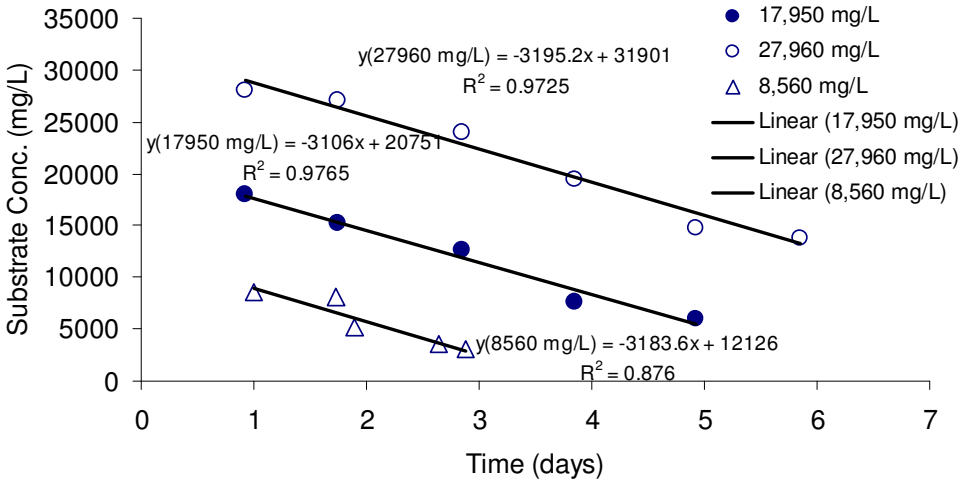
* Values given are the averages of duplicate measurements.

APPENDIX C

GRAPHS USED FOR DETERMINATION OF q, q_{max}, K_s



(a)



(b)

Figure C.1. Plots of substrate concentration vs. time to determine dS/dt values in Equation (2-7). (a) Plots for first batch run (b) Plots for second batch run.

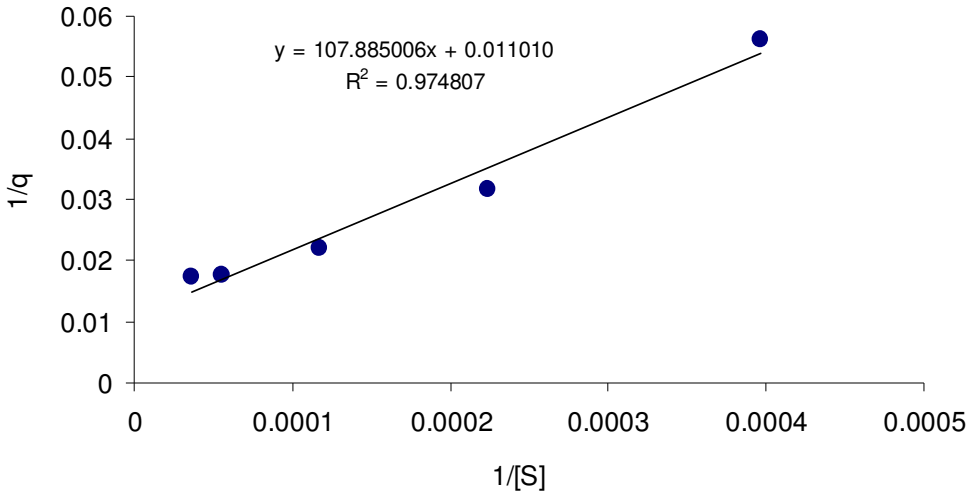


Figure C.2. Lineweaver-Burk plot to find K_s and q_{max}.

Table C.1. The values of q_{max} and K_s found by different models.

Model	Lineweaver-Burk	Nonlinear Regression by SigmaPlot
q_{max} (gCOD/gVSS.d)	91	74
K_s (mg/L)	9799	6135

* These values may not be compared with literature values because instead of data from exponential growth phase, initial seed VSS were used in calculation.

APPENDIX D

CHROMATOGRAMS OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY

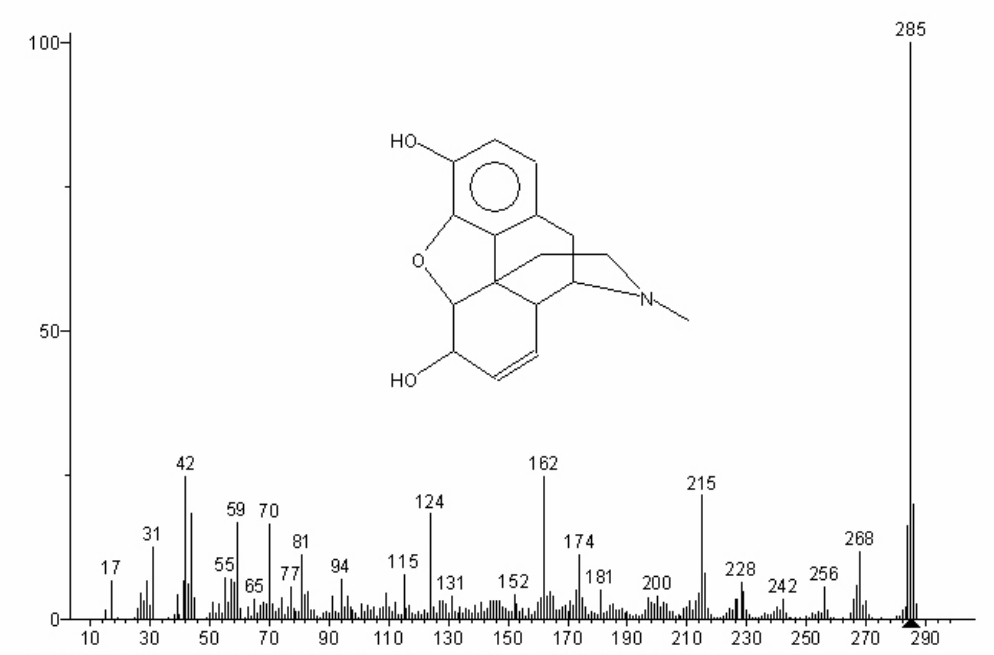


Figure D.1. Molecular structure and mass spectrum of morphine from instrument library. Chemical formula: $C_{17}H_{19}NO_3$; M.W. = 285.

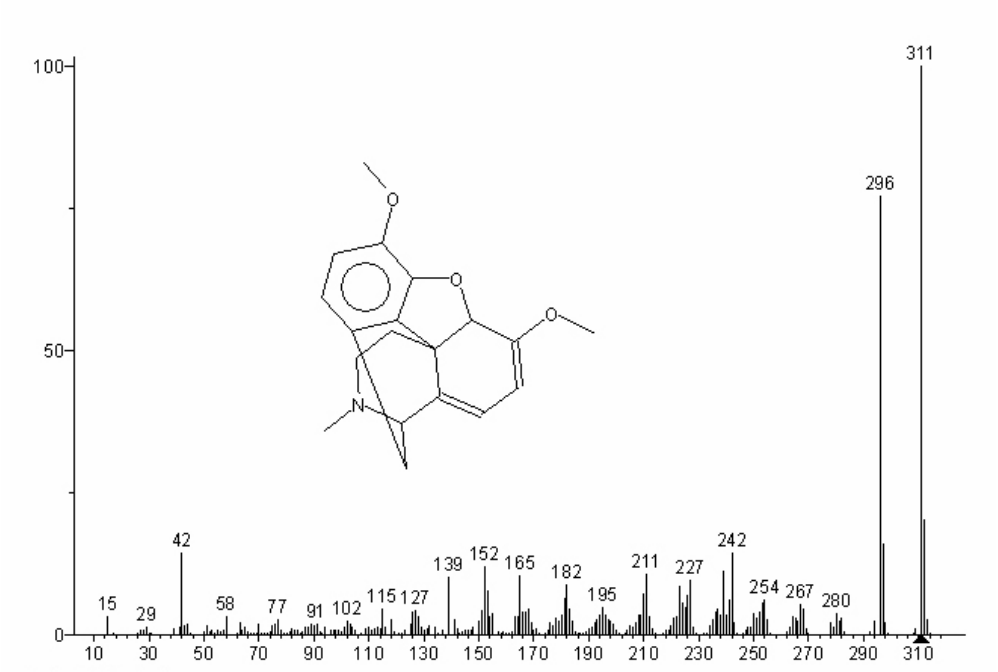


Figure D.2. Molecular structure and mass spectrum of thebaine from instrument library. Chemical formula: $C_{19}H_{21}NO_3$; M.W. = 311.

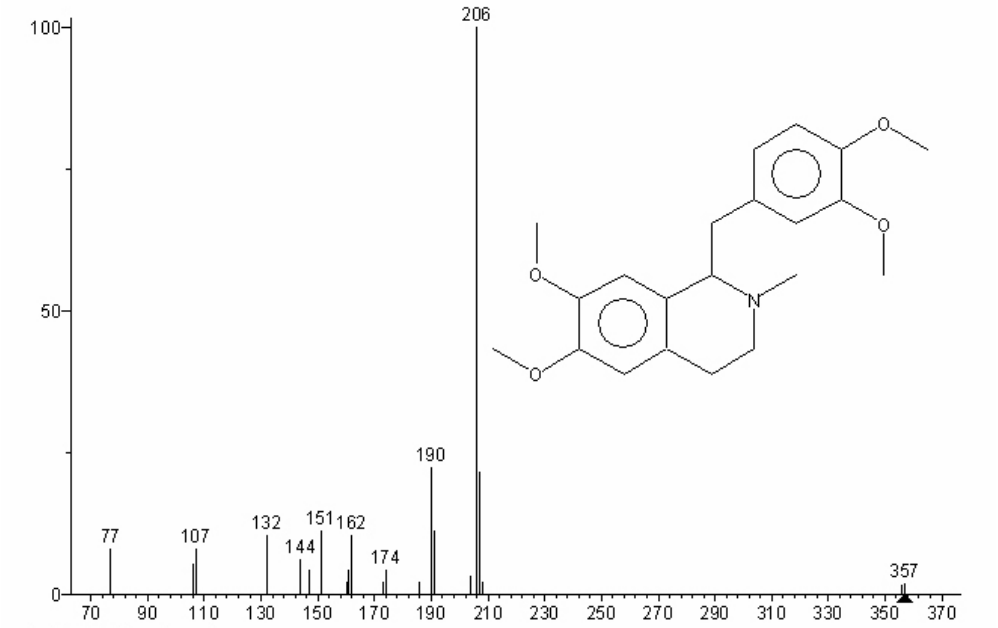


Figure D.3. Molecular structure and mass spectrum of laudanosine from instrument library. Chemical formula: $C_{21}H_{27}NO_4$; M.W. = 357.

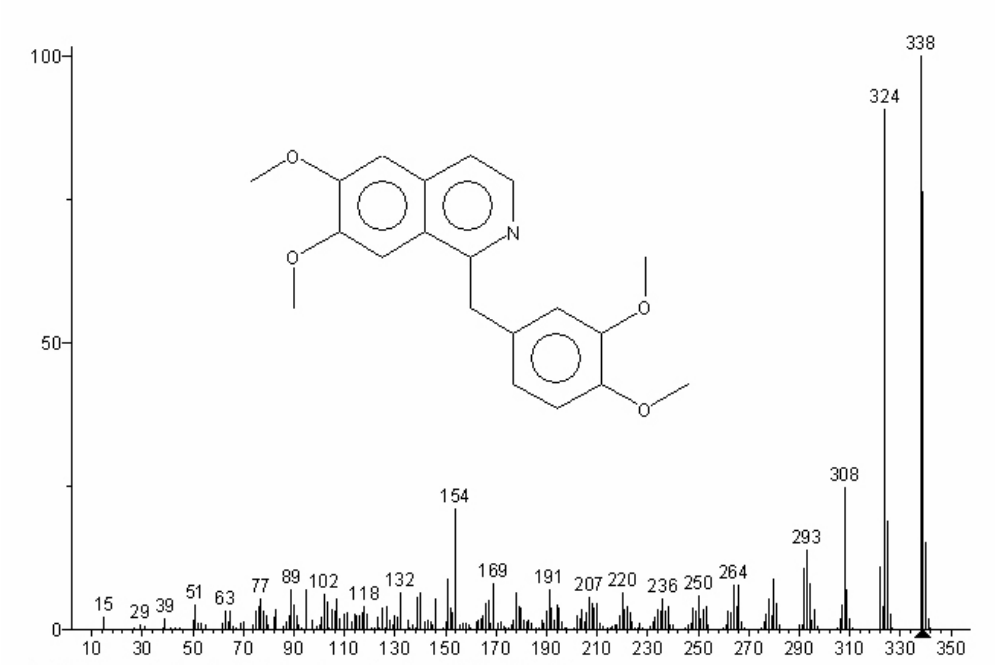


Figure D.4. Molecular structure and mass spectrum of papaverine from instrument library. Chemical formula: $C_{20}H_{21}NO_4$; M.W. = 339.

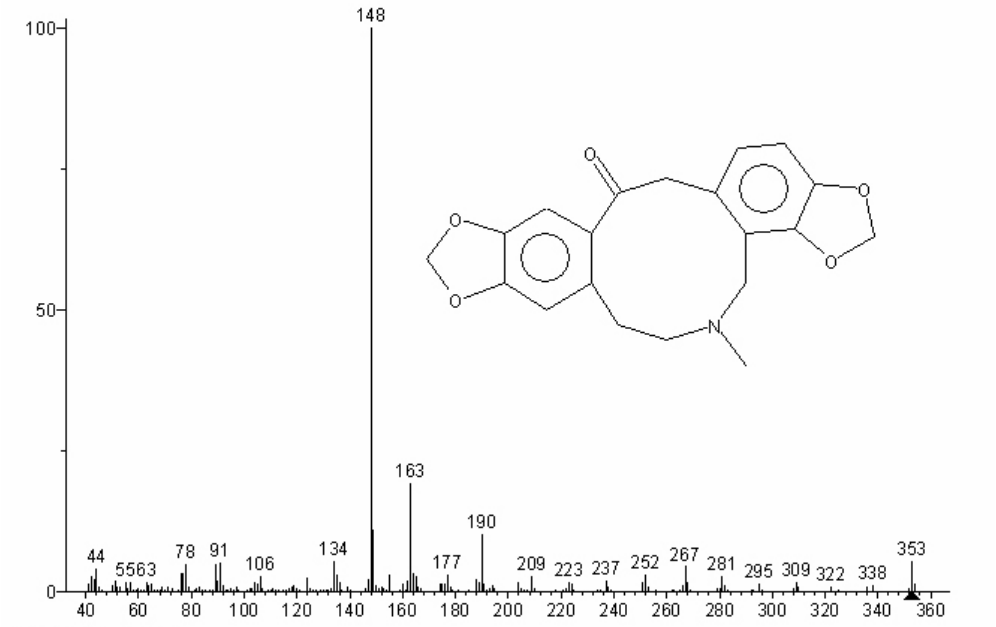


Figure D.5. Molecular structure and mass spectrum of protopine from instrument library. Chemical formula: $C_{20}H_{19}NO_5$; M.W. = 353.

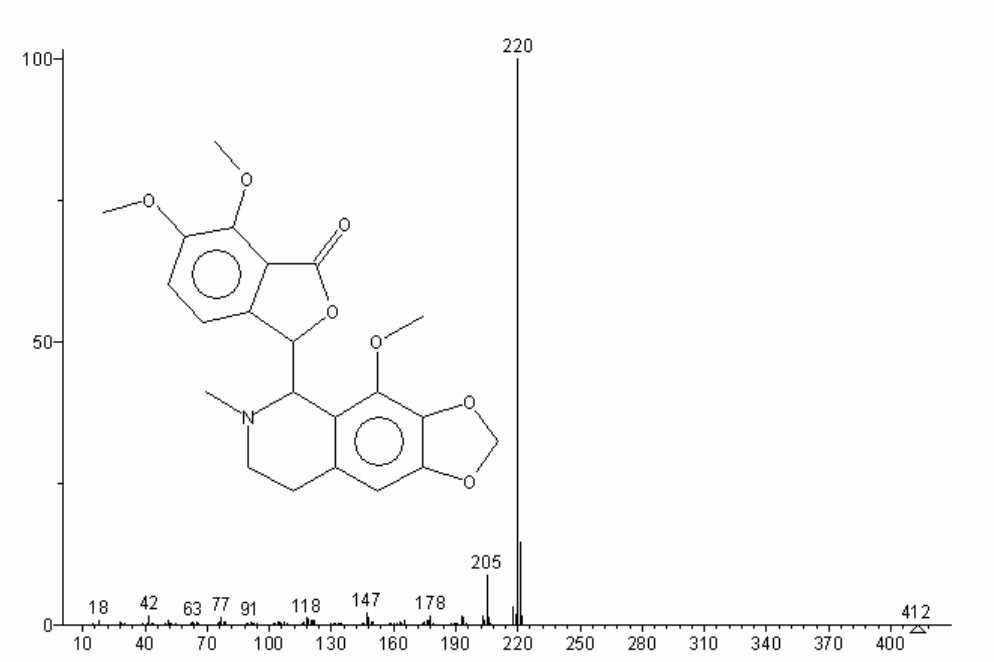


Figure D.6. Molecular structure and mass spectrum of noscapine from instrument library. Chemical formula: $C_{22}H_{23}NO_7$; M.W. = 413.

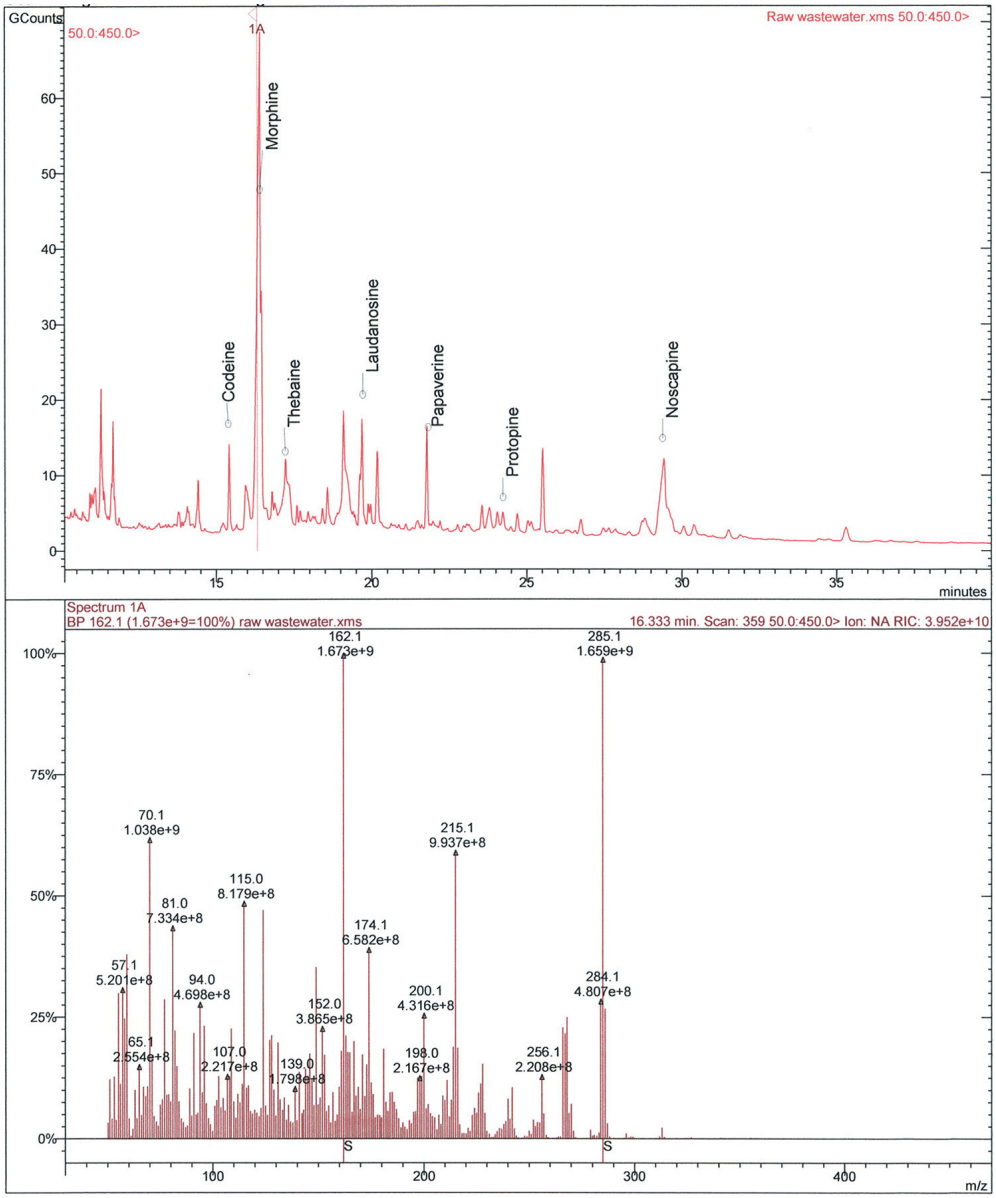


Figure D.7. GC Chromatogram of raw wastewater and Mass Spectra for morphine identified in wastewater.

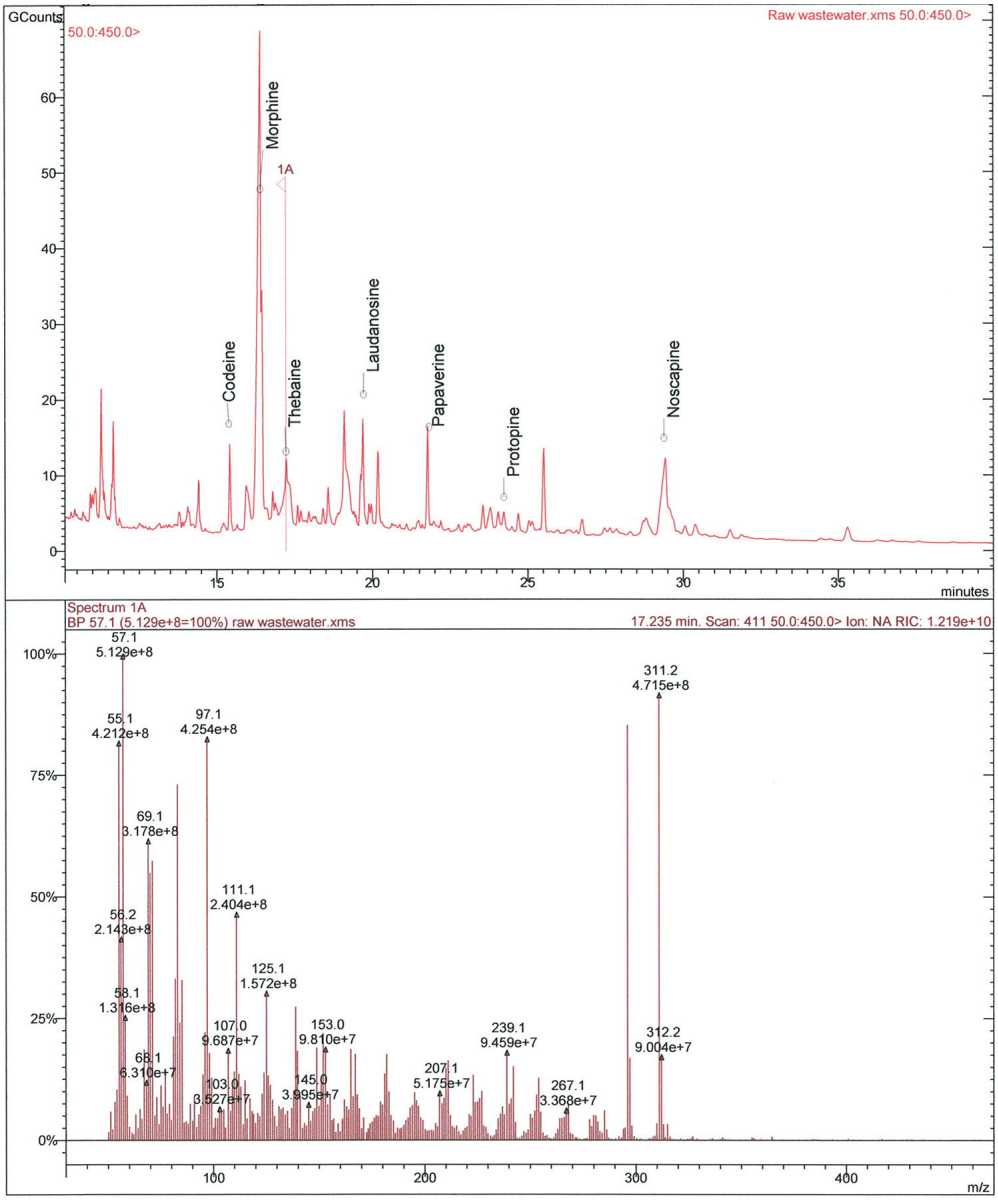


Figure D.8. GC Chromatogram of raw wastewater and Mass Spectra for thebain identified in wastewater.

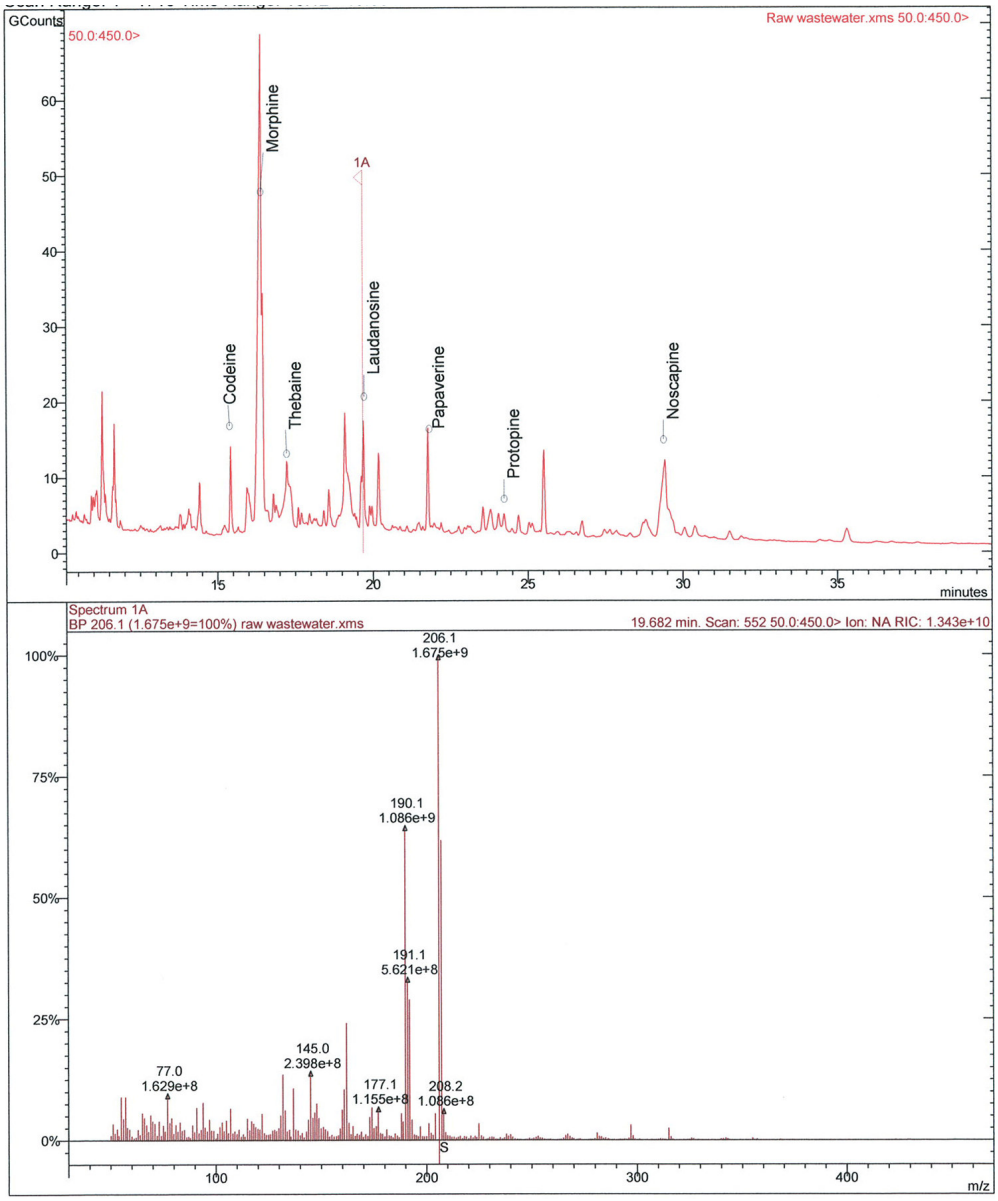


Figure D.9. GC Chromatogram of raw wastewater and Mass Spectra for laudanosine identified in wastewater.

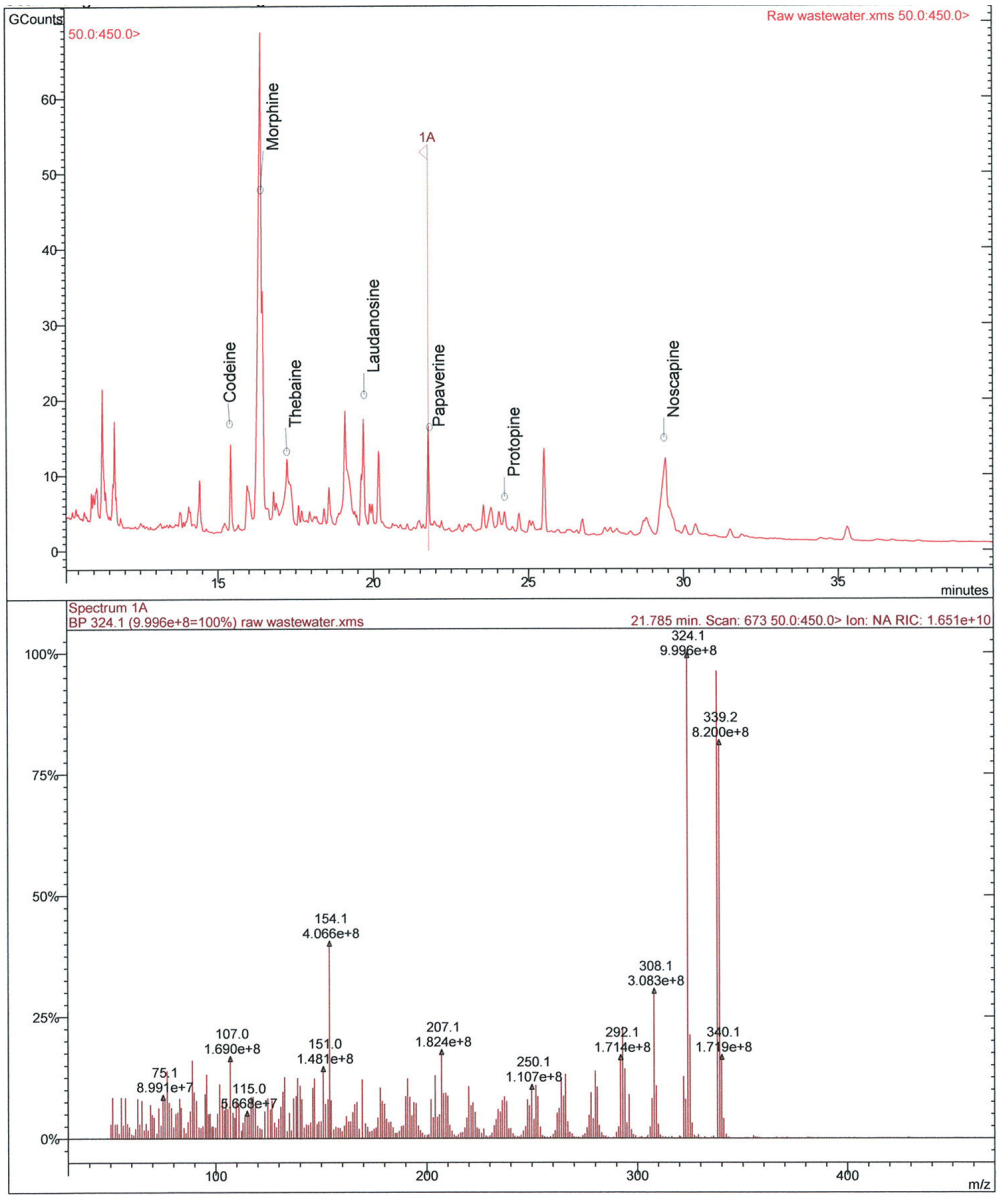


Figure D.10. GC Chromatogram of raw wastewater and Mass Spectra for papaverine identified in wastewater.

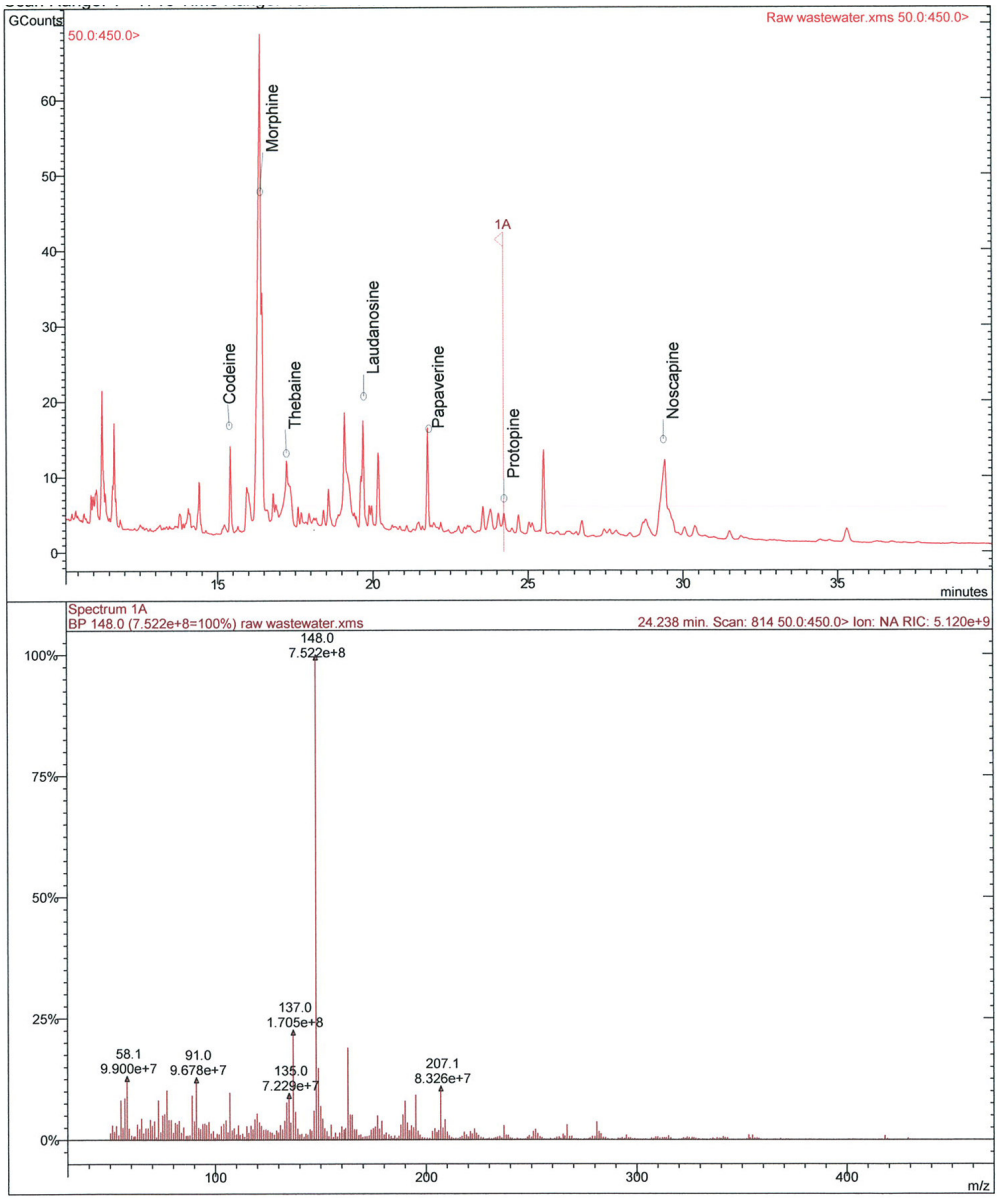


Figure D.11. GC Chromatogram of raw wastewater and Mass Spectra for protopine identified in wastewater.

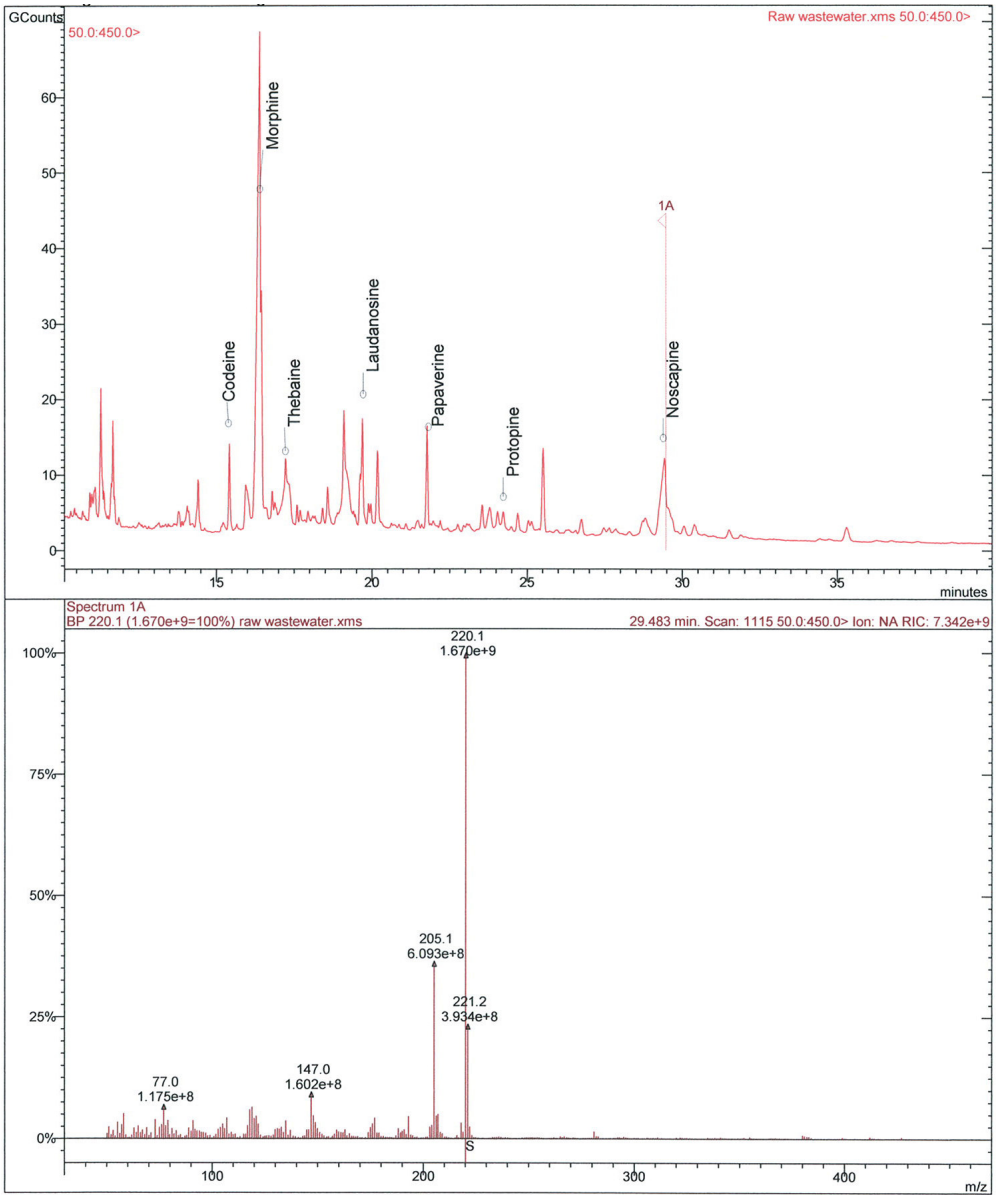


Figure D.12. GC Chromatogram of raw wastewater and Mass Spectra for noscapine identified in wastewater.

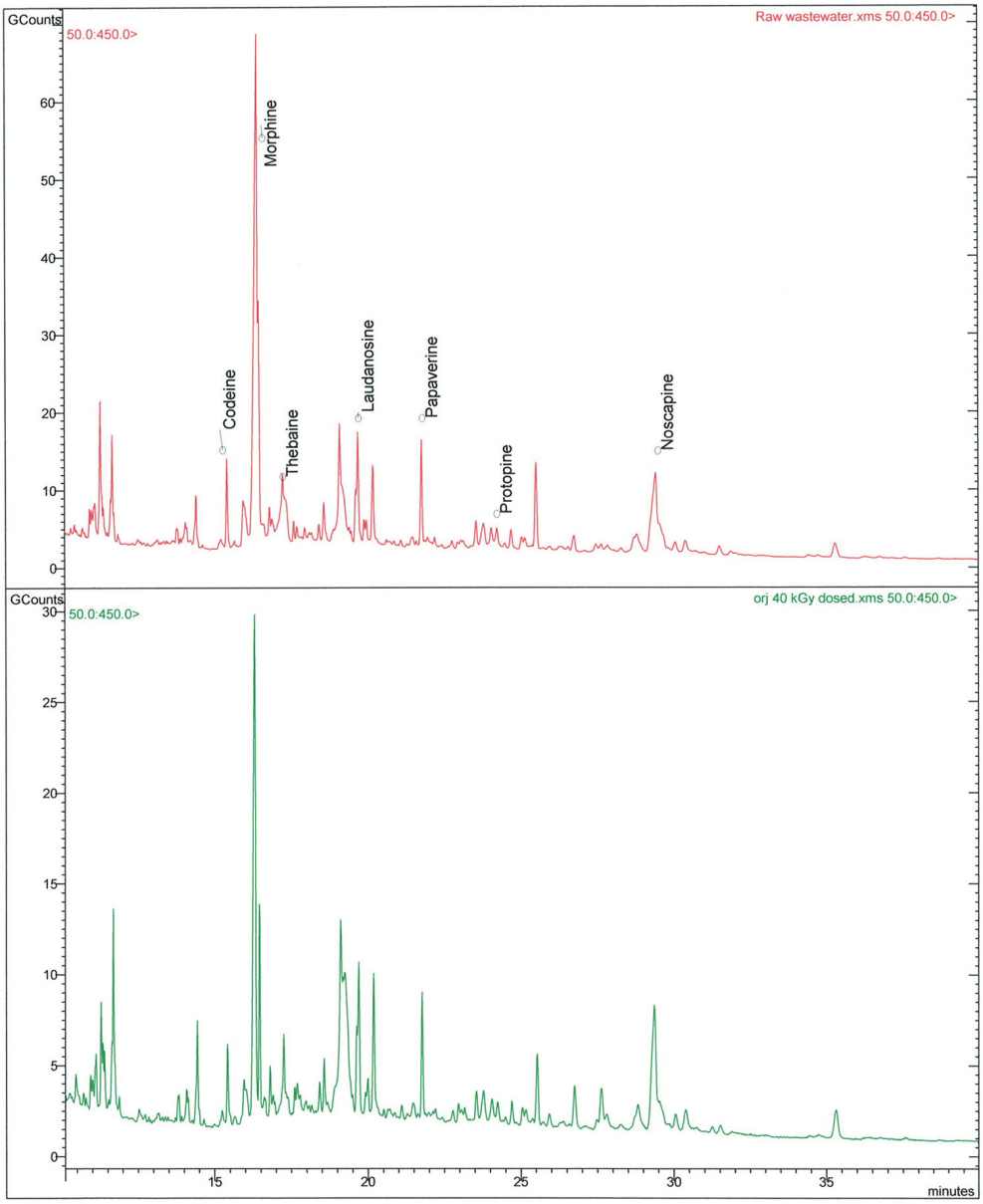


Figure D.13. Chromatogram plots for raw and 40 kGy irradiated wastewater.

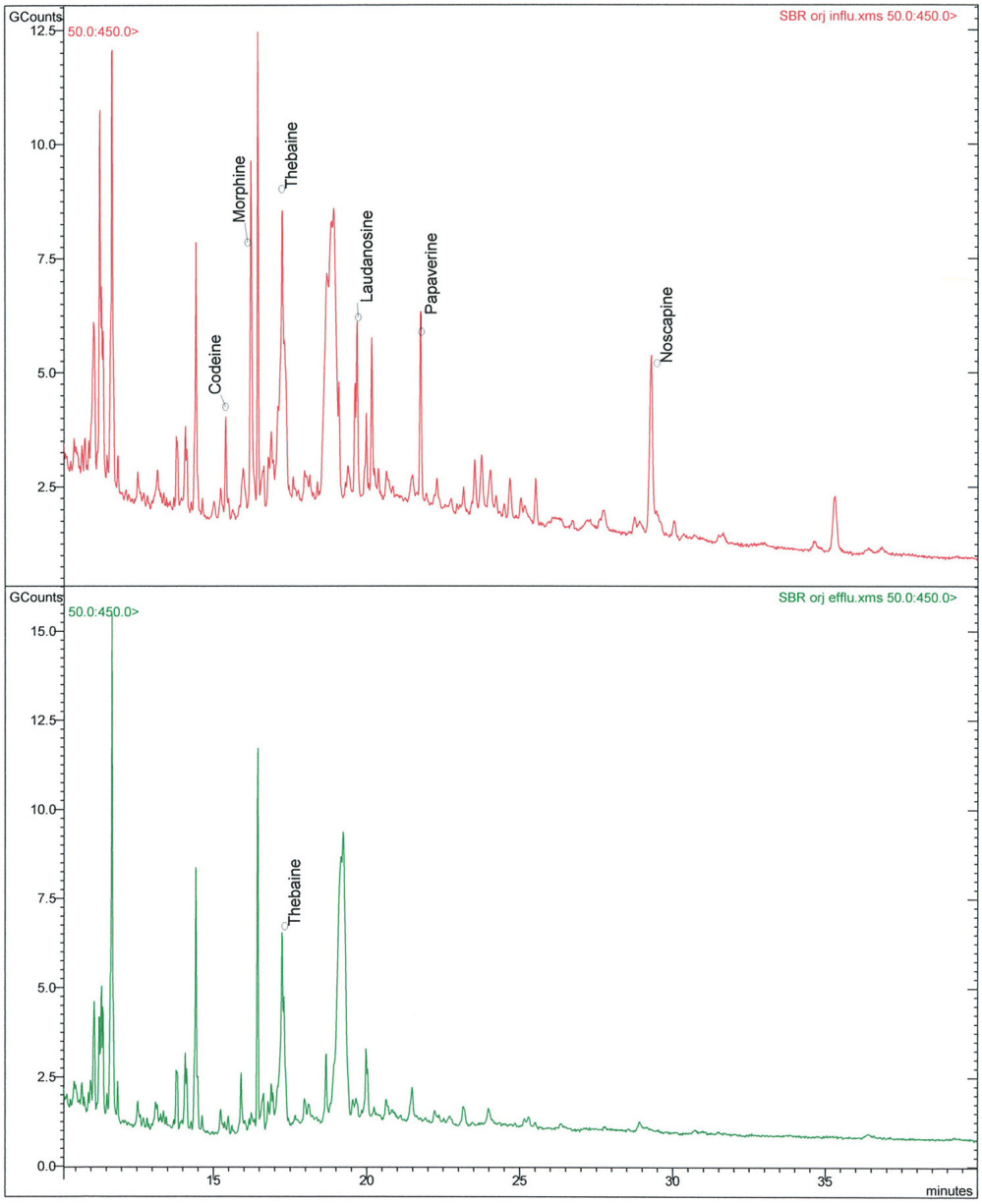


Figure D.14. GC chromatogram of samples taken from influent and effluent of SBR fed by original wastewater (influent COD: 5,000 mg/L).

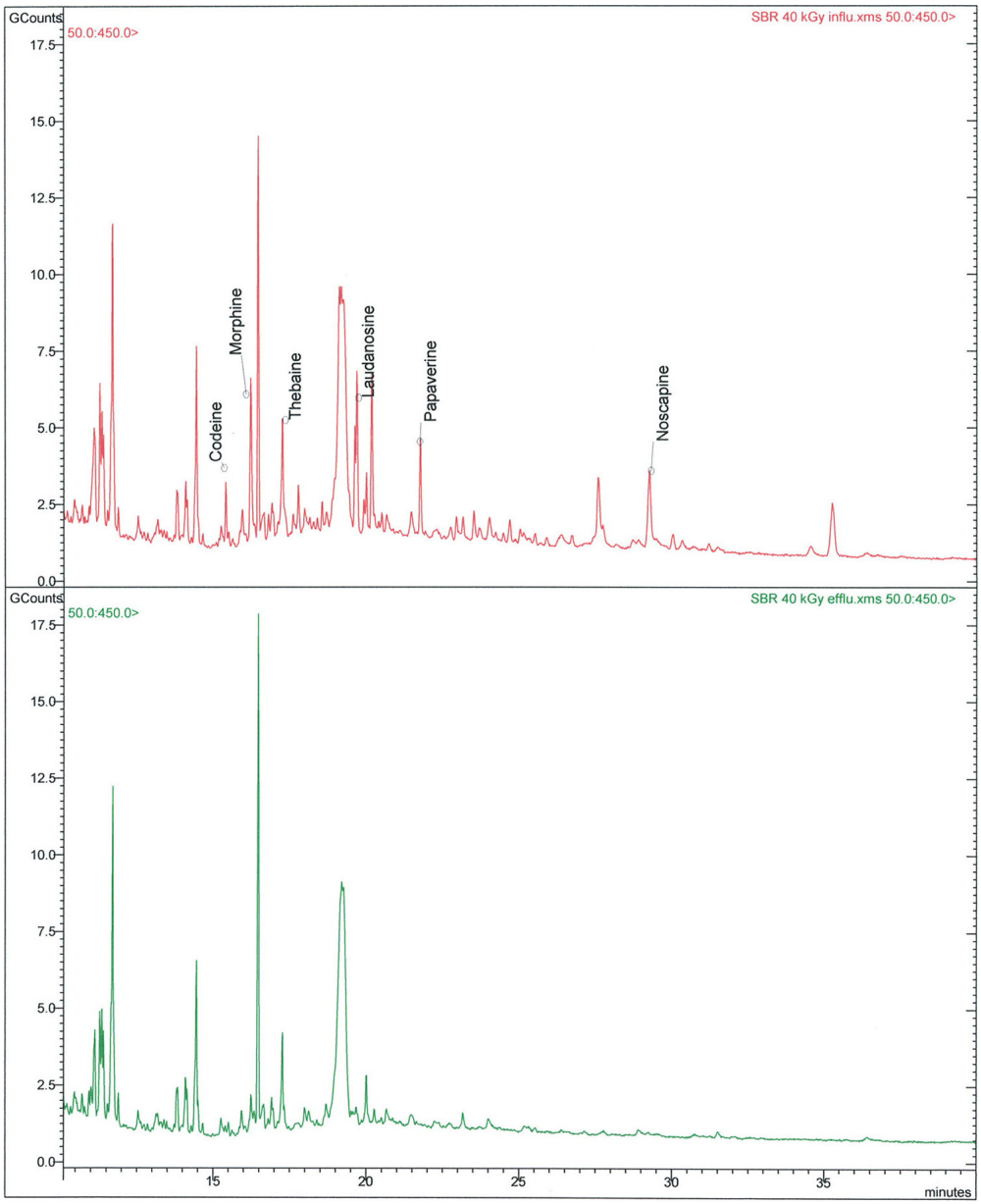


Figure D.15. GC chromatogram of samples taken from influent and effluent of SBR fed by 40 kGy irradiated wastewater (influent COD: 5,000 mg/L).

APPENDIX E

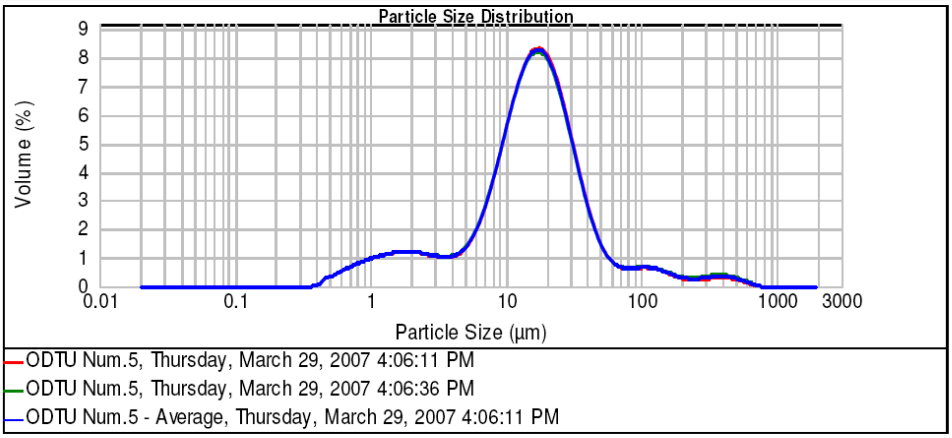
PARTICLE SIZE DISTRIBUTION ANALYSIS REPORTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: ODTU Num.5 - Average	**SOP Name:**	**Measured:** Thursday, March 29, 2007 4:06:11 PM	
Sample Source & type:	**Measured by:** MasterSizer	**Analysed:** Thursday, March 29, 2007 4:06:12 PM	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Default	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 1.520	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 11.86 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 1.022 %	**Result Emulation:** Off
Concentration: 0.0117 %Vol	**Span :** 2.620	**Uniformity:** 1.33	**Result units:** Volume
Specific Surface Area: 0.963 m²/g	**Surface Weighted Mean D[3,2]:** 6.230 um	**Vol. Weighted Mean D[4,3]:** 29.052 um	

d(0.1): 2.411 um **d(0.5):** 15.768 um **d(0.9):** 43.729 um



Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.010	0.00	0.105	0.00	1.096	3.97	11.482	34.41	120.226	96.12	1258.925	100.00
0.011	0.00	0.120	0.00	1.259	4.94	13.183	40.76	138.038	96.69	1445.440	100.00
0.013	0.00	0.138	0.00	1.445	5.97	15.136	47.82	158.489	97.16	1659.587	100.00
0.015	0.00	0.158	0.00	1.660	7.05	17.378	55.26	181.970	97.54	1905.461	100.00
0.017	0.00	0.182	0.00	1.905	8.15	19.953	62.67	208.930	97.83	2187.762	100.00
0.020	0.00	0.209	0.00	2.188	9.25	22.909	69.68	239.883	98.08	2511.886	100.00
0.023	0.00	0.240	0.00	2.512	10.31	26.303	75.93	275.423	98.32	2884.032	100.00
0.026	0.00	0.275	0.00	2.884	11.33	30.200	81.18	316.228	98.60	3311.311	100.00
0.030	0.00	0.316	0.00	3.311	12.29	34.674	85.34	363.078	98.91	3801.894	100.00
0.035	0.00	0.363	0.00	3.802	13.22	39.811	88.44	416.869	99.24	4365.158	100.00
0.040	0.00	0.417	0.00	4.365	14.19	45.709	90.61	478.630	99.55	5011.872	100.00
0.046	0.00	0.479	0.19	5.012	15.29	52.481	92.06	549.541	99.80	5754.399	100.00
0.052	0.00	0.550	0.52	5.754	16.69	60.256	93.03	630.957	99.94	6606.934	100.00
0.060	0.00	0.631	0.97	6.607	18.57	69.183	93.73	724.436	100.00	7585.776	100.00
0.069	0.00	0.724	1.56	7.586	21.13	79.433	94.33	831.764	100.00	8709.636	100.00
0.079	0.00	0.832	2.26	8.710	24.56	91.201	94.91	954.993	100.00	10000.000	100.00
0.091	0.00	0.955	3.07	10.000	28.97	104.713	95.52	1096.478	100.00		

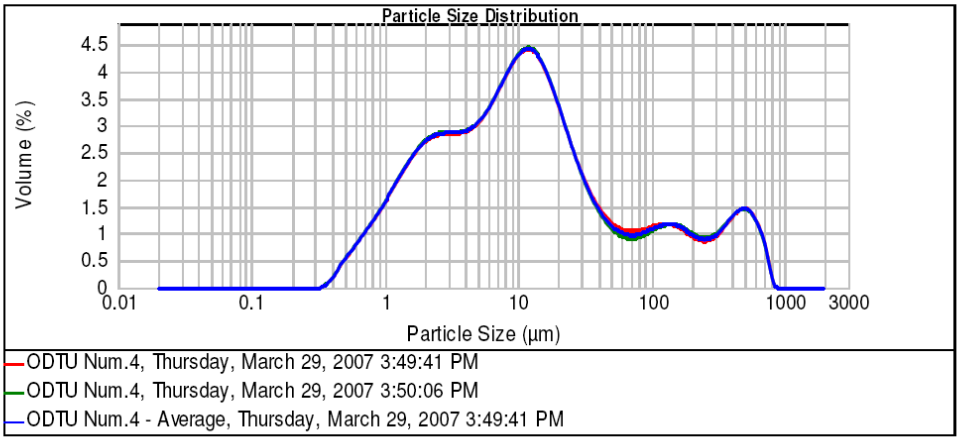
Figure E.1. Particle size distribution of raw wastewater

MASTERSIZER 2000

Result Analysis Report

Sample Name: ODTU Num.4 - Average	**SOP Name:**	**Measured:** Thursday, March 29, 2007 3:49:41 PM	
Sample Source & type:	**Measured by:** MasterSizer	**Analysed:** Thursday, March 29, 2007 3:49:43 PM	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: Default	**Accessory Name:** Hydro 2000MU (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 1.520	**Absorption:** 0.1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 12.38 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 1.250 %	**Result Emulation** Off
Concentration: 0.0080 %Vol	**Span :** 18.857	**Uniformity:** 5.58	**Result units:** Volume
Specific Surface Area: 1.48 m^2/g	**Surface Weighted Mean D[3,2]:** 4.049 um	**Vol. Weighted Mean D[4,3]:** 61.735 um	

d(0.1): 1.447 um **d(0.5):** 10.327 um **d(0.9):** 196.182 um



Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.010	0.00	0.105	0.00	1.096	6.37	11.482	53.05	120.226	86.38	1258.925	100.00
0.011	0.00	0.120	0.00	1.259	8.06	13.183	57.04	138.038	87.45	1445.440	100.00
0.013	0.00	0.138	0.00	1.445	9.98	15.136	60.92	158.489	88.50	1659.587	100.00
0.015	0.00	0.158	0.00	1.660	12.11	17.378	64.55	181.970	89.49	1905.461	100.00
0.017	0.00	0.182	0.00	1.905	14.43	19.953	67.83	208.930	90.40	2187.762	100.00
0.020	0.00	0.209	0.00	2.188	16.89	22.909	70.72	239.883	91.24	2511.886	100.00
0.023	0.00	0.240	0.00	2.512	19.43	26.303	73.18	275.423	92.05	2884.032	100.00
0.026	0.00	0.275	0.00	2.884	22.01	30.200	75.26	316.228	92.90	3311.311	100.00
0.030	0.00	0.316	0.00	3.311	24.60	34.674	76.99	363.078	93.87	3801.894	100.00
0.035	0.00	0.363	0.01	3.802	27.19	39.811	78.44	416.869	95.01	4365.158	100.00
0.040	0.00	0.417	0.15	4.365	29.81	45.709	79.67	478.630	96.29	5011.872	100.00
0.046	0.00	0.479	0.47	5.012	32.51	52.481	80.73	549.541	97.61	5754.399	100.00
0.052	0.00	0.550	0.99	5.754	35.36	60.256	81.68	630.957	98.80	6606.934	100.00
0.060	0.00	0.631	1.69	6.607	38.40	69.183	82.58	724.436	99.69	7585.776	100.00
0.069	0.00	0.724	2.57	7.586	41.71	79.433	83.46	831.764	100.00	8709.636	100.00
0.079	0.00	0.832	3.64	8.710	45.28	91.201	84.38	954.993	100.00	10000.000	100.00
0.091	0.00	0.955	4.90	10.000	49.09	104.713	85.35	1096.478	100.00		

Figure E.2. Particle size distribution of 40 kGy irradiated wastewater