ANAEROBIC TREATMENT OF OPIUM ALKALOID WASTEWATER AND EFFECT OF GAMMA-RAYS ON ANAEROBIC TREATMENT

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ABSTRACT

ANAEROBIC TREATMENT OF OPIUM ALKALOID WASTEWATER AND EFFECT OF GAMMA-RAYS ON ANAEROBIC TREATMENT

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In this study, anaerobic treatability of opium alkaloid wastewater and the effect of radiation pretreatment (gamma-rays) on anaerobic treatability were investigated. Biochemical Methane Potential (BMP) assay was performed with alkaloid wastewater having initial COD values of 2400, 6000 and 9600 mg/L with and without basal medium (BM). The highest anaerobic treatment efficiency of 77% was obtained in the BMP reactor containing alkaloid wastewater with initial COD of 9600 mg/L and BM.

Co-substrate use was investigated by using BMP assay. Alkaloid wastewater having initial COD concentrations of 9000, 13000 and 18000 mg/L were used with glucose, acetate and glucose-acetate as co-substrates. Results revealed that co-substrate use did not improve alkaloid removal efficiency significantly but it abrogated the acclimation period of anaerobic bacteria to alkaloid wastewater.

Continuous reactor experiments were carried out in Upflow Anaerobic Sludge Blanket (UASB) reactors. Highest overall efficiencies (above 80%) were obtained in the reactor fed with co-substrate (R2) for all initial COD concentrations. Up to 78% removal efficiency was obtained in R1 (fed with alkaloid wastewater only) at initial COD concentration of 19 g/L.

Effect of radiation was sought by using BMP assay 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.

Keywords: Opium alkaloid wastewater, Biochemical Methane Potential, Radiation, UASB, Two-phase, Co-substrate

AFYON ALKALOİDLERİ ATIKSUYUNUN ANAEROBİK ARITIMI VE GAMA IŞINLARININ ANAEROBİK ARITIMA ETKİSİ

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Bu çalışmada, afyon alkaloidleri atıksuyunun anaerobik olarak arıtılabilirliği ve ışınlamanın (gama ışınları) anaerobik arıtıma etkisi araştırılmıştır. 2400, 6000 ve 9600 mg/L KOİ değerlerine sahip afyon alkaloid atıksuyu ile Biyokimyasal Metan Potansiyeli (BMP) analizi gerçekleştirilmiştir. Bu analizde hem BM içeren hem de içermeyen reaktörler kullanılmıştır. En yüksek anaerobik arıtım verimi (%77) 9600 mg/L KOİ giriş değerine sahip BM içeren reaktörde elde edilmiştir.

BMP deneyi kullanılarak eş-substrat kullanımı araştırılmıştır. Bu deneyde 9000, 13000 ve 18000 mg/L KOİ konsantrasyonuna sahip alkaloid atıksuyu ve glükoz, asetat ve glükoz-asetat (eş substrat) kullanılmıştır. Sonuçlar eş substrat kullanımının alkaloid arıtım verimini önemli ölçüde arttırmadığını ancak aklimasyon süresini ortadan kaldırdığını açığa çıkarmıştır.

Sürekli reaktör deneyleri Yukarı Akışlı Çamur Yataklı Anaerobik (YAÇYA) reaktörlerde gerçekleştirilmiştir. En yüksek genel arıtım verimleri (%80'in üstünde), bütün ilk KOİ değerleri için eş substrat ile beslenen reaktörde (R2) elde edilmiştir. R1'de 19 g/L KOİ değerinde %78'e varan giderim verimleri elde edilmiştir.

BMP deneyi kullanılarak ışınlamanın etkisi araştırılmıştır. Bunun için giriş KOİ değerleri 14 ve 25 g/L olan alkaloid atıksuyu ve 40 ve 140 kGy olmak üzere iki ışınlama dozu kullanılmıştır. 14 g/L giriş KOİ'sinde her iki doz için de ışınlamanın herhangi bir etkisi olmamıştır. Ancak, 25 g/L giriş KOİ'sinde 140 kGy ve 40 kGy doz almış atıksu içeren reaktörlerdeki gaz üretim hızı belli bir zamandan sonra orjinal atıksu içeren reaktöre oranla yüksek olmuştur.

Anahtar kelimeler: Afyon alkaloid atıksuyu, Biyokimyasal Metan Potansiyeli, Işınlama, YAÇYA, İki aşamalı arıtım, Eş substrat

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ABBREVIATIONS

BM	: Basal medium	
BMP	: Biochemical methane potential	
BOD	: Biochemical oxygen demand	
CGP	: Cumulative gas production	
COD	: Chemical oxygen demand	
DoA	: Degree of acidification	
HRT	: Hydraulic retention time	
MLVSS	: Mixed liquor volatile suspended solids	
NCGP	: Net cumulative gas production	
OLR	: Organic loading rate	
SBR	: Sequencing batch reactor	
SRT	: Solid retention time	
TDS	: Total dissolved solids	
TKN	: Total kjeldahl nitrogen	
TOC	: Total organic carbon	
TS	: Total solids	
TSS	: Total suspended solids	
TVFA	: Total volatile fatty acids	
UASB	: Upflow anaerobic sludge blanket	
VSS	: Volatile suspended solids	

CHAPTER 1

INTRODUCTION

Industrial applications generate a wide range of wastes released to environment via air, soil, and water. Disposal of waste in large quantities without appropriate treatment results in a deteriorated environment.

Microbial-based technologies for the destruction of naturally occurring organic compounds have historically been the treatment of choice, relative to physical and chemical methods. Over the last century, development of microbial-based technologies for the treatment of liquid domestic waste streams has provided excellent processes for the destruction of waste constituents and then followed by application of biological wastewater treatment to industrial and hazardous wastewater (Sayles and Suidan, 1993).

The anaerobic treatment of industrial wastewater has become a viable technology in recent years due to the rapid development of high-rate reactors, such as the anaerobic filter and upflow anaerobic sludge blanket (UASB) (Perez *et al.*, 2006). Anaerobic biotechnology has been successfully applied for treatment of certain toxic compounds as well as most common organic pollutants in industrial wastewaters (Speece, 1996).

Opium alkaloid industry produces mainly morphine to be used in medical field. Licensed opium poppy cultivation for medical purposes currently takes place in 12 countries around the world. The four main producers are India, Australia, Turkey and France. Opium Alkaloid Plant in Afyon, Bolvadin produces around 75 tons of morphine each year corresponding to about 30% of global morphine production. Consequently, alkaloid industry is important for Turkey from financial and prestigious point of view.

Opium alkaloid plant generates 480 m³/day wastewater with an average flow rate of 27.5 m³/hr (Sevimli *et al.*, 1999). Effluent from the factory is being discharged to Eber Lake through Akarçay River. Pollution in Eber Lake has become intimidating to bird and fish species which use the lake as their habitat and even to people living in district. Besides alkaloid industry, sugar, cement and other factory wastewaters are also discharged to Eber Lake resulting in growing pollution in the lake.

Previous treatment studies on both biological (Sevimli *et al.*, 1999; Sevimli *et al.*, 2000; Kunukcu *et al.*, 2004) and physicochemical (Kınlı, 1994; Aydin *et al.*, 2002; Koyuncu, 2003) treatment of alkaloid wastewater could not present a remedy to the problem. Except these studies, no further information is available in the literature on the treatability of such wastewaters other than Afyon Alkaloid. Due to complex nature of this wastewater solution to this problem becomes challenging.

Ionizing radiation has been shown to be effective for destroying environmental contaminants from sewage, sludge and wastewater (Borrely *et al.*, 2000). The main effect of ionizing radiation is to generate a combination of reactive oxidizing and reducing species, free to interact with pollutants in the medium in both primary (direct effects) and secondary ionizations (indirect effects) (Meeroff *et al.*, 2004). Radiation treatment in combination with biological treatment is a promising option for industrial wastwaters (Jo *et al.*, 2006).

The aim of this study was to investigate anaerobic treatability of opium alkaloid wastewater in batch and continuous systems and to determine the effect of radiation on anaerobic treatment of alkaloid wastewater.

The tasks undertaken are:

- Investigation of anaerobic treatability of alkaloid wastewater in batch systems,
- Determination of the effect of nutrient supplementation and co-substrate addition to anaerobic treatability,
- Research of continuous anaerobic treatment of alkaloid wastewater in UASB reactors with different influent concentrations,
- Implementation of batch studies to find out the effect of radiation (60 Co- γ irradiation) on anaerobic treatability of opium alkaloid wastewater.

In the following chapter, information regarding anaerobic biotechnology, opium alkaloid industry and wastewater, previous treatability studies on alkaloid wastewater, co-substrate usage in anaerobic treatment, two-phase systems, UASB reactors and environmental applications of radiation are given. Materials and methods used in this study are presented in the third chapter. Results of experiments are presented and discussed in the fourth chapter. The fifth chapter covers conclusions of this study.

CHAPTER 2

THEORETICAL BACKROUND

2.1. Anaerobic Biotechnology

The production of biogas, which is mainly methane and carbon dioxide, was discovered in the seventeenth century after the observation of "marsh gas" burning on the surface of swamps. This occurs due to fact that mass transfer of oxygen from atmosphere is unable to maintain measurable concentrations of oxygen under stagnant water conditions and high concentrations of organic matter. Wastewaters, unless applied to a broad surface area or supplied from artificial aerators, naturally give rise to anaerobic treatment (Droste, 1997).

Anaerobic digestion has many advantages when compared to aerobic treatment such as process stability, reduction of waste biomass disposal costs, reduction of nitrogen and phosphorus supplementation costs, reduction of installation space requirements, conservation of energy, ensuring ecological and economical benefits, minimization of operational attention requirements, elimination of off-gas air pollution, avoidance of foaming with surfactant wastewaters, biodegradation of some of the aerobic nonbiodegradables, reduction of chlorinated organic toxicity levels and provision of seasonal treatment. Taking these advantages into consideration anaerobic biotechnology is a noteworthy option for industrial wastewater treatment especially for the industries with highly polluted effluents (Speece, 1996).

Anaerobic digestion has metabolic steps in which different classes of microorganisms interact to satisfactorily degrade complex organic compounds to methane and carbon dioxide. These metabolic steps and the microbial groups involved are shown in Figure 2.1.



Figure 2.1. Metabolic steps and microbial groups involved in anaerobic digestion 1) Fermentative bacteria; 2) H₂-producing acetogenic bacteria;

3) H₂-consuming acetogenic or homo acetogenic bacteria; 4) CO_2 -reducing methanogenic bacteria;

5) Acetoclastic methanogenic bacteria (Novaes, 1986; Speece, 1996).

The first step is the hydrolysis of complex organic compounds (carbohydrates, proteins, lipids) into their smaller molecular material by fermentative bacteria. Simple organic compounds are then transformed into volatile fatty acids by acidogens. Hydrogen producing acetogenic bacteria converts volatile fatty acids into hydrogen and acetate. Methanogenic bacteria are responsible for producing methane which is considered as the final step for anaerobic degradation (Novaes, 1986).

The anaerobic bacteria can also be divided into three subgroups with respect to their optimum thermal conditions: psychrophiles (<20 °C), mesophiles (25-40 °C) and thermophiles (>45 °C). The temperature range for each species has its own trend

where the growth rate increases exponentially with temperature and then rapidly decreases above the optimum temperature. The conversion rates among these three species is generally highest for thermopiles and lowest for psychrophiles (Lier *et al.*, 1997). Duran and Speece (1997) operated two CSTR reactors at mesophilic and thermophilic temperatures treating a common substrate. They ended up with a higher initial substrate utilization rate (26%) for thermophiles compared to mesophilic bacteria whereas mesophiles produced better quality effluent, in other words lower effluent COD concentrations (Duran and Speece, 1997). Anaerobic specific removal rate as a function of temperature is given in Figure 2.2.



Figure 2.2. Anaerobic specific removal rate as a function of temperature (The process is typically unstable or fails between 45°C and 50°C.) (Henze *et al.*, 2002)

The reactor configurations for biological wastewater treatment can be divided into two main groups depending on the microorganism retention mechanisms, namely attached and suspended growth systems. In suspended growth reactors, microorganisms responsible for treatment are maintained in liquid suspension by appropriate mixing methods. In attached growth processes, on the other hand, microorganisms are attached to an inert packing materials (Metcalf and Eddy, 2003). Among these two general reactor types attached growth has two major advantages over suspended growth systems, especially while treating wastewaters containing toxicants:

- 1. higher inherent solids retention times with relatively low hydraulic retention times
- 2. the potential for a quasi-plug flow hydraulic regime that allows more rapid elution of toxicants

The so called disadvantages of anaerobic treatment (low cell yield, temperature, hydraulic and organic fluctuations, toxicity) can be defeated by proper attention to solid retention time (SRT). The required extended SRT can be obtained by attached growth systems. In addition, organics which are difficult to degrade are more susceptible to biodegradation at longer SRT values (Parkin and Speece, 1983; Speece, 1996; Rajeshwari *et al.*, 2000).

High rate anaerobic processes utilize bioreactor configurations that provide significant retention of active biomass resulting in large differences between solid retention time (SRT) and hydraulic retention time (HRT) (Speece, 1996). Several high rate anaerobic reactor configurations have been developed for treatment of wastewaters at relatively high HRTs. In Figure 2.3 some of the anaerobic reactor configurations are depicted.



Figure 2.3. Various anaerobic reactor configurations (Speece, 1996)

2.2. Opium Alkaloid Industry

Opium, which is harvested in the province of Afyon, is used to produce alkaloids mainly morphine. Opium is the dried sap of the unripe seed capsule of the poppy *Papaver somniferum*. Morphine is by far the most prevalent and important alkaloid in opium, consisting of 10%-16% of the total. An alkaloid is any basic, nitrogen-containing plant product, often with a complex structure and significant pharmacological properties (Hart *et al.*, 1995). The alkaloids produced in the factory are base morphine, base codeine, base ethyl morphine, codeine phosphate and dionin. 3.3 kg morphine is produced per ton of opium processed (Sevimli *et al.*, 1999; Sevimli *et al.*, 2000).

Currently, opium poppy is the only commercially valuable source of the pain relief drugs morphine and codeine. Another pharmaceutically important chemical found in opium poppy is thebaine, which is used for the production of codeine (Warner *et al.*, 2005). Molecular structures of morphine, codeine and thebaine are given in Figure 2.4. Codeine has a density of 1.32 g/cm^3 and solubility of 7.9 g/L in water. Morphine is classified as insoluble in water (solubility = 0.15 g/L). Thebaine, which has a density of 1.305 g/L is also classified as insoluble (0.7 g/L) (Lide, 2006).



Figure 2.4. Molecular structures of alkaloids which were possible to be found in the effluent of opium alkaloid wastewater (Lide, 2006)

Licensed opium poppy cultivation for medical purposes currently takes place in 12 countries around the world. The four main producers are India, Australia, Turkey and France. The poppy cultivated in Turkey is morphine-rich, and about 95% of the opiate alkaloids produced are exported (Warner *et al.*, 2005). In the year of 2003, Australia produced 151 tons of poppy straw (M) (measured in terms of morphine equivalent), or 33.5% of worldwide production, followed by Turkey at 145 tons, or 32% of worldwide production. France and Spain accounted for 15 and 10% of worldwide production, respectively, having produced 68 and 44 tons (INCB, 2005).

The Alkaloid Factory in Afyon which is located in Bolvadin was commissioned in 1980. The factory processes 20,000 tons of opium straw and produces 75 tons of morphine each year. The factory is owned by the Ministry of Agriculture. In 2003 Turkey cultivated an area of almost 100,000 hectares, yielding 145 tons of CPS (M), which was about 30% of the global morphine production. It is estimated that

Turkey earns \$60 million annually from the export of poppy seeds and morphine (Gecin and Hakbilen, 2005).

The flow scheme of the plant is given in Figure 2.5. The factory has two sections. The first is the extraction unit, which works continuously extracts the resin from plants using the concentrated poppy straw method. In the second section the raw product is turned into various drugs by mixing and reacting with chemical agents (Kunukcu *et al.*, 2004). The chemicals used at the production line during extraction are given in Table 2.1.

Name of the chemical used	Amount of the chemical used (kg/tons capsule)
Lime	92.5
Toluene	7.5
Sodium carbonate	94.6
Acetic acid	22.3
Sulfuric acid	48.3
Ammonia (25%)	5.6
Butanol	5.1
Caustic	1.1

Table 2.1. Chemicals used during extraction and their amounts (Aydın, 2002)



Figure 2.5. Afyon Alkaloid Factory production flow scheme (Aydın, 2002)

2.3. Opium Alkaloid Wastewater

The wastewater generated from alkaloid factory is highly polluted having low pH, high COD and BOD values and a characteristic dark brown color. The main pollutant present in alkaloid wastewater is reported to be phytine which is found in the shell of opium poppy and solubilizes in basic environment (Aydın, 2002). Phytin is a water-insoluble accumulation of a mixed salt of magnesium, calcium, and potassium ionically bound to myo-inositol hexaphosphoric acid (phytic acid) (Downie, 2005). Phytic acid is a strong chelator of important minerals such as calcium, magnesium, iron and zinc. The molecular structure of phytic acid is given in Figure 2.6.



Figure 2.6. Molecular structure of phytic acid

Other pollutants that contribute to alkaloid wastewater are cellulose, color causing substances, oilly materials and morphine, codein and thebaine in trace amounts (Aydın, 2002).

The wastewater characteristics that are obtained in the previous studies are given in Table 2.2. Among these studies Aydın (2002) included analysis results from both alkaloid factory laboratories and other researchers' studies.

Wastewater generation is about 9 m³ per ton of the opium capsule processed (Sevimli *et al.*, 1999; Kunukcu and Wiesmann, 2004). The average process effluents generated from alkaloid extraction and processing is 480 m³/day and average flow rate is about 27.5 m³/hr (Sevimli *et al.*, 1999). The effluent of alkaloid factory is being discharged to Eber Lake through Akarçay River.

Parameter	Unit	Çil et al. (1994)	Kınlı (1994)	Sevimli et al. (1999)	Aydın (2002)
Total COD	mg/L	21200	23251	36500	18300-42500
Soluble COD	mg/L	-	-	32620	17050-39470
TOC	mg/L	-	9000	-	7335-14000
BOD ₅	mg/L	14700	14450	-	4250-22215
COD/BOD ₅	-	1.44	1.61	-	1.37-6.11
pН	-	5.10	4.89	-	4.9-6.3
Total alkalinity	mg CaCO ₃ /L	5294	2300	-	315-4450
Color	Pt-Co	-	-	-	2150-2550
TS	mg/L	-	-	-	27235-29750
TDS	mg/L	-	-	-	26220-29120
TSS	mg/L	1214	1450	1400	565-2295
VSS	mg/L	-	-	-	320-1775
TKN	mg/L	404	203	1030	550-841
NH ₄ -N	mg/L	147	62	140	73-141
Total-P	mg/L	15	29.2	65	3.1-15.0
PO ₄ -P	mg/L	-	-	-	20-30
SO ₄	mg/L	-	-	-	8-912
Na	mg/L	-	-	-	700-10445
К	mg/L	-	-	-	315-457
Ca	mg/L	-	-	-	10-41
Mg	mg/L	-	-	-	7.3-36
Cu	mg/L	-	-	-	0.508-0.564
Zn	mg/L	-	-	-	2.057-2.226
Pb	mg/L	-	-	-	0.572-0.624
Fe	mg/L	-	-	-	5.201-6.075
Conductivity	µmhos/c m	-	-	-	18900-22800
Temperature	°C	-	-	40	Summer: 35- 38 Winter: 26-31

Table 2.2. Opium alkaloid wastewater characteristics

About 2% of alkaloid wastewater COD was found to be inert (Sevimli *et al.*, 2000). Discharge standards for this industry, in fact for only this factory is presented in Table 2.3.

Fable 2.3. Alkaloid production plant wastewater discharge standard	ls (*)
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Parameter	Unit	Composite Sample (24 hr)
COD	(mg/L)	1500
TKN	(mg/L)	15
TSS	(mg/L)	200
pH	-	6-9

(*) Turkish Water Pollution Regulation, 2004, Table 14.17.

The plant had a wastewater treatment plant which is a serial activated sludge system with diffusers. At the early times of the plant as high as 90-95 % COD removal efficiencies were obtained. However, because of some operational problems and high costs of aeration, the existing plant is not operating efficiently any more. The flow scheme of the existing treatment plant is given in Figure 2.7. The most important operating problem was an uncontrolled temperature increase in the aeration basins which was mainly due to the cover effect of the thick scum layer and a long hydraulic detention time (Sevimli *et al.*, 2000).



Figure 2.7. Flow scheme of existing wastewater treatment plant (Sevimli *et al.*, 2000)

2.4. Previous Treatability Studies on Opium Alkaloid Wastewater

In literature treatment studies were performed on alkaloid wastewater mainly by İstanbul Technical University (İTÜ) and TÜBİTAK Marmara Research Center (MAM). These studies both covered treatability studies of raw wastewater and effluent from existing wastewater treatment plant. Previous treatability studies on opium alkaloid wastewater are summarized in Table 2.4 and described below:

<u>Anaerobic treatability studies (Sevimli et al., 2000)</u> A 36 L of UASB pilot reactor was operated for about 5 months. The seed was acquired from İzmit PAKMAYA wastewater treatment plant. The initial COD fed to the reactor was between 7000 and 13000 mg/L which was about 0.25-0.4 of the original COD of alkaloid wastewater. The removal efficiency was between 50-75 %. Also lab scale anaerobic studies were performed with alkaloid wastewater having initial COD values of 8000-16000 mg/L. In these studies 62-90% COD removal efficiencies were obtained. The results are given in Table 2.4.</u>

- <u>Ozone oxidation (Sevimli *et al.*, 2000):</u> Ozone oxidation was applied to effluent from WWTP. Optimum COD removal was achieved at pH 2.5 for 40 minutes of ozonation which happened to be 43%. Initial COD values were not given for wastewater used in this study.
- <u>Color removal by lime (Sevimli *et al.*, 2000):</u> Applied to effluent from WWTP. 40 g/L lime was used to achieve 84% color and 57% COD removal.
- <u>Fenton process (Aydin et al., 2002)</u>: Applied to effluent of lab scale biological reactor effluent with a COD of 650 mg/L and color of 4,950 Pt-Co. Optimum pH and reaction time were found to be 4 and 30 minutes, respectively. 200 mg Fe^{2+/}L and 400 mg H₂O₂/L was used in combination to achieve %91.3 COD and %99.1 color removal.
- <u>Membrane technology (Koyuncu, 2003)</u>: With both nano-filtration (NF) and reverse osmosis (RO) a full color removal and a 95% COD removal was achieved with previously treated wastewater having COD = 950-2000 mg/L.
- <u>TÜBİTAK Marmara Research Center (MAM) studies (Kınlı, 1994)</u>: These studies were applied to effluent of WWTP. They can be classified as physicochemical treatment (alum, FeCl₃, Fe₂(SO₄)₃), adsorption studies (activated carbon, perlite, cement powder) and chemical oxidation (potassium permanganate, hydrogen peroxide). The most significant results obtained were alum, Fe₂(SO₄)₃ and potassium permanganate oxidation which resulted in COD removals around 43-45% by a concentration of 1000 mg/L. Initial COD values were not given for wastewater used in this study.

Method	Raw or treated	Initial COD of	COD removal	Process	Reference
appneu	waste water	(mg/L)	(%)	parameters	
Anaerobic treatment (pilot)	Raw	13,000	45	$t_d = 2.5 \text{ days},$ OLR = 5.2 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (pilot)	Raw	7,000	70	t_d = 2.5 days, OLR = 2.8 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (lab)	Raw	5,000	90	$t_d = 1.6$ days, OLR = 3.0 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (lab)	Raw	8,000	87	$t_d = 1.6 \text{ days},$ OLR = 5.0 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (lab)	Raw	12,000	83	$t_d = 1.6$ days, OLR = 7.5 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (lab)	Raw	14,400	68	$t_d = 1.6 \text{ days},$ OLR = 9.0 kg COD/m ³ .day	Sevimli et al. (2000)
Anaerobic treatment (lab)	Raw	16,000	62	$t_d = 1.6$ days, OLR = 10.0 kg COD/m ³ .day	Sevimli et al. (2000)
Ozone oxidation	Effluent of WWTP	-	43	pH = 2.5, 40 minutes of ozonation	Sevimli et al. (2000)
Lime	Effluent of WWTP	2250	57	40 g/L lime	Sevimli et al. (2000)
Fenton	Effluent of lab scale reactor	650	91.3	pH = 4, Reaction time = 30 min, $200 \text{ mg Fe}^{2+/}L$ & 400 mg H ₂ O ₂ /L	Aydin et al. (2002)
Membrane (NF and RO)	Treated	950-2,000	95	-	Koyuncu (2003)
Alum	Effluent of WWTP	-	43	1000 mg/L alum pH = 6.5	Kınlı (1994)

 Table 2.4. Previous treatability studies on opium alkaloid wastewater

	Raw or	Initial COD	COD		
Method	treated	of	removal	Process	Deference
applied	waste	wastewater	efficiency	parameters	Kelefence
	water	(mg/L)	(%)		
FeCl ₃	Effluent			1000 mg/L	Kınlı
	of	-	41	FeCl ₃	(1994)
	WWTP			pH = 6.5	
$Fe_2(SO_4)_3$	Effluent			1000 mg/L	Kınlı
	of	-	43	$Fe_2(SO_4)_3$	(1994)
	WWTP			pH = 6.5	
Activated carbon	Effluent				Kınlı
	of	-	16	-	(1994)
	WWTP				
Perlite	Effluent				Kınlı
	of	-	15	-	(1994)
	WWTP				
Cement powder	Effluent				Kınlı
	of	-	10	-	(1994)
	WWTP				
Potassium perman ganate	Effluent			1000 mg/L	Kınlı
	of	-	45	potassium	(1994)
	WWTP			permanganate	
Hydrogen peroxide	Effluent			$20 \text{ mL/L } H_2O_2$	Kınlı
	of	-	7		(1994)
	WWTP				

Table 2.4 (continued)

Highest initial COD concentration used in these treatability studies was 16000 mg/L which is about half of the original alkaloid wastewater average COD value (30000 mg/L). Consequently, these studies could not be able to solve the problem completely.

2.5. Co-substrate Use in Anaerobic Treatment

Researchers studied effects of co-substrates which are easily biodegradable by anaerobic bacteria with wastes and wastewater. Co-substrate is an alternate growth substrate which when supplied to a bio-reactor can enhance the degradation of some wastes or pollutants that cannot alone support the microbial growth (Atlas, 1993).

Significant amount of studies focused on color removal since co-substrates act as carbon source which lacks in dye removal applications (Donlon *et al.*, 1997; Chinwetkitvanich *et al.*, 2000; Kapdan *et al.*, 2003; Şen and Demirer, 2003).

Vidal *et al.* (1999) used volatile fatty acids and as co-substrates in batch and continuous experiments for treatment of synthetic wastewater containing formaldehyde (FA). Two UASB reactors were initially fed with a standard glucose solution (1.6 g glucose/l, 3.36 g NaHCO3/l) and different COD/FA ratios were used in order to determine the minimum COD/FA ratio at which stable operation could be maintained. From continuous operation with FA and glucose it was found that a stable and efficient operation could be obtained when working at an OLR of about 6 g COD/L.d and with a COD/FA ratio of 1.4 (Vidal *et al.*, 1999).

Atuanya and Chakrabarti (2003), studied biotreatability of chlorophenol pollutants. UASB reactor was fed with a synthetic wastewater containing 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol at concentration range of 10 to 50 mg/L. Glucose was used as a co-substrate at a COD concentration of 250 mg/L. They concluded that addition of alternative utilizable substrate (such as glucose) can mitigate toxic effects and enhance degradation (Atuanya and Chakrabarti, 2003).

Biodegradation of tech-hexachlorocyclohexane in an upflow anaerobic sludge blanket (UASB) reactor with methanol as co-substrate was also studied (Bhat *et al.*, 2006). Methanol acted co-substrate as well as electron donor in this case. The UASB reactor under continuous mode of operation was able to degrade more than 85% of tech-HCH up to an inlet feed concentration of 175 mg/l and constant HRT of 48 h with methanol as co-substrate.

She *et al.* (2005), studied biodegradation of 2,4-dinitrophenol and 3-nitrophenol with glucose and volatile fatty acids as co-substrates. Batch experiments were performed and as high as 80% efficiencies were achieved (She *et al.*, 2005).

In the study conducted by Garcia *et al.* (2000), glucose was supplemented in order to determine anaerobic toxicity of commercial cationic surfactants, namely di(hydrogenated tallow) dimethyl ammonium chloride (DHTDMAC) and two esterquats. Toxic effects on anaerobic sludge were determined as a decreased level of biogas production in relation to co-substrate biogas production (Garcia *et al.*, 2000).

Sanz *et al.* (2003) investigated anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in UASB reactors. Co-substrate fed to system consisted of acetate, propionate, butyrate, lactate, methanol, ethanol and sucrose (1:1:1:1:1:1:1:1 COD ratio). The anaerobic biomass used in the tests was able to degrade LAS in the presence of co-substrates as well as in absence of added external co-substrates. The removal of LAS was always higher in the absence of alternative sources of carbon. Researchers suggested the reason for this behavior being the primary biodegradation of LAS did not occur through co-metabolism but that LAS was used as a source of energy and carbon (Sanz *et al.*, 2003).

Tay *et al.* (2001) worked on anaerobic degradation of phenol with glucose as cosubstrate. Both batch and continuous experiments were performed. For the continuous case two lab scale UASB reactors were operated. From the batch tests with feed phenol concentrations ranging from 420 to 2,100 mg/L, a glucose supplement of 500– 4,000 mg/L promoted biodegradation of phenol. At higher feed phenol concentrations, a correspondingly higher glucose supplement was required. The continuous reactors were fed with glucose for two weeks with a concentration of 1000 mg/L. After the addition of phenol the efficiency dropped drastically and recovered to 96% by day 52. The reactor which is only fed with phenol has efficiencies of 28% at these days (Tay *et al.*, 2001).

Removal of 2,4,6-Trichlorophenol and phenol supplemented with sucrose methanogenic and partially-aerated methanogenic conditions in a fluidized bed reactor was investigated. The biological fluidized bed reactor removed 99.9% of

TCP, in both the methanogenic and partially-aerated methanogenic conditions (Garibay-Orijel *et al.*, 2005).

A review about treatment of phenol and cresols in UASB reactors (Veeresh *et al.*, 2005) indicated that with phenols whose degradation rates are slow, co-substrate aids in granulation and early acclimation. It is stated that co-substrates (glucose, sucrose, volatile fatty acids (VFA), methanol, etc.), in the presence of phenols in phenolic wastewater, keep methanogens in active phase and acclimatized biomass bring about the hydrogenation, fission and fragmentation of the phenolic ring.

2.6. Two-Phase Anaerobic Treatment

Single phase anaerobic treatment incorporates both acid formation and methane production in the same reactor whereas two phase systems attempt to separate acid formation from methane production, usually by providing two separate reactors. A pH range of 4-6.5 was accepted as optimal for first stage namely acidification while pH range of 6.5-8.2 was reported optimal for second stage, namely methane production (Speece, 1996).

Acid forming and methane forming microorganisms differ in terms of their environmental conditions (Pohland and Ghosh, 1971). Pohland and Ghosh (1971) were first to propose to separate two phases in two different reactors where optimum conditions for microorganisms responsible for acidification and methanogenation would be provided.

Ghosh et.al. (1975) operated a mesophilic chemostat as acidification reactor with HRT 10-24 hr and obtained a 77% acidification which was ratio of COD in VFA form to total COD (Ghosh *et al.*, 1975). Maharaj and Elefsiniotis (2001) worked with starch rich wastewater and produced maximum VFA concentrations at HRT of 30 h (Maharaj and Elefsiniotis, 2001).

Lettinga and Hulshoff (1991), stated that HRT of 6-24 hours for pre-acidification and 20-40% acidification was suitable for the enhancement of process performance (Lettinga and Hulshoff, 1991).

Another important operational parameter for acidification was pH. pH range of 4-6.5 was reported as optimum for acidification reactor (Speece, 1996). McCarty and Mosey (1991) stated that 5.5-5.9 for acidogens; 6.6-7.6 for methanogens (McCarty and Mosey, 1991).

Zoetemeyer *et al.* (1982) studied with glucose at pH 4.5 to 7.9 recommended 5.7-6.0 for stable operation and overall process performance (Zoetemeyer *et al.*, 1982). Yu and Fang (2003) studied gelatine-rich wastewater at different pH ranges. Operation at pH of 4.0–5.0 favored propionate and hydrogen production, whereas operation at pH 6.0–7.0 favored acetate, butyrate, and i-butyrate production. The region between pH 5.0 and 6.0 was the transition zone.

Elefsiniotis and Oldham (1994) investigated effect of SRT using CSTR and UASB. Percentage VFA distribution slightly affected while organic matter degradation seemed independent of SRT for the range 10-20 days (Elefsiniotis and Oldham, 1994). In Table 2.5 some studies performed with single and two-phase anaerobic systems were presented. The use of two-phase system increased the removal efficiency for some wastewaters (i.e. instant coffee) and has almost no effect on removal of others (i.e. alcohol stillage).
Process	Reactor	Waste	HRT (h)	OLR (kg COD/m ³ .d)	Temp (°C)	Removal (%)	Reference
Two- phase	Contact	Confectionery	166		Meso	50 (COD)	(Massey and Pohland, 1978)
Two- phase	CSTR	Palm oil			Meso	85 (COD)	(Ng <i>et al.</i> , 1985)
Two- phase	CSTR+ AF	Soft drink	177.6		Meso	96 (COD)	(Ghosh <i>et al.</i> , 1985)
Two- phase	CSTR+ UASB	Instant coffee	12	16	Thermo/ meso	77 (COD)	(Dinsdale <i>et al.</i> , 1997b)
Two- phase	CSTR	Alcohol stillage			Thermo	65 (COD)	(Yeoh, 1997)
Two- phase	CSTR+ upflow digester	Sludge			Meso	53 (TVS)	(Lin and Ouyang, 1993)
Two- phase	CSTR	Solid waste			Meso/ thermo	83.5 (TVS)	(Pavan <i>et</i> <i>al.</i> , 2000)
Two- phase	Pilot	Sludge			Thermo/ meso	61 (VS)	(Huyard <i>et al.</i> , 2000)
Two- phase	AF+ UASB	Thermo mechanical pulp wastewater	21	11	Meso	90 (COD)	(Vinas <i>et al.</i> , 1993)
Two- phase	CSTR+ AF	Dairy	48	5	Meso	90 (COD)	(Ince, 1998)
Single- phase	Upflow digester	Sludge			Meso	<53 (TVS)	(Lin and Ouyang, 1993)
Single- phase	CSTR	Alcohol stillage	216		Thermo	65.2 (COD)	(Yeoh, 1997)
Single- phase	UASB	Instant coffee			Thermo	68-70 (COD)	(Dinsdale <i>et al.</i> , 1997a)
Single- phase	UASB	Bean blanching wastewater		8-10	Meso	90-95 (COD)	(Lettinga <i>et al.</i> , 1980)
Single- phase	UASB	Potato		15-18	Meso	95 (COD)	(Lettinga <i>et al.</i> , 1980)

Table 2.5. Performances of single- and two-phase anaerobic digestionprocesses (Demirel and Yenigün, 2002)

2.7. Upflow Anaerobic Sludge Blanket Reactor

UASB reactor concept was first introduced by Lettinga and co-workers (Lettinga *et al.*, 1980). Since 1982, the applicability of UASB systems for the direct treatment of wastewater has been investigated (Lettinga *et al.*, 1980; Pol and Lettinga, 1986).

Principal of operation of UASB reactors is distribution of feed at the base of the reactor, percolation through a naturally forming granular microbial sludge blanket, with integrated solid–liquid–gas separation at the surface of the reactor. The sludge blanket is partially fluidized and mixed by gas flow, which also mixes the liquid volume (Batstone *et al.*, 2005).

The advantages of UASB reactors are

- High efficiency
- Simplicity
- Flexibility
- Low space requirements
- Low energy consumption
- Low sludge production
- Low nutrient and chemical requirements (Seghezzo *et al.*, 1998)

Different researchers studied different wastewaters with UASB reactor. The wastewater used and efficiencies obtained were given in Table 2.6.

The use of UASB reactors for wastewaters containing toxic substances was investigated by many researchers. Tham and Kennedy (2004), worked with aircraft deicing fluid which happened to be toxic to anaerobic bacteria. They ended up with COD removal efficiencies above 90% at 0.73 g COD/g VSS/d (Tham and Kennedy, 2004).

Type of wastewater	Operational	Efficiency	Reference
	parameters		
Obaque beer brewery	HRT = 1 day	57%	(Parawira <i>et al.</i> , 2005)
Formaldehyde and urea	OLR = 6 g COD/L.d	90-95%	(Vidal <i>et al.</i> , 1999)
Liquid residues from grass bioraffination	OLR = 12-15 kg $COD/m^3.day$	80%	(Baier and Delavy, 2005)
Tapioca starch wastewater	OLR = 10-16 kg $COD/m^3.day$	95%	(Annachhatre and Amatya, 2000)
Dairy wastewater	OLR lower than 2.5 g COD/L.d	70-80%	(Nadais <i>et al.</i> , 2005b/2005a)
Slaughterhouse wastewater	OLR = 13-39 kg SCOD/m ³ .d	75-90%	(Caixeta <i>et al.</i> , 2002; Torkian <i>et al.</i> , 2003)
Carbon tetrachloride	OLR = 12.5 g COD/L.d	96%	(Sponza, 2002)
Sunflower oil factory effluent	OLR = 1.6-7.8 kg $COD /m^3.day$	70%	(Saatci <i>et al.</i> , 2003)
Mono and diazo dyes	HRT = 24 hr	88% (color)	(Bras <i>et al.</i> , 2005)
Canning factory effluent	OLR = 3.95-10.95 kg $COD/m^3.d$	90-93%	(Trnovec and Britz, 1998)
Pulp and paper industry	$OLR = 5.75 \text{ kg}$ $COD/m^3.d, HRT = 20$ hr	80-85%	(Chinnaraj and Rao, 2006)
Corn starch wastewater	OLR = 90 g COD/L.d	95.3%	(Kwong and Fang, 1996)
Cheese whey	HRT = 2-3 days	95-97%	(Ergüder <i>et al.</i> , 2001)
Tech- hexachlorocyclohexane	Initial conc. = 100- 200 mg/L, HRT = 48 hr	85%	(Bhat <i>et al.</i> , 2006)

Table 2.6. UASB reactor studies in the literature

UASB reactor was used for the treatment of synthetic substrates that simulate bleached and unbleached cellulose pulp plant wastewaters (Buzzini *et al.*, 2005). This wastewater contains chlorinated organics such as chlorophenol, dichlorophenol (DCP), trichlorophenol (TCP) and pentachlorophenol (PCP). An average overall

COD removal efficiency of 79% and minimum overall chlorinated organics removal efficiency of 71% were obtained in UASB reactor.

Biodegradation of phenolic compounds, namely phenol and p-cresol, was investigated in UASB reactor. Above 90% total COD removal was achieved at loading rates as high as 7 kg COD/m³.d (Razo-Flores *et al.*, 2003).

Dieldrin and lindane were used to be treated by UASB reactors (Ergüder *et al.*, 2003a; Ergüder *et al.*, 2003b). The maximum lindane loading rate and minimum hydraulic retention time (HRT) possible for the UASB system were found as 10 mg/l day and 18 h, respectively, which resulted in the overall chemical oxygen demand (COD) removal efficiency of 89%. For dieldrin at a maximum loading rate of 0.5 mg/l.d, an overall COD removal efficiency of 85% was obtained.

2.8. Environmental Applications of Radiation

The most widely used commercial radiation sources in use at the present time are cobalt-60 for γ -irradiation and electron accelerators for electron beam (EB) irradiation. The γ sources are used mainly to sterilize medical products and for food irradiation, applications where the greater penetration of γ -radiation is an advantage. The most important parameter in radiation processing is the energy transferred from the incident radiation to material being irradiated. This is called as "absorbed dose" which is responsible for producing ions and excited species in the material irradiated and thence for the radiation-induced chemical and biological changes observed. The SI unit for the absorbed dose is joule per kilogram (J/kg) which has a special name gray (symbol Gy). The absorbed dose rate is the absorbed dose per unit time (Gy/s) (Cooper *et al.*, 1998).

Radiation treatment was used for disinfection of drinking water (Blatchley, 1999; Gazso, 2000; Pikaev, 2002), for municipal wastewater and sludges (Lessel and

Suess, 1984; Miyata *et al.*, 1990; Lee *et al.*, 2000) and industrial wastewater treatment (Kimura *et al.*, ; Duarte *et al.*, 2002; Mucka *et al.*, 2003; Jo *et al.*, 2006).

Treated system	Treatment goal	Method and output	Notes
Drinking water	Removal of chlorine-containing substances and disinfection	Electron-beam treatment + ozonation (108 m ³ /h)	Project of a commercial plant (Austria)
Municipal wastewater	Purification and disinfection	Electron-beam treatment + aerosolation (500 m ³ /day)	Pilot plant (Russia)
Industrial wastewater	Removal of isobutyl- naphthalene sulfonates	Electron-beam + biological treatment (1200 t/day)	Commercial plant (Russia)
Molasses distillery slops + municipal wastewater	Purification	Electron-beam treatment +coagulation (7000 m ³ /day)	Project of a commercial plant (Russia)
Wastewater from a dyeing complex	Purification	Electron-beam + biological treatment (1000 m ³ /day)	Pilot plant (Republic of Korea)
Sewage sludge	Disinfection and partial purification	γ -Irradiation + aeration (60 m ³ /h)	Pilot plant (India)
Sewage sludge	Disinfection and partial purification	γ -Irradiation+ aeration (240 m ³ /h)	Pilot plant at commissioning stage (Argentina)

Table 2.7. Some examples of radiation process use in water, wastewater andsewage treatment (Pikaev, 2002)

In Table 2.7 some examples of radiation process used in water, wastewater and sewage treatment were tabulated. As indicated in the table both electron beam and γ -irradiation were used in combination with other treatment methods. For industrial wastewaters, especially for the ones containing toxic substances (e.g. salts of mercury and bismuth, cyanides, phenols, etc), high doses are generally required.

Therefore, combined processes were developed in which radiation treatment is used in combination with a conventional process such as chemical, biological or thermal treatment, floatation and so on (Cooper *et al.*, 1998).

Jo *et al.* (2006) investigated the effects of gamma-irradiation on biodegradability (BOD₅/COD) of textile and pulp wastewaters. They used a high-activity ⁶⁰Co source and measured biodegradability by a ratio of BOD₅ to COD: The improvement of biodegradability was found to be highly dependent on chemical properties of wastewater and irradiation dose of gamma rays. The absorbed doses studied, ranged from 5 to 20 kGy. For pulp wastewater the change of biodegradability of cooking and bleaching C/D effluent was trivial. But, bleaching E1 and final effluents showed large improvement of biodegradability by gamma-ray treatment at a dose of 5 kGy (E1 means first alkaline stage and C/D indicates an acid stage including chlorine and chlorine dioxide (Jo *et al.*, 2006).

Duarte *et al.*(2002) studied advanced oxidation of effluents from an industrial complex composed of eight separated production units namely: Intermediary Organic Products (IOP), Poly Vinyl Acetate (PVA), Resins (RES), Especial Products (SP), Detergents (DET), Sulfonation (SULF), Thiodan (THIO) and Azodyes (AZO).by electron beam irradiation. The electron beam irradiation was efficient in destroying the organic compounds delivered in these effluents, mainly, chloroform, dichloroethane, methyl isobutyl ketone, toluene, xylene and phenol. The necessary dose to remove 90% of the most organic compounds from industry effluent was 20 kGy (Duarte *et al.*, 2002).

Mechanism of radiation treatment of polluted water and wastewater was studied for four groups systems (Pikaev, 2001). The systems identified and mechanisms involved for each group is tabulated in Table 2.8.

Group No.	Group Description	System
1	Reduction of pollutants by H atoms, e_{aq} , and radicals from added compounds	Water containing heavy metal ions (Cd(II), Pb(II), Hg(II), and Cr(VI))
2	Oxidation of pollutants by OH ⁻ radicals and radicals from dissolved compounds	Water containing dissolved petroleum products or dyes; wastewater with Nekal (a mixture of isobutyInaphthalene sulfonates)
3	Reduction of pollutants by H atoms and e_{aq} and their oxidation by OH ⁻ radicals	Water polluted with chlorinated organic compounds or carboxylic acids
4	Removal of pollutants by reactions with water radiolysis products and by the effect of irradiation on the physicochemical properties of a system (precipitate formation, aggregation of colloid particles, etc.)	Water containing H_2O_2 (in the presence of activated carbon), heavy metal ions (in the presence of a sorbent), or dyes (in dissolved and dispersed forms); molasses distillery slops; municipal wastewater in an aerosol flow; landfill leachate; water-petroleum product heterogeneous system; highly colored river water; and wastewater from a dyeing complex and a paper mill

Table 2.8. Assignment of the test systems to groups with different

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Kurucz *et al.* (2002) compared large-scale electron beam and bench-scale ⁶⁰Co irradiations of simulated aqueous waste streams (benzene, toluene, phenol, PCE, TCE and chloroform) with doses at most 8 kGy. In general, removal efficiencies were greater for gamma irradiation. The lower dose rate of the ⁶⁰Co irradiations produced lower concentrations of the important reactive species OH⁻ and e_{aq} which resulted in a higher fraction of radical/contaminant reaction and thus better removal. They stated that extremely high dose rates may be better at high contaminant concentrations (Kurucz *et al.*, 2002).

CHAPTER 3

MATERIALS AND METHODS

In this chapter, materials and methods which were used throughout this study are presented.

3.1. Chemicals and Laboratory Apparatus

Chemicals: Calcium acetate (extra pure), glucose monohydrate and the chemicals used in the basal medium were obtained from Merck Chemical Co., Germany. EUTECH Instrument pH buffer solutions (4, 7 and 10) were used for the calibration of pH-meter and pH-controller.

Laboratory apparatus: The laboratory apparatus used in the experiments are as follows:

- Thermo gas chromatograph equipped with a flame ionization detector and a 30 m column
- Shimadzu 8A gas chromatograph equipped with thermal conductivity detector
- LaborBrand magnetic stirrers (model L-71)
- Variable speed peristaltic pumps (Model No: 77120-52, 7521-10 Cole Parmer Instrument Co., USA)
- Standard pump heads (Model No: 7514-20 Cole Parmer Instrument Co., USA)
- Masterflex Norprene pump tubing (Model No: 6404-14)
- pH meter (Model 2906, Jenway Ltd, UK)
- Photometer (AquaLytic PC Multidirect)
- AquaLytic HR Total Nitrogen Set

- AquaLytic Total Phosphate Reagent Set
- HACH spectrophotometer (model: P:N 45600-02)
- HACH COD Digestion Solution Set (0-1500 ppm)
- Cable ties (Cole Parmer Instrument Co., USA)
- Connectors and fittings (World Precision Instrument Inc., USA)
- Teflon sealer tape
- Latex rubber tubing
- 110 mL and 250 mL serum bottles, rubber stoppers
- 100 µL gas tight glass syringes
- Tedla gas collection bags
- 2 mL vials with PTFE/silicone septa (Cole Parmer Instrument Co., USA)
- 0.45 and 0.22 µm filter paper (Milipore)
- Laboratory glass apparatus

3.2. Inocula

3.2.1. Mixed anaerobic cultures

Mixed anaerobic cultures, which were used in Biochemical Methane Potential (BMP) assays and batch reactors, were obtained from anaerobic sludge digesters of the Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant. The digesters have a retention time of 14 to 20 days and pH in the digester ranges from 7 to 7.5. The inocula was mixed and filtered through a screen with a mesh size of 1 mm before being used.

3.2.2. Anaerobic granular cultures

Anaerobic granular cultures used in the continuous upflow anaerobic sludge bed reactors (UASB) were obtained from the Wastewater Treatment Plant of Kazan Tekel Brewery Factory UASB reactors.

3.3. Opium Alkaloid Wastewater

Wastewater used in the experiments was obtained from the Opium Alkaloid Factory in Afyon, Turkey. Characteristics of wastewater varied throughout this study. Ranges for parameters were given in Table 3.1. Initial COD of wastewater used in each experiment was specified in the corresponding method sections. Wastewater was preserved in fridge at 4 ± 2 °C before used.

Parameter	Unit	Value
pH	-	4.5-5.2
Total COD	mg/L	22000-34780
BOD ₅	mg/L	21250
TSS	mg/L	1120-1700
VSS	mg/L	580-990
TKN	mg/L	1001.2
NH ₄ -N	mg/L	61.6-172.5
Total P	mg/L	4-5.21
Alkalinity	mg/L as CaCO ₃	144-1050
Color	Pt-Co	4750
Protein	mg/L	5330
Carbohydrate	mg/L	10000

Table 3.1. Characteristics of opium alkaloid wastewater

3.4. Basal Medium (BM)

The composition of the basal medium was as follows (mg/L): NH₄Cl (1200), MgSO₄·7H₂O (400), KCl (400), Na₂S·9H₂O (300), CaCl₂·2H₂O (50), (NH₄)2HPO₄ (80), FeCl₂·4H₂O (40), CoCl₂·6H₂O (10), KI (10), MnCl₂·4H₂O (0.5), CuCl₂·2H₂O (0.5), ZnCl₂ (0.5), AlCl₃·6H₂O (0.5), NaMoO₄·2H₂O (0.5), H₃BO₃ (0.5), NiCl₂·6H₂O (0.5), NaWO₄·2H₂O (0.5), NaWO₄·2H₂O (0.5), cysteine (10), NaHCO3 (6000). This basal medium contained all the necessary micro and macro nutrients required for optimum anaerobic microbial growth (Demirer and Speece, 1998).

3.5. Experimental Procedure

3.5.1. Biochemical methane potential (BMP)

BMP is a screening assay which is employed to give a preliminary estimate of potential amount of COD which can be bio-transformed to CH₄ and a preliminary estimate of any toxicity inherent in a wastewater (Owen *et al.*, 1979). If unacclimated biomass is assayed, however, the results will not be conclusive and therefore one's interpretation of BMP data should be tempered with considerable caution. No treatability assay should be curtailed because of negative results from the BMP, since biomass may not be properly acclimated and the toxicity may be eventually biodegraded. The BMP assay yields preliminary results. The BMP assays the amount of the organic contaminant in the wastewater which can potentially be converted to methane. Whereas BOD measures the depletion of an oxidized product (dissolved oxygen or DO), the BMP measures the production of a reduced product (methane gas). Since 395 mL of methane at 35 °C is equivalent to 1 g of COD removed from the wastewater, there is a stoichiometric relationship which allows calculation of the COD reduction in the liquid phase (Speece, 1996).

First BMP test was performed for three different COD concentrations namely 2400, 6000 and 9600 mg/L with and without basal medium (12 bottles, two duplicate for each) by using 250 ml serum bottles. These concentrations were chosen as 0.1, 0.25 and 0.4 times original COD of wastewater used and chosen in order to identify treatability of alkaloid at this low concentrations. The liquid volume of the bottles was 100 ml. Serum bottles were seeded with mixed anaerobic cultures obtained from anaerobic sludge digesters of the Ankara Tatlar Domestic Wastewater Treatment Plant. First six bottles had no basal medium whereas the last six had. Four control bottles were used with and without basal medium. Bottles were then purged with a gas mixture of 75% N_2 and 25% CO₂ for 3–4 min to supply anaerobic conditions and to adjust the pH to an appropriate value, after which they were

sealed with rubber stoppers. Finally, the bottles were incubated in a temperaturecontrolled room at 35 ± 2 °C.

The results of five different determinations of gas content according to method described in Section 3.6 indicated that the average methane content of the biogas was $66\%\pm5$.

Amount of seed, BM and wastewater in each BMP bottle for BMP experiment were given in Table 3.2. It should be noted that the concentration of BM added to serum bottles were 5 times original basal medium concentration in order to provide original BM concentration in test reactors. The initial total COD of alkaloid wastewater used in this experiment was 24000 mg/L.

TEST	Seed (ml)	BM (ml)	Wastewater (ml)	Distilled Water (ml)
2400 mg/L without BM	40	-	10	50
6000 mg/L without BM	40	-	25	35
9600 mg/L without BM	40	-	40	20
2400 mg/L with BM	40	20	10	30
6000 mg/L with BM	40	20	25	15
9600 mg/L with BM	40	20	40	-
Seed control without BM	40	-	-	60
Seed control with BM	40	20	-	40

 Table 3.2. Amount of seed, BM and wastewater in each BMP bottle for

 BMP test

BMP experiment performed for co-substrate test differentiated from first BMP assay by serum bottles used, wastewater composition (initial COD of 33,000 mg/L) and co-substrate supplementation. Bottles used in this experiment were 110 mL

with 50 mL liquid volume. Co-substrates, glucose and acetate, were supplemented from stock solutions of 250 g/L in different amounts to obtain desired concentrations. Amount of seed, BM, co-substrate and wastewater in each BMP bottle for co-substrate experiment was given in Table 3.3.

TEST	Seed (mL)	BM (mL)	Waste water (mL)	Distilled Water (mL)	Glucose (mL)	Acetic acid (mL)
Seed control	10.0	10.0	-	30.0	-	-
Seed + Glucose Control	10.0	10.0	-	29.0	1.0	-
Seed + Calcium acetate Control	10.0	10.0	-	29.0	-	1.0
Seed + Glucose + Calcium acetate Control	10.0	10.0	-	28.0	1.0	1.0
9000 mg/L COD + Glucose	10.0	10.0	13.6	15.4	1.0	-
9000 mg/L COD + Calcium acetate	10.0	10.0	13.6	15.4	-	1.0
9000 mg/L COD + Glucose + Calcium acetate	10.0	10.0	13.6	14.4	1.0	1.0
13000 mg/L COD + Glucose	10.0	10.0	19.7	9.3	1.0	-
13000 mg/L COD + Calcium acetate	10.0	10.0	19.7	9.3	-	1.0
13000 mg/L COD + Glucose + Calcium acetate	10.0	10.0	19.7	8.3	1.0	1.0
18000 mg/L COD + Glucose	10.0	10.0	27.3	1.7	1.0	-
18000 mg/L COD + Calcium acetate	10.0	10.0	27.3	1.7	-	1.0
18000 mg/L COD + Glucose + Calcium acetate	10.0	10.0	27.3	0.7	1.0	1.0

 Table 3.3. Amount of seed, BM, glucose, calcium acetate and wastewater in

 each BMP bottle for co-substrate experiment

3.5.2. BMP experiment for radiation effect

BMP experiment performed in order to identify effect of radiation on anaerobic treatability of opium alkaloid wastewater differed from first BMP assay by wastewater composition and by employed serum bottles (50 mL liquid volume). Wastewater having initial COD of 34,000 mg/L was sent to Turkish Atomic Energy Authority (TAEK) for ⁶⁰Co- γ -irradiation and two doses of radiation, namely 40 and 140 kGy were applied to this wastewater. Initial COD values for original and radiated wastewater were 35000 mg/L. There were two kinds of wastewater treated with 140 kGy dose radiation sent at different times. Both were used in analysis.

Two different initial COD values (about 25000 and 14000 mg/L) were used for all wastewater in this BMP test. Amount of seed, BM and wastewater in each BMP bottle for radiation effect experiment was given in Table 3.4. The basal medium supplied was 12 times more concentrated than the original BM concentration in order to provide original BM concentration in test reactors.

TES	Т				Distilled	
Concentration	Raw or	Seed	BM (ml)	Wastewater	Wotor	
of wastewater	irradiated	(ml)	DIVI (IIII)	(ml)	(ml)	
(COD)	(dose)				(IIII)	
25 g/L	Raw	10	4	36	-	
14 g/L	Raw	10	4	20	16	
25 g/L	40 kGy	10	4	36	-	
14 g/L	40 kGy	10	4	20	16	
25 g/L (1)	140 kGy	10	4	36	-	
14 g/L (1)	140 kGy	10	4	20	16	
25 g/L (2)	140 kGy	10	4	36	-	
14 g/L (2)	140 kGy	10	4	20	16	

 Table 3.4. Amount of seed, BM and wastewater in each BMP bottle for radiation effect experiment

3.5.3. Batch reactors

Two reactors of 5L volumes with and without basal media were used. They had initial COD concentrations of 8900 mg/L. Gas was collected in plastic bags and measured by water displacement device. The reactors were continuously mixed with magnetic stirrers. Generated gas was collected by gas bags and measured using water displacement device.

3.5.4. Acidification reactor

The culture used in the acidification reactor was prepared by feeding a 2 L fedbatch reactor with alkaloid wastewater and maintaining pH at 5-5.5 by a pHcontroller. HRT and SRT of this fed-batch system were two days and this reactor was operated for 1 month. Obtained culture was preserved and acidogenic activity assay was carried out with these inocula. Acidogenic activity test was applied according to literature (Soto *et al.*, 1993), pH and glucose was determined accordingly.

Acidification reactor was a Sequencing Batch Reactor (SBR) type and operated with initial COD values of 10, 15, 19 and 27.5 g/L with HRT of 2 days for about 110 days. After day 110, HRT was decreased to 1 day. Reactor was agitated for 23 hours, let to settlement for 1 hour; effluent was then collected followed by feed addition. pH was maintained between 5 and 5.5 by a pH controller connected to an ON/OFF type centrifugal pump providing NaOH. Volatile fatty acid (VFA) concentration in the effluent of acidification reactor was measured once in three days.

3.5.5. Upflow Anaerobic Sludge Blanket (UASB) reactors

Three UASB reactors were operated for 138 days. The first reactor, Reactor 1 (R1), was fed with opium alkaloid wastewater. The second reactor, Reactor 2 (R2), had

feed composition of 25% (in terms of COD) calcium acetate as co-substrate and 75% alkaloid wastewater (in terms of COD). The third reactor, Reactor 3 (R3), was the second stage of a two phase system where the first phase (acidification) was a SBR reactor fed with alkaloid wastewater.

Reactors have liquid volumes of 790 mL. They were seeded with anaerobic granular sludge resulting in an effective volume of 500 mL (about 60% of reactor volume). This effective volume was used in calculations for HRT throughout the study. The feed was supplied from feed bottles by a centrifugal pump with varying speed. Effluent was collected in gradual cylinders and measured every day in order to assure that desired HRT was obtained in the reactors. Total COD and reactor pH was measured twice a week and every other day, respectively. Gas was also collected and measured volumetrically.

Initial COD concentrations of the reactors were 10 g/L at the start-up and were increased gradually. Total COD was the parameter used to decide on organic loading rate (OLR) changes.

3.6. Analytical Methods

<u>pH:</u> pH measurements were performed with a pH meter (Model 2906, Jenway Ltd, UK) and a pH probe.

<u>Total Volatile Fatty Acids:</u> Thermo gas chromatograph equipped with a flame ionization detector and a 30 m column was used for VFA analyses. The column temperature was started at 100 °C with 2 min. holding time and then increased to 250 °C with 8 °C/min ramping, and the injector/detector temperature was kept at 200/350 °C with nitrogen as the carrier gas and a flow rate of 30 mL/min. The gas flow rates were gauged at 350 mL/min for air and 35 mL/min for hydrogen. Liquid samples were prepared by centrifuging for 15 min at 3,000–4,000 rpm and by filtering 5 mL of the supernatant through a 0.22 mm glass fiber filter (Whatman

Co.). The filtered samples were acidified with 99% formic acid to a pH less than 3 to convert the fatty acids to their undissociated forms (i.e., acetic acid, propionic acid, butyric acid, etc.) before injecting 1 μ L of the acidified samples into the GC.

Suspended solids and volatile suspended solids: Suspended solids and volatile suspended solids were determined according to Standard Methods (2540 D-E, 1998).

<u>Chemical oxygen demand (COD)</u>: COD concentration was measured with a Hach spectrophotometer (model: P:N 45600-02) and vials for COD 0–1500 ppm according to an EPA approved reactor digestion method as given in Hach Analysis Handbook (HACH, 2000).

<u>5-Day Biochemical Oxygen Demand (BOD₅)</u>: BOD₅ concentration was determined according to Standard Methods (5210 B,1998).

<u>Total kjeldahl nitrogen and ammonia nitrogen:</u> Total kjeldahl nitrogen and ammonia nitrogen were determined according to Standard Methods (4500-N_{org} B, 4500-NH₃ B-C,1998).

<u>Total phosphorus:</u> Total phosphorus was determined according to Standard Methods (4500-P B-E, 1998).

<u>Carbohydrate:</u> Carbohydrate concentration was measured using the method of Dubois (Dubois *et al.*, 1956).

<u>Protein:</u> Protein concentration was measured using the Hartree (Hartree) modification of the Lowry (Lowry *et al.*) method.

<u>Color:</u> Color was measured with a Hach spectrophotometer (model: P:N 45600-02) according to platinum-cobalt standard method adapted from Standard Methods as given in Hach Analysis Handbook (HACH, 2000).

<u>Headspace gases:</u> Gas produced in BMP bottles was measured by a water displacement device consisting of a 50 mL of burette and 250 mL water reservoir.

<u>Methane content of gas:</u> The content of CH_4 in biogas was determined for BMP assay for treatability of alkaloid wastewater and for co-substrate test as follows: A known volume of the headspace gas (*V*1) produced in a serum bottle was syringed out and injected into another serum bottle which contained KOH solution. This serum bottle was shaken manually for 3–4 min so that all the CO_2 and H_2S was absorbed in the concentrated KOH solution. The volume of the remaining gas (*V*2) which was 99.9% CH₄ in the serum bottle was determined by means of a syringe. The ratio of V2:V1 provided the content of CH₄ in the headspace gas (Ergüder *et al.*, 2000; Ergüder *et al.*, 2001).

The content of CH₄ in biogas was determined for BMP assay for effect of radiation on treatability as follows: Gas samples for gas composition analysis were taken by a 100 μ L Hamilton gas-tight glass syringe. The gas composition was determined by a gas chromatography (GC) unit (Shimadzu 8A) equipped with thermal conductivity detector. Methane, nitrogen and carbon dioxide were separated through a 3 m Porapak Q, 5 mm I.D. column. Column was operated with helium as the carrier gas at a constant pressure of 20 kPa at 40°C. The injector was maintained at 100°C, and the detector temperature was set to 100°C. The calibration was carried out by using a standard gas mixture composed of N₂, CH₄, and CO₂.

<u>Glucose</u>: Glucose concentration in acidogenic activity assay was determined using the dinitro salicylic acid (DNS) reactive method (Miller, 1959).

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the batch anaerobic treatability experiments and continuous anaerobic reactor experiments for opium alkaloid wastewater are presented and discussed.

4.1. Batch Anaerobic Treatability Experiments

These experiments were performed in order to investigate anaerobic treatability of opium alkaloid wastewater. Biochemical methane potential (BMP) assay was conducted for three different initial COD concentrations of alkaloid wastewater. Two lab-scale batch reactors are then operated for about 50 days. Two different co-substrates, namely glucose and calcium acetate, were used in a BMP assay for the determination of effect of co-substrates on treatability of alkaloid wastewater.

4.1.1. BMP assay for treatability of opium alkaloid wastewater

Treatability of opium alkaloid wastewater was investigated by means of BMP assay for initial COD concentrations of 2400, 6000 and 9600 mg/L with and without basal medium. The cumulative gas production for these three concentrations and control reactors were given in Figure 4.1. It can be seen from Figure 4.1 that no significant gas production was observed for both seed control reactors with and without basal medium (BM) throughout the experimental period of 48 days (6.6 and 4.8 mL for seed control with and without BM respectively). This result pointed out that basal medium and seed cultures did not have a significant contribution to the cumulative gas production, though gas produced in the control reactor with BM was slightly higher than gas produced in the control reactor that does not contain BM. The difference was due to presence of basal medium. Gas produced in other reactors was emanated from biodegradation of alkaloid wastewater and net cumulative gas production (NCGP) for these reactors were calculated by subtracting seed control gas.



Figure 4.1. BMP experiment result for opium alkaloid wastewater (2400, 6000 and 9600 mg COD/L) with and without basal medium

The effect of basal medium for NCGP is not significant for the concentration of 2400 mg COD/L of alkaloid since on day 15 they both produced 75 mL of gas and

reached to a gas production value of around 85 mL on day 48. At this initial COD concentration (2400 mg/L) nutrients present in the wastewater itself were sufficient for the treatment. However, for the second and third initial COD concentrations (6000 and 9600 mg/L respectively), the difference between the gas produced in the reactors with BM and the reactors without BM was remarkable. COD:N:P ratio (average) for alkaloid wastewater was 6000:200:1. COD:N:P ratio of not less than 100:1.1:0.2 was recommended by McCarty (1964) for anaerobic treatment. Therefore a nutrient deficiency for alkaloid wastewater exists. For all initial COD concentrations ame amount of basal medium was supplied. Therefore, at low COD value of wastewater carbon became limiting whereas at high COD concentrations nutrient deficiency was more important hence explaining the effect of BM at high COD values.

Day 15 was the point where for all test reactors an average of 84% of the total gas production was ceased. On this day the reactor containing 6000 mg COD/L alkaloid with BM produced 225.6 mL doubling NCG produced in the reactor without BM (109 mL). The same result was observed for the reactors containing 9600 mg COD/L as the one with BM produced 167.2 mL NCG whereas the reactor without BM generated 363.9 mL by 15th day. A previous study on malt whisky wastewater demonstrated similar results. For lower initial COD concentrations of 5.07 and 10.1 g/L total gas production did not show any difference for the reactors with and without basal medium but for 15.2 g COD/L the nutrient supplemented set produced about 20% more gas than the one without basal medium (Uzal *et al.*, 2003). If nutrient content is not enough; carbon availability, itself, cannot increase the efficiency of treatment. Considering the results of BMP assay, nutrient supplementation was therefore a requirement for the anaerobic treatment of opium alkaloid wastewater especially at higher concentrations.

There was a lag period of about 5 days (the acclimation period for the anaerobic cultures) for all the reactors as seen in Figure 4.1. This was a relatively short time of acclimation and this short acclimation period may have resulted from high

carbohydrate content of the alkaloid wastewater (10 g/L in 35 g COD/L alkaloid wastewater) since growth constant for carbohydrate is much higher than that of proteins and fatty acids (McCarty, 1964). For the influent COD concentration of 2400 mg/L, cultures exerted 90% and 87% of the total gas production with and without basal medium, respectively, in the first 15 days. Similarly; for influent COD concentrations of 6000 mg/L the percentages were 92% and 81% and for 9000 mg/L they were 80% and 76%. At these concentrations of opium alkaloid wastewater, there was no indication of inhibition at all.

Figure 4.2 shows COD removal efficiencies calculated from NCGP for all reactors. For the lowest COD concentration of BMP assay which was 2400 mg/L removal efficiencies and NCGP for reactors with BM and without BM were almost same as stated earlier (54 and 56%, 78.1 and 81 respectively). The removal efficiencies are both below 60%. For such a problematic waste as alkaloid wastewater, removal efficiencies were expected to decrease by increasing initial COD concentration (Sevimli *et al.*, 2000). However, in this BMP experiment, lowest efficiency was obtained at the lowest initial COD concentration of 2400 mg/L. To explain this phenomena, it can be speculated that anaerobic treatment is generally considered unsuitable for low concentrated wastewaters because of the low utilization rate of substrate at low concentrations (Ndon and Dague, 1997). Half velocity constant is lower for anaerobic bacteria that at lower substrate concentrations, as in the case for alkaloid wastewater, so that removal (biodegradation) happened to be smaller.

The NCGP for the reactor with alkaloid wastewater having initial COD of 6000 mg/L with and without BM at the end of experiment were 239.5 and 130.5 mL, respectively. The corresponding theoretical removal efficiencies were 66 and 36%. The same result holds for this concentration also that lower removal efficiency was obtained for initial COD concentration of 6000 mg/L with BM than the initial COD concentration of 9600 mg/L with BM.



Figure 4.2. COD removal efficiencies for BMP assay

The highest NCGP (447.7 mL at the end of the experiment) was obtained in the reactor having initial COD concentration of 9600 mg/L with BM. The calculated removal efficiency for this reactor was 77%. On the other hand, the analogous reactor excluding BM has a NCGP value of 214.5 mL corresponding to a theoretical COD removal efficiency of 37%. The effect of BM at this initial COD concentration was very remarkable that addition of nutrients increased theoretical COD removal efficiency from 37% to 77%.

Sevimli *et al.* (2000), operated pilot and lab scale anaerobic reactors with opium alkaloid wastewater. In contradiction to present BMP experiment highest COD removal efficiency obtained was at initial COD concentration of 5000 mg/L which was the lowest concentration among those reactors (90% COD removal). Increasing initial COD concentration led to decreasing efficiency of COD removal. At 16000 mg/L the efficiency dropped to a value of 62%. This previous anaerobic studies

demonstrated that above some initial COD concentration, the efficiency of the treatment of alkaloid wastewater decreased drastically.

4.1.2. Batch reactors

The lab-scale mixed batch reactors having 5 L effective volume were operated for 51 days. One of the reactors was supplied with basal medium whereas the other was not. The experiment was terminated at day 51 since gas production was ceased. The cumulative gas production observed is depicted in Figure 4.3.

Gas production emanated from the reactor with BM is more than gas production from the reactor without BM. The resultant theoretical COD removal efficiencies, calculated from the cumulative gas production, were 38 and 41% for the reactors without and with BM, respectively. The actual removal efficiencies which were found by measuring final COD concentrations at the end of the test period were 40 and 50% for the reactors without and with BM, respectively. The resultant efficiencies once again proved the need for basal medium for anaerobic treatment of alkaloid wastewater.



Figure 4.3. Cumulative gas production for the lab-scale batch reactors (8900 mg COD/L with and without BM)

Removal efficiency in the batch reactor with BM was lower when compared to BMP test reactor having an initial COD concentration of 9600 mg/L. It was expected to obtain a better treatment at the batch reactors since agitation was provided. The experiments were both performed with same MLVSS concentration and BM supplied was also the same. The only factor that could lead the difference in the removal efficiencies was the wastewater. The original alkaloid wastewater used in BMP experiments had an original COD value of 30 g/L whereas the one used in batch reactors had COD value of 40 g/L. Effluent COD of the Opium Alkaloid Factory changes seasonally between 18000 and 42500 mg/L (Sevimli *et al.*, 1999; Aydın, 2002). Batch reactors proved to be insufficient for the treatment of opium alkaloid wastewater since at initial COD values similar to concentration provided in batch reactors higher treatment efficiencies were obtained. Sevimli *et al.* (2000) obtained COD removal efficiencies of 87 and 83% by continuous

anaerobic systems at 8000 and 12000 mg/L initial COD concentrations, respectively. Also for concentration 18000 mg/L another BMP experiment was conducted and efficiency below 30% was obtained.

4.1.3. BMP assay with glucose and/or calcium acetate as co-substrates

Previous studies in the literature (Sevimli *et al.*, 2000; Aydın, 2002) and this study indicated that above certain threshold level, opium alkaloid wastewater became inhibitory to anaerobic cultures and the treatment efficiency decreased drastically. To overcome this barrier co-substrate use was suggested (Vidal *et al.*, 1999; Tay *et al.*, 2001). Atuanya and Chakrabarti (2003) stated that addition of alternative utilizable substrate (such as glucose) can mitigate toxic effects and enhance degradation. Tay *et al.* (2001) studied degradation of phenol with glucose (4000 mg/L) as co substrate. She *et al.* (2005), investigated biodegradation of 2,4-dinitrophenol and 3-nitrophenol with glucose and volatile fatty acids as co-substrates. Batch experiments were performed and as high as 80% efficiencies were achieved.

The cumulative gas productions were depicted in Figure 4.4 and 4.5 for the cosubstrate experiment. In Figure 4.4, cumulative gas production was plotted for three different co-substrates which were glucose, acetate and glucose-acetate mixture. Three different initial alkaloid wastewater COD values were used namely 9000, 13000 and 18000 mg/L. These values were chosen to represent treatability of alkaloid wastewater at the concentrations where the inhibition tends to start (9000 mg/L), where no other anaerobic treatability data existed (18000 mg/L) and a point in between (13000 mg/L). The seed control reactor produced 7.9 mL gas at the end of the test period which was 42 days. As a result of this observation it can be stated that basal medium and seed cultures did not have significant contribution to gas production in all the reactors. The net cumulative gas production (NCGP) values, which were obtained from the subtraction of seed control gas from the gas production in test reactors, were used in the analysis of the data. The acclimation period of the mixed anaerobic culture which was found as 5 days in the BMP experiment explained before seemed to be disappeared in co-substrate test. The gas production started rapidly from day 1 and ceased at the end of a period of around 40 days. The reason for this disappearance was because of the addition of co-substrates to test reactors. Co-substrates, glucose and acetate, were easily biodegradable by anaerobic bacteria. The initial gas production in test reactors was mainly originated from the degradation of co-substrates. Co-substrates act as carbon source while acclimation of the anaerobic culture to alkaloid wastewater took place.

Other researchers proved the effect of glucose and acetate as co-substrates on shortening of acclimation period and on improvement of removal efficiency of toxic substrates (Pereboom *et al.*, 1994; Tay *et al.*, 2001; She *et al.*, 2005; Veeresh *et al.*, 2005). The reactors can also recover from the shock loads with co-substrates in use more easily (Veeresh *et al.*, 2005).

Theoretical gas productions of glucose and calcium acetate were calculated from the stoichiometry of the mentioned chemicals to methane and carbon dioxide (Battersby and Wilson, 1989). Theoretical gas productions found for 5000 mg/L glucose and 5000 mg/L calcium acetate were 210 and 160 mL respectively. The net cumulative gas productions of glucose, calcium acetate and glucose-calcium acetate mixture controls were observed as 166.1, 100.1 and 273.1. Comparing these values with the theoretical gas productions calculated, it was concluded that 21% of glucose, 37% of calcium acetate and 26% of glucose-calcium acetate mixture were not degraded.

In Figure 4.4 it is clearly seen that all the reactors with alkaloid wastewater produced NCGP more than the control reactors. This additional gas production emanated from the degradation of alkaloid wastewater. The net cumulative gas productions, obtained by subtracting gas generated in control reactors from gas produced in test reactors, for three concentrations of alkaloid wastewater were depicted in Figure 4.6.



Figure 4.4. Cumulative gas production for co-substrate experiment a) Glucose and calcium acetate as co-substrates, b) Calcium acetate as co-substrate, c) Glucose as co-substrate



Figure 4.5. Cumulative gas production for co-substrate experiment a) 9000 mg COD/L alkaloid wastewater, b) 13000 mg COD/L alkaloid wastewater, c) 18000 mg COD/L alkaloid wastewater

<u>Glucose-calcium acetate as co-substrates:</u> In Figure 4.4a, cumulative gas productions for 9000, 13000 and 18000 mg COD/L alkaloid wastewater with glucose-calcium acetate mixture as co-substrate can be seen. Glucose-acetate control produced a NCGP of 273.1 mL. The other three test reactors showed similar patterns up to day 21 which was half of the experiment period of 42 days and generated 366.7 (9000 mg COD/L), 340.5 (13000 mg COD/L) and 378.5 (18000 mg COD/L) mL of NCGP. Then, the cumulative gas production started to differentiate and at the end of experimental period NCGPs were 435, 504.5 and 556 mL for 9000, 13000 and 18000 mg COD/L test reactors, respectively.

<u>Calcium acetate as co-substrate:</u> In Figure 4.4b, CGPs of acetate control and test reactors containing acetate as co-substrate were depicted. Acetate control produced 100.1 mL of NCG. The result encountered in this co-substrate was similar to glucose-acetate case. At day 22, test reactors generated 300.2, 300 and 309 mL NCG, which were almost same, for initial COD concentrations of 9000, 13000 and 18000 mg/L, respectively. Then as in case of glucose-acetate mixture, the reactors were ended up with different NCGP values of 306.4, 342.5 and 492.1 mL, respectively.

<u>Glucose as co-substrate</u>: In Figure 4.4c, the results for the test reactors where glucose was used as co-substrate were represented. Glucose control generated NCG of 166.1 mL. In contrast to previous co-substrate results, the reactor with the highest initial COD of alkaloid wastewater produced more gas in the middle of experimental period. The day 22 NCGP for the test reactors with glucose as co-substrate were 311.8, 317.4 and 411.6 mL for initial COD concentrations of 9000, 13000 and 18000 mg/L, respectively. The final NCGP values were 324.2, 456.7 and 501.9 mL, respectively.

Among these three different cases the highest gas productions were observed at glucose-acetate mixture since both glucose and calcium acetate were supplied in equal concentrations of 5000 mg/L. For all co-substrates, gas production increases

with increasing initial COD of alkaloid wastewater. For case (a) where glucoseacetate mixture was supplied as co-substrate, 93% of the glucose-acetate control gas was generated by the 24th day. This result was valid for other two cases where for case (b) 96% and for case (c) 98% of the total gas production in the control reactors was ceased by the 24th day. Actually, it can be stated that for the control reactors gas production ceased by half of the experimental period. This supports the idea that source of gas generation in test reactors in the beginning was mainly due to degradation of co-substrates.

In Figure 4.5, CGP was plotted for three different initial COD concentrations of wastewater namely 9000, 13000 and 18000 mg/L.

<u>9000 mg COD/L</u>: The highest gas production (435 mL) was observed when glucose-acetate mixture was used as co-substrate. For this concentration of wastewater glucose and acetate assisted reactors generated approximately equal amounts of NCG (311.8 and 306.4 mL).

<u>13000 mg COD/L</u>: Same result holds for the case of glucose-acetate mixture for this concentration where highest NCGP of 504.5 mL occurred. Glucose supplemented reactor produced 456.7 mL of NCG which was close to this highest value. The reactor with acetate generated 342.5 mL of net cumulative gas.

<u>18000 mg COD/L</u>: At this concentration of opium alkaloid wastewater, NCGPs emanated from three different reactors with three different co-substrates were relatively close to each other. The values were 556, 492.1 and 501.9 mL for glucose-acetate, calcium acetate and glucose, respectively.

Among these three concentrations, in reactors supplied with calcium acetate, CGP tend to increase less sharply in the beginning of experiment. In order to present a more clear picture of the situation; net cumulative gas productions, obtained by subtracting gas generated in control reactors from gas produced in test reactors, for

three concentrations of alkaloid wastewater were depicted in Figure 4.6. Average values were taken for control reactors during subtraction in order to avoid negative data.

In Figure 4.6a (9000 mg COD/L alkaloid concentration), effect of glucose for treatment of alkaloid wastewater was relatively scarce that control subtracted net cumulative gas (CSNG) of 209 mL was obtained. On the other hand, reactors supplied with the other two co-substrates produced similar CSNG of 261.8 mL (calcium acetate) and 278.8 mL (glucose-acetate).

In Figure 4.6b (13000 mg COD/L alkaloid concentration), glucose and glucoseacetate supplied test reactors generated almost equal CSNG of 341.6 and 348.3 mL, respectively. The CSNG observed in the test reactor with acetate was 297.8 mL.

In Figure 4.6c (18000 mg COD/L alkaloid concentration), the highest CSNG was obtained in reactor with acetate and found as 447.5 mL. The other two reactors generated CSNG of 386.7 mL (glucose) and 399.8 mL (glucose-acetate).

The results depicted in Figure 4.6 varied with initial COD concentration of the wastewater. The NCGP data were then normalized with respect to control reactors by dividing the gas produced in test reactors to gas produced in control reactors. The normalized gas productions were given in Figure 4.7. Points above 1 on the y-axis of graph in Figure 4.7 indicate a positive gas production of test reactors with respect to control reactors.



Figure 4.6. Control subtracted net cumulative gas production for co-substrate experiment a) 9000 mg COD/L alkaloid wastewater, b) 13000 mg COD/L alkaloid wastewater, c) 18000 mg COD/L alkaloid wastewater

In Figure 4.7b, similar to case (a), normalized gas production for reactor with calcium acetate as co-substrate was 8.3 by day 13 but dropped to 3.3 by day 42. The other two reactors had normalized gas values of 2.7 (glucose) and 1.8 (glucose-acetate).

In Figure 4.7c, the acetate added reactor had a normalized gas production of 10.2 by day 12 and 4.6 by the end of the experimental period. Glucose assisted reactor generated a normalized gas value of 2.9 and glucose-acetate supplemented reactor resulted in 2.0 at day 42.

For all the concentrations acetate supplemented reactors have very high normalized gas data in the beginning up to around day 15. The reason is that acetate control developed a lag phase during this period. After day 15 normalized gas productions started to decrease. However, among these three co-substrates, reactors with calcium acetate had the highest normalized gas productions for all concentrations at the end of the experimental period.

Overall removal efficiencies were calculated for control and test reactors by using theoretical COD values of glucose and acetate. 5000 mg/L of glucose is equivalent to 5333 mg COD/L whereas 5000 mg/L acetate is equivalent to 4745 mg COD/L. In this context, glucose-acetate mixture has a COD value of 10078 mg/L. These values were added up to initial alkaloid COD values in test reactors (e.g. 14333 for test reactor with initial COD of 9000 mg/L and glucose as co-substrate). The normalized removal efficiencies which are defined as the ratio of % COD removal for test reactors to % COD removal for control reactors were depicted in Figure 4.8.



Figure 4.7. Normalized net cumulative gas production for co-substrate experiment a) 9000 mg COD/L alkaloid wastewater, b) 13000 mg COD/L alkaloid wastewater, c) 18000 mg COD/L alkaloid wastewater

As in Figure 4.7, normalized removal efficiencies indicated very high values in the beginning for test reactors containing calcium acetate. Other reactors with glucose and glucose-acetate provided similar results and normalized COD removal efficiencies remained around ratio of 1 throughout the experimental period. For the highest initial alkaloid wastewater concentration of 18000 mg COD /L, reactors with glucose and glucose-acetate both ended up with ratios below 1 while the one with acetate had a ratio of approximately 1 (Figure 4.8c). This situation held for the other two cases where reactors supplied with acetate had ratios around 1 and reactors supplied with glucose or glucose-acetate had ratios below 1.

In Figure 4.8a and 4.8b corresponding to initial COD values of 9000 and 13000 mg/L, respectively, normalized efficiencies for acetate supplemented reactors were also above 1 up to day 15. However, they both ended with values around 1 by the end of the experimental period.

Using co-substrates did not improve alkaloid removal efficiency significantly but it had a positive effect on reducing the acclimation period of the microorganisms to alkaloid wastewater. Normalization rather than subtracted COD removal efficiencies were used in analysis of data since synergistic effects of co-substrates with alkaloid wastewater should have effected cumulative gas productions in test reactors.


Figure 4.8. Normalized COD removal efficiencies for co-substrate experiment a) 9000 mg COD/L alkaloid wastewater, b) 13000 mg COD/L alkaloid wastewater, c) 18000 mg COD/L alkaloid wastewater

4.2. Continuous Anaerobic Reactor Experiments

These experiments were performed in order to investigate treatment of alkaloid wastewater in continuous high-rate anaerobic reactors, explicitly UASB. For this purpose, three UASB reactors were operated for 138 days. The aim was to examine the effect of pre-acidification and co-substrate use on continuous anaerobic treatment of alkaloid wastewater as well as to find out anaerobic treatability limit of alkaloid wastewater with UASB system.

In this section, results of acidification reactor and UASB reactor experiments were presented and discussed.

4.2.1. Acidification reactor

Culture which was used in acidification reactor was obtained using the method mentioned in Chapter 3. Acidogenic activity assay (used to determine the acidogenic activity of cultivated inocula) results were presented in Figure 4.9.

As it is seen in Figure 4.9, substrate composition and pH were monitored throughout the acidogenic activity assay. After the first feeding and once the substrate (glucose) was completely consumed, a second addition was carried out. During the first addition of acidogenic activity assay, 8 hours of lag period was observed. This lag phase was also observed in the previous study of Soto *et al.* (1993), in which the acidogenic activity assay methodology (used in present study) was developed.



Figure 4.9. Substrate concentration and pH change in acidogenic activity assay for cultivated culture

The lag phase disappeared in the second day. As depicted in Figure 4.9, a sharp increase in pH from 7.1 to 5.3 was observed at day 2 while substrate was consumed from 2 g/L. The consumption was indeed rapid that by the first hour around 70% of glucose was vanished. By pre-feeding (day 1), sludge adaptation was achieved and it was possible to obtain a correct activity value in second feeding (Soto *et al.*, 1993). Acidogenic activity of sludge which is the ratio of substrate utilization rate to

microorganism concentration was found from Figure 4.9 second feeding data. First consumption rate in second feeding was used to estimate the activity as 22.6 g COD/g VSS.d. Acidogenic activity was determined from the substrate (glucose) removal rate. To calculate specific activity the value obtained was divided by the VSS concentration in each case.

Soto *et al.* (1993) found specific acidogenic activity of sludge from an anaerobic filter at different filter heights. The highest activity occurred at 5 cm with a value of around 24 g COD/g VSS.d and activity dropped to 17 g COD/g VSS.d at filter height of 40 cm. Also in the study mentioned above, sludge from an anaerobic digester treating wastewater from the industrial processing of mussel was examined for its acidogenic activity and a value of 13.6 was obtained. In another study, maximum specific acidogenic activity was found as 38.1 g COD/g VSS.d for a labscale anaerobic baffled reactor sludge (first compartment). The activity decreased to a value below 5 g COD/g VSS.d in the second compartment of ABR (Punal *et al.*, 1999). Comparing the values in literature with the one found here, acidogenic activity of culture cultivated was considerably high and suitable for usage in pre-acidification.

Acidification reactor was operated at pH range 5-5.5 with different loading rates. In Figure 4.10, total volatile fatty acid production (TVFA) and degree of acidification (DoA) (VFA in COD bases / Total COD of influent) were depicted.

<u>Influent COD = 10 g/L, HRT = 2 days</u>: In Figure 4.10a, organic loading rate (OLR) of 5 g/L.d was supplied to acidification reactor. TVFA concentration tended to increase from 1424 (day 3) to 2114 (day 4) mg/L as HAc. An average DoA of 19% was obtained at this OLR.

<u>Influent COD = 15 g/L, HRT = 2 days:</u> In Figure 4.10b, OLR was 7.5 g/L.d. TVFA production was between 2091-3142 mg/L as HAc excluding the lowest value of 1411 mg/L. The degree of acidification for this OLR was found as 17%.



Figure 4.10. Total volatile fatty acid and acidification ratio for acidification reactor with initial COD-HRT values of a) 10 g/L-2 days, b) 15 g/L-2 days, c) 19 g/L-2 days, d) 27.5 g/L-2 days, and e) 27.5 g/L-1 day

<u>Influent COD = 19 g/L, HRT = 2 days</u>: In Figure 4.10c, OLR for acidification reactor was 9.5 g/L.d. TVFA production was between 3093-3718 mg/L as HAc excluding the lowest value of 2316 mg/L. The average degree of acidification for this OLR was found as 17%.

Influent COD = 27.5 g/L, HRT = 2 days: In Figure 4.10d, OLR was kept as 13.75 g/L.d. TVFA production values were between 3505-4771 mg/L as HAc excluding the lowest value of 2959 mg/L. The average DoA for this OLR was 16%.

Influent COD = 27.5 g/L, HRT = 1 day: In Figure 4.10e, OLR was increased to 27.5 g/L.d. Two TVFA values were obtained which were 2517 and 2720 mg/L as HAc. The average DoA for this OLR was found as 10% using these two VFA data.

Total VFA production in acidification reactor varied with changing OLR and HRT. Increasing loading rate resulted in increasing VFA concentration up to OLR = 27.5 g/L.d. Modifying HRT decreased VFA production as well as degree of acidification. Highest average DoA (19%) was obtained at lowest OLR of 5 g/L.d. Then DoA values remained around 16-17% for OLRs 7.5 to 13.75 g/L.d. Total COD change was insignificant in the acidification reactor.

For two phase systems DoA and HRT of acidification reactor were accepted as important factors influencing overall efficiency (Lettinga and Hulshoff, 1991; Alexiou and Anderson, 1997; Ahn *et al.*, 2001a; Ahn *et al.*, 2001b). Ahn et al. (2001b) studied brewery wastewater in UASB reactor and concluded that long hydraulic retention time (HRT) for the acidification phase was detrimental to the sludge granulation process in the UASB reactor. Lettinga and Hulshoff (1991) claimed that pre-acidification should be partial (20–40%) in achieving higher loading rates, reporting on completely mixed acidification at 6–24h HRT. Alexiou and Anderson (1997) suggested 40–50% acidification.

Partial acidification (around 20%) was obtained in this study at 2 days of HRT whereas 10% was achieved at 1 day of HRT. The composition of VFA produced for different OLRs were depicted in Figure 4.11.

<u>Influent COD = 10 g/L, HRT = 2 days</u>: In Figure 4.11a, 90% of TVFA consisted of acetic acid in the beginning and decreased gradually to 80% by 18^{th} day. The second dominant species was butyric acid which accounted for 13-26% of TVFA.

<u>Influent COD = 15 g/L, HRT = 2 days</u>: In Figure 4.11b, highest acetic acid percentages were obtained as 87-98%. As in the case (a), butyric acid followed a similar pattern but with lower percentage values (2-12%).

Influent COD = 19 g/L, HRT = 2 days: In Figure 4.11c, acetic acid percentage decreased while butyric acid percentage conjugately increased. The values were 57-88% and 12-41% for acetic and butyric acid, respectively.

<u>Influent COD = 27.5 g/L, HRT = 2 days</u>: In Figure 4.11d, the decrease of acetic acid amount and increase of butyric acid amount in TVFA were more obvious. Acetic acid percentage decreased from 87% to 48% by 24^{th} day while butyric acid amount in TVFA was increasing from 11% to 48%. TVFA was consisting of approximately equal amounts acetic and butyric acid by 24^{th} day.

<u>Influent COD = 27.5 g/L, HRT = 1 days</u>: In Figure 4.11e, values simulated a similar result as previous cases where almost all of TVFA was formed from acetic acid (93%) in the beginning and decreased by time (76% at 6^{th} day). On the other hand butyric acid concentration in TVFA increased accordingly (6% at 3^{rd} day and 21% at 6^{th} day). The experiment was terminated after 6^{th} day.



Figure 4.11. Percentages of organic acids produced in acidification reactor with initial COD-HRT values of a) 10 g/L-2 days, b) 15 g/L-2 days, c) 19 g/L-2 days, d) 27.5 g/L-2 days, and e) 27.5 g/L-1 day

For all OLRs, acetic and butyric acids were dominant species. Other acid components of TVFA as i-butyric, i-valeric, valeric, caproic and heptanoic acids were present in trace amounts with percentages below 1%. Propionic and i-caproic acids were below the detection limit.

In a previous study conducted with gelatin-rich wastewater (Yu and Fang, 2003), influence of temperature and pH on acidification was investigated using UASB reactor. Operation at pH 4-5 favored production of propionate and hydrogen whereas at pH 6-7 acetate, butyrate and i-butyrate was dominant. Region between pH 5-6 was determined as transition zone. In present study for opium alkaloid wastewater pH range 5-5.5, however, favored the production of acetate and butyrate acids.

4.2.2. Upflow Anaerobic Sludge Blanket (UASB) reactors

Results for UASB reactors were depicted in Figures 4.12 and 4.13. From now on, reactors will be called as R1, R2 and R2 corresponding to Reactor 1, 2 and 3, respectively.

Influent COD for start-up of UASB reactors were chosen as 10 g/L taking batch experiment results into consideration. HRT of reactors in the beginning was 4 days. Accordingly OLR was maintained at 2.5 g/L.d. The first 6 day data were discarded since wastewater entering to reactors did not reach to effluent port of the reactors until 7th day. Effluent COD was 6410, 6940 and 7230 mg/L for R1, R2 and R3, respectively at day 7 and decreased by time. R2 came to steady state by 21st day (1690 mg/L) whereas the other two reactors reached to steady state by 24th day. Co-substrate usage decreased the acclimation period as in batch experiments. The acclimation periods for the reactors R1 and R3 was 6 times HRT and R3 had a value around 5 times HRT.

The lowest effluent COD concentrations were observed in R2 at this OLR. The overall COD removal efficiency for this reactor at this HRT after acclimation period was 80-85%. R3 had also removal efficiencies above 80%. The highest effluent COD at this OLR was obtained in R1.

Influent pH at this OLR was around 6.5 for all the reactors. The alkalinity addition was the main reason for this relatively high pH from pH of original wastewater (pH = 5). pH in reactors had a significant tendency to increase at the start-up. Reactor pH values increased from 7 to 8 by 30^{th} day. Gas production also increased during this period to values around 0.5 L/day. Operational parameters and effluent COD values indicated that acclimation of granules to alkaloid wastewater was obtained around day 30.

OLR was increased at day 34 to 3.75 g/L.d with influent COD = 15 g/L and HRT = 4. As in case for the start-up COD data for this OLR was taken after around 7 days since wastewater entering to reactors did not reach to effluent port of the reactors until 7th day. At day 42, effluent COD values and removal efficiencies for R1, R2 and R3 were 2630, 2040 and 2850 mg/L and 82, 86 and 81% respectively. The best effluent quality was still in R2 and the other two reactors had similar effluent COD results.

Influent pH dropped to 6 for R1 while remaining reactors had influent pH data around 6.3. Gas production was highest at R2 probably because of acetate supplementation. However, for all reactors gas production did not improve significantly.



Figure 4.12. Effluent COD concentrations and COD removal efficiencies for UASB reactors with respect to organic loading rate



Figure 4.13. Daily gas production, influent and reactor pH values for UASB reactors with respect to organic loading rate

Between day 53 and 72, OLR was maintained at 4.75 g/L.d (influent COD = 19 g/L, HRT = 4 days). Effluent COD values at this OLR ascended such as 4155, 2470 and 4690 mg/L effluent COD was observed on day 67 for R1, R2 and R3 respectively. As in other cases removal efficiency for R2 was the highest among three reactors differentiating more. Above 85% overall removal efficiency was obtained for R2 at this OLR. 78% and 75% were the average efficiencies for R1 and R3 respectively. Effluent quality decline for R3 may have resulted from TVFA composition from acidification reactor such that acetic acid percentage was decreased while butyric acid concentration was increasing.

At this OLR influent pH for all reactors were around 6. On the other hand reactor pH remained steady. Gas production rate increased for reactors especially R2. At day 64 and 65 1.2 and 1.3 L of gas was released.

On day 73, OLR was increased to 6.875 g/L.d by increasing influent COD to 27.5 g/L. This was a break point where effluent COD values started to increase for R1 and R3 drastically up to values 23620 and 18360 on day 100. However R3 indicated a better performance than R1. Around 16-20% removal efficiencies were obtained in R1 whereas in R3 removal efficiencies were around 40%. On the other hand R2 had effluent COD values of around 6000 mg/L corresponding to overall efficiency of 78%.

Influent and reactor pH descended to 5.5 and 7.5 for R1, 5.8 and 7.9 for R2 and 5.9 and 7.7 for R3, respectively. Gas production was lower than expected for this influent COD with values around 0.5, 1 and 0.8 for R1, R2 and R3, respectively. It was obvious that this OLR negatively affected the performances of reactors R1 and R3, especially R1.

In order to change fate of the reactors, OLR for R1 was decremented by increasing HRT to 6 days. Acidification reactor of two phase system was modified such that HRT for this reactor was decreased to 1 day to observe effect of a different

acidification to UASB reactor performance. Influent COD for R3, on the other hand, was increased to 36.6 g/L so that corresponding 75% of influent to this reactor which was alkaloid wastewater should have a COD value of 27.5 g/L. By this way comparison between R3 and R1-R2 for this initial COD may have been possible.

These changes took place on day 112. The new OLR for R1, R2 and R3 became 4.6, 9.2 and 6.9, respectively. Changing HRT of R1 did not hinder the collapse of the reactor. Finally R1 was turned off on day 127 with and effluent COD of 25000 mg/L. Reducing HRT of acidification reactor negatively affected R3. Removal efficiencies dropped to values around 20%. It can be stated that lower acidification ratio (0.1) decreased effluent quality of two phase system. In reactor R2, increasing OLR eventually resulted in increasing effluent COD. Overall average COD removal efficiency was 40%. One should regard that this was overall removal efficiency. In order to find out removal for alkaloid wastewater in R2, acetate concentration was measured in effluent and found that almost all acetate was degraded in the reactor. Consequently, remainder COD in effluent of R2 was originated from alkaloid wastewater. Actual average removal efficiency for alkaloid wastewater with influent COD in R2 was around 20% close to R1 at OLR of 6.9 g/L.d.

Gas production rate at these OLRs for R1, R2 and R3, were around 0.05, 1 and 0.2 L/day, respectively. pH drop for all reactors were significant and by the end of the experimental period all reactor pHs were below 7. Operational parameters also dictated that all three reactors broke down by the end of experimental period.

Comparing the results obtained in continuous reactor experiment to batch experiments, higher removal efficiency (78%) was obtained at a higher initial COD concentration (19 g/L). At this initial COD concentration there was no data for anaerobic treatment of alkaloid wastewater. It can be stated that successful treatment of alkaloid wastewater by UASB reactor without any other process modification was accomplished for this COD value. However, previous results have

high efficiency values (above 80%) again by UASB reactors at OLRs higher than the one supplied in our case. Increasing HRT, in other words, increasing OLR to UASB reactor results in process failure.

4.3. Effect of Radiation on Anaerobic Treatment of Alkaloid Wastewater

BMP results for alkaloid wastewater exposed to radiation with 60 Co- γ -irradiation source for two different doses, namely 40 and 140 kGy was depicted in Figure 4.14. Cumulative gas production for seed control was insignificant (9 mL) indicating that gas production in test reactors were due to biodegradation of wastewater. The highest NCGP (420.6 mL) was observed at highest initial COD concentration of 25 g/L which absorbed 40 kGy of radiation. Two wastewater samples were sent to TAEK for irradiation at 140 kGy dose at different times and these two samples were defined as old and new in Figure 4.14. These two test reactors generated same amount of NCG at the end of the experimental period (399 and 398 mL at 25 g/L initial COD and 230 and 231 mL at 14 g/L initial COD) and followed similar CGP patterns for both initial COD concentrations. The NCGP for these test reactors at 25 g/L was almost same with 40 kGy absorbed wastewater.

At initial COD of 14 g/L all test reactors produced approximately same amount of cumulative gas with similar trends. NCGPs at this concentration were 252.3, 243.7, 231 and 230 for test reactors with original wastewater, 40 kGy absorbed wastewater, 140 kGy absorbed old and new wastewater, respectively. Effect of radiation on anaerobic treatment at this concentration was insignificant. At this initial COD concentration of alkaloid wastewater biodegradation was possible without any radiation treatment. The radiation could have decreased inhibition effect of alkaloid wastewater which happened to have an attenuator effect above some initial COD concentration (around 10-15 g/L) as indicated in previous sections.

The rate of CGP up to 8th day was similar for test reactors with initial COD of 25 g/L. After this point, at reactors with 140 kGy radiated wastewater (1 and 2), rate of gas production increased and departed from other two reactors containing original alkaloid wastewater and 40 kGy radiated wastewater. Between day 8 and 32 the highest gas production rate was observed at reactor with wastewater which absorbed the highest radiation of 140 kGy. At this specified period 40 kGy absorbed wastewater reactor had a rate lower than 140 kGy one and original wastewater reactor had the lowest. This indicated once more that alkaloid wastewater was inhibitory to anaerobic microorganisms for concentrations above certain threshold.

Methane contents of all test reactors reached around 80% by day 32. As in the case for cumulative gas production methane content of the headspace gas for test reactor with original wastewater having initial COD of 25 g/L attained this percentage after the other test reactors.

As Jo *et al.* (2006) stated, change of biodegradability was largely dependent on the chemical property of wastewaters and the absorbed dose of gamma-rays. Borrely *et al.* (2000), found that the complex mixture of industrial effluents required doses from 30.0 kGy to 100.0 kGy, especially when raw samples were extremely toxic (Borrely *et al.*, 2000).

High doses used in this study were able to decrease the inhibitory effect of alkaloid wastewater at high initial COD concentration of 25 g/L. Radiation altered the rate of gas production above some point but the NCGP values by the end of the experimental period were close. The effect was insignificant at a lower concentration of 14 g/L where inhibition was not observed.



Figure 4.14. Cumulative gas production and methane content of gas for BMP experiment of radiation effect

CHAPTER 5

CONCLUSION

In this study, anaerobic treatment of alkaloid wastewater with batch and continuous systems were investigated. BMP experiments were performed with and without co-substrate and using radiated wastewater. UASB reactors with three different feeds were operated.

The results of batch experiments revealed that;

- Above certain threshold level, opium alkaloid wastewater inhibits anaerobic cultures. This level was determined as 10-15 g/L COD taking into present and previous studies on alkaloid wastewater. Up to this threshold level alkaloid wastewater could be treated anaerobically in batch systems with removal efficiencies 50-80%.
- Nutrient and alkalinity supplementation were necessary for anaerobic treatment of alkaloid wastewater.
- Glucose and acetate usage as co-substrate with alkaloid wastewater did not improve removal efficiency significantly but acclimation period was positively affected by both.

The results of continuous reactor experiments performed in acidification and UASB reactors revealed that;

• In acidification reactor, TVFA and acidification ratio increased by increasing influent COD to reactor while HRT = 2 days. Decreasing HRT of

acidification reactor resulted in descent of acidification ratio (0.16 to 0.10) meaning less acidification could be achieved in the reactor.

- In UASB reactor, alkaloid wastewater with initial COD 19 g/L (corresponding to about 2/3 concentration of original wastewater) was successfully treated (80%) without any other modification. Highest overall COD removal efficiencies were obtained in reactor fed with alkaloid wastewater-calcium acetate.
- Influent COD of 27.5 g/L was the break point for R1 and R3. The efficiencies dropped to 20 and 40% for these reactors, respectively. Although removal efficiency for two phase system was low, it doubled the efficiency of one phase UASB.
- Changing acidification reactor HRT from 2 to 1 day and consequently altering acidification ratio (0.16 to 0.10) negatively affected performance of two phase system. Removal efficiency decreased to 20%.
- At the highest OLR of 9.2 g/L.d, R2 had overall COD removal efficiency of 40%. However, since acetate was not present in the effluent, remaining pollution was thought to be originating from alkaloid wastewater and actual COD removal efficiency for alkaloid wastewater at this OLR (influent alkaloid wastewater COD = 27.5 g/L) was found as 20%. This indicated that usage of co-substrate does not improve the anaerobic treatment of alkaloid wastewater.
- Radiation has a positive effect on anaerobic treatment of alkaloid wastewater, especially at higher COD concentration.

Alkaloid wastewater can be treated by anaerobic biotechnology with continuous reactor systems. Treatment efficiencies obtained in this study up to certain initial COD value were higher than other treatability studies conducted by other researchers. Radiation as a pretreatment option for anaerobic treatment of industrial wastewaters is a new subject of area since radiation treatment is a relatively fresh subject. This study can contribute to this developing research field.

The future studies may be required to;

- Investigate the effect of acidification ratio, HRT and pH in an acidification reactor to overall anaerobic two phase treatment of alkaloid wastewater
- Study other high rate anaerobic treatment technologies for treatment of alkaloid wastewater

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APPENDIX

EXAMPLE COD REMOVAL CALCULATION FROM CUMULATIVE GAS PRODUCTIONS OF BMP ASSAY

BMP Experiment 1 – Test reactor with 9600 mg/L COD and BM

1000 mg COD is equivalent to 395 ml CH₄

BMP bottle liquid volume = 100 mL

Methane ratio in total gas = 0.66

Net cumulative gas produced at the end of experimental period = 447.7 mL

Methane produced = NCGP (mL) *Methane ratio = 447.7*0.66 = 295.5 mL

COD removed = Methane produced (mL)*1000/395 = 748 mg

COD in reactor = Initial COD concentration (mg/L) *(100mL/1000mL)

= 9600*0.1 = 960 mg

COD removal efficiency (%) = (COD removed/COD in reactor)*100 = 77 %