Anaerobic Co-Treatability of Olive Mill Wastewaters and Domestic Wastewater

By

Ahmet Avni ATAYOL

A Dissertation Submitted to the
Graduate School in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

Department: Environmental Engineering
Major: Environmental Engineering
(Environmental Pollution and Control)

İzmir Institute of Technology
İzmir, Turkey

September, 2003

We approve the thesis of **Ahmet Avni ATAYOL**

Date of Signature

08.09.2003

-- -----------------------

Asst.Prof.Dr. Aysun SOFUOĞLU
Supervisor
Department of Chemical Engineering

08.09.2003

-- -----------------------

Prof.Dr. Şebnem HARSA
Co-Supervisor
Department of Food Engineering

08.09.2003

-- -----------------------

Assoc.Prof.Dr. Günnur KOÇAR
Department of Energy Technologies
Solar Energy Institute, Ege University

08.09.2003

-- -----------------------

Asst.Prof.Dr. Fikret İNAL
Department of Chemical Engineering

08.09.2003

-- -----------------------

Asst.Prof.Dr. Gülden GÜNERHAN
Department of Mechanical Engineering

08.09.2003

-- -----------------------

Asst.Prof.Dr. Aysun SOFUOĞLU
Head of Interdisciplinary Environmental Engineering
(Environmental Pollution and Control) Programme

ACKNOWLEGEMENTS

I am grateful to ***Assoc.Prof.Dr. Göksel N. Demirer***, from Environmental Engineering Department of Middle East Technical University, for the courage he gave me and his helpful guidance throughout this study.

I am thankful to my advisors ***Asst.Prof.Dr. Aysun Sofuoğlu*** and ***Prof.Dr. Şebnem Harsa*** for their support and recommendations.

I would like to express my gratitude to my friends ***Res.Asst. Naz Gültekin*** and ***Res.Asst. Oktay Yıldırım*** for their valuable help in laboratory studies.

I would like to thank ***Mrs. Nükhet Akkaya*** from PEKTİM for providing the standard gas mixture used in biogas composition analysis.

Finally, and most important, I am grateful to my wife ***Gülcan*** and my son ***Doğu Toprak*** for their tolerance and support. This study could not be finished without the courage and love they gave me.

This study was carried out for ***Doğu Toprak*** and for ***all children of the world***, with the most sincere wishes for them to live in a more healthy environment. Even the smallest contribution to this objective will be the highest pleasure for me.

This study was partially funded by ***Research Fund of İzmir Institute of Technology***.

ABSTRACT

Handling of olive mill wastewater (OMW) constitute an important problem for Mediterranean countries in general and western parts of Turkey in particular. This wastewater is seasonally produced and has high organic pollutant concentrations. Many treatment methods have been tried for its treatment, but a method having both sufficient treatment efficiency and economical feasibility could not be offered. Anaerobic treatment, having the advantages of applicability to high-strength and/or seasonal wastewaters, energy production and reduced costs, may be a good option. But olive mill wastewater is considered still to be highly polluted and not to have sufficient nutrients while domestic wastewater (DW) is assessed to be dilute and to have nutrients in excess for anaerobic treatment. Therefore anaerobic co-treatment of these wastewaters may be a better solution.

In this study, the anaerobic co-treatability of OMW and DW was investigated by means of anaerobic treatability assays called Biochemical Methane Potential (BMP) tests. These tests were applied to OMW and DW mixtures of different ratios (OMW:DW of 1:9, 1:5, 1:3, 1:1, 1:0) at different organic loadings (4553 mg/l, 9107 mg/l, 13660 mg/l, 22767 mg/l and 34150 mg/l Chemical Oxygen Demand (COD) loading) with two different seed cultures. In order to determine the effect of nutrient addition, and the complementarity of these wastewaters, all tests were carried out both with (BM+) and without (BM-) the addition of a nutrient solution (basal medium). Results of the experiments showed treatment efficiencies around 80% at 4553 mg/l COD loading and around 70% at higher loadings for samples seeded with the culture obtained from the treatment plant of Efes Pilsen Brewery in İzmir, Turkey. For samples seeded with the culture obtained from the anaerobic digesters of Ankara Municipal Wastewater Treatment Plant, results were between 61% and 82% with the higher efficiencies at medium COD loading levels. A meaningful relation between DW content

and treatment efficiency could not be observed; but the performance ratio of BM- reactors to BM+ reactors increased with increasing DW content in the mixture, indicating the positive effect of DW addition. Reactors seeded with Efes culture did not require an acclimation period, while those seeded with Ankara culture did so. Efes seeded reactors, at all COD loadings, performed most of the gas production, around 75-80%, in about 15-20 days. Ankara seeded BM+ reactors required about 25 days for 80% production, while this period was about 35-45 days for BM- reactors.

ÖZ

Zeytinyağı imalathanesi atıksularının (‘karasu’yun) bertarafı genelde Akdeniz ülkeleri, özelde ülkemizin batı bölgeleri için önemli bir sorundur. Bu atıksu mevsimsel olarak üretilir ve yüksek miktarda organik kirletici içerir. Karasuyun arıtımı için birçok yöntem denenmesine karşın hem yeterli kirlilik giderimi sağlayan hem de ekonomik uygulanabilirliği olan bir metod önerilememektedir. Yoğun kirlilik yüküne sahip ve/veya mevsimsel olarak üretilen atıksulara uygulanabilme, enerji üretimi ve düşük giderler gibi avantajları ile anaerobik arıtma uygun bir seçenek olabilir. Ancak karasu, anaerobik arıtma için bile yüksek kirlilik yüküne sahiptir ve yeterli besi maddesi içeriğine sahip değildir. Bunun yanında evsel atıksular, anaerobik arıtma için seyreltik ve fazla besi maddesi içeriğine sahip olarak değerlendirilmektedir. Bu yüzden anaerobik birlikte arıtma daha iyi bir seçenek olabilir.

Bu çalışmada, karasu ve evsel atıksuyun birlikte arıtılabilirliği, Biyokimyasal Metan Potansiyeli (BMP) testi olarak adlandırılan anaerobik arıtılabilirlik testleri kullanılarak araştırılmıştır. Bu testler karasu ve evsel atıksuyun değişik oranlardaki karışımlarına (1:9, 1:5, 1:3, 1:1 ve1:0 karasu:evsel atıksu oranlarında) değişik organik yükleme değerlerinde (4553 mg/l, 9107 mg/l, 13660 mg/l, 22767 mg/l ve 34150 mg/l Kimyasal Oksijen İhtiyacı (KOİ)) iki farklı aşı kültürü kullanılarak uygulanmıştır. Besi maddesi eklemesinin etkilerini ve bu atıksuların birbirini tamamlayabilme özelliklerini belirleyebilmek amacıyla, tüm testler hem bir besi çözeltisi eklemesi (BM+) hem de ekleme olmadan (BM-) gerçekleştirilmiştir. Deney sonuçları, Efes Pilsen İzmir Bira Fabrikası’nın arıtma tesisinden alınan kültürle aşılanan reaktörler için, 4553 mg/l KOİ yüklemesinde %80, diğer yükleme değerleri için ise %70 civarında kirlilik giderimi göstermektedir. Ankara Evsel Atıksu Arıtma Tesisi’nden alınan kültürle aşılanan reaktörler için ise bu değerler, daha yüksek değerler orta yoğunlukta KOİ yüklemelerinde olmak üzere, %61 ile %82 arasında değişmektedir. Karışımdaki evsel atıksu oranı ile kirlilik giderimi arasında anlamlı bir ilişki saptanamamış, ancak besi maddesi eklemesiz reaktörlerin arıtma veriminin besi maddesi eklemeli olanlarınkine oranının, karışımdaki evsel atıksu oranıyla doğru orantılı olarak arttığı gözlenmiştir. Bu durum evsel atıksu eklemesinin olumlu etkilerini ortaya koymaktadır. Efes kültürü ile aşılanan reaktörler herhangi uyum süreci gerekliliği yaşamamışken, Ankara kültürü ile aşılanan reaktörler bu sürece gereksinim duymuştur. Efes kültürü ile aşılanan reaktörler, tüm KOİ yüklemelerinde, biyogaz üretiminin çoğunu, %75 civarı, yaklaşık 15-20 gün içinde gerçekleştirmiştir. Ankara kültürü ile aşılanan besi maddesi eklemeli reaktörler toplam üretimin %80'ine ulaşmak için yaklaşık 25 güne gereksinim duymuş, besi eklemesi yapılmayanlarda ise bu süre 35 ile 45 günü bulmuştur.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

If not properly handled, wastewaters may cause serious environmental hazards. Therefore, wastewater treatment constitutes an important problem for the whole world. The method to be employed for the treatment of a wastewater must be selected in convenience with its characteristics. Usually biological methods are preferred when the wastewater mainly contains organic, biodegradable pollutants.

Although aerobic processes have been more widely used, anaerobic ones began to find more acceptance within the last two decades, with the development of high-rate reactors. Anaerobic treatment processes have apparent advantages over aerobic ones, such as applicability to high-strength wastewaters, reduced operational costs, provision of seasonal treatment, reduced waste biomass production, and in addition methane production as a significant energy source. Therefore, they should be employed wherever possible.

For deciding the applicability of anaerobic treatment to a wastewater, the anaerobic biodegradability of that wastewater should be determined. This is held by Biochemical Methane Potential (BMP) tests. This test may also provide useful information on some treatment parameters such as optimum feed concentration and nutrient limitations, and can be used for assessing the actual efficiency of an applied process. Although the necessity for this assay was clearly pointed far ago, there is a lack of treatability studies in the literature.

Olive mills are important sources of pollution for Mediterranean region, which is responsible for 95% of the total olive oil production of the world. Olive mill wastewaters (OMW) are high-strength, seasonally produced wastewaters with very high organic content, and anaerobic treatment is assessed to be the most convenient method for OMW treatment. However, there are still problems: nutrient deficiency and need for dilution.

The application of anaerobic processes to domestic wastewaters (DW), with energy production and reduced costs, would be beneficial especially for developing countries; but DW, which is nutrient-rich and has low strength, is not considered to be suitable for anaerobic treatment, particularly at low temperatures.

The combined anaerobic treatment of OMW and DW can be a good option while one is concentrated and nutrient-deficient, and the other is dilute and has nutrients in excess. Co-treatment also may have the economical advantage of preventing the use of several distributed treatment facilities.

Most of the olive mills in Turkey, and settlements near the mills have no treatment facility. These settlements are also generally touristic areas. Prevention of the environmental problem caused by improper wastewater handling in these areas is very crucial. The opportunity of producing a fraction of their energy demand by using biogas which is a valuable end-product of anaerobic treatment will also be a good gain for olive mills.

Despite above stated facts, there is a clear deficiency in anaerobic co-treatment of OMW and DW in the literature. In this study, the effect of combining these wastewaters on their anaerobic treatability was investigated by means of BMP assays. For this purpose 1. various mixtures, with OMW:DW of 1:9, 1:5, 1:3, 1:1 and 1:0, were used in the experiments for determining the optimum ratio of these wastewaters; 2. the experiments were carried out at five different organic loading levels, namely 4553 mg/l, 9107 mg/l, 13660 mg/l, 22767 mg/l, 34150 mg/l chemical oxygen demand (COD) concentrations, in order to see the variation of removal efficiency with increasing load, and decide the optimum feed concentration which is a valuable parameter in applications; 3. also for monitoring the complementary effect of co-treatment and nutrient requirements, the experiments were run with both nutrient addition and no nutrient addition; 4. two different anaerobic mixed cultures were used to seed the duplicate reactors in the study.

This study contains five chapters: a brief introduction about the study was given in Chapter 1; followed by Chapter 2 containing background information on the subject including the review of related literature; in Chapter 3 materials and methods used in the experiments are described; then obtained results are presented and discussed in Chapter 4; and finally conclusions based on the results of the study are stated in Chapter 5.

Chapter 2

BACKGROUND INFORMATION

Handling of wastewaters is one of the major problems our world is facing. If not properly treated and/or discharged they may have serious negative impacts on both physical and living environment. Usually, the characteristics of wastewaters vary according to their source, and they can be classified with respect to these sources: domestic wastewater, agricultural wastewater, and industrial wastewater etc.

Various methods are employed for wastewater treatment. These methods can be grouped into three: physical treatment (e.g. screening, grit removal, sedimentation), chemical treatment (e.g. precipitation, ion exchange, disinfection), and biological treatment (e.g. activated sludge, nitrification-denitrification, stabilization ponds) [1]. In the selection of the convenient method or combination of methods, the type and the composition of the wastewater is the primary factor. Usually aerobic or anaerobic biological methods are preferred when the pollutants to be removed are mainly biodegradable. The characteristics of these two are quite different, and they have advantages over each other.

2.1 Anaerobic Treatment

Anaerobic treatment, or methane fermentation process, is the use of biological phenomena which result in the conversion of organic materials to methane (CH_4) and carbondioxide (CO_2) in the absence of molecular oxygen (O_2). It has many applications such as septic tanks, sludge digesters, industrial wastewater treatment, domestic wastewater treatment, hazardous waste management, municipal solid waste management, and agricultural waste management/ biogas generation [2].

Early applications like septic tank were successful in capturing solids, but not that good in BOD (biochemical oxygen demand) removal. They required long retention times and large volumes, therefore were not economical. Major progress was after the development of high-rate reactors providing high solids residence times and high biomass concentrations for process efficiency and stability, with low hydraulic retention times (HRTs) for system economy [2]. After this progress, the advantages of anaerobic over aerobic treatment began to overweigh in many cases. The main advantages are higher loading rates, applicability to high-strength wastewaters, reduced waste biomass production, biogas production (energy source), lower endogenous decay (provision of seasonal treatment), no off-gas air pollution, no oxygen transfer (reduced operational cost and attention). However, it still has some disadvantages such as long start-up periods, insufficient effluent quality for surface water discharge in some cases, and low kinetic rates at low temperatures [3].

In order to decide the applicability of anaerobic treatment to a wastewater, the anaerobic treatability of that wastewater should be determined [3]. Anaerobic treatability, in other words the anaerobic biodegradability, of a wastewater is determined by an assay named BMP (biochemical methane potential) test [3,4]. This is a simple assay which does not require sophisticated equipment, and provides a realistic measure of potential process efficiency for anaerobic treatment. The principle of the BMP assay is measuring the methane production from the anaerobic degradation of a sample, under optimum conditions in a batch reactor (a serum bottle), and determining the degradable portion of the pollutant by using the stochiometric relation between substrate utilization and methane production. Treatability studies conducted in other types of reactors may cause interferences due to improper design and/or operation[4].

2.2 Domestic Wastewater and Anaerobic Treatment

Domestic wastewater (DW), by definition, is the wastewater generated mainly from residential, commercial and public facilities. It is usually rich in nutrients, and its components are mainly organic. Generation rates and composition vary considerably from place to place, basically depending on economic aspects, social behavior, climatic conditions, water consumption, type and conditions of sewer system etc. As it was in the past, the most common way of handling DW is still direct discharge to the environment, especially in developing countries [5]. Typical composition of untreated DW is given in Table 2.1.

Several methods, including anaerobic ones, are employed for DW treatment, but mainly aerobic biological processes such as conventional aerobic treatment in ponds, trickling filters, and activated sludge plants are preferred [5].

Table 2.1 Typical composition of untreated domestic wastewater [6]

Parameter	***unit***	***concentration***		
		weak	***medium***	***strong***
COD	**mg/l**	250	500	1000
BOD$_5$	**mg/l**	110	220	400
SOLIDS, total	**mg/l**	350	720	1200
Dissolved	**mg/l**	250	500	850
Fixed	**mg/l**	145	300	525
Volatile	**mg/l**	105	200	325
Suspended	**mg/l**	100	220	350
Fixed	**mg/l**	20	55	75
Volatile	**mg/l**	80	165	275
NITROGEN, total as N	**mg/l**	20	40	85
Organic	**mg/l**	8	15	35
Free Ammonia	**mg/l**	12	25	50
Nitrites	**mg/l**	0	0	0
Nitrates	**mg/l**	0	0	0
PHOSPHORUS, total as P	**mg/l**	4	8	15
Organic	**mg/l**	1	3	5
Inorganic	**mg/l**	3	5	10

One of the major challenges for anaerobic technology is the extension of its applicability to low-strength wastewaters, i.e. DW. The direct treatment of sewage by an anaerobic process is undoubtedly an attractive and appropriate option, especially for developing countries, since it requires low energy for operation and low initial investment, and also provides lower sludge production and easier maintenance than conventional aerobic processes [7]. However, the low nutrient removal by anaerobic treatment and the required post-treatment constitute an important disadvantage for its direct application to DW [5]. To overcome the mentioned disadvantages, extensive research is being held, and successive results, chemical oxygen demand (COD) removal efficiencies up to 95%, are reported [5,7].

In many developing tropical countries confronting rapidly increasing water pollution problems, this technology becomes even more favorable and promising, since the warm climate in these countries makes the process more efficient [7].

2.3 Olive Mill Wastewater and Anaerobic Treatment

Olive mills are small agro-industrial units located mainly around the Mediterranean Sea. More than 95% of the worldwide olive oil production is carried out in this region. Olive oil is extracted from the processed olives either by means of a discontinuous press (classical process) or a solid/liquid centrifuge (continuous process) [8]. The centrifugal process has two types: two-phase and three-phase. Two types of wastes are produced by classical and three-phase centrifugal processes: olive mill residual solids (OMRS) and OMW [8]. Two-phase centrifugal process uses less process water than the three-phase one and produce only one waste stream, generally called ‘alperujo’ for its Spanish origin, which is accepted to be a mixture of OMW and OMRS but lower in volume. The characteristics of OMW show a very high variation according to many factors such as process type, origin of the olive, harvesting time, annual climatic conditions, age of the olive tree etc. Characteristics of some OMW samples given in the literature are shown in Table 2.2.

Table 2.2 Characteristics of some OMW samples in the literature

parameter	***reference***			
	[8]	**[9]**	**[10]**	**[11]**
COD (mg/l)	138250	104958	73600	52500
TKN (mg/l)	579	878	80	(NH_4^+) 90
Tot-P (mg/l)	56	316	-	-
SS (mg/l)	42833	7706	7500	13800
VSS (mg/l)	42633	7350	6800	10000
Phenols (mg/l)	-	-	-	750
pH	4.63	-	5.31	5.2

It is estimated that OMW production is more than 30 million m^3 per year in the the Mediterranean area. This production constitute a significant source of potential or existing environmental pollution in the region. *"The difficulties of treatment of olive mill effluents are mainly related to (a) high organic loading, (b) seasonal operation, (c) high territorial scattering, and (d) presence of organic compounds which are hard to biodegrade such as long-chain fatty acids and phenolic compounds"* [8].

Although OMW can be assumed to be completely biodegradable, some constituents like polyphenols and lipids have lower degradation rates. This causes a problem for biological treatment due to longer retention times and larger reactor volumes that mean higher costs. For this reason non-biological processes also have been tested on OMW. Some of these processes are thermal processes such as distillation-evaporation and incineration, flocculation/clarification, ultrafiltration, and reverse osmosis; but these methods were not found to be convenient in terms of insufficient treatment efficiency, further treatment and/or disposal requirement, or atmospheric pollution emissions. Aerobic biological processes are also not applicable because of the intolerance of aerobic microorganisms to high COD concentrations, and the huge oxygen requirements and excess sludge volumes (3 to 20 times more than anaerobic ones) to be produced [12].

The seasonal production and high organic content of OMW make anaerobic treatment a very attractive option for these wastes. Furthermore, production of much less biosolids (sludge) and biogas as a valuable end product, which may offset the associated treatment costs, are additional positive aspects of anaerobic treatment of OMW [8]. There are several studies on the anaerobic treatment of OMW, showing COD removal efficiencies in the range of 65% to 92% [8,12-14].

Although anaerobic treatment is concluded as the best method for the treatment of OMW, attention must be paid to the nutrient requirements since OMW is found to be nutrient (N, P) deficient [8,12,15]. It must also be stated that although anaerobic treatment is applicable to high-strength wastewaters, OMW has still to be diluted in order to prevent the inhibitory effect of the extremely high COD and/or phenolic content on the microorganisms [12].

At this point, co-treatment seems to be a good option for the anaerobic treatment of OMW and DW. A proper mixture of these wastewaters may eliminate the problems mentioned before, since OMW must be diluted and is nutrient deficient, while DW is too dilute for anaerobic treatment and contains nutrients in excess.

2.4 Anaerobic Co-Treatment

The purpose of co-treatment can be stated as exploiting the complementarity in wastewater characteristics, and reducing the treatment costs by avoiding distributed treatment facilities. It also provides economically favorable stable year-round operation for seasonally produced wastewaters like most of agro-industrial ones [9,16,17].

In the literature, many studies on anaerobic co-treatment of various types of wastes/wastewaters exist [9,12,15-23]. Some of these are on the anaerobic co-treatment of OMW with other wastes/wastewaters such as sewage sludge, cattle manure, piggery and dairy wastewaters, but not with raw DW [9,12,15-17,42,43].

In this thesis study, anaerobic treatability assays (BMP tests) were applied to an OMW sample from Tariş Mordoğan Olive Mill and to its mixtures with a DW sample from Çiğli Municipal Wastewater Treatment Plant of İzmir at various proportions. By this method, the complementary effect of combining these wastewaters on their anaerobic treatability, and the optimum proportion of the wastewaters in the mixture were investigated. The samples were characterized, prior to the tests, in terms of parameters that may be required for further studies. This study may be a future option for the areas in which no treatment has been applied to OMW and DW such as most of the rural parts of Western Turkey.

2.5 Studies On Anaerobic Treatment

In this section studies in the literature on the anaerobic treatment of the wastewaters subject to this study will be reviewed.

2.5.1 Anaerobic Treatment of Domestic Wastewater

Anaerobic processes (such as septic tank) have been employed for DW treatment for over a century. The first anaerobic tank reactor, similar to septic tank, dates back to 1857 [5]. But these early applications, having low process efficiency, were abandoned as the effluent standards had got stricter [2]. Although DW is generally anaerobically treatable, process efficiency and cost-efficiency problems prevented the application of the process. In the late 70's, with the development of high-rate reactors, such as upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactors, it has been a viable option for DW treatment again [2,5,7]. However, still some problems exist for the widespread application of anaerobic treatment of DW, such as increased but still insufficient process efficiency (due to the low strength of DW), low nutrient removal, lower kinetic rates at low temperatures, long start-up periods etc. Present studies focus on these problems [2,5].

In a review paper by Seghezzo *et al.* [5], over 40 studies on anaerobic DW treatment are reported. Some of these studies were performed at low temperatures; de Man *et al.* [24], studied on different UASB reactors in the Netherlands, and concluded that DW could be treated anaerobically at 12-18°C applying HRTs of 7-12 hours, with a COD removal of 40-60% and a BOD removal of 50-70%; while Lettinga *et al.* [25] obtained COD removal efficiencies up to 85% and 70% respectively at 20°C and 13-17°C in a lab scale UASB reactor with HRTs of 14-17 hours. Results reported for higher treatment temperatures are more couraging; in three lab-scale studies, in South Africa [26], Indonesia [27] and Thailand [28], 90% removal of COD was achieved; by pilot-scale studies carried out in Colombia [29] and Brazil [30], COD and BOD removal efficiencies higher than 75% (up to 93%) were obtained with significantly lower HRTs (4-6 hours). These results led to several full-scale anaerobic DW treatment plants, especially in tropical countries. For instance in India a 1200 m^3 UASB reactor, is reported to treat 5000 m^3 sewage per day (25 000 population equivalent), achieving COD and BOD removal efficiencies of 74% and 75% respectively, at 20-30°C with an HRT of 6 hours [31]. Another UASB reactor in India, 6000 m^3 in volume, operates at 18-32°C with an HRT of 8 hours, and has average removal efficiencies of 62-72% for COD and 65-71% for BOD [32]. Although the decrease in removal efficiencies with the reactor size should be noticed, the results of full-scale applications seem to be promising. Treatment efficiencies of sewage by the UASB process reported in the literature vary significantly, ranging from 7% to 90% of total COD removals within 2-360 hours of HRTs and 7-32°C of temperatures.

Uemura and Harada [7] had run a UASB reactor treating DW for 6 months with an HRT of only 4.7 hours, gradually decreasing the temperature from 25°C to 13°C. The obtained COD removal efficiencies were 70%, 70%, 72%, 71% and 64% at 25°C, 22°C, 19°C, 16°C, and 13°C respectively. They concluded that these relatively better results might be due to the characteristics of the wastewater. As a result, this study showed that anaerobic bacteria may acclimate to low temperatures.

The most important point is that treatability studies for DW are generally not employed. The reviewed studies on process configurations and effects of operational parameters were carried out without treatability assays, although they are very important. The importance of treatability studies is also pointed out by some researchers [3].

2.5.2 Anaerobic Treatment of Olive Mill Wastewaters

The polluting load of olive mills in the Mediterranean region is estimated as 3800-4800 tons of BOD per day, assuming a milling season 100 days, by Rozzi and Malpei [12]. They discussed possible methods for the treatment of OMW. They concluded that biological methods, particularly anaerobic ones, are the best alternatives among all other methods. In the review, the need for dilution of OMW is stated and proposed particularly for biological processes. They also presented a review of anaerobic treatment of OMW by various process configurations. By anaerobic lagooning, COD removals of 20-30% to 75-80% were obtained in 2-4 months by Balice *et al.* [33] and Cabello and Ursinos [34]. However, the method used in the study contribute to atmospheric methane emissions, since the produced biogas is not collected. Several studies on the anaerobic contact process, a CSTR with recycle, are reported [35-38]. Operational parameters in these studies vary between 15-70 g COD/l for influent concentrations, 2-4 kg COD/m^3.day for loading rates, and 20-25 days for HRTs. Achieved COD removals were up to 80-85%. Although the HRTs are quite long, the results were promising. Brunetti *et al.*[39], by a lab-scale UASB reactor, achieved 70% COD removal for an influent COD concentration of 15 g/l, obtained by gradually increasing after beginning with 5g COD/l for the purpose of microbial acclimation and by the addition of alkalinity for pH control and urea for nutrition into the reactor. Finally they report studies with anaerobic filters; COD removals of 60-65% by a lab-scale [40], and 80% by a 2 m^3 pilot-scale filter with an HRT of 3-4 days [41] were obtained, applying loading rates of 9-18 and 3-4 kg COD/m^3.day respectively.

Ubay and Öztürk [14] investigated the treatability of OMW by a UASB reactor under various operating conditions. They obtained COD removal efficiencies around 75% with 1 day HRT and influent concentrations between 5 and 19 g COD/l. By ranging the feed COD concentrations between 15 and 22.6 g/l and the HRT between 0.83 and 2 days, the removal was around 70%. The term “treatability” used by Ubay and Öztürk in this study does not reflect to the ultimate treatability which can be decided by BMP asssays.

Dalis *et al.* [13] worked on the anaerobic treatment of OMW by a two-reactor, up-flow and fixed bed, system operating in series. The system operated for 390 days at 35±1°C with loading rates varying between 2.8-12.7 kg COD/m^3.day. Maximum removal (83%) was obtained at 11 kg COD/m^3.day loading, and an HRT of 10.7 days.

An important study on the anaerobic treatability of OMW, employing BMP assays, was carried out by Ergüder *et al.* [8]. In the same study, they also performed a screening study for determining the most important nutrients for the anaerobic digestion of OMW. COD removal efficiencies were ranging between 85.4 and 93.3% for varying initial COD concentrations. They claim that these are the minimum efficiency values that could be obtained, since the microorganisms employed were not previously acclimated to OMW. The ultimate gas production was reached in 44 days, and the required acclimation period increased from 13 to 23 days as the initial COD concentration increased. They concluded that this was due to the inhibitory effect of phenolic compounds present in OMW. In the second part, the screening study, they prepared 5 reactors by adding alkalinity and nutrients (N, P, K) one by one in each step, therefore each reactor contained an additional nutrient with respect to the previous one. Reactors containing only OMW, and OMW+alkalinity are reported to show poor COD removal rates when compared to nutrient containing reactors. Therefore they concluded that OMW did not contain sufficient nutrients, especially N.

2.5.3 Anaerobic Co-Treatment

Mata-Alvarez *et al.* [17] defined co-digestion as the use of a co-substrate in digestion, that in most cases improves removal efficiency due to positive synergism established in the digestion medium, and the supply of missing nutrients by the co-substrate. The nutrient, especially N, deficiency and dilution requirement of OMW was mentioned before. To overcome these problems, co-digestion of OMW with various wastes/wastewaters have been studied [9,12,15-17,42,43].

Mata-Alvarez *et al.* [17] report a study carried out by Schmidt *et al.* [42]. In this study, OMW was co-digested with pig manure and sewage sludge at ratios of 1/5 and 1/1 respectively. With pig manure, a COD removal of 75% is reported, but no other information is provided.

Gavala *et al.* [16] developed a mathematical model for co-digesting piggery, olive mill, and dairy wastewaters. Their model was based on batch kinetic experiments carried out by them before. By this model, they calculated an optimal COD loading rate for a digester operating on a year-round basis, fed sequentially with the mentioned wastewaters.

Gavala *et al.* [9] made another research on the same subject later. They carried out batch kinetic experiments for the co-digestion of the same wastewater types with different cultures acclimated to each wastewater. The microbial growth kinetics are calculated and compared. Then they developed a unified mathematical model describing the co-digestion process, which is capable of predicting the short-term response of a digester subjected to seasonal feed changes. That is an important point for centralized, whole year operating treatment plants.

Another study including a mathematical model was carried out by Angelidaki *et al.* [15]. They developed a model describing the anaerobic co-digestion of complex organic material, such as manure, and a lipid containing additive, such as OMW, based on another model developed previously by them. They simulated the co-digestion of the mentioned wastewaters, and compared the data with experimental data. The two types of data fitted well, and indicated that lack of ammonia, as a N source for microorganisms and an important pH buffer, may be the cause of problems faced in the single digestion of OMW. They showed that the amount of N required can be supplied by cattle manure in co-digestion.

Rozzi and Malpei [12] also point to the alkalinity and N deficiency, and the dilution requirement of OMW. They suggest DW as an optional source for diluting OMW. In the paper, anaerobic co-digestion of OMW and sewage sludge is discussed as a treatment option. Also a study, with a pilot-scale (5 m^3) UASB reactor, on co-digestion of OMW with settled sewage (for providing ammonia and dilution) is reported [43]. A maximum loading of 15 kg COD/m^3.day was applied and COD removal efficiencies of between 70 and 75% were achieved in this study.

It should be worth mentioning that treatability determinations are disregarded within the reviewed co-digestion studies. This situation seems to cause a lack of information in process assessment.

Chapter 3

MATERIALS AND METHODS

In this chapter materials, analytical methods, experimental set-up and procedures used in this study are explained.

3.1 Materials

In this section materials used in BMP experiments, namely olive mill wastewater, domestic wastewater, seed cultures, N_2/CO_2 mixture and apparatus, are described.

3.1.1 Olive Mill Wastewater

The olive mill wastewater was obtained from a mill owned by Tariş in Mordoğan, İzmir. The mill uses a 3-phase continuous process for oil production.

3.1.2 Domestic Wastewater

The domestic wastewater was obtained from İZSU Çiğli Municipal Wastewater Treatment Plant which receives over 95% of municipal wastewater, including pretreated industrial wastewater, generated in İzmir.

3.1.3 Seed Cultures

Two different anaerobic mixed cultures were used as seed culture -or inocula- in the BMP experiments. The first culture was a granular sludge obtained from the wastewater treatment plant of Efes Pilsen Brewery located in Pınarbaşı, İzmir. The plant uses a UASB process having an HRT of 3 hours. The second culture was obtained from the anaerobic sludge digester unit of ASKİ Municipal Wastewater Treatment Plant, Ankara. The digesters receive an average sludge flow of 805 m^3/day from the primary thickeners and are operating with an HRT of 14 days.

3.1.4 Basal Medium

The basal medium used in the BMP experiments was prepared in the laboratory. An eight times concentrated basal medium was prepared in order to have the final concentrations in the reactors as follows (mg/l): NH_4Cl (1200), $MgSO_4{\cdot}7H_2O$ (400), KCl (400), $Na_2S{\cdot}9H_2O$ (300), $CaCl_2{\cdot}2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2{\cdot}4H_2O$ (40), $CoCl_2{\cdot}6H_2O$ (10), KI (10), $MnCl_2{\cdot}4H_2O$ (0.5), $CuCl_2{\cdot}2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3{\cdot}6H_2O$ (0.5), $NaMoO_4{\cdot}2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2{\cdot}6H_2O$ (0.5), $NaWO_4{\cdot}2H_2O$ (0.5), Na_2SeO_3 (0.5), cysteine (10), $NaHCO_3$ (6000). This basal medium contains all the necessary micro and macro nutrients required for optimum anaerobic microbial growth [8].

3.1.5 N_2/CO_2 mixture

The gas mixture containing 75% N_2 and 25% CO_2, used for purging the reactors in order to provide anaerobic conditions, was bought from Karbogaz Ltd. in a 10 liter pressurized steel cylinder. It was custom prepared by the company and a certificate of analysis was provided with the gas.

3.1.6 Apparatus

The following apparatus were used in the BMP experiments:

- incubator (for providing a temperature of 35±1^{o}C)
- 100 ml serum bottles (as reactors)
- natural rubber sleeve stoppers
- plastic cable ties
- a water displacement device consisting of a separatory funnel, a 50 ml graduated pipette, a 28 gauge hypodermic syringe needle and 8 mm OD silicone tubing

3.2 Analytical Methods

In this section analytical methods used for the characterization of the wastewater samples and for biogas composition determinations are described. Characterization parameters used in this study were chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), total phosphorus (TP), phenols, total and volatile suspended solids (TSS, VSS), pH and temperature (T).

3.2.1 Chemical Oxygen Demand

COD values of the samples were determined by Closed Reflux Colorimetric Method (Standard Method 5220.D). The principle is stated as "*Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strong acid solution with a known excess of potassium dichromate....Colorimetric reaction vessels are sealed glass ampules or capped culture tubes. Oxygen consumed is measured against standards at 600 nm with a spectrophotometer.*" [44]. For the triplicate analysis, a Hach DR-2010 spectrophotometer was used.

In order to stay in the range of the method, 0-1500 mg/l, 1/100 diluted OMW and raw DW samples were used. The analysis were run against a reagent blank prepared with deionized water.

3.2.2 Biochemical Oxygen Demand

BOD values of the samples were determined by 5-Day BOD Test (Standard Method 5210.B). The principle is stated as "*The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 days. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference of initial and final DO.*" [44]. During the triplicate analysis, a WTW DO-meter was used.

In the analysis of OMW sample, the DW sample was used as seed to assure the presence of required microorganisms in the test bottles. In order to stay in the range of the method, 0-8 mg/l, 1/100 diluted OMW and raw DW samples were used. Sample sizes were selected to give a final dilution of 1/10000 for OMW and 1/100 for DW samples. The analysis were run against a reagent blank prepared with distilled water.

3.2.3 Total Kjeldahl Nitrogen

TKN contents of the samples were determined by Macro-Kjeldahl Digestion Method (Standard Method 4500-N_{org}.B). The principle is stated as "*In the presence of sulfuric acid, potassium sulfate, and cupric sulfate catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium.*" [44]. For the triplicate analysis, a Hach DR-2010 spectrophotometer was used.

In order to stay in the range of the method, 0-80 mg/l, 1/10 diluted OMW and raw DW samples were used. The analysis were run against a reagent blank prepared with ammonia-free water.

3.2.4 Total Phosphorus

TP contents of the samples were determined by Vanadomolybdophosphoric Acid Colorimetric Method (Standard Method 4500-P.C). The principle is stated as "*In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.*" [44]. For the triplicate analysis, a Hach DR-2010 spectrophotometer was used.

In order to stay in the range of the method, 0-100 mg/l as PO_4 and 0-33 mg/l as P, 1/100 diluted OMW and raw DW samples were used. The analysis were run against a reagent blank prepared with distilled water.

3.2.5 Phenols

Phenol content of the OMW sample was determined by Direct Photometric Method (Standard Method 5530.D). The principle is stated as "*Phenolic compounds react with 4-aminoantipyrine at pH 7.9±0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.*" [44]. For the triplicate analysis, a Hach DR-2010 spectrophotometer was used.

In order to stay in the range of the method, 0.1-5 mg/l, a 1/1000 diluted OMW sample was used. The analysis were run against a reagent blank prepared with freshly boiled and cooled distilled water.

3.2.6 Total and Volatile Suspended Solids

TSS contents of the samples and the seed cultures were determined by Total Suspended Solids Dried at 103-105^{o}C Method (Standard Method 2540.D). The principle is stated as "*A well mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103-105^{o}C. The increase in weight of the filter represents the total suspended solids.*" [44].

VSS content of the OMW sample was determined by Fixed and Volatile Suspended Solids Ignited at 550^{o}C Method (Standard Method 2540.E). The principle is stated as "*The residue from Method D is ignited to constant weight at 550^{o}C. The remaining solids represent the fixed suspended solids while the weight loss on ignition is the volatile suspended solids.*" [44]. For the triplicate analysis, 47mm Whatman Grade-C standard glass-fiber filter papers were used.

3.2.7 Temperature

Temperature values of the samples were determined at collection sites using a mercury-filled Celcius thermometer of –10-60^{o}C range.

3.2.8 pH

pH values of the samples were determined at collection sites using pH strips. In the triplicate analysis, 0-6 range strips for OMW and 0-14 range strips for DW were used. The values determined at site were controlled by measuring the values again using a WTW pH-meter immediately after reaching the laboratory -about 1 hour-.

3.2.9 Biogas Composition

The composition of the biogas samples produced throughout the study were determined by Gas Chromatographic Method (Standard Method 2720.C). For the determinations a Shimadzu GC17A Version3 gas chromatograph equipped with a direct injection port and a thermal conductivity detector (TCD), a Chrompack porous polymer (Hayesep®Q, 80/100 mesh) filled 8ftx1/8'' stainless steel packed column and a Hamilton gas-tight syringe (Series 1000, 2.5 ml capacity) were employed.

3.3 Experimental Set-Up and Procedures

In this section the works prior to BMP experiments, the procedure followed in the BMP experiments and set-up of reactors used in this study are described.

3.3.1 Sampling and Characterization

The OMW sample was collected into a 5 liter polyethylene bottle as a grab sample in the beginning of March 2003.

The DW sample was a 24 hour-composite sample taken from the outlet of the grit removal unit of the treatment plant. It was taken into a 5 liter polyethylene bottle .

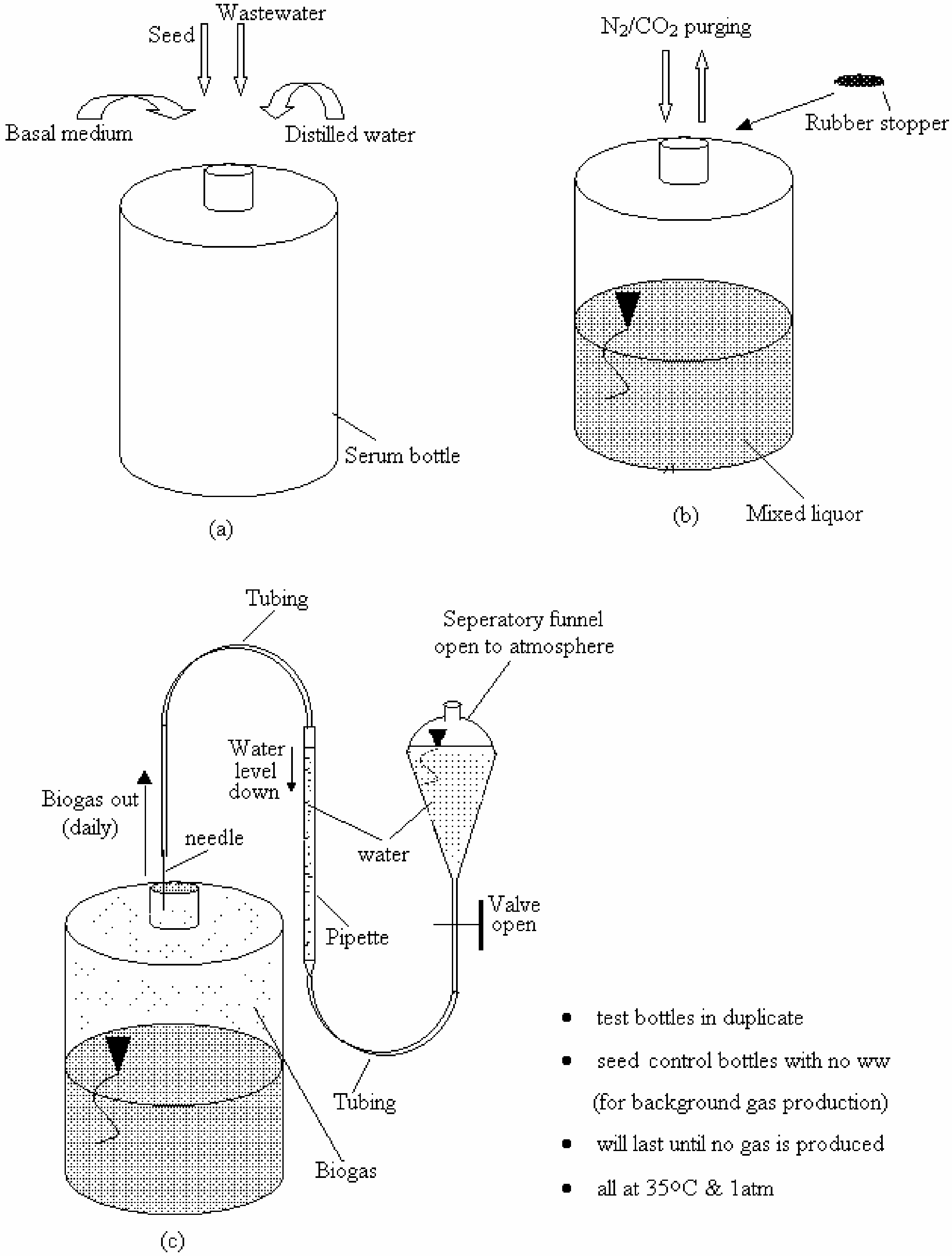
Temperature and pH measurements were performed at site, and then the collected samples were immediately taken to the laboratory for characterization.

Required dilutions for each parameter were prepared and the samples were characterized, according to aforementioned methods, both on the day of collection and at the beginning of the experiments. After the preparation of dilutions, remaining raw samples were stored at 4^oC until the preparation of the reactors on the next day.

3.3.2 Biochemical Methane Potential Experiments

BMP experiments are carried out by placing a sample in a serum bottle which is a reactor provided with all requirements of anaerobic microbial activity: removal of oxygen, an anaerobic seed culture, optimal constant temperature, micro and macro nutrients, and buffer against a decrease in pH. Net methane production of the reactor, total production minus background production observed in a control reactor containing only seed, is measured and corresponding pollutant decomposition is found. A schematic explanation of the procedure can be seen in Figure 3.1.

In this study BMP experiments were used to determine the anaerobic treatability of the olive mill and domestic wastewater samples and their mixtures of different proportions. 100 ml amber colored serum bottles with 40 ml effective volume were used as anaerobic (batch) reactors. Serum bottles were seeded with the mixed anaerobic cultures. The seed culture from ASKİ was thoroughly mixed, filtered through a 1 mm-mesh size screen while these procedures were not applied to the granular culture from Efes Pilsen. Then their SS and VSS contents were determined prior to use. Predetermined amounts of nutrient and trace metal solution (basal medium), distilled water, and wastewater mixtures were added into serum bottles. Bottles were then purged with a gas mixture of 75% N_2 and 25%CO_2 for 3–4 minutes to supply anaerobic conditions and to adjust the pH to an appropriate value. After purging they were sealed with rubber stoppers and the stoppers were locked in place using plastic cable ties. Finally, the bottles were incubated in an incubator at 35±1°C. Reactors were run for 5 different mixtures (having OMW:DW ratios of 1:9, 1:5, 1:3, 1:1 and 1:0) and at 5 different COD loading levels (4553, 9107, 13660, 22767 and 34150 mg/l) for mixtures having adequate COD concentrations, with two different seed cultures. Control reactors (seed-blanks), for background gas production, and non-BM reactors of each composition, for monitoring the complementarity of OMW and DW in their mixtures and the effect of nutrition, were employed . All reactors were run in duplicates. The composition of the reactors and BMP sets used in this study can be seen in Table 3.1 and Table 3.2 respectively.



- test bottles in duplicate
- seed control bottles with no ww (for background gas production)
- will last until no gas is produced
- all at 35°C & 1atm

Figure 3.1 Procedure of biochemical methane potential (BMP) experiment

Table 3.1 Content and COD loading (COD_r) of the reactors, and OMW:DW ratio and COD values (COD_{mix}) of the mixtures used

seed source	***reactor#***	***omw:dw***	***COD_{mix} (mg/l)***	***COD_r (mg/l)***	***BM (ml)***	***Seed (ml)***	***wastewater (ml)***	***distilled water (ml)***	***total volume (ml)***
EFES	***E1&E2***	Control	0	0	0	10	0	30	40
EFES	***E3&E4***	Control	0	0	5	10	0	25	40
EFES	***E5&E6***	1:9	9882	4553	0	10	18.4	11.6	40
EFES	***E7&E8***	1:9	9882	4553	5	10	18.4	6.6	40
EFES	***E9&E10***	1:5	15895	4553	0	10	11.5	18.5	40
EFES	***E11&E12***	1:5	15895	4553	5	10	11.5	13.5	40
EFES	***E13&E14***	1:5	15895	9107	0	10	22.9	7.1	40
EFES	***E15&E16***	1:5	15895	9107	5	10	22.9	2.1	40
EFES	***E17&E18***	1:3	23413	4553	0	10	7.8	22.2	40
EFES	***E19&E20***	1:3	23413	4553	5	10	7.8	17.2	40
EFES	***E21&E22***	1:3	23413	9107	0	10	15.6	14.4	40
EFES	***E23&E24***	1:3	23413	9107	5	10	15.6	9.4	40
EFES	***E25&E26***	1:3	23413	13660	0	10	23.3	6.7	40
EFES	***E27&E28***	1:3	23413	13660	5	10	23.3	1.7	40
EFES	***E29&E30***	1:1	45964	4553	0	10	4	26	40
EFES	***E31&E32***	1:1	45964	4553	5	10	4	21	40
EFES	***E33&E34***	1:1	45964	9107	0	10	7.9	22.1	40
EFES	***E35&E36***	1:1	45964	9107	5	10	7.9	17.1	40
EFES	***E37&E38***	1:1	45964	13660	0	10	11.9	18.1	40
EFES	***E39&E40***	1:1	45964	13660	5	10	11.9	13.1	40
EFES	***E41&E42***	1:1	45964	22767	0	10	19.8	10.2	40
EFES	***E43&E44***	1:1	45964	22767	5	10	19.8	5.2	40
EFES	***E45&E46***	1:0	91067	4553	0	10	2	28	40
EFES	***E47&E48***	1:0	91067	4553	5	10	2	23	40
EFES	***E49&E50***	1:0	91067	9107	0	10	4	26	40
EFES	***E51&E52***	1:0	91067	9107	5	10	4	21	40
EFES	***E53&E54***	1:0	91067	13660	0	10	6	24	40
EFES	***E55&E56***	1:0	91067	13660	5	10	6	19	40
EFES	***E57&E58***	1:0	91067	22767	0	10	10	20	40
EFES	***E59&E60***	1:0	91067	22767	5	10	10	15	40
EFES	***E61&E62***	1:0	91067	34150	0	10	15	15	40
EFES	***E63&E64***	1:0	91067	34150	5	10	15	10	40
ANKARA	***A1&A2***	Control	0	0	0	10	0	30	40
ANKARA	***A3&A4***	Control	0	0	5	10	0	25	40
ANKARA	***A5&A6***	1:5	15895	4553	0	10	11.5	18.5	40
ANKARA	***A7&A8***	1:5	15895	4553	5	10	11.5	13.5	40
ANKARA	***A9&A10***	1:5	15895	9107	0	10	22.9	7.1	40
ANKARA	***A11&A12***	1:5	15895	9107	5	10	22.9	2.1	40
ANKARA	***A13&A14***	1:3	23413	9107	0	10	15.6	14.4	40
ANKARA	***A15&A16***	1:3	23413	9107	5	10	15.6	9.4	40
ANKARA	***A17&A18***	1:3	23413	13660	0	10	23.3	6.7	40
ANKARA	***A19&A20***	1:3	23413	13660	5	10	23.3	1.7	40
ANKARA	***A21&A22***	1:1	45964	13660	0	10	11.9	18.1	40
ANKARA	***A23&A24***	1:1	45964	13660	5	10	11.9	13.1	40
ANKARA	***A25&A26***	1:1	45964	22767	0	10	19.8	10.2	40
ANKARA	***A27&A28***	1:1	45964	22767	5	10	19.8	5.2	40

Table 3.2 BMP sets used in the study

seed culture		***EFES***					***ANKARA***				
COD (mg/l)		***4553***	***9107***	***13660***	***22767***	***34150***	***4553***	***9107***	***13660***	***22767***	***34150***
OMW:DW	***1:9***	√*	-	-	-	-	-	-	-	-	-
	1:5	√	√	-	-	-	√	√	-	-	-
	1:3	√	√	√	-	-	-	√	√	-	-
	1:1	√	√	√	√	-	-	-	√	√	-
	1:0	√	√	√	√	√	-	-	-	-	-

*Each '√' represents 4 reactors (duplicates of BM- and BM+ reactors)

Gas produced in each serum bottle was taken out and measured daily by means of a water displacement device. The device consists of a 50 ml graduated pipette and a separatory funnel, which serves as a reservoir open to atmospheric pressure, connected with a piece of silicon tubing from the bottom, and a hypodermic needle connected to the upper end of the pipette with another piece of silicon tubing. The pipette and the funnel are fixed on a support in the position that water level shows the zero mark of the pipette. When the needle is inserted through the rubber stopper of a reactor, produced gas causing excess pressure inside flows out and pushes the fluid until the pressure comes back to 1 atm. So the water level indicates the volume of produced gas. For the fast release of the gas taken into the system, in order to pass to the next measurement in a quick manner, a third piece of tubing was attached to the second tubing, between the pipette and the needle, using a T-connector and it was fitted with a valve at the open end. Measurements were carried out daily for each reactor until no biogas production was observed.

Raw daily gas production data, obtained at the recorded room temperature, were corrected for 35^{o}C using the ideal gas law.

Composition of produced gas was determined by GC analysis for each reactor using the average of at least 2 determinations throughout the experimental period. In the GC analysis, operating temperatures of injection port, column oven and detector were 180^{o}C, 50^{o}C and 100^{o}C respectively. Sample size was selected as 1 ml with a carrier gas (helium) flowrate of 30 ml/min. Definition and quantification of observed peaks (CH_4, CO_2, N_2, H_2S) was carried out by analyzing a standard gas mixture containing 73.63% CH_4, 23.88% CO_2, 1.99% N_2 and 0.50% H_2S then preparing a calibration curve for each gas. Calibrations, peak area vs. concentration, were performed using the results of triplicate analysis of standard gas mixture injections of 0.5, 0.8, 1, 1.2 and 1.5 ml.

Cumulative CH_4 production values were obtained by combining temperature corrected gas production results and gas composition analysis for each reactor. Then mean values for each reactor pair were taken and background production was subtracted. Resultant values were recorded as net CH_4 production. Finally, the ratio of these actual net values to theoretical maximum CH_4 values, calculated by using the stochiometric relation of "1 g COD destruction corresponds to 395 ml CH_4 production at 35 ^{o}C", gave the average theoretical COD removal efficiency of each reactor pair.

At the end of the study, final COD concentrations were obtained for reactor pairs by triplicate spectrophotometric determinations. Background COD concentrations, COD of control reactors, were subtracted from reactor COD concentrations and residual values were used to determine the actual COD removal efficiency for each reactor pair. These efficiency values were used in the assessment of the study, instead of the ones obtained from CH_4 determinations, since direct measurement of COD is more reliable.

Chapter 4

RESULTS AND DISCUSSION

In this chapter, the results of the studies mentioned in the preceding chapter are presented and discussed.

4.1 Characterization

Results of the characterization studies for olive mill and domestic wastewater samples used in this anaerobic treatability study are given below in Table 4.1.

These results approve the expected nutrient deficiency of OMW, particularly for nitrogen. Generally accepted minimum nutrient requirement expressed as COD/N/P ratio, calculated from stochiometric relations, vary between 350/7/1 and 1000/7/1

Table 4.1 Characteristics of OMW and DW samples used in the study

Parameter	OMW	DW
COD (mg/l)	91067 ± 2 836	681 ± 73
BOD (mg/l)	30679 ± 693	364 ± 38
TKN (mg/l)	373 ± 18	24.7 ± 2.3
Tot-P (mg/l)	424 ± 65	7.4 ± 0.2
Phenol (mg/l)	1351 ± 21	-
SS (mg/l)	11347 ± 579	196 ± 17
VSS (mg/l)	11235 ± 493	124 ± 13
VSS/SS (%)	99.0	63.3
pH	5.35	7.24
Temperature (^{o}C)	29	19

according to loading conditions [3]. For the measured COD of the OMW sample, calculated nitrogen and phosphorus requirements are 637-1821 mg/l and 91-260 mg/l respectively. Compared to present concentrations in the OMW sample, these requirements show a significant deficiency of nitrogen and an excess of phosphorus. Even using the lowest requirement ratio and disregarding that all nitrogen cannot be utilized, the self dependency for nitrogen is about 59%. The high temperature of the sample presents an advantage for potential applications of heated mesophilic digesters, which is the case in this study, with a minimal necessity of heating.

The characteristics of the domestic wastewater sample fit typical values of strong wastewaters in terms of COD and BOD, and average wastewaters for the other parameters.

The SS and VSS concentrations of the seed cultures were found to be 28700 and 11000 mg/l for the culture from ASKİ treatment plant (Ankara), and 65548 and 60333 mg/l for the culture from Efes Pilsen Brewery (Efes) respectively.

4.2 Biochemical Methane Potential Experiments

4.2.1 General

In this section the results of the BMP experiments, consisting of gas measurements, biogas composition analysis and raw data processing, are presented and discussed.

After characterization, mixtures with OMW:DW ratios of 1:9, 1:5, 1:3, and 1:1 corresponding to 10%, 17%, 25% and 50% OMW contents were prepared. Predetermined amounts of these mixtures and raw OMW, basal medium, seed cultures and distilled water were filled into the reactors, and the experiments were successfully initiated. The biomass concentrations in Efes seeded reactors (E#1-E#64) and Ankara seeded ones (A#1-A#28) (as VSS of seed culture, regarding 10ml/40ml dilution in the reactors) were 15083 and 2750 mg/l respectively.

The first gas production measurement was conducted on the first day. The consistency of duplicate reactors and also equally loaded reactors proved the success of initiating.

A summary of the experimental results, including total biogas production, methane content, total, net and theoretical methane production, COD removal efficiency values calculated by methane production, net final COD concentrations and actual COD removal efficiency values for each pair of reactors are given in Table 4.2. In Table 4.3 net biogas and methane yields are also given for grams of COD added, grams of COD removed and grams of microbial population (as VSS) added.

Results for reactor E#24 are given separately and they were not considered in comparisons because of its very high gas production and COD removal efficiency values. Theoretical and actual COD removal efficiency values of this reactor were 102.7% by gas production and 94.0% by COD determination, while the average values for equally loaded reactors were 64.0±12.3% and 72.2±6.9% respectively.

COD removal efficiencies achieved in the experiments, around 67-82% for both BM- and BM+ reactors, are found to be in the mid-range of results reported in the literature, from around 70% up to 85-95% [7,9,10,13]. COD removal efficiencies obtained by BM- and BM+ reactors were generally very close for all reactors except 34150 mg/l COD loaded ones. Especially at low COD loadings, some BM- reactors performed slightly better than BM+ ones. BM- reactors seeded with Ankara culture were not as successful as those seeded with Efes culture when compared with respect to their performance against their BM+ pairs. This fact may be a result of a possible higher requirement of Ankara culture microorganisms for trace elements (micronutrients) which are not found in DW in adequate amounts. Gas production trends followed by BM- and BM+ reactors also give a similar opinion. All Efes culture

Table 4.2 Results of the BMP Experiments

reactor#	***description***	***COD_r (mg/l)***	***total gas production (ml)***	***CH_4 content (%)***	***total CH_4 production (ml)***	***net CH_4 production (ml)***	***theoretical CH_4 production (ml)***	***theoretical removal efficiency (%)***	***final COD (mg/l)***	***removal efficiency (%)***
E1&E2	control-	-	99.3	67.6	67.1	-	-	-	-	-
E3&E4	control+	-	93.6	65.5	61.3	-	-	-	-	-
E5&E6	(1:9)-	4553	166.6	66.7	111.2	44.0	71.9	61.2	1166	74.4
E7&E8	(1:9)+	4553	156.8	66.5	104.3	43.0	71.9	59.8	1368	70.0
E9&E10	(1:5)-	4553	176.7	67.5	119.2	52.1	71.9	72.4	813	82.2
E11&E12	(1:5)+	4553	161.8	66.6	107.8	46.5	71.9	64.6	1043	77.1
E13&E14	(1:5)-	9107	229.1	66.5	152.4	85.3	143.9	59.3	2888	68.3
E15&E16	(1:5)+	9107	227.5	66.0	150.1	88.8	143.9	61.7	2818	69.1
E17&E18	(1:3)-	4553	171.9	66.6	114.5	47.4	71.9	65.8	838	81.6
E19&E20	(1:3)+	4553	171.5	66.8	114.5	53.1	71.9	73.9	805	82.3
E21&E22	(1:3)-	9107	244.6	67.1	164.1	97.0	143.9	67.4	2388	73.8
E23	(1:3)+	9107	233.8	67.0	156.5	95.2	143.9	66.2	2568	71.8
E24	(1:3)+	9107	318.2	65.7	209.0	147.7	143.9	102.7	550	94.0
E25&E26	(1:3)-	13660	294.2	66.6	196.0	128.9	215.8	59.7	4050	70.4
E27&E28	(1:3)+	13660	304.2	65.6	199.6	138.3	215.8	64.1	3793	72.2
E29&E30	(1:1)-	4553	177.4	67.9	120.4	53.3	71.9	74.0	913	80.0
E31&E32	(1:1)+	4553	164.7	68.8	113.3	52.0	71.9	72.3	893	80.4
E33&E34	(1:1)-	9107	234.2	66.2	155.0	87.9	143.9	61.1	2713	70.2
E35&E36	(1:1)+	9107	230.1	66.1	152.1	90.7	143.9	63.1	2680	70.6
E37&E38	(1:1)-	13660	299.2	66.2	198.2	131.0	215.8	60.7	4400	67.8
E39&E40	(1:1)+	13660	295.6	65.5	193.7	132.4	215.8	61.3	4343	68.2
E41&E42	(1:1)-	22767	442.5	66.1	292.3	225.1	359.7	62.6	7025	69.1
E43&E44	(1:1)+	22767	435.2	65.0	283.1	221.7	359.7	61.6	6893	69.7
E45&E46	(1:0)-	4553	170.8	67.2	114.7	47.6	71.9	66.2	1025	77.5
E47&E48	(1:0)+	4553	165.9	66.5	110.4	49.0	71.9	68.2	968	78.8
E49&E50	(1:0)-	9107	233.7	66.5	155.4	88.3	143.9	61.3	2838	68.8
E51&E52	(1:0)+	9107	232.0	66.3	153.8	92.5	143.9	64.3	2705	70.3
E53&E54	(1:0)-	13660	307.3	65.9	202.4	135.2	215.8	62.7	4113	69.9
E55&E56	(1:0)+	13660	300.4	65.3	196.3	135.0	215.8	62.5	3843	71.9
E57&E58	(1:0)-	22767	467.6	66.5	310.8	243.7	359.7	67.7	5863	74.3
E59&E60	(1:0)+	22767	428.1	65.1	278.6	217.3	359.7	60.4	7018	69.2
E61&E62	(1:0)-	34150	114.4	28.1	32.3	-34.9	539.6	-6.5	33700	1.3
E63&E64	(1:0)+	34150	597.0	65.9	393.7	332.4	539.6	61.6	10718	68.6
A1&A2	control-	-	9.4	66.1	6.2	0	-	-	-	-
A3&A4	control+	-	9.6	67.7	6.5	0	-	-	-	-
A5&A6	(1:5)-	4553	65.4	67.4	44.1	37.9	71.9	52.7	1610	64.6
A7&A8	(1:5)+	4553	60.4	69.6	42.0	35.6	71.9	49.4	1650	63.8
A9&A10	(1:5)-	9107	122.6	70.1	86.0	79.8	143.9	55.4	2880	68.4
A11&A12	(1:5)+	9107	124.8	72.0	89.8	83.3	143.9	57.9	2540	72.1
A13&A14	(1:3)-	9107	119.7	69.7	83.4	77.2	143.9	53.6	2990	67.2
A15&A16	(1:3)+	9107	125.4	72.1	90.4	84.0	143.9	58.4	2370	74.0
A17&A18	(1:3)-	13660	171.5	69.2	118.6	112.5	215.8	52.1	3885	71.6
A19&A20	(1:3)+	13660	185.4	72.5	134.4	127.9	215.8	59.3	3315	75.7
A21&A22	(1:1)-	13660	172.0	70.9	121.9	115.8	215.8	53.6	3775	72.4
A23&A24	(1:1)+	13660	190.6	72.6	138.3	131.9	215.8	61.1	2435	82.2
A25&A26	(1:1)-	22767	287.1	65.2	187.2	181.0	359.7	50.3	8865	61.1
A27&A28	(1:1)+	22767	299.9	71.5	214.4	207.9	359.7	57.8	7495	67.1

Table 4.3 Net biogas and CH_4 yields for grams of COD added, grams of COD removed and grams of microbial population (as VSS) added

reactor#	***description***	***CODr (mg/l)***	***ml biogas / g COD added***	***ml CH4 / g COD added***	***ml biogas / g COD removed***	***ml CH4 / g COD removed***	***ml biogas / g VSS added***	***ml CH4 / g VSS added***
E5&E6	(1:9)-	4553	369.8	241.8	497.2	325.1	111.6	73.0
E7&E8	(1:9)+	4553	347.0	236.1	496.0	337.4	104.8	71.3
E9&E10	(1:5)-	4553	425.0	286.2	517.3	348.3	128.3	86.4
E11&E12	(1:5)+	4553	374.5	255.2	485.7	331.0	113.0	77.0
E13&E14	(1:5)-	9107	356.5	234.1	521.9	342.7	215.2	141.3
E15&E16	(1:5)+	9107	367.6	243.8	532.2	353.0	221.9	147.2
E17&E18	(1:3)-	4553	398.9	260.0	488.8	318.7	120.4	78.5
E19&E20	(1:3)+	4553	427.7	291.8	519.6	354.5	129.1	88.1
E21&E22	(1:3)-	9107	398.9	266.2	540.6	360.8	240.8	160.7
E23	(1:3)+	9107	385.0	261.4	536.2	364.0	232.5	157.8
E24	(1:3)+	9107	616.7	405.5	656.3	431.5	372.4	244.8
E25&E26	(1:3)-	13660	356.8	235.9	507.2	335.3	323.1	213.6
E27&E28	(1:3)+	13660	385.4	253.2	533.6	350.5	349.1	229.3
E29&E30	(1:1)-	4553	428.8	292.5	536.3	365.8	129.5	88.3
E31&E32	(1:1)+	4553	390.7	285.7	485.9	355.4	117.9	86.2
E33&E34	(1:1)-	9107	370.5	241.3	527.6	343.7	223.7	145.7
E35&E36	(1:1)+	9107	374.7	249.1	531.0	353.0	226.2	150.4
E37&E38	(1:1)-	13660	365.8	239.8	539.7	353.8	331.3	217.2
E39&E40	(1:1)+	13660	369.7	242.3	542.0	355.2	334.8	219.4
E41&E42	(1:1)-	22767	376.9	247.2	545.0	357.5	568.9	373.2
E43&E44	(1:1)+	22767	375.1	243.5	538.0	349.2	566.2	367.5
E45&E46	(1:0)-	4553	392.6	261.5	506.7	337.5	118.5	78.9
E47&E48	(1:0)+	4553	397.0	269.3	504.1	342.0	119.8	81.3
E49&E50	(1:0)-	9107	369.1	242.3	536.1	352.0	222.9	146.3
E51&E52	(1:0)+	9107	379.9	253.8	540.5	361.0	229.4	153.2
E53&E54	(1:0)-	13660	380.8	247.5	544.8	354.1	344.8	224.2
E55&E56	(1:0)+	13660	378.5	247.0	526.6	343.7	342.8	223.7
E57&E58	(1:0)-	22767	404.5	267.6	544.8	360.4	610.5	403.9
E59&E60	(1:0)+	22767	367.3	238.6	531.0	344.9	554.4	360.2
E61&E62	(1:0)-	34150	11.1	-	838.9	-	25.0	-
E63&E64	(1:0)+	34150	368.6	243.3	537.1	354.6	834.5	550.9
A5&A6	(1:5)-	4553	307.8	208.3	476.1	322.3	509.5	344.9
A7&A8	(1:5)+	4553	279.2	195.3	437.9	306.3	462.3	323.4
A9&A10	(1:5)-	9107	310.7	219.0	454.5	320.3	1029.1	725.2
A11&A12	(1:5)+	9107	316.4	228.8	438.7	317.2	1047.7	757.6
A13&A14	(1:3)-	9107	302.8	211.9	450.8	315.5	1002.7	701.7
A15&A16	(1:3)+	9107	318.0	230.5	429.9	311.6	1053.2	763.5
A17&A18	(1:3)-	13660	296.7	205.8	414.6	287.6	1473.6	1022.3
A19&A20	(1:3)+	13660	321.7	234.1	424.8	309.1	1598.2	1162.8
A21&A22	(1:1)-	13660	297.7	211.9	411.4	292.8	1478.6	1052.4
A23&A24	(1:1)+	13660	331.3	241.4	403.1	293.7	1645.5	1198.9
A25&A26	(1:1)-	22767	304.9	198.8	499.4	325.5	2524.5	1645.5
A27&A28	(1:1)+	22767	318.8	228.3	475.2	340.3	2639.1	1890.1

seeded reactors show similar trends except 34150 mg/l COD loaded ones, while Ankara culture seeded BM- reactors differ from BM+ ones as will be seen in following sections. COD removal efficiencies of BM- and BM+ reactors and their comparison can be viewed in Figure 4.1 and Figure 4.2 with respect to seed cultures. In addition to the data of these figures, a comparison of BM- and BM+ reactors in terms of total gas production and net CH_4 production is given in tabular form in Table 4.4.

No toxic effect which may be caused by the phenol and/or high organic content of the OMW sample was observed up to a COD loading of 22767 mg/l. At 34150 mg/l COD loading, only in BM- reactors microbial activity has nearly stopped after a few days of gas production at low rates. Since 22767 mg/l COD loaded reactors operated similar to lower loadings and they had closer COD removal efficiencies, this toxic effect is not thought to be caused by high organic loading. This phenomenon shows that BM addition does not only help for dealing with nutrient deficiency, but also improves the resistance of microorganisms to toxic/inhibitory effects.

Methane content of biogas samples produced by Efes culture seeded reactors and Ankara culture seeded reactors were in the range of 65-67% and 67-72% respectively. For the first group BM+ reactors and for the second group BM- ones produced slightly higher methane containing biogas. Since the main composition (biomass and substrate) is identical for BM- and BM+ reactors, by stochiometric relations, the resultant products were expected to be identical. Nutrient addition may affect the rate and total amount of substrate consumption, but it is not expected to change the stochiometry. This topic may be subject to further investigations.

A general linear relation, presenting increased COD removal efficiency with increased DW content in wastewater mixture, could not be established by the results. On the other hand, this type of relation was observed for COD removal efficiency ratio of BM- to BM+ reactors seeded with the Efes culture. The only exception was caused by the unexpectedly high performance of 22767 mg/l COD loaded BM- reactors.

Ankara seeded reactors showed increasing performance with increasing loading levels until 13660 mg/l loading. However, performance COD removal ratio of BM- to BM+ reactors decreased with increasing loading. Then at 22767 mg/l loading, a viable decrease was observed for both efficiency values and their ratio. This decrease show that the optimum COD loading level may be between 13000-20000mg/l; and when compared to Efes seeded reactors, it can be concluded that reactors containing Efes culture can operate at higher loadings which means lower reactor volumes, therefore reduced initial costs.

Cumulative gas production patterns of the reactors are given in Figures 4.2-4.6 under relevant headings with respect to COD loading : Figure 4.3 4553mg/l, Figure 4.4 9107 mg/l, Figure 4.5 13660 mg/l, Figure 4.6 22767 mg/l, Figure 4.7 34150 mg/l.

Since the seed cultures were not acclimated before the experiments, a lag period for acclimation followed by a logarithmic increase period reaching to a plateau could be expected. But in the contrary high gas production rates in the first days were followed by lower rates for all reactors. This might be due to the fast utilization of easily biodegradable portion of substrate in soluble form in the first days. Then slow hydrolysis rates might have been the rate limiting step in organic material degradation. Also the decrease in COD concentration, which is the driving force of microbial activity, may cause a reduced substrate utilization rate.

All Efes reactors showed no significant demand for an acclimation period. The only exception was the 34150 mg/l COD loading with no nutrition case, in which strong inhibition with stopped gas production was observed.

At all organic loading levels, reactors with equal loading, same nutritional conditions (BM+ or BM-) and the same seed followed similar trends resulting in gas production values close to each other. At all loadings, all Efes reactors, either BM- or BM+, showed similar trends and achieved most of their gas production between 15-20 days increasing with loading. In the case of Ankara seeded reactors, BM- reactors required acclimation periods as mentioned before, and produced most of the biogas in 25-30 days, while this period was 20-25 days for BM+ reactors. Length of the period

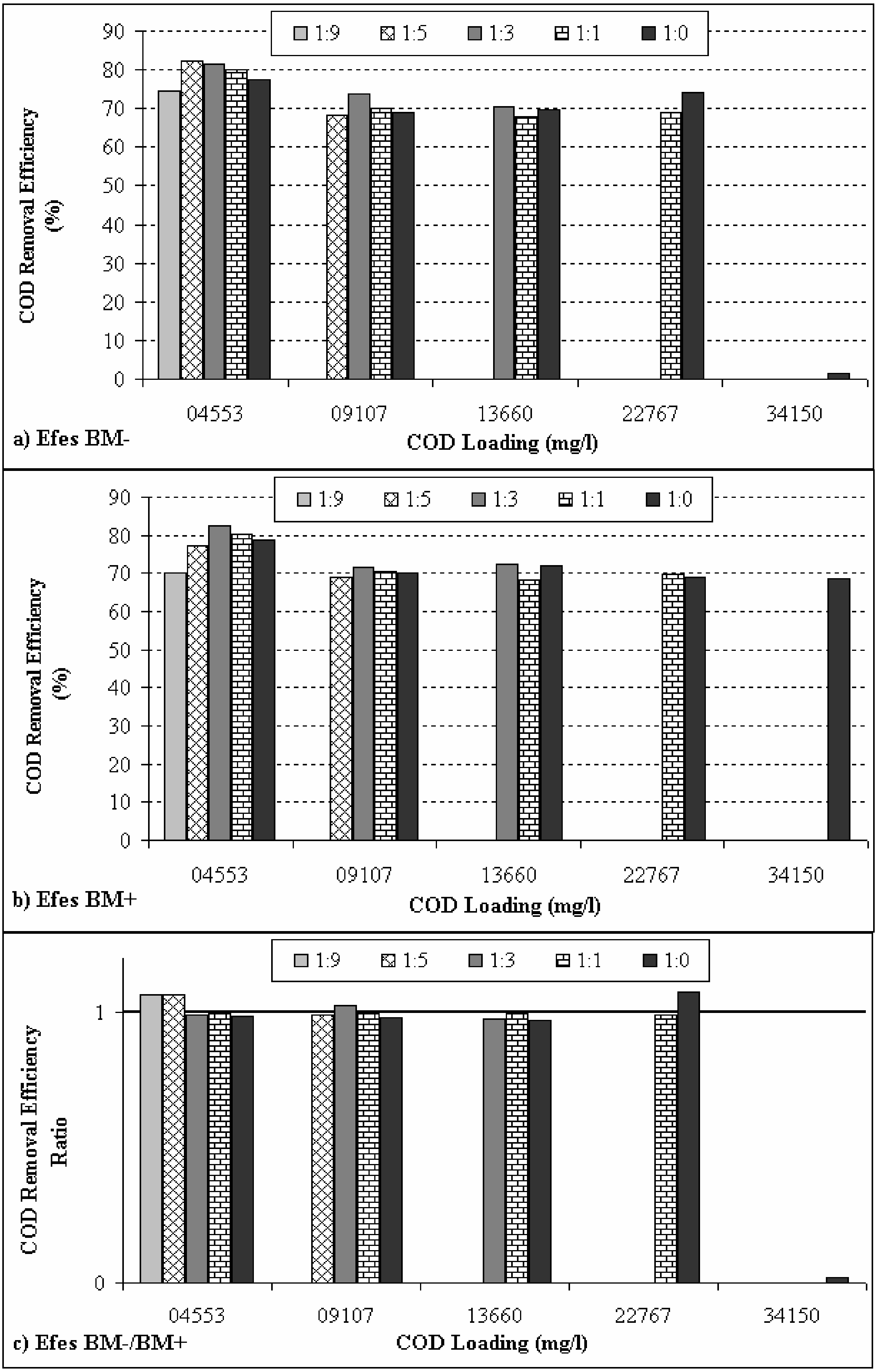


Figure 4.1 COD removal efficiencies of Efes culture seeded BM-(a) and BM+(b) reactors and their ratio(c)

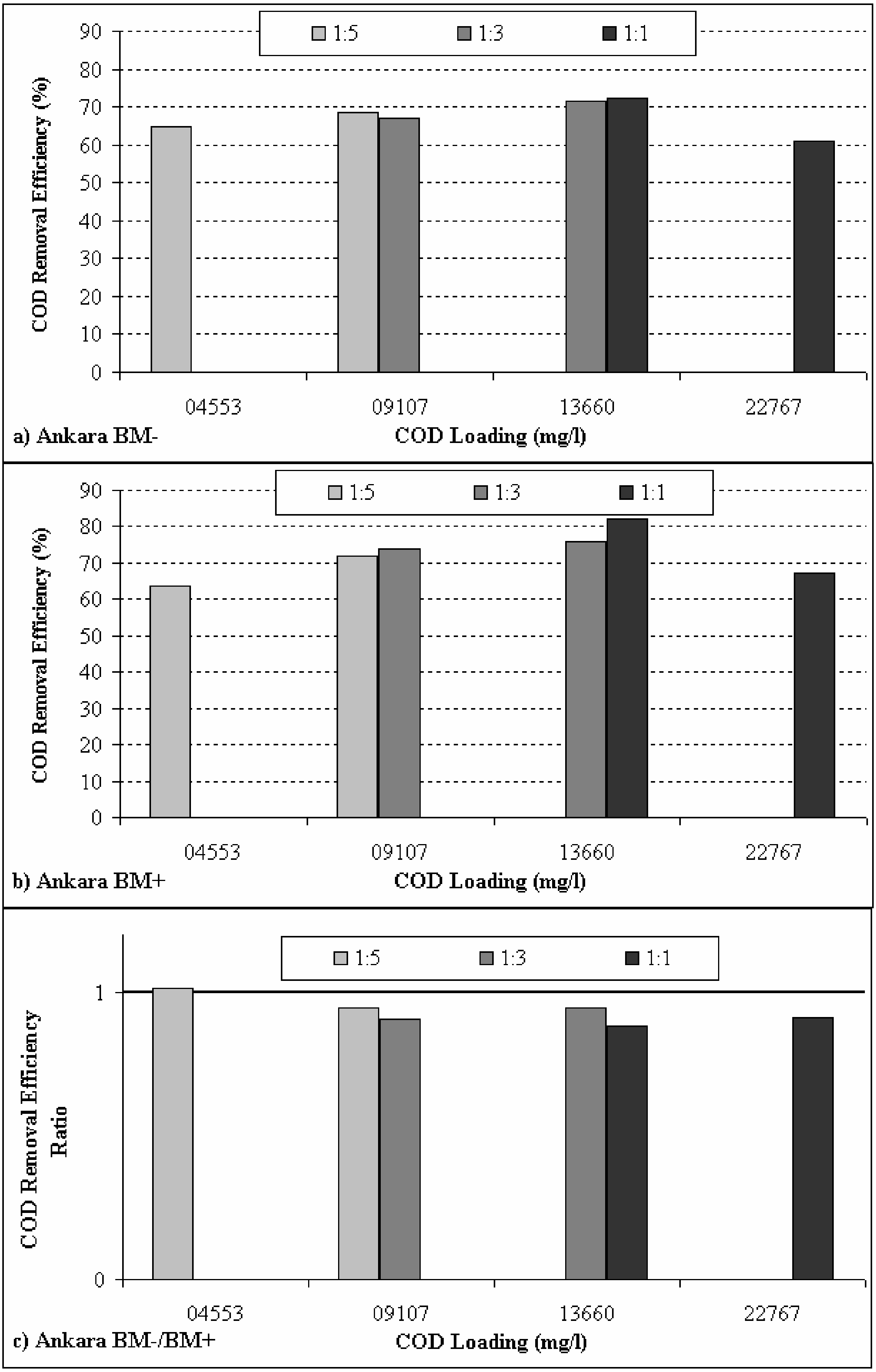


Figure 4.2 COD removal efficiencies of Ankara culture seeded BM-(a) and BM+(b) reactors and their ratio(c)

Table 4.4 COD removal efficiencies of BM- and BM+ reactors; and COD removal efficiency ratio total gas production ratio and net CH_4 production ratio of BM- to BM+ reactors

a) COD removal efficiencies of BM- reactors (%)

COD (mg/l)	***4553***		***9107***		***13660***		***22767***		***34150***	
OMW:DW	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara
1:9	74.4	-	-	-	-	-	-	-	-	-
1:5	82.2	64.6	68.3	68.4	-	-	-	-	-	-
1:3	81.6	-	73.8	67.2	70.4	71.6	-	-	-	-
1:1	80.0	-	70.2	-	67.8	72.4	69.1	61.1	-	-
1:0	77.5	-	68.8	-	69.9	-	74.3	-	1.3	-

b) COD removal efficiencies of BM+ reactors (%)

COD (mg/l)	***4553***		***9107***		***13660***		***22767***		***34150***	
OMW:DW	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara
1:9	70.0	-	-	-	-	-	-	-	-	-
1:5	77.1	63.8	69.1	72.1	-	-	-	-	-	-
1:3	82.3	-	71.8	74.0	72.2	75.7	-	-	-	-
1:1	80.4	-	70.6	-	68.2	82.2	69.7	67.1	-	-
1:0	78.8	-	70.3	-	71.9	-	69.2	-	68.6	-

c) COD removal efficiency ratio of BM- to BM+ reactors

COD (mg/l)	***4553***		***9107***		***13660***		***22767***		***34150***	
OMW:DW	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara
1:9	1.063	-	-	-	-	-	-	-	-	-
1:5	1.066	1.014	0.989	0.948	-	-	-	-	-	-
1:3	0.991	-	1.028	0.908	0.974	0.945	-	-	-	-
1:1	0.995	-	0.995	-	0.994	0.881	0.992	0.910	-	-
1:0	0.984	-	0.979	-	0.972	-	1.073	-	0.019	-

d) Total gas production ratio of BM- to BM+ reactors

COD (mg/l)	***4553***		***9107***		***13660***		***22767***		***34150***	
OMW:DW	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara
1:9	1.063	-	-	-	-	-	-	-	-	-
1:5	1.092	1.083	1.007	0.982	-	-	-	-	-	-
1:3	1.003	-	1.046	0.954	0.967	0.925	-	-	-	-
1:1	1.077	-	1.018	-	1.012	0.903	1.017	0.957	-	-
1:0	1.030	-	1.008	-	1.023	-	1.092	-	0.192	-

e) Net CH4 production ratio of BM- to BM+ reactors

COD (mg/l)	***4553***		***9107***		***13660***		***22767***		***34150***	
OMW:DW	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara	Efes	Ankara
1:9	1.024	-	-	-	-	-	-	-	-	-
1:5	1.121	1.067	0.960	0.957	-	-	-	-	-	-
1:3	0.891	-	1.018	0.919	0.932	0.879	-	-	-	-
1:1	1.024	-	0.969	-	0.990	0.878	1.015	0.871	-	-
1:0	0.971	-	0.955	-	1.002	-	1.121	-	-0.105	-

required for reaching most of the production, which means required for degradation, is a very important factor in the economy of applications. As the degradation rate increases, hydraulic retention time (HRT) of the reactor required to achieve targeted removal efficiency decreases. Reactor construction is the main initial cost in treatment plants, and the size/cost of a reactor is directly proportional to the required HRT.

4.2.2 COD Loading of 4553 mg/l

This set contained samples seeded with Efes culture of all wastewater mixtures having different OMW content and a 1:5 (OMW:DW) sample seeded with Ankara culture. Organic load on biomass was 0.302 and 1.656 gCOD/gVSS in Efes culture and Ankara culture seeded reactors respectively.

Gas production trends of all Efes reactors were similar. As mentioned before, there was no lag period required for acclimation; they followed a parabolic curve with very high initial removal rates as can be seen in Figure 4.3. They produced 17-18% of their total biogas in 1 day and reached about 28-32% on the third day. This is thought to be a consequence of the relatively high VSS concentration in the reactors. There were no breakpoints or plateaus on the gas production curves; production achieved in 30 days was around 70% of total and on 71st day production was slowly going on.

The case was different for the Ankara reactors which were loaded with 1:5 (OMW:DW) mixture. After going together for 10 days up to 40% of their total gas production, BM+ reactors made a jump and reached a stationary phase on 16th day with 83% of their total. On the same day BM- reactors could reach only 59%, and they came to their stationary phase on 21st day with 80% of gas production. This variation shows the positive effect of BM addition on the acclimation problem of Ankara culture that may be mainly caused by its relatively low VSS concentration.

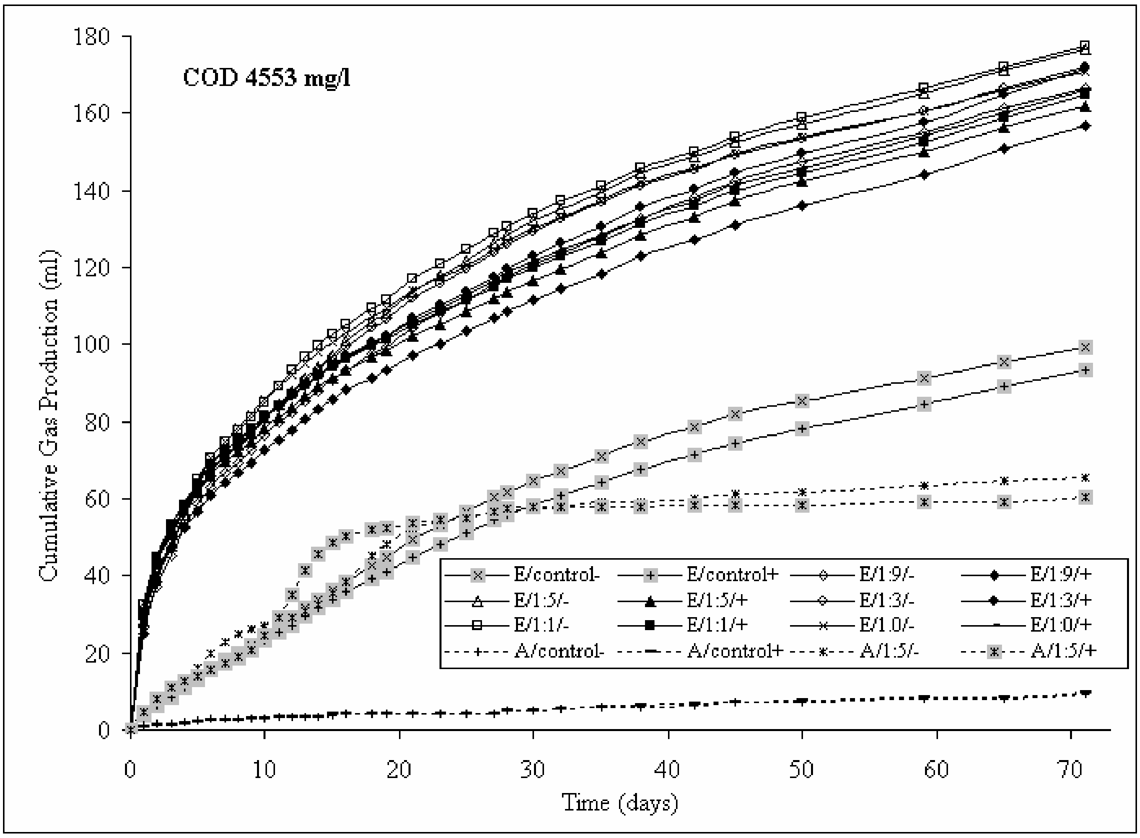


Figure 4.3 Cumulative gas productions of reactors loaded with 4553 mg/l COD

Although average gas production of Efes reactors was nearly two times more than Ankara reactors, 168.4 and 62.9 ml respectively, for average COD removal efficiencies the difference was 14.2%; 78.4% for Efes and 64.2% for Ankara reactors. This is mainly caused by the high background gas production of Efes reactors of 96.5 ml in average which is more than one and a half of average production of COD loaded Ankara reactors. Net gas productions and COD removal efficiencies of BM- and BM+ reactors with their ratio is given in Table 4.5. Results in the table indicate the positive effect of higher DW content in wastewater in terms of BM-/BM+ ratio as expected.

Table 4.5 Cumulative gas production and COD removal efficiency values of BM- and BM+ reactors and their ratio at 4553 mg/l COD loading

OMW:DW	***gas production (ml)***			***COD removal (%)***		
efes	***BM-***	***BM+***	***ratio***	***BM-***	***BM+***	***ratio***
1:9	166.6	156.8	1.063	74.4	70.0	1.063
1:5	176.7	161.8	1.092	82.2	77.1	1.066
1:3	171.9	171.5	1.003	81.6	82.3	0.991
1:1	177.4	164.7	1.077	80.0	80.4	0.995
1:0	170.8	165.9	1.030	77.5	78.8	0.984
ankara						
1:5	65.4	60.4	1.083	64.6	63.8	1.014

4.2.3 COD Loading of 9107 mg/l

This set contained samples seeded with Efes culture of all wastewater mixtures having different OMW content except 1:9 (OMW:DW) and two samples, 1:5 and 1:3 mixtures, seeded with Ankara culture. Organic load on biomass was 0.604 and 3.312 gCOD/gVSS in Efes culture and Ankara culture seeded reactors respectively.

All Efes reactors followed a similar trend that was a parabolic curve with a sharp beginning and a very slight breakpoint about 15th day as seen in Figure 4.4. Observed gas productions were about 25%, 32%, 40% and 45% of totals on 1st, 2nd, 3rd and 4th days respectively. Until 15th day, the breakpoint, about 68% of the total productions were carried out; 80% and 90% were reached on 30th and 45th days respectively.

For Ankara reactors, gas production trends of either BM- or BM+ ones loaded with 1:5 and 1:3 (OMW:DW) mixtures were identical. As in 4553 mg/l loaded set, they went closer for 10 days up to 35-40% of their total gas production; but at this point instead of a jump by BM+ reactors, a decrease in gas production rates of BM- reactors, which indicates the positive effect of BM addition on that culture, was observed. BM+ reactors reached a stationary phase on 23rd day with 86-87% of their total production,

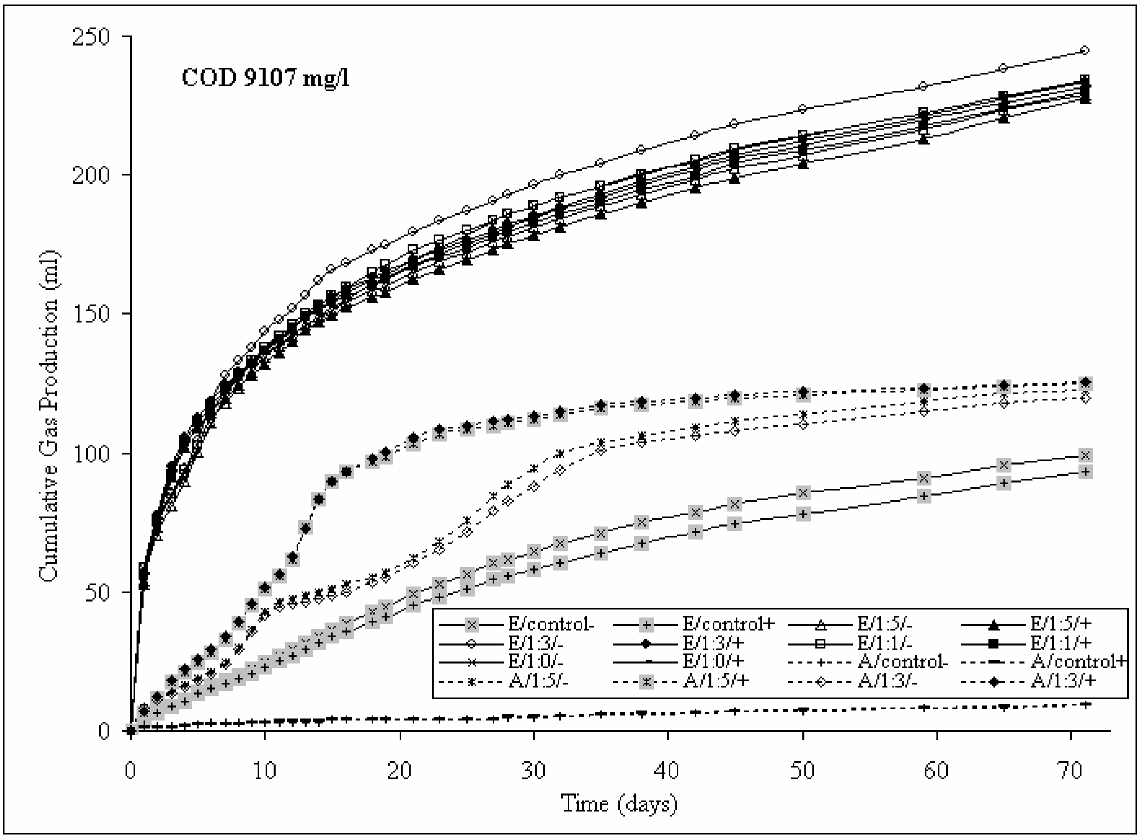


Figure 4.4 Cumulative gas productions of reactors loaded with 9107 mg/l COD

while BM- ones could reach a plateau on 35th day with 84-85% of production. Until that day BM+ reactors had produced 93-94% of the total gas.

BM- Efes reactors achieved slightly lower COD removal efficiencies than BM+ ones except the 1:3 (OMW:DW) mixture loading case as given in Table 4.6. Raw OMW loaded reactors had the lowest COD removal efficiency ratio of BM- reactors to BM+ ones. This fact shows the positive effect of DW addition. Except the 1:5 (OMW:DW) mixture loaded reactors, which unexpectedly showed the lowest performance in terms of COD removal efficiency, observed increase in efficiency values were directly proportional to the DW content of wastewater mixtures.

Average COD removal efficiencies of Ankara reactors were close to those of Efes ones; 2.5% lower for BM- reactors (67.8% and 70.3%) and 2.6% higher for BM+ ones (73.0% and 70.4%). These differences correspond to a lower average BM-/BM+ ratio of Ankara reactors than that of Efes reactors (0.928 and 0.998).

Table 4.6 Cumulative gas production and COD removal efficiency values of BM- and BM+ reactors and their ratio at 9107 mg/l COD loading

OMW:DW	***gas production (ml)***			***COD removal (%)***		
Efes	***BM-***	***BM+***	***Ratio***	***BM-***	***BM+***	***ratio***
1:5	229.1	227.5	1.007	68.3	69.1	0.989
1:3	244.6	233.8	1.046	73.8	71.8	1.028
1:1	234.2	230.1	1.018	70.2	70.6	0.995
1:0	233.7	232.0	1.008	68.8	70.3	0.979
Ankara						
1:5	122.6	124.8	0.982	68.4	72.1	0.948
1:3	119.7	125.4	0.954	67.2	74.0	0.908

4.2.4 COD Loading of 13660 mg/l

This set contained 3 samples, 1:3, 1:1 and 1:0 (OMW:DW) mixtures, seeded with Efes culture and two samples, 1:3 and 1:1 (OMW:DW) mixtures, seeded with Ankara culture. Organic load on biomass was 0.906 and 4.968 gCOD/gVSS in Efes culture and Ankara culture seeded reactors respectively.

As the 9107 mg/l COD loaded set, all Efes reactors in this set followed a similar trend consisting of a sharply beginning parabolic curve with a very slight breakpoint about 16th day as shown in Figure 4.5. The only diffence was the little lower cumulative production values of BM- reactors than BM+ ones between the 3rd and the 8th days. All reactors produced 26-28% of the total gas in the 1st day and about 50% in 6 days. Until the breakpoint they produced 73-75% of their gas, then achieved 80% and 90% on 25th and 45th days respectively. On 71st day they were still producing biogas.

Unlike the first two sets with lower COD loading, Ankara BM- reactors could not go along with BM+ reactors even at the beginning, and a lag period for acclimation was observed. BM+ reactors had two breakpoints at 16th and 27th days corresponding to 68% and 88% of total gas production. BM- reactors produced 37% of the total gas until their first breakpoint after acclimation at 13th day, and reached their stationary phase on 38th day with 90% of overall gas production. The higher variations of gas production

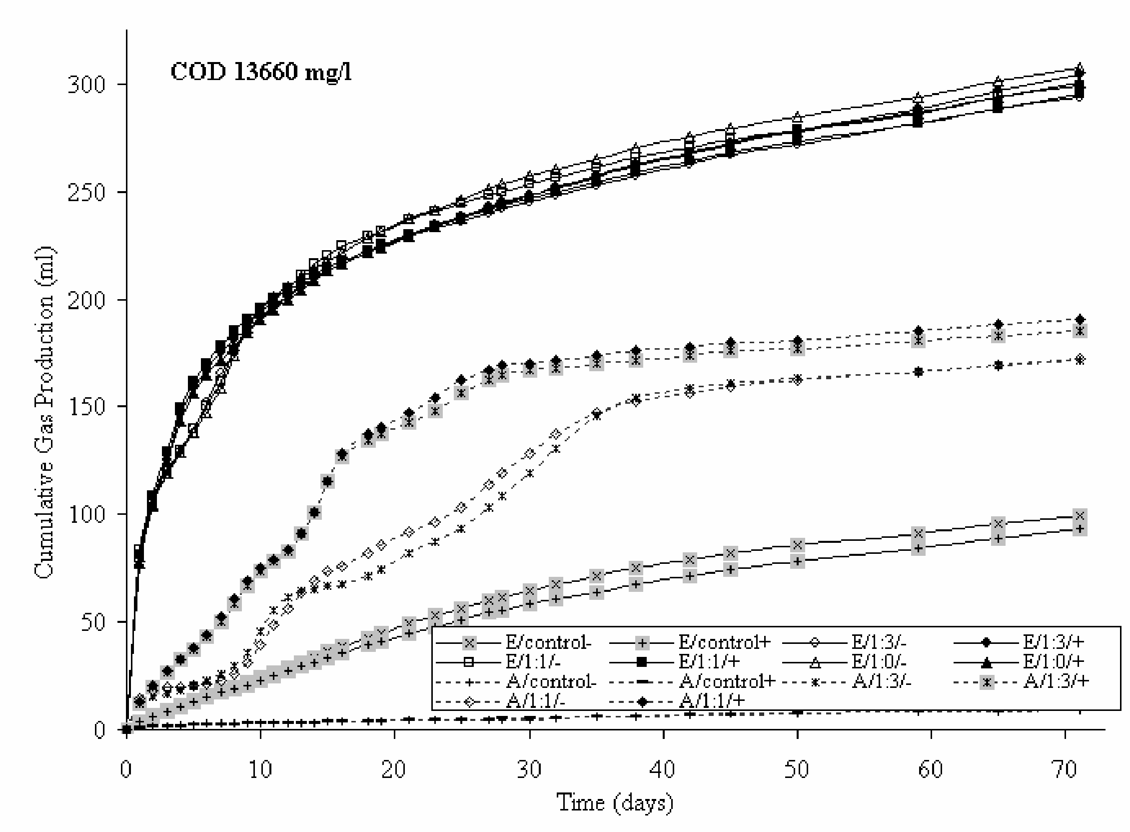


Figure 4.5 Cumulative gas productions of reactors loaded with 13660 mg/l COD

rates when compared to lower COD loadings present a clear sign of stability problems of Ankara culture at higher loadings. This fact must be considered, since stability is a major factor of proper operation in full scale applications.

Total gas productions and COD removal efficiencies achieved in this set are given in Table 4.7. BM- Efes reactors performed quite similar to BM+ ones; 69.3% and 70.8% of COD removal efficiencies on average respectively, corresponding to a ratio of 0.980.

Although their average BM-/BM+ ratio of 0.913 is lower than that of Efes reactors, Ankara reactors performed better in terms of COD removal efficiency. The average values achieved were 72.0% for BM- and 79.0% for BM+ reactors.

Table 4.7 Cumulative gas production and COD removal efficiency values of BM- and BM+ reactors and their ratio at 13660 mg/l COD loading

OMW:DWW	***gas production (ml)***			***COD removal (%)***		
Efes	***BM-***	***BM+***	***ratio***	***BM-***	***BM+***	***ratio***
1:3	294.2	304.2	0.967	70.4	72.2	0.974
1:1	299.2	295.6	1.012	67.8	68.2	0.994
1:0	307.3	300.4	1.023	69.9	71.9	0.972
ankara						
1:3	171.5	185.4	0.925	71.6	75.7	0.945
1:1	172.0	190.6	0.903	72.4	82.2	0.881

4.2.5 COD Loading of 22767 mg/l

This set contained samples of 1:1 and 1:0 (OMW:DW) wastewater mixtures seeded with Efes culture and a sample of 1:1 (OMW:DW) mixture seeded with Ankara culture. Organic load on biomass was 1.510 and 8.290 gCOD/gVSS in Efes culture and Ankara culture seeded reactors respectively.

As seen in Figure 4.6, gas production trends of BM- and BM+ Efes reactors were quite similar except the lower rates of BM- ones between 3rd and 8th days. BM+ reactors achieved 28%, 37% and 44% of its total gas production on 1st, 2nd and 3rd days respectively; then reached its breakpoint at 8th day with a production of 66% of the total. BM- reactors also began with high gas productions; 25%, 35% and 40% on 1st, 2nd and 3rd days respectively. After 3rd day, a decrease in production rates followed by an increase was observed; then until 13th day, which was their breakpoint, they produced 71% of their overall production.

Ankara reactors showed increased instability in gas production rates at this COD loading. Major breakpoints were 19th and 38th days for BM+ reactors, corresponding to 70% and 91% of total gas production, and 15th, 21st and 45th days for BM- ones, corresponding to gas productions of 31%, 49% and 93% of total respectively.

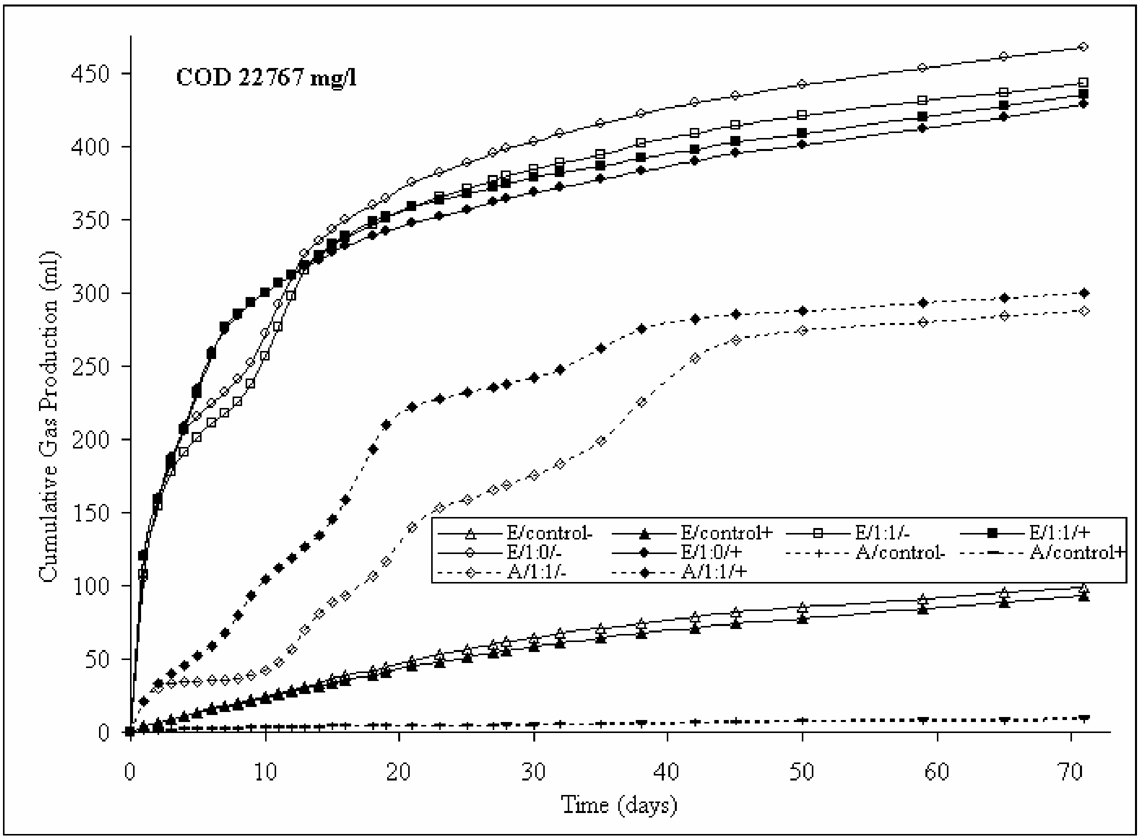


Figure 4.6 Cumulative gas productions of reactors loaded with 22767 mg/l COD

COD removal efficiency values given in Table 4.8 show that Efes reactors performed similar to each other, about 69%, except BM- reactors loaded with raw OMW which had higher efficiencies of 74%. Although it was not an expected situation, the similar gas productions of each reactor of that pair, 459 and 476 ml, indicate that this is possibly not an experimental error. It may be claimed that BM addition had adversely affected the culture, but this is not the general case for other reactors and BM+ reactors reflected positively by showing increased stability.

Ankara reactors had lower COD removal efficiencies than former COD loading levels for both BM- and BM+ reactors; but BM-/BM+ ratio remained about 0.900.

Table 4.8 Cumulative gas production and COD removal efficiency values of BM- and BM+ reactors and their ratio at 22767 mg/l COD loading

OMW:DWW	***gas production (ml)***			***COD removal (%)***		
efes	***BM-***	***BM+***	***ratio***	***BM-***	***BM+***	***ratio***
1:1	442.5	435.2	1.017	69.1	69.7	0.992
1:0	467.6	428.1	1.092	74.3	69.2	1.073
ankara						
1:1	287.1	299.9	0.957	61.1	67.1	0.910

4.2.6 COD Loading of 34150 mg/l

This set contained only the raw OMW sample seeded with Efes culture. Organic load on biomass was 3.020 gCOD/gVSS.

Gas productions trends of this set are presented in Figure 4.7. BM+ reactors reached 28%, 38% and 43% of the overall gas production on 1st, 2nd and 3rd days respectively. Then a slight decrease in gas production rate of BM+ reactors was observed between 3rd and 7th days. After 7th day, increased production rates were observed until the breakpoint at 11th day and 74% of total gas was produced. 80%, 85%, 90% and 95% gas productions of overall were achieved on 15th, 21st, 32nd and 50th days respectively.

BM- reactors presented the trend of a typical inhibition. This inhibition is possibly caused by the phenol content of the OMW sample which is reported to be toxic/inhibitive at high concentrations in the literature [7-10]. Although BM- reactors were strongly inhibited, on the last gas measurements, on 71st day, a restart of gas production in only one of these reactors was observed. These reactors were followed for a few more days, and an increasing gas production was observed in one of them but not for its duplicate. This situation indicates the possibility of acclimation of the culture to high loading conditions without nutrient addition; but a clear statement cannot be made since it was not the case for both of the duplicates and further research is required. Achievement of biogas production in BM+ reactors show that the addition of BM,

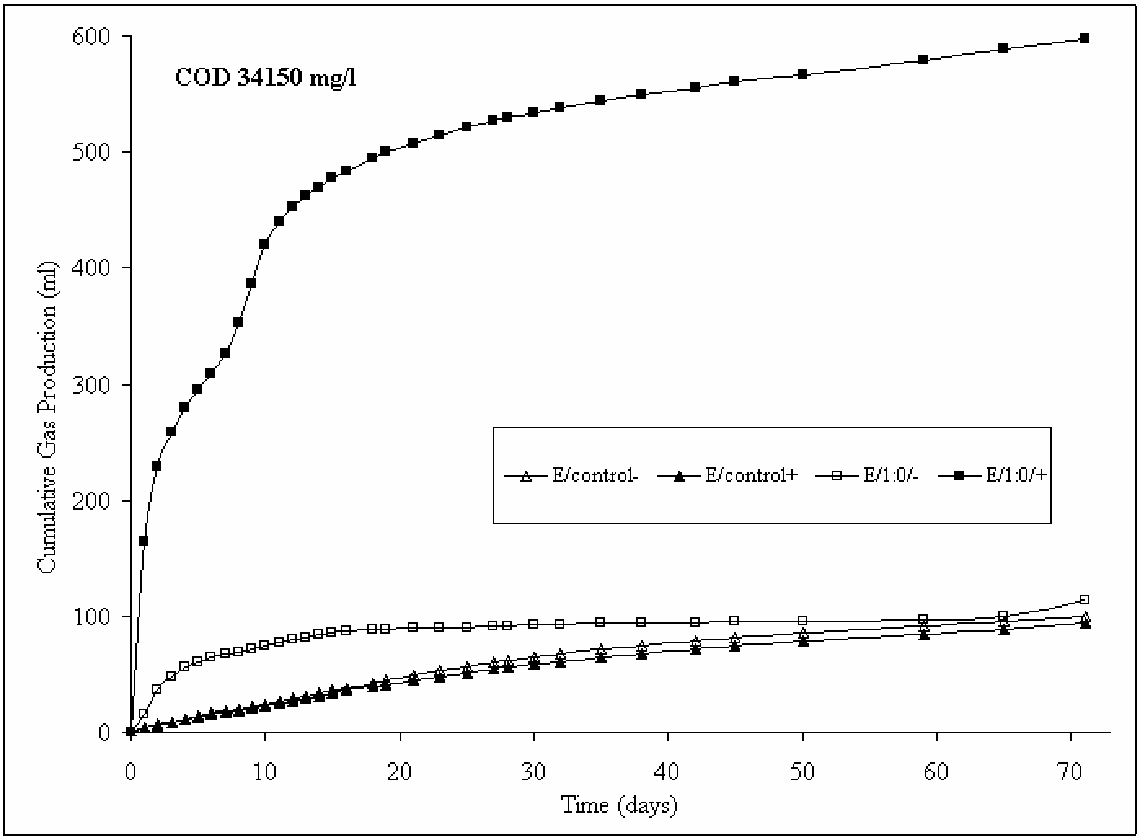


Figure 4.7 Cumulative gas productions of reactors loaded with 34150 mg/l COD

nutrients and trace metals, is helpful to anaerobic bacteria for increased resistance to inhibition.

Gas production and COD removal efficiency values for the reactors are given in Table 4.9. BM+ reactors performed quite well by the help of BM, despite the clear existence of a strong inhibitor in the medium.

Table 4.9 Cumulative gas production and COD removal efficiency values of BM- and BM+ reactors and their ratio at 34150 mg/l COD loading

OMW:DWW	***gas production (ml)***			***COD removal (%)***		
Efes	***BM-***	***BM+***	***ratio***	***BM-***	***BM+***	***ratio***
1:0	114.4	597.0	0.192	1.3	68.6	0.019

4.2.7 Biogas Composition Analysis

The biogas composition analysis were carried out by gas chromatographic method as described in section 3.3.2. After preparing calibration curves for CH_4, CO_2, H_2S and N_2, at least two analysis were carried out for biogas samples produced by each reactor throughout the study. Results of biogas composition analysis with respect to seed culture and BM condition, and also 'R^2' values of the prepared calibration curves are given in Table 4.10. Also biogas compositions of each reactor pair can be seen in Table 4.11.

N_2 contents are not given in the tables due to some problems which had been faced in its analysis. After subtracting background N_2 and CO_2 values coming from the purging gas from the raw analysis data, very high or negative N_2 content results, which were expected to be at ppm levels, were obtained. Two possible reasons of this situation were the relatively low R^2 value, 0.95008, and the excessive amounts of background N_2 content especially in reactors with low biogas production. The lowest and highest calibration points for N_2 were 1.00% and 3.98% respectively, while calculated background concentrations were up to 40%. Therefore, N_2 contents of biogas samples, if any, were neglected and detected values were assumed to completely come from background.

CH_4 was the main gas considered in the analysis. Average CH_4 contents of 66.43% and 70.24%, for Efes and Ankara cultures respectively, are a little less than values achieved in other studies and given in the literature, from 72% up to 85% [7,9,10,13].

In the analysis, no peak other than standard gases except H_2O was observed and this peak was not considered. Therefore results given in Table 4.10 and 4.11 are on dry basis.

Table 4.10 Average composition of biogas with respect to seed culture and nutrient addition, and R^2 values of calibration curves

reactors	***CH_4 (%)***		***CO_2 (%)***		***H_2S (%)***	
	average	***standard deviation***	***average***	***standard deviation***	***average***	***standard deviation***
EFES	66.43	0.91	33.20	0.95	0.37	0.21
EFES/BM-	66.74	0.60	33.00	0.66	0.26	0.14
EFES/BM+	66.15	0.91	33.37	1.00	0.48	0.21
ANKARA	70.24	2.24	29.44	2.31	0.32	0.25
ANKARA/BM-	68.38	2.16	31.42	2.18	0.20	0.15
ANKARA/BM+	71.14	1.83	28.45	1.83	0.41	0.26
R^2 of calibration curve	0.99634		0.99774		0.97848	

Table 4.11 Average composition of biogas produced in reactor pairs

reactor#	***CH_4 (%)***	***CO_2 (%)***	***H_2S (%)***	***reactor#***	***CH_4 (%)***	***CO_2 (%)***	***H_2S (%)***
E1&E2	67.62	32.05	0.33	***E47&E48***	66.55	33.08	0.38
E3&E4	65.54	33.49	0.97	***E49&E50***	66.49	33.42	0.10
E5&E6	66.72	32.78	0.50	***E51&E52***	66.31	33.34	0.35
E7&E8	66.54	32.68	0.78	***E53&E54***	65.85	34.07	0.08
E9&E10	67.53	32.12	0.35	***E55&E56***	65.35	34.38	0.27
E11&E12	66.64	32.72	0.64	***E57&E58***	66.48	33.41	0.12
E13&E14	66.51	33.09	0.39	***E59&E60***	65.09	34.69	0.22
E15&E16	66.01	33.43	0.56	***E61&E62***	28.06	71.54	0.40
E17&E18	66.59	32.86	0.55	***E63&E64***	65.95	33.88	0.17
E19&E20	66.76	32.66	0.58	***A1&A2***	66.10	33.67	0.23
E21&E22	67.10	32.64	0.26	***A3&A4***	67.70	32.13	0.17
E23	66.96	32.49	0.55	***A5&A6***	67.43	32.19	0.38
E24	65.69	33.80	0.51	***A7&A8***	69.59	29.54	0.87
E25&E26	66.63	33.12	0.25	***A9&A10***	70.14	29.55	0.30
E27&E28	65.65	33.88	0.47	***A11&A12***	72.01	27.36	0.63
E29&E30	67.89	31.87	0.25	***A13&A14***	69.67	30.08	0.25
E31&E32	68.84	30.54	0.62	***A15&A16***	72.12	27.42	0.46
E33&E34	66.20	33.60	0.20	***A17&A18***	69.19	30.58	0.23
E35&E36	66.10	33.48	0.42	***A19&A20***	72.49	27.22	0.28
E37&E38	66.24	33.56	0.20	***A21&A22***	70.90	29.10	-
E39&E40	65.54	34.11	0.35	***A23&A24***	72.60	27.16	0.24
E41&E42	66.05	33.78	0.17	***A25&A26***	65.20	34.80	-
E43&E44	65.05	34.68	0.27	***A27&A28***	71.49	28.32	0.19
E45&E46	67.18	32.61	0.20				

Chapter 5

CONCLUSION

In this study, biochemical methane potential experiments (BMP) were applied to an olive mill wastewater (OMW) sample taken from Tariş Mordoğan Olive Mill in İzmir and its mixtures with a domestic wastewater (DW) sample taken from İZSU Çiğli Municipal Wastewater Treatment Plant, İzmir, Turkey. OMW:DW ratio of the mixtures were 1:9, 1:5, 1:3 and 1:1. In addition, the experiments were carried out at five different chemical oxgen demand (COD) loading levels of 4553 mg/l, 9107 mg/l, 13660 mg/l, 22767 mg/l and 34150 mg/l for either nutrient addition or no nutrient addition (BM+/BM-) conditions with two different anaerobic seed cultures in duplicates.

Characterization of OMW proved its nutrient, particularly nitrogen, deficiency. The nitrogen content of the sample could be theoretically sufficient for maximum 59% COD removal. Determined characteristics of the DW sample were typical for an average DW, except the relatively high COD and BOD concentrations. This means some part of the expected extra nutrients had to be used for itself.

Although a clear linear relation between COD removal and DW content could not be established, positive effects of co-treatment were observed. Performance ratio of BM- reactors to BM+ ones varied between 0.970-1.090 and 0.900-1.080 in terms of gas production and 0.970-1.070 and 0.880-1.010 in terms of COD removal efficiency, for Efes and Ankara seeded cultures respectively.

COD removal efficiencies obtained by Efes seeded reactors were between 68% and 82% for both BM+ and BM- conditions. At 4553 mg/l COD loading, the efficiencies were about 80%, while at higher loadings this value was about 70%. Although not very high, these efficiency values may be enough for a feasible pretreatment application, also are in the mid-range of the results cited in the literature. And it must be noted that results of this study are the minimum efficiencies to be obtained since an unacclimated culture was used in batch reactors with no mixing.

Nutrient addition can be concluded to be beneficial not only for increasing the removal efficiency, but for preventing toxic/inhibitory effects on anaerobic bacteria and for achieving faster removal. At 34150 mg/l COD loading Efes BM+ reactors achieved 68.6% removal efficiency while in BM- ones microbial activity has stopped in a few days. Also, Ankara BM+ reactors showed faster acclimation than BM- ones. All Efes reactors produced most of the gas, about 75%, in 15-20 days. Ankara BM+ reactors produced about 80% or the biogas in 20-25 days, while this period lasted up to 35-45 days for BM- reactors. This period is important for reducing the hydraulic retention time in applications which directly reflects to reactor volume and therefore initial costs.

Methane (CH_4) content of the biogas was about 66% for Efes and about 70% for Ankara culture seeded reactors. The rest of the gas was mainly carbondioxide (CO_2) with less than 1%, generally below 0.5%, hydrogen sulphide (H_2S). H_2S content of biogas is important in the use of obtained biogas for its corrosive effect. Considering this composition, there is no inconvenience for most types of direct usage, e.g. in stoves, of the produced biogas, whereas some pretreatment may be required for utilization in engines.

On the above stated conclusions, it can be said that co-treatment of OMW and DW may result in improved treatment efficiencies according to their characteristics, and better results for this study could be obtained by the use of an acclimated anaerobic seed culture.

Solution of OMW handling problem in the western parts of Turkey is very crucial to the environment and to the economy (when regarding the effect of pollution on tourism). On the base of such treatability studies, laboratory and pilot scale studies should be implemented for the anaerobic treatment of OMW or its co-treatment with some possible waste/waters. These studies then should lead to full scale applications.

REFERENCES

[1] T.D.Reynolds, *Unit Operations and Processes in Environmental Engineering*, MA: PWS-KENT, Boston, 1982.

[2] M.S.Switzenbaum, "Obstacles in the Implementation of Anaerobic Treatment Technology", *Bioresource Technology* 53 (1995), pp 255-262.

[3] R.E.Speece, *Anaerobic Biotechnology for Industrial Wastewaters*, Archae Press, USA, 1996.

[4] W.F.Owen, D.C.Stuckey, J.B.Healey, L.Y.Young, P.L.McCarty, "Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity", *Water Research* 13 (1979), pp 485-492.

[5] L.Seghezzo, G.Zeeman, J.B.van Lier, H.V.M.Hamelers, G.Lettinga, "A Review: The Anaerobic Treatment of Sewage in UASB and EGSB Reactors", *Bioresource Technology* 65 (1998), pp 175-190.

[6] Metcalf & Eddy, *Wastewater Engineering: Treatment, Disposal, and Reuse*, McGraw-Hill Inc., 3rd Edition, Singapore,1991.

[7] S.Uemura, and H.Harada, “Treatment of Sewage by a UASB Reactor Under Moderate to Low Temperature Conditions”, *Bioresource Technology* 72 (2000), pp 275-282.

[8] T.H.Ergüder, E.Güven, G.N.Demirer, “Anaerobic Treatment of Olive Mill Wastes in Batch Reactors”, *Process Biochemistry* 36 (2000), pp 243-248.

[9] H.N.Gavala, I.V.Skiadass, N.A.Bozinis, G.Lyberatos, “Anaerobic Codigestion of Agricultural Industries’ Wastewaters”, *Water Science and Technology* 34(11) (1996), pp 67-75.

[10] M.Hamdi, C.Festino, C.Aubart, “Anaerobic Digestion of Olive Mill Wastewaters in Fully Mixed Reactors and in Fixed Film Reactors”, *Process Biochemistry* 27 (1992), pp37-42.

[11] R.Borja, A.Martin, C.J.Banks, V.Alonso, A.Chica, “A Kinetic Study of Anaerobic Digestion of Olive Mill Wastewater at Mesophilic and Thermophilic Temperatures”, *Environmental Pollution* 88 (1995), pp13-18.

[12] A.Rozzi and F.Malpei, “Treatment and Disposal of Olive Mill Effluents”, *International Biodeterioration and Biodegradation* (1996), pp 135-144.

[13] D.Dalis, et al., “Anaerobic Digestion of Total Raw Olive-Oil Wastewater in a Two-Stage Pilot Plant (Up-Flow and Fixed-Bed Bioreactors)”, *Bioresource Technology* 57 (1996), pp 237-243.

[14] G.Ubay and I.Öztürk, “Anaerobic treatment of olive mill effluents”, *Water Science and Technology* 36(2-3) (1997), pp 287-294.

[15] I.Angelidaki, L.Ellegaard, B.K.Ahring, " Modelling Anaerobic Codigestion of Manure with Olive Oil Mill Effluent", *Water Science and Technology* 36(6-7) (1997), pp 263-270.

[16] H.N.Gavala, I.V.Skiadas, G.Lyberatos, "On the Performance of a Centralised Digestion Facility Receiving Seasonal Agroindustrial Wastewaters", *Water Science and Technology* 40(1) (1999), pp 339-346.

[17] J.Mata-Alvarez, S.Macé, P.Llabrés, "Anaerobic Digestion of Organic Solid Wastes: An Overview of Research Achievements and Perspectives", *Bioresource Technology* 74 (2000), pp 3-16.

[18] W.Edelmann, "Co-Digestion of Organic Solid Waste and Sludge From Sewage Treatment", *Water Science and Technology* 41(3) (2000), pp 213-221.

[19] H.Kübler, "Full Scale Co-Digestion of Organic Waste", *Water Science and Technology* 41(3) (2000), pp 195-202.

[20] H.M.Poggi-Varaldo, "Solid Substrate Anaerobic Co-Digestion of Paper Mill Sludge, Biosolids, and Municipal Solid Waste", *Water Science and Technology* 35(2-3) (1997), pp 197-204.

[21] F.J.Callaghan, D.A.J.Wase, K.Thayanithy, C.F.Forster, "Co-Digestion of Waste Organic Solids: Batch Studies", *Bioresource Technology* 67 (1999), pp 117-122.

[22] R.M.Dinsdale, "Two-Stage Anaerobic Co-Digestion of Waste Activated Sludge and Fruit/Vegetable Waste Using Inclined Tubular Digesters", *Bioresource Technology* 72 (2000), pp 159-168.

[23] M.R.Al-Masri, "Changes in Biogas Production Due to Different Ratios of Some Animal and Agricultural Wastes", *Bioresource Technology* 77 (2001), pp 97-110.

[24] A.W.A.de Man, P.C.Grin, R.E.Roersma, K.C.F.Grolle, G.Lettinga, "Anaerobic Treatment of Municipal Wastewater at Low Temperatures", *Anaerobic Treatment: a Grown-up Technology Conference Papers (Aquatech'86),* Amsterdam, 1986, pp 451-466.

[25] G.Lettinga, S.W.Hobma, P.Hulshoff, W.de Zeeuw, P.de Jong, P.Grin, R.Roersma, "Design, Operation, and Economy of Anaerobic Treatment", *Water Science and Technology* 15 (1983), pp 177-195.

[26] W.A.Pretorius, "Anaerobic Digestion of Raw Sewage", *Water Research* 5 (1971), pp 681-687.

[27] G.Lettinga, A.W.A.de Man, A.R.M.van der Last, K. Van Knippenberg, J.Frijns, J.C.L.van Buuren, "Anaerobic Treatment of Domestic Sewage and Wastewater", *Water Science and Technology* 27 (9) (1993), pp 67-73.

[28] A.Gninanapathy, C.Polprasert, "Treatment of Domestic Wastewater with UASB Reactors", *Water Science and Technology* 27 (1) (1993), pp 195-203.

[29] J.L.Kooijmans, E.M.van Velsen, "Application of the UASB Process for Treatment of Domestic Sewage Under Sub-tropical Conditions, the Cali Case", *Anaerobic Treatment: a Grown-up Technology Conference Papers (Aquatech'86)*, Amsterdam, 1986, pp 423-436.

[30] R.A.Barbosa, G.L.Jr.Sant'Anna, "Treatment of Raw Domestic Sewage in an UASB Reactor", *Water Research* 23 (12) (1989), pp 1483-1489.

[31] H.Draaijer, J.A.W.Maas, J.E.Schaapman, A.Khan, "Performance of the % MLD UASB Reactor for Sewage Treatment at Kanpur, India", *Water Science and Technology* 25 (7) (1992), pp 123-133.

[32] Haskoning Consulting Engineers and Architects, *"36 MLD UASB Treatment Plant in Kanpur, India. Evaluation Report on Process Performance"*, Internal Report, 1996.

[33] V.Balice, C.Carrieri, O.Cera, A.Di Fazio, "Natural Biodegradation in Olive Mill Effluents Stored in Open Basins", *Proceedings of International Symposium on Olive Byproducts Valorization*, March 4-7, Sevilla, 1986.

[34] R.L.Cabello, J.A.F.R.des Ursinos, "Evaluacion de Alpechines en Balsas de Evaporacion", *Proc. Congreso Sobre Biotecnologiasde Bajo Costo para la Depuracion de Aguas Residuales*, November 18-20, Madrid, FAO, 1981.

[35] J.A.F.R.des Ursinos, "Anaerobic Fermentation of Wastewater with High Organic Load", *Ingenieria Quimica* 47 (1981), pp 85-91.

[36] R.Antonacci, A.Brunetti, A.Rozzi, M.Santori, "Trattamento Anaerobica di Acque di Vegetazione di Frantoio Risultati Preliminari", *Ingegneria Sanitaria* 6 (1981), pp 357-363.

[37] A.Di Dona, A.Valentini, "Biogas e Depurazione in un Oleficio", *Esp. Risparmi Energetici Boll A.P.R.E* 1 (1982), pp 20-23.

[38] A.Aveni, "Biogas Recovery from Olive-Mill Waste Water by Anaerobic Digestion", in Anaerobic Digestion and Carbohydrate Hydrolysis of Waste, Eds. G.L.Ferrero, M.P.Ferranti and H.Naveau, Elsevier Applied Science, Barking, 1984.

[39] A.Brunetti, A.Rozzi, R.Antonacci, G.Labellarte, C.Longobardi, "Trattamento Anaerobica di Acque di Vegetazione di Frantoio", *Ingegneria Sanitaria* 31 (4) (1983), pp 18-24.

[40] A.Rozzi, R.Passino, N.Limoni, "Anaerobic Treatment of Olive Mill Effluents on Polyurethane Foam Bed Reactors", *Process Biochemistry* 24 (1989), pp 68-74.

[41] S.Rigoni-Stern, R.Rismondi, L.Spyrkowicz, F.Zilio-Grandi, "Digestione Anaerobica su Letto Battorico dei Liquami Provenienti dalla Molitura delle Olive con Produzione di Biogas", *Ingegneria Ambientale* 17 (1988), pp 267-271.

[42] J.E.Schmidt, I.Angelidaki, B.K.Ahring, "Anaerobic Digestion of Olive Mill Effluents Together with Different Wastes", *Proceedings of the2nd International Symposium on Anaerobic Digestion of Solid Wastes*, June 15-18, Vol.2, Barcelona, 1999, pp 169-174.

[43] A.Boari, A.Brunetti, R.Passino, A.Rozzi, "Anaerobic Digestion of Olive Oil Mill Wastewaters", *Agricultural Wastes* 10 (1984), pp 161-175.

[44] APHA, *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Washington D.C., 1995.