SEQUENTIAL (ANAEROBIC/AEROBIC) BIOLOGICAL TREATMENT OF MALT WHISKY WASTEWATER

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ABSTRACT

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In this study, anaerobic treatability of Ankara Tekel Alcohol Factory malt whisky wastewater was investigated. To this purpose, both Biochemical Methane Potential (BMP) experiments and continuous reactor experiments were conducted. BMP experiments were conducted both with and without Basal Medium (BM) to observe the effect of nutrient supplementation. The serum bottles were seeded both with anaerobic mixed cultures and acetate enriched *Methanosarcina* cultures at a volumetric ratio of 1:1. For the batch anaerobic reactors (serum bottles) containing no nutrients but only $NaHCO_3$, net total gas productions at the end of 29 days were observed as 98.7, 220.8 and 260.5 mL for the COD concentrations of 5070, 10140 and 15210 mg/L, respectively. For the nutrient supplemented set of serum bottles the net total gas production for the COD concentrations of 5070, 10140 and 15210 mg/L were observed as 98, 214.1 and 332.6 mL, respectively. For the COD concentrations

of 5070 and 10140 mg/L acclimation period of 10 days was observed for the serum bottles with no nutrient supplementation. However, the acclimation period needed was about 15 days for the initial COD concentration of 15210 mg/L. After the acclimation period, the gas production rates increased significantly. The delay in gas production observed for the no-nutrient supplemented set of serum bottles was not observed for the nutrient supplemented set.

The continuous reactor experiments were carried out in single stage anaerobic filter (AF) with celite support material, two stage AF with pumice support material reactors and two stage upflow anaerobic sludge blanket (UASB) reactors. In AF reactor experiments startup periods for the attachment of microorganisms on pumice or celite were not applied. In AF (celite) reactor experiments, influent COD concentrations up to 11087 mg/L were treated effectively. When 11087 mg/L influent COD concentration was applied to the reactor, biomass washout was observed because of high loading and gas production rates. In AF (pumice) reactor experiments, influent COD concentrations up to 11087 mg/L were treated effectively. Above this concentration biomass washout also observed in the two stage AF (pumice) reactors as in the case of the single stage AF (celite) reactor. Two stage UASB reactor experiments indicated that two stage UASB reactor configuration is an efficient system for malt whisky wastewater treatment. Up to 33866 mg/L influent COD concentration was treated efficiently. When an influent COD concentration of 33866 mg/L was applied, the color of the granular culture of the first stage UASB reactor changed from black to brownish. The granular culture was also deteriorated in the first stage. This was probably due to the dominance of acidogenic culture in the first stage against methanogenic culture. However in the second stage of the UASB system, no operational problem was observed. After UASB reactor experiments batch aerobic experiments were conducted and COD and BOD removal efficiencies were 55% and 70%, respectively in this part.

Key Words: Anaerobic treatment, Whisky wastewater, BMP experiments, AF, UASB

ÖZ

MALT VİSKİ ATIKSUYUNUN ARDIŞIK (ANAEROBİK/AEROBİK) BİYOLOJİK ARITIMI

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Bu çalışmada, Ankara Tekel İçki Fabrikası malt viski atıksuyunun anaerobik olarak arıtılabilirliği araştırılmıştır. Bu amaçla Biyokimyasal Metan Potansiyeli (BMP) ve sürekli reaktör deneyleri gerçekleştirilmiştir. BMP deneyleri Basal Ortam (BO) içeren ve içermeyen ortamlarda besin eklemesinin etkisini gözlemek amacıyla gerçekleştirilmiştir. Serum şişeleri eşit hacimsel oranlarda anaerobik karışık kültürle ve asetatla zenginleştirilmiş *Methanosarcina* kültürüyle aşılanmıştır. Besin içermeyen yalnızca $NaHCO_3$ bulunduran kesikli anaerobik reaktörlerde 29 günün sonunda 5070, 10140 ve 15210 mg/L KOİ derişimlerine karşılık net toplam gaz üretimi sırasıyla, 98,7, 220,8 ve 260,5 mL olarak gözlenmiştir. Besin eklenmiş serum şişelerinde ise 29 günün sonunda 5070, 10140 and 15210 mg/L KOİ derişimlerine karşılık net toplam gaz üretimi sırasıyla, 98, 214,1 ve 332,6 mL olarak gözlenmiştir. Besinsiz ortamda 5070 ve 10140 mg/L KOİ derişimleri için 10 günlük bir alışma

evresi gözlenmiştir. Ancak 15210 mg/L'lik KOİ derişimi için gereken alışma süresi 15 gündü. Alışma döneminden sonra gaz üretimi önemli ölçüde artmıştır. Besinsiz ortamdaki gaz üretiminde gözlenen gecikme besin eklenen ortamda gözlenmemiştir.

Sürekli reaktör deneyleri tek aşamalı Anaerobik Filtre (AF) celite destek ortamlı, iki aşamalı AF ponza destek ortamlı ve Yukarı Akışlı Çamur Yataklı Anaerobik (YAÇYA) reaktörlerde gerçekleştirilmiştir. AF reaktör deneylerinde, mikroorganizmaların celite veya ponza maddelerine tutunması için devreye alma süreci uygulanmamıştır. AF (celite) reaktör deneylerinde giriş KOİ derişimleri 11087 mg/L'ye kadar arıtılmıştır. 11087 mg/L giriş KOİ derişimi reaktöre uygulandığında yüksek yükleme ve gaz üretim hızlarına bağlı olarak reaktörde biyokütle yıkanması gözlenmiştir. AF (ponza) reaktör deneylerinde, giriş KOİ derişimleri 11087 mg/L'ye kadar etkili biçimde arıtılmıştır. Bu derişimlerin üstünde biyokütle yıkanması da yine AF (celite) reaktörde olduğu gibi AF (ponza) reaktörde de gözlenmiştir. İki aşamalı YAÇYA reaktör deneyleri, iki aşamalı YAÇYA reaktör tipinin malt viski atıksuyu arıtımında iyi çalışan bir sistem olduğunu göstermiştir. 33866 mg/L giriş KOİ derişimine kadar iki aşamalı YAÇYA reaktörde verimli olarak arıtılmıştır. Giriş KOİ derişimi olarak 20920 mg/L uygulandığında, YAÇYA reaktörün birinci aşamasındaki granüler kültürün renginde siyahtan kahverengiye bir değişim olmuştur. Birinci aşamada granüler kültür bozulmaya uğramıştır. Bu birinci aşamada asidojenik kültürün methanojenik kültüre karşı baskın olmasına bağlı olabilir. Ama YAÇYA sisteminin ikinci aşamasında hiçbir işletim problemi gözlenmemiştir.YAÇYA reaktör deneylerinin ardından kesikli aerobik deneyler gerçekleştirilmiştir ve bu kısımda KOİ ve BOİ derişimleri sırasıyla %55 ve % 70 olarak bulunmuştur.

Anahtar Kelimeler: Anaerobik arıtım, Viski Atıksuyu, BMP deneyleri, AF, YAÇYA

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ABBREVIATIONS

BM	: Basal Medium
BOD	: Biochemical oxygen demand
COD	: Chemical oxygen demand
TKN	: Total Kjeldahl Nitrogen
VFA	: Volatile fatty acids
MLSS	: Mixed liquor suspended solids
MLVSS	: Mixed liquor volatile suspended solids
HAc	: Acetic acid
HRT	: Hydraulic retention time
BMP	: Biochemical Methane Potential
ATA	: Anaerobic Toxicity Assay
SRT	: Solid retention time
OLR	: Organic Loading Rate
CSTR	: Contact Stirred Tank Reactor
AF	: Anaerobic Filter
UASB	: Upflow anaerobic sludge blanket

CHAPTER 1

INTRODUCTION

Treatment of high strength industrial wastewaters is still a problem to be solved in several regions of the world. High initial and operating costs of conventional treatment systems served as the stimulus for the development of innovative and cost effective treatment alternatives.

Existing aerobic treatment systems such as modifications of the activated sludge process require high oxygen transfer rates and are not the most economical option. Although attached growth systems, such as trickling filters or aerobic rotating biological contactor systems, can be used as an alternative to activated sludge processes in order to reduce the cost of oxygen transfer, yet they are insufficient for the treatment of high strength wastewaters. However, aerobic treatment may effectively be used as secondary treatment to polish effluents after other types of treatment.

Alternatively anaerobic systems are effective in reducing the organic contents of wastewaters and producing usable energy in the form of by product methane gas. A low energy requirement, a lower excess sludge production and lesser demand for nutrients (N, P) are the primary advantages of anaerobic wastewater treatment as compared to the aerobic processes. This often allows a cost effective means of reducing the pollution load. The anaerobic treatment processes operate most efficiently under specific conditions of temperature, pH and nutrient supply.

Sequential biological systems are preferred when effluent quality of the treatment methods (aerobic or anaerobic) was not appropriate to the discharge limits.

Conventional anaerobic treatment by itself is not a complete treatment and the treated effluents are not usually suitable for discharge to watercourses. This is partly because of the nature of the process and partly because the starting material is so high in pollutants (frequently several hundred times more polluting than domestic wastewater) that a process efficiency of well over 99% has to be attained before the pollutants are reduced to concentrations suitable for discharge. However, much of the polluting material can be removed (50-90% depending on what is measured) economically and the remainder is stabilized so that the problems of odor and dangerous gas production on storage and spreading are virtually eliminated. By combining digester systems with other technologies such as solids separation and secondary treatments it is possible to produce effluents suitable for discharge.

Much of the interest in anaerobic digestion is not so aged and is based on the possibility of generating power which could be used elsewhere and thus give a return on the costs of pollution control. The impetus in industrial countries came from the 1970-74 oil crisis when the cost of oil increased sharply. In the past much research was conducted to develop the process as an energy supply system. However energy generation is still seen as an important aspect today; anaerobic treatment is considered mainly as a cost effective pollution control technology. The biogas can be used to produce energy in the form of heat, electricity or motive power, singly or in combination. This energy can compensate for a lack of other easily accessible sources of energy such as wood, or replace these rapidly diminishing supplies.

Distillery wastes are frequently very strong (COD and solid concentrations of 10-60000 and 10000 mg/L, respectively). These wastes have previously been treated aerobically but only after dilution with other wash waters and recycling. Aerobic waste treatment plants have always been difficult to operate because of the acidity of

the waste, high temperatures and high oxygen demands. Thus, distillery wastes are ideal for anaerobic treatment (Wheatley, 1991).

Objective of this study is to investigate the biological treatibility of the wastewater of the Ankara Tekel Factory producing malt whisky by using a sequential system consisting of an anaerobic filter (AF) or an upflow anaerobic sludge blanket (UASB) reactor and a batch aerobic reactor.

CHAPTER 2

THEORETICAL BACKGROUND

2.1. Anaerobic Biotechnology

Over the past twenty years there has been an increasing demand for more efficient systems for the treatment of wastewaters due to increasingly stringent discharge standards now widely adopted by various national and international agencies. Anaerobic digestion has proven over recent years to be a better alternative to aerobic processes, especially for the treatment of high strength wastewaters (Akkunna and Clark, 2000).

Anaerobic biological treatment is a process in which complex organics are converted to methane and carbondioxide, in the absence of free oxygen. Due to its proven capacity to degrade certain toxic components as well as most common organic pollutants, anaerobic biotechnology today has advanced to a high level of usefulness in the restoration of many industrial effluents (Speece, 1996). Especially, energy considerations and environmental concerns have increased the interest in direct anaerobic treatment of industrial wastes.

In addition to biogas anaerobic digestion may generate other products, which can be valued or sold as well as having a number less tangible benefits such as pathogen control. The most widely used byproduct of anaerobic digestion is the effluent which, depending on residual solids, nitrogen content or water purity, may be used as fertilizer, soil conditioner or for irrigation (Wheatley, 1991).

Anaerobic biotechnology cancels the need for aerobic oxygen transfer with the associated high microbial synthesis characteristics, thus significantly lowering the disposal costs involved with excess biomass synthesis. And a high degree of stabilization is possible with anaerobic biotechnology.

All anaerobic biological treatment involves a consortium of bacteria and is based on series reactions, the slowest of which will determine the overall safety factor for that system. If the substrate consists of complex organic compounds, they must be first hydrolyzed to simpler organics after which they are fermented to volatile acids by acidogens. Finally acetate and H_2 gas are converted to CH_4 by methanogens. Figure 2.1 shows series of metabolisms resulting in methanogenesis.

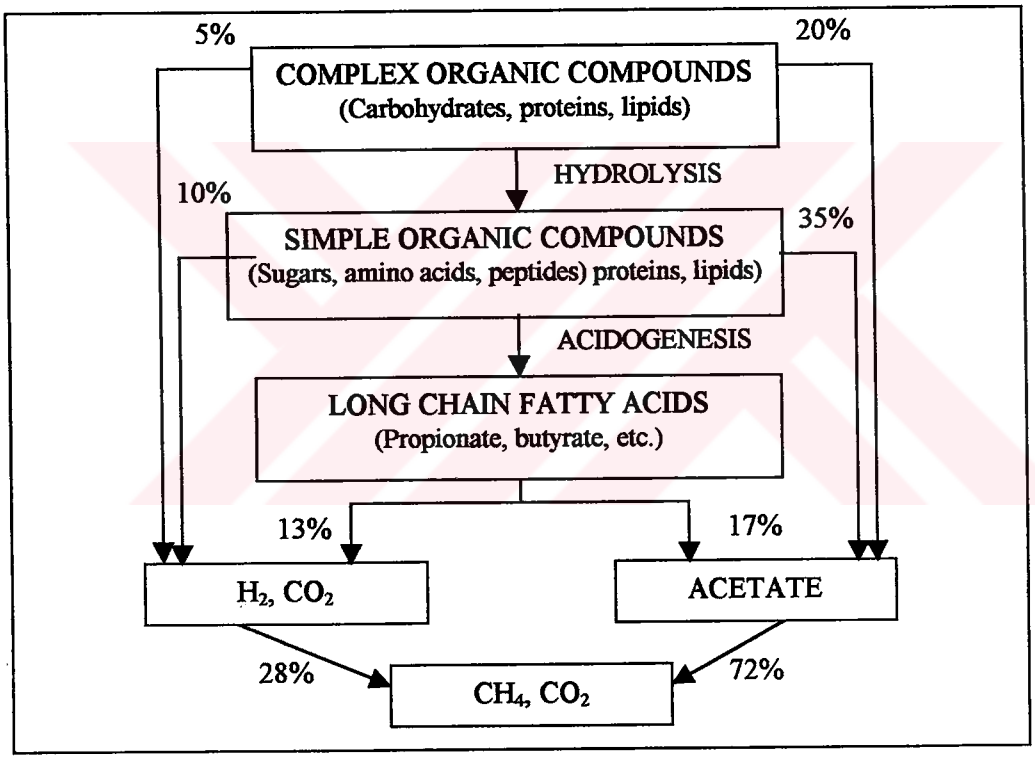

Figure 2.1. Series metabolism resulting in methanogenesis (Speece, 1996)

Two classes of methanogens metabolize acetate to methane. *Methanothrix* has a high affinity for acetate, K_s=20 mg/L, but a relatively low maximum specific utilization rate, k_{max}= 2 to 4 g COD/g VSS day. On the other hand *Methanosarcina* has a much

lower substrate affinity K_s=400 mg/L, but a higher maximum specific utilization rate, k_{max}= 6 to 10 g COD/g VSS day. Thus it would be anticipated that predominance of *Methanothrix* would be favored at low acetate concentrations and *Methanosarcina* would tend to predominate at high acetate concentrations. According to Figure 2.2 at acetate concentrations below approximately 70 mg/L, *Methanothrix* has a competitive advantage; above this level, *Methanosarcina* would have a competitive advantage.

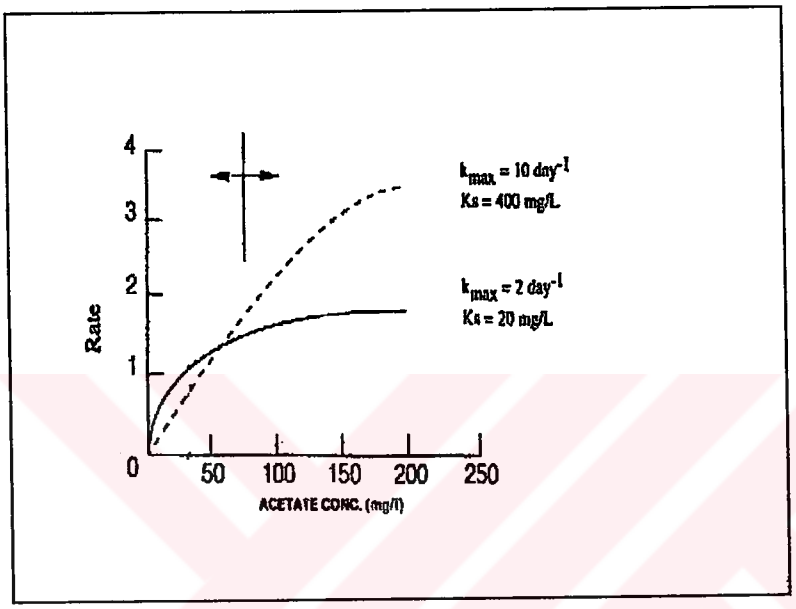

Figure 2.2. Competition for predominance of microorganisms (Speece, 1996)

The anaerobic process may be applied to seasonally produced wastewaters, such as winery or sugar operations, which normally produce effluent during only 2-4 months each year. Biomass viability is maintained, due to the unique feature of drastically reduced endogenous decay during starvation.

In weighing the merits of anaerobic treatment for a given wastewater certain disadvantages also need to be kept in mind. Sometimes it would not be practical to use anaerobic treatment, as might be the case in processing low temperature, or dilute

wastewaters, insufficient alkalinity wastewaters, or effluents requiring exceptionally low BOD for final discharge regulations (Speece, 1996). Long startup requirement for development of biomass inventory and odor generation are other disadvantages of anaerobic treatment.

Methanogens prefer nearly neutral pH conditions with a generally accepted optimum range of approximately 6.5-8.2. *Methanosarcina mazei,* a commonly observed methanogen, is reported to be able to operate at a pH range lower than other classes of methanogens. Conditions above or below this range decrease the rate of methane production rather steeply. Methanogenesis will continue at pH 6.0 and even lower at reduced rates but the bicarbonate alkalinity does not buffer well under such conditions, and this characteristic tends to result in considerable instability.

In addition to the two elements required for both aerobic and anaerobic microbial systems (N and P), some sulfide precursor must be added, commonly in the sulfate form to the anaerobic system. The methanogens manifest an obligate requirement for sulfide and phosphorus even though this need may be satisfied by maintaining very low concentrations of both ions in the reactor. However nitrogen concentration between 40 to 70 mg/L must be provided to prevent nitrogen limitation (Speece, 1996).

As with most microbially mediated processes, methanogenesis has been shown to be strongly temperature dependent, with reaction rates generally increasing with temperature up to 60^0C. Two optimal temperature ranges, mesophilic (near 35 ^{0}C) and thermophilic (55 to 60 ^{0}C), with decreased rates between these optima, have often be cited. With temperatures at or above 70 ^{0}C, methanogenic rates have been reported to decrease, although a larger pool of substrate may be available for conversion when higher temperatures are present (Malina and Pohland, 1992).

Toxicity and inhibition of methanogenic processes can be consequenced by a variety of circumstances, including the generation of intermediary products such as volatile

fatty acids, which may also manifest an adverse pH effect. Methanogenic microbial growth has been often shown to be restricted in the presence of excessive amounts of volatile fatty acids, particularly when propionate accumulate, and sudden increase in concentration of either acetate or butyrate have also exhibited stimulation of the process (Malina and Pohland, 1992).

In order to evaluate the potential toxicity of a wastewater sample to the anaerobic biomass, McCarty's group at Stanford developed a very useful and simple assay termed as Anaerobic Toxicity Assay (ATA) (Owen *et al.*, 1979). In fact, it is now possible to anaerobically biodegrade many additional organic toxicants when appropriate precautions are provided to protect the biomass, i.e. to carefully increase the toxicant concentration and to prevent loss of biomass from the system until the biodegradation/acclimation commences. With acclimation (the adaptation of the microorganisms to the new environment or conditions) the toxicity of a compound may be greatly reduced or may disappear. For instance, cyanide, trichloroetylene, chloroform, formaldehyde, acrolein, acrylate and a host of other toxic organics have been demonstrated to be biodegradable in properly acclimated anaerobic processes. The major advantage of anaerobic treatment may be toxicity reduction in the process compared to the aerobic counterpart. The strongly reducing conditions within the anaerobic system favor toxicity reduction (Speece, 1996).

2.2. Anaerobic Process Configurations

Anaerobic system design has evolved from that of a simple chemostat to the modern high-rate anaerobic processes that permit operation at very low HRT's. In all cases, the applicability, performance, and economy of the systems are related to the SRT's that can be maintained in each process. However, the kinetics of biodegradation of specific wastewater constituents and practical operating considerations still dictate the selection of a specific process for a given treatment application (Malina and Pohland, 1992).

The type of the design depends on the waste to be treated. Most of the organic pollutants in domestic sludge and animal slurry are as solids. The organic matter in industrial waste is in solution or colloidal suspension and therefore amenable to rapid treatment. The anaerobic bacteria grow slowly. If there is no special system for keeping and reusing the bacteria, then the minimum retention time in the reactor is limited by the microbial growth. The doubling time of the anaerobic methanogenic bacteria is 5 days and this is too long a retention time for reasonable commercial treatment of industrial effluent. New reactor designs have been developed for industrial wastes, which hold back most of the organisms inside the reactor or which recycle the bacteria after separation.

Bacteria are retained in the reactor by four basic methods;

a) Physical separation of the biomass from the effluent by filtration or sedimentation, followed by recycle back to the reactor. This type of the reactor is known as the Contact Stirred Tank Reactor (CSTR)
b) Retention and attachment of the bacteria by an internal packing to reduce the upflow velocity: anaerobic filters
c) Natural bacterial flocculation assisted by low upflow velocities, known as UASB.
d) Attachment of the bacteria to a small support particle and fluidization to produce mixing (fluidized or expanded bed)

There are also a variety of other reactor types: either bulk volume low rate systems such as lagoons, and large stirred tanks as well as some hybrids (Wheatley, 1991).

High rate anaerobic biological reactors may be classified into three broad groups depending on the mechanism used to achieve biomass detention, and these are fixed film, suspended growth, and hybrid. There are currently 900 full-scale installations in the world today and they are distributed as follows: upflow anaerobic sludge blanket (UASB) 67%, CSTR 12%, Anaerobic Filter 7%, other 14% (Barber and Stuckey, 1999). Figure 2.3 shows the main reactor configurations of anaerobic biotechnology.

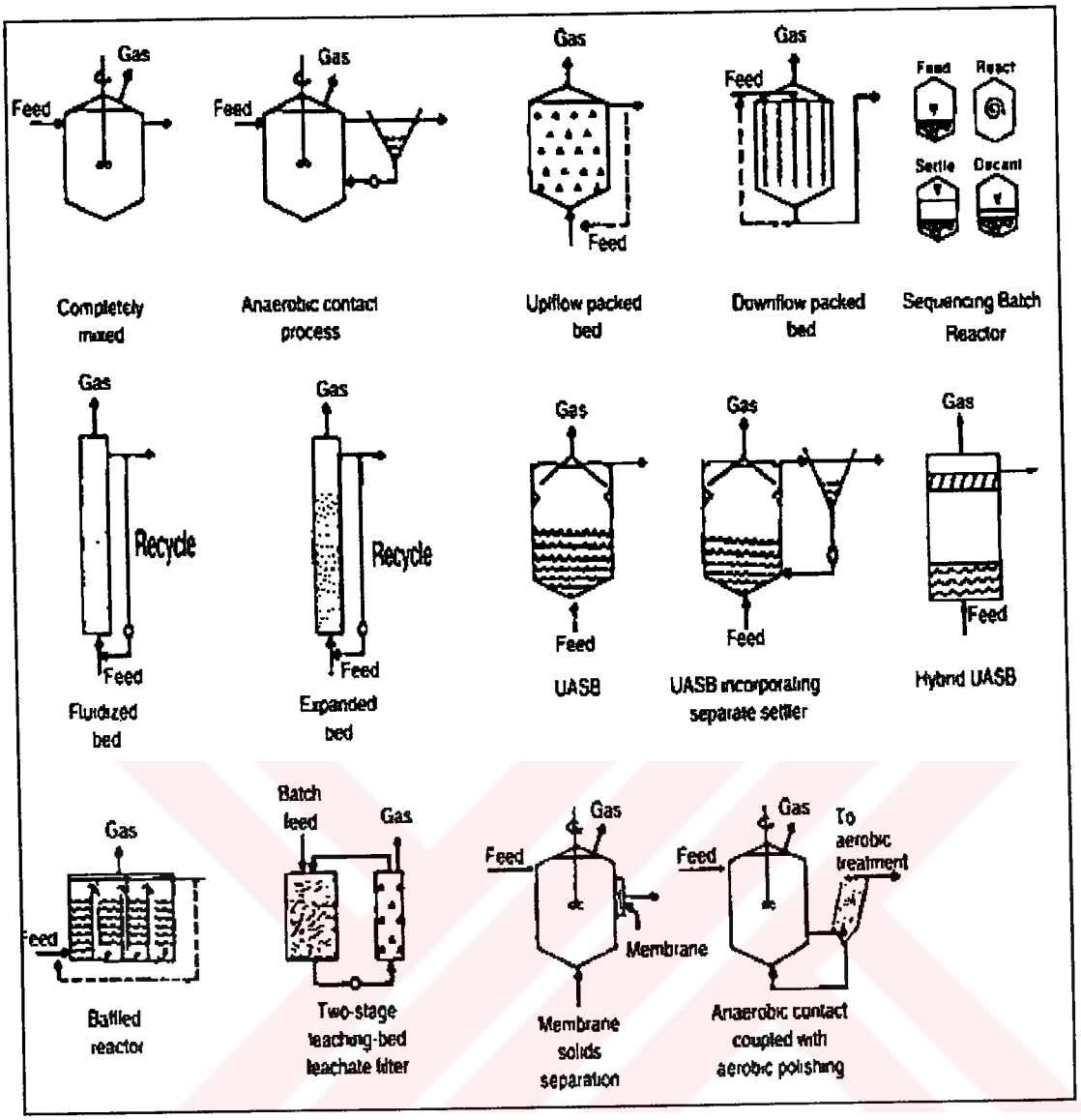

Figure 2.3. The main reactors developed for anaerobic biotechnology (Speece, 1996)

2.2.1. Anaerobic Contact Reactor

The principle involved is the same as in the activated sludge process settling of microbiological floc and other suspended solids and contacting the raw waste with the anaerobic sludge. The reactor's performance depends therefore markedly on the efficiency with which the microorganisms and suspended solids settle. The operation of the process suggests that the process is especially suited for wastes with a certain

amount of hard to digest solids that settle readily or attach themselves readily to settleable solids. The anaerobic contact process has little advantage for very concentrated wastes because the long hydraulic retention times already required make settling of microorganisms unnecessary (Berg and Kennedy, 1981).

2.2.2. Anaerobic Filter (AF)

This reactor was developed by Young and Mc Carthy (1967) and resembles an upflow trickling filter. Waste enters in the bottom and flows upwards through the packing, composed of rocks or plastic media with biomass collecting in void spaces and surfaces.

The process is particularly suitable for dilute soluble wastes or soluble wastes which can be made dilute by recirculating effluent. The main limitation of the process is due to accumulation of solids in the packing material. The solids can be waste suspended solid; materials precipitated from the waste (e.g. calcium carbonate) or suspended growth. In large reactors, an inadequate liquid distribution system may cause channeling and short-circuiting (Berg and Kennedy, 1981).

Different packing materials were tried as a support media in anaerobic filters. Attachment to hydrophilic surfaces is very rapid. If the surface is hydrophobic, like the plastics, then the extra-cellular polymeric secretions of bacteria act as bridge between the surface of the cell and the plastic. The attachment process occurs in two stages: a) an initial electrostatic attraction to the surface with surface roughness providing shelter against the liquid shear forces, followed by b) permanent binding by the extra-cellular secretions (Wheatley, 1991).

The two stage cyclic process can achieve much lower effluent soluble organic concentrations than single stage systems operating at the same organic loadings. During the first cycle of series operation, solids synthesis is high in the lead reactor because of the high waste strength and high loading. The net cell yield in the

following reactor is low, or even negative, because of low influent waste concentration. A major performance characteristic of the two stage cyclic process is its ability to receive extreme shock loads without major efficiency loss. Solids lost from the lead stage because of gas flotation and dispersion would be captured in the following stages, which further adds to improved performance and increased stability (Howerton and Young, 1987).

The biomass accumulation rate is related to the yield (Y) of substrate synthesized. High synthesis aerobic systems therefore accumulate biomass at much higher rates than low synthesis anaerobic systems. All anaerobic processes involve prolonged startup times before the design biomass inventory is reached. Startup efficiency parameters for anaerobic filters were reported by Bonastre and Paris (1989), (cited in Speece, 1996).

In their experience careful attention should be given to the following categories: quantity and quality of inoculum, substrate composition, nutrient and buffer potential of substrate, initial HRT, flow direction, recycle rate, type of reactor, supplementation of methanol to promote higher synthesis of methanogens. Sugar cane molasses treatment startup (Camilleri, 1988) took only 3 months and was similar in characteristics to beet sugar refinery processing wastewater in behavior. In the same study the wine distillery took 6-8 months startup period to reach the nominal loading rates of 15 kg/m^3d. Cheese making and casein factory operations required 8 months initial time but this time period can probably be reduced to a little over 5 months (Speece, 1996). Anaerobic startup research observations are summarized in Table 2.1.

Table 2.1. Anaerobic startup research observations (Speece, 1996)

Researcher	Observation
Camilleri (1988)	• Sugar substrate immobilization quick, starch substrates much slower • Toxicity effects greatest during startup • *Methanosaeta* immobilize more easily than *Methanosarcina* • *Methanospirillum* attach to polyethylene quicker than *Methanosaeta*, but the opposite is true for PVC
Young and McCarty (1967)	• Startup 4 times slower for municipal anaerobic waste biomass than for other types
Saslawsky *et al.* (1988)	• High sulfide effluents pose problems for seeding, nutrient introduction, inhibition and biomass loss • SO_2 and furfural were the main inhibitors in sulfite Rich pulp mill effluent
Saslawsky (1988)	• Sulfide-rich effluent responded positively to whey substitution for trace metals
Tait end Freidman (1980)	• Addition of methanol promoted methanogen growth
Colleran *et al.* (1992)	• OLR of 4 to 9 kg/m3day performed without problem and with less control than at lower levels; startup periods varied from 3 to 9 months based on substrate and OLR differences
Van den Berg and Kennedy (1981)	• Startup period dependent upon type of inert media

2.2.3. Upflow Anaerobic Sludge Blanket Reactor (UASB)

This reactor type was developed to avoid the main problem of the anaerobic filter, namely plugging of the packing. The UASB reactor has been able to work with high concentrations of biomass and this has resulted in very high loading rates and excellent COD removals. The major similarity between the UASB and the anaerobic

filter, namely dependence on suspended growth for high performance, would suggest that the same types of wastes are suitable for both reactors. The limitation of the process is based on problems associated with the development of the granular sludge (Berg and Kennedy, 1981).

Sludge granulation is a complex and not fully understood process. Comprehensive and detailed studies have been made in a number of countries but principally in Holland where the UASB process was devised (Lettinga *et al.*, 1987). Granulation is a natural process and is due to a combination of microbial morphology, nature of the substrate and accumulation of inorganic salts. Formation of rapidly settling granules from an ordinary inoculum may take 50 days. Key elements in the feed substrate for successful formation are calcium, phosphorus, aluminium and silicon (Wheatley, 1991).

Dense granules in the UASB, with their high settling velocity, avoid the costly packing, which is otherwise necessary in other configurations to provide quiescent conditions for efficient biomass retention. A distinctive feature of successful UASB operations is the very high loading rate achieved by the systems. Good settleability, high biomass concentration (30000 to 80000 mg/L) and excellent solid/liquid separation are realized with proper granulation (Speece, 1996).

In UASB reactors, the sludge stabilization that can be accomplished depends strongly on the biodegradability of the entrapped, sorbed and/or precipitated substrate ingredients, on the operational temperature and the average sludge hold-up time (Lettinga and Hulshoff Pol., 1991).

2.2.4. Anaerobic Fluidized and Expanded Bed Reactors

These reactors are similar to suspended growth reactors in that the active biomass is present in the form of a bed of readily settleable aggregates. These aggregates are obtained by having the biomass grow on small inert particles such as fine sand and

alumina. A rapid and even a flow of liquid is used to keep the particles in suspension. The rate of liquid flow and the resulting degree of expansion of the bed determine whether the reactor is called a fluidized or an expanded bed reactor.

The preferred waste substrate for these reactors is soluble or at least the suspended material should be easily degradable. The limitation of the process lies in the need for a high and very uniform upflow of liquid. The capital cost of the flow distribution system and the pumps is high and also the net energy yield is lower than for other reactors (Berg and Kennedy, 1981).

2.2.5. Downflow Stationary Fixed Film Reactor

This reactor was also developed from the anaerobic filter to avoid plugging problems. Loading rates of the reactor are limited by the amount of active biomass that can be retained in the reactor. It is not suitable for the treatment of very dilute waste streams (Berg and Kennedy, 1981).

2.2.6. Novel Reactor Systems: Membrane Bioreactors

Efficient liquid solid separation is the basis of any anaerobic high rate reactor system for wastewater treatment. Solid separation may be improved distinctly by the combination of a digester with a membrane process, where the separated solids (biological and non-biological) are continuously recycled. The sludge retention time can be easily adjusted through the amount of waste sludge withdrawal, and is highly dependent on the amount of inert solids loaded to the reactor. With a low inert solid loading, these systems can be operated even at approximately infinite SRT, thus allowing them to reach very low effluent soluble concentrations. In addition, allowing the growth of slow growing microorganisms this reactor concept could be particularly suited for the treatment of recalcitrant compounds. However, one of the major drawbacks of the high-pressure physical separation device is the disruption of

the microbial conglomerates needed for the efficient conversion of complex organic matter (Brockman and Seyfried, 1997).

Nonetheless considering their potential anaerobic membrane bioreactors maybe very beneficial for specific applications, e.g. when biomass granulation proceeds with great difficulty. Also with respect to slurry digestion where a solid liquid separation step is needed after digestion, membrane bioreactors show interesting perspectives another application is the treatment of wastewaters with high concentrations of suspended solids (Nagono *et al.*, 1992). The advantage of this configuration can be the lower energy need. The drawback for an anaerobic reactor could be the difficulty of maintaining the membranes, which can be subjected to fouling and scaling with e.g. typical precipitates such as calcium carbonate (Van Lier *et al.*, 2001).

When we consider at the factors governing reactor choice; a technology is acceptable to an industry if it requires less capital, less land area and is more reliable when compared to the other well-established options. For an anaerobic digestion system, this translates into the process being able to run at high organic and hydraulic loading rates with minimum operation and maintenance requirements. To choose the most appropriate reactor type for a particular application, it is essential to conduct a systematic evaluation of different reactor configurations with the wastewater stream.

The organic and hydraulic loading potential of a reactor depends on three factors:

- Amount of active biomass that can be retained by a reactor per unit volume.
- Contact opportunity between the retained biomass and the incoming wastewater.
- Diffusion of substrate within the biomass.

With these considerations, granular sludge UASB reactor stands out distinctively as the best choice with the only limitations being the tendency of granules to float and shearing of granules at high loading rates. These constraints are also valid to a lesser

degree for attached biomass reactors (such as fixed film, fluidized bed and rotary biological contactors). In addition, due to the space occupied by the media, the attached biomass reactors possess comparatively lower capacity for biomass retention per unit volume of the reactor. The latter depends on the film thickness, which would be the highest in a fluidized bed reactor due to large surface area available for biomass attachment (Rajeshwari *et al.*, 2000).

2.3. Anaerobic Digestion of High Strength Wastewaters

Anaerobic digestion is the most suitable option for the treatment of high strength organic effluents. The presence of biodegradable components in the effluents coupled with the advantages of anaerobic process over other treatment methods makes it an attractive option (Rajeshwari *et al.*, 2000)

The BOD content of many high strength effluents from the food, fermentation, beverage and pulp and paper industries can be reduced by anaerobic digestion. The various industrial effluents also differ in chemical composition ranging from the sugar factory in which the BOD consists mainly of volatile fatty acids in solution, starch effluents from potato and cereal processing which contain colloidal solids to those from canning factories and alcohol production, which may contain insoluble particulate material. In all cases the digester has to cope with a high volume of dilute solution making a short HRT essential to reduce capital costs. As a consequence of the high liquid throughput retention of the active biomass in the reactor by some means is also essential. There is therefore widespread use of advanced designs such as, UASB reactors, anaerobic filters and fluidized bed reactors (Wheatley, 1991).

Wastewater from a slaughterhouse arises from different steps of the slaughtering process such as washing of animals, bleeding out, skinning, cleaning of animal bodies, cleaning of rooms, etc. The typical COD concentration of a slaughterhouse wastewater is between 5.2-11.4 g COD/L (Rajeshwari *et al.*, 2000). Nunez and Martinez (1999) reported that the use of an expanded granular sludge bed (EGSB)

reactor was effective in the treatment of slaughterhouse wastewater for up to organic loading rates of 15 kg COD / m^3 day. Removal efficiencies of COD were dependent on HRT. Total COD removal efficiencies averaged 65-80 % according to HRT.

The liquid waste in a dairy originates from manufacturing process utilities and service sections. The various sources of waste generation from a dairy are spilled milk, spoiled milk, skimmed milk, whey, and wash water from milk cans, equipment, bottles and floor washing. Whey is the most difficult high strength waste product of cheese manufacture. The treatment of cheese whey wastewaters by anaerobic degradation is constrained by the drop in pH that inhibits further conversion of acids to methane. However, with proper startup, UASB reactors can cope with cheese whey wastewaters at low pH of 4 even at high OLR of 6.5 kg COD/m^3day. A high treatment efficiency with 90 % COD reduction has been achieved in laboratory and pilot scale reactors at both mesophilic and submesophilic temperatures with a maximum organic loading rate of 28.5 kg COD/m^3day and 9.5 kg COD/m^3day, respectively. But there is a problem in the treatment of cheese whey wastewater that as the substrate loading is increased; the acidogenic region extends into the methanogenic. This makes the entire region acidic, ultimately resulting in the failure of the reactor. Thus, two-stage reactor becomes essential for improving the biogas production and methane yield (Rajeshwari *et al.*, 2000).

Ergüder *et al.* (2001) reported that high rate anaerobic treatment of undiluted cheese whey in UASB reactors is a very efficient and cost effective method. HRT values as low as 2-3 days were sufficient for a COD removal efficiency of 95-97% at influent COD concentration of 42700-55100 mg/L.

The manufacturing process in a distillery involves dilution of molasses with water followed by fermentation. The product is then distilled to obtain rectified spirit or neutral alcohol. The distillation process results in the generation of a strong organic effluent. The source of other wastes is from floor washing, recovery units of yeast and other byproducts. For the treatment of sugar cane molasses using an UASB

reactor, the dilution has a significant effect on the loading rate. In a 100 L reactor for stillages with COD ranging from 35 to 100 g/L, an OLR of 24 kg COD/m^3day resulted in 75% COD removal. Feeding with undiluted stillage resulted in a tremendous increase in the concentrations of acetic and propionic acids, thus affecting the stability of the reactor (Rajeshwari *et al.*, 2000).

In the pulp and paper industry, there are various types of wastewater generation. Some wastewater coming from leaks and spills from the digester. Pulp washing and bleaching gives wastewaters of various characteristics depending on the bleaching sequence. Bleaching section results in wastewater and chlorolignins. Wastewater is also generated from paper machine section, caustic chlorine manufacture and black liquor recovery. There are variations in the COD, inhibitors and the degradability depending upon the source of wastewaters (Rajeshwari *et al.*, 2000). A laboratory scale study was carried out by Korczak *et al* (1991) for the anaerobic treatment effluents from acid hydrolysis of wood from sulfate cellulose production and from the sulfite cellulose fiber washing. The efficiency was about 80 % in terms of COD reduction and methane production, 0.34 kg COD/m^3day was removed from the high strength effluent (63000 mg/L) from acid hydrolysis. However, for the effluent from cellulose washings, the COD reduction was only 20-30% and the methane yield was 0.27-0.36 m^3/kg COD removed. This was due to the fact that the effluent contained refractory compounds such as lignin derivatives, resins and tannins apart from sugars.

Also Tezel *et al* (2001) reported that application of a sequential biological (anaerobic/aerobic) system to treat the Dalaman SEKA Pulp and Paper Industry wastewater resulted in approximately 91% COD and 58% AOX removals at a HRT of 5 and 6.54 h for anaerobic and aerobic stages, respectively.

Although most of the high rate reactors have proved their applicability for different high strength wastewaters over a range of organic loading rates, there exists certain differences in the preference of a particular type of anaerobic reactor over others in

terms of various factors such as requirement of pre-treatment, dilution, control of operating conditions, etc. In the case of slaughterhouse wastewater, an anaerobic contact reactor can be used without pre-treatment whereas for the usage of high rate digester such as UASB, a pre-treatment step for removal of the suspended solids and fats is essential prior to anaerobic treatment. Two phase digestion with pH and temperature control results in a higher biogas production rate with cheese whey wastewater digestion. Distillery effluent due to its high strength appears to be having maximum potential in comparison to other effluents. UASB and fixed film reactors are more commonly used for distillery effluent due to their ability to withstand high OLR. An aerobic post-treatment is necessary to attain the permissible COD and BOD level before discharge. Due to the generation of wastewater from various sections of pulp and paper industry, there are variations in the composition and the treatability of effluents.

Effluent treatment plants based on the UASB principle have been widely adopted by the sugar and starch industries in particular. By 1986 over 60 mesophilic plants of between 30 and 4600 m^3 reactor volume had been built in Europe, the US and Thailand designed to handle between 5 and 20 kg COD / m^3 day. More recently over 20 UASB systems have been built in Brazil for treatment of stillage generated by distilleries associated with the National Alcohol Programme, as well as effluents from processing of meat, starch and dairy products (Hirata and Craveiro, 1988). These plants, all of which were commissioned between 1986 and 1989, are large with over two thirds of the digesters in excess of 1000 m^3. In addition one contact digester system (1800 m^3) and two anaerobic filters have been built.

Although China is best known for its small rural anaerobic digesters a number of larger industrial digesters have also been built. For instance Zuxuan and Zepeng (1984) describe a two-phase mesophilic (35 ^{0}C) process, based on anaerobic filter (30 m^3) combined with a UASB (100 m^3) which removed over 70% of the BOD from molasses derived stillage from an alcohol plant. With a feed COD of 26.5 g/L and a HRT of less than a day in the first (acidogenic phase) (cited in Wheatley, 1991).

Anaerobic treatment processes operate at low redox potential (-350mV) and this means that organic nitrogen and sulphur compounds are reduced by the process to ammonia, amines and various sulphides. The smell, oxygen demand of these compounds and their toxicity means that anaerobically treated effluents are unsuitable for discharge to inland watercourses. Rearetion is required. There are two convenient methods of rearetion: aerobic polishing treatment or discharge to sewer for aerobic treatment in combination with domestic watewater (Wheatley, 1991).

2.4. Whisky and Whisky Production

Whisky is an alcoholic beverage, prepared from fermented cereals normally in matured oak barrels. There are many possible ways of producing whisky, within the limitations set by the materials and processes available, and details vary depending on custom and regulation in producing countries. (Lea and Piggott, 1995)

2.4.1. Raw Materials of Whisky

Corn (maize), rye, barley and wheat are the major cereals used for whisky. These grains have traditionally been the major sources of starch for whisky production and meet the main criterion of a high starch content to permit the greatest yield of spirit. Corn is most used for whisky production in the USA and was the prime cereal used for Scotch grain whisky.

Rye is a minor crop in the USA and Canada and, major production being in Eastern Europe and states of the former USSR, and is used for its flavor contribution in whiskies, since it contains less starch than corn and wheat. Rye malt is also occasionally used. Barley is used primarily in the form of malt for the flavor characteristics it provides in the spirit. In this case the enzyme content is a major quality criterion, irrespective of the starch content, which is rather low. Malts are normally classified on the basis of the content of phenols. Wheat is a major in USA,

CIS (Confederation of Independent States) and EU crop, total production being approximately equal to maize (Lea and Piggott, 1995).

2.4.2. Manufacturing Processes of Whisky Production

2.4.2.1. Malting

Most malts used in alcoholic beverage production are produced from barley although other cereals are malted for production of certain specialty beers and North American spirits. Grain is graded and then steeped in water, with air rests to assist respiration, and allowed to germinate at moisture contents between 43 and 49%. The precise manner in which this hydration is effected may be important as certain barleys exhibit water sensitive in that submerged grain fails to germinate. In the embryo the moisture content will rise to 60-65%. During this germination, synthesis of depolymerising enzymes takes place in both the aleurone and scutellum in response to secretion of plant hormones by the embryo (Lea and Piggott, 1995).

2.4.2.2. Mashing

Mashing is the process of forming a fermentable extract. Two major routes may be followed, depending on whether a malted or unmalted cereal is used (Lea and Piggott, 1995).

2.4.2.3. Fermentation

The fermentation stage is similar to that used for many other alcoholic beverages, and in most regulations yeast (*Saccharomyces cerevisiae*) is specified as the only organism. (Lea and Piggott.1995). *Saccharomyces cerevisiae*, being the most widely used due to its robust growth rate and high ethanol tolerance. With proper nutrient and growth conditions, it has been showed that *Saccharomyces cerevisiae* can tolerate ethanol conditions up to 23 % (Wilkie *et al.*, 2000). While malt and other

cereals may be contaminated with a wide variety of organisms (yeast and bacteria), whisky fermentations are started by pitching the worth with a known yeast culture, normally a specific strain of high performance distilling yeast. (Lea and Piggott, 1995).

The fermentation process is normally operated batch, but the process may also be continuous. In a conventional batch process, an inoculum of yeast culture often-close 10 % of the fermenter volume is added to the cool mash (Wilkie *et al.*, 2000). A typical fermentation will run for 40-48 hours, a very much shorter time has traditionally been allowed. Shorter fermentations may be detrimental to spirit quality, and excessively long fermentations allow considerable bacterial growth with the consequent loss of ethanol yield and danger of flavor defects (Lea and Piggott, 1995).

2.4.2.4. Distillation

Two distinct distillation systems have been used for production of whiskies; the batch or pot still, normally a double distillation (occasionally triple), to produce high flavored spirit, and the continuous column still to produce lighter flavored spirits normally used as the base for blending (Lea and Piggott.1995). With efficient distillation, the stillage should contain less than 0.1-0.2 % ethanol, but at times when distillation is not optimal, the stillage may contain a significant ethanol content. For each 1 % ethanol left in the stillage, the COD of the stillage is incremented by more than 20 g/L.Due to the potential impact of residual ethanol content; therefore, proper control over distillation can greatly affect the COD of the stillage (Wilkie *et al.*, 2000).

2.4.2.5. Maturation

Maturation is an important step in the development of whisky flavor. Freshly distilled whisky generally has an unacceptable sensory characteristic and is matured

in oak casks to produce an acceptable product. During the maturation period, the new distillate becomes highly modified as a result of its contact with the cask (Lea and Piggott, 1995).

2.4.2.6. By-Products

The two major by-products of whisky production are the residues of the cereals used as the source of carbohydrate (spent grains), and the residues of the distillation (pot ale) (Lea and Piggott, 1995).

2.5. Studies On Stillage Treatment and Utilization

Stillage, also termed distillery wastewater, distillery pot ale, and distillery slops, distillery spent wash, etc., is the aqueous by product from the distillation of ethanol following fermentation of carbohydrates. An early means of treatment and disposal included evaporation of the stillage, neutralization with alkali, followed by incorporation into road building materials. While the fertilizer value of molasses stillage was well recognized.

A potentially viable use of stillage is for single cell protein (SCP) production, where a second aerobic culture is employed to remove residual sugars and soluble proteins in the stillage and lower the COD and nutrient content. Also a proportion of the stillage can be used to produce inoculum for ethanol production. Finally, the sludge from biological treatment of stillage could be processed into feed materials. A mixed culture of *Geotrichum candidum, Candida crusei, Hansenula anomala* was used to reduce the COD of whiskey stillage by 54.9 %, which was higher than achieved by any of the organisms in pure culture (Wilkie *et al.*, 2000).

When we consider the applicability of aerobic treatment for ethanol wastewaters, the high COD of stillage means that significant aeration power would be required for aerobic treatment and that about 50 % of the COD would be converted to sludge

requiring further disposal. Anaerobic digestion can convert a significant portion (>50 %) of COD to biogas, which maybe used as an in plant fuel, and also saves the energy that would be required for aeration using aerobic treatment. In addition anaerobic digestion has about 10% of the sludge yield and lower nutrient requirements compared to aerobic treatment. (Wilkie *et al.*, 2000).

Harada *et al.*(1996) studied the anaerobic treatment of an alcohol distillery wastewater by using an upflow anaerobic sludge blanket reactor for a period of 430 days. Organic loading rates were applied up to 28 kg COD/m^3day by reducing HRT at a fixed influent concentration of 10 g COD/L. COD removals during the entire experimental period were relatively low between 39-67%, while BOD removals were more satisfactory more than 80 %. In the period of days 50-160 days, COD removal tended to worsen as the loading increased from 4.1 to 28 kg COD/ m^3day.

Akunna and Clark (2000) have studied the performance of a granular bed anaerobic baffled reactor (GRABBR) in the treatment of whisky distillery wastewater. COD and BOD concentrations of whisky distillery wastewater were 16600-58000 mg/L and 8900-30000 mg/L, respectively. The ABR was fed with diluted whisky distillery wastewater containing 9500 mg/L COD at different values of HRT (10, 7, 4 and 2 days) corresponding to organic loading rates of 0.99, 1.33, 2.37 and 4.75 kg COD/ m^3day, respectively. The removal of total BOD and COD from the wastewater were 80-92 % and 90-96 %, respectively. The best performance was observed with a HRT of 4 days and loading rate of 2.37 kg COD/ m^3day. When the retention time was decreased to 2 days the efficiency of the GRABBR dropped, but the removal rates were still comparatively good. The poor performance observed at 2 days retention time was attributed principally to the instability created by the sudden doubling of the influent loading rate. After nine months of operation, a significant change in the nature of the sludge bed was observed, especially in the two compartments closer to the influent port. The original granules in these first compartments were appeared broken up and replaced by non-granular fluffy sludge. This is because of that acidogens are mostly hydrophilic (or non-granule-forming)

while methanogens are hydrophobic (or granule forming). Thus, the GRABBR system encouraged the occurrence of an acidogenic, non-granular sludge zone upstream of the methanogenic granular zone, which produced an overall effect of reduced sludge washout and improved process stability.

Many distilleries particularly in the United Kingdom, where they also are small and rural have been able to dispose of effluents by land irrigation or discharge to sea. A small Scottish highland distillery, for example, will produce 500 m^3 of effluent a week. Large industrialized distilleries, however, have major waste disposal problems. One French cognac producer, with an anaerobic digester, generates 10000 m^3 of effluent a week, equivalent to the waste from a population of 0.5 million. Many plants are contact stirred tank reactors but there are a significant number of AF in France (Wheatley, 1991).

Tokuda *et al.* (1999) performed a pilot scale anaerobic treatment test for non-diluted pot ale using an upflow AF reactor. The support medium was a module structure composed of multi layer plates. COD removal efficiency was exceeded 76 % with a 20 kg COD/ m^3day organic loading rate and 80 % or more of organic nitrogen content was converted into NH_4^+-N, and 90 % or more of the organic phosphorus content into PO_4^{3} -P. Approximately 70% of the total nitrogen content was removed by biological denitrification nitrification treatment with recirculation.

Goodwin *et al.* (1994) used two identical UASB reactors operated in paralell as duplicates for the treatment of malt whisky pot ale and achieved COD reductions up to 90% for influent concentrations of 3526-52126 mg/L. This study was conducted for 327 days. When the organic loading rates of 15 kg/ m^3day and above were used , COD removal efficiency dropped to less than 20 %, in one of the duplicate reactors.

In a further study, Goodwin *et al.* (2001) stated that digester failure occured when undiluted pot ale was used. Stable operation was observed at OLRs of 5.46 kg COD/m^3 day or less. Again this study was conducted for 279 days.

CHAPTER 3

MATERIALS AND METHODS

In this chapter, chemicals, laboratory apparatus and support materials, inocula, the analytical methods, experimental setups and procedures used in this study are described.

3.1. Chemicals, Laboratory Apparatus and Support Materials

Chemicals: Malt whisky wastewater was obtained from the Ankara Tekel Factory., Turkey.

Laboratory Apparatus: The laboratory apparatus used in the experiments were as follows; 110 mL glass serum bottles, natural rubber sleeve stoppers, cable ties (Cole Parmer Instrument Co., USA), latex rubber tubing; Teflon tubing (Cole Parmer Instrument Co., USA); Teflon connectors/fittings (World Precision Instrument Inc., USA); 10 mL centrifuge tubes; magnetic stirrer (Heidolph, Germany); peristaltic pumps (Model No 77120-30, Cole Parmer Instrument Co., USA); pH controller (Cole Parmer Instrument Co., USA Model No: 5656-00) and a pH probe (Cole Parmer Instrument Co., USA Model No: 59500-81).

Support Materials: Support materials used in anaerobic filter experiments were pumice stone and Celite R-632. Pumice stone was obtained from HESS Pumice Products, Inc. (Malan, Idaho, USA) and has a diameter and density of 0.25-1.4 mm and 1.764 g/cm^3, respectively. Celite R-632 was obtained from Health, Safety and

Environment Department Celite Corporation (California, USA) and has a diameter and density of 0.7-1.2 mm and 2.1 g/ cm^3, respectively.

3.1.2. Basal Medium

Basal medium (BM) containing all the necessary micro- and macronutrients for an optimum anaerobic microbial growth was used in the experiments. The composition of BM used in all the experiments is as follows (concentrations of the constituents are given in parentheses as mg/L): NH_4Cl (1200), $MgSO_4.7H_2O$ (400), KCl (400), $Na_2S.9H_2O$ (300), $CaCl_2.2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2.4H_20$ (40), $CoCl_2.6H_20$ (10), KI (10), $MnCl_2.4H_20$ (0.5), $CuCl_2.2H_20$ (0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_20$ (0.5), $NaMoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_20$ (0.5), $NaWO_4.2H_2O$ (0.5), Na_2SeO_3 (0.5), cysteine (10), $NaHCO_3$ (6000) (Demirer and Speece, 1997).

3.2. Inocula

3.2.1. Mixed Anaerobic Cultures

Mixed anaerobic cultures, which were used in Biochemical Methane Potential (BMP) experiments and in the anaerobic filter (AF) experiments, were obtained from the anaerobic sludge digesters of the Greater Municipality of Ankara Domestic Wastewater Treatment Plant. The digesters have a retention time of 14 days. The average sludge flow from primary thickeners to each digester is 805 m^3/day. The pH in the digesters ranges from 7 to 7.7.

Before being used as inocula in the experiments, mixed anaerobic cultures were thoroughly mixed and filtered through a screen with a mesh size of 1 mm. MLSS and MLVSS concentrations of the mixed anaerobic culture were 58±0.7 g/L and 18.6±0.4 g/L, respectively.

3.2.2. Acetate Enriched *Methanosarcina* Cultures

Acetate enriched *Methanosarcina* cultures were also used in BMP and AF experiments. The acetate enriched *Methanosarcina* cultures were obtained from a pH-Stat CSTR operating for about 89 days at a constant pH of 6.8±0.2. MLSS and MLVSS concentrations of the acetate enriched *Methanosarcina,* cultures were 36±0.11 g/L and 12.4±0.15 g/L, respectively. pH-Stat CSTR consisted of a magnetically stirred glass erlenmayer of 1.6 litres of effective volume with a head space of 400 mL. The erlenmayer was sealed with black rubber stopper with ports for probe penetration, feeding, sample withdrawal and gas venting.The pH-Stat CSTR had no recycle and incorparated with an pH controller and a probe (Figure3.1). The pH increased when the substrate was consumed by the microorganisms. After detecting a signal above the pH set point of 6.8, the pH controller sent a signal to turn on the peristaltic substrate feed pump for 1 second. Then a fixed amount of substrate was delivered to the reactor and lowered the pH not more than 0.2 pH units. By this way, the pH value in the reactor was kept constant at 6.8±0.2. Photographs of acetate enriched *Methanosarcina* cultures are given in Appendix A.

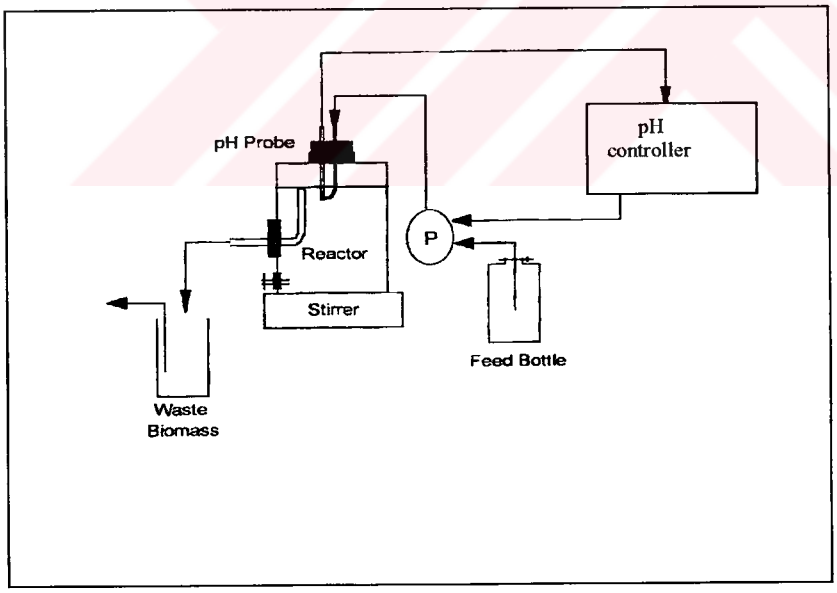

Figure 3.1. Schematic diagram of a pH-Stat CSTR (Demirer and Speece,1999)

3.2.3. Anaerobic Granular Cultures

Anaerobic granular cultures, which were used in the upflow anaerobic sludge blanket UASB) reactor experiments, were obtained from the UASB (reactors of the Wastewater Treatment Plant of İstanbul Tekel Paşabahçe Factory. The organic loading rate of the UASB reactors were 30 kg COD/m^3.day. The pH of UASB reactors ranges between 6-9. The anaerobic granular sludge used in the UASB reactor experiments had MLSS and MLVSS concentrations of 61.5±1.25 g/L and 54.3±1.04 g/L, respectively. All the cultures were kept in anaerobic conditions at 35±2°C until used.

3.3. Analytical Methods

pH: pH values were determined with a pH meter (Model 2906, Jenway LTD., UK) and a pH probe (G-05992-55, Cole Parmer Instrument Co., USA).

Suspended solids (SS) and volatile suspended solids (VSS): SS and VSS were determined by following standard methods (2540 D, E) (Standard Methods, 1997).

Volatile fatty acids (VFA) and bicarbonate alkalinity: Volatile fatty acids (as HAc) and bicarbonate alkalinity (as mg/L $CaCO_3$) were determined according to the titration procedure given by Anderson and Yang (1992).

Chemical oxygen demand (COD): COD values of samples were determined according to an EPA approved reactor digestion method (for a COD range of 0-1500 mg/L) as given in Hach Water Analysis Handbook (1988). For COD analysis, Hach Spectrophotometer (Model No 45600-02, Cole Parmer Instrument Co., USA) and vials were used.

Biological Oxygen Demand (BOD): BOD values of samples were determined by following standard methods (5210 B. 5 day BOD Test) (Standard Methods, 1997).

Gas Production: Gas production in serum bottles were determined by a water displacement device consisting of a 50 mL burette and a 250 mL water reservoir. A needle connected to the burette via latex rubber tubing was inserted through the rubber stoppers of the serum bottles. The volume of the water displaced in the burette was recorded as the produced gas volume. Figure 3.2. shows the schematic diagram of water displacement device.

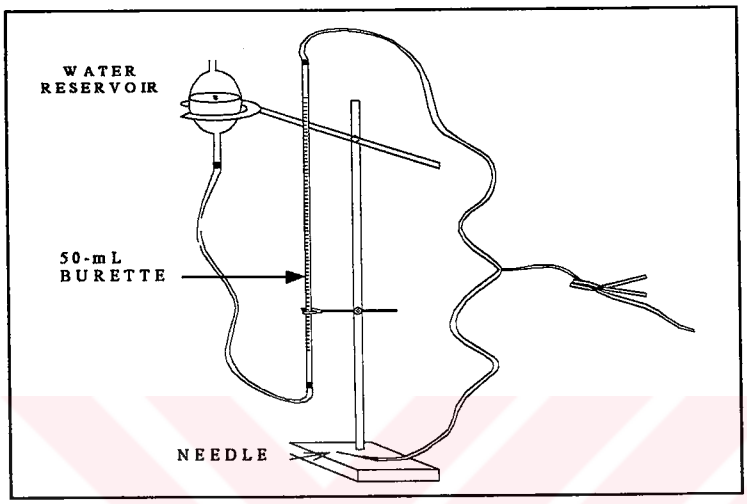

Figure 3.2. Schematic diagram of water displacement device (Güven, 1999)

The content of CH_4 in biogas was determined as follows. A known volume of the headspace gas (V_1) produced in a serum bottle used in BMP experiments was syringed out and injected into another serum bottle, which contained 20 g/L KOH solution. This serum bottle was shaken manually for 3-4 min so that all the CO_2 and H_2S were absorbed in the concentrated KOH solution. The volume of the remaining gas (V_2), which was 99.9%, CH_4 in the serum bottle was determined by means of a syringe. The ratio of V_2 /V_1 provided the content of CH_4 in the headspace gas. The results of three different determinations indicated that the average methane content of the biogas was 77±7% (Ergüder *et al.*, 2000).

Total Kjeldahl Nitrogen (TKN): TKN values of samples were measured by following standard methods (4500-Norg B. Macro Kjeldahl Nitrogen) (Standard Methods, 1997).

Total PO_4-P and PO_4: Total PO_4-P and PO_4 values of samples were measured by following standard methods (4500-P F. Automated Ascorbic Acid Reduction Method) (Standard Methods, 1997).

3.4. Experimental Setups and Procedures

In this study the experiments conducted can be grouped into four parts; characterization of Malt Whisky Wastewater, BMP experiments, continuous anaerobic rector experiments (UASB and AF) and batch aerobic experiments.

3.4.1. Characterization of Malt Whisky Wastewater

The Malt Whisky Wastewater, which was obtained from Ankara Tekel Alcohol Factory, was characterized. To this purpose pH, COD, BOD, TKN, Total PO_4 and PO_4-P, MLSS and MLVSS of this wastewater were measured and tabulated in Table 3.1.

Table 3.1. Characterisation of Ankara Tekel Factory Malt Whisky Wastewater

Parameter	Concentration (mg/L)
COD	37,060-50,700
BOD	15,600-22,100
TKN	45.4-71.68
MLSS	2,040-2,820
MLVSS	2,005-2,800
Total PO_4	222-665
Total PO_4-P	72.4-216.7

3.4.2.BMP Experiments

In order to observe the anaerobic treatability and the effect of nutrient supplementation on the anaerobic treatment of malt whisky wastewater, BMP experiments were conducted both with and without BM. In the first set only 6000 mg/L of $NaHCO_3$ was delivered to serum bottles as the source of alkalinity and no other nutrients were supplemented. However second set of serum bottles received all the necessary micro and macronutrients by using the BM as described in section 3.1.2. The mixed anaerobic cultures and acetate enriched *Methanosarcina* cultures were used together as seed. The cultures were present in the reactors at a volumetric ratio of 1:1.

3.4.2.1. BMP without Basal Medium

This experiment was conducted to investigate the biodegradability of malt whisky wastewater in the absence of nutrient supplementation. The serum bottles contained 6000 mg/L of $NaHCO_3$ as the source of alkalinity. Experiments were conducted in 110 mL serum bottles with 50 mL effective volume. After seeding and delivering all necessary chemicals, serum bottles were flushed with 25% CO_2 and 75% N_2 gas mixture and incubated in a temperature-controlled room at 35 ± 2^0C. Gas measurements were conducted daily for 30 days. The serum bottles were seeded with anaerobic mixed cultures, which had MLSS and MLVSS concentrations 58±0.7 g/L and 18.6±0.4 g/L, respectively, and acetate enriched *Methanosarcina* cultures which had MLSS and MLVSS concentrations 36±0.11g/L and 12.4±0.15 g/L, respectively, at a volumetric ratio of 1:1. The initial COD concentrations in three different serum bottles were 5.07, 10.140, 15.210 g COD/ L.

3.4.2.2. BMP with Basal Medium

This experiment was conducted to investigate the effect of nutrient supplementation on anaerobic biodegradability of malt whisky wastewater. Experiments were

conducted in 110 mL serum bottles with 50 mL effective volume. In this experiment BM (Section 3.1.2) was used as the nutrient source. The serum bottles were seeded with anaerobic mixed cultures, which had MLSS and MLVSS concentrations 58±0.7 g/L and 18.6±0.4 g/L, respectively, and acetate enriched *Methanosarcina* cultures which had MLSS and MLVSS concentrations 36±0.11 g/L and 12.4±0.15 g/L, respectively, at a volumetric ratio of 1:1. The initial COD concentrations in three different sets of serum bottles were 5.07, 10.140, 15.210 g COD/ L.

After seeding and delivering all necessary chemicals, serum bottles were flushed with 25% CO_2 and 75% N_2 gas mixture with a flow rate of 4L/min for 4 minutes to maintain anaerobic conditions and proper pH and then capped with natural rubber sleeve stoppers. The serum bottles were incubated in a temperature controlled room at 35±2^{0}C. Gas produced was measured daily for 30 days with a water displacement device (Fig 3.2) in each serum bottle.

3.4.3. Continuous Anaerobic Reactor Experiments

3.4.3.1. AF Reactors

This part of the study was performed to determine the anaerobic treatability of malt whisky wastewater in single and staged AF reactors with two different types of support materials. To this purpose two-stage AF reactor system with pumice support material (Figure 3.3 a) and single-stage AF reactor with celite support material (Figure 3.3 b) were operated. Figure 3.3 shows the experimental set-up of the two stage AF with pumice support medium and single stage AF with celite support medium.

Each rector was constructed of cylindrical plexiglass columns with a height and inner diameter of 50 and 2.5 cm, respectively. Total and effective volumes of each reactor of the AF reactor with pumice support media were 245 and 87 mL, respectively. For AF reactor with celite support media total volume was 245 mL and effective volume

was 96 ml. Wastewater was continuously fed to the inlet of the first stage AF reactor. The effluent of the first stage AF with pumice support material was fed to the inlet of the second stage AF reactor. The reactors were operated in a temperature controlled room at 35±2^{0}C.

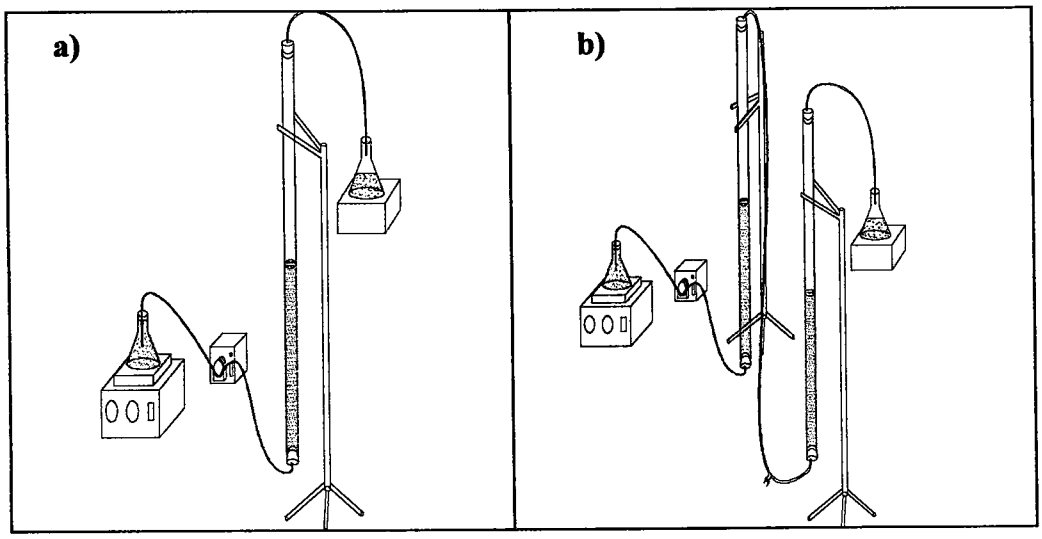

Figure 3.3. Schematic diagrams of the single stage AF with celite support material (a) and the two-stage AF with pumice support material (b) reactor systems.

The reactors were seeded with mixed anaerobic cultures (18.6±0.4 g MLVSS/L) and acetate enriched *Methanosarcina* cultures (12.4±0.15 g MLVSS /L) resulting in a volume ratio of 1:1. The wastewater, which was mixed by a magnetic stirrer, was continuously fed into the inlet of the first stage AF reactor.

In the effluent of reactors, pH, alkalinity (as $CaCO_3$) and VFA, COD, BOD, TKN, total phosphate, phosphorus and MLSS and MLVSS were measured. The sampling frequency for COD, pH, alkalinity and VFA analyses were two or three times a week. The other parameters were measured when the influent COD concentration was increased.

Day to day influent COD concentrations, loading rates and HRTs applied to the systems given in Table 3.3 and Table 3.4.

Table 3.2. Influent COD concentration, loading rate and HRT applied to the single-stage AF with celite support medium system.

Day No	Influent COD conc. (mg/L)	COD Loading rate (kg/m^3d)	HRT(h)
0-15	1000	0.9-3.4	7.0-12.9
15-30	3638	5.9-11.6	7.2-13.2
30-36	3320	8.6-12.6	6.1-9.2
36-39	12060	10.8-14.1	6.7-7.7
39-53	11087	20.9-58.3	3.4-8.7

Table 3.3. Influent COD concentration, loading rate and HRT applied to the first-stage AF with pumice support medium system

Day No	Influent COD conc. (mg/L)	COD Loading rate (kg/m^3d)	HRT(h)
0-15	1000	1.0-8.0	6.3-11.2
15-30	3638	0.8-12.0	7.3-32.3
30-36	3320	4.4-15.3	5.2-18.3
36-39	12060	4.8-36.0	8-16.7
39-53	11087	18.1-56.1	4.8-14.7

The influent COD concentrations seen in Tables 3.3 and 3.4 were obtained by diluting the malt whisky wastewater (Table 3.1) by tap water.

3.4.3.2. UASB Reactors

This set of experiments were conducted to determine the anaerobic treatability of malt whisky wastewater in single and two-staged continuous anaerobic reactors. For this, two stage upflow anaerobic sludge blanket (UASB) reactors with anaerobic

granular sludge were operated. Figure 3.4 shows the experimental set-up of the two stage UASB reactor system used in this study.

Day to day influent COD concentrations, loading rates and HRTs applied to the system are given in Table 3.2.

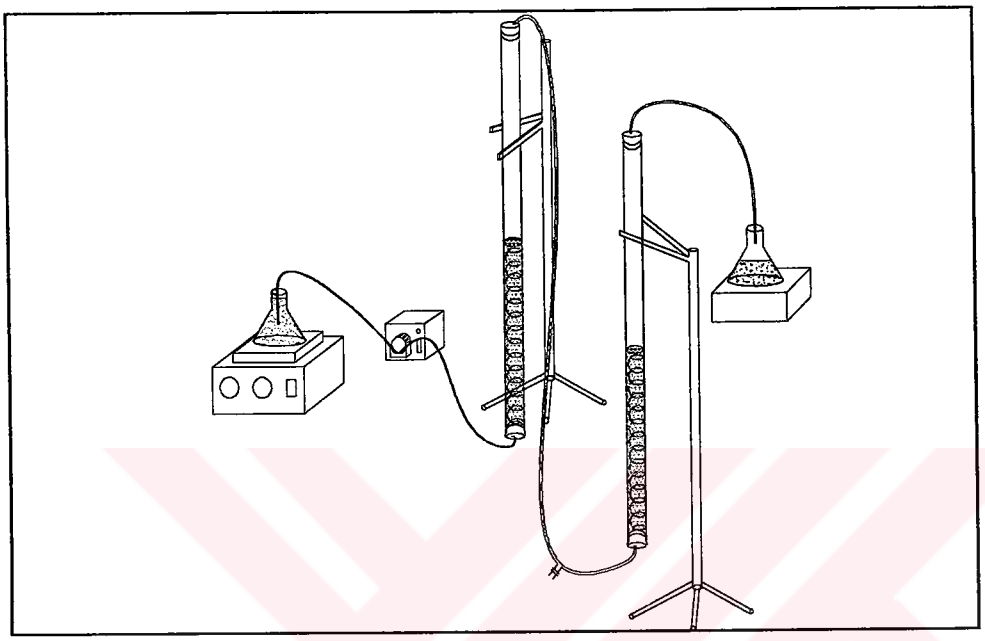

Figure 3.4 Schematic diagram of the two stage UASB reactor system

Each rector was constructed of cylindrical plexiglass columns with a height and inner diameter of 50 and 2.5 cm, respectively. The volumes of each reactor were 245 mL and effective volumes were 113 mL.Wastewater was continuously fed to the the inlet of the first UASB reactor.The effluent of the first stage UASB was fed to the inlet of the second stage. Spiral shaped wires with a length of 60 cm and a cross section of 1.5 mm^2 were placed into the reactors to avoid floating of the granular sludge. The reactors were operated in a temperature controlled room at 35 ± 2^0C.

The reactors were seeded with anaerobic granular sludge resulting in a sludge volume of 113 mL in each stage. Biomass inventory in each reactor was

approximately 6.1±0.12 g. The wastewater, which was mixed by a magnetic stirrer, was continuously fed into the inlet of the first stage UASB reactor.

In the effluent of reactors, pH, alkalinity (as $CaCO_3$) and VFA, COD, BOD, TKN, total phosphate, phosphorus, MLSS and MLVSS were measured.

The sampling frequency for COD, pH, alkalinity and VFA analyses two or three times a week. The other parameters were measured when the influent COD concentration was increased/

Table 3.4. Influent COD concentration, loading rate and HRT applied to the first-stage UASB system.

Day No	Influent COD conc. (mg/L)	COD Loading rate (kg/m^3d)	HRT(h)	Dilution With
0-9	1000	0.6-4.2	5.7-23.8	Tap water
9-24.0	3638	3.3-14.7	6.0-26.6	Tap water
24-30	3320	4.3-15.3	5.2-18.6	Tap water
30-33	12060	7.7-44.8	6.5-10.4	Tap water
33-46	11087	12.2-58.1	5.5-21.7	Tap water
46-73	20920	7.7-50.0	10.0-65.3	Tap water
73-89	33866	4.6-55.5	9.0-49.3	BM

The influent COD concentrations were obtained by diluting the original malt whisky wastewater (Table 3.1) either by tap water or BM as seen in Table 3.4. BM was used in the dilution as a nutrient support because of deterioration in the granular culture in the first stage of the UASB reactor system observed on Day 71.

3.4.4. Batch Aerobic Reactor Experiments

This part of the study was conducted to determine the aerobic treatibility of malt whisky wastewater after anaerobic treatment processes as a post treatment to satisfy

discharge standards. The experiments were conducted in 500 mL volumetric flasks. Sample volumes were 100 mL. The batch anaerobic reactors were continuously shaked for 15 days in a shaker with a 380 rpm and at 25° C.

Aerobic cultures were obtained from the semi-continuous reactor operated at 8 days SRT (base-line reactor). Initial aerobic cultures were obtained from the aeration tanks of the activated sludge units of the Ankara Municipal Wastewater Treatment Plant, with a sludge age and organic loading of 2.8 days and 165000 kgBOD_5/day, respectively. In batch aerobic reactor experiments, the effluent of anaerobic reactors were used as the feed BOD and COD values were measured at Days 0, 5, 10,15.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the BMP, continuous anaerobic reactor experiments and batch aerobic reactor experiments are presented and discussed.

4.1. BMP Experiments

BMP experiments were conducted to investigate the anaerobic treatability of malt whisky wastewater both in the presence and absence (only alkalinity addition) of nutrient supplementation.

In BMP experiments, mixed anaerobic cultures and acetate enriched *Methanosarcina* cultures were used together as seed. The serum bottles were incubated in a temperature controlled room at 35±2 ^{0}C. Gas produced was measured daily for 30 days by a water displacement device (Fig 3.2) in each serum bottle. BMP experiments were performed for three different COD concentrations, namely 5070, 10140, 15210 mg/L. Serum bottles for three COD concentrations were run as duplicates. Control serum bottles were also run in all experiments to determine the background gas production. The average gas productions observed in each serum bottles are presented in Figure 4.1.

For the batch anaerobic reactors (serum bottles) containing no nutrients but only $NaHCO_3$, gas production of control sample was 12.75 mL as seen from Figure 4.1. (a). For the COD concentrations of 5070, 10140, 15210 mg/L net total gas

productions at the end of 29 days were observed as 98.7, 220.8 and 260.5 mL, respectively (Figure 4.1 a).

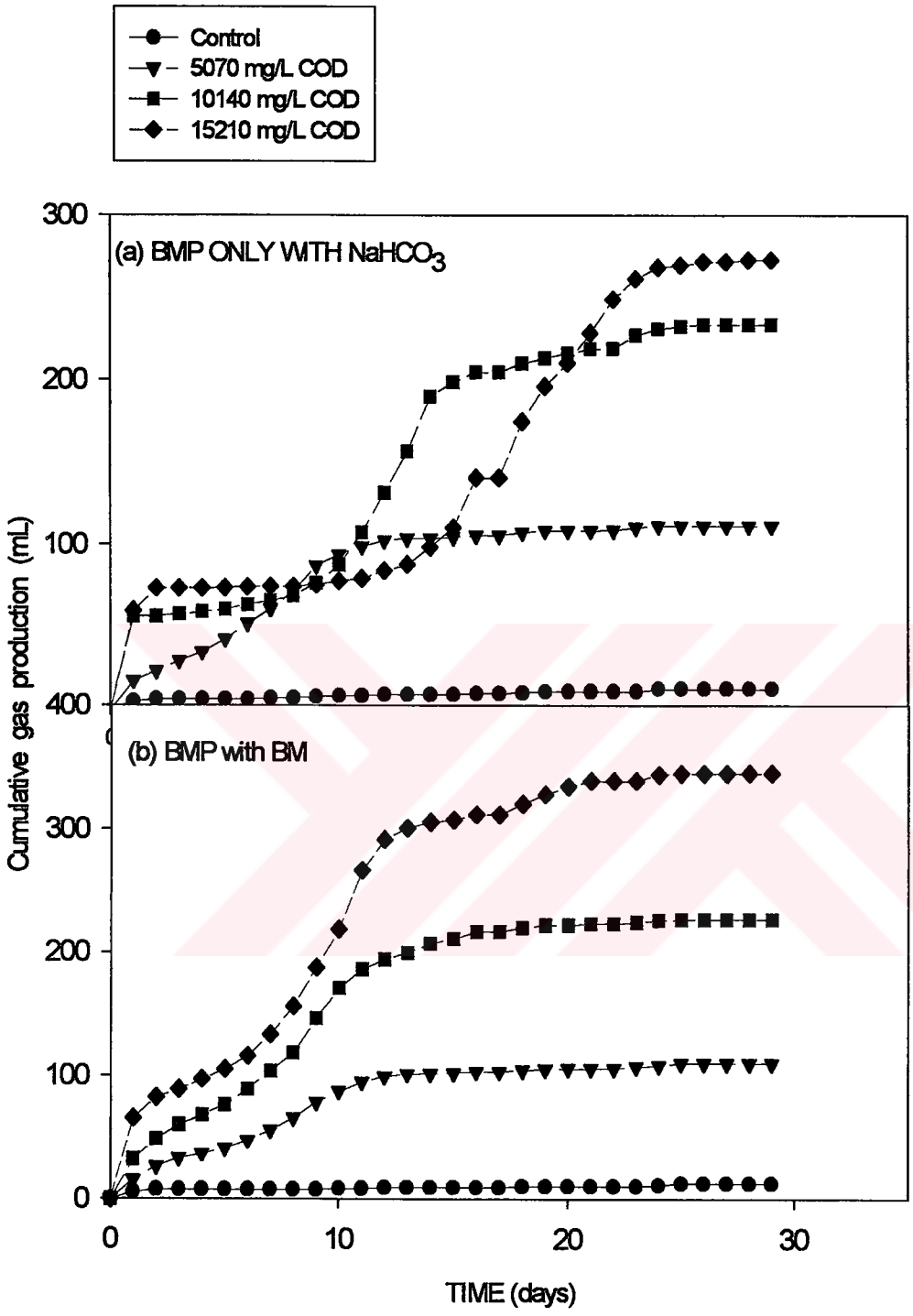

Figure 4.1. BMP experiment results for instead of BM with only $NaHCO_3$ set-up (a), and for with only BM set-up (b)

For the nutrient supplemented set of serum bottles the total gas production of control sample was 12.6 mL. The net total gas production for the COD concentrations of 5070, 10140, 15210 mg/L were observed as 98, 214.1 and 332.6 mL, respectively. For the COD concentrations of 5070 and 10140 mg/L acclimation period of 10 days was observed for the serum bottles with no nutrient supplementation as seen in Figure 4.1 a. However, the acclimation period needed was about 15 days for the initial COD concentration of 15210 mg/L. After the acclimation period, the gas production rates increased significantly (Figure 4.1 a). The delay in gas production observed for the no-nutrient supplemented set of serum bottles (Figure 4.1a) was not observed for the nutrient supplemented set (Figure 4.1 b). So, the delay in gas production (or acclimation phase) for the first set of serum bottles was thought to be due to lack of nutrients in the reactors.

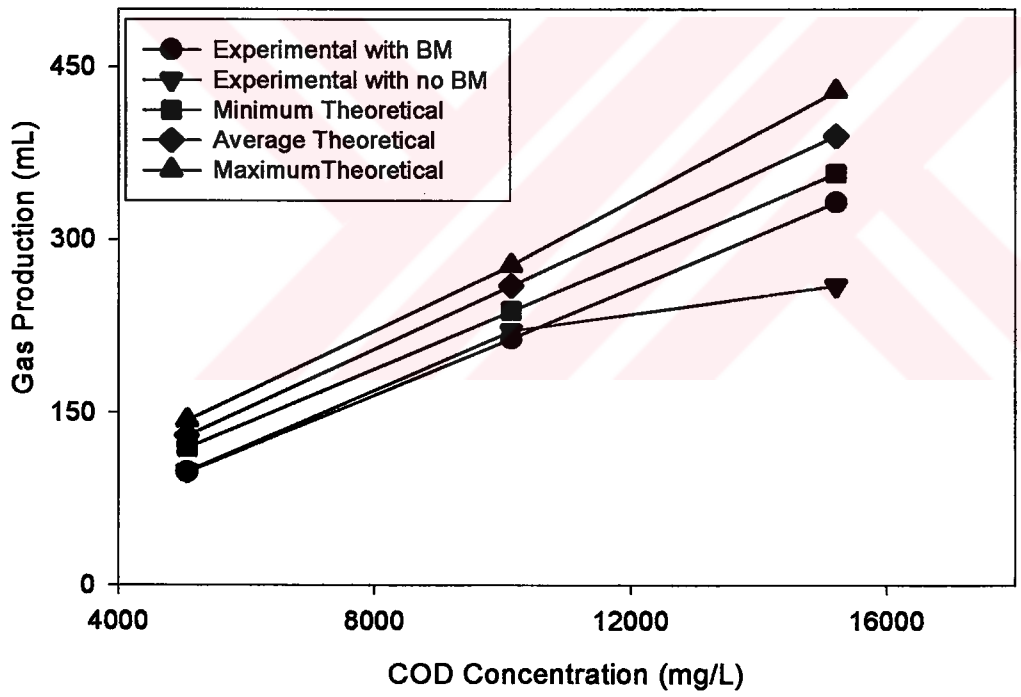

Figure 4.2. Comparison of the theoretical and experimental gas production for biochemical methane potential experiments

The theoretical gas production values were calculated and compared with experimental (observed) values (Figure 4.2). The amount of gas produced by the cultures with BM or without BM supplementation was closed to but not in the range of theoretical gas production for the same COD concentrations. This lower gas production observed for the same COD concentrations by the cultures with BM or without BM can be explained by the refractory COD content of the wastewater. Furthermore, the portion of the substrates, which was used for metabolic activities such as energy, and growth of biomass might have added this difference. Finally the sensitivity of the method for the CH_4 content determination (Section 3.3) might be a factor. The most reliable method in CH_4 content determination is gas chromatograph (GC) method. However, in this study KOH absorption method that was not as sensitive as GC method was used. Addition to sensitivity of CH_4 content determination method some experimental errors caused lower gas calculations in the study.

For the initial COD concentrations of 5.07 and 10.1 g/L the total gas productions for the nutrient supplemented (98 and 214.1 mL) and not supplemented (98.7 and 220.8 mL) serum bottles were almost the same. However, the net total gas production for the COD concentration of 15.2 g COD/L was 332.6 mL for nutrient supplemented set while it was 260.5 mL for the alkalinity only set. The difference between total gas productions could again be because of nutrient deficiency.

The results of BMP experiments were indicated that Ankara Tekel Whisky Factory wastewater could be treated anaerobically.

4.2. Continuous Anaerobic Reactor Experiments

This part of the study was carried out to determine the biological treatability of the Ankara Tekel Whisky Factory wastewater in single and two-stage continuous anaerobic reactors. To this purpose, single-stage anaerobic filter with celite support material, two-stage anaerobic filter with pumice support material and two-stage

UASB reactors were used. It was aimed to investigate the effect of reactor configuration and support material. It was also aimed to determine the maximum loading rate achievable with the minimum HRT possible for these reactors. In this section, the results of each reactor experiments are presented and discussed.

4.2.1. Single-Stage AF (with Celite) Reactor Experiments

The operational conditions such as organic loading rates, HRTs applied to the AF (celite) reactor system and the influent-effluent pH, influent-effluent COD concentrations, COD removal efficiencies, effluent MLSS and MLVSS concentrations and alkalinity values (as $CaCO_3$) are presented in Figure 4.3 a, b, c, d, e, f and g, respectively. VFA (as HAc), influent-effluent BOD concentrations, BOD removal efficiencies, influent-effluent TKN concentrations, Total PO_4 and Total PO_4-P concentrations of AF (celite) reactor system are presented in Figure 4.4 a, b, c, d, e and f.

Along the entire operation of the reactor, the HRT was reduced gradually from about 10 to 5 hours (Figure 4.3 a). On Days 37 and 53, uncontrolled increases in the HRT were encountered due to operational problems.

Organic loading rate values of AF (celite) reactor system increased from 1 kg/m^3day day to 58.3 kg/m^3day in a stepwise manner (Figure 4.3.b). In this period, the influent COD concentrations were increased from 1000 mg/L to 11087 mg/L (Figure 4.3.d). Gradually increased influent COD concentrations (1000, 3638, 3320, 12060, 11087 mg/L) were applied to the system. On Day 35, HRT and OLR were 8.2 h and 9.7kg/m^3d, respectively.

On Day 35, the influent and effluent COD and BOD concentrations were 3320 and 1199 mg/L (Figure 4.3 d), 1397.5 and 520 mg/L (Figure 4.4.b), respectively. Removal efficiencies were 64% and 63%.

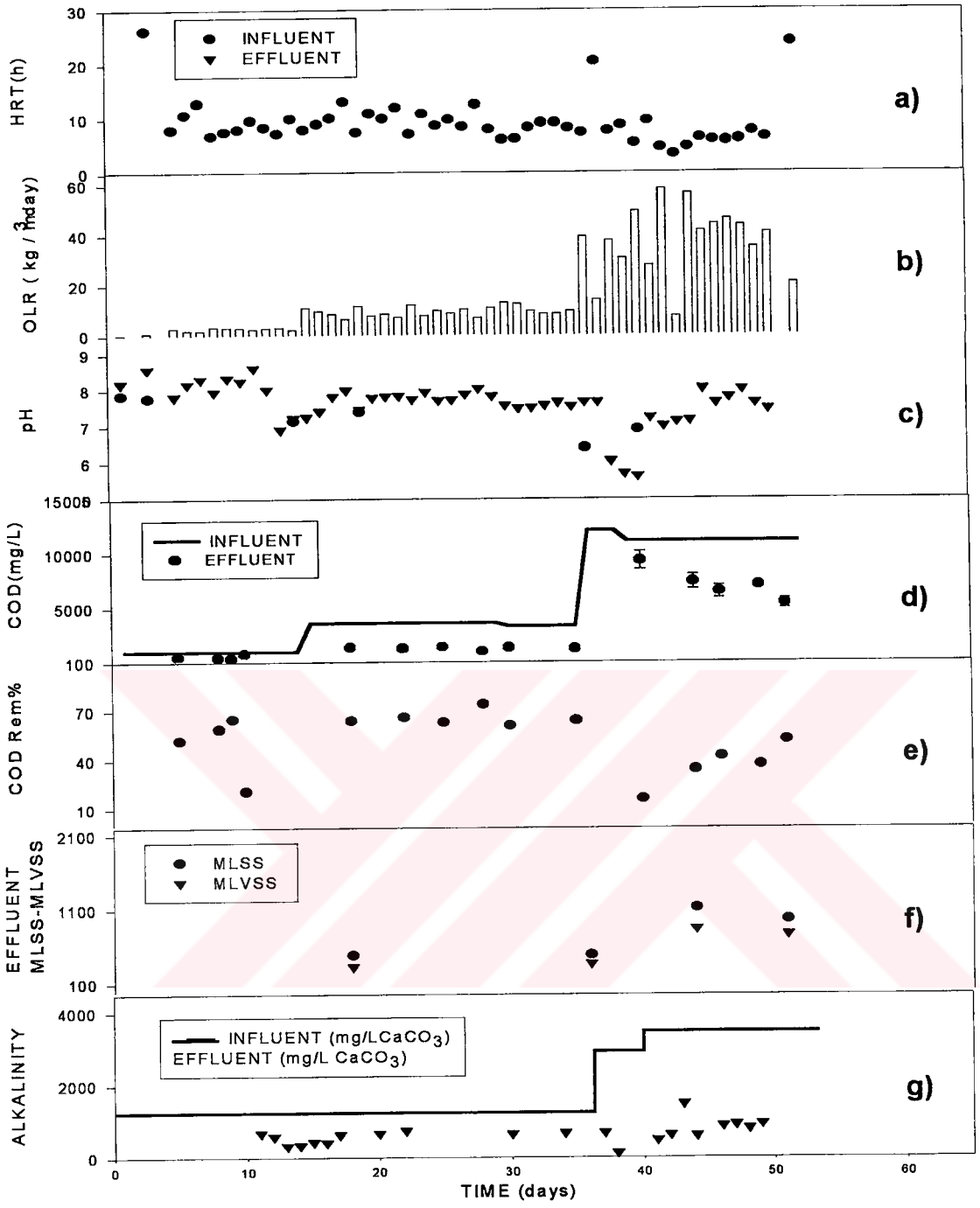

Figure 4.3. The operational conditions and results of the anaerobic filter with celite support media; a) HRT b) Organic Loading Rate c) pH d) COD concentrations e) COD removal efficiencies f) MLSS and MLVSS concentrations g) Alkalinity (as $CaCO_3$)

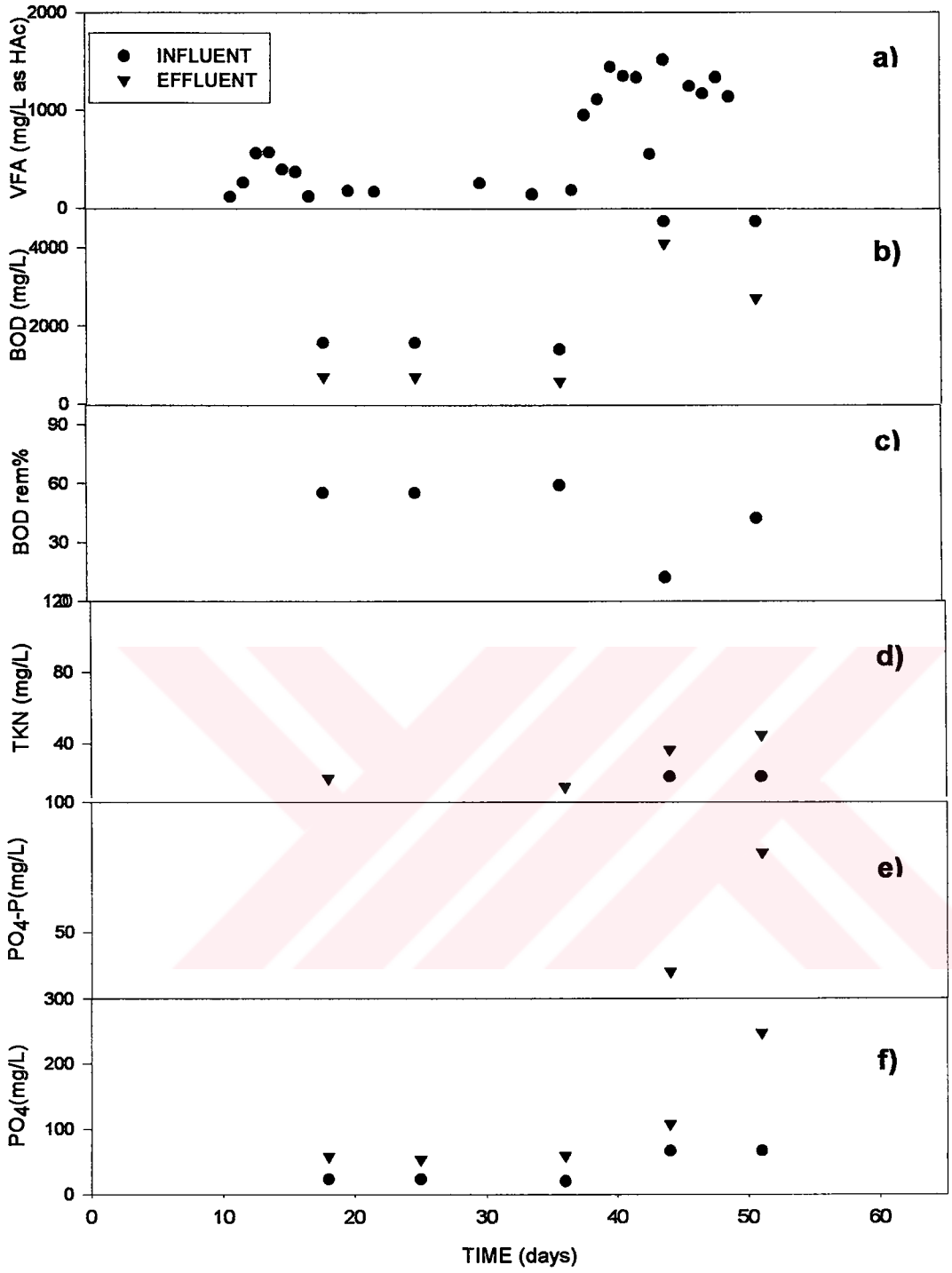

Figure 4.4. The operational conditions and results of the anaerobic filter with celite support media; a) VFA (as HAc) b) BOD concentrations c) BOD removal efficiencies d) TKN concentrations e) PO_4-P concentrations f) PO_4 concentrations.

As can be seen from Figure 4.3.d, the influent COD concentration was below 11087 mg/L until the Day 36. When COD concentration of 11087 mg/L was maintained in the influent on Day 38 (Figure 4.3.d), the effluent quality of the system started to deteriorate. On Day 40, COD removal efficiency decreased from 64% to 16%. On Day 51 COD removal efficiency was increased up to 52%. However, on Day 53 the reactor was stopped because of biomass washout.

On Day 44, MLSS and MLVSS concentrations increased up to 1117 and 833 mg/L (Figure 4.3 f), respectively. Higher loading rates and higher gas production rates caused biomass washout in the AF celite reactor system. This should be because of lack of biomass attachment on to the support medium. As discussed previously in Section 2.2.2, for anaerobic filter reactors startup period is so important to attach the biomass on to the support media. In this study, startup period was not carried out for anaerobic filter reactors to reveal the effect of higher loading rates without any startup period. Biomass washout observed during the operation of anaerobic filter reactors confirmed that startup period is so important and should be applied to resist higher loading rates.

Due to higher MLSS and MLVSS concentrations in the effluent, effluent TKN and phosphorus concentrations are higher than influent concentrations. On Day 36, in the influent and effluent TKN and phosphorus concentrations of the AF (celite) reactor were 6.4 and 17.5 mg/L and 19.9 and 59.1 mg/L, respectively.

The influent pH values of the system were between 6.4-7.86 and the effluent pH values were between 5.61-8.63. Along the operation period uncontrolled increases were occurred in VFA concentrations because of reduced metabolic activity of methanogenic culture. For instance, the VFA concentration (as HAc) was increased from 181.2 mg/L to 1133 mg/L (as HAc) on Day 49. This observation indicated that the system was not operating properly. Up to 11087 mg/L influent COD concentration alkalinity added to the influent was 1190 mg/L (as $CaCO_3$) however,

when the influent COD concentration increased up to 11087 mg/L alkalinity concentration was not sufficient and was increased up to 3571 mg/L (as $CaCO_3$).

The concentrations between 1000-3638 mg/L had no negative effect on AF (celite) reactor system. As a result of these AF (celite) reactor experiments, without applying any startup period up to 11087 mg/L influent COD concentration the system operated effectively. When 11087 mg/L COD concentration was applied because of higher loading and gas production rates biomass washout was observed in the reactor. OLR and HRT were increased up to 12.6 kg/ m^3day 6.3 h, respectively. The COD removal efficiency increased up to 74 % in this operation period.

4.2.2. Two Stage Anaerobic Filter (with Pumice) Reactor Experiments

The operational conditions such as organic loading rates, HRTs applied to the first stage AF (pumice) reactor system, influent-effluent COD concentrations, COD removal efficiencies, effluent MLSS and MLVSS concentrations, influent-effluent BOD concentrations, BOD removal efficiencies are presented in Figure 4.5 a, b, c, d, e, f and g, respectively.

In Figure 4.6 a, b, c, d, e and f organic loading rates, HRTs applied to the second stage AF (pumice) reactor system and influent-effluent COD concentrations, COD removal efficiencies, influent-effluent BOD concentrations, BOD removal efficiencies are presented, respectively. VFA (as HAc), effluent MLSS and MLVSS concentrations of the second stage AF (pumice) reactor system are presented in Figure 4.7 a and b.

Figure 4.5 a and 4.6 a depict that the HRT was reduced gradually from about 10 to 5 hours. On Days 22 and 28, uncontrolled increases in the HRT were encountered due to operational problems.

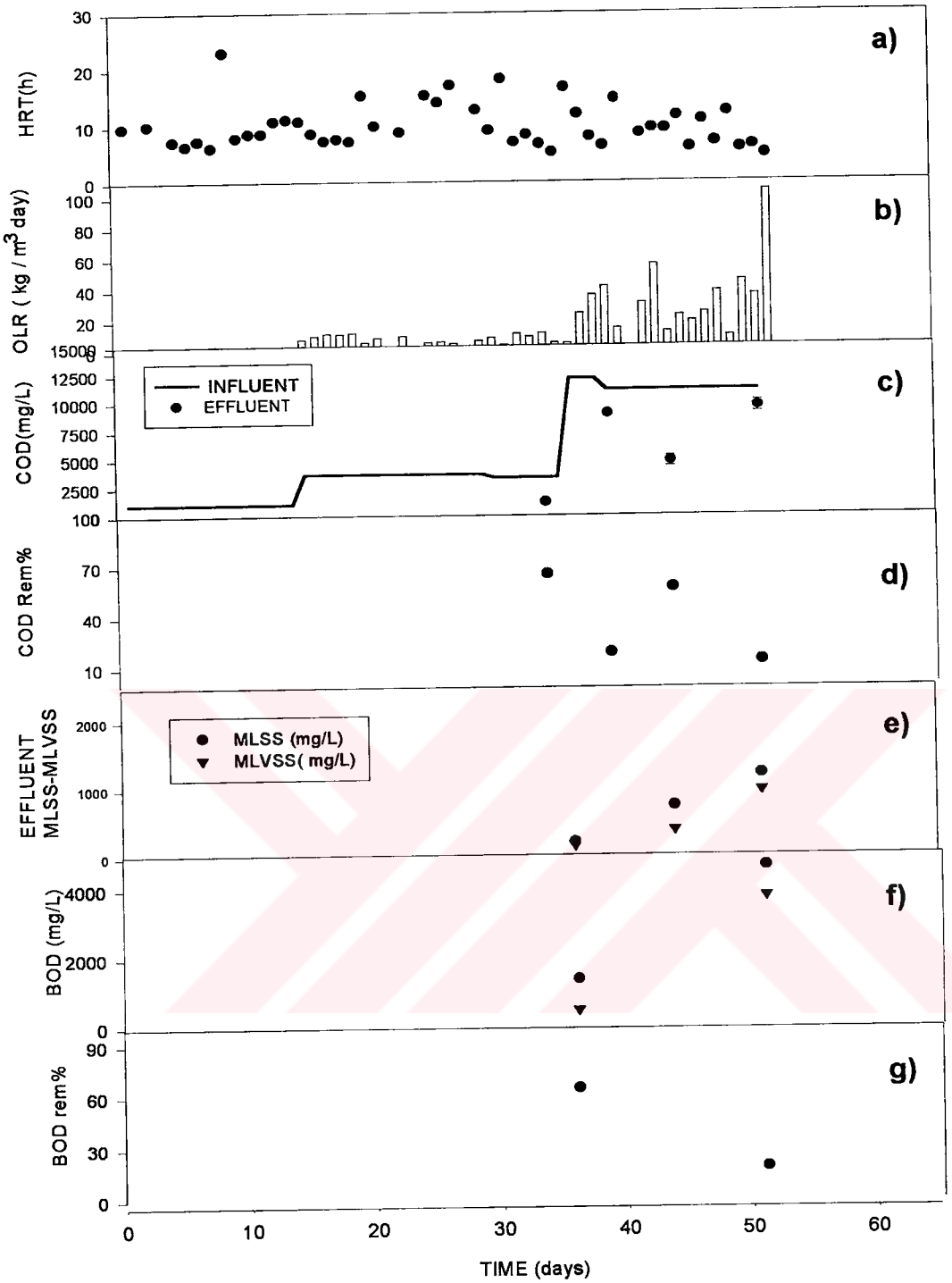

Figure 4.5. The operational conditions and results of the first stage anaerobic filter with pumice support media; a) HRT b) Organic Loading Rate c) COD concentrations d) COD removal efficiencies e) effluent MLSS-MLVSS concentrations f) BOD concentrations g) BOD removal efficiencies

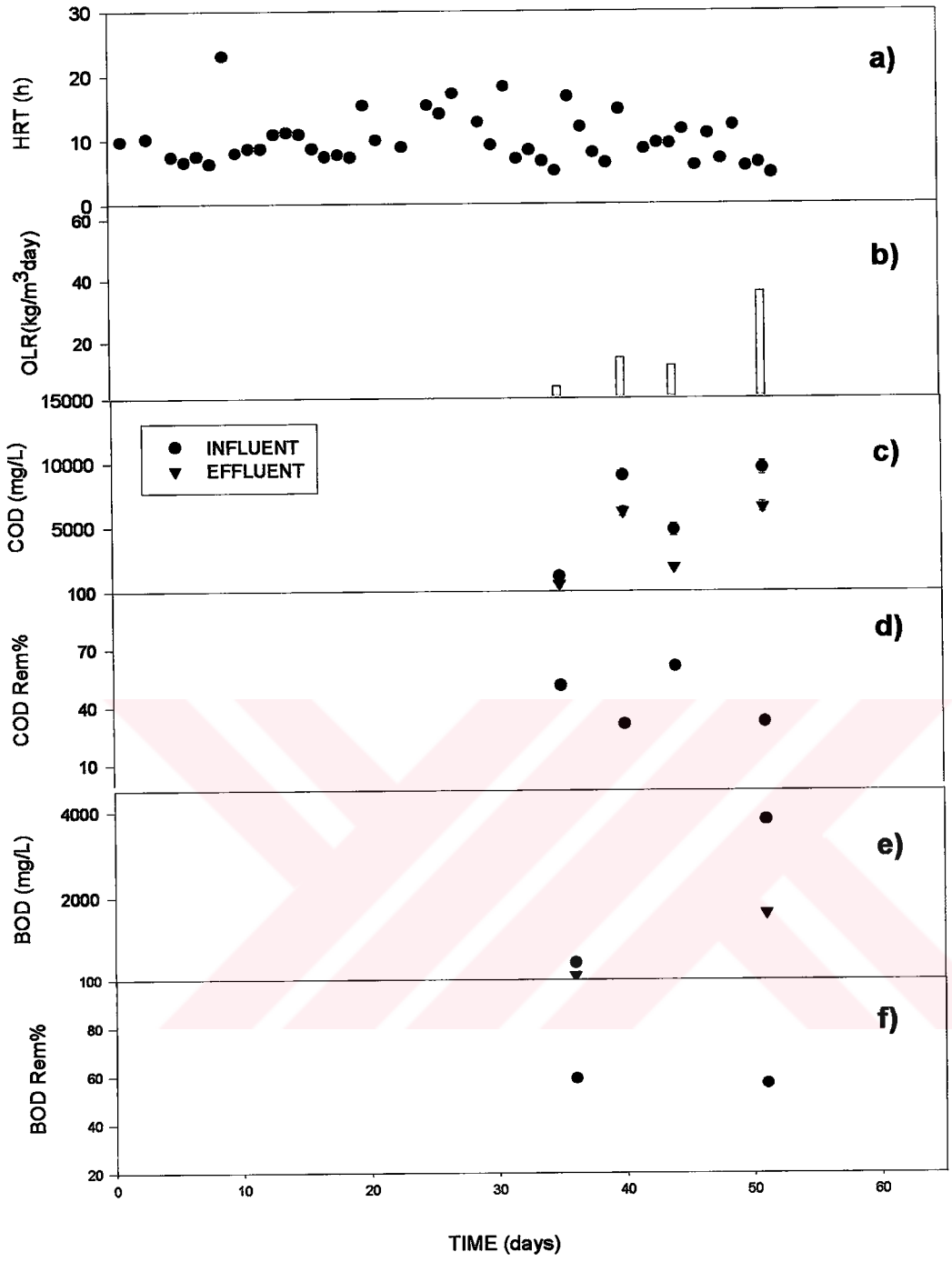

Figure 4.6. The operational conditions and results of the second stage anaerobic filter with pumice support media; a) HRT b) Organic Loading Rate c) COD concentrations d) COD removal efficiencies e) BOD concentrations f) BOD removal efficiencies

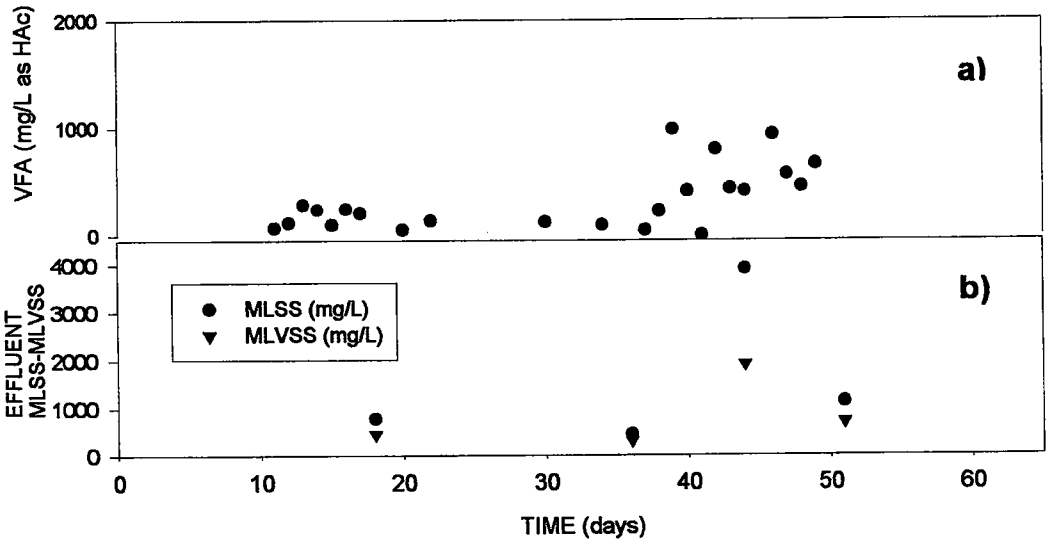

Figure 4.7. The operational conditions and results of the second stage anaerobic filter with pumice support media; a) VFA (as HAc) b) effluent MLSS and MLVSS concentrations

Organic loading rates of the two-stage AF (pumice) reactor system increased from 1 kg/m^3 day to 56.1 kg/m^{3}day in a stepwise manner (Figure 4.5.b and 4.6 b). In this period, the influent COD concentrations were increased from 1000 mg/L to 11087 mg/L for the first stage of the AF (pumice) reactor system (Figure 4.5.c). Gradually influent COD concentrations (1000, 3638, 3320, 12060, 11087 mg/L) were applied to the first stage of the AF (pumice) reactor system.

On Day 36, HRT and OLR of the first stage of the system were 16.7 h and 4.9 kg/m^{3}d, respectively. COD and BOD removal rates were 65% and 65% in the first stage and 51% and 59% in the second stage for the influent concentrations of 3.32 gCOD /L and 1.17 gCOD /L, respectively.

On Day 51, HRT and OLR of the first stage of the system were 6.4 h and 41.8 kg/m^{3}d, respectively. COD and BOD removal rates were 14% and 19% in the first stage and 32% and 57% in the second stage for the influent concentrations of

11.087 gCOD /L and 9.5 gCOD /L, respectively. COD removal efficiencies declined because of higher loading rates and higher gas production, which resulted in biomass washout as in the case of the single stage AF (celite) reactor.

MLSS and MLVSS concentrations increased during the operation time. On Day 44, the effluent MLSS and MLVSS concentrations in the first stage AF reactors were 1220 980 mg/L, respectively (Figure 4.5 e). On Day 52, the effluent MLSS and MLVSS concentrations in the second stage AF reactors were 3900 and 1947 mg/L, respectively (Figure 4.7 b). Higher loading rates and higher gas production rates cause biomass washout in the two-stage AF (pumice) reactor. This was because of lack of biomass attachment on to the support medium. As discussed previously in Section 2.2.2, for AF reactors startup period is so important to attach the biomass on to the support media.

In the first stage of the AF (pumice) reactor system up to 11087 mg/L influent COD concentration alkalinity added to the influent was 1190 mg/L (as $CaCO_3$) but when the influent COD concentration increased up to 11087 mg/L alkalinity concentration was not sufficient and it was increased to 3571 mg/L (as $CaCO_3$). The influent pH values of the system were between 6.4-7.86 and the effluent pH values were between 6.77-9.08. Along the operation period uncontrolled increases observed in VFA concentrations in the second stage of the reactor due to reduction in metabolic activity of the methanogenic culture. On Day 46, VFA concentration increased from 124.7 mg/L to 931.6 mg/L (as HAc) and alkalinity of the system was 1213 mg/L (as CaCO3) in the second stage of the AF (pumice) reactor system. These observations indicated that the system was not operated properly.

Effluent TKN and phosphorus concentrations were found higher than influent TKN and phosphorus concentrations in some measurements; this was probably because of the loss of biomass or increase in MLSS and MLVSS concentrations in the effluent. For instance, on Day 44 influent and effluent TKN concentrations in the first stage of the reactor system were 19.7 and 57.6 mg/L, respectively. On Day 51, influent and

effluent TKN concentrations in the second stage of the reactor system were 21.4 and 55.4 mg/L respectively. On Day 51, influent and effluent phosphorus concentrations in the first and second stage of the AF (pumice) reactor system were 66.4 and 222.61 mg/L and 222.61 and 236.7 mg/L, respectively.

On Day 25, HRT and OLR of the overall system of the two-stage AF(pumice) reactors were 14.1 h and 6.2 kg/m^{3}d, respectively. COD and BOD removal rates were 85% and 86% for the influent concentrations of 3.6 gCOD /L, respectively. On Day 49, HRT and OLR of the overall system of the two-stage AF (pumice) reactors were 12.3 h and 21.7 kg/m^{3}d, respectively. COD and BOD removal rates were 74% for the influent concentrations of 11.087 gCOD /L, respectively.

As a result of AF (pumice) reactor experiments, in the first stage of the AF (pumice) reactor up to 11087 mg/L influent COD concentration the system operated effectively. When 11087 mg/L COD concentration was applied, the system was not operated properly either in the first or in the second stage of AF (pumice) reactor. OLR and HRT were 11.8 kg/m^3 day and 6.7 h, respectively in the first stage of the AF (pumice) reactor. In the second stage of the AF (pumice) reactor up to 4760 mg/L influent COD concentration the system was operated effectively. OLR and HRT were 12.2 kg/m^3 day and 9.4 h, respectively.

4.2.3. Two Stage UASB Reactor Experiments

The operational conditions such as organic loading rates, HRTs applied to the first stage UASB reactor system, influent-effluent COD concentrations, COD removal efficiencies, effluent MLSS and MLVSS concentrations, influent-effluent BOD concentrations, BOD removal efficiencies are presented in Figure 4.8 a, b, c, d, e, f and g, respectively.

In Figure 4.9 a, b, c, d, e and f organic loading rates, HRTs applied to the second stage UASB reactor system and influent-effluent COD concentrations, COD removal

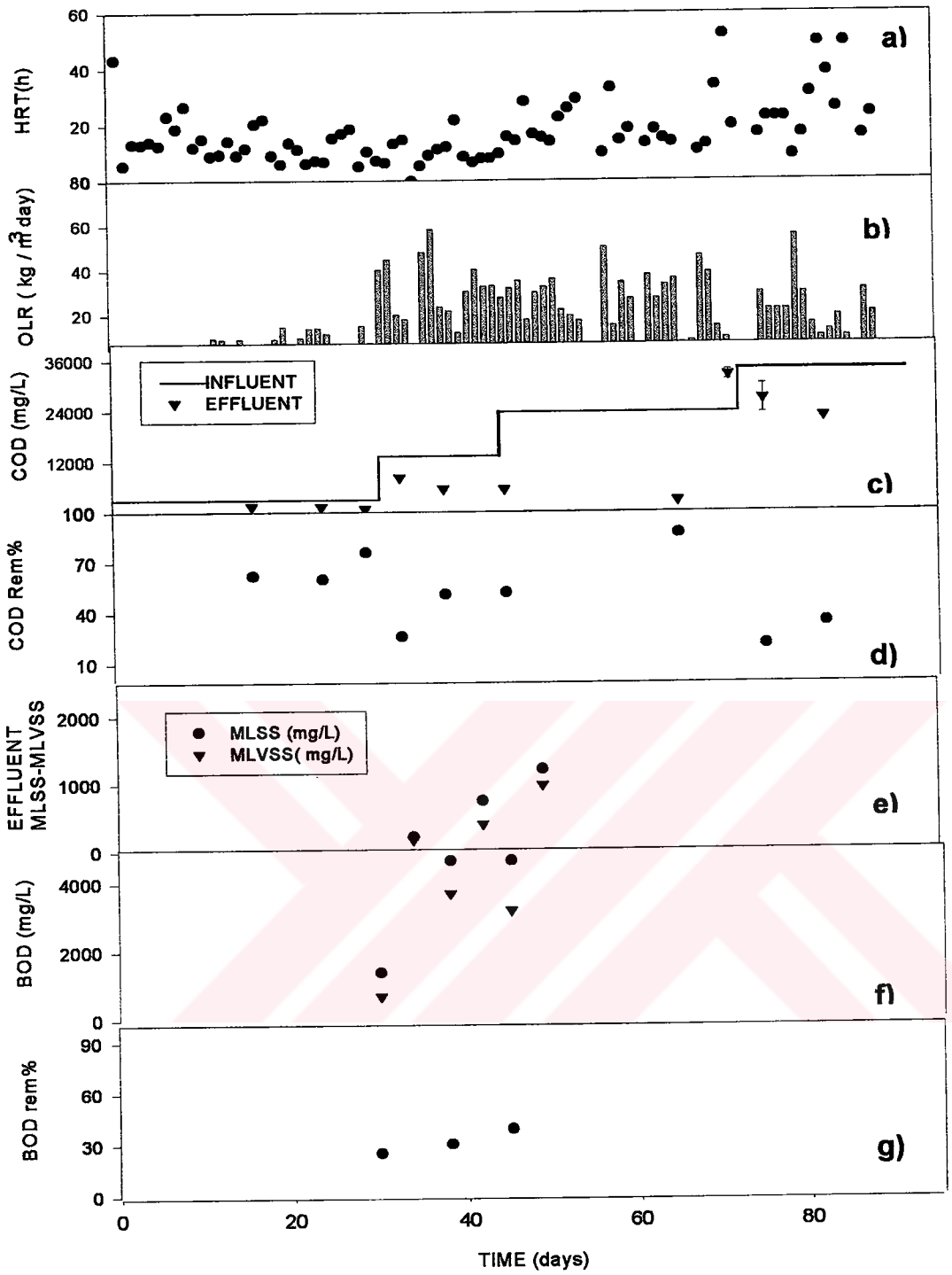

Figure 4.8. The operational conditions and results of the first stage UASB reactor; a) HRT b) Organic Loading Rate c) COD concentrations d) COD removal efficiencies e)effluent MLSS-MLVSS concentrations f)BOD concentrations g) BOD removal efficiencies.

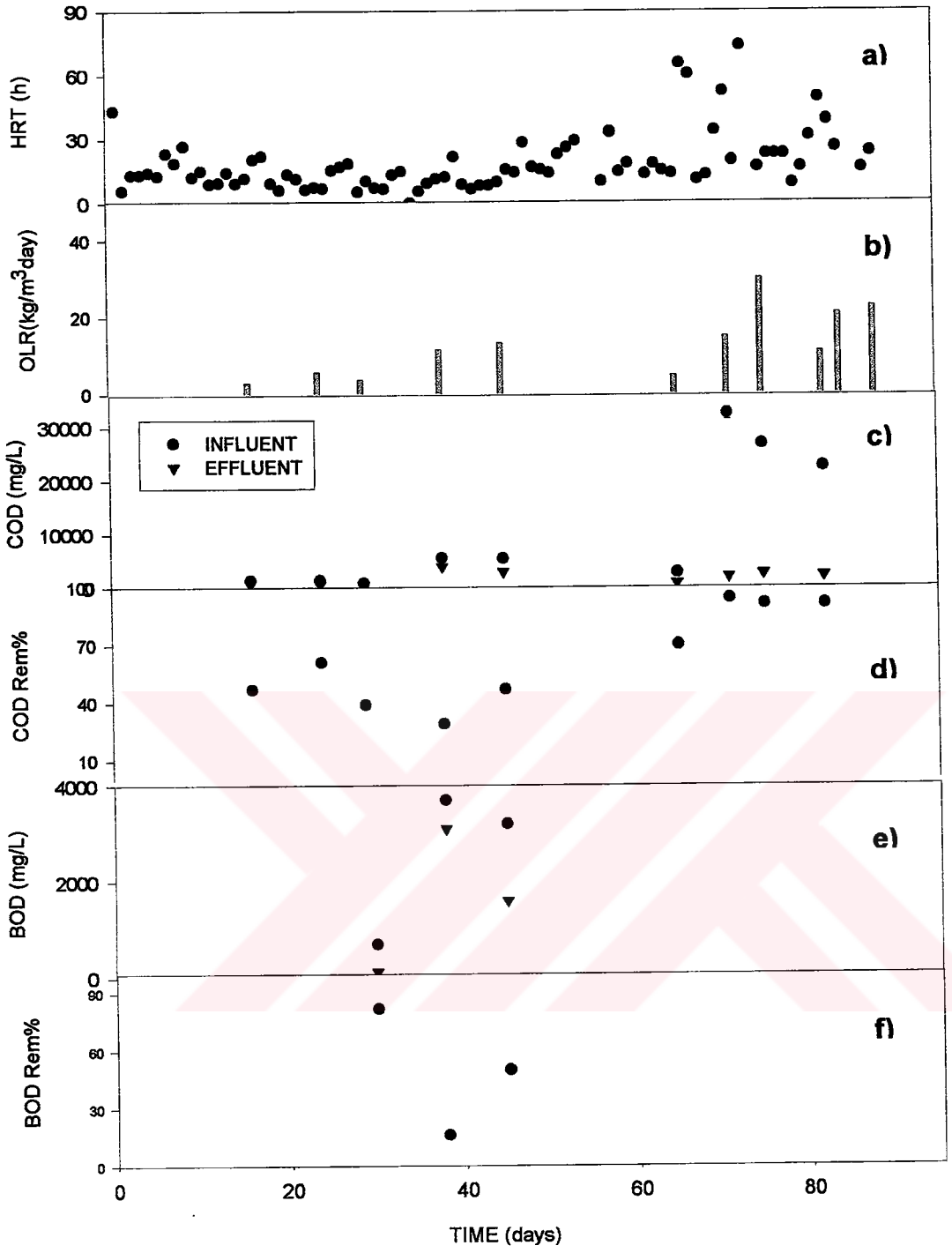

Figure 4.9. The operational conditions and results of the second stage UASB reactor; a) HRT b) Organic Loading Rate c) COD concentrations d) COD removal efficiencies e) BOD concentrations f) BOD removal efficiencies

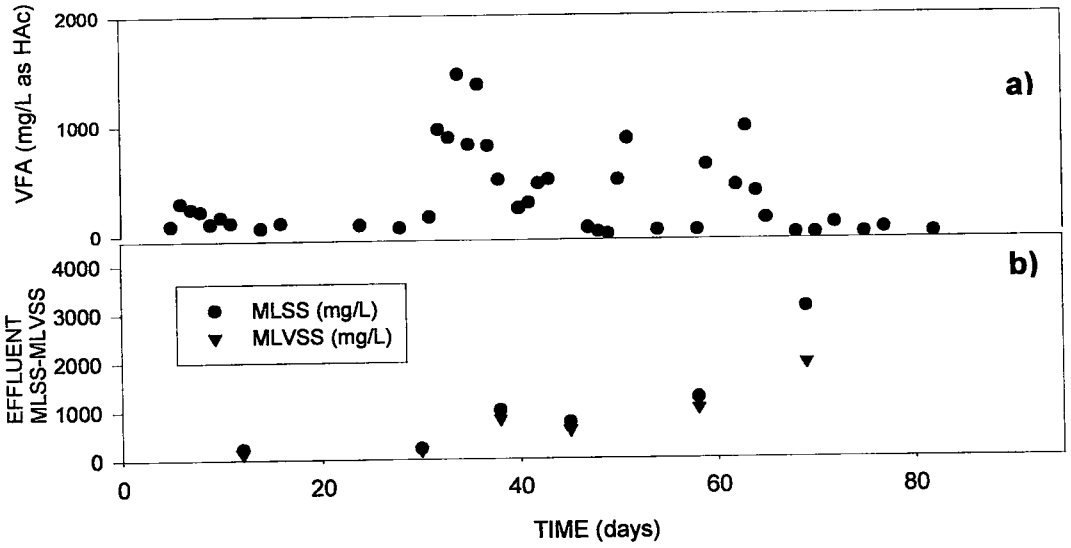

Figure 4.10. The operational conditions and results of the second stage UASB reactor; a) VFA (as HAc) b) effluent MLSS and MLVSS concentrations

efficiencies, influent-effluent BOD concentrations, BOD removal efficiencies are presented, respectively. VFA (as HAc), effluent MLSS and MLVSS concentrations of the second stage UASB reactor system are presented in Figure 4.10 a and b.

During the entire operation of the reactors, the HRT was reduced gradually from about 40 to 5 hours (Figure 4.9 a). On Days 66, 67, 71, 73, 74, 82, and 83, uncontrolled increases in the HRT were encountered due to some operational problems.

Organic loading rate values of two-stage UASB reactor system increased from 0.6 kg/m^3 day to 48.1 kg/m^3day in a stepwise manner (Figure 4.8 b and 4.9 b). In this period, the influent COD concentrations were increased from 1000 mg/L to 33866 mg/L in the first stage of the UASB reactor system (Figure 4.8 c). Different influent COD concentrations (1000, 3638, 3320, 12060, 11087, 20920, 33866 mg/L) were applied to the first stage of the system.

On Day 29, HRT and OLR of the first and second stage of the UASB reactor system were 5.2 h and 15.3 kg/m^{3}day and 5.2 h and 3.7 kg/m^{3}day, respectively. COD removal rate of the first stage UASB system was 76% and COD and BOD removal efficiencies of the second stage UASB system were 39% and 82% for the 3320 and 795 mg/L influent COD concentrations, respectively. On Day 45, HRT and OLR of the first and second stage of the UASB reactor system were 9.7 h and 27.5 kg/m^{3}day and 9.7 h and 13.3 kg/m^{3}day, respectively. COD removal efficiency of the first stage UASB system was 52% and COD and BOD removal efficiencies of the second stage UASB system were 47% and 50% for the 11087 and 5363 mg/L influent COD concentrations, respectively.

When the COD concentration of 20920 mg/L was maintained in the influent of the first stage, the effluent quality of the first stage of the two-stage UASB reactor system started to deteriorate. Furthermore, a significant color change was observed (from black to brownish black and brown) which was thought to be due to the reduced metabolic activity thus increased oxidation-reduction potential resulted from the toxic effect of wastewater on granular biomass. The two-stage set-up used is suitable for anaerobic treatment of Malt whisky wastewater, obtaining control of acidogenesis in the first stage and greater stability of methanogenesis in the second stage .

Higher loading rates and higher gas production rates cause an increase in the MLSS and MLVSS concentration of the reactor effluent. Because of biomass in the effluent, effluent COD concentration was higher than the influent COD concentration in the first stage of the reactor. These observations indicated that the first stage of the reactor was not operating properly. However, in the second stage no problem was observed. To this purpose, the influent COD concentration was increased up to 33866 mg/L in the first stage of the UASB reactor system. The previous dilutions of wastewater applied to the system were prepared with tap water. However, for the 33866 mg/L influent COD concentration in the first stage of the UASB reactor

system dilution was prepared with BM as a nutrient support to the system due to deterioration of the granular culture in the first stage.

In the second stage of the reactor no negative effect observed due to the increase in the influent COD concentration of the first stage of the UASB reactor up to 33866 mg/L. On Day 65, HRT and OLR of the first and second stage of the UASB reactor system were 13.8 h and 36.3 kg/m^{3}day and 13.8 h and 4.7 kg/m^{3}day, respectively. COD removal efficiencies of the first stage and second stage of the UASB system were 87% and 70% for the 20920 and 2693 mg/L influent COD concentrations, respectively. On Day 82, HRT and OLR of the first and second stage of the UASB reactor system were 49.3 h and 10.2 kg/m^{3}day and 49.3 h and 11 kg/m^{3}day, respectively. COD removal efficiencies of the first stage and second stage of the UASB system were 34% and 91% for the 33866 and 22500 mg/L influent COD concentrations, respectively. The operation of the system was stopped on Day 89, because of lack of wastewater. The Ankara Tekel Factory had a renovation and the operation of the factory stopped.

The influent pH values of the system were between 6.2-7.86 and the effluent pH values were between 5.25-9.38. As same as in the filter reactors, up to 11087 mg/L influent COD concentration in the first stage of the UASB system alkalinity added to the influent was 1190 mg/L (as $CaCO_3$) but when the influent COD concentration increased up to 11087 mg/L alkalinity concentration was not sufficient and it is increased up to 3571 mg/L (as $CaCO_3$). During the operation period instead of some operational problems no problem was observed in the alkalinity and VFA concentrations in the second stage of the system.

For the overall system of the two-stage UASB reactors, on Day 54, HRT and OLR of the system were 29.2 h and 17.2 kg/m^{3}d, respectively. COD and BOD removal rates were 92% and 98% for the influent concentrations of 20.92 gCOD /L, respectively. On Day 84, HRT and OLR of the system were 25.8 h and 19.4 kg/m^{3}d, respectively.

COD and BOD removal rates were 96% and 99% for the influent concentrations of 33.86 gCOD /L, respectively.

Effluent TKN and phosphorus concentrations were higher than influent TKN and phosphorus concentrations. High TKN and P values observed in the effluents were evidently due to excessive column bleed at high organic loading rates. On Day 30 influent and effluent TKN and phosphorus concentrations in the first stage of the UASB reactor system were 6.4 and 25.3 mg/L and 19.9 and 49.8 mg/L and in the second stage of the UASB reactor system were 25.3 and 61.4 mg/L and 49.8 and 72.3 mg/L, respectively.

The study of anaerobic digestion of distillery waste in the two-stage reactor enabled clearly to identify the two main phases of the process- fermentative (acidogenic) and acetogenic/methanogenic phase. The acidogenic reactor performed satisfactory in terms of conversion of initial COD to VFAs. VFAs produced in the first stage were readily used as a substrate in the acetogenic/methanogenic stage (Blonskaja *et.al.*,2001).

Two stage UASB reactor experiments proved that two stage UASB reactor configuration is efficient for Malt whisky wastewater treatment. Up to 20920 mg/L influent COD concentration the first stage of the UASB reactor was operated efficiently. When 20920 mg/L COD concentration was applied the black color of the granular culture of the first stage UASB reactor was changed to brownish color. The granular culture was also deteriorated. This is due to the acidogenic culture dominated in the first stage against methanogenic culture.

4.2.4. Aerobic Batch Experiments

Anaerobic continuous reactor experiment results indicated a good performance in the treatment of whisky wastewater however; the results were not satisfying the

discharge limits. Aerobic batch experiments were conducted to achieve further COD removal.

Four batch reactors used for the experiments of aerobic treatment of whisky wastewater. The reactors were conducted with a 100 mL volume, and shaked continuously at a constant temperature 25^0C. No nutrient supplementation was done due to enough nitrogen and phosphorus concentration in the effluent of the UASB reactors. Operation time of the reactors was 15 days. During the operation time, COD concentrations were measured on the Days 0, 1, 6, 10, 15 and BOD concentrations were measured on the Days 0, 10, 15.

On Day 15, influent and effluent COD concentrations were 1476 mg/L and 649 mg/L, respectively. Also BOD influent and effluent concentrations were 323 mg/L to 90 mg/L, respectively. COD and BOD removal efficiencies were 55% and 70% respectively.

As seen from Table 4.1 in anaerobic treatment COD and BOD removal efficiencies for the influent COD concentrations of 33866 mg/L. were 96 % and 98%, respectively. In aerobic treatment, which is used after anaerobic treatment, COD and BOD removal efficiencies were 55% and70%, respectively. The overall COD and BOD removal of the anaerobic and aerobic treatment were 98% and 99.5%, respectively.

After anaerobic digestion the treated effluent COD and BOD concentrations are not usually suitable for discharge according to our discharge limits. This is because of the starting with high strength wastewater. According to the Su Kirliliği Kontrol Yönetmeliği discharge standards for alcoholic beverages are 200 mg/L for COD and 40 mg/L for BOD parameters.

Aerobic treatment effluent concentrations can provide BOD limits appropriate for the discharge limits but for COD limits overall system could not provide suitable

concentrations. COD and BOD concentrations after aerobic treatment were 641 mg/L and 38 mg/L, respectively.

Table 4.1. Operational Results after Anaerobic Treatment and Aerobic Treatment

Raw Wastewater		After Anaerobic Treatment				After Aerobic Treatment				Overall System	
COD (mg/L)	BOD (mg/L)	COD (mg/L)	Rem %	BOD (mg/L)	Rem %	COD (mg/L)	Rem %	BOD (mg/L)	Rem %	COD Rem%	BOD Rem%
33866	17680	1332	96	250	99	605	55	30	88.06	98.21	99.50
33866	17680	1633.5	95	400	98	635.5	61	45	88.79	98.12	99.50
33866	17680	1461	96	320	98	683.5	53	40	87.55	97.98	99.50
33866	**17680**	**1475.5**	**95.6667**	**323.333**	**98.3333**	**641.333**	**56**	**38.1833**	**88.13**	**98.11**	**99.50**

CHAPTER 5

CONCLUSIONS

The results of the BMP experiments conducted both in the presence and absence (only alkalinity addition) of nutrient supplementation revealed that;

- Malt whisky wastewater with initial COD concentrations of 5070, 10140, 15210 mg COD/L could be treated anaerobically.
- Net total gas production values in nutrient supplemented set of serum bottles were higher than no-nutrient supplemented set of serum bottles, especially in the highest concentrations of 15210 mg/L. The delay in gas production observed for the no-nutrient supplemented set of serum bottles was not observed for the nutrient supplemented set. So, the delay in gas production (or acclimation phase) for the first set of serum bottles was thought to be due to lack of nutrients in the reactors.

The results of the continuous reactor experiments performed in single and staged AF reactors with celite and pumice support materials, respectively revealed that;

- In the AF (celite) reactor, influent COD concentrations were increased from 1000 mg/L to 11087 mg/L. The COD removal efficiency increased up to 74% in this period.
- In the first stage of the AF (pumice) reactor up to 11087 mg/L influent COD concentration the system operated efficiently. When 11087 mg/L

COD concentration was applied, the system did not operate properly either in the first or in the second stage of AF (pumice) reactor. In the second stage of the AF (pumice) reactor, up to 4760 mg/L influent COD concentration the system operated effectively.

- In AF reactors biomass washout was observed, which was probably due to lack of biomass attachment on to support media and due to higher organic loading and gas production rates. However, in 52 days operation time celite support material showed a better performance than pumice support material.

The results of the continuous reactor experiments performed in staged UASB reactors revealed that;

- In the two-stage UASB system, influent COD concentrations were increased from 1000 mg/L to 33866 mg/L. Up to 20920 mg/L influent COD concentration, the first stage of the UASB system operated efficiently. Above this influent COD concentration the first stage was not operated properly. In the second stage no problem was observed even for the highest influent COD concentrations of 33866 mg/L.
- Higher loading rates also affected one-stage UASB reactor adversely but two-stage could overcome this problem.
- Two-stage UASB reactor configuration is efficient for Malt whisky wastewater treatment even at organic loading rates as high as 39 kg/m^3day. This was nearly 8 folds higher than pumice-AF and 4 folds higher than celite-AF.
- High TKN and P values observed in the effluents were evidently due to excessive column bleed at high organic loading rates in all continuous reactor experiments.

The results of the aerobic reactor experiments revealed that;

- In aerobic treatment, which is used after two-stage UASB reactors, COD and BOD removal efficiencies were 55% and 70%, respectively.

For the overall system (anaerobic/aerobic) treatment COD and BOD removal efficiencies were 99.5% and 98.1%, respectively.

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APPENDIX A

PHOTOGRAPHS OF ACETATE ENRICHED *METHANOSARCINA* CULTURES

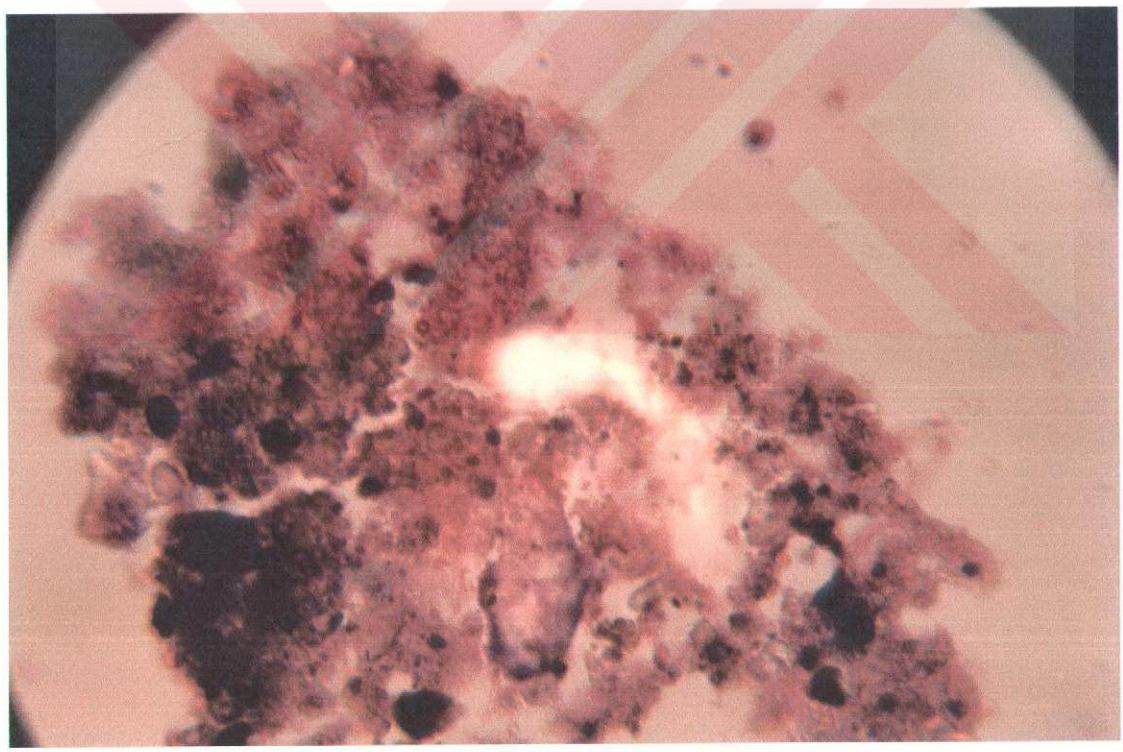

Figure A.1. Photographs of acetate enriched *Methanosarcina* cultures

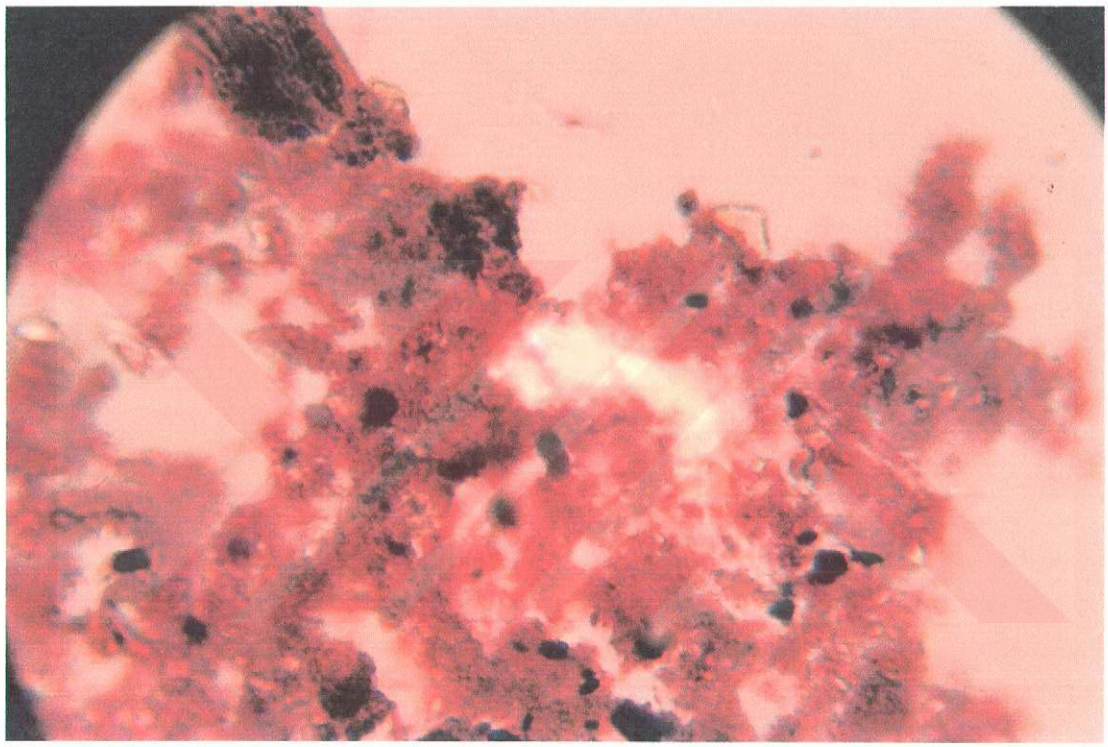

Figure A.2. Photographs of acetate enriched *Methanosarcina* cultures