İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

FORMALDEHYDE AND ISO-PROPANOL TREATMENT IN AN ANAEROBIC FLUIDIZED BED REACTOR AND KINETIC EVALUATIONS

PhD Thesis by

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JUNE 1999

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

HAVASIZ AKIŞKAN YATAKLI BİR REAKTÖRDE FORMALDEHİT VE İSO-PROPANOL ARITIMI VE KİNETİK DEĞERLENDİRMELER

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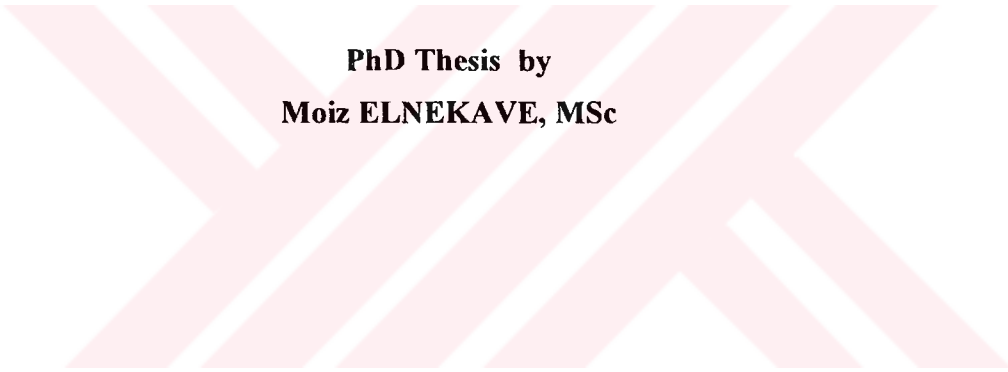
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Department :   **Chemical Engineering**

Programme: **Chemical Engineering**

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PREFACE

Formaldehyde and iso-propanol are the common compounds that can be encountered in many industrial wastewaters in high concentrations. Formaldehyde is definitely a toxic and inhibitory compound, which can react directly with the protein, DNA, and RNA of organisms and damage cells, which may cause death of microorganisms or inhibit their activity in anaerobic processes. High concentrations of iso-propanol are also very dangerous for living organisms, for reclamation systems and for environment. For today, the anaerobic biodegradation of these compounds by using other high-rate anaerobic processes than fluidized bed process seems very difficult because of their own process capabilities. So, in the beginning of the present study, our assumption was that the fluidized bed bioreactor would be useful in treating such kind of wastewaters containing high concentrations of organic pollutants, and toxic and inhibitory compounds.

Therefore, in this present study, the determination of the effectiveness of an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor in treating a high-strength wastewater including high concentrations of iso-propanol and an inhibitory and toxic compound such as formaldehyde, the determination of the process parameters, and the kinetics of anaerobic biodegradation were under investigation. In this manner, obtaining of reliable and useful data in enlightening the use of the biosystem on pilot- and full-scale applications has been aimed.

I would like to express my deepest gratitude and appreciation to Professor Nuran Deveci for her guidance, encouragement and for initially stimulating my interest in this area.

I am grateful to Professor Makram T. Suidan, Head of the Department of Civil and Environmental Engineering of University of Cincinnati, Ohio, USA, for his interest, collaboration and support. Special thanks to Professor Gedahlia Shelef and Dr. Shlomo Kimchie, Faculty of Environmental and Water Resources Engineering of Technion, Haifa, Israel, for their real contribution to my study.

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June, 1999   Moiz ELNEKAVE

CONTENTS

ABBREVIATIONS

AF	: Anaerobic filter
AFBGAC	: Anaerobic Fluidized Bed Granular Activated Carbon
BMP	: Biochemical Methane Potential
BOD	: Biological Oxygen Demand
COD	: Chemical Oxygen Demand
DNA	: Deoxyribonucleic acid
DOC	: Dissolved Organic Carbon
DSFF	: Downflow Stationery Fixed Film
EB	: Expanded Bed
FB	: Fluidized Bed
GAC	: Granular Activated Carbon
HRT	: Hydraulic retention time for liquid
ID	: Inner diameter
MPB	: Methane-producing bacteria
OLR	: Organic Loading Rate
PLFA	: Phospholipid Fatty Acids
RR	: Recycle ratio
RNA	: Ribonucleic acid
RTD	: Retention Time Distributions
SRB	: Sulfate-reducing bacteria
SRT	: Solids Retention Time
T	: Temperature
TOC	: Total Organic Carbon
UASB	: Up flow Anaerobic Sludge Blanket
VFA	: Volatile Fatty Acids
WWTP	: Wastewater Treatment Plant

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General Symbols

A_r	: Arrhenius constant
A_c	: Cross-sectional area of bioreactor
B	: Microbial endogenous decay coefficient
E	: Activation energy
k_a	: Rate constant for Arrhenius equation
k	: Maximum specific substrate utilization rate
K	: Equilibrium constant
K_c	: Competitive inhibition coefficent
K_i	: Substrate inhibition coefficient
K_s	: Half-velocity (saturation) coefficient
L_H	: Hydraulic loading rate
L_O	: Organic loading rate
mM	: millimolar (mmol/L)
M	: Produced methane rate
N	: Nitrogen concentration
Q	: Inlet flow rate
R_G	: Gas constant
R_c	: Recycle biomass flow rate
R	: Reaction rate
R_v	: COD removal capacity (volumetric)
S	: Effluent substrate concentration (COD)
S_{min}	: Minimum substrate concentration
S_0	: Influent substrate concentration (COD)
TS	: Total solids concentration
TSS	: Total suspended solids concentration
V	: Reactor volume
VSS	: Volatile suspended solids concentration
W	: Waste biomass flow rate
X	: Biomass concentration in the reactor
X_e	: Biomass concentration in effluent
X_0	: Biomass concentration in influent
X_r	: Biomass concentration in the recycle line
Y	: Growth yield coefficient

Specific Symbols

B_1 : the endogenous decay coefficient of iso-propanol-utilizing acetogens
B_2 : the endogenous decay coefficient of propionate-forming bacteria
B_3 : the endogenous decay coefficient of hydrogen-utilizing methanogens
B_4 : the endogenous decay coefficient of acetate-utilizing methanogens
B_5 : the endogenous decay coefficient of propionate-utilizing bacteria
k_1 : the maximum specific transformation rate for formaldehyde
k_2 : the maximum specific transformation rate for formic acid
k_3 : the maximum specific transformation rate for methanol
k_4 : the maximum specific transformation rate for iso-propanol
k_5 : the maximum specific transformation rate for propionate formation
k_6 : the maximum specific transformation rate for the methanogenesis of hydrogen
k_7 : the maximum specific transformation rate for the methanogenesis of acetate
k_8 : the maximum specific transformation rate for propionate
K_{c2} : the competitive inhibition coefficient of formaldehyde against formic acid
K_{c3} : the competitive inhibition coefficient of formaldehyde against methanol
K_{c4a} : the competitive inhibition coefficient of formaldehyde, formic acid, and methanol against iso-propanol
K_{c5a} : the competitive inhibition coefficient of formaldehyde, formic acid, and methanol against acetate for propionate formation
K_{c6a} : the competitive inhibition coefficient of formaldehyde, formic acid, and methanol against hydrogen
K_{c7a} : the competitive inhibition coefficient of formaldehyde, formic acid, and methanol against acetate
K_{c7b} : the competitive inhibition coefficient of hydrogen against acetate
K_{c8a} : the competitive inhibition coefficient of hydrogen against propionate
K_{c8b} : the competitive inhibition coefficient of acetate against propionate
K_{i1} : the substrate inhibition coefficient for formaldehyde
K_{i2} : the substrate inhibition coefficient for formic acid
K_{i3} : the substrate inhibition coefficient for methanol
K_{i4} : the substrate inhibition coefficient for iso-propanol
K_{s1} : the half saturation coefficient of formaldehyde
K_{s2} : the half saturation coefficient of formic acid
K_{s3} : the half saturation coefficient of methanol
K_{s4} : the half saturation coefficient of iso-propanol
K_{s6} : the half saturation coefficient of hydrogen
K_{s65} : the half saturation coefficient of hydrogen for propionate formation
K_{s7} : the half saturation coefficient of acetate
K_{s75} : the half saturation coefficient of acetate for propionate formation
K_{s8} : the half saturation coefficient of propionate
R_1 : the rate of formaldehyde biotransformation
R_2 : the rate of formic acid biodegradation
R_3 : the rate of methanol biodegradation

R_4	: the rate of iso-propanol biodegradation
R_5	: the rate of propionate formation
R_6	: the rate of hydrogen utilization
R_7	: the rate of acetate utilization
R_8	: the rate of propionate biodegradation
S_I	: formaldehyde concentration
S_{II}	: formic acid concentration
S_{III}	: methanol concentration
S_V	: iso-propanol concentration
S_{VI}	: hydrogen concentration
S_{VII}	: acetate concentration
S_{VIII}	: propionate concentration
X_1	: the concentration of iso-propanol-utilizing acetogens
X_2	: the concentration of propionate-forming bacteria
X_3	: the concentration of hydrogen-utilizing methanogens
X_4	: the concentration of acetate-utilizing methanogens
X_5	: the concentration of propionate-utilizing bacteria
Y_1	: the growth yield coefficient of iso-propanol-utilizing acetogens
Y_2	: the growth yield coefficient of propionate-forming bacteria
Y_3	: the growth yield coefficient of hydrogen-utilizing methanogens
Y_4	: the growth yield coefficient of acetate-utilizing methanogens
Y_5	: the growth yield coefficient of propionate-utilizing bacteria

Greek Symbols

μ	: Specific growth rate
μ_m	: Maximum Specific growth rate
μ^*_m	: Maximum Specific growth rate in the presence of inhibition
θ	: Hydraulic retention time
θ_s	: Solids retention time

FORMALDEHYDE AND ISO-PROPANOL TREATMENT IN AN ANAEROBIC FLUIDIZED BED REACTOR AND KINETIC EVALUATIONS

SUMMARY

In this present study, an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor (10 L in volume) was used to determine the biotreatability of a synthetically prepared high-strength wastewater (~ 7000 mg/L of Chemical Oxygen Demand-COD) containing formaldehyde as an inhibitory and toxic compound (maximum concentration of 1474 mg/L) and high concentrations of iso-propanol (maximum concentration of 2948 mg/L). For individual, double and triple substrate combinations, biodegradation experiments (biochemical methane potential-BMP) were performed in an anaerobic respirometer by applying different initial concentrations to determine the correlation between different substrate concentrations and their biodegradabilities. Kinetic parameters were obtained by manipulating the results which had been obtained from the BMP tests, to the corresponding Haldane and competitive inhibition kinetic model expressions to determine the mechanism of the substrate biodegradations.

The AFBGAC bioreactor followed by an activated sludge reactor was used to biodegrade the high strength organic waste stream. After having achieved 99.9 % of pollutant removal with respect to formaldehyde and iso-propanol mostly in the first stage alone the activated sludge reactor was discontinued on the day of 325 and the rest of the experiments were conducted in the AFBGAC bioreactor alone. Feed flow rates of the buffer and nutrient solutions, and organic waste stream, temperature, effluent pH, and gas production were monitored daily while the off-gas composition was analyzed weekly. Aqueous effluent samples were analyzed weekly for alcohols, formaldehyde, Volatile Fatty Acids (VFA), Chemical Oxygen Demand (COD), Dissolved Organic Carbon (DOC), and ammonia.

Kinetic evaluations have been established based on the experimental results. Initially, the kinetic parameters for each substrate (formaldehyde, iso-propanol, acetate) were obtained individually. Intrinsic substrate kinetic parameters for the double and triple substrate combinations were obtained to determine the toxic and/or inhibitory effect of formaldehyde on the other substrates and on itself. At the last stage of the kinetic investigation, the anaerobic biotransformation of formaldehyde was applied to the competitive inhibition kinetic model. In each case, estimation and optimization was performed using corresponding modified Haldane kinetic expression and for all cases, Levenberg-Marquardt algorithm was used in order to obtain the corresponding kinetic data.

Formaldehyde and iso-propanol removal efficiencies were obtained as 99.9 % and the total COD reduction was found more than 98.8 % in the system. In the anaerobic bioreactor the average biogas production rate was 51.6 liters per day and the methane gas production efficiency was determined as 0.38 m^3/kg $COD_{rem.}$. During performing the BMP tests, it was observed that all the substrates were biodegraded, however, most of the substrates exhibited strong to moderate inhibition effects upon the biodegradation process.

HAVASIZ AKIŞKAN YATAKLI BİR REAKTÖRDE FORMALDEHİT VE İSO-PROPANOL ARITIMI VE KİNETİK DEĞERLENDİRMELER

ÖZET

Bu çalışmada, sentetik olarak hazırlanan ve içerisinde toksik ve inhibe edici bileşen olarak formaldehit (maksimum konsantrasyonu 1474 mg/L) ve yüksek konsantrasyonlarda iso-propanol (maksimum konsantrasyonu 2948 mg/L) içeren kuvvetli bir atıksuyun (~7000 mg/L Kimyasal Oksijen İhtiyacı-KOİ) arıtılabilirliğinin belirlenmesinde havasız akışkan yataklı biyolojik bir reaktör kullanılmıştır. Değişen substrat konsantrasyonları ile bu substratların ayrışabilirlikleri arasındaki ilişkinin tespiti maksadıyla tekil ve ikili ve üçlü substrat kombinasyonlarının değişik giriş konsantrasyonlarında biyolojik olarak ayrışabilirlik deneyleri (Biyokimyasal Metan Potansiyeli-BMP) bir havasız respirometre cihazında gerçekleştirilmiştir. Substrat ayrışabilirliklerinin mekanizmasının belirlenmesi için, BMP testlerinden elde edilmiş olan sonuçların ilgili Haldane ve yarışmalı inhibisyon kinetik model ifadelerinde kullanılması ile kinetik parametreler elde edilmiştir.

Kuvvetli organik atıksuyun arıtımında, içerisinde granül aktif karbon bulunan havasız akışkan yataklı biyolojik bir reaktör ve bunu takip eden bir aktif çamur reaktörü kullanılmıştır. Sadece ilk kademede formaldehit ve iso-propanol için % 99.9 oranında kirletici giderimi sağlandığından, 325. günden itibaren aktif çamur reaktörü devreden çıkarılmış ve geriye kalan bütün deneyler sadece havasız akışkan yatak biyolojik reaktöründe yürütülmüştür. Organik atık akımının ve tampon ve besi çözeltilerinin besleme akış debileri, sıcaklık, çıkış pH'sı ve gaz üretimi günlük olarak takip edilirken, gaz bileşimi haftalık olarak analiz edilmiştir. Sıvı çıkış numunelerinin; alkoller, formaldehit, uçucu yağ asitleri, kimyasal oksijen ihtiyacı, çözünmüş organik karbon ve amonyak içerikleri haftalık olarak analiz edilmişlerdir.

Kinetik değerlendirmeler, deneysel sonuçlara dayandırılarak yapılmıştır. Başlangıçta, kinetik parametreler her bir substrat için (formaldehit, iso-propanol, asetat) tekil olarak elde edilmiştir. İkili ve üçlü substrat kombinasyonlarının tekil substrat kinetik parametreleri ise formaldehit'in kendi ve diğer substratlar üzerindeki toksik ve/veya inhibe edici etkisini belirlemek amacı ile elde edilmiştir. Kinetik araştırmanın son kademesinde, formaldehit'in havasız biyolojik dönüşümü, yarışmalı inhibisyon kinetik modeline uygulanmıştır. Her durumda, tahmin ve optimizasyon, ilgili modifiye edilmiş Haldane kinetik ifadesinin kullanılmasıyla yapılmıştır ve bütün durumlar için ilgili kinetik dataların elde edilmesinde Levenberg-Marquardt algoritması kullanılmıştır.

Formaldehit ve iso-propanol giderim verimleri % 99.9 olarak elde edilmiş ve sistemdeki toplam KOİ azalması % 98.8'den daha fazla olarak bulunmuştur. Havasız biyolojik reaktörde, günlük biyolojik gaz üretimi ortalama 51.6 litre ve metan gazı üretim verimi 0.38 m^3/kg KOİ$_{uzak.}$ olarak belirlenmiştir. BMP testlerinin yürütülmesi

sırasında, bütün substratlar biyolojik olarak ayrıştırılmışlardır, ancak substratların çoğunun biyolojik ayrışma prosesi üzerinde kuvvetli derecelerden orta derecelere kadar inhibisyon etkileri gösterdikleri gözlenmiştir.

1. INTRODUCTION

1.1. Significance of the Study

Biodegradation and biotransformation have been extensively studied for the treatment of organic compounds in wastes including wastewaters, solid wastes, hazardous wastes and contaminated ground waters and soils. Much effort has been expended in collecting experimental data on the biodegradability of individual organic compounds because the environmental scientists and engineers in industries and regulatory agencies need these data to assess their fate in natural and synthetic environments.

Anaerobic wastewater purification processes are increasingly used on industrial scale. Since these processes have the advantages of high organic load, low energy consumption, small amount of sludge produced and simple equipment requirements, and can recover methane gas as useful energy, it is widely investigated and generally recognized to have great potential as an economical and effective approach to wastewater treatment.

In the last twenty years some new processes and devices which are generally known as "high-rate processes", have been developed for anaerobic treatment, such as the anaerobic filter, the up-flow anaerobic sludge blanket reactor, the anaerobic expanded or fluidized bed reactor, etc. Several kinds of industrial wastewater have been successfully treated by means of these new anaerobic processes in laboratory research, pilot-scale studies and full-scale operations.

Many studies have been carried out during the last 15 years on the application of fluidized bed for the treatment of different substrates, enlightening of the circumstances which provide good efficiencies and description of the performance. Although the application of the fluidized bed technology in the anaerobic treatment of wastewaters has been studied to a great extent, only a few full-scale reactors have

been constructed and tried in industry and limited success has been accomplished. Some of the reasons for this imbalance stem from the difficulties of process control and periodic substrate perturbations during the process. Besides, biological treatment of industrial wastes containing high levels of toxic and inhibitory compounds can be difficult and can affect the performance of the system.

On the other hand, increased water usage in recent years has resulted in higher volumes of wastewater, rich in organic pollutants. Formaldehyde and iso-propanol are the major compounds that can be encountered very frequently in the industrial wastewater.

Formaldehyde has been classified as a "Probable Human Carcinogen" by the Environmental Protection Agency (EPA) and is a chemical that is widely used in many products and processes in industry such as chemical industry, textile processing, paper industry, wood processing and etc.

Iso-propanol is also one of the commonly used solvents and easily found in an industrial effluent. Wastewaters from pharmaceutical, cosmetic, textile, rubber and other industries will inevitably contain solvents, often in high concentrations. The presence of solvents is undesirable in an industrial effluent. Direct disposal of them in a river or to the sewer system is prohibited in almost every country of the world because of their effect in living organisms, on sewage reclamation system itself and also because of their flammability.

The Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor has been proven effective in the treatment of the toxic and inhibitory waste such as coal gasification wastewater and wastewaters containing chlorinated hydrocarbons, phenols, catechol, p-cresol, indole and quinoline both under steady-state and shock loading experiments.

The adsorptive capacity of GAC acts as a buffer for the liquid phase organic concentration, which promotes greater reactor stability relative to shock loads and perturbations in operation. GAC was also found to be a superior attachment medium.

1.2. Purpose of the Research

The purpose of this study was to determine the effectiveness of an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor system for treating a synthetic high-strength industrial wastewater bearing toxic and inhibitory concentrations of formaldehyde and iso-propanol.

The biodegradation of formaldehyde and iso-propanol was investigated under anaerobic conditions.

The performance of the bioreactor was evaluated mainly by; detecting the contaminant levels in the effluent, checking the operating parameters at both ascending and constant formaldehyde and iso-propanol influent concentrations and determining the viable potential of the microorganisms by means of periodic GAC extraction from the reactor.

The main subjects that were under investigation during the experiments can be summarized as follows:

Determination of;

- the effect of the continuous feeding on the efficiency of the system,

- the effect of the toxic and inhibitory compounds on the performance of the system

- while applying high concentrations,

- the relationships between the hydraulic parameters and the treatment process,

- the degradation kinetics of the main constituents of the wastewater,

- the viable biomass activity,

- the biochemical methane potential of the system.

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2. BIOLOGICAL TREATMENT OF INDUSTRIAL WASTEWATERS

2.1. Objectives of Biological Treatment

The objectives of the biological treatment of wastewater are to coagulate and remove the non-settleable colloidal solids and to stabilize the organic matter. For domestic wastewater, the major objective is to reduce the organic content and, in many cases, the nutrients such as nitrogen and phosphorus. In many locations, the removal of trace organic compounds that may be toxic is also an important treatment objective. For agricultural return wastewater, the objective is to remove the nutrients, specifically nitrogen and phosphorus, that are capable of stimulating the growth of aquatic plants. For industrial wastewater, the objective is to remove or reduce the concentration of organic and inorganic compounds. Because many of these compounds are toxic to microorganisms, pretreatment may be required [1,p 359].

2.2. Biological Treatment Processes

The major biological processes used for wastewater treatment and their applications are identified in Table 2.1. Aerobic processes, anoxic processes and anaerobic processes are the major groups. The individual processes are further subdivided, depending on whether treatment is accomplished in suspended-growth systems, attached-growth systems, or combinations thereof [1,p 378, 2].

The principle applications of these processes, also identified in Table 2.1, are for (1) the removal of the carbonaceous organic matter in wastewater, usually measured as biochemical oxygen demand (BOD), total organic carbon (TOC), chemical oxygen demand (COD); (2) nitrification; (3) denitrification; (4) phosphorus removal; and (5) waste stabilization [1,p 378, 3].

Table 2.1. Major biological treatment processes used for wastewater treatment [1,p 380-381].

Type	Common Name	Use[a]
Aerobic		
processes:	Activated Sludge process	Carbonaceous BOD removal (nitrification)
Suspended-growth	Suspended-growth nitrification	Nitrification
	Aerated lagoons	Carbonaceous BOD removal (nitrification)
	Aerobic Digestion	Stabilization, Carbonaceous BOD removal
	Trickling filters	Carbonaceous BOD removal, nitrification
Attached-growth	Rotating biological contactors	Carbonaceous BOD removal (nitrification)
	Packed-bed reactors	Carbonaceous BOD removal (nitrification)
	Activated biofilter process	Carbonaceous BOD removal (nitrification)
Combined		
suspended- and		
attached-growth		
Anoxic processes:		
Suspended growth	Suspended-growth denitrification	Denitrification
Attached growth	Fixed-film denitrification	Denitrification
Anaerobic		
processes:		
Suspended-growth	Anaerobic digestion	Stabilization, Carbonaceous BOD removal
	Anaerobic contact process	Carbonaceous BOD removal
	Up-flow anaerobic sludge blanket	Carbonaceous BOD removal
Attached-growth	Anaerobic filter process	Carbonaceous BOD removal, waste stabilization, denitrification
	Fluidized/Anaerobic bed	Carbonaceous BOD removal, waste stabilization

[a] Major uses are presented first; other uses are identified in parentheses.

2.2.1. Anaerobic digestion

2.2.1.1. Process description

Anaerobic digestion (or methanogenic fermentation) is an effective method for the treatment of many organic wastes. This method has been widely and successfully used in a number of both developed and developing countries. This treatment is affected by facultative and obligate anaerobic microorganisms, which in the absence of oxygen,

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convert complex organic materials (both soluble and insoluble) into gaseous end products called "biogas". Biogas consists of a mixture of CH_4 and CO_2 (about 99 % of biogas) and traces of other gaseous products including NH_3, H_2, H_2S, H_2O and N_2. The ratio of methane to carbon dioxide in biogas is typically 1:1.4, and can vary according to the original composition of the organic material, the microflora present and the fermentation regimen employed [4].

Anaerobic digestion reduces the odors and bacteria levels in the sludge feed, leaving the stabilized sludge relatively inert. Stabilization also reduces the amount of solids present in the sludge, although the overall sludge volume remains unchanged unless the supernatant is removed. Anaerobic digestion is often the most economical stabilization option because this process can actually produce energy in the form of methane gas [5,p 1066]. The process is carried out in an airtight reactor. Sludge, introduced continuously or intermittently, is retained in the reactor for varying periods of time. The stabilized sludge, withdrawn continuously or intermittently from the reactor, is reduced in organic and pathogen content and is non-putrescible [1,p 420].

2.2.2. Steps of anaerobic digestion

The anaerobic conversion of polymeric organic substrates into methane and carbon dioxide is a complex process in which various microbial populations play a part because of their different substrate and product specifications (food chains) [6,p 447]. Any description can only be simplified of the real and complex processes taking place in an anaerobic system. Such an account is given in Figure 2.1[7].

ORGANIC MATTER

Carbohydrates, Proteins, Lipids

Hydrolysis and Fermentation   (1)

76 %

Fatty acids

20 %

Acetogenic
Dehydrogenetion   (2)

4 %

52 %   24 %

Acetate $H_2 + CO_2$

72 %

Acetogenic
Hydrogenation (4)

28 %

Acetate
Decarboxylation   (3)

Reductive Methane
Formation   (3)

$CH_4 + CO_2$ $CH_4 + H_2O$
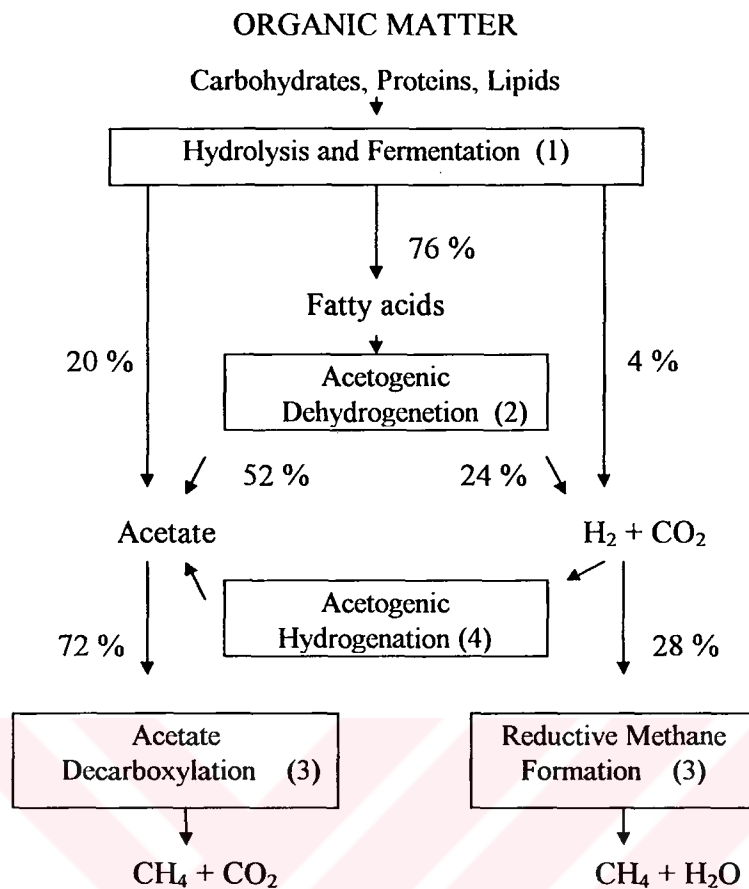
Figure 2.1. The stages of anaerobic digestion [7].

Hydrolysis and acidogenesis phase:

This phase is performed by a wide variety of species like mesophilic, thermophilic, obligate or facultative anaerobs. This phase results in a mixture of volatile fatty acids, such as acetic, lactic, propionic, butyric, etc., neutral compounds such as ethanol, gaseous products such as carbon dioxide and hydrogen, and ammonium.

These microorganisms often have shorter generation times than those in the following phases.

Acetogenesis phase:

This singular acetogenesis (acetate production) phase is brought about by bacteria that are obligate producers of hydrogen (or obligate proton reducers). The process uses reduced metabolites from the hydrolysis and fermentation phases (lactate, ethanol, propionate, butyrate). The dehydrogenation of these compounds is a process

which, in the absence of microorganisms capable of using hydrogen (or a combination of hydrogen with sulfur, for example, to form H_2S) is thermodynamically unfavorable, if not impossible:

$$\text{Lactate} + H_2O \leftrightarrow \text{acetate} + 2\ H_2 + CO_2 + 4.18\ \text{kJ. mole}^{-1} \tag{2.1}$$

$$\text{Ethanol} + H_2O \leftrightarrow \text{acetate} + 2\ H_2 - 9.6\ \text{kJ. mole}^{-1} \tag{2.2}$$

$$\text{Butyrate} + 2\ H_2O \leftrightarrow \text{acetate} + 2\ H_2 - 48.1\ \text{kJ. mole}^{-1} \tag{2.3}$$

$$\text{Propionate} + 2\ H_2O \leftrightarrow \text{acetate} + 3\ H_2 + CO_2 - 76\ \text{kJ. mole}^{-1} \tag{2.4}$$
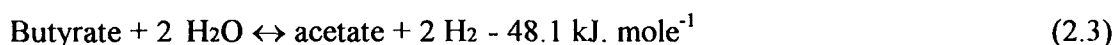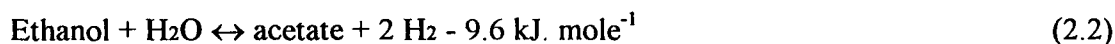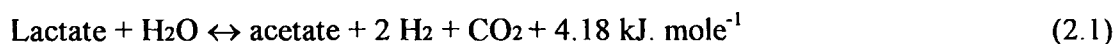
Methanogenesis phase:

Two general methods of methanogenesis are identifiable. In the first, the H_2/CO_2 couple forms H_2O and CH_4. In the second, which is known as acetate-cleaving, acetate is broken down into CO_2 and CH_4. The second method produces about 70% methane.

Other sources of carbon such as methanol, formaldehyde, and methylamines may also be used by methane-producing microorganisms [8,p 316].

If sulfate is present, sulfate-reducing bacteria can partially or completely mineralize the fermentation products to hydrogen sulfide and carbon dioxide via sulfate reduction. Some species can also perform as syntrophic acetogens, especially in the case of ethanol degradation.

The rate-limiting step in anaerobic degradation depends on the nature of the waste undergoing treatment.

It may be hydrolysis for suspended, polymeric organic particles; fermentation for aromatic compounds; syntrophic acetogenesis for propionate, or even methane production from acetate [9]. In general hydrolysis is considered to be the rate limiting step for fermentation of soluble substrates [10].

2.2.3. Important microorganisms in biological treatment

2.2.3.1. Bacteria

2.2.3.2. Cell structure

Bacteria are single-celled procaryotic organisms. Their general forms are spherical, cylindrical (rods), and helical (spiral). As shown in Figure 2.2, the interior of the cell, called the cytoplasm, contains a colloidal suspension of proteins, carbohydrates, and other complex organic compounds [1,p 364].
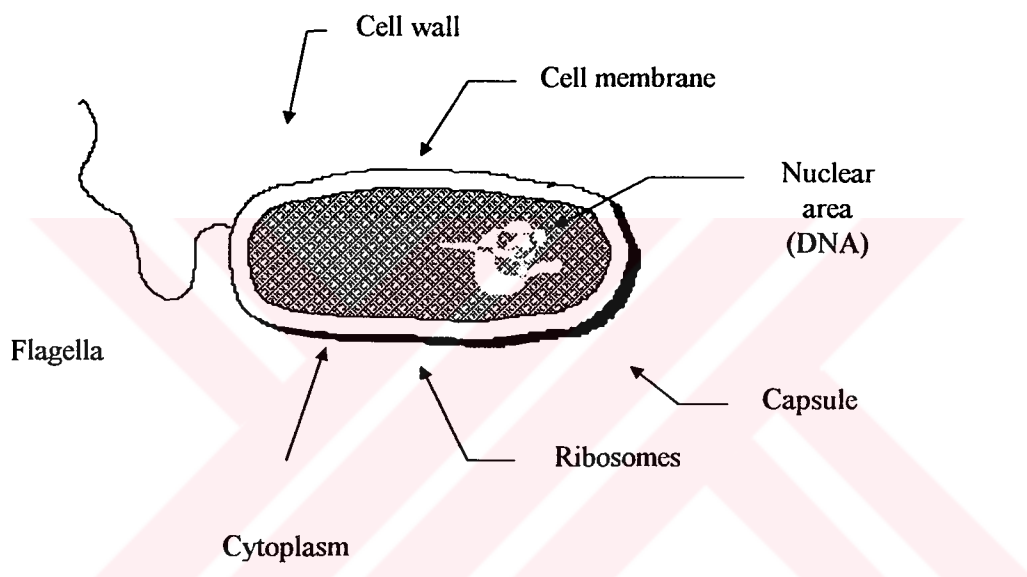
Figure 2.2. Generalized schematic of a bacteria cell [1,p 364].

The cytoplasmic area contains ribonucleic acid (RNA), whose major role is in the synthesis of proteins. Also within the cytoplasm is the area of the nucleus, which is rich in deoxyribonucleic acid (DNA). DNA contains all the information necessary for the reproduction of all the cell components and may be considered the blue print of the cell.

2.2.3.3. Environmental requirements

Environmental conditions of temperature and pH have an important effect on the survival and growth of bacteria. According to the temperature range in which they function best, bacteria may be classified as *psychrophilic, mesophilic, or*

thermophilic. Typical temperature ranges for bacteria in each of these categories are presented in Table 2.2 [1,p 365-366].

The pH of the environment is also a key factor in the growth of organisms. Most bacteria cannot tolerate pH levels above 9.5 or below 4.0. Generally, the optimum pH range for bacterial growth is between 6.5 and 7.5 [1,p 366].

Table 2.2. Some typical temperature ranges for various bacteria [1,p 366].

Type	Temperature, (°C)	
	Range	Optimum
Psychrophilic[a]	(−10)-(30)	12-18
Mesophilic	20-50	25-40
Thermophilic	35-75	55-65

[a] Also called Cryophilic.

2.2.4.   The microbiology of anaerobic digestion

Anaerobic methanogenic digestion can be considered as a three-stage process, which requires the syntrophic interaction of metabolically different groups of bacteria (Figure 2.3) [4].

1) **Hydrolytic and fermentative bacteria:** This first stage (full line in Fig. 2.3) of the anaerobic biodegradation is carried out by a diverse cluster of anaerobic and facultative bacteria of the genera *Clostrida, Bacteroides, Selenomonas, Butyrovibrio* and *Eubacterium*.

These bacteria, using extracellular enzymes, hydrolyze biopolymers such as protein, carbohydrate and lipids into their respective amino acids, sugars and fatty acids.

Protein   Carbohydrate   Lipid

Cellulose
Starch
Pentosan (Hemicellulose)
Pectin

Glycerol   Fatty acids

Amino acids   Methanol   Sugars

NH₃

Ethanol
Lactate
Succinate
Propionate
Butyrate

Formate
CO_2
H_2 Acetate

CH_4
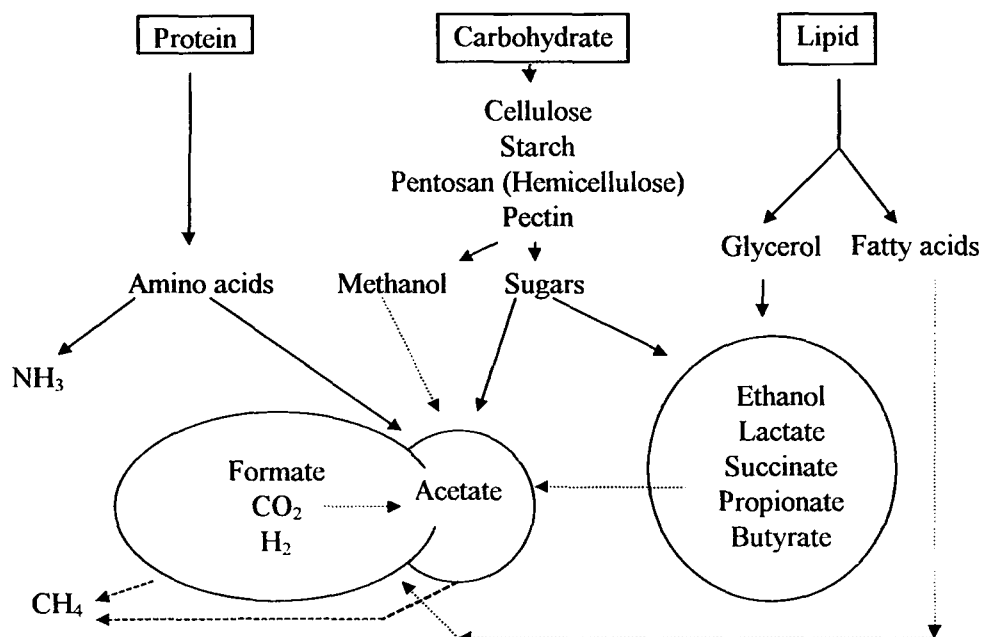
Figure 2.3. Anaerobic breakdown of complex organic matter [4].
Primary: hydrolytic and fermentative fermentation
processes (⸺); Secondary: acetogenic fermentation process (---)
Tertiary: methanogenic fermentation processes (—).

These are then broken down further in this primary fermentative stage (see Fig. 2.3) to yield acetate, formate, CO_2 and H_2, in addition to ethanol, lactate, succinate, propionate and butyrate [4].

2) **Acetogenic bacteria:** Two types of reactions yielding acetate are included within this fermentation stage, H_2-producing and H_2-consuming, which serve as the basis for further subdivision.

a) **H_2-producing acetogenic bacteria:** These include both anaerobic and facultative H_2-reducers species that can ferment organic acids larger than acetate (e.g., propionate, butyrate, succinate) and neutral compounds larger than methanol (e.g., ethanol, propanol) to H_2 and CO_2 (secondary fermentation processes, indicated by dashed line in Fig. 2.3). An example of such organisms is S-organism that grows in obligate symbiotic association with *Methanobacterium bryantii*, an association which for many years was thought to be a single organism, *Methanobacillus omelianskii*, which is able to ferment ethanol to acetate, CO_2 and CH_4 directly. The fatty acid degrading bacteria *Syntrophomonas sp.* (e.g., *S. wolfei* which degrades butyrate, caproate and caprylate to acetate. *S. wolinii* which degrades propionate to acetate)

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are additional examples of obligate proton reducers found in methanogenic fermentors. Other bacteria belonging to the acetogenic group include members of the genus *Desulfovibrio* (e.g., *D. vulgaris, D. desulphuricans*) which oxidize ethanol and butyrate to acetate [4].

b) **Homoacetogenic bacteria:** These are H_2-oxidizing, acetate-producing bacteria that can ferment a wide spectrum of multi or one carbon compounds by C_1-C_1 condensation to acetic acid. For example, members of the genera *Acetobacterium* (e.g., *A. wodii, A. aceticum*), and the genus *Clostridium* (e.g., *C. thermoaceticum, C . formiaceticum*) and *Butyribacterium* (e.g., *B. methylotrophicum*) belong to the homoacetogenic group [4].

3) **Methanogenic bacteria:** The tertiary and final fermentation process in biodegradation involves the methanogenic bacteria (dotted line in Fig. 2.3). They are strict obligate anaerobes which can only use a limited range of substrates for growth and energy production, including one carbon compounds (e.g., CO_2/H_2, formate, methanol, methylamines, CO) and acetate. In anaerobic digesters two major pathways of methane generation operate: (a) acetate decarboxylation (or aceticlastic methanogenesis) and (b) carbon dioxide reduction (or hydrogenophilic methanogenesis) [4, 11].

2.2.5. Environmental effects on anaerobic digestion

2.2.5.1. The effect of temperature

Anaerobic digestion is subject to temperature influences just as any other biological or chemical process [7]. Typically, microorganisms growth and activity increase as temperatures increase; the microorganisms therefore process food faster. Faster growth allows more sludge withdrawals (shorter detention times) while maintaining an adequate supply of microorganisms in the digester for stable operation.

Digestion almost ceases at approximately 10°C. Most digesters operate in the mesophilic temperature range of 32 to 38°C. The thermophilic range of 50 to 60°C digester operation is also used by wastewater treatment plants (WWTPs) for its improved dewatering efficiency. Regardless of the operating temperature selected, the

tank temperature must not deviate more than 0.6°C per day from that temperature. Each group of methane-forming microorganisms has an optimum temperature for growth. If the temperature fluctuates, no group of methane formers can develop the large, stable population required to optimize the process. It is good practice to log the digester temperature twice a day and watch for temperature swings [5,p 1070, 12].

The Arrhenius equation in the logarithmic form is used to describe the effect of increasing temperature on the rate limiting reaction as follows:

$$\ln (k_a) = \ln (A_r) - E/R_G \cdot (1/T) \tag{2.5}$$

where k_a is the rate constant at a given temperature T (°K), R_G is the gas constant, 8.314 J/K/mole, E is the activation energy (kJ/mole), and A_r is the Arrhenius constant. Thus the activation energy E can be obtained from the slope of the linear plot of ln (k) against the reciprocal of the absolute temperature. The Arrhenius law is applicable in biological systems within a limited temperature range , because enzymes present in these systems are deactivated at high temperatures, due to the denaturation of the protein [7].

2.2.5.2. The effect of pH

Anaerobic digestion requires a stable pH within the range of 6.5-7.5, which is maintained in anaerobic reactors by the adjustment of a carbonic acid/bicarbonate system [13].

The need for pH control in anaerobic digestion also arises from the nature of the process. Although several different conversion processes have been identified in the degradation of complex organic matter to methane [14] the entire process is generally considered to be di-phasic, dominated by the activities of two major groups of microorganisms that are very different from each other in terms of physiology, nutritional requirements, growth rate, and sensitivity to environmental stresses [15]. Whenever the equilibrium of the activities of these two groups of bacteria is upset, a sudden increase in volatile fatty acid concentration and a subsequent decrease in pH in the reactor may result. It is necessary that some measure of pH control be taken to

prevent a further fall in pH. Without adequate pH control, a prolonged imbalance in the digester could lead to total inhibition [16].

There are four groups of chemical and biochemical reactions that are significant in regulating the pH of a digester. These reactions are (1) Ammonia consumption and release; (2) volatile fatty acid production or consumption; (3) sulfide release by sulfate or sulfite dissimilatory reduction; (4) conversion of neutral carbonaceous organic carbon to methane and carbon dioxide [13]. If the pH falls below 6, unionized volatile acids become toxic to methane-forming microorganisms. Above pH of 8, unionized aqueous ammonia (dissolved ammonia) becomes toxic to methane-forming microorganisms. These key pH values reflect the ionization constants for ammonia and acetic acid [5,p 1071].

2.2.5.3. Chemical effects

Alkalinity

Calcium, magnesium, and ammonium bicarbonates are examples of buffering substances typically found in a digester. The digestion process produces ammonium bicarbonate; the others are contained in raw sludge. A well-established heated digester has a total alkalinity of 2,000 to 5,000 mg/L [5,p 1070].

Volatile Acids

Volatile acids are intermediate digestion products. Although typical volatile acid concentrations range from 50 to 300 mg/L, higher levels are possible if sufficient alkalinity exists to buffer the acid level. Because of the required balance between volatile acids and alkalinity, the volatile acid: alkalinity ratio provides an excellent indicator of the efficiency of the digester. Careful monitoring of the rate of change of that ratio can indicate a problem before a critical change in pH occurs. If the ratio exceeds 0.8, pH depression and inhibition of methane production occurs. Increases above ratios of 0.3 to 0.4 indicate the inefficiency and the need for corrective action [5,p 1070-1071, 17].

Toxicity

The most typical types of toxins are organic compounds, heavy metals, ammonia, sulfide, oxygen, and salt. While typical inhibitory levels are shown in Table 2.3 through 2.6, a given digester can, over a period of time, acclimate to a higher tolerance level. Most of the toxins affect the microorganisms only if they are in solution. Otherwise, the toxin will not penetrate the cell wall and inhibit performance. Precipitation of the toxin from solution will eliminate its inhibitory effect [5,p 1071, 18, 19].

Table 2.3. Effect of ammonia nitrogen on anaerobic digestion [5,p 1071].

Ammonia concentration, as N [a], mg/L	Effect
50 - 200	Beneficial
200 - 1000	No adverse effects
1500 - 3000	Inhibitory at pH 7.4 to 7.6
> 3000	Toxic

[a] Nitrogen

Table 2.4. Total concentration of individual metals required to severely inhibit anaerobic digestion [5,p 1072].

Metal	Concentration in the contents of the digester		
	Dry solids, %	Moles metal / kg dry solids	Soluble metal, mg/L
Copper	0.93	150	0.5
Cadmium	1.08	100	-
Zinc	0.97	150	1.0
Iron	9.56	1710	-
Chromium			
6+	2.20	420	3.0
3+	2.60	500	-
Nickel	-	-	2.0

Table 2.5. Stimulating and inhibitory concentrations of light metal cations [5,p 1072].

Cation	Concentration, mg/L		
	Stimulatory	Moderately inhibitory	Strongly inhibitory
Calcium	100 - 200	2500 - 4500	8000
Magnesium	75 - 150	1000 - 1500	3000
Potassium	200 - 400	2500 - 4500	12000
Sodium	100 - 200	3500 - 5500	8000

Table 2.6. Synergistic and antagonistic cation combinations [5,p 1072].

Toxic cations	Synergistic cations	Antagonistic cations
Ammonium	Calcium, magnesium, potassium	Sodium
Calcium	Ammonium, magnesium	Potassium, sodium
Magnesium	Ammonium, calcium	
Potassium	-	Ammonium, calcium, magnesium
Sodium	Ammonium, calcium, magnesium	Potassium

Anaerobic Toxicity of Formaldehyde

The processes that deal with formaldehyde release wastewaters with high COD and high concentrations of formaldehyde. Even these wastewaters are diluted with effluents from processing, the treatment of these wastewaters still consume high amounts of energy and may lead to inhibition of degradation and disturbance of the process due to the high organic and toxicant concentration [20, 21]. Formaldehyde, as a disinfectant in the wastewater, was found to be toxic for the anaerobic biological digestion process [22-24]. In addition, formaldehyde can react directly with protein, DNA, RNA of organisms and damage cells which may cause death of microorganisms or inhibit their activity in anaerobic processes [20]. Mostly, the toxicity depends not only on the formaldehyde concentration, however on the type of substrate, test time, and possibly other conditions. The gas production rate and COD removal efficiency decreases generally with the increase in formaldehyde concentration. Sometimes the inhibition changes with time, if the formaldehyde is under the critical concentration, but when it is over the critical concentration, the inhibition becomes high, and it would not decrease with time. Formaldehyde degradation rate is also affected by its concentration. At low formaldehyde concentration, a great portion of the

formaldehyde can be degraded. But particularly, at high formaldehyde concentration, removal efficiency drops significantly.

High formaldehyde concentrations can lead to inhibitory effects on the anaerobic methanogenic activity as well as acidogenic activity. If a wastewater with high concentration of formaldehyde is treated directly by an anaerobic process, the process could be unstable and could need a very long hydraulic retention time. At low concentration the formaldehyde can be removed in an anaerobic system by the formaldehyde-resistant bacteria. If the process is overloaded, the anaerobic treatment system may be disrupted [20, 22].

2.2.5.4. Effect of inhibitors

Environmental conditions such as pH, HRT, TS, and OLR, influence the sensitivity the response to toxicity and acclimatization characteristics of the bacteria [25]. For example, long HRT maximizes the potential for acclimatization and, in general, minimizes the severity of response to toxicity. Another important environmental factor involves toxicities of excessive quantities of many common, relatively non-toxic, organic or inorganic substances which become inhibitory at high OLR values. The threshold toxic levels of inorganic substances vary depending on whether these substances act singly or in combination. Certain combinations have synergistic effect, whereas others display antagonistic effect [26, 27].

Many substances are mentioned in the literature as being toxic or inhibitory to anaerobic digestion. The chlorinated analogues of CH_4, carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and methylene chloride (CH_2Cl_2) have been reported as specific inhibitors of methanogenic bacteria [28].

Activity of methane forming bacteria was reduced to zero by 3 μM $CHCl_3$, while acetogenic bacteria remained unaffected. Other organic compounds reported as being toxic to methanogenesis at low concentrations include ethylene and other unsaturated hydrocarbons, azides, amines and hyrazines, and antibiotics. Cyanide was reported to inhibit methanogenesis at relatively low concentrations (10 μM). Bromoethanesulfonic acid was reported as a specific inhibitor of methanogens [4].

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There is ample evidence in the literature to indicate that heavy metals (e.g., Cd, Ni, Pb, Zn, Cu, Se) are toxic to anaerobic digestion even at quite low concentrations (10^{-3}-10^{-4} M).

Inorganic cations such as Ca^{++}, Mg^{++}, Na^+, K^+, Fe^{++} or NH_4^+ which have stimulatory effect at low or normal concentration, exhibit inhibitory effect at higher concentrations. Inorganic ions such as $SO_4^=$, NO_3^- are potential inorganic inhibitors of methanogenesis in regards to their ability of being alternative electron acceptors. Sulfide ($S^=$) which is essential for most methanogens, is toxic above 200 mg/L, however, can be rendered insoluble when heavy metals are present.

Another source of toxicity is the overproduction of VFAs (acetate, propionate and butyrate) which result in a drop of pH. At the present there has been considerable controversy in the literature as to whether VFA are toxic, or merely a byproduct of toxicity. On the other hand, the question if VFA directly inhibits methanogens or other bacterial groups is still under discussion. Acetate, of all the VFA produced in the digester, appears to be the least toxic. It has been found that a concentration of 12 g/L acetate did not inhibit methanogenesis and neither acetate nor butyric acid had any significant toxic effect on methanogenesis at concentrations up to 10 g/L, whereas concentrations of propionic acid as high as 8 g/L were necessary for the inhibition of anaerobic digesters. VFA toxicity should be directly related to the concentration of the unionized VFA, an effect which increased when pH decreased. Excess of long chain fatty acids such as palmitic, stearic or oleic acids, were also reported to be toxic to anaerobic digester microorganisms [4].

2.2.6. Comparison of aerobic and anaerobic processes

There are basic differences between the aerobic and anaerobic process sequences in eliminating the high-energy organic substances from wastewater. Aerobically, the organic impurities are largely reduced simultaneously to carbon dioxide and water during respiration by the mixed bacterial culture by means of "biological combustion".

Anaerobic degradation is similarly carried out by a mixed culture. However, in this case the wastewater constituents are largely fermented one after the other by various bacteria into methane and carbon dioxide by a type of "biological pyrolysis".

The synthesis of new cell substance depends on the energy released for the cell. Thus, during continuous anaerobic wastewater purification up to 50 % of the organic carbon obtained is incorporated into the cell mass, compared to only approximately 3 % during aerobic purification (Figure 2.4) [6,p 446].
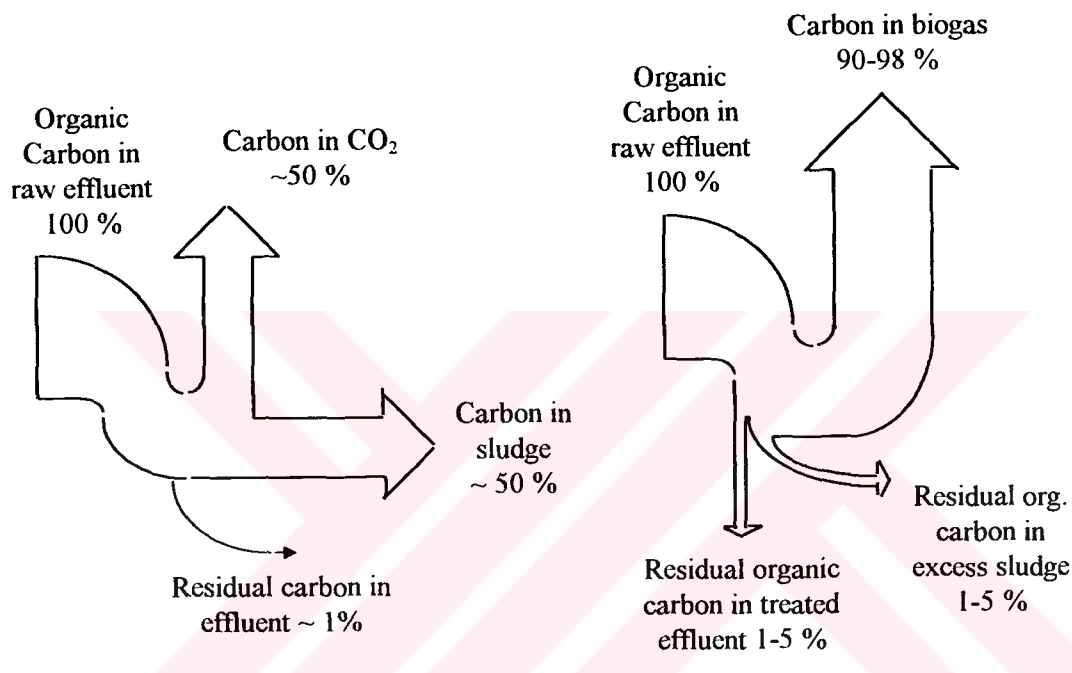
Figure 2.4. Comparison of the aerobic (left) and anaerobic (right) degradation of carbon [6,p 446].

2.2.7. Advantages and disadvantages of anaerobic digestion

The disadvantages and advantages of the anaerobic treatment of an organic waste, as compared to aerobic treatment, stem directly from the slow growth rate of the methanogenic bacteria. Slow growth rates require a relatively long detention time in the digester for adequate waste stabilization to occur.

However, the low growth yield signifies that only a small portion of the degradable organic waste is being synthesized into new cells. Typical kinetic coefficients for anaerobic digestion are reported in Table 2.7 [1,p 425]. With the methanogenic

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bacteria, most of the organic waste is converted to methane gas , which is combustible and therefore a useful end product. If sufficient quantities are produced, as is customary with municipal wastewater sludge, the methane gas can be used to operate dual-fuel engines to produce electricity and to provide building heat.

Table 2.7. Typical kinetic coefficients for the anaerobic digestion of various substrates [1,p 425].

	Coefficient	Basis	Value[b]	
			Range	Typical
Domestic sludge	Y	mg VSS/mg BOD_5	0.040-0.100	0.06
	B	d^{-1}	0.020-0.040	0.03
Fatty acid	Y	mg VSS/mg BOD_5	0.040-0.070	0.05
	B	d^{-1}	0.030-0.050	0.04
Carbohydrate	Y	mg VSS/mg BOD_5	0.020-0.040	0.024
	B	d^{-1}	0.025-0.035	0.03
Protein	Y	mg VSS/mg BOD_5	0.050-0.090	0.075
	B	d^{-1}	0.010-0.020	0.014

[b] Values reported are for 20°C

Because of the low cellular growth rate and the conversion of organic matter to methane gas and carbon dioxide, the resulting solid matter is reasonably well stabilized. After drying or de-watering, the digested sludge may be suitable for disposal in sanitary landfills, for composting, or for other applications on land. Because of the large proportion of cellular organic material, the sludge solids resulting from aerobic processes are most commonly digested, usually anaerobically.

The high temperatures necessary to achieve adequate treatment are often listed as disadvantages of the anaerobic treatment process; however, high temperatures are necessary only when sufficiently long mean cell-residence time cannot be obtained at nominal temperatures. In the anaerobic treatment systems (standard-rate and high-rate) the mean cell-residence time of the microorganisms in the reactor is equivalent to the hydraulic detention time of the liquid in the reactor. As the operation temperature is increased, the minimum mean cell-residence time is reduced significantly. Thus, heating of the reactor contents lowers not only the mean cell-

residence time necessary to achieve adequate treatment but also the hydraulic detention time, so a smaller reactor volume can be used [1,p 425-427].

Advantages for anaerobic wastewater purification are: little excess sludge (less biomass is produced per unit of substrate utilized which also means a decrease in the requirements for nitrogen and phosphorous), no aeration necessary (low energy consumption, and simpler reactors), energy obtained by utilizing the biogas (direct combustion, electricity generation, or input into the natural gas grid after methane enrichment), heavy metal precipitation in the reactor by conversion into insoluble sulfides (landfill sites), and higher organic loadings because the process is not limited by oxygen transfer capacity at high oxygen utilization rates [4, 6,p 447].

Disadvantages of the anaerobic process, in particular in the case of insoluble substrates, are the elevated temperatures (55-60 °C) required to maintain microbial activity at a reasonable rate and the incompleteness of organic stabilization (25-50 % degradation) at economical treatment times and digester volumes, the slow growth of the microorganisms (time-consuming start-up procedure) and the sequential product degradation (unstable system). Anaerobic processes are more sensitive to variations in pH, organic overloads, and toxic shocks. The instabilities may result from interactions among several specialized microbial populations, which must be closely balanced [4, 6,p 447, 29].

Anaerobic wastewater purification plants are becoming increasingly interesting due to their simplicity and low energy requirements [6,p 447].

2.2.8. Quality, performance and control of anaerobic digestion

Gas production is the most representative and simplest criterion to measure the quality of digestion. Gas production depends on two main factors:

- Temperature

- Retention time

The main components of the gas are CH_4 (65 to 70 %) and CO_2 (25 to 30 %). Other constituents -CO, N_2, O_2, hydrocarbons, and H_2S- may exist in small quantities.

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The temperature is critical to the digestion process, affecting the start-up speed, the stabilization of fermentation, and the gas output. The sludge heating facilities can only be bypassed on small units-a few thousand-population equivalents - with very long retention times.

The process generally used is mesophilic fermentation at temperatures around 35°C. Thermophilic fermentation is rarely used because the gains in reactor size and removal of pathogenic germs are cancelled out by high power consumption and the increased sensitivity to variations in load.

The net calorific value of gas produced by digestion varies according to the methane content, and usually increases in line with digestion time. The figure is usually between 22,600 and 25,100 kJ. N. m^{-3} , which corresponds to 5,400 and 6,000 kcal. N. m^{-3}.

Over and above the temperature, **the retention time,** relative to the daily input of fresh sludge, is a very important design factor for two reasons. First, because the settled and digested sludge is not recycled in the reactor, and the second, because the sludge thickens only very slightly in the reactors.

Satisfactory retention times can be achieved by :

- a sufficiently high reactor volume;

- highly concentrated fresh sludge.

Biochemical reactions are encouraged by high sludge concentration levels. A concentration of 15 g/L dense sludge is the minimum value for the industrial plants handling municipal waste sludge.

Two other parameters can be used to improve the performance and control of the sludge digesters:

- **intensive mixing,** which is more useful in methane fermentation of (viscous) sludge than that of the effluents. Thorough mixing minimizes the differences in temperature and organic matter concentration between the fresh and the digested

sludge in the digester, and improves the chances of contact between microorganisms and the organic matter to be broken down. The difference in performance between laboratory digesters and full-scale versions is often due to a difference in the intensity of mixing;

- **regular feed,** the input of fresh sludge and the extraction of the digested sludge must be regular to avoid sudden variations in the development of microorganisms.

The removal of organic matter is also affected by the organic matter content of the fresh sludge and the type of the organic matter. Removal is usually improved when the initial organic matter content is high. Removal rates may drop as far as 35-40 % with certain fresh sludge containing only 50 to 55 % organic matter [8,p 934].

3.   HIGH-RATE ANAEROBIC WASTEWATER TREATMENT

3.1.   Introduction

Although anaerobic biological systems have been used for the treatment of wastes for a long time, the processes called "high rate" have been developed over the last twenty-five years. As implied by the name, high rate processes require a much smaller reactor to treat a given amount of waste than do conventional anaerobic treatment processes [30].

High-rate anaerobic treatment has emerged as a viable alternative for the treatment of many industrial and municipal wastewaters. A number of different process options have been reduced to practice, although some configurations are clearly more well developed than others. One common thread that links these various processes (principally the Anaerobic Filter, Up-flow Anaerobic Sludge Blanket and Expanded/Fluidized Bed Reactors), is the ability to effectively separate solids and reduce the hydraulic retention times. This permits design to be based upon the degradative capacity of the anaerobes, not growth rate and results in reduction of the treatment times from days (typical for conventional digester systems) to hours. Separation of the hydraulic and solids retention times allows accumulation of high biomass concentrations and use of relatively low hydraulic treatment times. In anaerobic filters and expanded/fluidized beds, this is accomplished by the development of granules or flocs that have extremely good settling properties [31].

3.2.   High-Rate Anaerobic Processes

- Up flow Anaerobic Sludge Blanket (UASB)-(Suspended-growth)
- Up flow Fluidized Bed (FB)-(Attached-growth)
- Fixed Bed Filters-up flow and down flow-(Attached-growth)
- Contact-Membrane Process-(Suspended-growth)

Typical process loading and performance data for the UASB, FB/EB, Fixed Bed Filters and Contact-Membrane processes are reported in Table 3.1 [1,p 428].

Table 3.1. Typical process and performance data for anaerobic processes used for the treatment of industrial wastes [1,p 428].

Process	Input COD, (mg/L)	Hydraulic detention time, (h)	Organic loading, (kg COD/m³.d)	COD removal, (%)
Anaerobic contact	1,500-5,000	2-10	0.48-2.40	75-90
Up-flow anaerobic Sludge blanket (UASB)	5,000-15,000	4-12	4.0-12.0	75-85
Fixed-bed	10,000-20,000	24-48	0.96-4.81	75-85
Fluidized/Expanded Bed	5,000-10,000	5-10	4.81-9.61	80-85

3.3. Comparison of Different Process Configurations

UASB-type systems, AF and Expanded/Fluidized reactor systems provide the means to attain long solids retention times at short hydraulic retention times. This translates to high efficiency and stability at high organic loading rates and, therefore, relatively small, economical systems. Efficient treatment at ambient temperatures and/or low organic strength feeds can be realized with all three systems. The comparison of these treatment systems is given in Table 3.2 and this table serves as a useful guide for the comparison [31].

According to Table 3.2 the fluidized bed system shows the most favorable process behavior, but the application in practice is scarce [32, 33].

Table 3.2. Comparison of the three reactor types [31].

	Anaerobic Filter	Fluidized/Expanded Bed	UASB
Biofilm structure of microorganism	(+)	+	(+)
Non-attached biomass important	(+)	$(+)^4$	+
Biofilm thickness control	0	+	0
Recycle necessary	0	+	0
Mixing necessary^3	0	0	(+)
Separation equipment necessary	0	0	+
Phasing possible^1	$(+)^2$	+	$(+)^2$
Suitable for wastes with suspended organics	(+)	+	(+)
Run-through of inerts in raw waste	0	(+)	(+)
Problems with foaming	0	(+)	
Problems with gas bubbles in reactor	(+)	(+)	(+)
High microbe/wastewater contact	0	+	(+)
Tolerates hydraulic overloading	+	+	(+)
Tolerates organic overloading	+	+	(+)
Suitable for high conc. of biodegradable toxins	$+^2$	$+^5$	$(+)^2$
Suitable for shock dose toxicants	0	$+^5$	+
Start-up problems	(+)	+	+
Re-start easy	+	(+)	+

+ = Yes; (+) = Partially; 0 = No/None

^{1}Two reactors with separate acid and gas phases, respectively; ^{2}Recycle or mixing mandatory; ^{3}In excess of mixing caused by gas production; ^{4}May be for wastes containing high solids levels; ^{5}Especially well suited if adsorbent carrier such as GAC used.

Based upon modeling results, fluidized beds can achieve removal efficiencies superior to the other systems at the same volumetric loading rates.

It has also been predicted that long narrow beds would give better performance and expanded/fluidized bed reactors represent the most efficient of the three process configurations because of the excellent mass transfer properties.

There is one advantage of the expanded/fluidized bed process that has become apparent over the last decade. If an adsorbent carrier is used, there is the ability to couple biological and physicochemical removal mechanisms.

While this is not a major concern or consideration for the treatment of readily degradable wastes, such as food-processing-related effluents, that most of the existing plants have been designed for, it appears to offer a considerable advantage for the treatment of wastes containing recalcitrant or inhibitory compounds. Laboratory-scale anaerobic expanded/fluidized beds using GAC as a carrier have been shown to provide extremely stable treatment for wastewaters (synthetic and real waste) containing biodegradable but inhibitory compounds such as catechol, phenol, p-cresol, indole and quinolin both under steady-state and shock loading experiments. When used to treat a phenol-based waste, adsorption, desorption and bioregeneration of the GAC media in the anaerobic fluidized bed, were observed to be operative. Significant adsorptive capacity of the GAC was retained even after development of a mature biofilm.

The treatment of wastewaters containing inhibitory compounds that are not biodegradable has also been shown for toxic effluents such as coal gasification wastewaters. The periodic replacement of GAC to prevent exhaustion of adsorptive capacity for the non-biodegradable, inhibitory compounds was found to be a necessary operating strategy [31].

All systems show individual bottlenecks in industrial operation. Difficulties, which often impede reactor operation in practice are listed in Table 3.3 [33].

3.4.   Research Needs For The High-Rate Anaerobic Treatment Systems

High-rate anaerobic treatment systems demonstrate that in full-scale operation, they are useful for the treatment of various wastewaters. However, especially fundamental work on granule formation and biofilm adhesion as well as practical work on process control and optimization, is needed in order to decrease start-up times and operation costs in steady-state operation. Some urgent research activities needed for each reactor are listed in Table 3.4 [33].

Table 3.3. Bottlenecks in the operation of the different high-rate systems [33].

UASB	AF	EB/FB
• control of bed expansion	• uniform distribution	• control of bed expansion
• process stability at fluctuating feed conditions	• clogging and channeling of the bed	• uniform distribution of the feed
• biomass retention at shock loads	• periodically backwash of the bed	• support flotation
• accumulation of inert particles	• accumulation of inert particles	• change of fluidization properties
• flotation of the biomass	• sludge separation from the effluent	• biofilm detachment and small experience with full-scale installations

Table 3.4. Research needs for the different high-rate anaerobic treatment systems [33].

UASB	AF	EB/FB
• more basic knowledge on granule formation	• more basic knowledge on biofilm adhesion	• more basic knowledge on biofilm formation
• methods to control granule size	• influence of carrier material and surface properties on biofilm formation	• influence of carrier material and surface properties on biofilm formation
• methods to hinder granule flotation	• role of additives to increase film formation	• general strategies for reactor start-up
• role of environmental factors on granule formation and stability	• strategies for reactor backwash	• simple methods for bed expansion control
• proper amount of granules for reactor start-up	• optimization of recycle rate	• role of GAC on process stability

4. KINETICS OF ANAEROBIC PROCESS

4.1. Introduction

Process kinetics plays a central role in the development and operation of anaerobic treatment systems [34]. The objective of a kinetic analysis is to obtain the removal rate as a function of both substrate and biological solids concentration and provide a rational basis for process analysis, control and design [34, 35].

Bacterial growth is one of the most important features on wastewater treatment. The main factors affecting growth rate are transfer of substrate from the medium, the maximum growth velocity, which is that, observed when no substrate limitations occur and environmental conditions that effect bacterial growth directly [36].

In a completely mixed continuous flow system with solids recirculation as shown in Figure 4.1, the rate of change of the bacterial mass and substrate concentration are described by two differential equations.
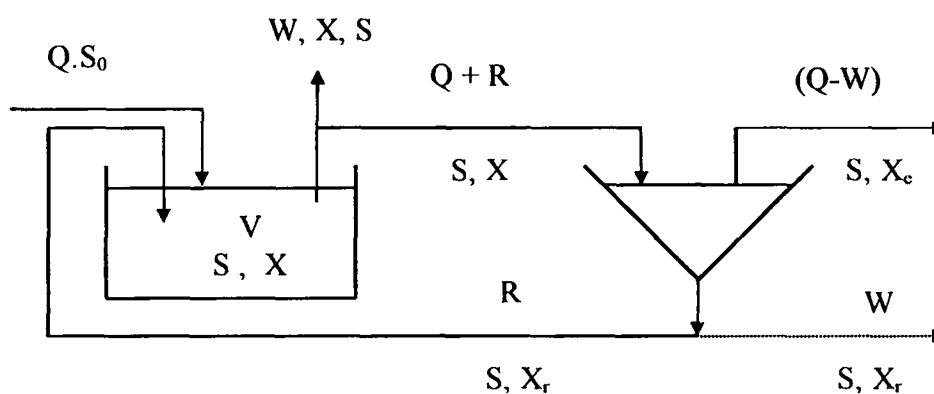
Figure 4.1. Flow scheme for continuous flow stirred reactor [7].

$$(dX/dt) = \mu.X.V - B\ X.V-[W.X + (Q - W)].\ X_e \qquad (4.1)$$

$$(dS/dt) = [Q.(S_0 - S)] - (\mu/Y).X.V \qquad (4.2)$$

Assuming that anaerobic digestion exhibits Monod type kinetics the bacterial growth rate can be linked with the concentration of the limiting substrate (S).

$$\mu = \mu_m.\ S/(K_s + S) \qquad (4.3)$$

Under steady -state conditions Equations 4.1 and 4.2 yield;

$$\theta_s^{-1} = \mu - B \qquad (4.4)$$

$$\mu = Y.[Q.(S_0 - S)]/\ (X.V) \qquad (4.5)$$

The growth (Y) and decay (B) parameters can be determined as the slope and the intercept, respectively, of the graphics of the Equation 4.4 as $1/\theta_s$ vs. μ. The maximum specific growth rate (μ_m) and half-velocity coefficient (K_s) can be determined by rearranging the Equation 4.3 to obtain linearity and plotting $1/\mu$ vs. $1/S$.

Expression for effluent substrate concentration (S) and bacterial mass (X) can be derived as a function of θ_s and these parameters are of chief interest to the plant operator of a biological treatment plant;

$$S = [K_s.(1 + \theta_s.\ B)]\ /\ [\theta_s.(\mu_m - B) - 1] \qquad (4.6)$$

$$X = (\theta_s/\theta).[Y.(S_0 - S)]\ /\ (1 + \theta_s.\ B) \qquad (4.7)$$

In order to sustain steady state bacterial growth a minimum substrate concentration is needed. This must be in the amount that is enough to support a growth rate of the bacterial population greater than its loss rate due to lysis and endogenous decay

mechanisms ($\mu \geq B$). This can be implemented if it is substituted in the Monod equation the endogenous decay constant (B) for the specific growth rate (μ):

$$B = \mu_m. \, S \, / \, (K_s + S) \tag{4.8}$$

solving for S,

$$S_{(min)} = K_s. \, B \, / \, (\mu_m - B) \tag{4.9}$$

but,

$$\mu_m - B = \theta_{s(min)}^{-1}, \tag{4.10}$$

and so,

$$S_{(min)} = K_s. \, B.\theta_{s(min)} \tag{4.11}$$

The minimum substrate concentration is defined as $S_{(min)}$ and it can be associated with $\theta_{s(min)}$. The $S_{(min)}$ represents the lower limit of the effluent substrate concentration, because at substrate concentration lower than $S_{(min)}$ the bacterial growth is always going to be less than the bacterial loss and the process cannot function. There is no way to achieve lower effluent substrate concentration than $S_{(min)}$ under any process loading at fixed environmental conditions [7, 37, 38].

A large number of studies, especially those dealing with undefined, complex substrates, have yielded kinetic parameters and a summary of kinetic data from these studies has been compiled. Based on an extensive literature review a set of kinetic values representing the acid-phase and the methane-phase of the anaerobic digestion is given in Table 4.1 [34].

Table 4.1. Representative values of kinetic constants for anaerobic digestion at 35°C [34].

Process	k, mg COD/mg VSS-d	Y, mg VSS/mg COD	Ks, mg COD/L	μ_{max}, d^{-1}
Acidogenesis	13	0.15	200	2.0
Methanogenesis	13	0.03	50	0.4
Overall	2	0.18	-	0.4

The anaerobic degradation of particulate substrates requires hydrolysis (or liquefaction) to render these substrates available to the anaerobic microflora. The hydrolysis step is usually assumed to follow the first-order kinetics. In most cases, the Monod model (or variations thereof) has been found to be inadequate in describing the heterogeneous reactions taking place during hydrolysis of complex, particulate substrates. With the exception of the hydrolysis step, all other subprocesses of anaerobic treatment have been successfully modeled by following Monod kinetics. A summary of reported values for the kinetic constants pertaining to each subprocess is given in Table 4.2 [34].

Table 4.2. Summary of values of kinetic constants for various substrates utilized in mesophilic anaerobic treatment processes [34].

Substrate	Process	K g COD/g VSS-d	Ks mg COD/L	μ_{max} d^{-1}	Y g VSS/g COD	B d^{-1}
Carbohydrates	Acidogenesis	1.33 - 70.6	22.5-630	7.2 - 30	0.14 - 0.17	6.1
Long-chain fatty acids	Anaerobic oxidation	0.77 - 6.67	105-3180	0.085 - 0.55	0.04 - 0.11	0.01-0.015
Short-chain fatty acids [a]	Anaerobic oxidation	6.2 - 17.1	12-500	0.13 - 1.20	0.025 - 0.047	0.01-0.027
Acetate	Aceticlastic methanogenesis	2.6 - 11.6	11-421	0.08 - 0.7	0.01 - 0.054	0.004-0.037
Hydrogen/Carbon dioxide	Methanogenesis	1.92 - 90	4.8×10^{-5}-0.60	0.05 - 4.07	0.017 - 0.045	0.088

[a] Except acetate

4.2. Inhibition Function

Little information is available concerning the functional relationship between an inhibitory substrate and the specific growth rate of the organism utilizing the substrate. In order to illustrate the dynamic behavior of microorganisms utilizing an

inhibitory substrate a function such as that proposed by Haldane can be used. Although there is no theoretical basis for the use of this function for microorganisms, it should be pointed out that the Monod relationship, which is empirical, is similar in form to the Michealis-Menten expression upon which the Haldane function is based.

Also, studies with *Nitrobacter winogradisky*, have shown that the rate of nitrite oxidation can be related to nitrite concentration by this type of inhibition function.

This inhibition function may be expressed as:

$$\mu = \frac{\mu^*}{1 + \dfrac{K_s}{S} + \dfrac{S}{K_i}} \tag{4.12}$$

where μ is the specific growth rate, time^{-1}; μ^* is the maximum specific growth rate in the absence of inhibition, time^{-1}, S is the limiting substrate concentration, mass/volume, K_s is the saturation constant, numerically equals lowest concentration of the substrate at which the specific growth rate is equal to one-half the maximum specific growth rate in the absence of inhibition, mass/volume, K_i is the inhibition constant, numerically equals the highest substrate concentration at which the specific growth rate is equal to one-half of the maximum specific growth rate in the absence of inhibition, mass/volume.

In the usual continuous culture, operated near steady state, substrate concentrations are low and the term S / K_i is therefore much less than the term K_s / S even for low values of K_i. Under these conditions the inhibition function reduces to the Monod function. In batch cultures, even for the higher values of K_i, the term S / K_i may be significant because of the higher substrate concentrations present during the early stages of the growth [39].

5. ANAEROBIC REACTORS

The two types of commonly used anaerobic digesters are identified as standard-rate and high-rate. In the standard-rate digestion process (Figure 5.1-a), the contents of the digester are usually unheated and unmixed. Detention times for the standard-rate process vary from 30 to 60 days. In a high-rate digestion process (Figure 5.1-b), the contents of the digester are heated and mixed completely. The required detention time for high rate digestion is typically 15 days or less. A combination of these two basic processes is known as the "two-stage process" (Figure 5.1-c). The primary function of the second stage is to separate the digested solids from the supernatant liquor; however, additional digestion and gas production may occur [1,p 420-423].

The concept of high-rate anaerobic reactors is based on three fundamental aspects.

(1) Accumulation, within the reactor, of biomass by means of settling, attachment to solids (fixed or mobile) or by re-circulation. Such systems allow the retention of slowly growing microorganisms by ensuring that the mean solids retention time becomes much longer than the mean hydraulic retention time.

(2) Improved contact between biomass and wastewater, overcoming problems of diffusion of substrates and products from the bulk liquid to biofilms or granules.

(3) Enhanced activity of the biomass, due to adaptation and growth.

The high-rate reactor designs retain biomass in either attached or suspended growth form or by a combination of both of these growth forms. The relative importance of attachment versus suspended growth may be graded as follows:

Attachment   Suspension

FB > DSFF > AF > AF (hybrid) > Granular UASB > Flocculant UASB > Contact

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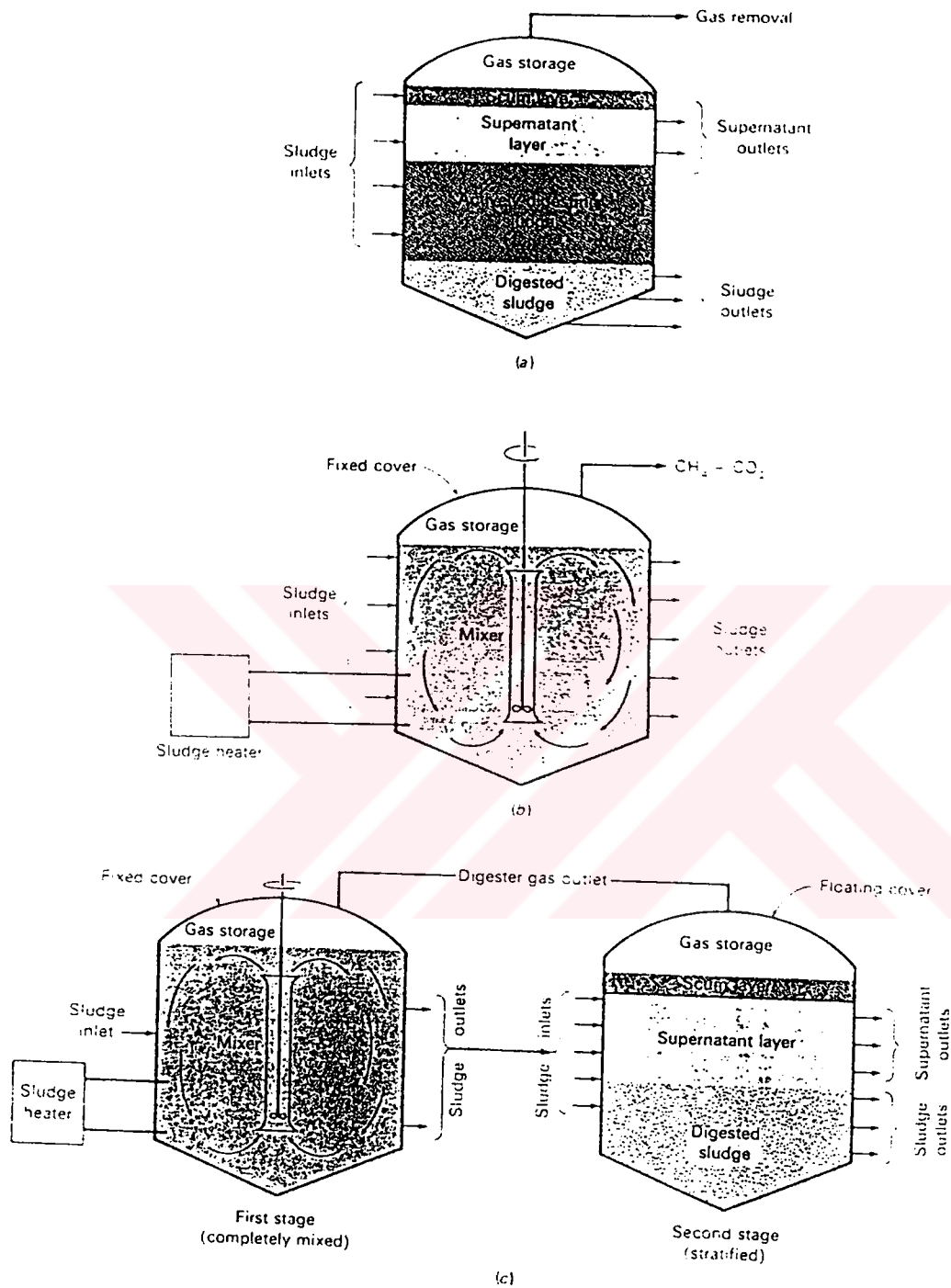
Figure 5.1. Typical anaerobic digesters:

    (a) conventional standard rate single-stage process,

    (b) high-rate, complete-mix, single -stage process,

    (c) two- stage process [1,p 422].

The more common reactors now in use are shown schematically in Figure 5.2.

Completely mixed anaerobic digestion

Anaerobic contact process

Upflow packed bed

Downflow packed bed

Fluidized bed

Expanded bed

Uplflow anaerobic sludge blanket (UASB)

UASB incorporating separate settler

Baffled reactor

Two-stage leaching-bed leachate filter

Membrane solids separation

Anaerobic contact coupled with aerobic polishing
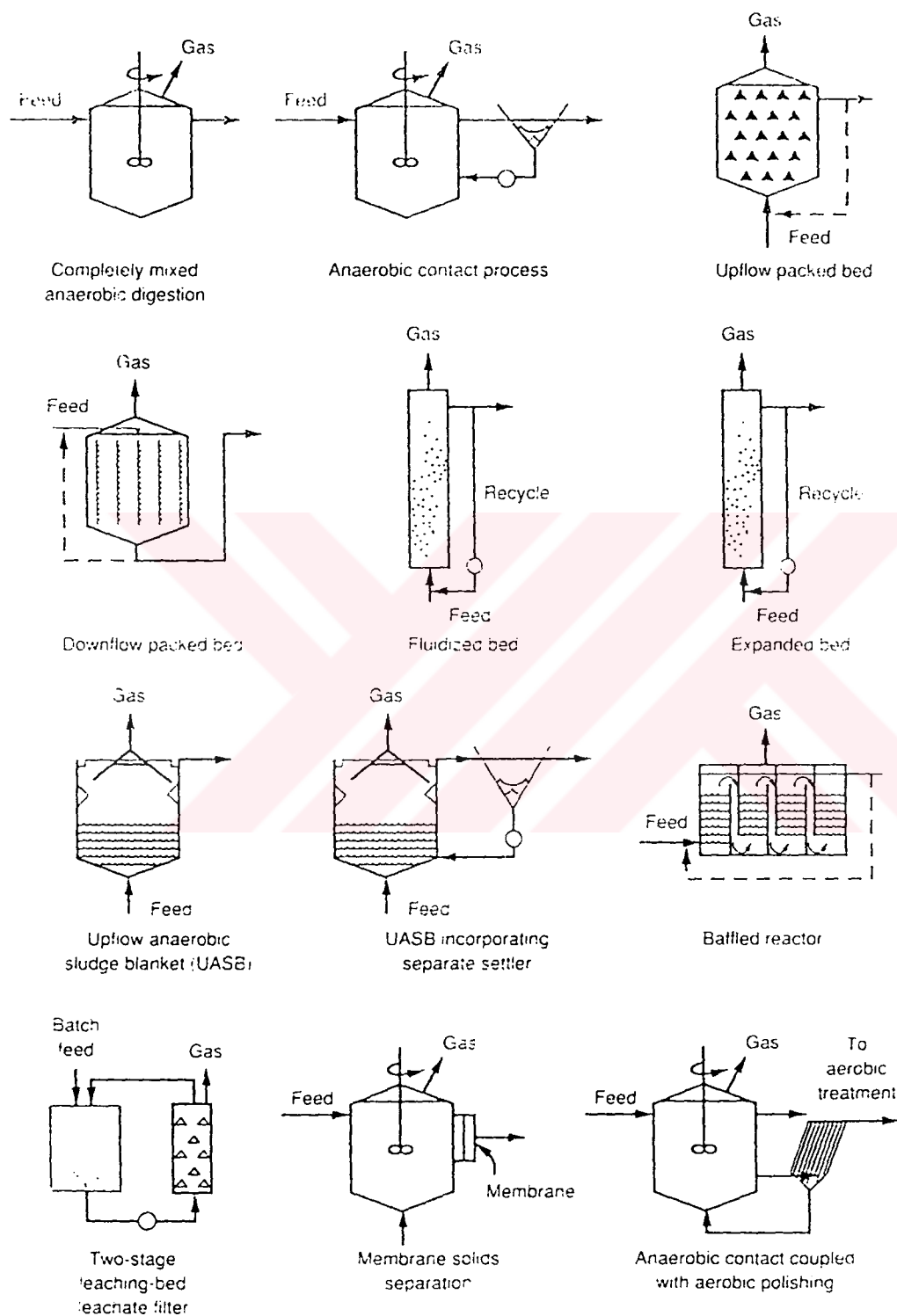
Figure 5.2. Typical reactor configurations used in anaerobic wastewater treatment [1,p 421, 31, 35]

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In order to ensure effective biomass retention by the different reactor types, both the design and operational conditions of the reactors must be optimized. Table 5.1 summarizes the requirements and conditions of the various reactor designs.

Table 5.1. Summary of biomass retention requirements [9].

REACTOR	REQUIREMENTS	CONDITIONS
Contact	Biomass capable of separation by - flotation - settlement - filtration	Proper de-gasification if settlement is applied
DSFF	Formation of stable biofilm	Support material of appropriate type and arrangement
AF	Formation of stable biofilm	Gas/Solid separation by means
Hybrid AF	Biomass with good settling characteristics	of internal random or oriented packing or by external settlement
UASB	Biomass with good settling characteristics	Efficient Gas/Solid/Liquid device
FB/EB	Formation of stable biofilm	Appropriate support material. Appropriate flow distribution

Another fundamental concept of high-rate anaerobic treatment technology concerns the provision and maintenance of optimum contact between the retained biomass and the waste undergoing treatment. A summary of these requirements and the conditions needed to implement them is presented in Table 5.2.

There are three important factors that adversely affect the contact between biomass and wastewater. These are (1) channeling, i.e. the formation of preferential paths through the reactor; (2) dead-zone formation caused by sludge compaction or clogging of matrix interstitial spaces by solids, and (3) clogging of poorly designed-or maintained-distributions systems. Channeling occurs especially when big gas bubbles are produced in tall up flow reactors.

These bubbles travel through the bed of particles in FB/EB reactors or the sludge blanket in UASB or hybrid AF systems causing a wake by which a large slug of liquid passes through the reactor without achieving contact with the biomass and without being treated.

Table 5.2. Requirements and conditions for effective biomass and wastewater contact [9].

REACTOR	REQUIREMENTS	CONDITIONS
Contact	Proper mixing.	Sludge that is mechanically stable. Appropriate mixing device.
DSFF	Reactor of sufficient height for distribution purposes.	Recycle.
AF	Even distribution of influent. No short circuiting.	Number and placement of feed inlets. Sufficient bed mixing. No channeling in packed section.
UASB	Even distribution of influent.	Number and placement of feed inlets. Sufficient bed mixing.
FB/EB	Even distribution of influent. Tall reactor.	Number and placement of feed inlets. Effective recycle ratio.

Dead zone formation is caused by lack of mixing and resultant compaction of the sludge; by accumulation of non-biodegradable solids carried in with the influent (inorganic solids, for example) or by internally produced inorganic solids (calcium precipitates, etc.). Formation of dead zones reduces the effective cross sectional area of the reactor and results in greater up flow liquid velocities. This, in turn, induces channeling and adversely affects the mass transfer of substrates [9].

5.1. Applications of High-Rate Anaerobic Reactors to Different Industrial Sectors

The wastewaters currently being treated at full-scale by high-rate anaerobic reactor configurations are listed in Table 5.3 [9].

Table 5.3. Application of anaerobic processes in full-scale wastewater treatment [9].

Wastewater	Contact	UASB	FB/EB	AF	DSFF
Alcohol distillery	+	+	+	+	+
Beet sugar		+			
Brewery		+	+		
Cellulose condensate	+			+	
Chemical		+		+	
Citric acid	+	+			
Confectionery		+			
Domestic sewage		+	+	+	
Enzyme manufacture				+	
Fish processing		+			
Guar gum				+	
Landfill leachate		+		+	+
Meat processing	+	+			
Organic acids		+		+	
Paper mill		+			
Pharmaceutical	+	+			
Pectin factory	+				
Pig manure		+		+	+
Potato processing		+		+	
Slaughterhouse		+			
Soft drink bottling		+	+	+	
Starch processing	+	+		+	
Surge factory	+				
Thermal sludge liquor		+	+	+	
Vegetable canning	+	+		+	
Yeast	+	+		+	
Milk processing/cheese pr.	+	+		+	

5.2.   Theory for Anaerobic Fluidized Bed Reactor

5.2.1.   Completely mixed reactor analysis

The fluidized bed reactor has an extremely high recycle ratio ($RR=Q_R/Q_I$) varying from 12,000 initially to 35 at the peak loading rates. Although significant gradient in

biological solids concentration exists within the fluidized bed, the average substrate concentration in the reactor is very close to the effluent concentration due to the high recycle ratio. This allows the reactor to be analyzed as a completely mixed system using the total biological mass of solids in the fluidized bed with the total liquid volume in the bed. A schematic representation of the system is shown in Figure 5.3 and uses the following nomenclature.

S_T = Total soluble COD concentration, subscripts i and e are influent and effluent, respectively,

V_l = liquid volume in bed

X_l = Total mass of biological (volatile) solids in bed

h = Fluidized bed height

h_l = Sludge material interface

Q_i = Influent flow, Q_e = Effluent flow

Q_R = Recycle flow

G = Gas flow
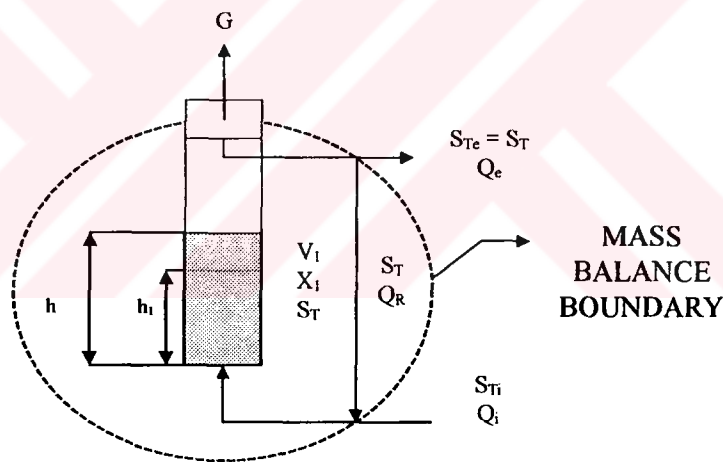
Figure 5.3. Schematic representation of fluidized bed reactor for a completely mixed system [35].

A mass balance for the total soluble COD concentration in the liquid phase of the reactor yields:

Rate of mass

In	-	Out	-	Reaction	=	Accumulation		

$$Q_i S_{Ti} \quad - \quad Q_i S_T \quad - \quad R_T V_1 \quad = \quad V_1 dS_T/dt \tag{5.1}$$

At steady-state, $dS_T / dt = 0$, and the reaction rate occurring in the bed liquid volume is:

$$R_T = \frac{Q_i (S_{Ti} + S_T)}{V_1} \tag{5.2}$$

Defining the hydraulic detention time in the bed based on the raw wastewater flow rate as $t_0 = V_1 / Q_i$ gives:

$$R_T = \frac{(S_{Ti} + S_T)}{t_0} \tag{5.3}$$

which is the basic equation defining the total soluble COD removal rate, R_T, in a completely mixed system. COD stripping to the gas phase is ignored in the above analysis since the raw wastewater consisted mainly of volatile acids which are not significantly stripped at the reactor pH of 6.5 to 7.4 since they are in the ionized form [35].

5.3. Fluidized Bed Reactors for Anaerobic Wastewater Treatment

The most recent of the high-rate anaerobic process configurations, the anaerobic expanded/fluidized bed reactor, was initially developed in the late 1970s [40].

5.3.1. Start-up of anaerobic fluidized bed reactor

The reduction of the start-up time is one of the key parameters to increase the competitiveness of high rate anaerobic reactors [33].

Selection of the carrier particles itself, which will often be dictated by other concerns such as cost, availability or specific needs (i.e. GAC for removal of inhibitory compounds), may influence the start-up procedure to be used and will certainly impact the length of the start-up period [31]. As shown in Figure 5.4, the start-up is influenced by the wastewater composition and strength, the volume, activity and adaptation of the inoculum, environmental parameters such as temperature, pH, nutrient and trace elements content, operation parameters like loading rate, retention time and liquid mixing and reactor configuration, geometry and size.
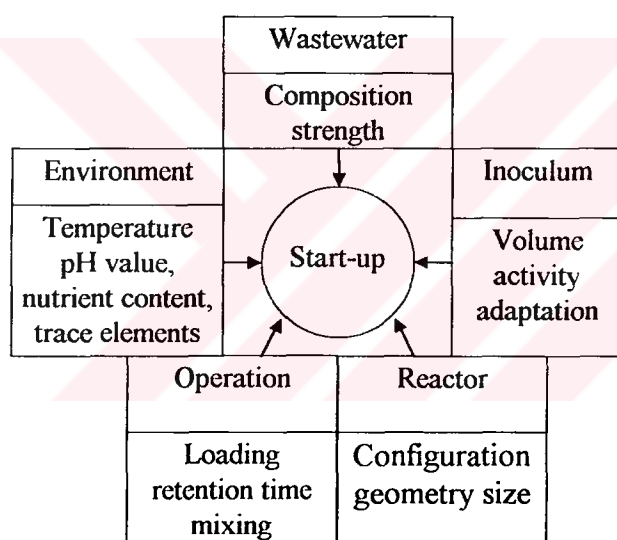
Figure 5.4. Important parameters for reactor start-up [33].

The rate of reactor start-up is markedly dependent on the properties of the microbial seed biomass (Table 5.4).

Table 5.4. Microbial factors which influence the reactor start-up [33].

- Dominating bacterial groups (hydrolytic, acidogenic, acetogenic/methanogenic)
- Growth rate of the methanogenic species
- Dominating shape of the methanogenic species (rods, sarcina, cocci)
- Biomass yield coefficient
- Half velocity constant (K_S) of the microorganisms
- Adaptation rate of the microorganisms on wastewater properties
- Ability to excrete polysaccharides (glycocalyx).

Trace elements such as iron, nickel, cobalt and molybdenum can considerably affect the duration of the start-up, because these elements are essential for methanogenic growth. Some approximate values for the trace elements demand of low and high strength wastewaters are given in Table 5.5.

Table 5.5. Minimum trace elements demand of wastewaters in different concentration [33].

Trace element	Concentration range (mg/L)	
	10 g COD/L	50 g COD/L
Fe	0.5	3
Ni	0.05	0.3
Co	0.05	0.3
Mo	0.01	0.05

The conditions necessary for the start-up of fluidized bed reactors (FB) are similar in several aspects with anaerobic filters (AF) and expanded bed reactors because the biofilm formation on the static or fluidized support media is the rate limiting step in both systems.

As shown in the Table 5.6, biofilm formation is a three-step process, which is strongly influenced by the surface properties of the support media, the genera of the starter culture and the shear stress on the support surface.

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Table 5.6. Steps in the biofilm formation [33].

- **LATENT PHASE**

  Loose deposits of microorganisms on the surface and the niches of the support media

- **STABILIZATION PHASE**

  Excretion of glycocalyx (polysaccharides) which leads to the formation of a fixed bacterial matrix on the support surface

- **GROWTH PHASE**

  Biofilm growth up to shear stress limited to the film thickness

In addition, the support media must fulfill many additional requirements, which are listed in Table 5.7.

Table 5.7. Characteristics of the ideal support media [33].

GENERAL REQUIREMENTS:

- High area to volume ratio
- Porous or rough surface to permit bacterial adhesion
- Biological inertia
- High mechanical stability
- Low cost per unit volume

FLUIDIZED BED:

- Uniform size distribution
- Optimum shape to avoid diffusion limitations

5.3.2. Difficulties in reactor start-up

The individual start-up problems of the fluidized bed reactors result from economical, biological and technical causes, as shown in Table 5.8.

Table 5.8. Typical difficulties in reactor start-up [33].

EB/FB
• Initial fluidization of the support media
• Particle interaction at low gas formation rates
• Loss of biomass
• Coagulation of the supports after shut-downs
• Selection of sessile microorganisms

5.3.3. Operation

High-rate reactors are accepted and applied in wastewater treatment only if a lot of requirements in the operation and the control can be fulfilled in industrial practice. For a given wastewater, most of these requirements, which are listed in Table 5.9, are mainly a function of the biomass concentration and activity and the mixing and flow behavior of the reactor, when all other environmental parameters (temperature, pH, buffer capacity etc.) are in the optimum range.

Table 5.9. Typical requirements on high-rate anaerobic systems [33].

• High organic loading rate	• Low energy demand
• Short hydraulic retention time	• High process reliability
• High COD removal efficiency	• Applicability to different wastewaters
• High tolerance to overloadings	• Easy operation and control
• Fast start-up and re-start after shut-downs	• Economy in materials and design

An independent control of the solids (SRT) and liquid retention time (HRT), the prevention of an accumulation of inert suspended solids within the reactor and favorable mass transport conditions are the most common objectives for the operation of all high-rate systems.

A comparison of the typical biomass concentrations in the different high-rate systems is shown in Figure 5.5 and the typical operation regimes of the different high-rate systems are shown in Figure 5.6.
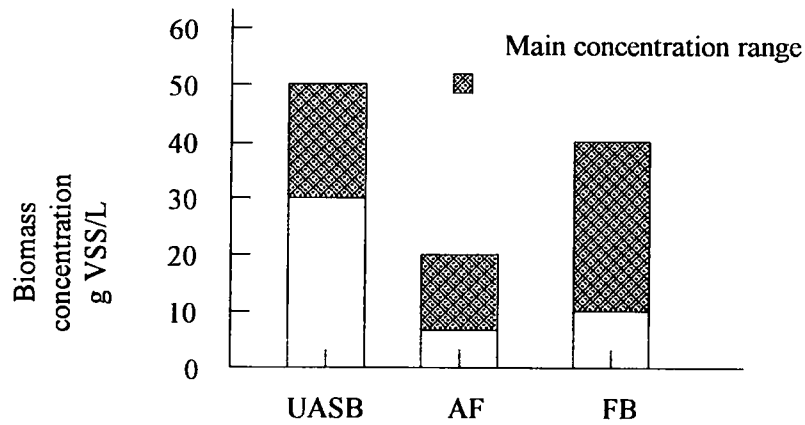
Figure 5.5. Typical biomass concentrations in different high-rate systems [33].
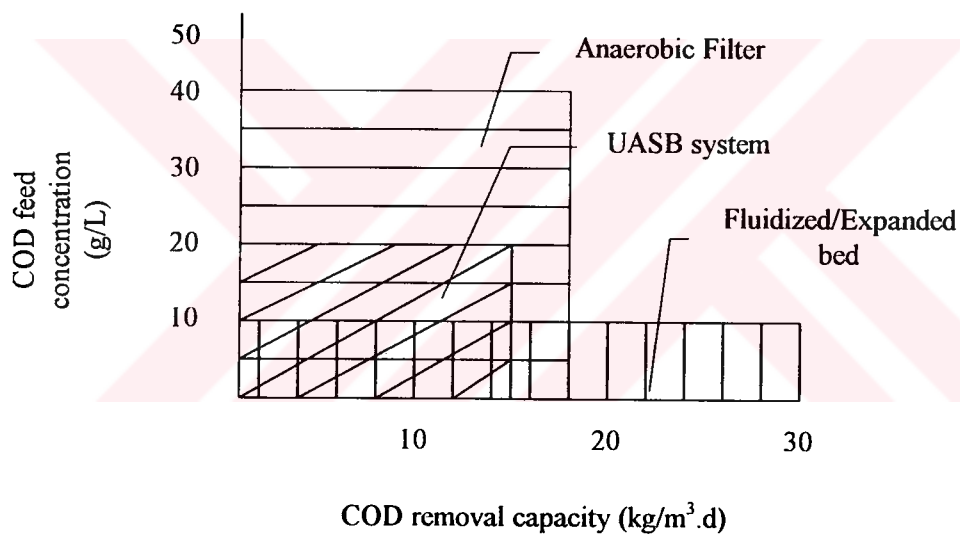
Figure 5.6. Typical operation regimes of high-rate anaerobic systems [33].

These figures demonstrate that the operation of UASB reactors is not restricted by the microbial capacity but is limited by hydraulic factors.

The operation of fixed bed and fluidized bed reactors is limited by the capacity of microorganisms [33, 41].

5.4. Characteristics of Fluidized Bed Reactors

Compared with other high-rate anaerobic treatment systems the fluidized bed reactors offer several advantages. The advantages and disadvantages of the fluidized beds are depicted in Table 5.10.

Table 5.10.   Characteristics of the fluidized bed reactors [33].

ADVANTAGES	DISADVANTAGES
• Extremely high removal capacity	• Difficult start-up
• Extremely short retention times	• High energy demand for
• Applicable to very high organic loadings	fluidization
• Good adaptation to various wastewaters	• Bed height difficult to control
• Applicable to solids-bearing wastewaters	• High TSS content in the effluent
• Insensitive against higher suspended solids concentrations	• Difficult reactor design and scale-up
• With GAC, applicable to inhibitory wastewaters	• Little full-scale experience
• Insensitive against organic and hydraulic shock loads	• Eventually high media costs
• Low area demand	
• High concentration of active biomass (no "wash out" problem)	
• Possible to treat very dilute influents	
• No problems of plugging and channeling	
• Easy to restart-up	

An additional advantage of fluidized bed systems, which becomes increasingly important, is the chance to treat toxic or recalcitrant wastewaters, if granular activated carbon (GAC) is used as support [1,p 155, 42, 43]. The integrated function of GAC for detoxification and biomass fixation can be found in no other high-rate system. Meanwhile, the use of the recycle for the fluidization purposes also offers other advantages for the process: it provides alkalinity to neutralize the influent,

reduces its concentration and smoothes spikes of toxicants or inhibitory compounds. Besides, long seasonal shut-downs do not affect the capacity of the system [18, 33, 41].

5.5.   Previous Studies at Laboratory, Pilot and Full-Scale

The anaerobic expanded/fluidized bed process has primarily been applied to industrial wastewaters. A listing of pilot results is presented in Table 5.11. Because of the stable hydraulics of the process, when dense carrier particles (i.e. sand) are used, extremely high organic loading rates can be applied to expanded/fluidized beds. Over 100 kg COD/m^3-day could be loaded to a 15 cm diameter by 3 m high fluidized bed [19, 31].

The pilot scale reactor commonly used ranges between 30 and 6000 L in volume, with high H/D (height/diameter) ratios, to reduce problems of flow distribution.

Sand and anthracite have been widely used as carriers, more recently, lighter products like ion exchange resin beads, natural or baked clays (kaolinite, sepiolite, Argex (R), Arlita (R)), granular activated carbon, pumice and reticulated polyurethane have been used.

A partial list of examples can be found in Table 5.12 [41].

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Table 5.11. Anaerobic expanded/fluidized bed pilot studies [31].

Waste	Media	Feed COD (g/L)	Loading (kg COD/m³-d)	Temp (°C)	% COD Removal
Acid whey	Sand	50-56	13.4-37.6	35	72-84
Acid whey	Sand	52-55.4	15-37	24	65-71
Acid whey (two stages in series)	Sand	52.2	10.5	35	94
Starch-based food processing	Sand	7.2-9.4	3.5-24.1	35	75-86
Chemical waste	Sand	12.0	4.1-27.3	35	79-93
Soft drink bottling	Sand	6.0	4-18.5	35	66-84
Thermal conditioning liquor	Sand	10.0	4.3-21.4	35	52-75
Whey permeate	Sand	6.8	8.6-10.4	30-35	68
Whey permeate	Sand	27.3	5.3-7.4	30-35	82
Soy whey	Sand	9.7-10.9	13-19.7	36	80-85
Acidified yeast waste	Sand	3.0	20-60	37	90
Brewery waste	Sand	1.0-12.0	1.0-14.6	35	72-96
Bakery waste	Sand	8.8	2.9-14.7	35	55-95
Paper mill foul condensate	Sand	8-16	25-48	35	88-92
Sewage	Sand	0.17-0.27	0.45-0.52	10-23	37-47
Sewage	Zeolite		0.8-7.3	35	27-44
Sewage	Sand	0.3	1.2-3.6	20-30	29-42
Sewage	Activated carbon	0.3	1.8-3.6	15-25	50-60
Corn starch waste	Activated carbon	2-6	14-50	35	70-95
Sugar beet waste	Sepiolite	3.0-6.5	38		85
Thermal conditioned sludge liquor	Sand		44	35	

Table 5.12. Pilot-scale reactors [41].

Volume (L)	H/D (m)	Wastewater
270	6/0.25	Yeast production
5380	6.85/1	Soft-drink waste
1000	6/0.5	Sugarbeet wastewater
7000	7/1.13	Sewage
4200	3/1.5	Sewage
70	2.5/0.144	Synthetic waste

The fluidized bed technology, however, has been applied with great success in other biotechnological processes, such as nitrification, denitrification, aerobic wastewater treatment, and alcohol, beer and vinegar production.

Some of the plants in operation are:

- Gist Brocades, n.v., in Delft, The Netherlands. Yeast production wastewater. 4 reactors 300 m^3 each.

- From the same company, there are other systems in Prouvy, France, (2 reactors 125 m^3 each) and Monheim, FRG, (125 m^3) this one operating as an Expanded granular sludge bed.

- Degremont (Cervezas El Aguila), Madrid Spain. Brewery wastewater. 5 reactors 165 m^3 each.

- From the same company, other plant in France.

- Reliance Industries, Bombay, India. (Dorr Oliver design). 1 reactor 850 m^3.

- Lansing Wastewater Treatment Plant. Lansing, Michigan, USA. For heat treatment liquor of sludges. 4 reactors of 180 m^3, and some other plants in the USA, by Envirex, such as the one in W.C.I., Hatfield, Pennsylvania [41, 44].

6. EXPERIMENTAL WORK

6.1. Introduction

In this study, mainly the determination of the effectiveness of an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor in treating the waste stream which was composed of formaldehyde and isopropyl alcohol, and the biodegradation kinetics of the main constituents of the waste which were formaldehyde, iso-propanol and acetate (one of the most significant intermediate products of the biodegradation of organic matter) were investigated.

6.2. Formaldehyde and Iso-Propanol Treatment in a Two Stage Biological System

An Anaerobic Fluidized Bed Granular Activated Carbon bioreactor (AFBGAC) followed by an activated sludge reactor was used to biodegrade a high strength organic waste stream (synthetically prepared) containing basically formaldehyde and iso-propanol.

Figure 6.1 shows a schematic representation of the two stage biological system and the characterization of the influent waste stream is given in Table 6.1.

The anaerobic bioreactor was charged with 1.0 kg of 16×20 U.S. Mesh Granular Activated Carbon (GAC) and seeded with anaerobic digester sludge from the Mill Creek Wastewater Treatment Facility (MCWTF), Cincinnati, Ohio.

The GAC serves as an excellent attachment media for the biological growth as well as a buffer against any shock loading and toxic compounds.
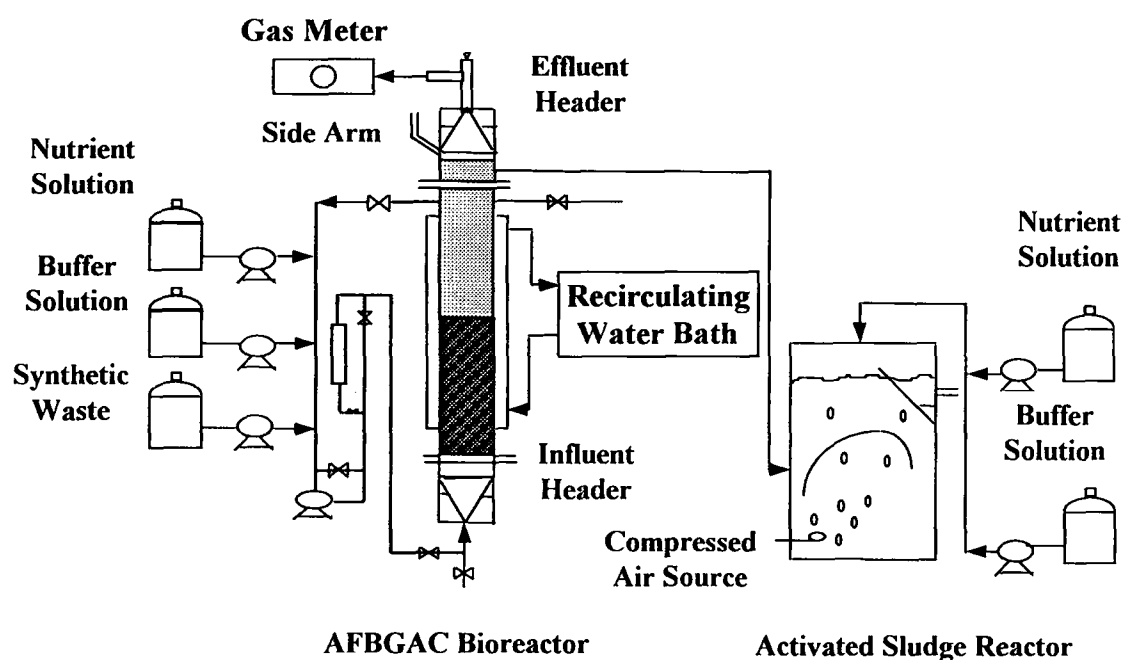
Figure 6.1. Sequential anaerobic/aerobic biological system.

Table 6.1. Characterization of the influent waste stream.

Parameter	Influent
Formaldehyde, mg/L	27.00 - 1474.00
Iso-propanol, mg/L	295.00 - 2950.00
COD, mg/L	~ 7000.00
DOC, mg/L	~ 1920.00
Ph	7.20
Temperature, (°C)	35
NH_3 - N, mg/L	70
NO_3 - N, mg/L	0

The anaerobic compartment of the bioreactor, 9.1 L in volume, is surrounded by a plexiglas jacket used for the passage of heated water to maintain the bioreactor at a constant temperature of 35 °C. An internal recycle stream provides bed-expansion and promotes a completely mixed flow regime in the GAC reactor. The combined volume of the anaerobic compartment and recycle stream is 10 L with a system Hydraulic Retention Time (HRT) of 1.5 days which was later decreased to 16 hours.

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The operating parameters of the Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor and design parameters are given as follows:

Reactor volume	: 10	L
H/D (height/inner diameter)	: 0.97/0.10	m
Hydraulic retention time	: 16	hours
Feed COD (soluble)	: ~ 7000.00	mg/L
Organic loading rate	: 10.50	kg COD/m^3.day
Hydraulic loading rate	: 1.84	m^3/m^2.day
COD (%) removal	: ~ 99 %	
COD removal capacity (volumetric)	: 10.34	kg $COD_{removed}$ /m^3.day
Methane gas efficiency in the bioreactor	: 0.38	m^3 methane/kg $COD_{removed}$

Hydraulic retention time	: $\theta = V / Q$	(day)
Organic loading rate	: $L_O = (Q . S_0) / V = S_0 / \theta$	(kg COD/ m^3.day)
Hydraulic loading rate	: $L_H = Q / A_c$	(m^3/m^2.day)
COD (%) removal	: $(S_0 - S) / S_0 \times 100$	
COD removal capacity (volumetric)	: $R_V = (S_0 - S) / \theta$	(kg $COD_{removed}$ / m^3.day)
Methane gas efficiency in the bioreactor	: $M / Q . (S_0 - S)$	(m^3 methane/kg $COD_{removed}$)

where θ is the hydraulic retention time (day), V is the volume of the bioreactor (m^3), Q is the volumetric flow rate of the waste, buffer and nutrient solutions (m^3/day), L_O is the organic loading rate (kg COD/m^3.day), S_0 is the initial COD (kg/m^3), L_H is the hydraulic loading rate (m^3/m^2.day), A_c is the cross-sectional area of the bioreactor (m^2), S is the final COD (kg/m^3), R_V is the volumetric COD removal capacity (kg $COD_{removed}$/m^3.day), M is the volumetric flow rate of the produced methane (m^3/day).

The operating parameters of the Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor exhibit a large correspondence with the operating parameters

of which have been given in the literature for anaerobic fluidized bed bioreactors [31, 33, 41].

The activated sludge reactor, 17 L in volume, has a sludge age of 20 days and HRT of 2.5 days.

Nutrient and carbonate buffer solutions, needed to sustain biological growth and maintain the pH at approximately 7.2 in the anaerobic bioreactor and 8.0 in the activated sludge are fed to the reactor system. The composition of the buffer solution and the feed concentrations of vitamins and salts in the nutrient solution, normalized to the total feed flow rate, are given in Tables 6.2 and 6.3 respectively.

The iso-propanol concentration was gradually increased from 295 mg/L to 2950 mg/L over a period of 4 months, after which formaldehyde was added as a co-substrate from an initial concentration of about 27 mg/L and gradually increased to 1474 mg/L over another 2 months period. Ammonia was fed to the AFBGAC bioreactor at 18 mg/L -N as the primary source of nitrogen needed for the biological growth. The ammonia concentration was later increased to 70 mg/L - N when the anaerobic system became nitrogen limited.

Table 6.2. Composition of the buffer solution.

Component	Concentration, mg/L
Na_2S	350
Na_2CO_3	7500
NaOH	7500

Table 6.3. Composition of minerals and nutrients in the feed for the Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) in which the anaerobic culture was acclimated (modification) [18].

Compound		Concentration (g/L)
$(NH_4)_6Mo_7O_{27}.4H_2O$		2.08
$Na_2B_4O_7.10H_2O$		1.15
$NiCl_2.6H_2O$	Stock Trace	3.00
$MnCl_2.2H_2O$	Salt Solution	4.74
$CoCl_2.6H_2O$		2.86
$ZnCl_2$		3.27
$CuCl_2.2H_2O$		2.05
Stock Trace Salt Solution		33.1 mL/L
$MgCl_2.6H_2O$		8.13
$NaH_2PO_4.H_2O$	Stock Salt	8.28
$KH_2PO_4.H_2O$	Solution	13.60
NH_4Cl		17.00
$CaCl_2.2H_2O$		5.88
p-Aminobenzoic acid		0.01
Biotin		0.0039
Cyanocabalimin (B_{12})		0.0002
Folic acid	Stock Vitamin	0.0039
Nicotinic acid	Solution	0.01
Pantothenic acid		0.01
Pyridoxine hydrochloride		0.002
Riboflavin		0.01
Thiamin hydrochloride		0.01
Thioctic acid		0.01

Values adjusted for total feed volume.

6.2.1. Materials and methods

6.2.1.1. Anaerobic fluidized bed granular activated carbon (AFBGAC) bioreactor

The 9.1 L bioreactor consisted of a jacketed main column, an influent header, and an effluent header is shown in Figure 6.2. The inner-jacketed tube (97 cm long, 10 cm inner diameter) was constructed of Plexiglas and was enclosed in an outer plexiglas tube. Water was circulated through the annular space from a constant temperature bath (model MW112 CA Magna Whirl, Blue M Electric Co., Blue Island, IL) to maintain a constant temperature of 35 °C within the column. The recycle tubes were constructed from polyvinyl chloride tubing and the feed and effluent lines were made of Tygon and neoprene tubing.

The column was charged with 1.0 kg of 16×20 U.S. Mesh Filtrasorb 400 GAC (Granular Activated Carbon; Calgon Corporation, Pittsburgh, PA). Effluent recycle was used to maintain a bed-expansion of 30 %. The influent header was filled with marbles to distribute the flow evenly across the column cross section. The effluent header served to separate and convey the liquid effluent and off-gas to respective effluent ports.

The column was equipped with a side arm. The entrance to this side arm was above the effluent header and the exit was below the recycle withdrawal port inside the main column. The purpose of this side arm was to provide a port for the introduction of preloaded GAC to the column during reactor start-up without being caught in the recycle stream. The column was also equipped with a GAC withdrawal port located at the top of the effluent header. This allowed the GAC to be withdrawn from the column using a constant volume cup that could be lowered to the desired level in the bed to ensure representative sampling of the GAC medium. The buffer and nutrient solutions were fed into the recycle lines using fixed rpm pump drives (Masterflex Pump Model 7543-02 with model 7015-20 pump head for the buffer and model 7016-20 pump head for the nutrient, Cole-Palmer Instruments Co., Chicago, IL).
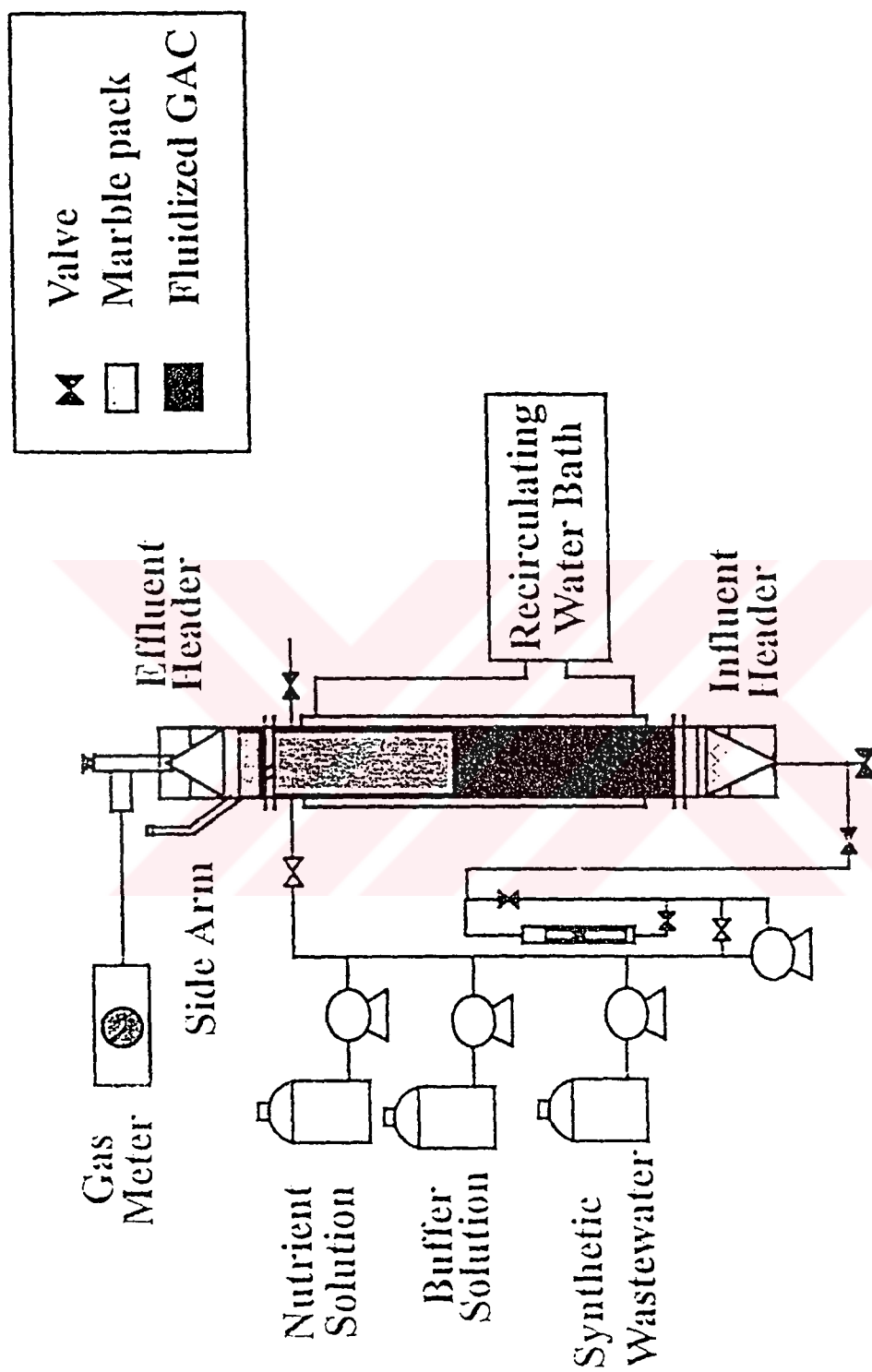
Figure 6.2. Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) Bioreactor

Bacterial growth in the feed reservoirs and feed lines was minimized by separating the growth nutrients and buffer solutions into different feed reservoirs and by pumping the nutrient and buffer solutions into the recycle line at different points in the recycle loop. Power to the Masterflex pumps was channeled through control timers. These on/off timers were used to adjust the flows and to obtain the proper hydraulic retention time. The solutions were supplied to the reactors at regular intervals to maintain uniform conditions in the reactor.

6.2.1.2. Activated sludge reactor

The activated sludge unit consisted of a 17 L plexiglas tank with an up-flow clarifier. Clarification was achieved by means of a plexiglas plate oriented approximately 25° from vertical position which was raised or lowered to adjust the width of the opening between the plate and the reactor wall. Aeration was supplied through a stainless steel diffuser connected to a compressed air source.

6.2.2. Analytical methods

The reactors were monitored daily for feed flow rates, temperature, and effluent pH. Daily anaerobic gas production was measured with a wet tip gas meter (Environmental and Water Research Engineering, Nashville, TN). The off-gas composition was determined weeklyAqueous effluent samples were withdrawn weekly from the anaerobic bioreactor to analyze for total Chemical Oxygen Demand (COD), alcohols, volatile fatty acids (VFAs) and Dissolved Organic Carbon (DOC). Aqueous samples were withdrawn twice weekly from the reactor to analyze for ammonia, and any biotransformation products. Aqueous samples for the various analyses were filtered through 0.45 μm Magna Nylon supported plain filters (Micron Separations Inc., Westboro, MA). DOC samples were filtered through similar 0.22 μm filters.

i. Gas composition: Effluent gas samples from the anaerobic reactor were analyzed for nitrogen, oxygen, carbon dioxide and methane with a Hewlett Packard 5890 Series II Gas Chromatograph with a thermal conductivity detector (TCD). Argon was

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used as the carrier gas through a 3.2 mm ID, 3 m steel column packed with 45/60 molecular sieve (Hewlett-Packard Company, San Fernando, CA).

ii. Chemical oxygen demand: Samples were analyzed for COD in accordance with Standard Method 508C (16th ed.) [45]. Filtered samples were acidified with 85 % o-phosphoric acid to a pH of 2 and purged with prepurified nitrogen for 10 minutes to strip out the sulfide. Prepared Hach COD test vials (range 0-150 mg/L) and a Bausch and Lomb Spectronic 70 spectrophotometer (Bausch & Lomb, U. S. A.) were used for analysis.

iii. Alcohols: Alcohols (iso-propanol, methanol, and ethanol) were analyzed by aqueous injection in a Hewlett Packard 5890 Gas Chromotagraph equipped with flame ionization detectors.

Alcohols were analyzed using a 2 mm ID, 1.83 m glass column packed with 5 % Carbowax 20 M on a 60/80 Carbopack B (Supelco, Inc., Bellefonte, PA) Nitrogen was the carrier gas (20 mL/min.)The oven temperature was maintained at 200 °C. The detection limit for all alcohols was 0.1 mg/L.

iv. Dissolved organic carbon: The pH of the aqueous sample was first reduced below 2 with hydrochloric acidSubsequent purging with nitrogen stripped inorganic carbon as CO_2. The DOC was then determined by injection into a Shimadzu DOC analyzer, model 5000 (Shimadzu Corp., Tokyo, Japan).

v. Ammonia: The ammonia concentration was measured with model 720A Orion pH meter (Orion Research Co., Boston, MA) using a model 13-620-505 ammonia ion selective electrode (Fisher Scientific, Pittsburgh, PA) and an Orion model 215284-A01 ATC probe.

vi. Formaldehyde: Formaldehyde was analyzed by aqueous injection in a Hewlett Packard 5890 Gas Chromatograph equipped with flame ionization detectors.

Formaldehyde was analyzed using a 1/8 inch OD metal packed column, HayesepT, (Supelco Inc., Bellefonte, PA)Nitrogen was the carrier gas (20 mL/min.). The detection limit was 1.0 mg/L. The oven temperature was maintained at 132 °C for 6

minutes and then ramped to 146 °C at a rate of 28 °C/minute. Acetonitrile was used as the internal standard.

vii. Volatile fatty acids: Acetate, propionate, butyrate, iso-butyric acid, and valeric acid were analyzed by a High Performance Liquid Chromatograph (HPLC), Hewlett Packard, 1050 Series, equipped with a Diode Array Detector. Aminex HPX-87H column, 300 mm × 7.8 mm, was used (BIO-RAD, Hercules, CA). Mobile phase employed was 0.01 H_2SO_4 at a flow rate of 0.75 mL/minute. The operating temperature was 45 °CDetection limit of acetate was 1.0 mg/L.

6.3.   Biochemical Methane Potential (BMP) Test

6.3.1.   Materials and methods

6.3.1.1. Anaerobic respirometer

After steady-state operation was obtained in the Anaerobic Fluidized Bed with Granular Activated Carbon (AFBGAC), i.e. the effluent composition of formaldehyde, iso-propanol, methanol, ethanol, acetate, propionate, butyrate, methane production, pH, COD, and the viable biomass concentration (lipid phosphate analysis) in the AFBGAC had been constant, mixed culture from the AFBGAC was transferred to 600 mL batch reactors (N-Con Systems Co., Inc., Larchmont, NY) under anaerobic conditions. All the preparative working steps that had to be necessarily done under absolute anaerobic conditions were carried out in a 1024 Model anaerobic chamber (glove box; Forma Scientific, USA). Anaerobic conditions were established by automatically flushing the working chamber with a gas mixture composed of 93 % N_2 and 7 % H_2. Traces of O_2 were removed by a Pd-catalyzed reaction with H_2, to form water, which was then absorbed on silica gel. The Pd catalyst and drying silica agent that were contained in two wafers, were regenerated weekly by heating them at 160 °C for two hours. Loss of anaerobic conditions was detected by passing the gas through a solution containing 0.5 % yeast extract, 0.1 % cystein-sulfide reducing agent and 0.001 % resazurin indicator. Resazurin was repeatedly added into all batch reactors during their filling in order to be sure that anaerobic conditions were

completely established inside the reactors. The entrance to the anaerobic chamber was preceded by a pre-chamber, in which three successive evacuations and regassing with N_2 were completed. Only then all materials were transferred into the working chamber. Before the acclimated culture was transferred to the batch reactors, each was purged for 3 minutes with a gas mixture of 70 % nitrogen and 30 % carbon dioxide to remove oxygen from the anaerobic chamber. Any traces of oxygen in the purge gas mixture were removed by passing the gas mixture through a heated (450 °C) silica glass tube filled with light copper fillings.

In total 48 grams of mixed acclimated culture (attached biomass on the granular activated carbon) which was collected from the distinct regions of the AFBGAC, mineral and nutrients (Table 6.3), a phosphate buffer (10.8 g/L KH_2PO_4 and 67.68 g/L $Na_2HPO_4.7H_2O$) to keep a constant pH of about 7.2 and in total 7.2 liters effluent from the main reactor was introduced into each batch reactor while purging with the oxygen-free gas mixture in the anaerobic chamber. During the entire experiment different substrates such as formaldehyde, acetate, iso-propanol and their double and triple combinations with different concentrations were injected into the reactors with different sizes of gas-tight syringes (Hamilton Co., Reno, Nev.) to result in different concentrations of substrates. 0.1 mg/L of resazurin was used as an indicator of anaerobic conditionsThe batch reactors were completely filled leaving no head-space remaining in the reactors. The batch tests were conducted in an anaerobic respirometer (Model WB512, N-Con Systems Co., Inc., Larchmont, NY). The temperature in the respirometer was maintained at 35 °C. The respirometer contained twelve 600 mL reactors, and each reactor was well mixed using a magnetic stirrer and stirring bar. When gas is produced in a batch reactor, it produces a positive pressure, which is sensed by a pressure sensor shown in the Figure 6.3.

The pressure sensor electronically signals the opening of a valve that is precisely calibrated with respect to gas volume. The valve is connected to a vacuum line set at - 0.5 psig., which withdraws the excess gas from the reactor in pulses. The computer keeps track of the number of pulses until the pressure returns to atmospheric. The number of pulses is converted into a gas volume and recorded by the computer. Thus, the pressure in the batch reactors is held constant. A computer program controls all

these operations. The gas produced in a reactor consists of methane, carbon dioxide, and moisture. To obtain methane production data, silica gel and KOH traps were used to remove moisture and carbon dioxide respectively (Fig. 6.3). This was done by placing silica gel in one tube followed by another tube containing KOH pellets plus silica gel. The two tubes were connected in line between the reactors and the pressure sensors. The silica gel removes the moisture, the KOH removes the CO_2, and thus, the gas volume recorded by the computer is the volume of methane produced in the reactor.
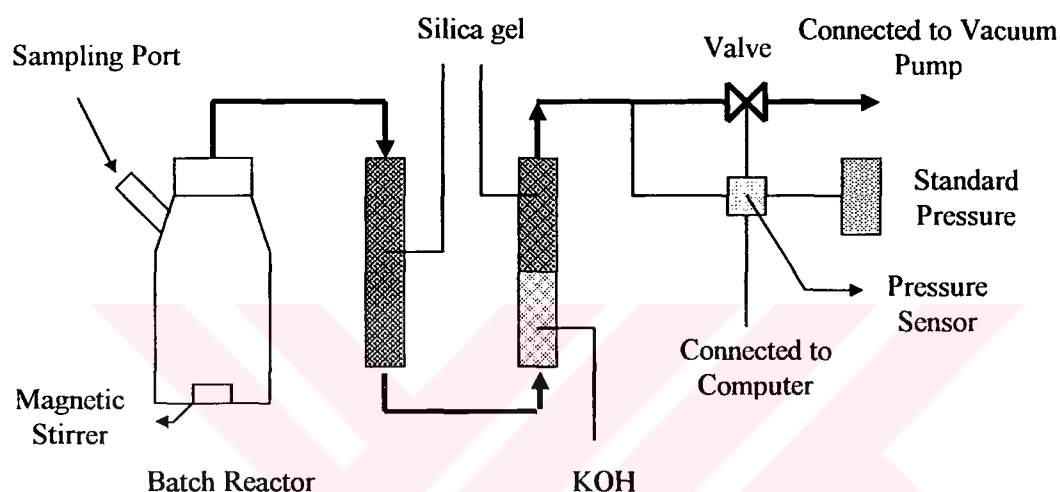
Figure 6.3. Schematic of a batch reactor system for Biochemical Methane Potential tests in anaerobic respirometer.

Different substrates and different concentrations were applied to the batch reactors. Duplicates were run for all samples, including the controls in which there was no main substrate.

6.3.2. Analytical methods

At preassigned intervals (every three hours), aqueous samples were taken from the sampling port of the reactor bottles (Fig. 6.3) with different sizes of gas-tight syringes (Hamilton Co., Reno, Nev.). The sample was replaced with an identical volume of nitrogen pre-purged deionized water to maintain the proper balance of pressure and volume in the reactorsEach sample was immediately acidified to pH 2 with one drop of 85 % phosphoric acid. After a pertinent filtration, formaldehyde, iso-propanol,

methanol, ethanol were quantified using a Hewlett Packard 5890 Series II Gas Chromatograph (GC) with a flame ionization detector (FID).

Formaldehyde was analyzed using a 1/8 inch OD metal packed column, HayesepT, (Supelco Inc., Bellefonte, PA). For 1 mL aqueous filtered sample 8 μL acitonitrile was used as the internal standard and 0.2 μL mixed sample was injected into the column.

A 2 mm ID, 1.83 m glass column packed with 5 % Carbowax 20 M on a 60/80 Carbopack B (Supelco, Inc., Bellefonte, PA) was used to separate the alcohols (iso-propanol, methanol, and ethanol). The mixture of 40 mg/L propanol and 10.5 mg/L standard ethanol served as the internal standard and 1 μL prepared sample was injected into the column. Nitrogen was used as carrier gas in both analyses.

Acetate, propionate and butyrate were quantified by using a High Pressure Liquid Chromatograph (HPLC), Hewlett Packard, 1050 Series, equipped with a Diode Array Detector. Aminex HPX-87H column, 300 mm × 7.8 mm, was used (BIO-RAD, Hercules, CA). Butyric acid served as the internal standard and each time 200 μL prepared sample was injected into the column.

6.4.   Kinetic Assessment

6.4.1.   Introduction

The establishment of kinetic models to describe the biodegradation processes and the estimation of the kinetic parameters can help us understand the intrinsic characteristics of the processes and predict the fate of the organic compounds in certain systems [46, 47], thereby saving significant experimental work and preventing much labor-intensive undertaking. Kinetic models and estimated parameters are also very important in developing the strategies of environmental management and in designing pilot and industrial-scale application systems [48].

The most widely used model for biodegradation kinetics is Monod [37] equation for a non-inhibitory substrate:

$$-\frac{dS}{Xdt} = \frac{\mu}{Y} = \frac{\mu_m}{Y} \cdot \frac{S}{K_s + S} = \frac{kS}{K_s + S} \qquad (6.1)$$

where S is the substrate concentration, X is the biomass concentration, t is the time of biodegradation, μ is the specific growth rate, Y is the yield coefficient, μ_m is the maximum specific growth rate, K_s is the half saturation coefficient, and k is the maximum specific substrate utilization rate. In a biological system there are a series of biochemical reactions catalyzed by enzymes. The Monod kinetic equation is based on an assumption that there is a key enzyme to catalyze the biodegradation in the system and the concentration of the enzyme is proportional to the biomass concentration. Therefore, Michealis-Menten kinetics can be applied to the biodegradation in the system. The enzyme-substrate interactions can be expressed as the following equations:

$$E + S \xleftarrow{\quad K_1 \quad} E.S \qquad (6.2)$$

$$E.S \xrightarrow{\quad k \quad} E + P \qquad (6.3)$$

The Monod kinetic model (Equation 6.1) is developed from the above enzymatic reactions.

The Haldene kinetic model [49] has been frequently used to describe the biodegradation of inhibitory compounds, such as phenolics and aromatics [50, 46, 47]. The Haldene model is also called the substrate inhibition model. It is based upon the hypothesis that the substrate can be combined with the enzyme-substrate complex to form a more complicated complex that inhibits the biodegradation of the substrate itself. The enzymatic reactions can be expressed as the following equations:

$$E + S \xleftarrow{\quad K_1 \quad} E.S \qquad (6.2)$$

$$E.S + S \xleftarrow{\quad K_2 \quad} S.E.S \qquad (6.4)$$

$$E.S \xrightarrow{\ k\ } E + P \tag{6.3}$$

where K_1 and K_2 are equilibrium constants. The Haldane kinetic equation is developed upon these enzymatic reactions with the following expression:

$$-\frac{dS}{Xdt} = \frac{k.S}{K_s + S + \dfrac{S^2}{K_i}} \tag{6.5}$$

where K_i is a substrate inhibition coefficient. K_s and K_i are equivalent to $1/K_1$ and $1/K_2$, respectively.

When there are more than one substrate in a biological system, the substrates may compete with each other for the enzymesThe specific utilization rate of Substrate 1 in a dual substrate biosystem can be expressed with the following competitive inhibition model:

$$-\frac{dS_1}{Xdt} = \frac{k.S_1}{K_s\left(1 + \dfrac{S_2}{K_c}\right) + S_1} \tag{6.6}$$

where S_1 and S_2 are the concentrations of Substrates 1 and 2, respectively, and K_c is the competitive inhibition coefficient. In dual substrate biosystem Substrate 2 competes with Substrate 1 for the active enzymes, thereby inhibiting the degradation of Substrate 1 [51].

In this present study, in order to have the kinetic data, the biodegradability of the formaldehyde, iso-propanol and acetate has been experimentally investigated in an anaerobic respirometer during the BMP (Biochemical Methane Potential) tests. Initially, the biodegradability of these compounds has been examined individually. Subsequently, double and triple substrate combinations were investigated for the same purpose. Finally, a competitive inhibition model has been developed for the anaerobic biodegradation of formaldehyde with addition of iso-propanol as a growth substrate.

6.4.2. Model development

The experimental results from the BMP (Biochemical Methane Potential) tests in an anaerobic respirometer have shown that acetate is converted to methane and carbon dioxide by acetate-utilizing methanogens. Formaldehyde is biotransformed to formic acid and methanol. Throughout the formaldehyde biotransormation experiments in the respirometer, both formic acid and methanol concentrations were very low. This fact signifies that the conversion rate of formic acid and methanol to the final products were very fast and both of them were easy to degrade. Formic acid is further biodegraded to hydrogen and carbon dioxide, while methanol is converted to methane and carbon dioxide. Iso-propanol is converted to acetate and hydrogen by acetogenic bacteria. Some of the hydrogen and acetate together with carbon dioxide already existing in the biosystem are converted by propionate-forming bacteria to form propionate. The rest of the hydrogen is converted to methane by hydrogen-utilizing methanogens. Propionate is converted by the propionate-utilizing bacteria back to hydrogen and acetate, which are further converted to methane by methanogenic bacteria. The biochemical reactions involved in the anaerobic biotransformation of formaldehyde, iso-propanol and acetate are listed in Table 6.4.

When the acclimated anaerobic culture was transferred from the AFBGAC bioreactor to the BMP reactors, there are five types of anaerobic bacteria in the culture: iso-propanol-utilizing acetogens, X_1; propionate-forming bacteria, X_2; hydrogen-utilizing methanogens, X_3; acetate-utilizing methanogens, X_4; and propionate-utilizing bacteria, X_5.

Table 6.4. Biochemical reactions involved in the anaerobic biotransformation of formaldehyde, iso-propanol and acetate.

1. $2 CH_2O$ (I) $+ H_2O \rightarrow CH_2O_2 + CH_3OH$
2. CH_2O_2 (II) $\rightarrow 2 H^+ + CO_2$
3. $4 CH_3OH$ (III) $\rightarrow 3 CH_4$ (IV) $+ CO_2 + 2 H_2O$
4. $2 C_3H_7OH$ (V) $+ 4 H_2O \rightarrow 3 CH_3COO^-$ (VII) $+ H^+ + 7 H_2$ (VI)
5. $3 H_2$ (VI) $+ CH_3COO^-$ (VII) $+ HCO_3^- + H^+ \rightarrow CH_3CH_2COO^-$ (VIII) $+ 3 H_2O$
6. $4 H_2$ (VI) $+ HCO_3^- + H^+ \rightarrow CH_4$ (IV) $+ 3 H_2O$
7. CH_3COO^- (VII) $+ H_2O \rightarrow CH_4$ (IV) $+ HCO_3^-$
8. $CH_3CH_2COO^-$ (VIII) $+ 3 H_2O \rightarrow 3 H_2$ (VI) $+ CH_3COO^-$ (VII) $+ HCO_3^- + H^+$

In describing many biochemical reactions, both reactants and products need to be considered when their concentrations are relatively high and t=0 is an initial condition. These cases happen in biochemical processes such as fermentation of ethanol, butanol, and acetic acid. However, in most environmental systems, both reactants and products are at low concentrations. Therefore, the effect of products is usually negligible. In this present study, all the kinetic expressions have been expressed by this way of thinking.

Concerning the inhibition models in this study, there are two types of inhibition involved in expressing the kinetic expressions. One of them is substrate inhibition and the other one is competitive inhibition. Substrate inhibition or Haldane inhibition occurs when the substrate inhibits its own biotransformation (Equation 6.5). Competitive inhibition happens when another substrate or compound competes for the enzymes against the substrate whose biotransformation is kinetically expressed (Equation 6.6). In the present kinetic expressions, K_i and K_c were used for the substrate and competitive inhibition coefficients, respectively.

Initially, iso-propanol and formaldehyde are the only substrates in the AFBGAC bioreactor and iso-propanol is the sole growth substrate that can support the growth of the bacteria. Therefore, iso-propanol-utilizing acetogenic bacteria are the dominant microorganisms in the initial period of the corresponding reactions. Formaldehyde is a toxic and inhibitory organic compound. Its inhibitory or toxic effect changes with concentration. It inhibits its own biotransformation as well as the acetogenesis of iso-propanol and the biodegradation of acetate. It is reasonable to assume that iso-propanol-utilizing acetogens provide enzymes to biotransform formaldehyde. Herein, the kinetic expression of formaldehyde has been defined by the Haldane-single substrate inhibition model.

The kinetics of formaldehyde biotransformation can be expressed as the following equation:

$$R_1 = k_1 . X_1 \frac{S_1}{K_{s1} + S_1 + \dfrac{S_1^2}{K_{i1}}} \qquad (6.7)$$

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where R_1 is the rate of formaldehyde biotransformation in mM/hr, k_1 is the maximum specific transformation rate in hr^{-1}, X_1 is the concentration of iso-propanol-utilizing acetogens in mM, S_I is the concentration of formaldehyde in mM, K_{s1} is the half saturation coefficient of formaldehyde in mM, K_{i1} is the substrate inhibition coefficient in mM.

Experimental results showed that formaldehyde is biotransformed to formic acid and methanol. During the sampling intervals (every three hours) of the respirometer experiments, it was observed that there was no accumulation of both formic acid and methanol in the system. Both formic acid and methanol concentrations were very low. Therefore, it was concluded that the conversion rate of formic acid and methanol to the final products were very fast. This fact signifies that there was no inhibition effect of formaldehyde on the intermediate compounds. Due to the easy biodegradation of formic acid and methanol in the biosystem, no individual substrate inhibition effects on both formic acid and methanol have been observed as well. Thus, simply Monod equations were applied for the biodegradation of formic acid and methanol. However, due to their very low production concentrations, both of them were omitted in the related kinetic expressions.

The kinetics of formic acid and methanol biotransformation are expressed as Equations 6.8 and 6.9, respectively.

$$R_2 = k_2.X_1 \frac{S_{II}}{K_{s2} + S_{II}}$$ (6.8)

$$R_3 = k_3.X_1 \frac{S_{III}}{K_{s3} + S_{III}}$$ (6.9)

where R_2 is the rate of formic acid biodegradation in mM/hr, k_2 is the maximum specific transformation rate for formic acid in hr^{-1}, S_{II} is the concentration of formic acid in mM, K_{s2} is the half saturation coefficient of formic acid in mM, R_3 is the rate of methanol biodegradation in mM/hr, k_3 is the maximum specific transformation rate for methanol in hr^{-1}, S_{III} is the concentration of methanol in mM, K_{s3} is the half saturation coefficient of methanol in mM.

The experimental data reveal that the biodegradation of iso-propanol is less inhibited when it couples with formaldehyde in the environment. The self-biodegradation of iso-propanol shows more inhibition nearly at the same concentration. Since there is not any accumulation of formic acid and methanol in the biosystem, it can be regarded that these compounds do not compete for the active enzymes against iso-propanol. Despite the inhibitions are at the same order of magnitude when the double substrate biosystem is concerned (iso-propanol (P)-formaldehyde (F)), the only difference appears in the biodegradation rate of iso-propanol. Higher formaldehyde initial concentrations prolong the biodegradation time of the iso-propanol as well as formaldehyde.

The kinetics of iso-propanol biodegradation can be expressed by the following equation:

$$R_4 = k_4 \cdot X_1 \frac{S_v}{K_{s4}\left(1 + \frac{S_1}{K_{c4a}}\right) + S_v + \frac{S_v^2}{K_{i4}}} \tag{6.10}$$

where R_4 is the rate of iso-propanol biodegradation in mM/hr, k_4 is the maximum specific biodegradation rate in hr^{-1}, S_v is the concentration of iso-propanol in mM, K_{s4} is the half saturation coefficient of iso-propanol in mM, K_{c4a} is the competitive inhibition coefficient of formaldehyde against iso-propanol in mM, K_{i4} is the substrate inhibition coefficient in mM.

The rate of propionate formation is dependent on the concentrations of acetate and hydrogen. Bicarbonate is overabundant in the biosystem. Formaldehyde may inhibit the main compound of acetate, thus at the same time, the formation of propionate by blocking the active sites of the enzymes, which catalyze the biochemical reaction. Due to the easy biodegradation of formic acid and methanol in the biosystem, these compounds do not exhibit inhibition to the formation of propionate. Based on the experimental results of the competitive inhibition model, it was determined that hydrogen didn't show inhibition to the formation of propionate (see Figure 7.56). Therefore, a simple Monod kinetic equation is used for the hydrogen as a co-substrate.

The kinetics of propionate formation can be expressed as in the following equation:

$$R_5 = k_5 . X_2 \frac{S_{VII}}{K_{s75}\left(1 + \dfrac{S_I}{K_{c5a}}\right) + S_{VII}} \cdot \frac{S_{VI}}{K_{s65} + S_{VI}} \qquad (6.11)$$

where R_5 is the rate of propionate formation in mM/hr, k_5 is the maximum specific reaction rate in hr^{-1}, X_2 is the concentration of propionate-forming bacteria in mM, S_{VII} is the concentration of acetate in mM, S_{VI} is the concentration of hydrogen in mM, K_{s75} is the half saturation coefficient of acetate for propionate formation in mM, K_{c5a} is the competitive inhibition coefficient of formaldehyde against acetate for propionate formation in mM, and K_{s65} is the half saturation coefficient of hydrogen for propionate formation in mM.

The hydrogen and acetate produced from the acetogenesis of iso-propanol are converted to methane by the hydrogen-utilizing and acetate-utilizing methanogenic bacteria, respectively.

Formaldehyde may inhibit both methanogeneses of hydrogen and acetate. Hydrogen might exhibit competitive inhibition to the methanogenesis of acetate if they compete for the same enzymes. Based on the literature [52], acetate was not reported as an inhibitor for methanogenesis of hydrogen. However, some researchers indicated that high hydrogen level could inhibit the methanogenesis of acetate. Therefore, no acetate inhibition term was used in Equation 6.12, but a hydrogen inhibition term was used in Equation 6.13. In this present study, it was concluded that hydrogen was not inhibiting the methanogenesis of acetate ($K_{c7b}=$ infinity).

The kinetics of both methanogeneses of hydrogen and acetate can be expressed as Equations 6.12 and 6.13, respectively.

$$R_6 = k_6 . X_3 \frac{S_{VI}}{K_{s6}\left(1 + \dfrac{S_I}{K_{c6a}}\right) + S_{VI}} \qquad (6.12)$$

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$$R_7 = k_7.X_4 \frac{S_{VII}}{K_{s7}\left(1 + \dfrac{S_I}{K_{c7a}} + \dfrac{S_{VI}}{K_{c7b}}\right) + S_{VII}} \tag{6.13}$$

where R_6 is the rate of hydrogen utilization in mM/hr, k_6 is the maximum specific utilization rate for the methanogenesis of hydrogen in hr^{-1}, X_3 is the concentration of hydrogen-utilizing methanogens in mM, K_{s6} is the half saturation coefficient of hydrogen in mM, K_{c6a} is the competitive inhibition coefficient of formaldehyde against hydrogen in mM, R_7 is the rate of acetate utilization in mM/hr, k_7 is the maximum specific utilization rate for the methanogenesis of acetate in hr^{-1}, X_4 is the concentration of acetate-utilizing methanogens in mM, K_{s7} is the half saturation coefficient of acetate in mM, K_{c7a} is the competitive inhibition coefficient of formaldehyde against acetate in mM, K_{c7b} is the competitive inhibition coefficient of hydrogen against acetate in mM.

Propionate is then converted back to hydrogen and acetate, which are further converted to methane.

Based on the experimental results of the competitive inhibition model, it was concluded that the biodegradation of propionate is much slower than the methanogenesis of either hydrogen or acetate (see Figure 7.57). Thus, it is the limiting reaction step of the process from propionate to methane. According to the experimental results, the presence of acetate strongly inhibits the biodegradation of propionate. In the kinetic expression of propionate biodegradation, a competitive inhibition term related with formaldehyde has not been used against propionate. Because both hydrogen and acetate form propionate and the competitive inhibition effect of formaldehyde had already been shown against to the propionate formation, hydrogen-utilizing methanogenesis, acetate-utilizing methanogenesis as well as the iso-propanol-utilizing acetogenesis. Based on the experimental results, it was concluded that formaldehyde has exhibited very strong competitive inhibition effects to the above-mentioned compounds.

The kinetics of propionate degradation can be expressed as in the following equation:

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$$R_8 = k_8 . X_5 \frac{S_{VIII}}{K_{s8} \left(1 + \frac{S_{VI}}{K_{c8a}} + \frac{S_{VII}}{K_{c8b}} \right) + S_{VIII}} \qquad (6.14)$$

where R_8 is the rate of propionate biodegradation in mM/hr, k_8 is the maximum specific biodegradation rate in hr^{-1}, X_5 is the concentration of propionate-utilizing bacteria in mM, S_{VIII} is the concentration of propionate in mM, K_{s8} is the half saturation coefficient of propionate in mM, K_{c8a} is the competitive inhibition coefficient of hydrogen against propionate in mM, K_{c8b} is the competitive inhibition coefficient of acetate against propionate in mM.

The reaction rates of all the substrates and the products and the microbial growth rates are expressed as the following equations:

Formaldehyde Reaction Rate: $dS_I / dt = - 2.0 . R_1$ $\qquad$ (6.15)

Formic acid Reaction Rate: $dS_{II} / dt = 0.5 . R_1 - R_2$ $\qquad$ (6.16)

Methanol Reaction Rate: $dS_{III} / dt = 0.5 . R_1 - 4.0 . R_3$ $\qquad$ (6.17)

Methane Production Rate: $dS_{IV} / dt = 0.75 . R_3 + 0.25 . R_6 + R_7 + 1.75 . R_8$ $\qquad$ (6.18)

Iso-propanol Reaction Rate: $dS_V / dt = - 2.0 . R_4$ $\qquad$ (6.19)

Hydrogen Reaction Rate: $dS_{VI} / dt = -3.0 . R_5 - 4.0 . R_6 + 7.0 . R_4 + 3.0 . R_8$ $\qquad$ (6.20)

Acetate Reaction Rate: $dS_{VII} / dt = 3.0 . R_4 - R_5 - R_7 + R_8$ $\qquad$ (6.21)

Propionate Reaction Rate: $dS_{VIII} / dt = R_5 - R_8$ $\qquad$ (6.22)

Growth Rate of Iso-propanol-Utilizing Acetogens:

$$dX_1 / dt = Y_1 . R_4 - B_1 . X_1 \qquad (6.23)$$

Growth Rate of Propionate-Forming Bacteria:

$$dX_2 / dt = Y_2 . R_5 - B_2 . X_2 \qquad (6.24)$$

Growth Rate of Hydrogen-Utilizing Methanogens:

$$dX_3 / dt = Y_3 . R_6 - B_3 . X_3 \qquad (6.25)$$

Growth Rate of Acetate-Utilizing Methanogens:

$$dX_4 / dt = Y_4 . R_7 - B_4 . X_4 \qquad (6.26)$$

Growth Rate of Propionate-Utilizing Bacteria:

$$dX_5 / dt = Y_5 . R_8 - B_5 . X_5 \qquad (6.27)$$

where Y_1, Y_2, Y_3, Y_4, and Y_5, are the yield coefficients of the corresponding anaerobic bacteria, and B_1, B_2, B_3, B_4, and B_5, are the endogenous decay coefficients of the corresponding bacteria in hr^{-1}.

6.5.   Lipid Phosphate Analysis

6.5.1.   Quantification of viable biomass

Determining the viable biomass of a microbial community provides an estimate of the amount of active microorganisms in a particular environment and, therefore, the capability for metabolic transformations in that environment. The viable biomass of a microbial community is determined by measuring a cellular component that is common to all cells of the microbiota and quickly degraded on cell death.

The method is based on the extraction of lipids from bacterial membranes. Phospholipids are cellular constituents that are universally distributed among micro-organismsThey form an essential component of the cell membrane, where they are arranged in a bilayer to form a fluid, liquid-crystalline matrix.

Contrary to the storage material, the cellular content of the phospholipids remains remarkably constant in a wide variety of monocultures under stresses compatible with survival conditions in nature. The phospholipids have an active metabolism, which was observed during the growth of bacteria in monocultures. The phosphate incorporated into the microbial phospholipids has a relatively rapid turnover (in the viable cells), i.e. a time to lose half of the lipid phosphate of about 2-10 days for

aerobic and 12 days for anaerobic bacteria. Therefore, phospholipids are considered to be related to the active (viable) microbial biomass content [53].

Determining the total phospholipid fatty acid (PLFA) composition can provide an estimate of microbial community biomass. A typical phospholipid contains 2 moles of fatty acids per mole of phospholipid: thus, by dividing the total fatty acid composition by 2, it is possible to determine the phospholipid biomass [54].

6.5.2. Lipid analysis in microbial ecology

6.5.2.1. Extraction of lipids

For the study of microbial communities, lipid analysis basically entails the extraction of lipids from a sample with organic solvents followed by analysis of certain fractions of the extracted material. The extraction and analysis are straightforward. In the field or laboratory, the sample is exposed to a single-phase mixture of chloroform, methanol, and water in an initial ratio of 1:2:0.8. When these solvents are added, the lipids dissolve almost instantly and further lipid metabolism stops. This technique provides a snapshot of the lipids at the time of extraction. After a short period of extraction (approximately 2 hours) in this mono-phasic system, water and chloroform are added to separate the phases by changing the polarity of the mixture. The total lipid fraction will be found in the lower chloroform phase, whereas the more polar proteins, nucleic acids, cell walls, and other components remain in the upper methanol-water phase or at the chloroform-water interface.

As diagrammed in Figure 6.4, the organic (lipid-containing) phase can be further fractionated into phospholipids for analysis of community structure and biomass, the residue at the interface can be used to measure the Gram negative, Gram positive, and total eubacterial biomass.

The original sample can also be incubated for a short time with ^{14}C isotopes and the lipids extracted to measure the incorporation of ^{14}C as an indication of microbial metabolic activity. Or the sample can be extracted with boiling chloroform and the intracellular storage molecules measured as an indication of metabolic or nutritional stress.

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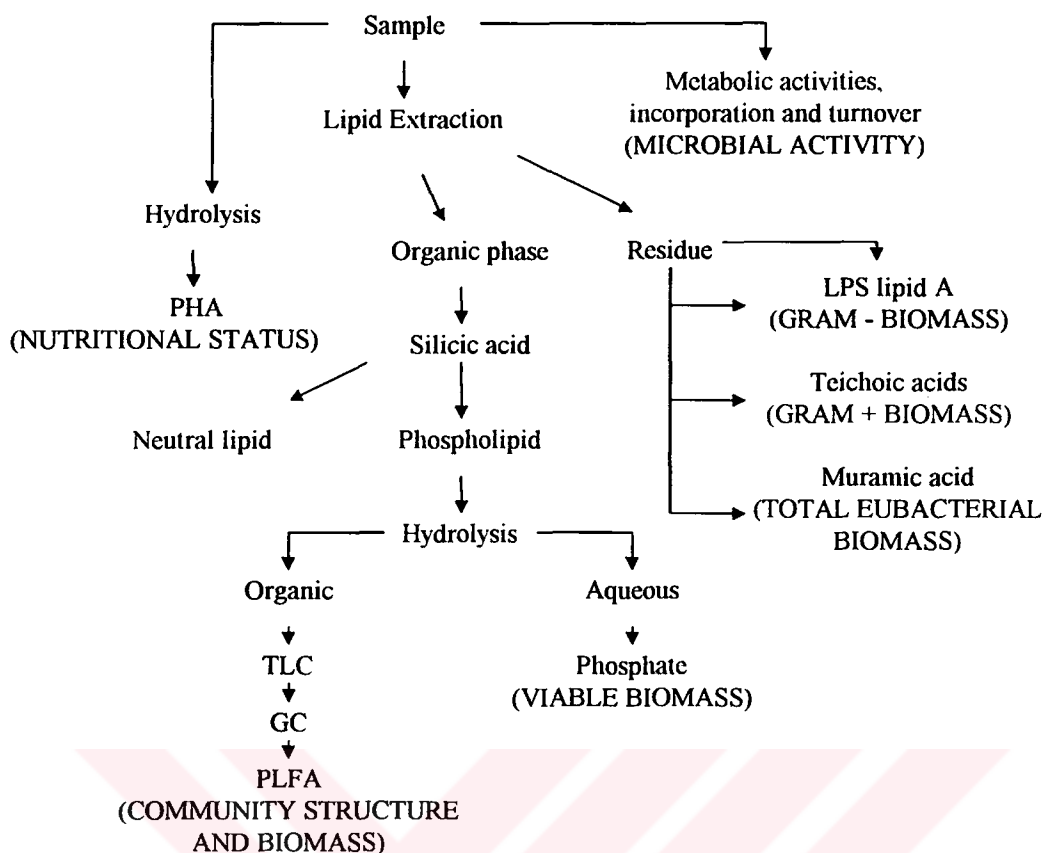
Figure 6.4. Flow diagram for the biochemical analysis of natural microbial communities using lipids [54].

6.5.3. The importance of Phospholipid Fatty Acids (PLFA) analysis

1) Polar lipids are critical membrane components of all cells. The polar lipids of bacterial cell membranes are exclusively phospholipids. When the cell dies internal and external phospholipase enzymes hydrolyze the phospholipid into a diglyceride and phosphate group. Therefore, PLFA analysis is able to define the "viable biomass" as the phospholipid fatty acids which are a measure of all cells present in the sample that have an intact membrane.

2) Phospholipid fatty acid analysis can also be used to identify the different classes and types of microorganisms giving the "community structure" of the sample. The relationship between the different communities can be compared and defined quantitatively by statistical analysis. It is also possible to follow trends within one community over any given time period. In addition, many important microbes

have unique PLFA by which they can be identified. These include sulfate-reducing bacteria, Actinomycetes, various pathogens (such as legionella) of algae, fungi and protozoa to name a few.

3) Phospholipid fatty acid analysis can also indicate the nutritional status of a microbial community. When microbes suffer nutritional stress they accumulate specific PLFA, with ratios of precursor to product providing an estimate of the degree of the stress. Calculation of the different PLFA ratios indicates the state of growth and the exposure level of the microbes to environmental stresses such as toxicity or starvation [55].

6.5.4. Lipid phosphate procedure

6.5.4.1. Reagents

1) Persulfate digestion reagent: 5 % potassium persulfate in 0.36 N sulfuric acid (5 g/100 mL 0.36 N sulfuric acid).

2) Ammonium molybdate reagent: 2.5 %, (2.5 g $(NH_4)_6Mo_7O_{24}$ - 4 H_2O/100 mL 5.72 N H_2SO_4).

3) Malachite green reagent: dissolve 0.111 % polyvinyl alcohol (PVA) in water at 80 °C, cool, then add 0.011 % malachite green.

4) 0.36 N sulfuric acid: CAREFULLY dilute concentrated sulfuric acid 1/100 (1 mL acid + 99 mL water).

5) 5.72 N sulfuric acid: CAREFULLY dilute concentrated sulfuric acid 1/6.3 (10 mL acid + 53 mL water).

6) 2 mL Wheaton glass ampoules.

6.5.4.2. Procedure

1. Except for that which is disposable, acid washed and well rinsed with deionized water glassware is used.

2. 0.25 g attached biomass onto GAC is dissolved by 2 mL deionized water and combined with 2.5 mL of reagent grade chloroform, and 5 mL of reagent grade methanol.

3. The above solution is agitated until it forms one phase and it is let to stay for at least 2 hours.

4. 2.5 mL reagent grade chloroform and 2.5 mL 0.0306 M H_2SO_4 are added.

5. The solution is agitated and is formed two-phase mixture. At least 18 hours is paused.

6. The chloroform layer is removed by using a disposable Pasteur pipet and is placed in a disposable 5 mL Wheaton ampoule.

7. The chloroform is dried off with oxygen free nitrogen gas by using a special gassing manifold (Figure 6.5).
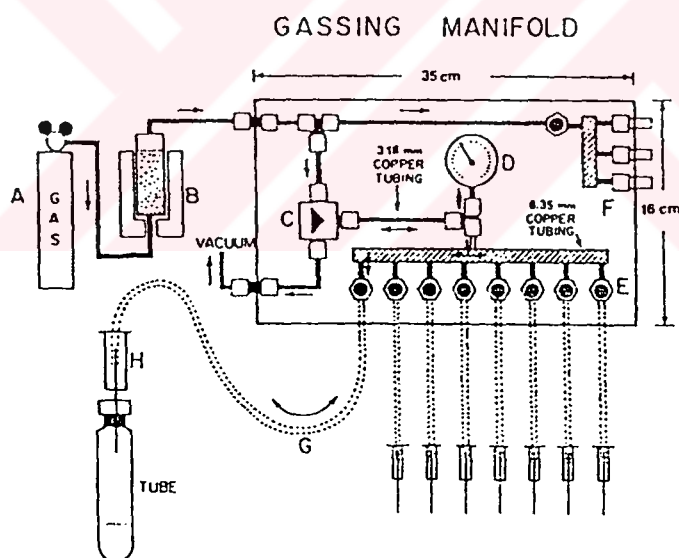
Figure 6.5. Gassing manifold for head-space gas flushing in growth and test vials [4].

8. 0.9 mL persulfate digestion reagent is added.

9. The ampoules (every trial must be done by using at least triplicates for each ampoule) are sealed and heated for at least 18 hours at 105 °C.

10. The ampoules are cooled to room temperatureThereafter, they are opened and 0.2 mL ammonium molybdate reagent is added.

11. The ampoules are allowed to sit for 10 minutes and 0.9 mL malachite green reagent is added. 30 minutes must be waited before analyzing the samples.

12. Samples are analyzed spectrophotometrically at 610 nm using a minimum 7 point standard curve in triplicateThe standard curve is produced using ampoules containing the following amounts of K_2HPO_4:

Stock standard: 0.05 M K_2HPO_4 (MW 174.18) = 8.709 g/L or 1.742 g/200 mL

Standard curve:

µL/ampoule:	Blank	10	20	30	50	70	100
nmol/ampoule:	0	5.0	10.0	15.0	25.0	35.0	50.0

The r^2 value of the standard curve should be at least 0.990 [56].

7. RESULTS AND DISCUSSION

7.1. Experiments Conducted in the Model Reactor

Experiments have been performed mainly in an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor with a 10 L combined volume of the anaerobic compartment and recycle line (see chapter 6, Figure 6.2). Feed flow rates of the buffer solution, nutrient solution and waste stream, temperature, effluent pH, and gas production were monitored daily in the anaerobic bioreactor. Off-gas composition was analyzed weekly by using a gas chromatograph equipped with a thermal conductivity detector (TCD). Aqueous effluent samples were taken weekly and analyzed for alcohols, formaldehyde, Volatile Fatty Acids (VFA), Chemical Oxygen Demand (COD), Dissolved Organic Carbon (DOC), ammonia, and nitrate in order to determine the effectiveness of the bioreactor in treating the waste stream which was synthetically composed of formaldehyde and iso-propanol. After steady state was achieved, effluent concentrations of both iso-propanol and formaldehyde were reduced by more than 99.9 % mostly in the first stage alone. Subsequently, the activated sludge reactor was discontinued on the 325th day and the rest of the experiments were conducted in the AFBGAC bioreactor alone.

The operational data and the effectiveness of the biological treatment system are shown in Tables 7.1 and 7.2, and in Figures 7.1 to 7.9, respectively. System (anaerobic + aerobic) reductions of iso-propanol and formaldehyde are shown in Figures 7.1 and 7.2, respectively. As can be seen in these figures, in the inception of the experiment, iso-propanol was the only substrate that was fed into the bioreactor with an initial concentration of approximately 295 mg/L, which was gradually increased, to approximately 2950 mg/L over a period of 4 months. Subsequently, formaldehyde feeding was launched with an initial concentration of about 27 mg/L which was gradually increased to 1474 mg/L over another 2 months period.

Table 7.1. Operational data in the Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor.

Day	Buffer Flow (L/day)	Waste Flow (L/day)	Nutrient Flow (L/day)	Waste: Iso - propanol				Waste: Formaldehyde			
				Influent (mg/L)	Influent (mg/day)	Effluent* (mg/L)	Effluent* (mg/day)	Influent (mg/L)	Influent (mg/day)	Effluent* (mg/L)	Effluent* (mg/day)
0											
3	0.31	4.90	1.00	294.80	1444.52	11.45	71.09	0.00	0.00	0.00	0.00
10	0.32	4.92	1.00	294.80	1450.42	27.41	171.04	0.00	0.00	0.00	0.00
17	0.31	4.94	1.00	294.80	1456.31	24.77	154.81	0.00	0.00	0.00	0.00
24	0.31	4.78	0.98	294.80	1409.14	24.66	149.69	0.00	0.00	0.00	0.00
31	0.31	4.78	0.98	294.80	1409.14	0.00	0.00	0.00	0.00	0.00	0.00
38	0.31	4.78	0.98	294.80	1409.14	12.50	75.88	0.00	0.00	0.00	0.00
45	0.31	4.78	0.98	294.80	1409.14	0.00	0.00	0.00	0.00	0.00	0.00
52	0.31	4.78	0.98	294.80	1409.14	0.00	0.00	0.00	0.00	0.00	0.00
59	0.30	4.90	1.10	294.80	1444.52	0.00	0.00	0.00	0.00	0.00	0.00
66	0.30	4.78	1.08	294.80	1409.14	0.00	0.00	0.00	0.00	0.00	0.00
73	0.33	4.77	1.04	294.80	1406.20	14.07	86.39	0.00	0.00	0.00	0.00
80	0.70	4.88	1.07	294.80	1438.62	0.00	0.00	0.00	0.00	0.00	0.00
87	0.69	4.75	0.98	589.60	2800.60	0.47	3.00	0.00	0.00	0.00	0.00
94	0.71	4.96	1.06	884.40	4386.62	0.33	2.22	0.00	0.00	0.00	0.00
101	0.71	4.95	1.07	1179.20	5837.04	0.22	1.49	0.00	0.00	0.00	0.00
108	0.72	4.88	1.00	1474.00	7193.12	0.15	0.99	0.00	0.00	0.00	0.00
115	0.70	4.74	1.07	2063.60	9781.46	0.44	2.86	0.00	0.00	0.00	0.00

Continuation:

122	0.72	5.00	1.03	2948.00	14740.00	0.48	3.24	26.80	134.00	0.00	0.00
129	0.71	4.97	1.01	2948.00	14652.00	0.77	5.15	67.00	332.99	0.00	0.00
136	0.70	4.50	0.97	2948.00	13266.00	2.62	16.17	67.00	301.50	1.95	12.03
143	0.75	4.63	1.06	2948.00	13649.00	0.95	6.12	67.00	310.21	4.24	27.29
150	0.74	4.88	1.06	2948.00	14386.00	0.32	2.10	67.00	326.96	7.15	47.76
157	0.78	4.94	1.02	2948.00	14563.00	0.00	0.00	67.00	330.98	0.14	0.92
164	0.81	4.94	0.86	2948.00	14563.00	1.55	10.26	134.00	661.96	26.13	172.72
171	0.94	4.86	1.04	2948.00	14327.00	0.67	4.56	268.00	1302.48	51.30	350.89
178	1.02	4.85	1.02	2948.00	14298.00	1.75	12.05	536.00	2599.60	3.63	25.70
185	1.52	4.92	1.03	2948.00	14504.00	1.10	8.21	1072.00	5288.18	38.04	278.41
192	1.34	4.93	1.02	2948.00	14542.00	2.99	21.81	1474.00	7134.16	26.62	199.03
199	1.76	4.86	1.01	2948.00	14327.00	7.87	60.09	1474.00	7163.64	1.33	10.16
206	1.99	4.88	1.05	2948.00	14395.00	1.36	10.78	1474.00	7197.54	1.26	9.96
213	1.60	4.94	1.04	2948.00	14563.00	1.27	9.66	1474.00	7281.56	3.03	23.01
220	0.80	4.86	1.03	2948.00	14327.00	0.35	2.33	1474.00	7163.64	1.28	8.59
227	0.83	5.00	1.03	2948.00	14740.00	0.49	3.33	1474.00	7370.00	3.88	26.64
230	0.78	4.80	1.03	2948.00	14150.00	0.84	5.58	1474.00	7075.20	1.67	11.03
234	0.75	5.00	1.00	2948.00	14740.00	0.66	4.47	1474.00	7370.00	1.59	10.73
237	0.78	4.93	1.03	2948.00	14543.00	0.52	3.53	1474.00	7271.73	1.25	8.40
241	0.80	5.00	1.05	2948.00	14740.00	0.35	2.42	1474.00	7370.00	0.00	0.00
244	0.79	4.98	1.01	2948.00	14691.00	0.00	0.00	1474.00	7345.38	0.00	0.00
248	0.83	4.97	1.03	2948.00	14640.00	0.48	3.28	1474.00	7319.88	0.00	0.00
251	0.75	4.90	1.08	2948.00	14445.00	1.60	10.76	1474.00	7222.60	0.00	0.00
255	0.83	4.35	0.90	2948.00	12824.00	79.61	483.63	1474.00	7370.00	63.72	428.52
257	-	-	-	-	-	295.50	-	-	-	297.00	-

Continuation:

258	-	-	-	-	-	136.15	-	-	-	24.37	-
259	-	-	-	-	-	73.27	-	-	-	-	-
260	-	-	-	-	-	44.58	-	-	-	6.01	-
261	-	-	-	-	-	68.17	-	-	-	5.59	-
262	0.80	4.80	0.90	1474.00	7075.20	166.63	758.10	67.00	321.60	0.50	3.23
263	0.00	5.00	1.00	1474.00	7370.00	153.20	919.20	67.00	335.00	0.46	3.10
264	0.80	5.00	1.00	737.00	3685.00	201.44	1369.79	67.00	335.00	0.32	2.19
265	0.15	5.00	0.20	737.00	3685.00	168.73	902.71	67.00	335.00	0.13	0.68
267	-	-	-	-	-	60.68	-	-	-	0.36	-
268	0.75	4.90	1.20	737.00	3611.30	53.86	368.93	67.00	328.30	3.82	26.17
269	0.80	4.90	1.00	737.00	3611.30	38.58	258.48	67.00	328.30	0.00	0.00
270	0.80	5.00	1.10	737.00	3685.00	18.01	124.27	67.00	335.00	0.00	0.00
271	0.80	5.00	1.10	737.00	3685.00	2.62	18.09	294.80	1474.00	10.57	72.91
272	0.80	5.00	1.10	1474.00	7370.00	8.67	59.82	294.80	1474.00	36.46	251.57
273	0.80	4.90	1.00	1474.00	7222.60	-	-	294.80	1444.52	0.00	0.00
274	0.80	4.70	1.00	1474.00	6927.80	4.55	29.56	294.80	1385.56	4.96	32.25
275	0.75	4.90	1.00	1474.00	7222.60	3.14	20.86	737.00	3611.30	2.64	17.54
276	0.85	5.00	1.10	2948.00	14740.00	15.10	104.93	1474.00	7370.00	2.04	14.14
277	0.80	5.00	1.00	2948.00	14740.00	10.29	69.99	1474.00	7370.00	-	-
278	0.80	5.00	1.10	2948.00	14740.00	5.11	35.27	1474.00	7075.20	1.70	11.70
283	0.83	4.80	1.00	2948.00	14150.00	1.70	11.23	1474.00	7075.20	0.00	0.00
286	0.80	4.70	1.03	2948.00	13856.00	1.33	8.67	1474.00	7222.60	0.00	0.00
290	0.80	4.90	1.03	2948.00	14445.00	1.01	6.80	1474.00	7443.70	2.45	16.48
297	0.83	5.05	1.00	2948.00	14887.00	0.65	4.50	1474.00	7266.82	0.00	0.00
300	0.79	4.93	1.00	2948.00	14534.00	-	-	1474.00	7296.30	1.01	6.79

Continuation:

304	0.80	4.95	1.06	2948.00	14593.00	0.89	6.05	1474.00	7443.70	0.96	6.57
311	0.80	5.05	1.03	2948.00	14887.00	0.70	4.80	1474.00	15330.00	1.97	13.56
314	1.60	10.40	1.30	2948.00	30659.00	3.49	46.40	1474.00	8917.70	1.85	24.61
316	0.98	6.05	1.13	2948.00	17835.00	1.11	9.07	1474.00	8210.18	2.01	16.38
318	0.93	5.57	1.20	2948.00	16420.00	0.62	4.74	1474.00	9698.92	2.14	16.50
321	1.12	6.58	1.43	2948.00	19398.00	1.06	9.67	1474.00	11424.00	2.42	22.05
325	1.30	7.75	1.43	2948.00	22847.00	1.39	14.51	1474.00	12345.00	0.00	0.00
328	1.43	8.38	1.47	2948.00	24690.00	0.85	9.62	1474.00	12345.00	2.98	33.64
332	1.60	9.83	1.83	2948.00	28979.00	1.10	14.53	1474.00	14489.00	2.76	36.61
339	1.66	9.85	2.00	2948.00	29038.00	1.11	15.02	1474.00	14519.00	0.47	6.31
342	1.73	10.43	2.03	2948.00	30748.00	0.78	11.07	1474.00	15374.00	2.99	42.43
346	1.60	10.35	1.95	2948.00	30512.00	0.59	8.24	1474.00	15256.00	2.99	41.53
349	1.70	10.23	1.90	2948.00	30158.00	0.65	8.98	1474.00	15079.00	1.92	26.50
353	1.70	9.87	1.93	2948.00	29097.00	0.64	8.67	1474.00	14548.00	0.61	8.19
356	1.67	10.13	2.05	2948.00	29863.00	0.55	7.66	1474.00	14932.00	0.56	7.77
360	1.67	10.30	1.90	2948.00	30364.00	0.75	10.43	1474.00	15182.00	0.60	8.35
363	1.70	10.35	1.95	2948.00	30512.00	1.54	21.50	1474.00	15256.00	0.87	12.12
367	1.68	10.43	2.03	2948.00	30748.00	0.89	12.64	1474.00	15374.00	1.33	18.75
370	1.75	10.70	1.90	2948.00	31544.00	0.65	9.37	1474.00	15772.00	1.30	18.64
374	1.75	10.90	1.90	2948.00	32133.00	0.60	8.67	1474.00	16067.00	0.00	0.00
375	1.10	7.10	1.50	2948.00	20931.00	56.83	551.29	1474.00	10465.00	2.01	19.54
376	1.80	10.40	2.00	2948.00	30659.00	401.21	5697.14	1474.00	15330.00	0.49	6.99
381	1.35	8.20	1.50	1474.00	12087.00	4.21	46.49	737.00	6043.00	2.57	28.39
382	1.75	11.00	2.20	1474.00	16214.00	8.28	123.85	737.00	8107.00	2.02	30.20
383	1.85	11.20	1.70	1474.00	16509.00	4.87	71.85	737.00	8254.00	-	-

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Continuation:

384	1.70	10.00	1.50	2211.00	22110.00	4.04	53.29	1106.00	11060.00	1.65	21.77
386	1.65	10.00	1.80	2211.00	22110.00	6.54	88.00	1106.00	11060.00	-	-
388	1.85	11.17	1.97	2948.00	32929.00	10.10	151.41	1474.00	16465.00	0.82	12.31
391	1.80	11.00	2.00	2948.00	32428.00	1.12	16.61	1474.00	16214.00	0.55	8.13
395	1.83	11.15	1.90	2948.00	32870.00	0.51	7.59	1474.00	16435.00	0.16	2.37
402	1.87	11.05	1.90	2948.00	32575.00	0.54	8.05	1474.00	16288.00	1.45	21.52
405	1.80	11.00	1.90	2948.00	32428.00	0.56	8.16	1474.00	16214.00	1.57	23.11
409	1.85	10.93	2.03	2948.00	32222.00	0.71	10.50	1474.00	16111.00	0.71	10.46
412	1.82	10.80	1.93	2948.00	31838.00	0.47	6.77	1474.00	15919.00	0.53	7.74
416	1.83	10.75	1.93	2948.00	31691.00	0.74	10.71	1474.00	15846.00	0.73	10.65
419	1.82	11.10	2.03	2948.00	32723.00	0.51	7.56	1474.00	16361.00	0.69	10.33
424	1.79	11.00	2.01	2948.00	32428.00	4.43	65.58	1474.00	16361.00	-	-
425	1.79	11.00	2.01	2948.00	32428.00	-	-	1474.00	16214.00	0.88	13.05
428	1.81	11.05	2.05	2948.00	32575.00	2.84	42.39	1474.00	16288.00	-	-
430	1.80	10.90	2.00	2948.00	32133.00	4.74	69.60	1474.00	16067.00	1.86	27.27
433	1.72	10.60	1.90	2948.00	31249.00	2.27	32.21	1474.00	15624.00	3.48	49.50
437	1.98	10.70	1.85	2948.00	31544.00	1.00	14.48	1474.00	15772.00	2.77	40.26
440	1.77	10.85	2.00	2948.00	31986.00	0.69	10.07	1474.00	15993.00	-	-
444	1.78	11.07	1.93	2948.00	32634.00	0.40	5.84	1474.00	16311.00	1.77	26.11
447	1.53	9.03	1.50	2948.00	26620.00	0.30	3.59	1474.00	13303.00	4.28	51.59
449	1.53	9.03	1.50	2948.00	26620.00	-	-	1474.00	13303.00	10.82	130.39
451	0.93	5.00	1.00	2948.00	14740.00	0.39	2.70	1474.00	7370.00	-	-
453	0.87	5.77	1.13	2948.00	17010.00	0.61	4.76	1474.00	8504.98	-	-
454	0.87	5.77	1.13	2948.00	17010.00	-	-	1474.00	8504.98	0.19	1.47
456	0.87	5.77	1.13	2948.00	17010.00	1.89	14.65	1474.00	8504.98	0.78	6.04

Continuation:

458	1.75	10.50	1.93	2948.00	30954.00	1.69	24.01	1474.00	15477.00	0.41	5.76
461	1.83	10.70	1.83	2948.00	31544.00	2.23	31.98	1474.00	15772.00	0.86	12.35
465	1.75	11.10	1.90	2948.00	32723.00	1.89	27.94	1474.00	16361.00	4.52	66.71
468	1.80	11.30	2.10	2948.00	33312.00	1.36	20.61	1474.00	16656.00	0.78	11.83
472	1.85	11.70	1.80	2948.00	34492.00	0.72	11.11	1474.00	17246.00	0.67	10.21
475	1.80	10.95	2.10	2948.00	32281.00	2.36	34.97	1474.00	16140.00	0.53	7.89
479	1.70	10.70	2.00	2948.00	31544.00	0.50	7.13	1474.00	15772.00	0.47	6.72
482	1.70	10.70	1.90	2948.00	31544.00	0.21	3.00	1474.00	15772.00	0.00	0.00
486	1.77	10.67	1.93	2948.00	31455.00	0.22	3.16	1474.00	15728.00	0.67	9.73
489	1.47	9.43	1.85	2948.00	27800.00	1.38	17.60	1474.00	13900.00	0.88	11.17
493	1.75	10.70	1.80	2948.00	31544.00	0.94	13.41	1474.00	15772.00	0.33	4.75
496	1.72	10.63	2.05	2948.00	31346.00	1.38	19.87	1474.00	15673.00	0.87	12.57
499	1.78	11.10	1.80	2948.00	32723.00	-	-	1474.00	16361.00	1.32	19.37
500	1.78	11.10	1.80	2948.00	32723.00	0.67	9.79	1474.00	16361.00	-	-
503	1.75	10.80	1.90	2948.00	31838.00	0.58	8.35	1474.00	15919.00	2.69	38.83
507	1.75	10.70	2.00	2948.00	31544.00	1.97	28.47	1474.00	15772.00	1.05	15.13
514	0.50	4.25	1.22	2948.00	12529.00	0.14	0.86	1474.00	6264.50	1.97	11.72
521	0.34	2.57	0.53	2948.00	7564.57	3.26	11.18	1474.00	3782.28	1.86	6.39
528	0.99	6.02	1.62	2948.00	17747.00	6.58	56.79	1474.00	8873.48	0.70	6.06
532	1.29	7.93	1.53	2948.00	23378.00	-	-	1474.00	11689.00	3.05	32.74
536	1.50	10.10	1.73	2948.00	29775.00	0.39	5.19	1474.00	14887.00	-	-
538	1.72	10.38	2.05	2948.00	30600.00	-	-	1474.00	15300.00	0.71	10.06
539	1.72	10.38	2.05	2948.00	30600.00	0.06	0.79	1474.00	15300.00	-	-
543	1.78	11.00	1.73	2948.00	32428.00	1.13	16.37	1474.00	16214.00	0.73	10.64
550	1.78	10.97	2.00	2948.00	32340.00	2.95	43.57	1474.00	16170.00	1.19	17.54

Continuation:

553	1.78	10.95	1.88	2948.00	32281.00	0.94	13.75	1474.00	16140.00	-	-
557	1.77	11.00	1.97	2948.00	32428.00	0.75	11.07	1474.00	16214.00	1.00	14.74
561	1.76	10.53	2.00	2948.00	31042.00	0.96	13.66	1474.00	15521.00	-	-
564	1.82	11.00	1.97	2948.00	32428.00	0.43	6.28	1474.00	16214.00	2.13	31.53
568	1.88	11.37	2.17	2948.00	33507.00	5.89	90.81	1474.00	16759.00	-	-
569	1.88	11.37	2.17	2948.00	33507.00	-	-	1474.00	16753.00	2.04	31.38
571	1.83	10.50	2.00	2948.00	30954.00	5.20	74.53	1474.00	15477.00	0.85	12.18
575	1.67	10.90	1.93	2948.00	32133.00	2.02	29.28	1474.00	16067.00	1.94	28.10
578	1.72	10.45	2.10	2948.00	30807.00	7.38	105.30	1474.00	15403.00	1.18	16.82
581	1.74	10.70	2.00	2948.00	31544.00	0.66	9.53	1474.00	15772.00	2.56	37.00
585	1.73	10.93	1.93	2948.00	32222.00	4.10	59.77	1474.00	16111.00	1.40	20.46
589	1.80	10.63	2.08	2948.00	31338.00	3.35	48.61	1474.00	15669.00	1.50	21.76
592	1.78	11.00	2.00	2948.00	32428.00	2.37	35.05	1474.00	16214.00	-	-
595	1.73	10.97	1.98	2948.00	32340.00	0.34	5.02	1474.00	16170.00	4.97	72.93
599	1.70	10.77	2.03	2948.00	31738.00	8.24	119.52	1474.00	15869.00	7.13	103.37
602	1.13	7.00	1.23	2948.00	20636.00	3.17	29.66	1474.00	10318.00	-	-
603	1.80	11.00	2.00	2948.00	32428.00	-	-	1474.00	16214.00	8.71	128.94
605	0.45	3.10	0.60	2948.00	9138.80	1.00	4.15	1474.00	4569.40	-	-
606	0.45	3.10	0.60	2948.00	9138.80	-	-	1474.00	4569.40	2.73	11.31
609	0.48	2.95	0.40	2948.00	8696.60	15.96	61.18	1474.00	4348.30	-	-
610	0.48	2.95	0.40	2948.00	8696.60	-	-	1474.00	4348.30	2.73	10.47
613	0.63	4.00	0.57	2948.00	11792.00	20.80	108.15	1474.00	5896.00	0.64	3.35
617	0.75	5.10	0.80	2948.00	15035.00	16.28	108.28	1474.00	7517.40	0.49	3.26
620	1.50	7.90	1.75	2948.00	23289.00	7.64	77.55	1474.00	11645.00	1.82	18.47
623	1.90	10.55	1.98	2948.00	31101.00	2.12	30.59	1474.00	15551.00	1.94	27.99

Continuation:

627	1.81	11.35	2.00	2948.00	33460.00	1.63	24.69	1474.00	16730.00	1.31	19.77
630	1.72	10.91	2.10	2948.00	32163.00	1.17	17.23	1474.00	16081.00	0.99	14.54
634	1.75	10.75	1.98	2948.00	31691.00	1.36	19.69	1474.00	15846.00	0.54	7.86
637	1.81	11.00	1.87	2948.00	32419.00	1.24	18.20	1474.00	16210.00	0.76	11.19
641	1.80	11.08	1.96	2948.00	32664.00	0.96	14.25	1474.00	16332.00	0.91	13.52
644	1.76	10.95	1.95	2948.00	32281.00	0.75	10.99	1474.00	16140.00	1.02	14.95
648	1.81	10.90	1.94	2948.00	32133.00	0.89	13.04	1474.00	16067.00	1.67	24.47
651	1.83	10.78	1.98	2948.00	31779.00	1.07	15.61	1474.00	15890.00	1.00	14.52
655	1.80	10.82	2.00	2948.00	31897.00	1.32	19.30	1474.00	15949.00	0.79	11.19
658	1.77	10.75	2.10	2948.00	31691.00	2.54	37.13	1474.00	15846.00	1.03	15.06
662	1.79	10.78	1.98	2948.00	31768.00	1.14	15.45	1474.00	15884.00	1.52	20.60
665	1.61	10.85	1.81	2948.00	31989.00	0.98	13.98	1474.00	15994.00	1.67	23.83
669	1.65	10.95	1.74	2948.00	32281.00	0.67	9.61	1474.00	16140.00	1.43	20.51
672	1.71	11.02	1.79	2948.00	32490.00	0.91	13.21	1474.00	16245.00	1.28	18.59
675	1.82	11.25	1.82	2948.00	33165.00	1.13	16.83	1474.00	16583.00	1.61	24.00
679	1.80	11.14	1.87	2948.00	32841.00	1.29	19.10	1474.00	16420.00	1.41	20.90
682	1.90	11.07	1.91	2948.00	32634.00	0.84	12.50	1474.00	16317.00	1.30	19.37
686	1.85	10.89	1.78	2948.00	32104.00	0.78	11.33	1474.00	16052.00	0.99	14.33
689	1.83	10.76	1.86	2948.00	31720.00	1.20	17.34	1474.00	15860.00	0.67	9.70

* The effluent concentrations of formaldehyde and iso-propanol were measured by taking the samples from the both bottom and top ports of the column. The average values of these readings are given in this Table.

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Table 7.2 Operational data in the Activated Sludge (AS) unit.

Day	Buffer Flow (L/day)	Nutrient Flow (L/day)	Waste: Iso-propanol Influent (mg/day)	Waste: Formaldehyde Influent (mg/day)	Final effluent Iso-propanol (mg/day)	Final effluent Formaldehyde (mg/day)
0						
3	0.28	1.16	71.09	0.00	0.00	0.00
10	0.25	1.14	171.04	0.00	0.00	0.00
17	0.22	1.12	154.81	0.00	0.00	0.00
24	0.21	1.12	149.69	0.00	0.00	0.00
31	0.16	1.14	0.00	0.00	0.00	0.00
38	0.17	1.12	75.88	0.00	0.00	0.00
45	0.18	1.13	0.00	0.00	0.00	0.00
52	0.20	1.12	0.00	0.00	0.00	0.00
59	0.22	1.09	0.00	0.00	0.00	0.00
66	0.18	1.14	0.00	0.00	0.00	0.00
73	0.25	1.12	86.39	0.00	0.00	0.00
80	0.05	1.15	0.00	0.00	0.00	0.00
87	0.00	1.40	3.00	0.00	0.00	0.00
94	0.00	1.32	2.22	0.00	0.00	0.00
101	0.24	1.42	1.49	0.00	0.00	0.00
108	0.23	1.35	0.99	0.00	0.00	0.00
115	0.21	1.29	2.86	0.00	0.00	0.00
122	0.00	1.41	3.24	0.00	0.00	0.00
129	0.00	1.42	5.15	0.00	0.00	0.00
136	0.02	1.40	16.17	12.03	0.00	0.00
143	0.00	1.46	6.12	27.29	0.00	0.00
150	0.00	1.44	2.10	47.76	0.00	0.00
157	0.00	1.42	0.00	0.92	0.00	0.00
164	0.00	1.28	10.26	172.72	0.00	29.71
171	0.00	0.97	4.56	350.89	0.00	214.59
178	0.00	0.85	12.05	25.70	0.00	0.23
185	0.00	0.85	8.21	278.41	0.00	262.63
192	0.00	0.81	21.81	199.03	0.00	33.77
199	0.00	0.82	60.09	10.16	0.00	5.66
206	0.00	0.82	10.78	9.96	0.00	3.18
213	0.00	0.57	9.66	23.01	0.00	22.65
220	0.00	0.77	2.33	8.59	0.00	7.60
227	0.00	0.78	3.33	26.64	0.00	21.79
230	0.00	0.78	5.58	11.03	0.00	5.08
234	0.00	0.78	4.47	10.73	0.00	7.68
237	0.00	0.78	3.53	8.40	0.00	4.78
241	0.00	0.75	2.42	0.00	0.00	0.00
244	0.00	0.77	0.00	0.00	0.00	0.00
248	0.00	0.77	3.28	0.00	0.00	0.00
251	0.00	0.70	10.76	0.00	0.00	0.00
255	0.00	0.48	483.63	428.52	0.00	408.54
257	0.00	0.05	0.00	2004.75	0.00	-

Continuation:

|						
258	0.00	0.00	0.00	164.50	0.00	-
259	0.65	0.75	0.00	-	0.00	0.00
260	1.00	0.75	0.00	40.56	-	-
261	0.40	0.85	0.00	37.73	-	-
262	0.55	0.80	758.10	3.23	0.00	-
263	0.45	0.80	919.20	3.10	-	-
264	0.00	0.85	1369.79	2.19	-	0.00
265	0.70	0.85	902.71	0.68	0.00	1.10
267	0.60	0.80	0.00	2.47	-	-
268	0.70	0.85	368.93	26.17	-	-
269	0.65	0.80	258.48	0.00	0.00	-
270	0.60	0.80	124.27	0.00	-	-
271	0.70	0.80	18.09	72.91	-	-
272	0.70	0.85	59.82	251.57	14.37	259.25
273	0.60	0.75	-	-	-	-
274	0.70	0.80	29.56	32.25	-	-
275	0.65	0.85	20.86	17.54	-	-
276	0.70	0.85	104.93	14.14	-	11.72
277	0.75	0.75	69.99	-	-	-
278	0.70	0.80	35.27	11.70	-	0.00
283	0.70	0.80	11.23	0.00	0.00	0.00
286	0.64	0.71	8.67	0.00	0.00	0.00
290	0.68	0.47	6.80	16.48	0.00	9.47
297	0.00	0.80	4.50	0.00	0.00	0.00
300	0.08	0.75	-	6.79	0.00	0.00
304	0.25	0.75	6.05	6.57	0.00	3.97
311	0.27	0.77	4.80	13.56	0.00	7.67
314	0.20	0.80	46.40	24.61	0.00	20.29
316	0.22	0.78	9.07	16.38	0.00	10.73
318	0.20	0.80	4.74	16.50	0.00	16.15
321	0.18	0.71	9.67	22.05	0.00	12.04
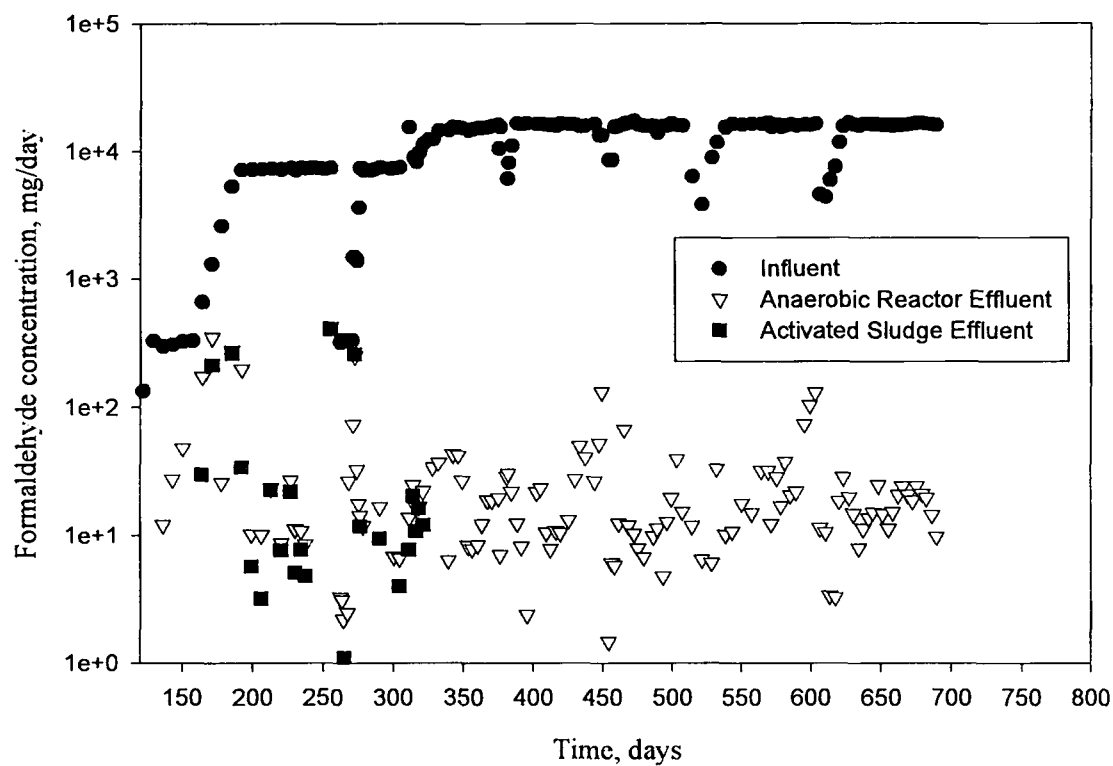
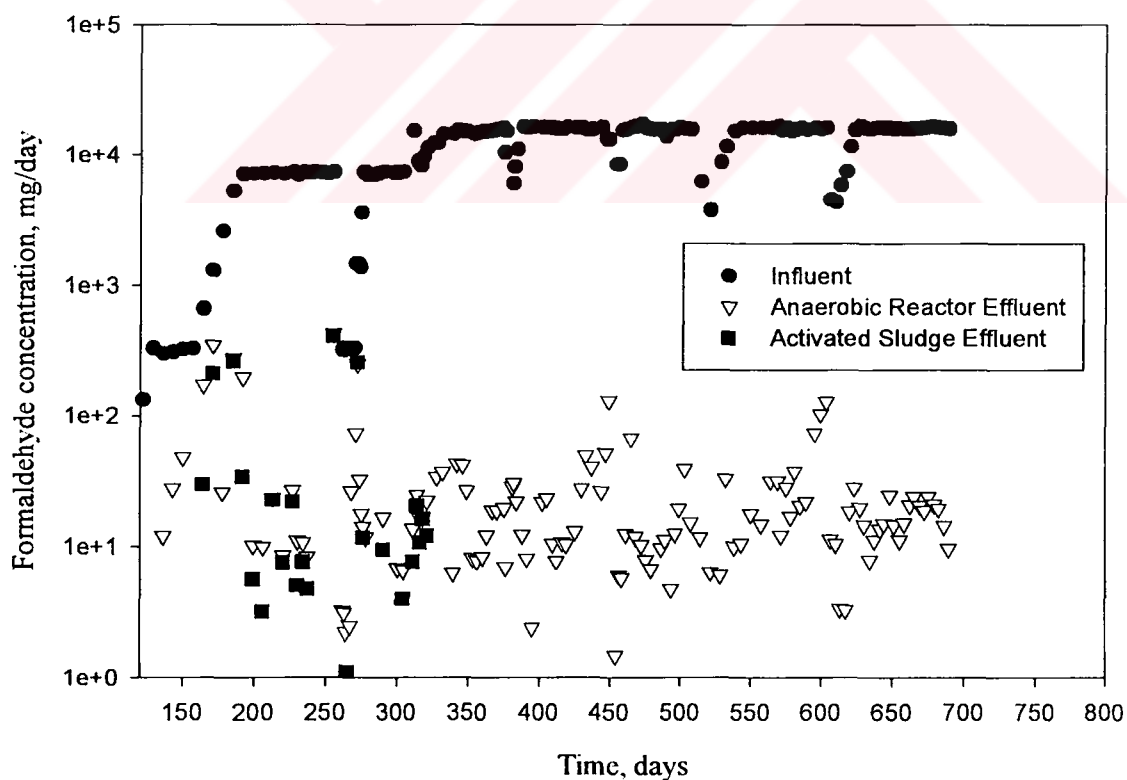**Figure 7.1.** System Iso-propanol Reduction.

Figure 7.2. System Formaldehyde Reduction.

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The anaerobic bioreactor failed on days 256, 375, 451, 508 and 602 because of the following reasons:

1) Failure in the recycle pump that caused oxygen leakage into the biosystem,

2) Failure of accumulation of the GAC particles in the bottom part of the bioreactor. When microorganisms grew sufficiently, the density of the GAC particles increased and started to accumulate in the bottom part of the bioreactor by the gravitational force. This resulted in fluidization problem by clogging the feed entrance of the bioreactor.

These failures have affected the performance of the biosystem as well as the quality of the effluent. That is why, during the days of failures, the anaerobic bioreactor was operated either in batch conditions or by applying lower feeding rates. Except for these periods, the performance and productivity of the biosystem were both outstanding.

Satisfactory biodegradation results of formaldehyde and iso-propanol were achieved rendering the removal efficiency of more than 99.9 % during stable operation of the anaerobic bioreactor. Figure 7.3 shows the substrate removal efficiencies (%) obtained in the anaerobic bioreactor.

Figure 7.4 exhibits the biosystem reduction of total COD. Initially, the high strength organic waste stream had ~ 7000 mg/L of total CODAfter the biotreatment, the effluent total COD value was decreased to approximately 73 mg/L as the mean value of the anaerobic bioreactor. The total COD removal efficiency was ~ 99 %.

Figure 7.5 shows the differentiation of the effluent concentration of acetate during the bioprocess of the substrates. Experimental data reveal that when low substrate feeding concentrations were introduced into the biosystem, the acetate utilization was high. After having increased concentrations, the acetate utilization stopped and accumulation of acetate was observed in the biosystem. However, when these increments of substrate feeding concentrations were continued, it was apparently determined that the biosystem easily tolerated these high concentrations and the acetate utilization augmented again.
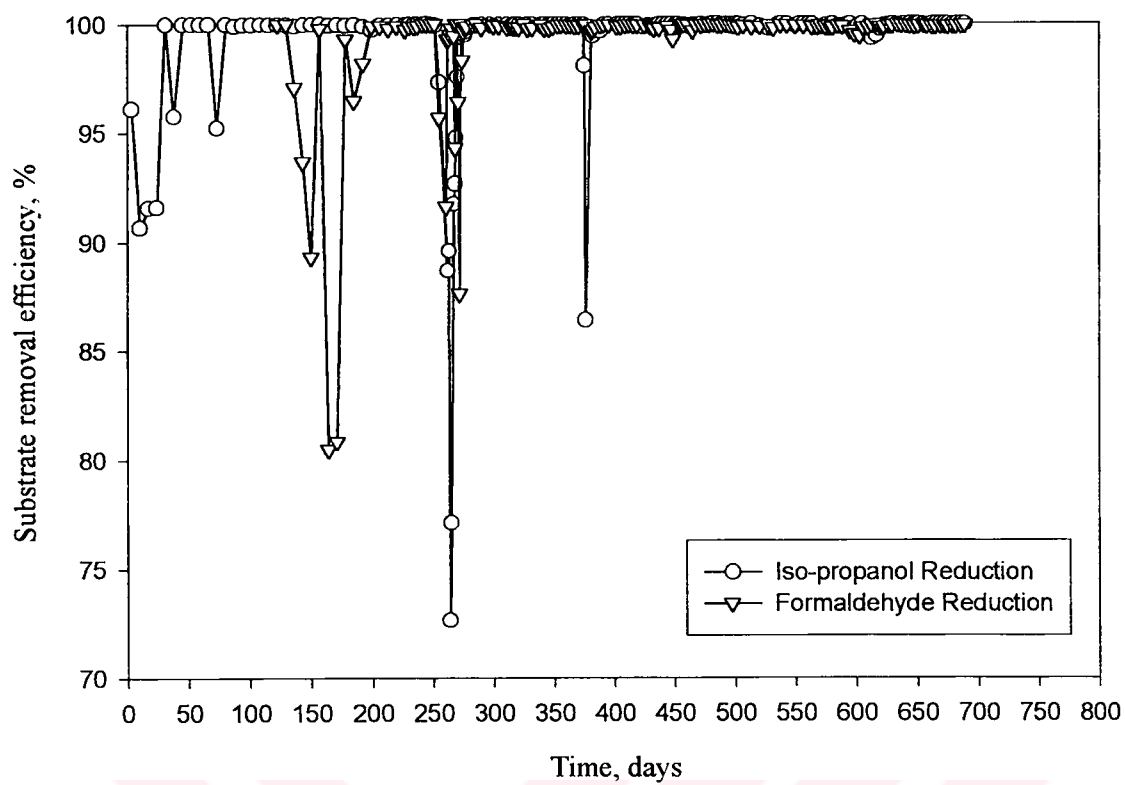
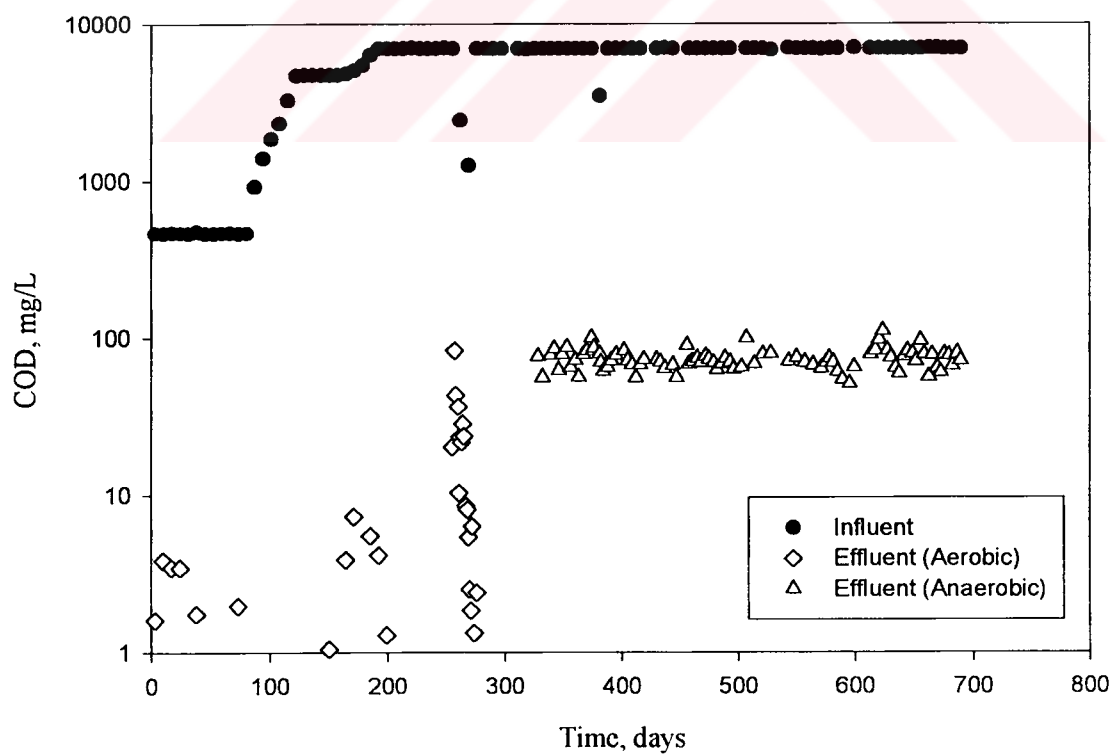**Figure 7.3.** Substrate removal efficiencies (%) in the anaerobic bioreactor.

Figure 7.4. System COD (soluble) reduction.

In Figure 7.5, there are three different periods of time that the acetate utilization rates were very low and their concentrations were over 150 mg/L. These high results were obtained for several times when the bioprocess failed due to the reasons of which were interpreted above.

Figure 7.6 shows the biosystem ammonia-nitrogen (NH_3-N) utilization. Initially, ammonia was fed to the AFBGAC bioreactor at 18.0 mg/L-N as the primary source of nitrogen needed for biological growth. However, in the ensuing period, the effluent ammonia dropped to less than 1.0 mg/L and the anaerobic bioreactor became nitrogen limited. This depletion of nitrogen and the increase in acetate formation were both observed at the higher formaldehyde loadings. This may have resulted from the growth of new formaldehyde metabolizing bacterial strain in the biosystem. The ammonia concentration was later increased to 70 mg/L-N in order to overcome the negative influence of nitrogen limitation.

Figure 7.7 shows the pH profile in the anaerobic bioreactor. The pH values given are based on the calculations of average weekly analysesAs can be seen, the pH values were always changing between 7.0 and 7.3 in properly operating biosystems. During the failures in biosystem operation, the pH tended to decrease due to the high acetate accumulations. The acidification effect was prevented by frequent checking of the pH and pH adjustments.

Figure 7.8 presents the total gas production values received from the anaerobic bioreactor. Total anaerobic gas production measurements were done daily with a wet tip gas meter. As can be seen, the total anaerobic gas production values increased as a result of increasing the substrate concentrations except for the cases that the biosystem failed. The total gas production of the AFBGAC bioreactor was 51.6 liters per day as the mean value. The gas compositions of the AFBGAC bioreactor were measured weekly by taking effluent gas samples from the anaerobic bioreactor and analyzing them in a gas chromatography. The average gas composition was computed by taking the averages of the weekly analyses given in Table 7.3.
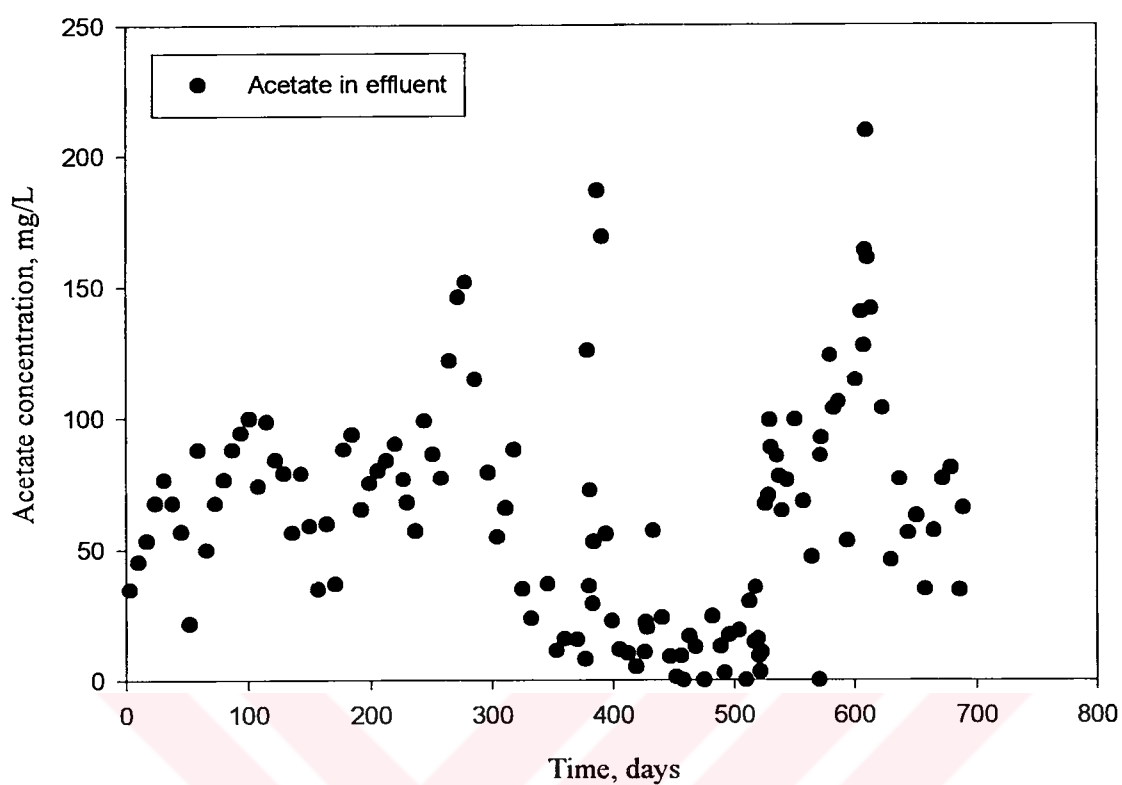
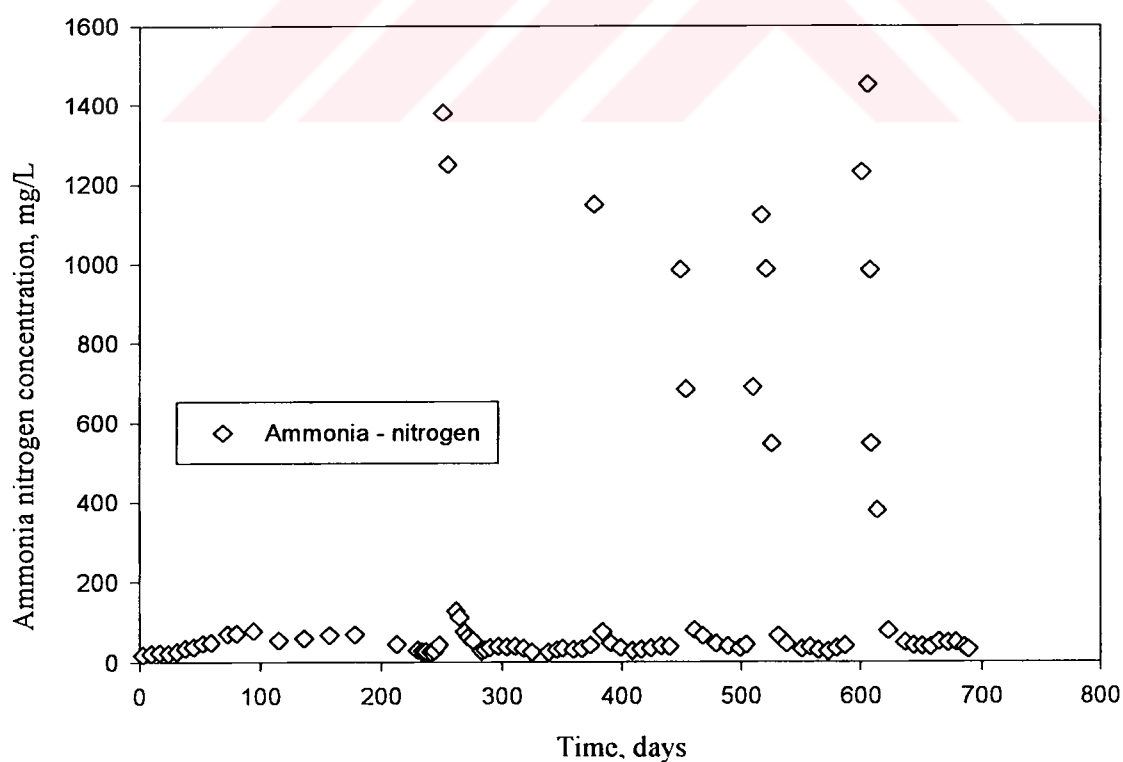**Figure 7.5.** Differentiation of acetate during the bioprocess as a representative compound of VFAs

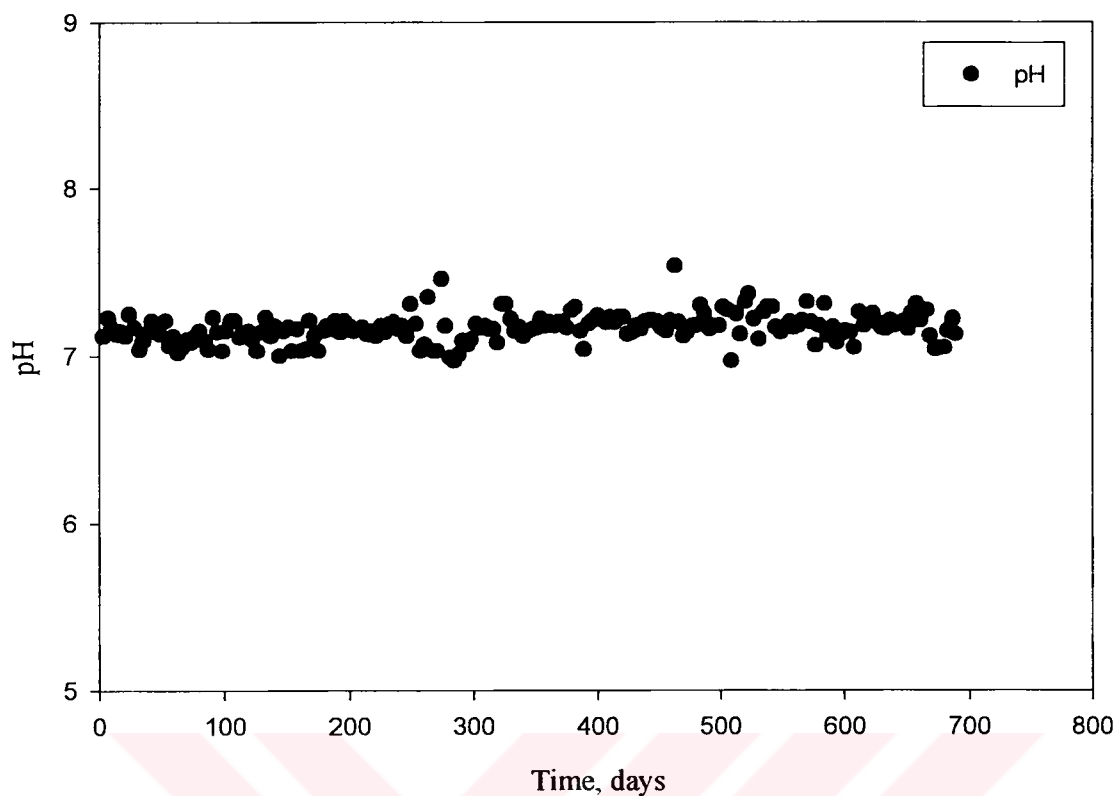**Figure 7.6.** System ammonia-nitrogen (NH_3-N) utilization

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Figure 7.7. Differentiation of pH in the anaerobic bioreactor during the entire course of experiment.
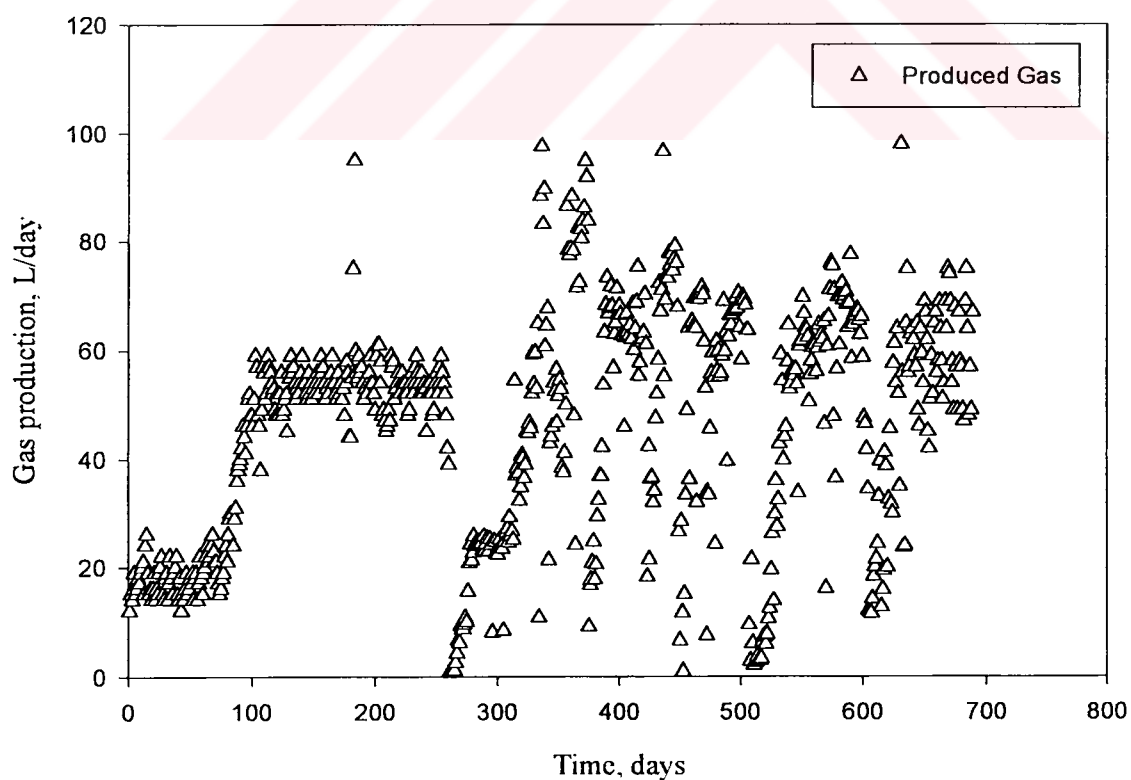

Figure 7.8. Total gas production from the anaerobic bioreactor.

Figure 7.9 exhibits the reduction in the Dissolved Organic Carbon (DOC) in the anaerobic bioreactor. The synthetically prepared wastewater was a well mixed solution which means that it had soluble pollutants. Therefore, the DOC measurements were carried out in order to determine the organic content of the effluent. High DOC removals were obtained which means that soluble pollutants were adsorbed easily onto the GAC and biotreated effectively.

7.2. Experiments Conducted in the Anaerobic Respirometer

Anaerobic respirometer experiments were performed mainly for two objectives. The first one was to determine the biotreatability of each substrate in different concentrations. For this purpose, acetate, formaldehyde and iso-propanol were used as the main substrates. Secondly, the data obtained from the respirometer experiments were used for elucidating the kinetic considerations of each substrate.

In the anaerobic respirometer, there were twelve ports to place the batch reactors. The first 4 ports were always used for the blank (control) samples in which there was no main substrate. In each experiment duplicates were run for all samples. Each batch reactor was containing the effluent of the main bioreactor (AFBGAC). This effluent has served as a good source of nutrients, minerals, and vitamins, which were essential for the microorganism's biological activity.

In the figures, which concern the biodegradation of each substrate, the biodegradation results of the blank samples have not been shown due to their very low values that actually were very close to zero.

Figures 7.10 and 7.11 show the biodegradation of acetate alone in different concentrations by using formaldehyde acclimated anaerobic culture. The experimental results reveal that without using Granular Activated Carbon (GAC) in the batch reactors, the biodegradation of acetate took a long time, proceeded very slowly and the results drifted frequently.

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Table 7.3. Gas Composition of the Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) Bioreactor.

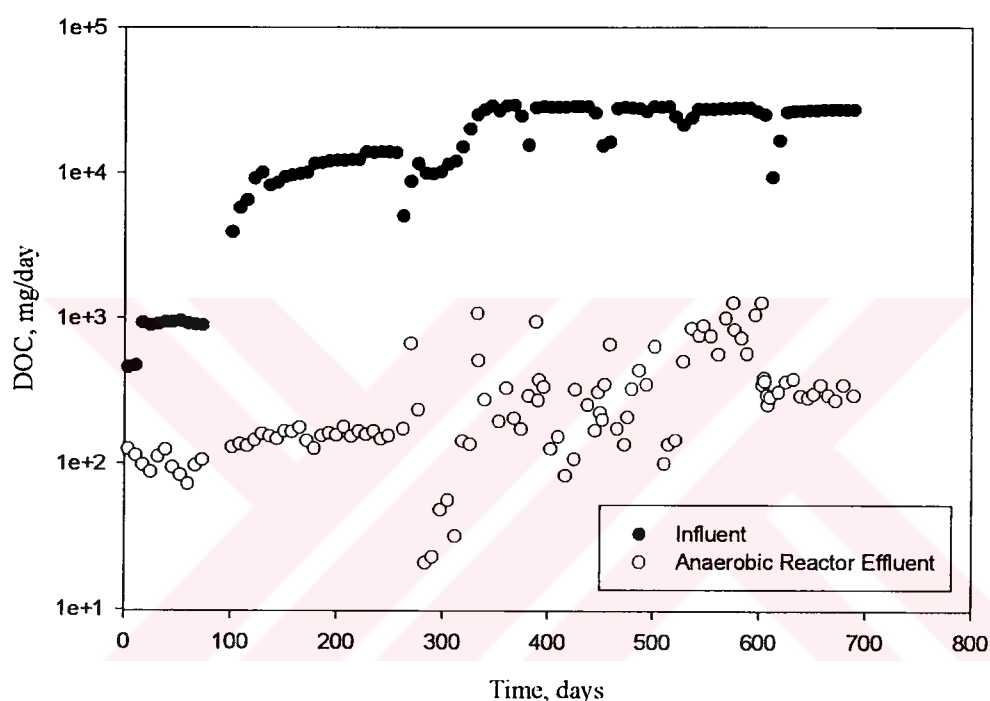| Component	Gas Composition (%)
CO_2	20.87
O_2	0.07
N_2	0.81
CH_4	78.25

Figure 7.9. DOC reduction in the anaerobic bioreactor.

The lowest initial concentration of acetate was biodegraded by anaerobic culture within 50 hours. After having increments of concentration of acetate, the results showed slight fluctuations but in spite of this, the rates of the biodegradation were quite similar for all three different acetate concentrations.

Figure 7.12 exhibits the methane production results of the biodegradation of acetate in different concentrations. According to the results, it is apparent that the cumulative methane production concentrations remained lower than the theoretical values. For

example, the sharpest difference was obtained for the case of the maximum initial concentration of acetate (1352.55 mg/L). In this case, the maximum methane production was around 11.7 mM. However, according to the stoichiometry of the biodegradation of acetate, this value was expected to be 22.52 mM. Similarly, methane concentrations lower than expected were obtained for the other different acetate concentrations. When the initial concentration of acetate was 476.43 mg/L, the obtained methane gas production was 5.5 mM (mean value) instead of 7.93 mM and when the initial concentration of acetate was 919.23 mg/L, the obtained methane gas production was 10.25 mM (mean value) instead of 15.31 mM. In case of no addition of GAC into the batch reactors, the produced methane gas concentrations were very low.

Figure 13 displays the variation of the methane production versus time. In case of no GAC in the batch reactors, the methane production changed slightly with time. When the initial concentration of acetate was 476.43 mg/L, the methane gas production increased evenly until the time of about 30 hours from the beginning and then tended to decrease almost with the same rate. For the other higher initial concentrations, the productions of methane did not change uniformly and the fluctuations became more clear. When the initial acetate concentration was 919.23 mg/L, the methane production values increased very quickly especially when it was compared with the initial acetate concentration of 1352.55 mg/L, which means that the lower concentrations were biodegraded more easily than the higher concentrations. In case of maximum concentration of acetate, the methane production rates became slower and the results fluctuated severely.
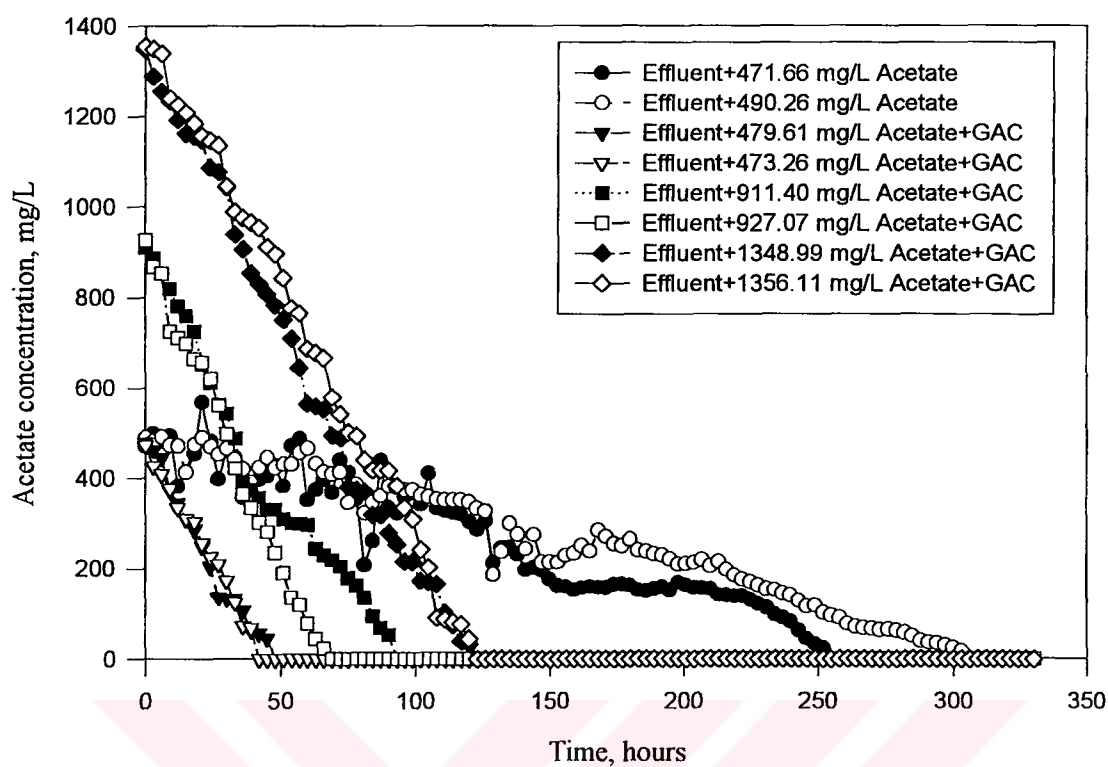
Figure 7.10. Acetate biodegradation by using formaldehyde acclimated culture.
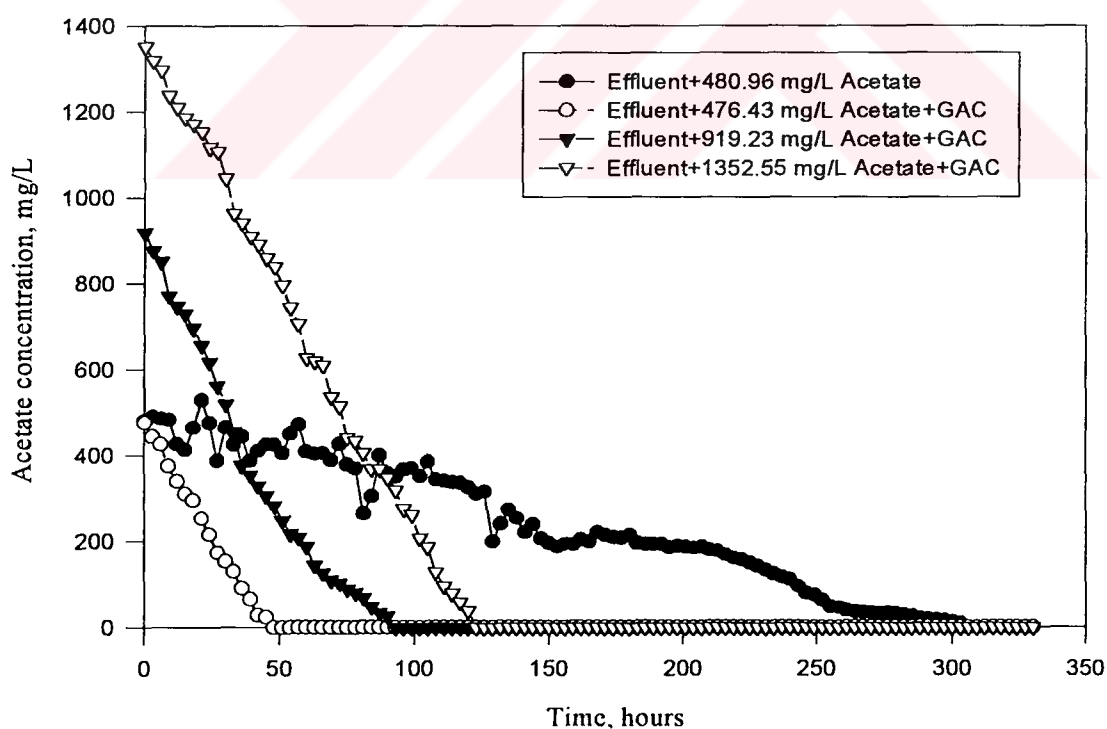

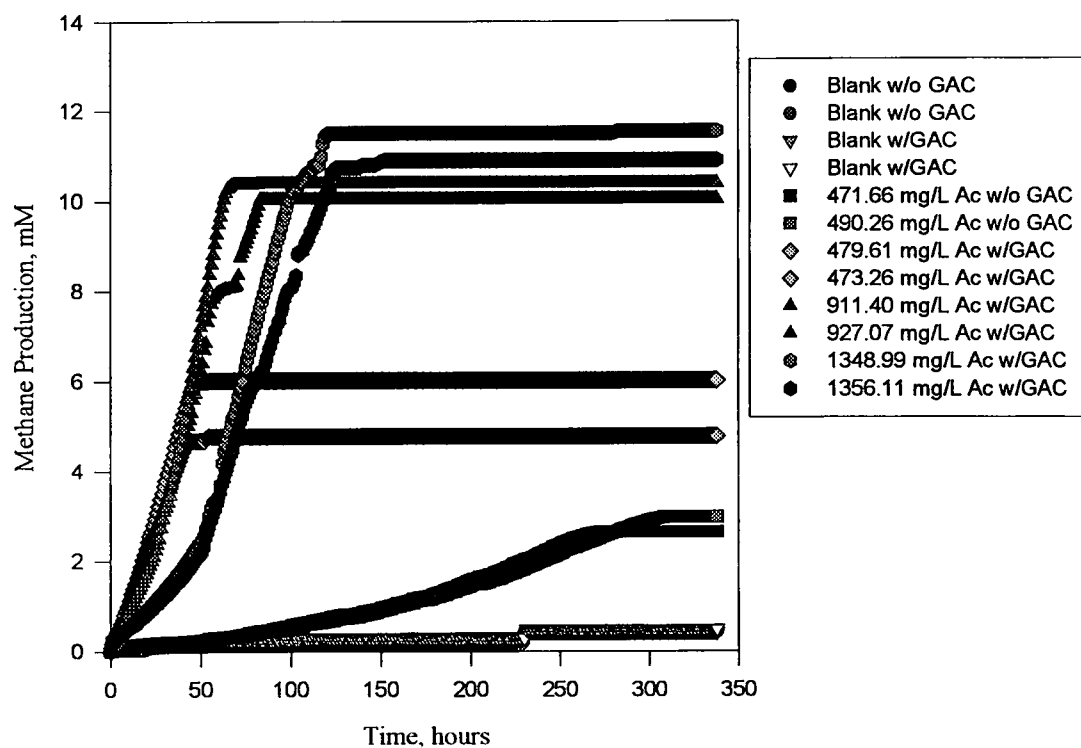**Figure 7.11.** Acetate biodegradation by using formaldehyde acclimated culture (averages).

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Figure 7.12. Methane production during the anaerobic biodegradation of acetic acid.
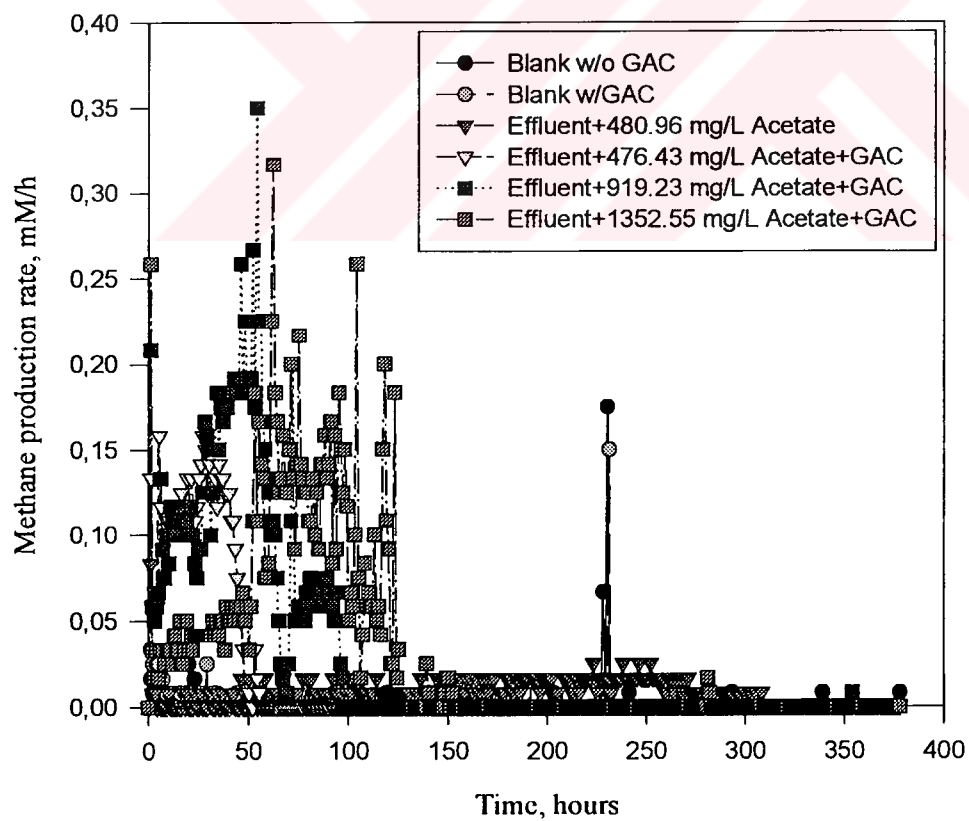

Figure 7.13. Methane production rates of acetate biodegradation (averages).

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Figures 7.14, 7.15, 7.17, and 7.18 show the results of the biodegradation of formaldehyde obtained from the duplicate experiments and in terms of averages. Again, different initial concentrations of formaldehyde were applied. According to the experimental results, the lower concentrations of formaldehyde (65.62 mg/L, 67.64 mg/L, and 97.39 mg/L) were tolerated more rapidly than the higher concentrations of formaldehyde (148.92 mg/L, 192.38 mg/L, and 386.85 mg/L). Although, the higher formaldehyde concentrations had lower lag time periods, in the ensuing period, their biodegradation rates became slower.

Figures 7.16 and 7.19 present the variation of the methane production for the distinct initial concentrations of formaldehyde. The methane productions were very low as expected. Low concentrations of formaldehyde produced low concentrations of methane. Up to the concentration of 148.92 mg/L of formaldehyde, no significant methane production was determined and the value obtained hardly reached 0.5 mM. For the concentration of 148.92 mg/L of formaldehyde, the produced methane concentration attained to 1.25 mM, while the other two highest concentrations of formaldehyde resulted in similar productions of methane (about 1.80 mM). From Figure 7.19, it is obvious that for the highest initial concentration of formaldehyde (386.85 mg/L), the methane production rate was very low and it began to show significant increments of methane productions only after 20 hours from the inception of the bioprocess.
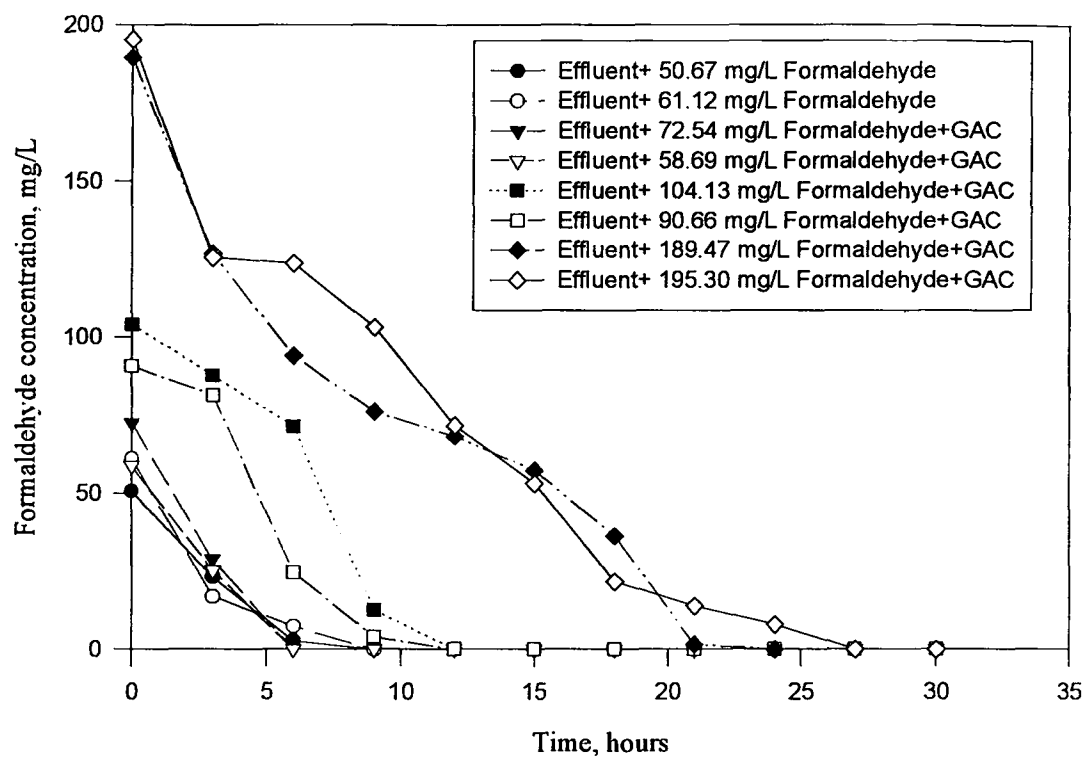
Figure 7.14. Formaldehyde biodegradation by using formaldehyde acclimated culture.
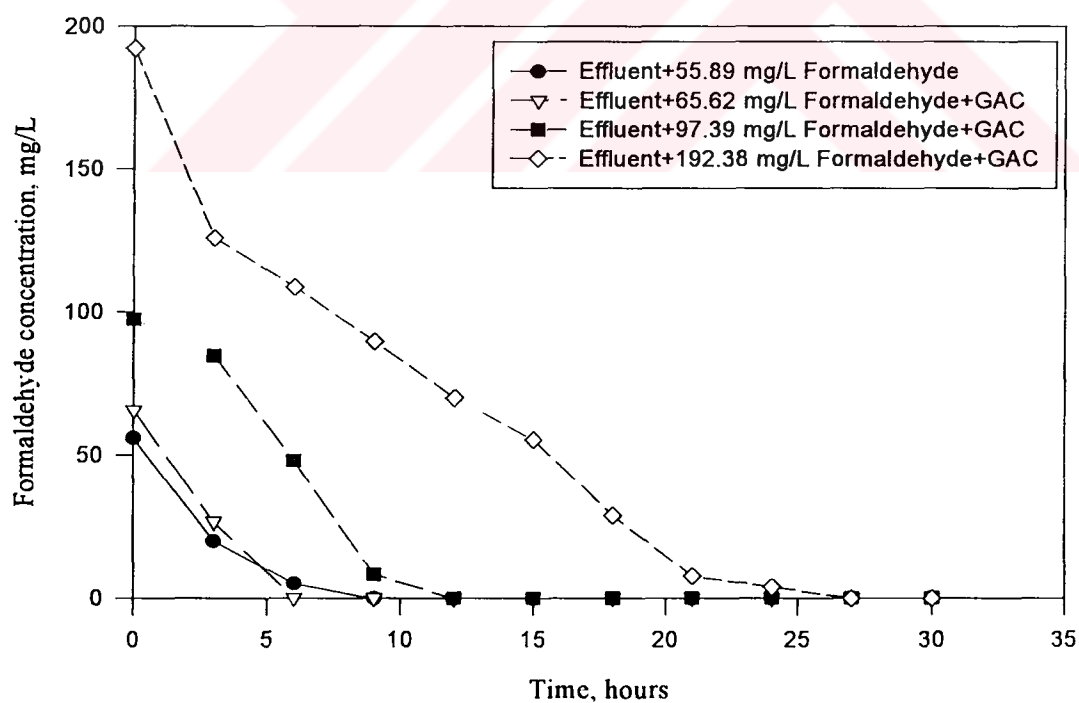

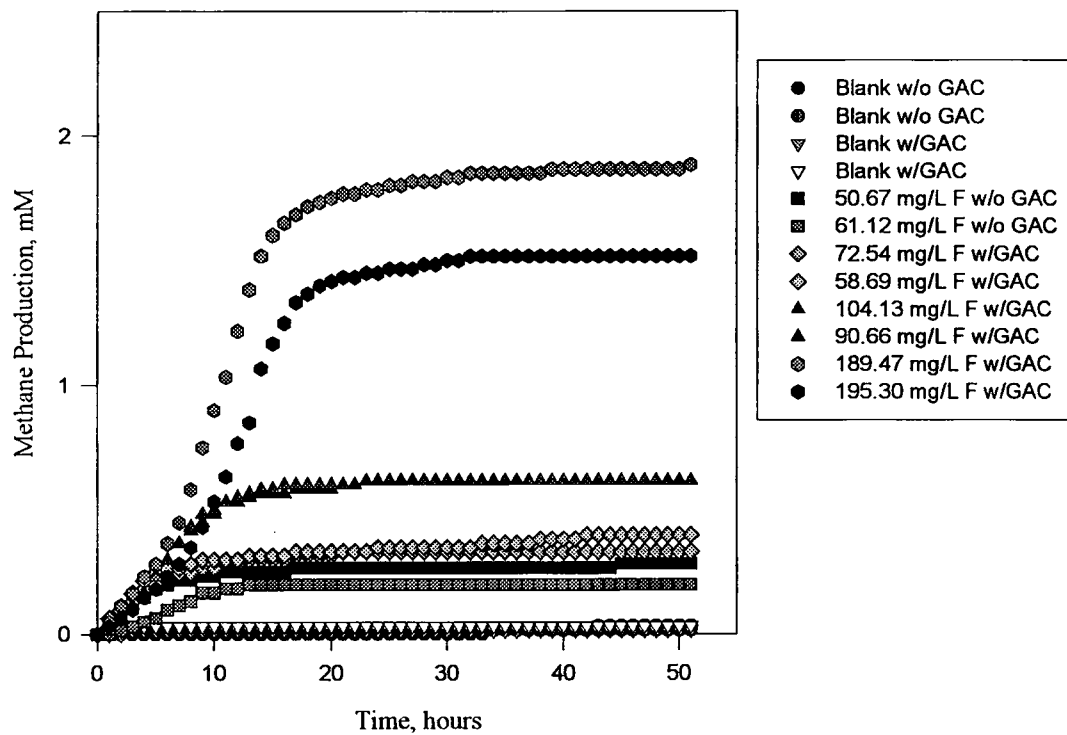**Figure 7.15.** Formaldehyde biodegradation by using formaldehyde acclimated culture (Averages).

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Figure 7.16. Methane production during the anaerobic biodegradation of formaldehyde.
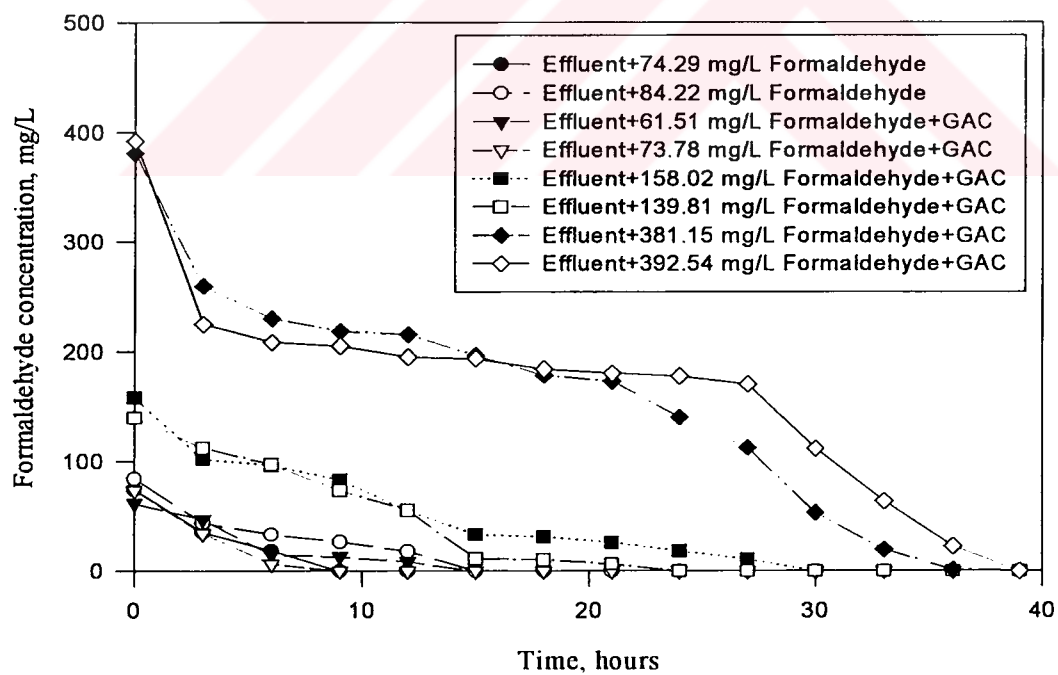

Figure 7.17. Formaldehyde biodegradation by using formaldehyde acclimated culture.

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Figure 7.20 exhibits the toxic effect of formaldehyde during its anaerobic biotransformation. In this case, the concentration of formaldehyde was 437.00 mg/L and no biotransformation of formaldehyde was detected. In the literature, the toxic effect of formaldehyde was also shown for the maximum concentration of 400.00 mg/L, where the wastewater was biotreated by anaerobic filter [57,p 78]. It can be concluded that for approximately up to 400.00 mg/L of formaldehyde concentration, the biotransformation is continued in decreasing order. However, when the formaldehyde concentration was greater than 400.00 mg/L, the inhibition effect turned to the toxic effect, and no biotransformation of formaldehyde was achieved which was evidenced by the experimental results.

During the experiments of formaldehyde biotransformation, the effect of the initial concentrations on the COD removal efficiency (%) was also investigated. The related results are shown in Table 7.4 and Figure 7.21. As can be seen in Figure 7.21, the COD removal efficiencies (%) exhibited a gradual decrease from the formaldehyde initial concentration of 65.62 mg/L to up to 192.38 mg/L, however, later the efficiencies decreased dramatically to lower values. When the toxic effect of formaldehyde on microbial activity was observed, the COD removal efficiency was only 1.3 (%) which was determined by the experimental results.

Figures 7.22 and 7.23 present the results for the biodegradation of iso-propanol obtained from duplicate experiments carried out with different initial concentrations. The results obtained for each of the duplicate experiments and the averages of duplicates are presented, respectively. In case of iso-propanol alone without GAC, the complete biodegradation of iso-propanol was accomplished in a long time (approx. in 150 hours). The initial iso-propanol concentrations of 436.29 mg/L and 772.78 mg/L were biodegraded evenly and their biodegradation curves decreased smoothly. The highest initial concentration of iso-propanol (1243.84 mg/L) showed fluctuations on its own biodegradation results, but the bioprocess was terminated almost at the same time with the case which the initial concentration of iso-propanol was 772.78 mg/L and its biodegradation had reached to completion after 83 hours.
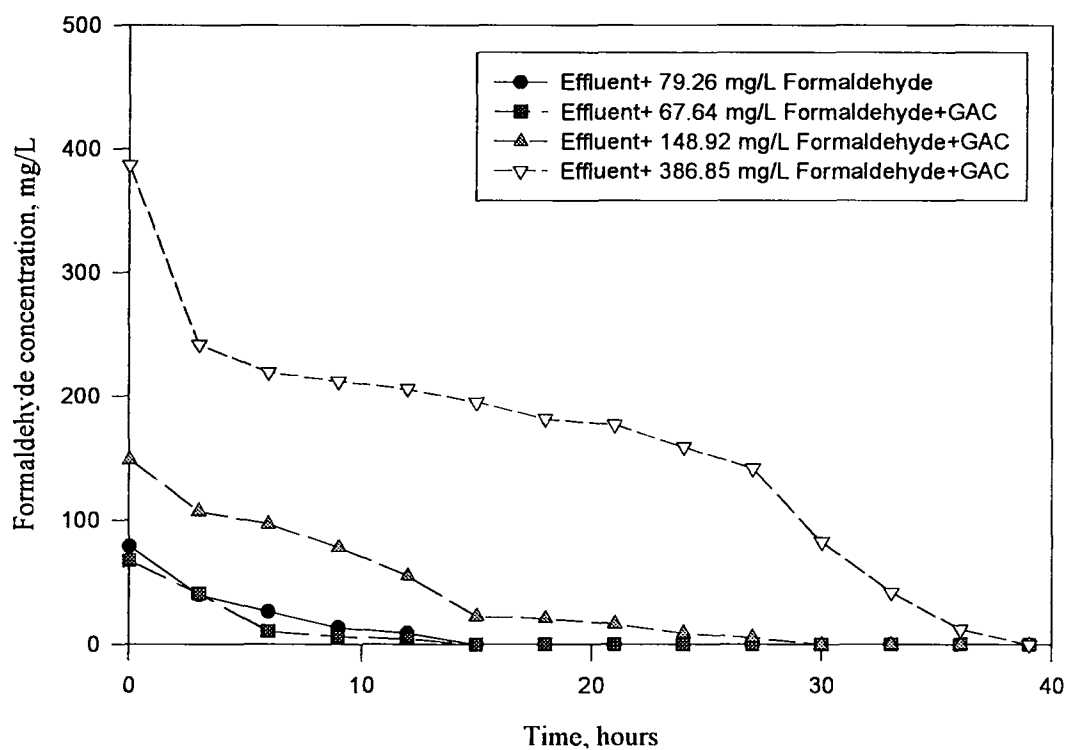
Figure 7.18. Formaldehyde biodegradation by using formaldehyde acclimated culture (Averages).
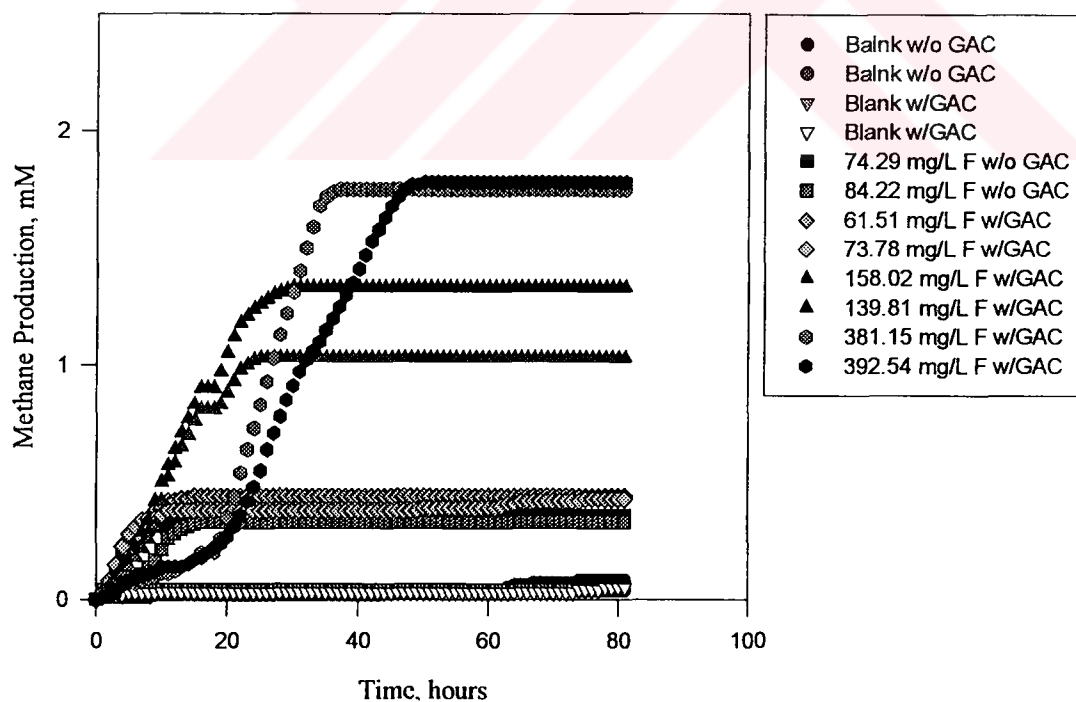

Figure 7.19. Methane production during the anaerobic biodegradation of formaldehyde.
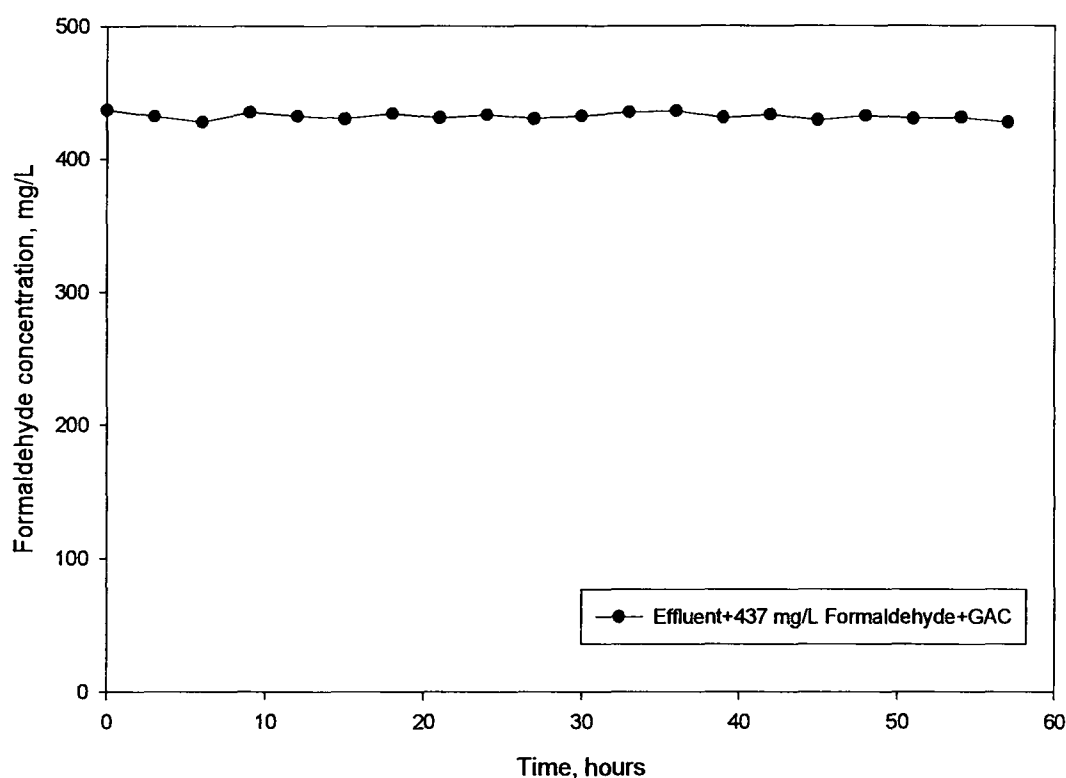
Figure 7.20. The toxic effect of formaldehyde during its anaerobic biodegradation.

Figure 7.24 presents the methane production values through the biodegradation of iso-propanol alone for the different initial concentrations investigated. In case of iso-propanol alone without GAC, the total concentration of produced methane was around 3.7 mM which was far from the theoretical value that must have been 10.13 mM. For the initial iso-propanol concentrations of 436.29 mg/L and 772.78 mg/L, the produced methane concentrations were approximately 11.25 mM and 18.18 mM. These values were very close to the expected theoretical values of 10.9 mM and 19.3 mM, respectively. For the highest initial concentration of iso-propanol, the produced total methane concentration reached to a maximum value of 23.13 mM which was quite different from the theoretical value expected to be 31.0 mM. The present data reveal that there was no evidence of a strong inhibition that effected the biodegradation of iso-propanol. Inhibition effect was smaller for the lower initial concentrations of iso-propanol.

Table 7.4. Effect of formaldehyde on COD removal efficiency in the BMP test.

Initial formaldehyde* Concentration (mg/L)	COD* $_{in}$ (mg/L)	COD* $_{out}$ (mg/L)	COD Removal* Efficiency (%)
65.62	102.87	36.00	65
67.64	114.65	44.71	61
97.39	126.44	50.58	60
148.92	150.00	63.00	58
192.38	173.59	78.12	55
386.85	267.87	211.62	21
437.00	285.31	281.60	1.3

* The figures are given as the mean values of the duplicates.
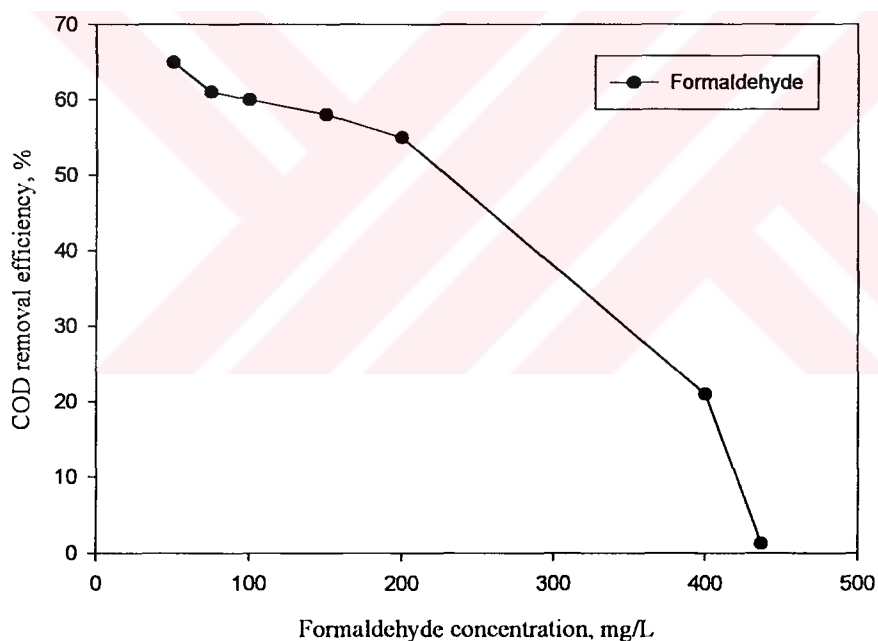

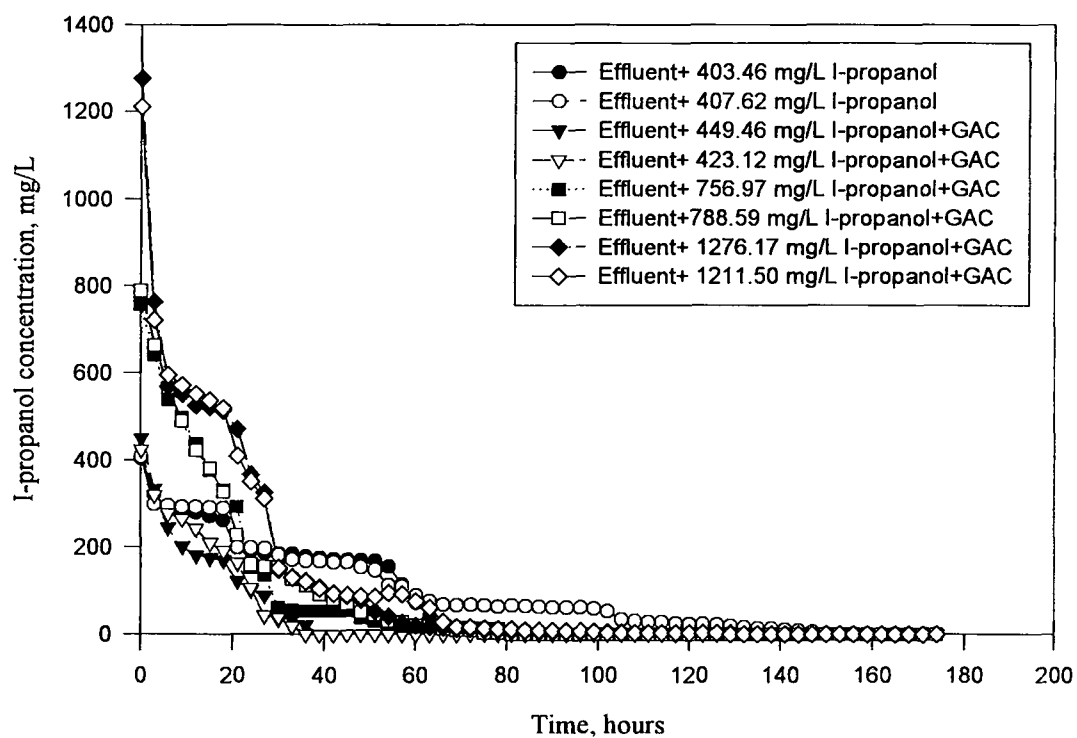**Figure 7.21.** Effect of formaldehyde on COD removal efficiencies during the BMP test.

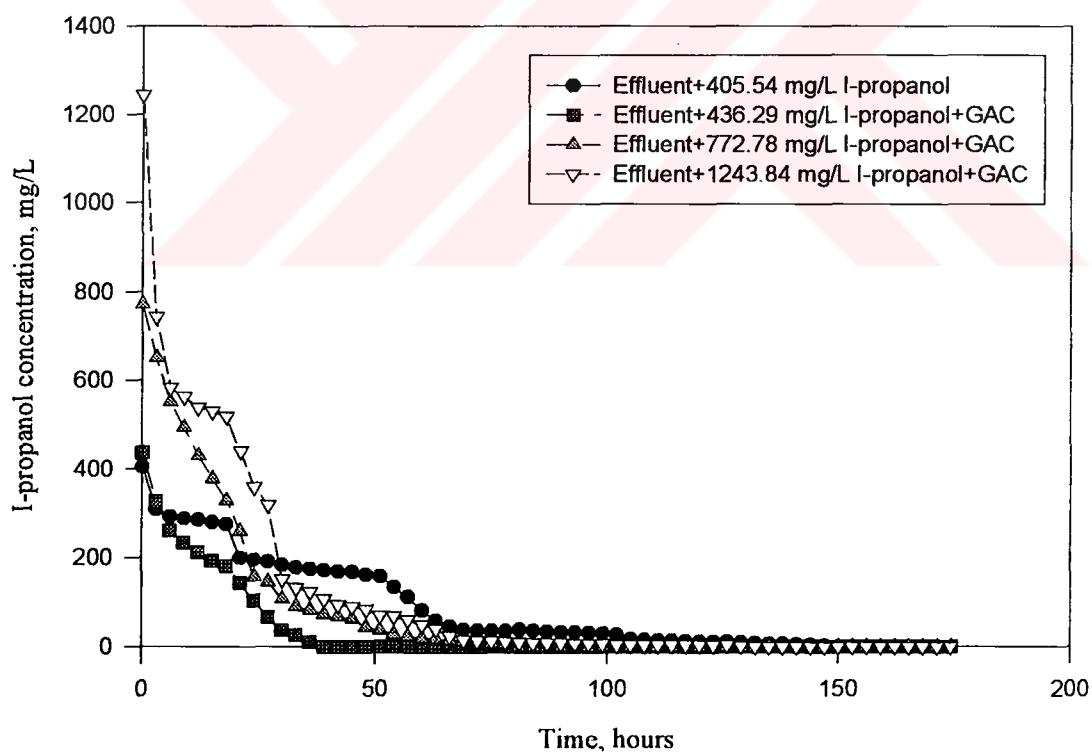**Figure 7.22.** I-propanol biodegradation by using formaldehyde acclimated culture.

Figure 7.23. I-propanol biodegradation by using formaldehyde acclimated culture (averages).

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The methane production rates that appertain to the biodegradation of iso-propanol are given in Figure 7.25. All the different initial concentrations of iso-propanol exhibited identical methane production rates in the inception of the bioprocess. Subsequently, the rates of methane production set off to decrease and displayed more fluctuations.

In Figures 7.26, 7.27, and 7.28, the variations of the substrate concentrations with time in a triple substrate biosystem are shown. In this stage of the study, the acetate and iso-propanol initial concentrations were kept constant while the formaldehyde initial concentration was changed. In this manner, the probable inhibition effect of the formaldehyde was investigated on the rest of the substrates and on itself. The acetate and iso-propanol initial concentrations ranged between 786.79 mg/L and 937.44 mg/L. The different initial concentrations of formaldehyde investigated were 101.45 mg/L, 201.33 mg/L, and 405.79 mg/L. When the lowest initial concentration of formaldehyde (101.45 mg/L) was used in the triple substrate biosystem, the biotransformation of formaldehyde was completed after 30 hours and there were slight fluctuations in the results of the biotransformation (Figure 7.26). For the case of the biotransformation of formaldehyde alone, the duration of the complete biotransformation was 12 hours for a similar concentration. This signifies that there was an inhibition effect on biotransformation of formaldehyde. Simultaneously, according to the results of the biodegradation of acetate and iso-propanol, the same inhibition effect appeared for both of these substrates as well. Particularly, as the three different substrates were in the biosystem at the same time, the acetate accumulation occurred and even only this effect engendered or incited the inhibition of the substrates.
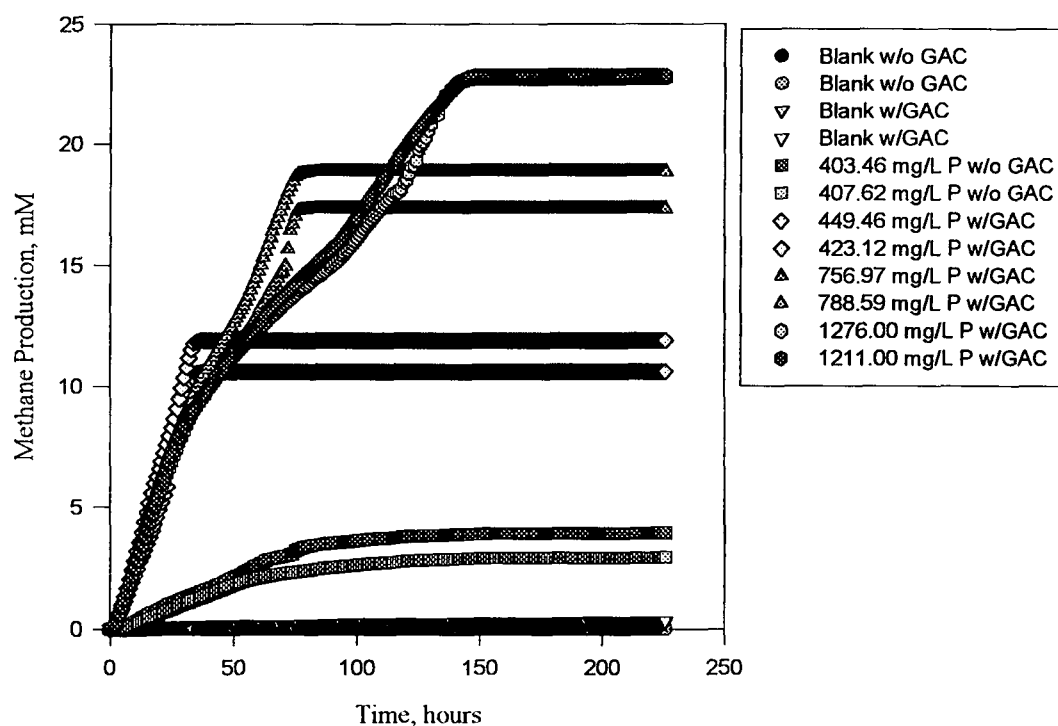
Figure 7.24. Methane production during the anaerobic biodegradation of i-propanol.
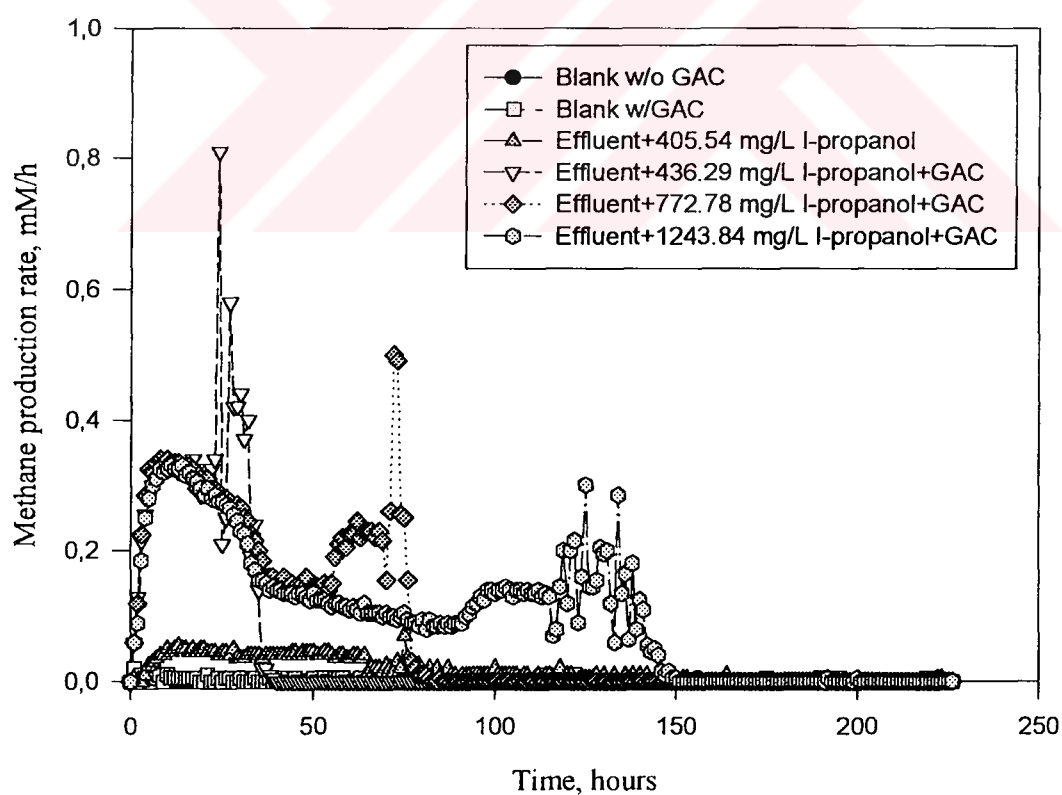
Figure 7.25. Methane production rates of i-propanol biodegradation (averages).

Figure 7.26. Mixed substrate biodegradation by using formaldehyde acclimated culture (averages) Case of 937.44 mg/L Ac., 818.52 mg/L I-propanol, 101.45 mg/L Formaldehyde utilization.

Figure 7.27. Mixed substrate biodegradation by using formaldehyde acclimated culture (averages) Case of 863.90 mg/L Ac., 817.00 mg/L I-propanol, 201.33 mg/L Formaldehyde utilizationzation.

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The similar conclusions can be made for the other higher initial concentrations of formaldehyde in the triple substrate biosystem. The related figures are given in Figure 7.27 and 7.28. Again, higher initial concentrations of formaldehyde caused inhibition on formaldehyde itself as well as on the biodegradations of acetate and iso-propanol. The acetate accumulation increased significantly and the substrate utilization rates damped for all of the three substrates.
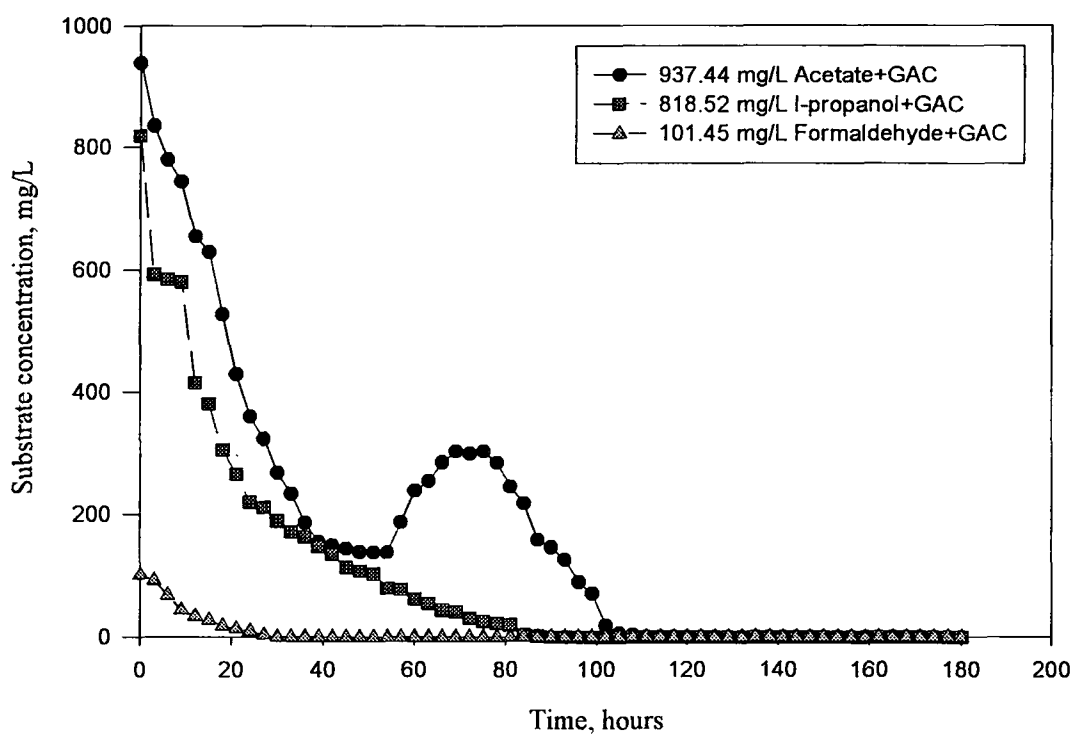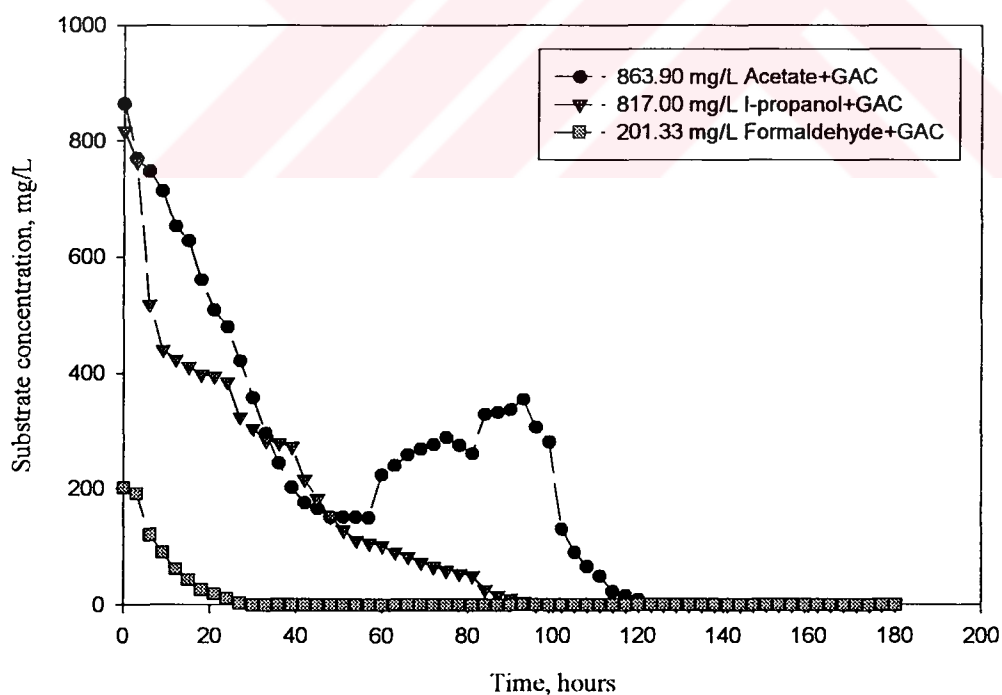

Figure 7.28. Mixed substrate biodegradation by using formaldehyde acclimated culture (averages) Case of 842.45 mg/L Acetate, 786.79 mg/L I-propanol, 405.79 mg/L Formaldehyde utilization.

Figures 7.29 to 7.32 show the biosystems which constituted of two different substrates. In these dual substrate biosystems, the probable inhibition effect of formaldehyde was investigated on the biodegradations of both acetate and iso-propanol, and on formaldehyde itself. Again, the acetate and iso-propanol initial concentrations were constant and the initial concentration of formaldehyde was varied. Two different initial concentrations of formaldehyde were applied to the dual substrate biosystems. According to the results shown in Figures 7.29 and 7.30, it can be concluded that acetate and formaldehyde utilization rates were quite slow due to the competition of the microorganisms for both of the substrates. This competition

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inhibited the biodegradability of the acetate as well as formaldehyde. As can be seen by the figures, no acetate accumulation was observed for both of the cases. This indicates that the acetate accumulation occurs in the presence of iso-propanol in the biosystem. It is highly possible that iso-propanol alone is biotransformed to acetate and hydrogen during its own biodegradation and this elevates the concentration of acetate in the biosystem.

Figures 7.31 and 7.32 present the variation of the substrate concentrations with time for the iso-propanol biodegradation experiments coupled with formaldehyde in the dual substrate biosystem. Observations similar to those made for the case of acetate-formaldehyde dual substrate biosystem can be made for this case, too. According to the experimental results, both iso-propanol and formaldehyde were inhibited by the formaldehyde acclimated anaerobic culture. The biodegradation period increased from 57 hours to 99 hours by increasing the initial concentration of formaldehyde from 101.78 mg/L to 402.56 mg/L. Both of the substrates exhibited fluctuations in their biodegradation results and the utilization rates of iso-propanol and formaldehyde decreased substantially with time.

Figure 7.33 shows the concentrations of methane produced through the anaerobic biodegradation of the triple and dual substrate biosystems. The triple substrate biosystems exhibited high concentrations of methane. In this figure, the total methane gas concentrations were relatively high for all types of substrate combinations because of the contribution of each substrate.

Figure 7.34 shows the methane production rates of the dual and triple substrate biosystems. For the case of the highest initial concentration of formaldehyde (~ 400.00 mg/L) which was used one time in the triple and two times in the double substrate biosystems, the rates of the methane production were relatively slower than the other substrate combinations and showed fluctuations during their biodegradations. However, all substrate combinations displayed the highest methane productions at the end.

Figure 7.29. Mixed substrate biodegradation by using formaldehyde acclimated culture. Case of 804.14 mg/L Acetate, 105.72 mg/L Formaldehyde utilization (averages).
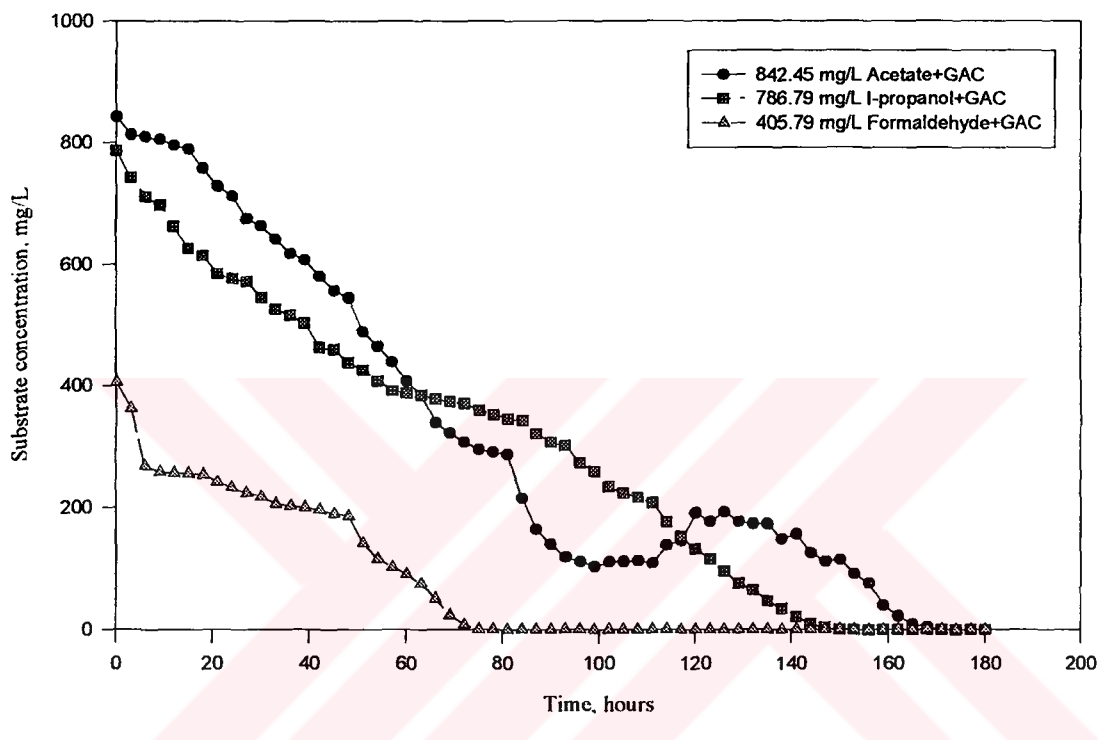

Figure 7.30. Mixed substrate biodegradation by using formaldehyde acclimated culture. Case of 840.43 mg/L Acetate, 398.09 mg/L Formaldehyde utilization (averages).

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Figure 7.31. Mixed substrate biodegradation by using formaldehyde acclimated culture. Case of 851.74 mg/L I-propanol, 101.78 mg/L Formaldehyde utilization (averages)
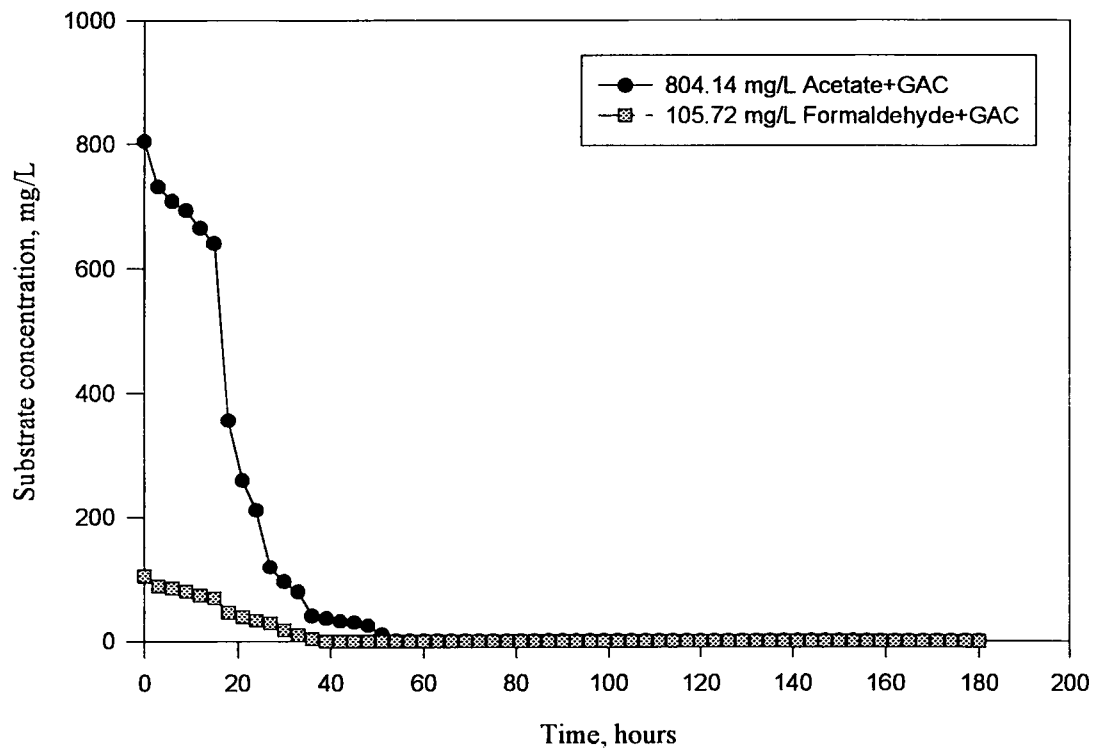

Figure 7.32. Mixed substrate biodegradation by using formaldehyde acclimated culture. Case of 850.62 mg/L I-propanol, 402.56 mg/L Formaldehyde utilization (averages).
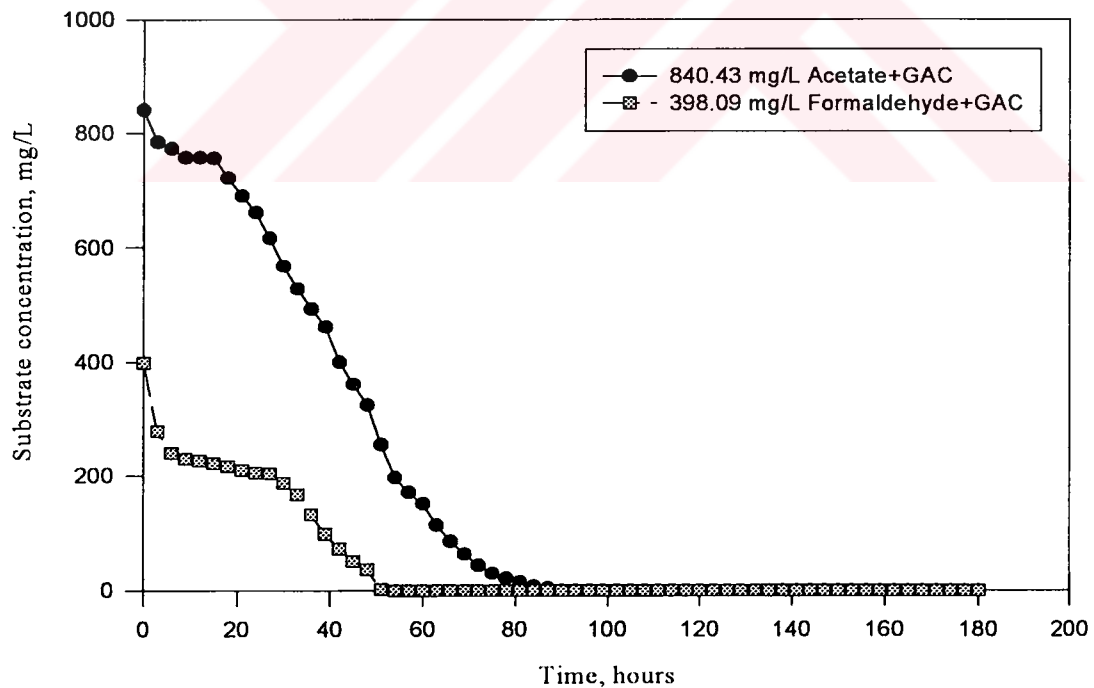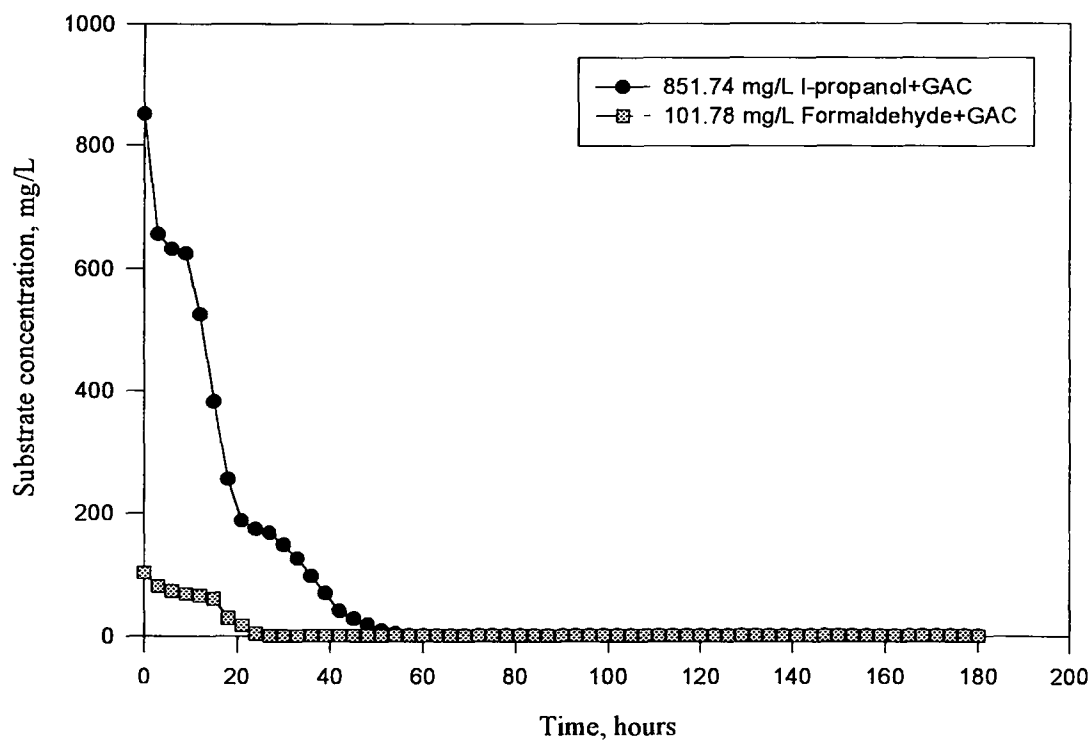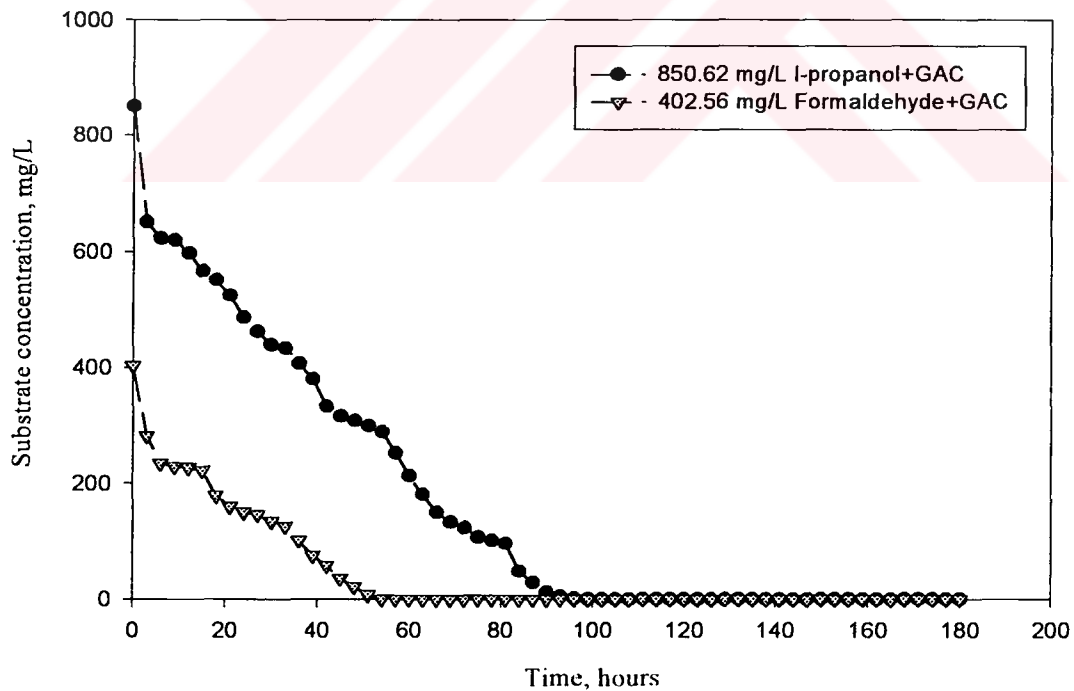
Figure 7.35 exhibits the comparison of the methane gas production results obtained from the respirometer and biodegradation experiments. All the values obtained experimentally are average values of the duplicates. During the calculation of the results (for the biodegradations) the theoretical substrate conversion considerations were taken into account for each corresponding substrate. According to the experimental results, it was found that, acetate was converted to methane and carbon dioxide by acetate-utilizing methanogens, iso-propanol was converted to acetate and hydrogen by iso-propanol-utilizing acetogens, formaldehyde was initially converted to formic acid (formate) and methanol by formaldehyde acclimated bacteria, subsequently, methanol was converted to methane and carbon dioxide while formate was converted to hydrogen and carbon dioxide by methanogenic microorganisms at the same time. Experimentally, it has been determined that the intermediate products of formaldehyde biotransformation, formic acid and methanol, were easily and rapidly biodegradable compounds and their measured concentrations were extremely low. Figure 7.35 shows that for the substrate combinations of having the maximum concentrations of formaldehyde, the results of the methane production obtained by the experiments of the biodegradation were quite lower than the results were obtained from the experiments of the respirometer. These differences stemmed from the high concentrations of hydrogen and carbon dioxide, which were in the media. They were generated by the conversion of the substrates and they were further transformed to the final products of methane and carbon dioxide by hydrogen- and hydrogen-carbon dioxide-utilizing methanogens. For the rest of the substrate combinations, the results of the biodegradation and respirometer showed very close conformity between each other.

The summarized results of the experiments, which were explained at this section so far, are given in Table 7.5.
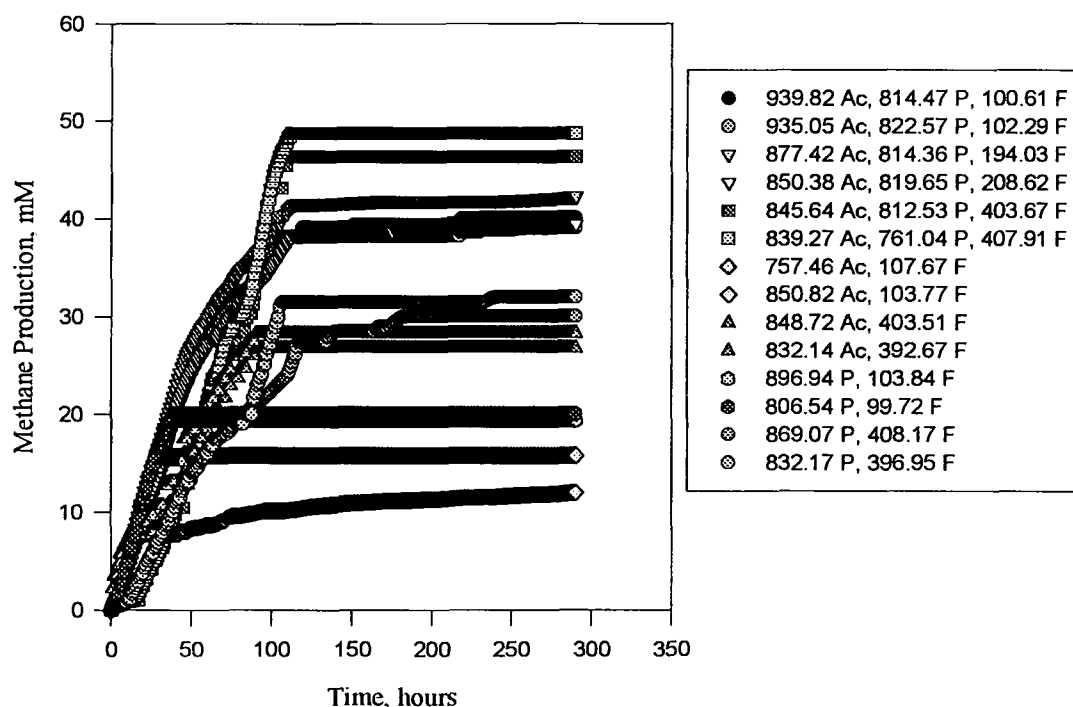
Figure 7.33. Methane production during the anaerobic biodegradation of mixed substrates Ac: Acetate, P: I-propanol, F: Formaldehyde The units of the figures are given as "mg/L".
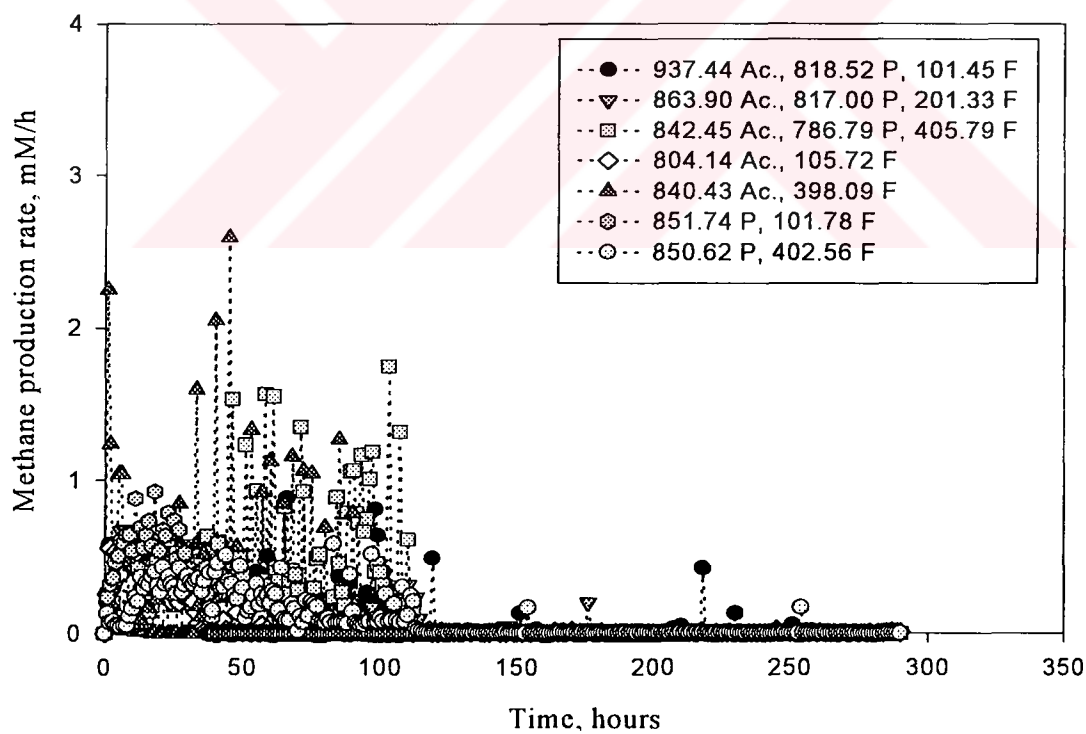
Figure 7.34. Methane production rates of mixed substrate biodegradation (averages) Ac: Acetate, P: I-propanol, F: Formaldehyde The units of the figures are given as "mg/L".

117
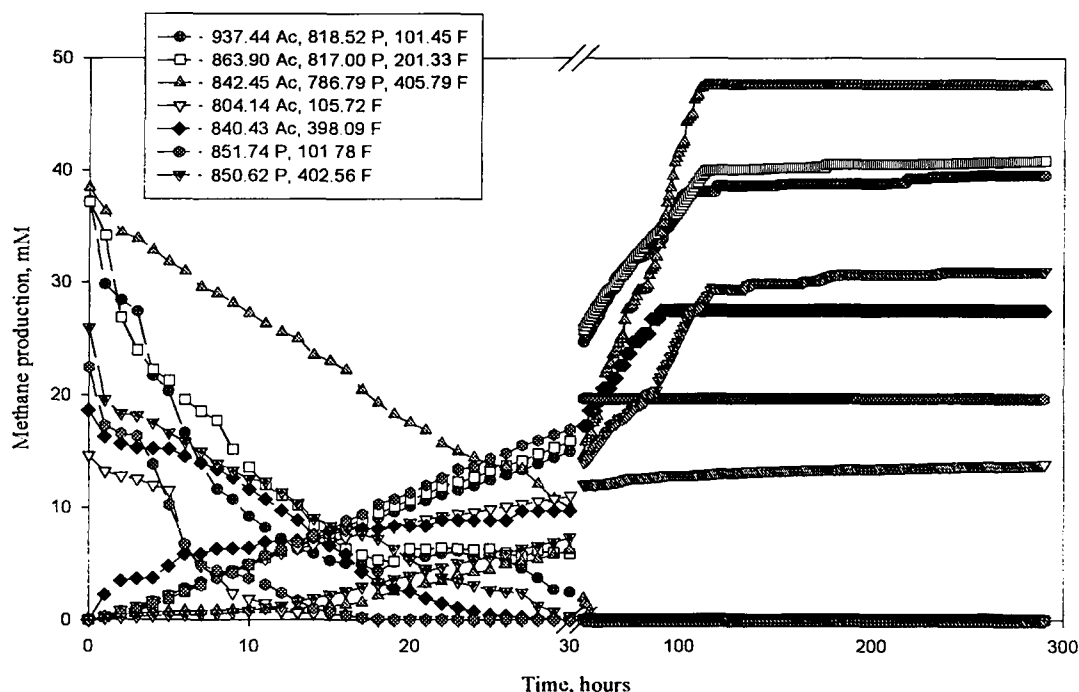
Figure 7.35. Comparison of methane gas production results between respirometer and biodegradation (averages)

Curves on the right hand side show the results after respirometer experiments,

Curves on the left hand side show the results after biodegradation experiments.

Ac: Acetate, P: I-propanol, F: Formaldehyde

The units of the figures are given as "mg/L".

Table 7.5. Anaerobic Biodegradation of Acetate, Formaldehyde and I-propanol during the BMP Tests.

Substrate Concentration (mg/L)			Complete Biodegradation Time of corresponding compound (hours)			Average Methane Production (mM)
Acetate	Formaldehyde	I-Propanol	Acetate	Formaldehyde	I-Propanol	
480.96*	-	-	306	-	-	2.80
476.43	-	-	48	-	-	5.50
919.23	-	-	93	-	-	10.25
1352.55	-	-	123	-	-	11.70
-	55.89*	-	-	9	-	0.24
-	65.62	-	-	6	-	0.37
-	67.64	-	-	15	-	0.44
-	79.26*	-	-	15	-	0.35
-	97.39	-	-	12	-	0.62
-	148.92	-	-	30	-	1.18
-	192.38	-	-	27	-	1.70
-	386.85	-	-	39	-	1.77
-	-	405.54*		-	150	3.70
-	-	436.29		-	39	11.25
-	-	772.78		-	81	18.18
-	-	1243.84		-	132	23.13
804.14	105.72	-	54	39	-	13.88
840.43	398.09	-	90	54	-	27.50
-	101.78	851.74	-	27	57	19.69
-	402.56	850.62	-	54	99	30.99
937.44	101.45	818.52	111	30	90	39.55
863.90	201.33	817.00	123	30	96	40.84
842.45	405.79	786.79	171	75	150	47.51

*without (w/o) - Granular Activated Carbon (GAC).

7.3. Kinetic Assessment

A kinetic model that describes the anaerobic biodegradation of formaldehyde, isopropanol, and acetate has been developed based on the experimental results. During the entire course of the study, three different types of kinetic evaluation were carried out. The aim of the first one was to determine the kinetic parameters for each substrate individually. For this purpose, biodegradations of formaldehyde alone, isopropanol alone and acetate alone have been accomplished in different concentrations by using formaldehyde acclimated anaerobic culture in an anaerobic respirometer.

In the second type of kinetic investigation the aim was to determine the intrinsic kinetic parameters for the double and triple substrate combinations. In this case, the main idea was to define the toxic and/or inhibitory effect of formaldehyde on the other substrates and on itselfTherefore, formaldehyde in different concentrations was applied to the both double and triple substrate combinations. In each case estimation and optimization was performed using the corresponding modified Haldane kinetic expression and for all cases, Levenberg-Marquardt algorithm (Figure 7.36) was used in order to obtain the corresponding kinetic parameters. At the last stage of the kinetic investigation, a competitive inhibition model was applied to the anaerobic biotransformation of formaldehyde. For this purpose, as a growth substrate, which is defined as carbon and energy sources for microbial growth and maintenance, isopropanol has been selected in the concentration of 12.858 mM. The kinetic expressions which were described in Chapter 6 (Equations 6.7 to 6.27) were resolved in order to get the corresponding kinetic parameters. There were totally 32 kinetic parameters and 10 ordinary differential equations to be solved simultaneously. The interpretation of this section is given broadly in Chapter 6 with the subtitle of "model development".
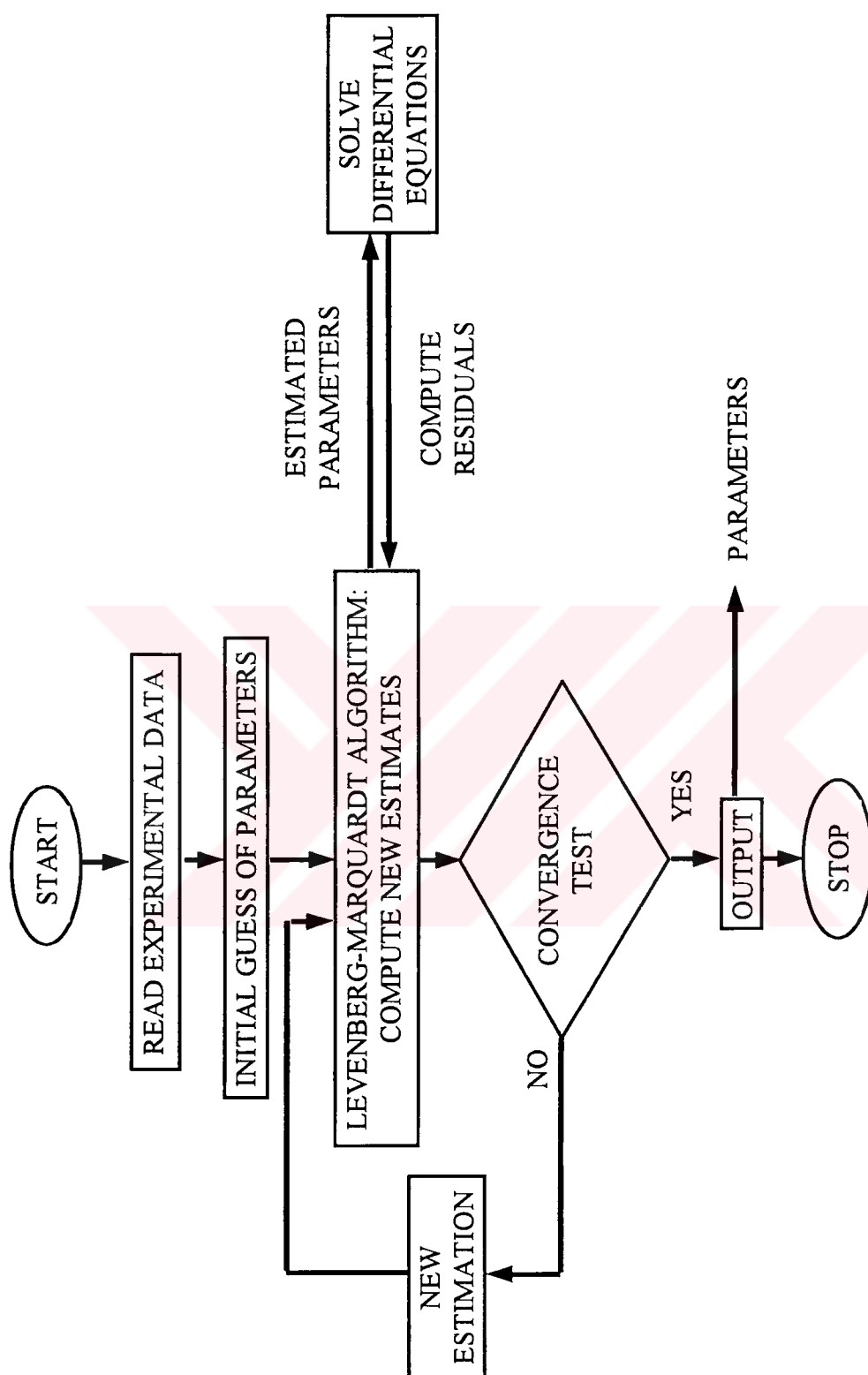
Figure 7.36. Flow Chart for Parameter Estimation and Optimization.

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7.3.1. Determination of intrinsic kinetic parameters

Intrinsic kinetic parameters estimated and optimized for each type of substrate are shown in Figures 7.37 to 7.55.

7.3.1.1. Kinetics for acetate biodegradation

In Figures 7.37, 7.38 and 7.39, the intrinsic kinetic parameters obtained for the acetate biodegradation are shown. In the beginning, the acetate concentration was 7.934 mM. Subsequently, the acetate concentration was ascended first from 7.934 mM to 15.31 mM and then from 15.31 mM to 22.52 mM. According to the experimental data, acetate was biodegraded directly to methane and carbon dioxide. The methanogenesis of acetate can be well expressed with an inhibition model. The numerical solutions fit the experimental data very well in all three cases. Based upon to the data obtained, it can be considered that methanogenesis of acetate was strongly inhibited by the formaldehyde acclimated anaerobic microorganisms and acetate probably inhibited its own methanogenesis. Although, no fluctuations were obtained during the biodegradation of acetate, the biochemical reaction rate of acetate was quite slow. The methanogenesis of acetate showed the same inhibition effect $(K_{ia}=2.07$ mM) on biodegradation of acetate for the all three different concentrations but the duration for complete biodegradation become longer for higher acetate concentrations. These three graphs indicate that the rate of acetate biodegradation depends on its concentration and higher acetate input or production result in longer biodegradation times. The immoderate acetate concentrations in the environment affect the pH balance of the biosystem, which can influence severely the activity of microorganisms. Each time longer lag time periods for the methanogenic bacterium was observed by increasing the acetate concentration.
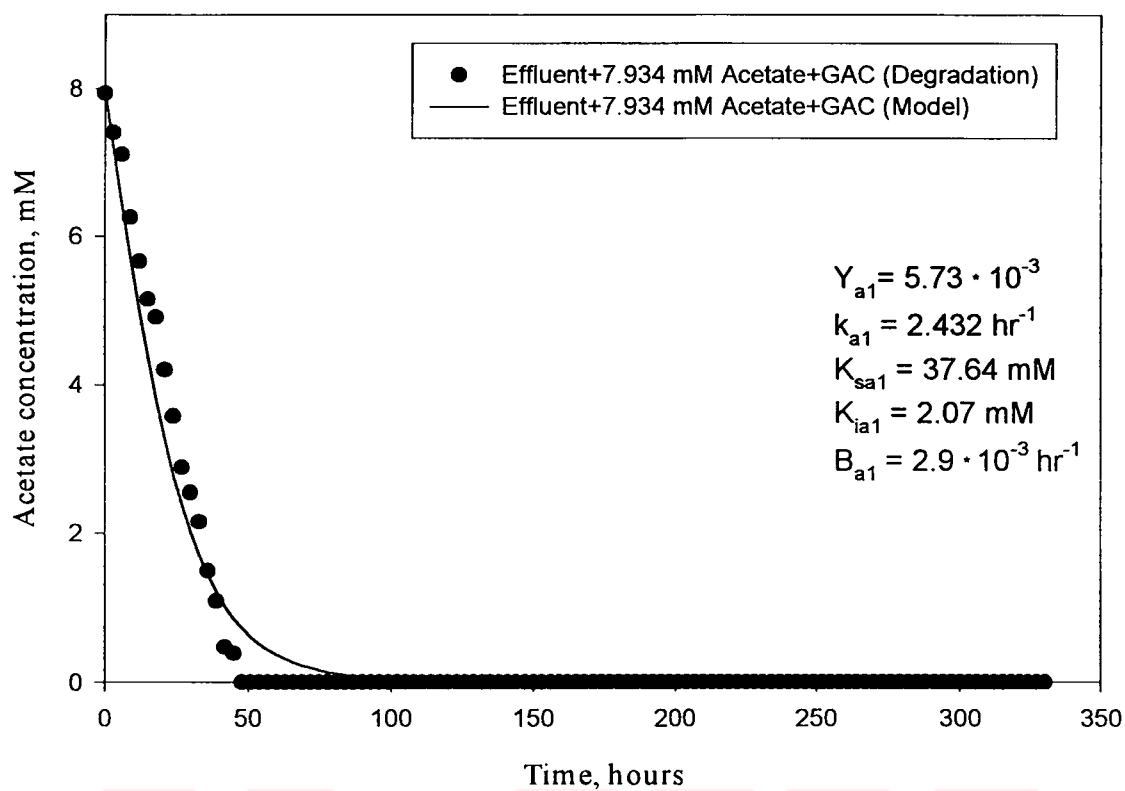
Figure 7.37. Comparison curves of the acetate biodegradation (a1)
Case of sole acetate: 476.43 mg/L (7.934 mM).
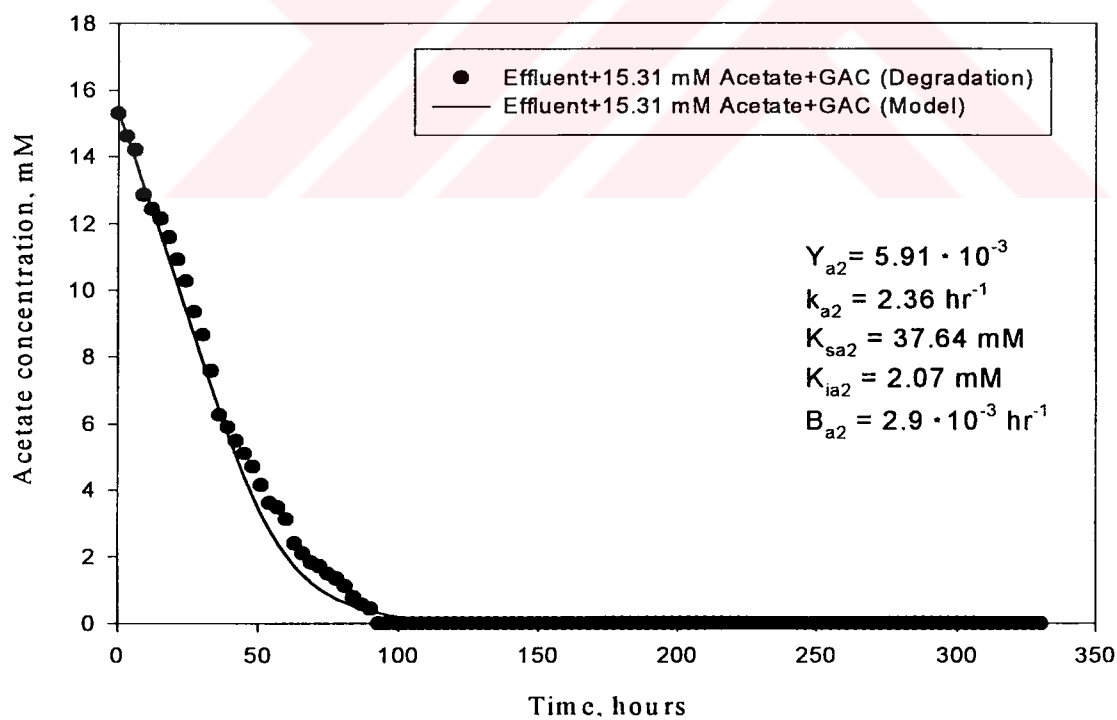
Figure 7.38. Comparison curves of the acetate biodegradation (a2)
Case of sole acetate: 919.23 mg/L (15.31 mM).
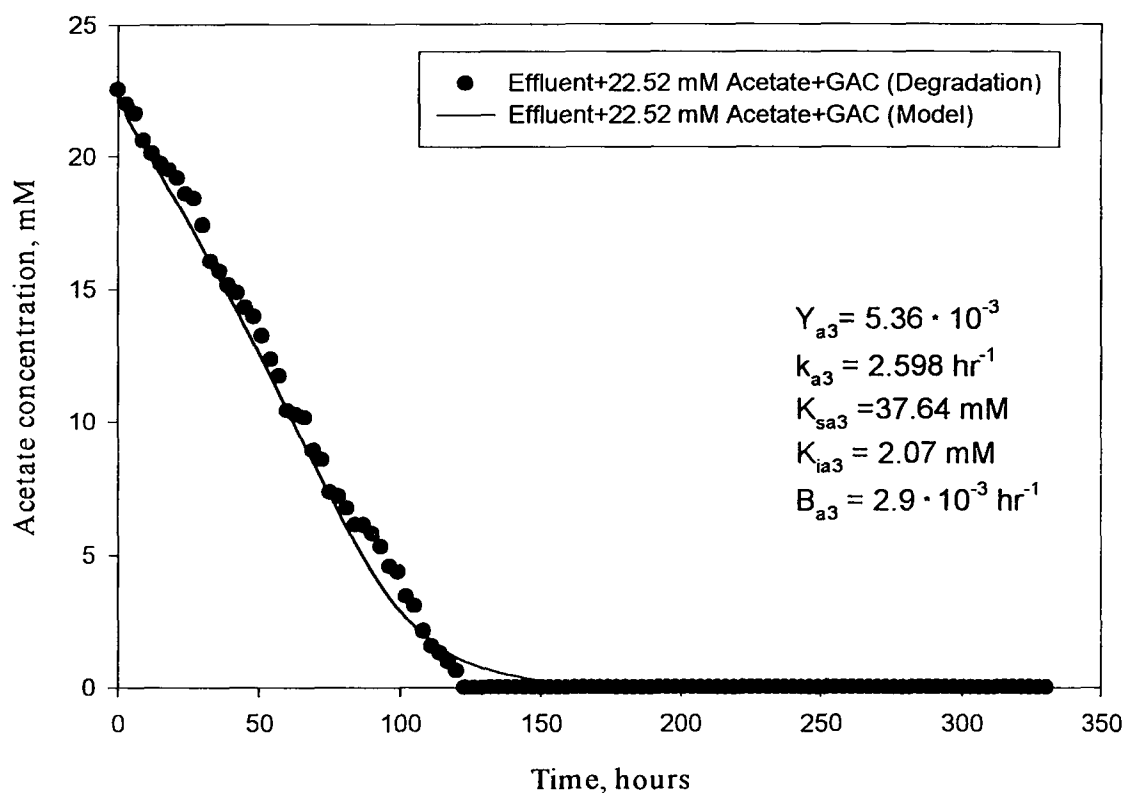
Figure 7.39. Comparison curves of the acetate biodegradation (a3)
Case of sole acetate: 1352.55 mg/L (22.52 mM)

7.3.1.2. Kinetics for formaldehyde biotransformation

During the experiments for biotransformation of formaldehyde alone, 6 different formaldehyde concentrations were studied which ranged from 2.185 mM to 12.882 mM (Figures 7.40 to 7.45). According to the experimental data, no inhibition effects were determined on formaldehyde biotransformations low formaldehyde concentrations. The low formaldehyde concentrations; 2.185 mM, 2.25 mM, and 3.24 mM showed similarity regarding both the biotransformation results and kinetic parameters. The inhibition coefficients were quite high, indicating that formaldehyde acclimated microorganisms tolerated the low formaldehyde concentrations very easily (Figures 7.40, 7.41 and 7.42). According to the complete biotransformation durations, there was no time difference for the concentrations of 2.25 and 3.24 mM of formaldehyde, and both of them were biotransformed around 15 hours while the lowest formaldehyde concentration (2.185 mM) was biotransformed within 6 hours. Thereafter, the formaldehyde concentrations were increased to 4.96, 6.41 and 12.882 mM, respectively (Figures 7.43, 7.44 and 7.45).

$Y_{f1} = 1.0 \cdot 10^{-2}$

$k_{f1} = 0.5046 \ hr^{-1}$

$K_{sf1} = 0.3591 \ mM$

$K_{if1} = 130.76 \ mM$

$B_{f1} = 4.6 \cdot 10^{-2} \ hr^{-1}$
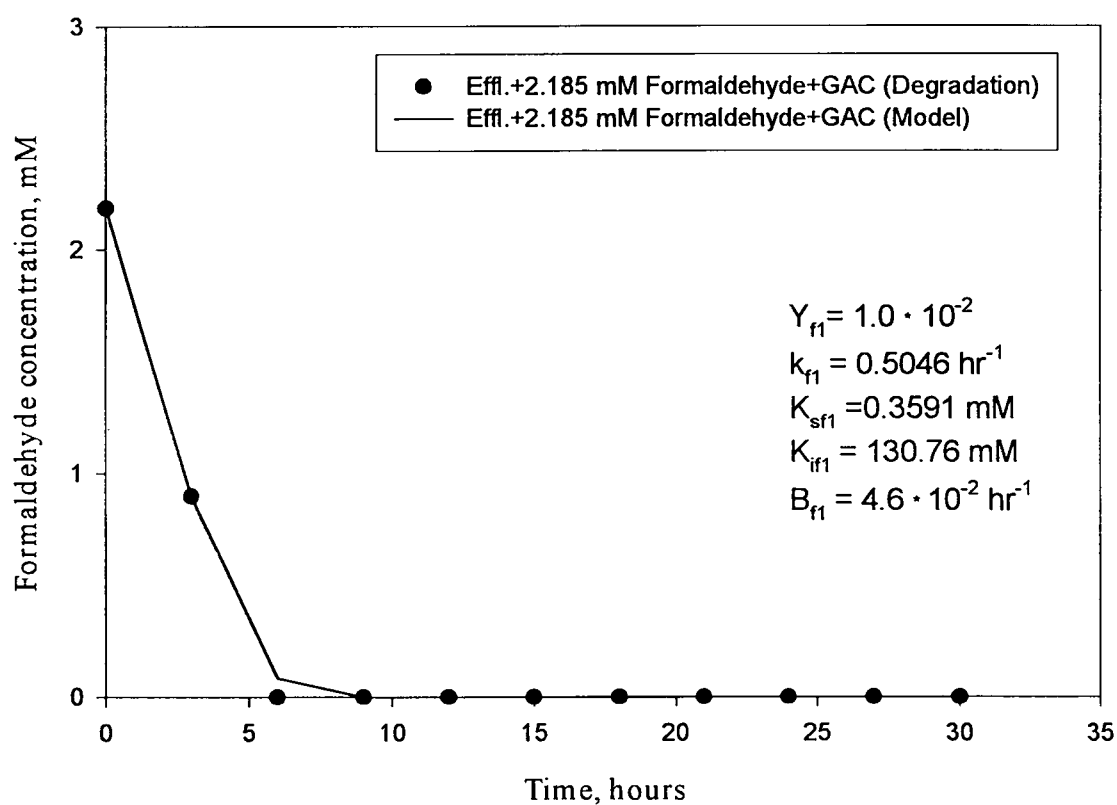
Figure 7.40. Comparison curves of the formaldehyde biodegradation (f1)
Case of sole formaldehyde: 65.62 mg/L (2.185 mM)

$Y_{f2} = 1.0 \cdot 10^{-5}$

$k_{f2} = 7.042 \ hr^{-1}$

$K_{sf2} = 22.25 \ mM$

$K_{if2} = 100.64 \ mM$

$B_{f2} = 3.63 \cdot 10^{-2} \ hr^{-1}$
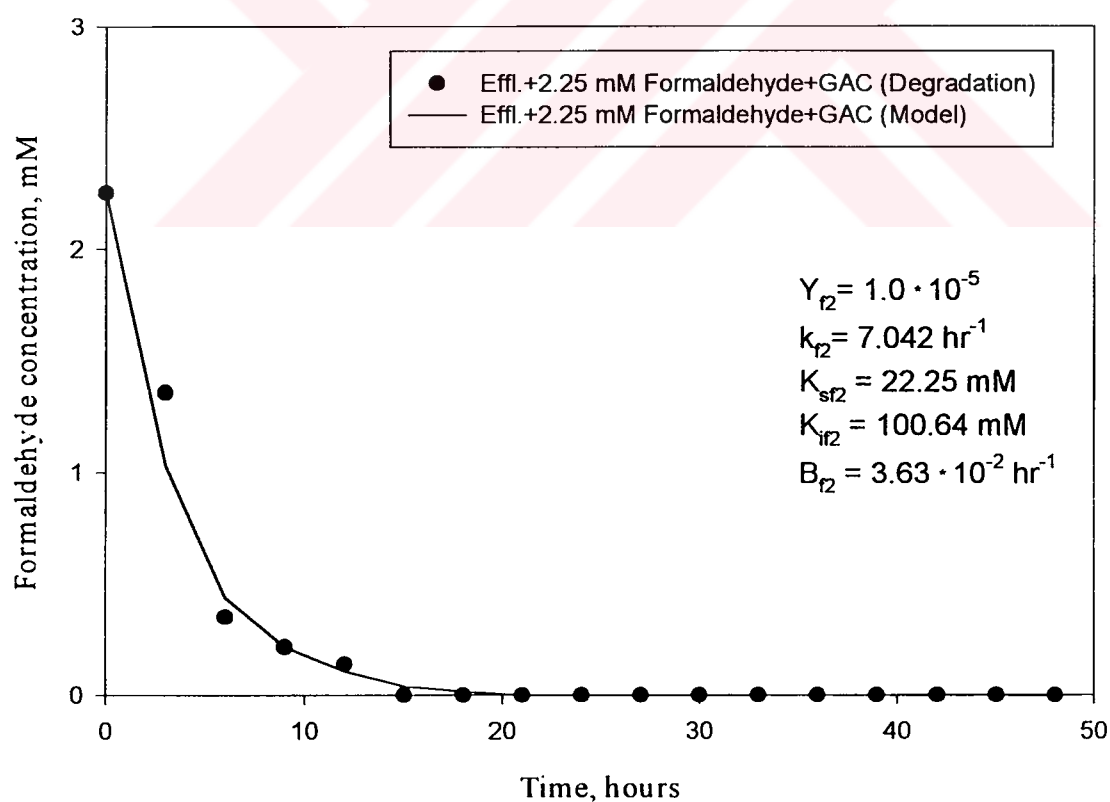
Figure 7.41. Comparison curves of the formaldehyde biodegradation (f2)
Case of sole formaldehyde: 67.64 mg/L (2.25 mM).

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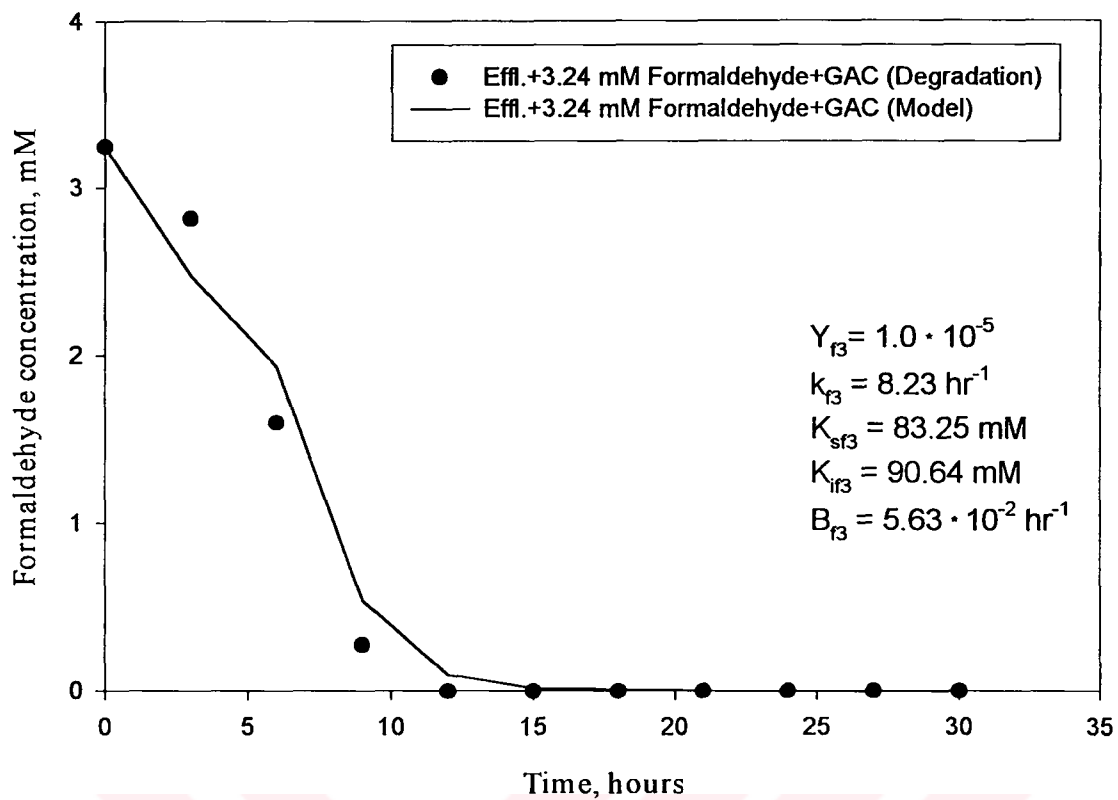
Figure 7.42. Comparison curves of the formaldehyde biodegradation (f3)
Case of sole formaldehyde: 97.39 mg/L (3.24 mM)

Constants shown on Figure 7.42:

$$Y_{f3} = 1.0 \cdot 10^{-5}$$
$$k_{f3} = 8.23 \ hr^{-1}$$
$$K_{sf3} = 83.25 \ mM$$
$$K_{if3} = 90.64 \ mM$$
$$B_{f3} = 5.63 \cdot 10^{-2} \ hr^{-1}$$
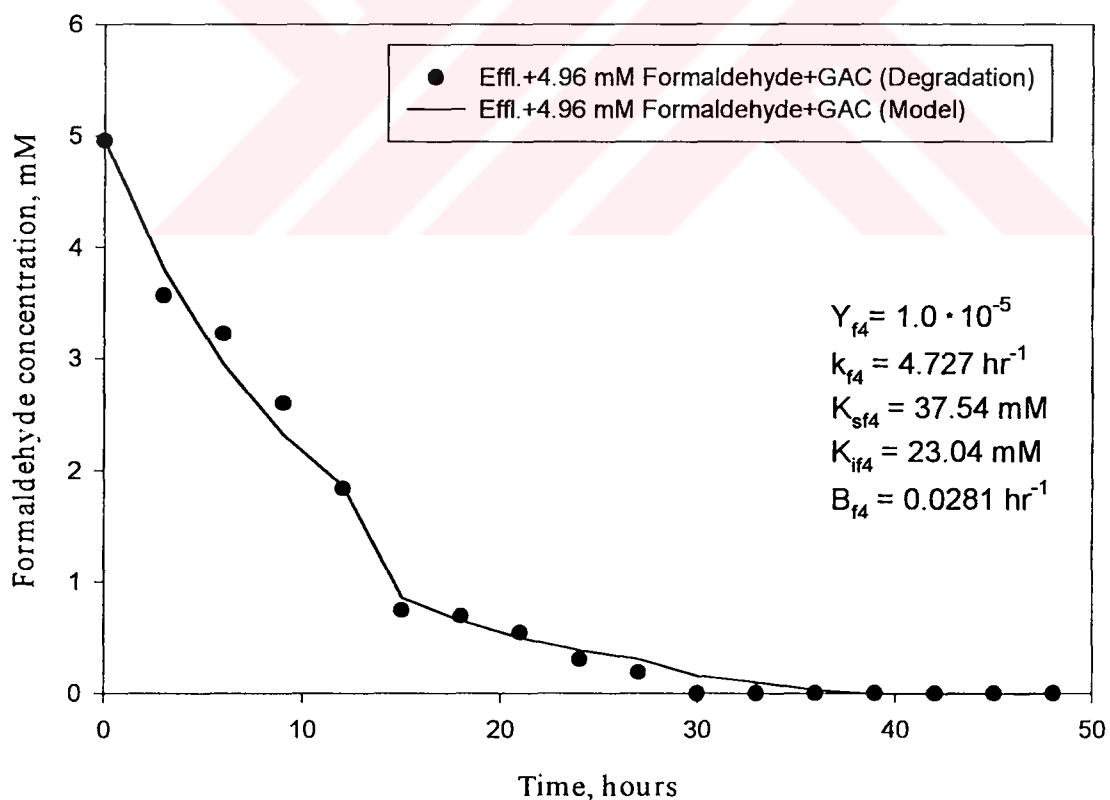
Figure 7.43. Comparison curves of the formaldehyde biodegradation (f4)
Case of sole formaldehyde: 148.92 mg/L (4.96 mM)

Constants shown on Figure 7.43:

$$Y_{f4} = 1.0 \cdot 10^{-5}$$
$$k_{f4} = 4.727 \ hr^{-1}$$
$$K_{sf4} = 37.54 \ mM$$
$$K_{if4} = 23.04 \ mM$$
$$B_{f4} = 0.0281 \ hr^{-1}$$

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These latter three cases reveal that the biotransformation of formaldehyde started to become moderately difficult for the microorganisms. The numerical solutions (models) fit the experimental data very well. The formaldehyde concentrations of 4.96 and 6.41 mM showed approximately the same inhibition effects, indicating that the inhibitions were not very strong. The inhibition effect started to become more obvious when the formaldehyde concentration attained its highest level. In this case, the formaldehyde concentration was 12.882 mM and the inhibition coefficient obtained from the numerical solution was K_{if6}=13.40 mM which means that inhibition was relatively higher than for the other concentrations. The duration of biotransformation increased to around 30 hours for the concentrations of 4.96 and 6.41 mM. For the concentration of 12.882 mM of formaldehyde, the complete biotransformation was accomplished approximately after 40 hours. Consequently, these 6 figures which give information about the formaldehyde biotransformation signify that in some measure, the formaldehyde, as an inhibitory and/or toxic compound, can be biotransformed and the biotransformation of formaldehyde is strongly dependent on its initial concentration.

$$Y_{f5}= 1.0 \cdot 10^{-2}$$
$$k_{f5} = 2.899 \text{ hr}^{-1}$$
$$K_{sf5} = 24.80 \text{ mM}$$
$$K_{if5} = 20.07 \text{ mM}$$
$$B_{f5} = 1.0 \cdot 10^{-5} \text{ hr}^{-1}$$

Figure 7.44. Comparison curves of the formaldehyde biodegradation (f5)
Case of sole formaldehyde: 192.38 mg/L (6.41 mM)

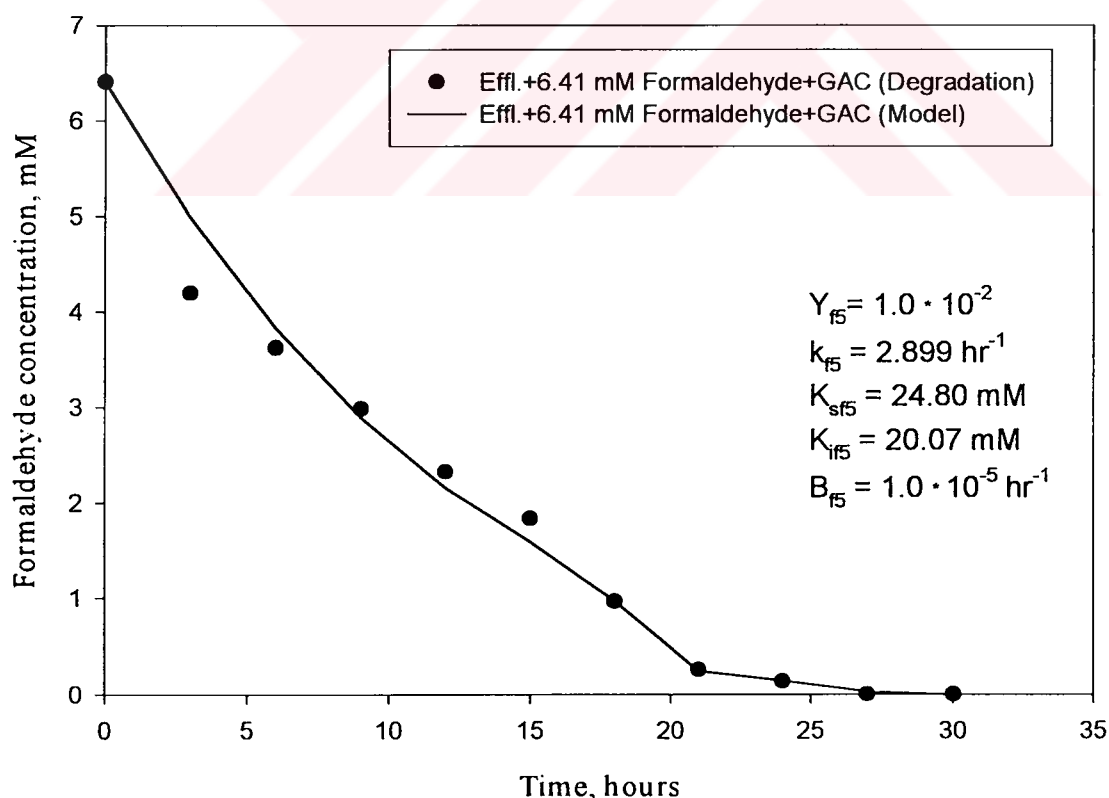If the initial concentration of formaldehyde is greater than a critical value, the formaldehyde begins to affect the active enzymes that catalyze the biotransformation process and constitutes more complex compounds, which are mostly recalcitrant to biotransformation process.

7.3.1.3. Kinetics for iso-propanol biodegradation

During the biodegradation experiments of iso-propanol alone, three different concentrations were investigated. The related figures are shown in Figures 7.46, 7.47, and 7.48, respectively. While applying the lowest concentration of iso-propanol (7.259 mM in Figure 7.46), the inhibition effect of formaldehyde acclimated microorganisms on iso-propanol biodegradation was not very strong (K_{ip1}=42.08 mM) and biodegradation was completed after 40 hours. When the iso-propanol concentration was increased to 12.858 mM, the inhibition effect raised as well but it was not so strong (K_{ip2}=20.13 mM) again. Although, the maximum specific transformation rates for iso-propanol biodegradation were at the same order of magnitude, the yield coefficients were different. For the concentration of 12.858 mM of iso-propanol, the yield coefficient was much lower which indicates that because of the inhibition effect, more complex insubordinate constituents occurred during the bioprocess and these constituents held up the utilization of iso-propanol. That is why, the biodegradation was completed after 80 hours. Eventually, the results obtained from the Figures 7.46 and 7.47 show very close agreement between each other. When the inhibition effect increased two-fold, the biodegradability of the substrate also decreased two-fold. In Figure 7.48, the iso-propanol concentration was the highest and its value was 20.696 mM. In this case, the maximum specific transformation rate for iso-propanol was higher than for the other concentrations (k_{p3}=4.4 hr^{-1}) due to the relatively higher inhibition effect (K_{ip3}=13.40 mM). This conclusion signifies that the biodegradation of iso-propanol proceeded more slowly, but consequently, the biodegradation was accomplished at the same time as in the case of the biodegradation of iso-propanol alone in the concentration of 12.858 mM which had required 80 hours for complete biodegradation.
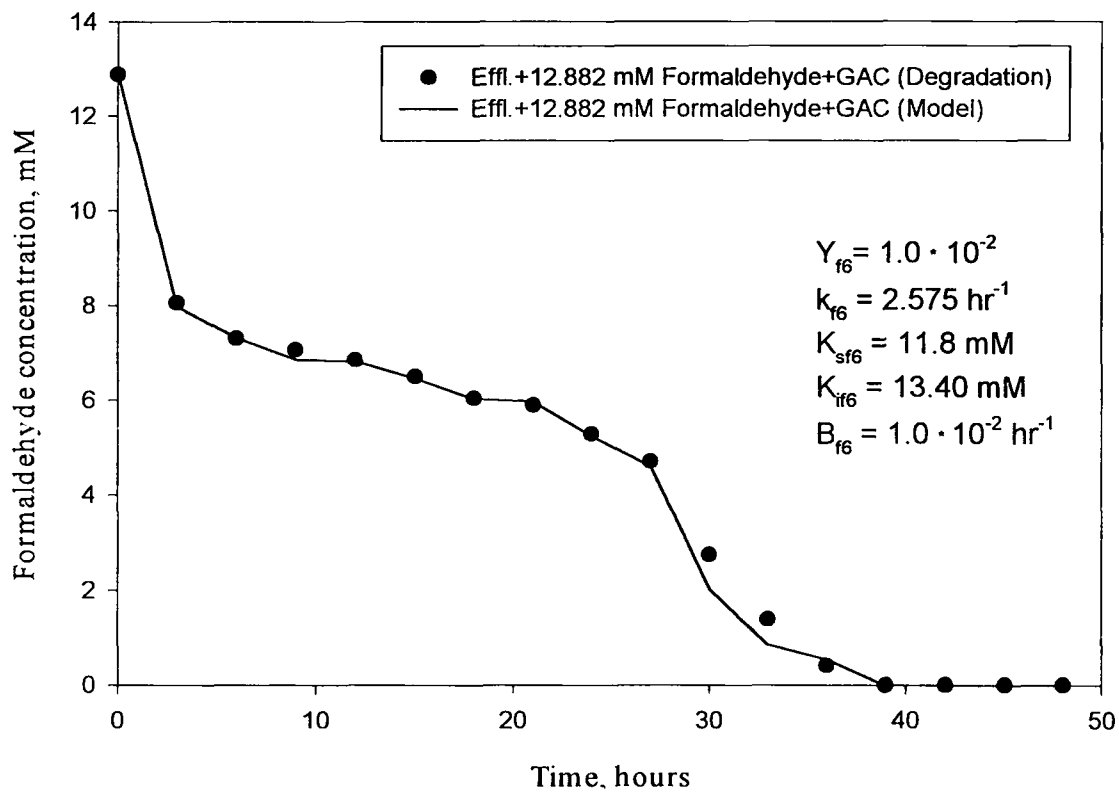
Figure 7.45. Comparison curves of the formaldehyde biodegradation (f6)
Case of sole formaldehyde: 386.85 mg/L (12.882 mM).

Figure 7.46. Comparison curves of the I-propanol biodegradation (p1)
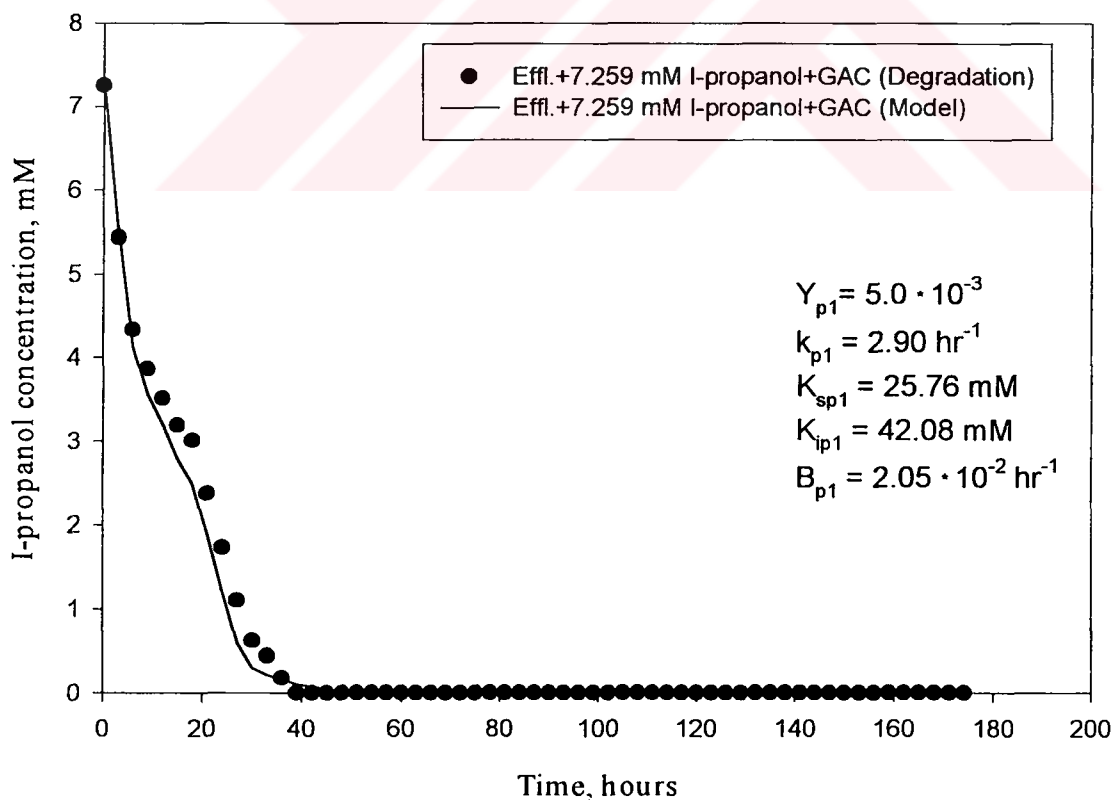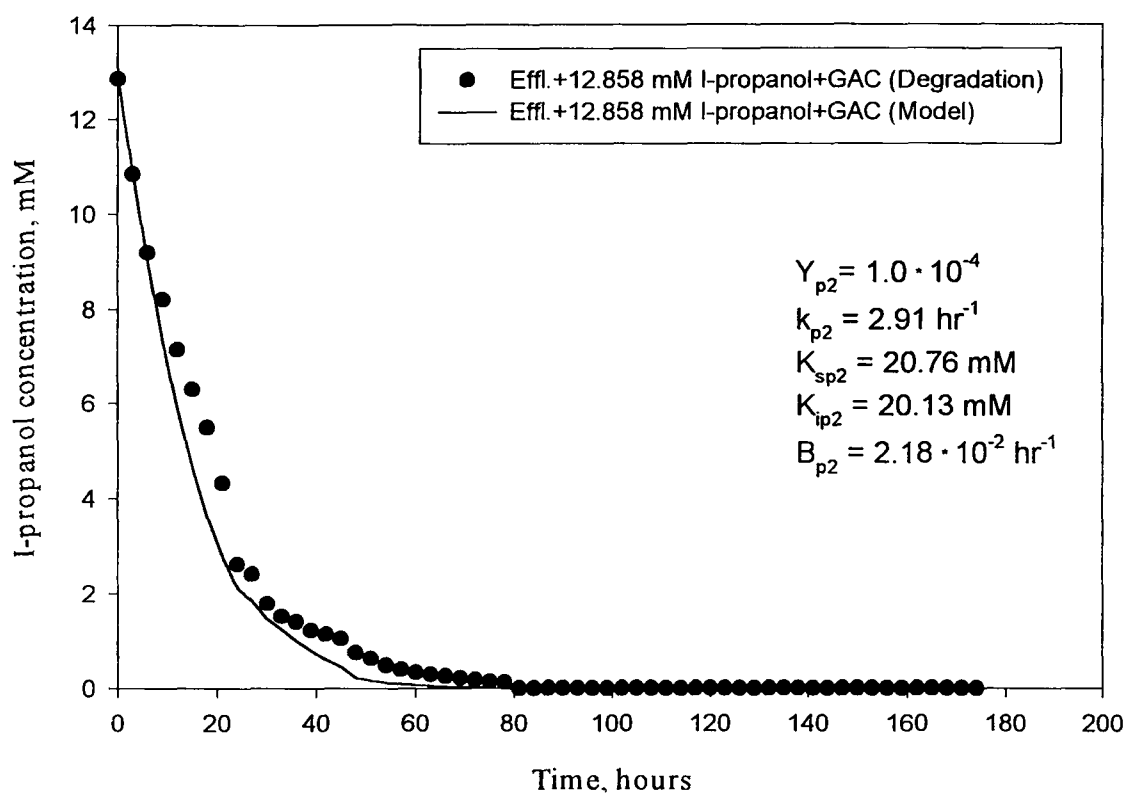Case of sole I-propanol: 436.29 mg/L (7.259 mM).

Figure 7.47. Comparison curves of the I-propanol biodegradation (p2)
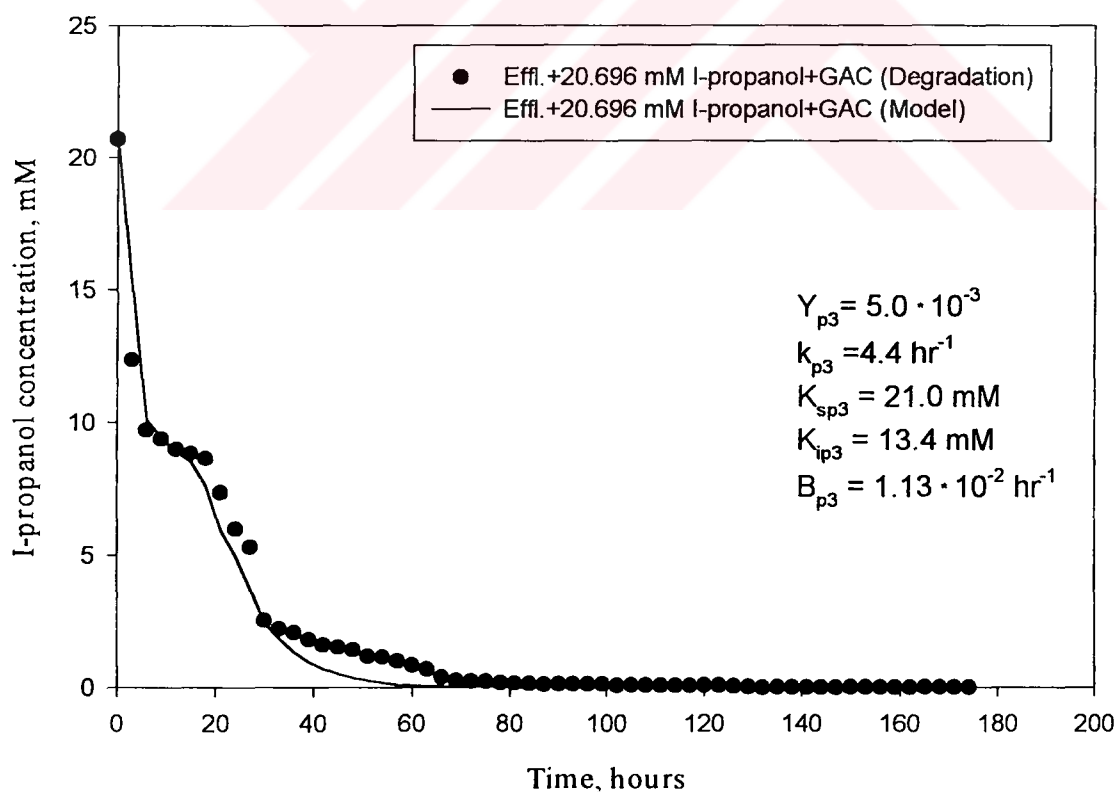Case of sole I-propanol: 772.78 mg/L (12.858 mM)

Figure 7.48. Comparison curves of the I-propanol biodegradation (p3)
Case of sole I-propanol: 1243.84 mg/L (20.696 mM)

7.3.1.4 Kinetics for triple substrate combinations

Three different triple substrate combinations were investigated. The related figures are shown in Figures 7.49, 7.50 and 7.51, respectively. In all three cases, the iso-propanol and acetate concentrations were kept at the same order of magnitude whereas only formaldehyde concentrations were changed. Initially, the formaldehyde concentration was 3.38 mM (Figure 7.49). For this case, the numerical solutions fit the experimental data very well except for acetate biodegradation. The best model has been tried for the best fit to the experimental data to see the span of inhibition. According to the experimental results, it was observed that, all the substrates showed strong inhibition effect. At the beginning, the substrate utilization rates were quite similar. Although, formaldehyde was consumed faster than the other substrates, even this biotransformation took about 32 hours to reach completion. In the case of formaldehyde alone that was used previously at the same concentration, the duration was 12 hours. After formaldehyde was depleted completely, the acetate started to accumulate in the biosystem. At this point, the acetate-utilizing methanogens were inhibited by the excess of acetate concentration and its biodegradation ceased for a certain period of time until the iso-propanol was consumed almost completely from the biosystem. Figure 7.50 presents the variation of the biodegradation of all substrates for the formaldehyde concentration of 6.70 mM. In this case, the formaldehyde utilization rate was greater than those of the other two substrates and it was consumed first. The complete biotransformation for formaldehyde was actualized within 28 hours. This duration shows consistency with the biotransformation duration of formaldehyde alone in the same concentration. However, due to the competition of microorganisms for the active enzymes and for the other substrates, the yield coefficient of formaldehyde biotransformation was decreased and the microbial endogenous decay coefficient was increased. The competition effect of microorganisms affected the biodegradability of both iso-propanol and acetate. After all of the formaldehyde was biotransformed, the biodegradation rates of iso-propanol and acetate started to increase.
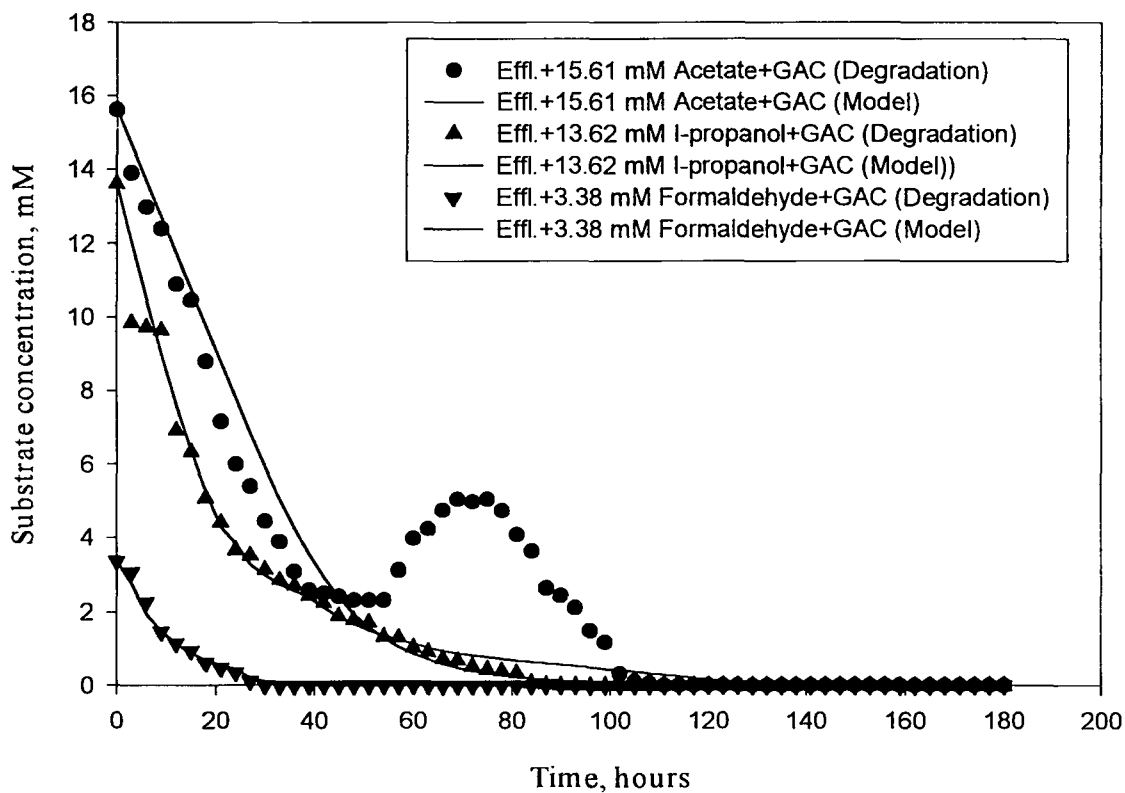
Figure 7.49. Comparison curves of the mixed substrate biodegradation (afp1)
Case of 937.44 mg/L (15.61 mM) Acetate, 101.45 mg/L (3.38 mM)
Formaldehyde, 818.52 mg/L (13.62 mM) I-propanol.

Figure 7.50. Comparison curves of the mixed substrate biodegradation (afp2)
Case of 863.90 mg/L (14.39 mM) Acetate, 201.33 mg/L (6.70 mM)
Formaldehyde, 817.00 mg/L (13.59 mM) I-propanol.

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The same acetate accumulation problem was encountered in this case as well. But at this time, acetate fluctuations were more pronounced. The biodegradation times became longer for both iso-propanol and acetate. The numerical solutions fit the experimental data very well except for acetate biodegradation. Again the best fitting has been tried in order to acquire the kinetic parameters. Experimental data and numerical solutions for the formaldehyde concentration of 13.51 mM are shown in Figure 7.51. As can be seen from the figure, the formaldehyde and acetate experimental data do not fit the numerical solutions well. Both showed marked fluctuations through the biochemical reactions. Again the best fits have been tried to be obtained. According to the kinetic data which appertains to formaldehyde biotransformation, the inhibition effect does not seem so strong but based upon the former results and evaluations, this result probably does not reflect the truth. On the contrary, the inhibition coefficient is expected to be higher than the previous corresponding results. Generally, the same remarks which have been made previously (in Figures 7.49 and 7.50), are applicable here as well. The only difference is based on the highest formaldehyde concentration, therefore the same effects became stronger by overlapping.

7.3.1.5. Kinetics for double substrate combinations

Figures 7.52 and 7.53 show the kinetics of acetate and formaldehyde where they were together in the biosystem. In the beginning, the initial formaldehyde concentration was set to 3.52 mM (Figure 7.52). Thereafter, the concentration was increased to 13.26 mM (Figure 7.53). In both cases, the initial acetate concentrations were kept almost constant. As can be seen in Figure 7.52, the numerical solutions fit the experimental data very well and neither of the substrates exhibited strong inhibition. Although at the beginning, the biodegradation rates were quite similar, later the acetate biodegradation rate grew faster than the formaldehyde biotransformation rate. After having increased the initial formaldehyde concentration, both of the substrates exhibited inhibition individually.

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Figure 7.51. Comparison curves of the mixed substrate biodegradation (afp3)
Case of 842.45 mg/L (14.03 mM) Acetate, 405.79 mg/L (13.51 mM)
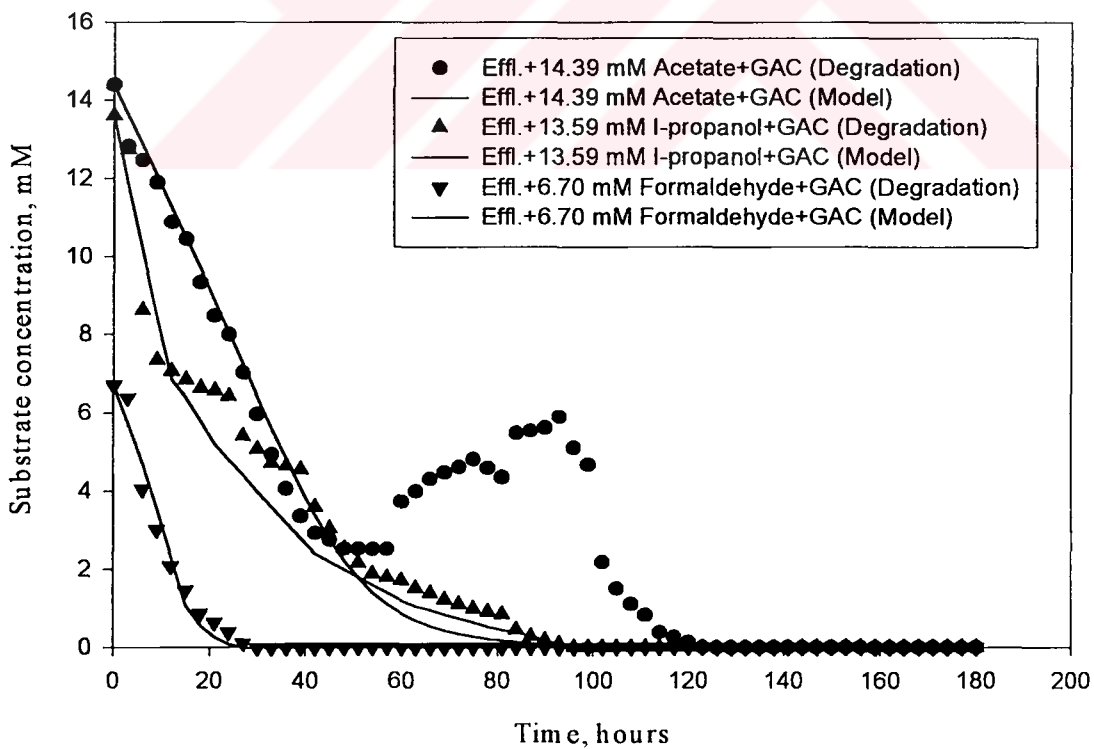Formaldehyde, 786.79 mg/L (13.09 mM) I-propanol
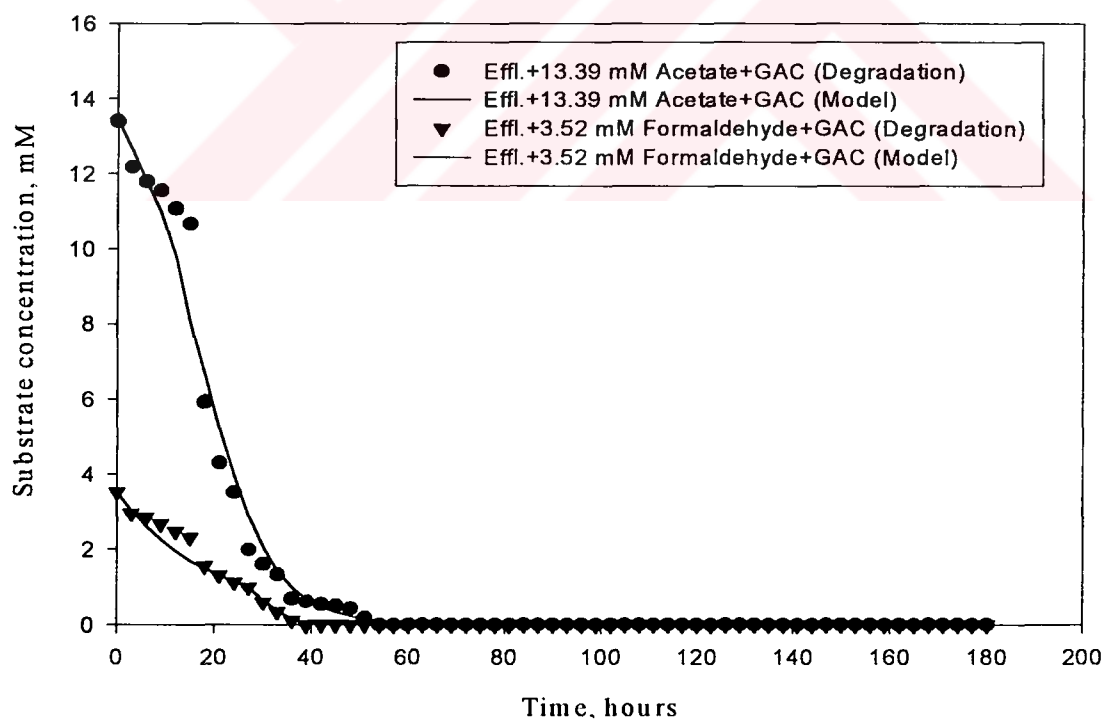
Figure 7.52. Comparison curves of the mixed substrate biodegradation (af1)
Case of 804.14 mg/L (13.39 mM) Acetate, 105.72 mg/L (3.52 mM)
Formaldehyde

Figure 7.53 displays the related biodegradation curves of acetate and formaldehyde. Again, the numerical solution for the formaldehyde biotransformation does not fit the experimental data very well, but in order to have a bias on formaldehyde biotransformation, the best fit has been tried to be obtained. Formaldehyde acclimated anaerobic bacteria showed rapid biotransformation rate for the formaldehyde at the initial stages of biochemical reactions, but thereafter, the acetate-utilizing methanogens began to be dominant in the biosystem. When both acetate and formaldehyde concentrations attained exactly the same concentration after 30 hours, the biodegradation rates become almost equal. Due to the absence of iso-propanol in the biosystem, the competition of microorganisms for the active sites of enzymes and substrates decreased. However, on the other hand, the inhibition effects caused lower biochemical reaction rates especially for the formaldehyde biotransformation. The influence of this can be observed from the kinetics of formaldehyde biotransformation (Y_{af2}=0.001 and k_{af2}=2.8 hr^{-1}). Figures 7.54 and 7.55 show the variation of iso-propanol and formaldehyde concentrations in the dual substrate biosystems. Again, the initial formaldehyde concentration was kept low (3.39 mM), after that its value was increased to 13.405 mM. Iso-propanol concentrations were sustained quiescent in both of the cases. The numerical solutions fit the experimental data very well and they are all in very close agreement as well as the kinetic parameters, which have been obtained from the models. The results reveal that in all cases the inhibition is not very strong but after having increased the formaldehyde concentration, the biodegradation rates of iso-propanol and formaldehyde become slower.

All the intrinsic kinetic parameters, which were obtained from the first two parts of the kinetic evaluation, are listed in Table 7.6.
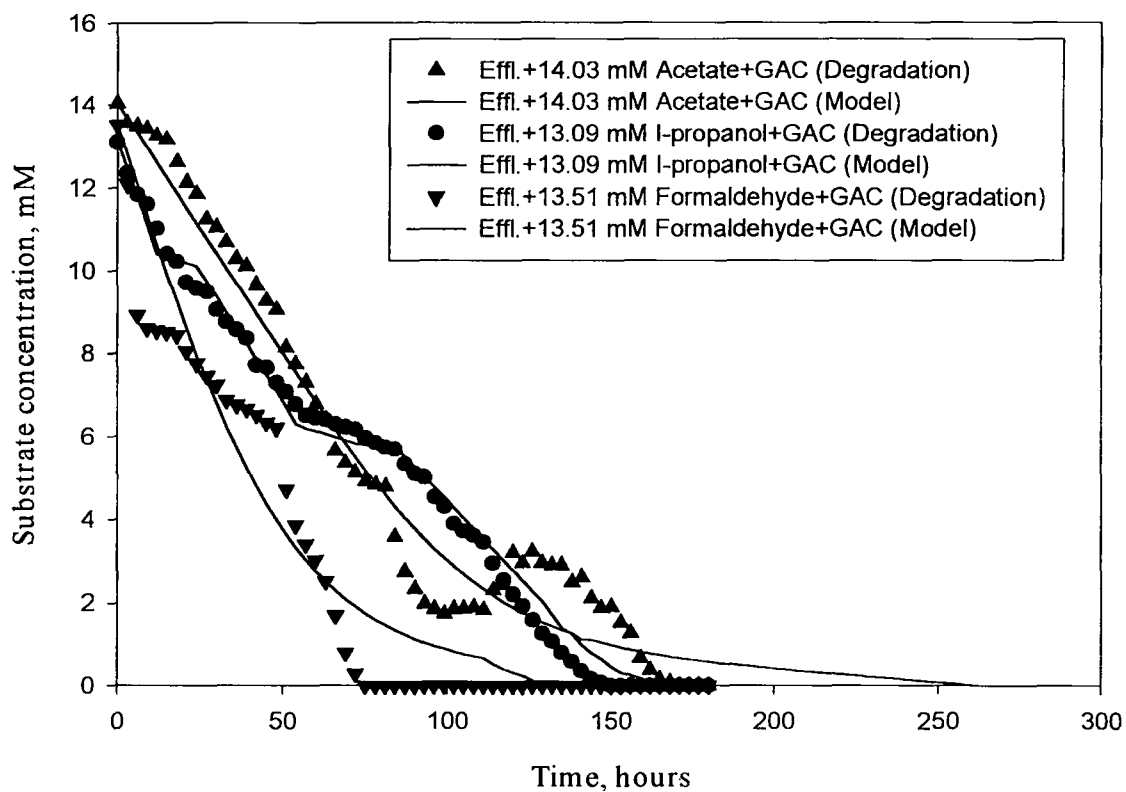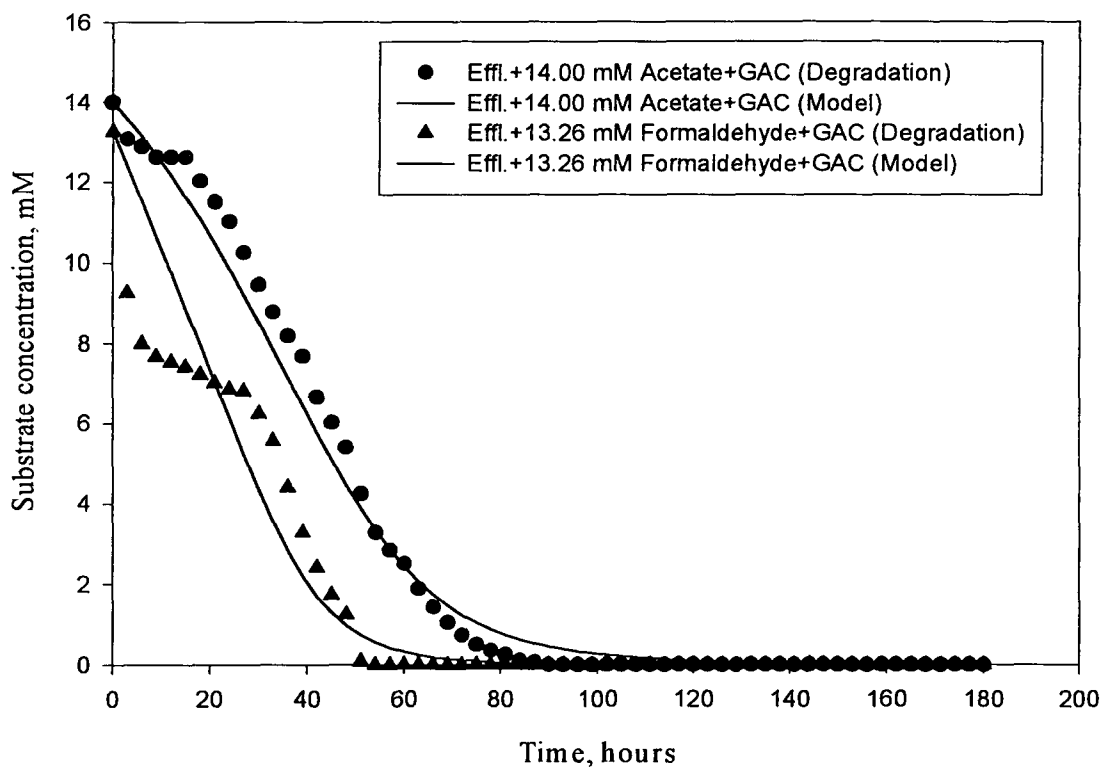
Figure 7.53. Comparison curves of the mixed substrate biodegradation (af2) Case of 840.43 mg/L (14.00 mM) Acetate, 398.09 mg/L (13.26 mM) Formaldehyde.

Figure 7.54. Comparison curves of the mixed substrate biodegradation (pf1) Case of 851.74 mg/L (14.17 mM) I-propanol, 101.78 mg/L (3.39 mM) Formaldehyde.

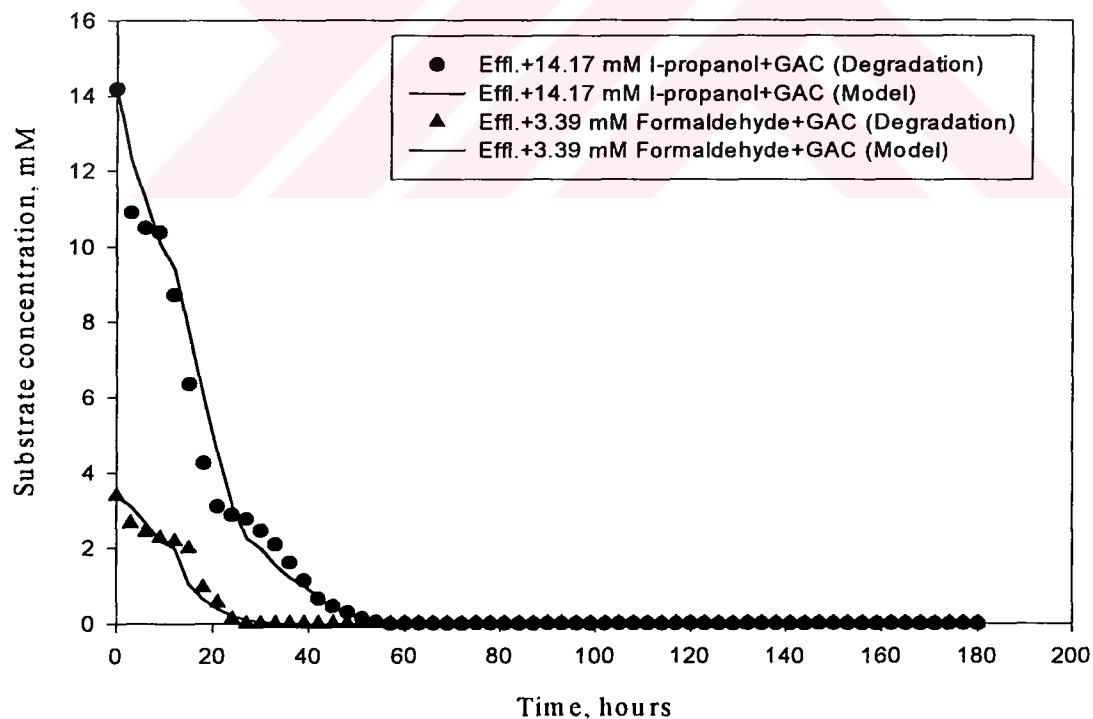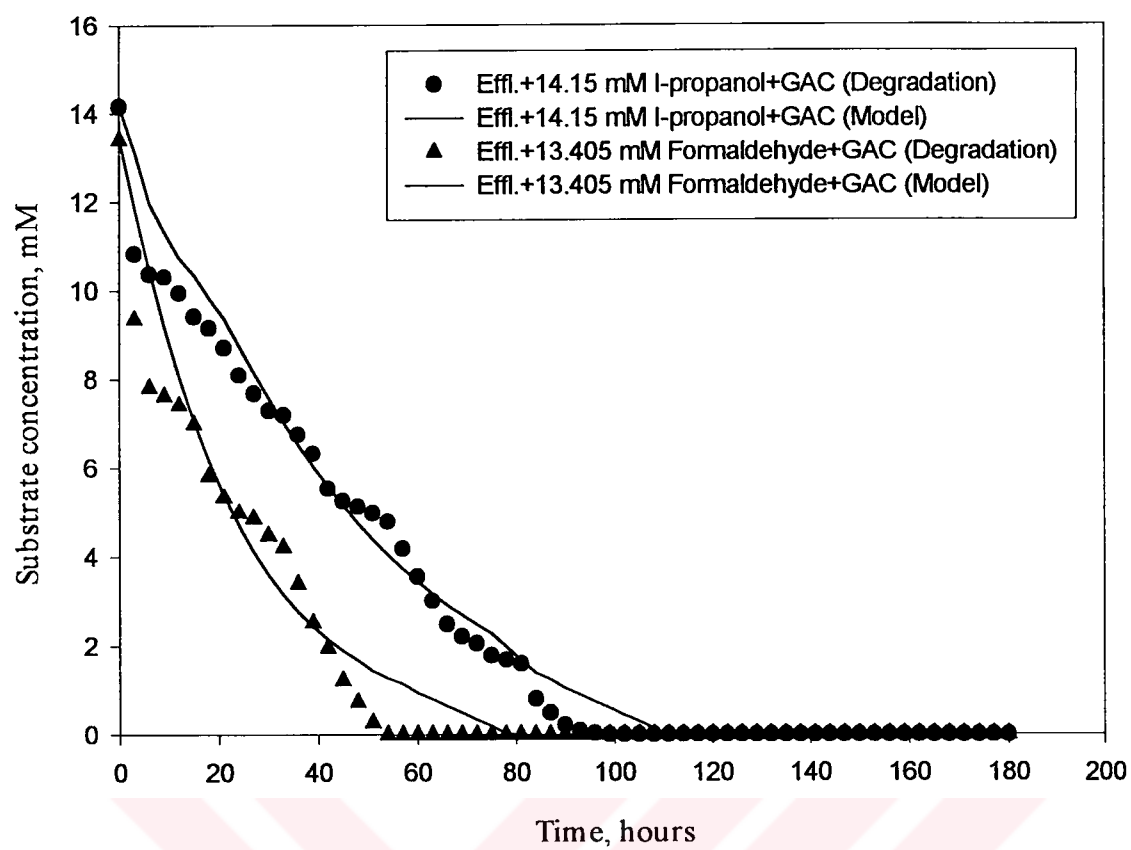**Figure 7.55.** Comparison curves of the mixed substrate biodegradation (pf2)
Case of 850.62 mg/L (14.15 mM) I-propanol,
402.56 mg/L (13.405 mM) Formaldehyde.

Table 7.6. Intrinsic kinetic parameters.

Substrate	Symbol	Conc. (mM)	Parameter				
			Y	k (hr^{-1})	K_s (mM)	K_i (mM)	B (hr^{-1})
Acetate (Ac)	a1	7.934	$5.73*10^{-3}$	2.43	37.64	2.07	$2.90*10^{-3}$
Acetate	a2	15.31	$5.91*10^{-3}$	2.36	37.64	2.07	$2.90*10^{-3}$
Acetate	a3	22.52	$5.36*10^{-3}$	2.60	37.64	2.07	$2.90*10^{-3}$
Formaldehyde (F)	f1	2.185	$1.00*10^{-2}$	0.51	0.359	130.76	$4.60*10^{-2}$
Formaldehyde	f2	2.25	$1.00*10^{-5}$	7.04	22.25	100.64	$3.63*10^{-2}$
Formaldehyde	f3	3.24	$1.00*10^{-5}$	8.23	83.25	90.64	$5.63*10^{-2}$
Formaldehyde	f4	4.96	$1.00*10^{-5}$	4.73	37.54	23.04	$2.81*10^{-2}$
Formaldehyde	f5	6.41	$1.00*10^{-2}$	2.90	24.80	20.07	$1.00*10^{-5}$
Formaldehyde	f6	12.882	$1.00*10^{-2}$	2.58	11.80	13.40	$1.00*10^{-2}$
Iso-propanol (P)	p1	7.259	$5.00*10^{-3}$	2.90	25.76	42.08	$2.05*10^{-2}$
Iso-propanol	p2	12.858	$1.00*10^{-4}$	2.91	20.76	20.13	$2.18*10^{-2}$
Iso-propanol	p3	20.696	$5.00*10^{-3}$	4.40	21.00	13.40	$1.13*10^{-2}$
Ac. - F. - P.	afp1 - a:	15.61	$1.00*10^{-3}$	3.70	30.18	1.78	$1.00*10^{-2}$
	afp1 - f:	3.38	$5.00*10^{-3}$	3.50	28.00	2.60	$2.05*10^{-2}$
	afp1 - p:	13.62	$5.00*10^{-3}$	3.50	34.50	3.40	$2.05*10^{-2}$
Ac. - F. - P.	afp2 - a:	14.39	$1.00*10^{-3}$	2.40	27.33	1.78	$1.00*10^{-2}$
	afp2 - f:	6.70	$5.00*10^{-3}$	1.60	2.180	1.95	$1.00*10^{-2}$
	afp2 - p:	13.59	$5.00*10^{-3}$	3.60	25.10	4.20	$2.05*10^{-2}$
Ac. - F. - P.	afp3 - a:	14.03	$7.00*10^{-2}$	0.85	26.33	2.62	$1.00*10^{-2}$
	afp3 - f:	13.51	$7.00*10^{-2}$	0.85	26.33	30.62	$1.00*10^{-2}$
	afp3 - p:	13.09	$5.00*10^{-3}$	1.18	2.180	1.78	$1.00*10^{-2}$
Ac. - F.	af1 - a:	13.39	$5.00*10^{-1}$	0.86	40.18	30.78	$1.00*10^{-2}$
	af1 - f:	3.52	$1.00*10^{-4}$	1.60	31.18	20.76	$1.00*10^{-2}$
Ac. - F.	af2 - a:	14.00	$2.50*10^{-1}$	0.86	40.18	4.78	$1.00*10^{-2}$
	af2 - f:	13.26	$1.00*10^{-3}$	2.80	27.33	1.78	$1.00*10^{-2}$
P. - F.	pf1 - p:	14.17	$5.00*10^{-1}$	0.85	37.05	30.63	$1.00*10^{-2}$
	pf1 - f:	3.39	$0.27*10^{1}$	0.85	37.05	30.63	$1.00*10^{-2}$
P. - F.	pf2 - p:	14.15	$7.00*10^{-2}$	0.85	30.05	30.62	$1.00*10^{-2}$
	pf2 - f:	13.405	$8.00*10^{-3}$	2.25	37.06	30.51	$1.00*10^{-2}$

7.3.2. A competitive inhibition model: Case of anaerobic biotransformation of formaldehyde with addition of iso-propanol as a growth substrate

The biosystem was chosen as a dual substrate biosystem and the main substrates were formaldehyde and iso-propanol with the concentrations of 12.81 mM and 13.27 mM, respectively.

Equation 6.15 to 6.26 can be solved with known parameters and the initial concentrations of the substrates and the anaerobic bacteria. In the BMP tests, the anaerobic culture from a steady-state AFBGAC bioreactor was used as the inoculum to initiate the biotransformation. The concentration of each type of anaerobic bacteria can be computed with the following formula:

$$-\frac{dS}{Xdt} = \frac{\mu_m}{Y} \cdot \frac{S}{K_s + S} = \frac{k.S}{K_s + S} \qquad (K_s << S) \tag{7.1}$$

where X is the concentration of a type of bacteria, Y is the yield coefficient of the bacteria, μ_m is the maximum specific growth rate, S is the substrate concentration, and K_s is the half saturation coefficient.

To estimate and optimize the kinetic parameters based on experimental results, the Levenberg-Marquardt algorithm was used. There are 36 parameters to be estimated and optimized and 12 ordinary differential equations (Equations 6.15 to 6.27 except for Equation 6.18) to be solved simultaneously. However, due to failure to determine the concentrations of formic acid and methanol produced through the biotransformation of formaldehyde, the number of unknown parameters was reduced to 32, S_{II}, S_{III} were assumed to be zero and 10 ordinary differential equations remained to be solved. To simplify this complicated problem, the parameter estimation and optimization was started with the experimental data obtained from the BMP reactors in which iso-propanol was the sole substrate without the addition of formaldehyde and/or acetate.

In this case, Equations 6.10 to 6.14 can be simplified and reorganized to the following equations:

$$R'_4 = k_4 . X_1 \frac{S_V}{K_{s4} + S_V + \dfrac{S_V^2}{K_{i4}}} \tag{7.2}$$

$$R'_5 = k_5 . X_2 \frac{S_{VII}}{K_{s75} + S_{VII}} . \frac{S_{VI}}{K_{s65} + S_V} \tag{7.3}$$

$$R'_6 = k_6 . X_3 \frac{S_{VI}}{K_{s6} + S_{VI}} \tag{7.4}$$

$$R'_7 = k_7 . X_4 \frac{S_{VII}}{K_{s7}\left(1 + \dfrac{S_{VI}}{K_{c7b}}\right) + S_{VII}} \tag{7.5}$$

$$R'_8 = k_8 . X_5 \frac{S_{VIII}}{K_{s8}\left(1 + \dfrac{S_{VI}}{K_{c8a}} + \dfrac{S_{VII}}{K_{c8b}}\right) + S_{VIII}} \tag{7.6}$$

Before solving the kinetic expressions, the following assumptions have been made:

1) The conversion rate of formic acid and methanol to the final products were very fast.

2) Formic acid and methanol concentrations which were produced through the biotransformation of formaldehyde have been assumed to be zero, and therefore, the related expressions and coefficients have been neglected.

3) The biosystem has been considered to be a dual substrate biosystem.

It is apparent that in this case iso-propanol reaction rate (Equations 6.18 and 7.2) is independent of any other substrates. Therefore, the kinetic equations (Equations 6.18 and 6.22) which describe iso-propanol biodegradation and the growth of iso-propanol-utilizing acetogenic bacteria together with Equation 7.2 have been solved independently of the other equations.

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The kinetic parameters for the biodegradation of iso-propanol alone (Equation 7.2) and for the growth of iso-propanol-utilizing acetogenic bacteria (Equation 6.22) have been estimated and optimized with the data obtained for the biodegradation of iso-propanol alone (Case of sole iso-propanol: 772.78 mg/L (12.858 mM)). The comparison of the experimental data and the numerical solution for the biodegradation of iso-propanol alone is shown in Figure 7.47. The numerical solution and the experimental data are in very close agreement. The kinetic parameters for iso-propanol biodegradation are listed in Table 7.7.

Table 7.7. The intrinsic kinetic parameters for iso-propanol biodegradation (p2) Case of sole iso-propanol: 772.78 mg/L (12.858 mM).

Parameter	Value	
Y_{1p2}	$1.00*10^{-4}$	
K_{4p2}	2.91	hr^{-1}
K_{s4p2}	20.76	mM
K_{i4p2}	20.13	mM
B_{1p2}	$2.18*10^{-2}$	hr^{-1}

Iso-propanol biodegradation by a formaldehyde acclimated anaerobic culture can be well expressed with Haldane substrate inhibition model. However, the substrate inhibition is not very strong because the substrate inhibition coefficient is relatively high (K_{i4p2}=20.13 mM).

The kinetic parameters for propionate formation, hydrogen utilization, acetate utilization, and propionate utilization have been estimated and optimized with the known kinetic parameters for iso-propanol only biodegradation. Equations 6.18 to 6.26 have been solved simultaneously together with the kinetic expressions in Equations 7.2 to 7.6. Again Levenberg-Marquardt algorithm and experimental data have been used for parameter estimation and optimization. The comparison of the numerical solution and the experimental data is shown in Figure 7.56 and they agree each other very well.

The kinetic parameters for propionate formation, hydrogen utilization, acetate utilization, and propionate utilization and for the growth of the corresponding bacteria are listed in Table 7.8.

Table 7.8. Kinetic parameters for propionate formation, hydrogen utilization, acetate utilization, propionate utilization and for the growth of the corresponding bacteria.

Parameter	Value	
k_5	21.04	hr^{-1}
K_{s75}	0.25	mM
K_{s65}	5.96	mM
k_6	30.20	hr^{-1}
K_{s6}	5.71	mM
K_7	0.49	hr^{-1}
K_{s7}	0.35	mM
K_{c7b}	∞	mM
K_8	0.14	hr^{-1}
K_{s8}	$1.00*10^{-5}$	mM
K_{c8a}	∞	mM
K_{c8b}	$2.76*10^{-6}$	mM
Y_2	$2.89*10^{-2}$	
B_2	$7.414*10^{-2}$	hr^{-1}
Y_3	$6.84*10^{-3}$	
B_3	$8.35*10^{-3}$	hr^{-1}
Y_4	$2.40*10^{-2}$	
B_4	$1.83*10^{-3}$	hr^{-1}
Y_5	0.092	
B_5	$5.72*10^{-3}$	hr^{-1}

The competitive inhibition coefficients of hydrogen to both acetate and propionate biodegradation are extremely large ($K_{c7b}= \infty$ and $K_{c8a}= \infty$), indicating that hydrogen does not inhibit the biodegradation of either acetate or propionate.

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The competitive inhibition coefficient of acetate to propionate biodegradation is very small (K_{c8b}=2.76*10^{-6} mM), indicating that acetate exhibits a strong inhibition to the biodegradation of propionate. The presence of acetate probably blocks the active sites of the enzymes, which catalyze the biodegradation of propionate.

The rest of the kinetic parameters have been estimated and optimized by solving Equations 6.15 to 6.27 with the kinetic expressions shown in Equations 6.7 to 6.14.

Again the Levenberg-Marquardt algorithm has been used for parameter optimization with the experimental data obtained from the BMP test for the case of 12.81 mM formaldehyde and 13.27 mM iso-propanol.

The number of unknown parameters was reduced to 7, since 25 parameters have already been estimated and optimized as shown in Table 7.7 and 7.8.

The results of kinetic parameters and the comparison of experimental data and numerical solutions for the anaerobic biotransformation of formaldehyde with addition of iso-propanol as a growth substrate are shown in Table 7.9, and Figure 7.57, respectively. The numerical solutions for the competitive inhibition model study fit the experimental data very well.

Table 7.9. Kinetic parameters for the anaerobic biotransformation of formaldehyde with addition of iso-propanol as a growth substrate.

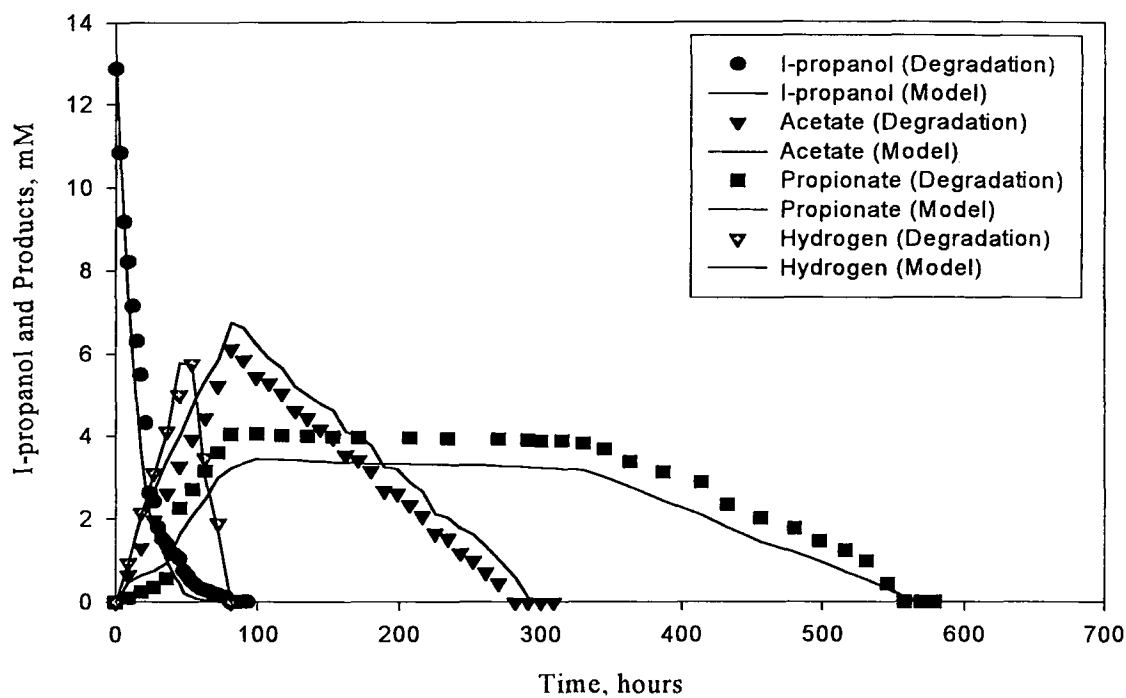| Parameter	Value	
k_1	2.575	hr^{-1}
K_{s1}	11.8	mM
K_{i1}	13.40	mM
K_{c4a}	1.28*10^{-4}	mM
K_{c5a}	1.00*10^{-11}	mM
K_{c6a}	1.12*10^{-2}	mM
K_{c7a}	1.10*10^{-11}	mM

Figure 7.56. Kinetics of I-propanol biodegradation and its products
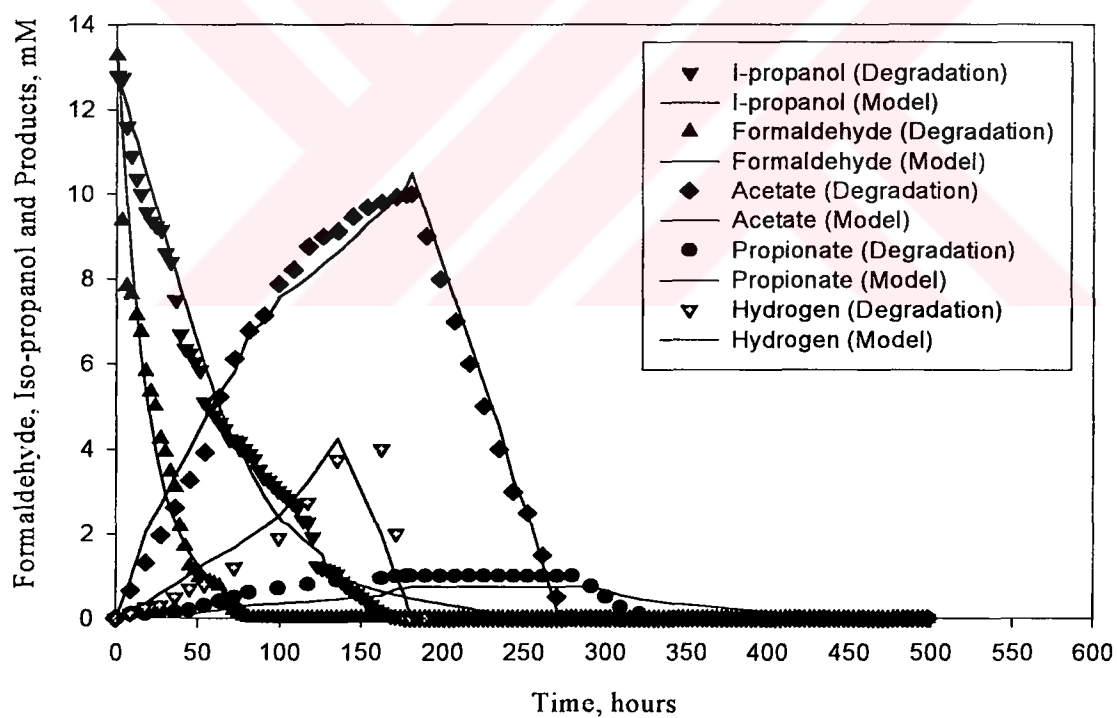Case of sole I-propanol: 772.78 mg/L (12.858 mM).

Figure 7.57. Kinetics of anaerobic biodegradation of formaldehyde
with addition of iso-propanol as a growth substrate
Case of 797.53 mg/L (13.27 mM) I-propanol,
384.68 mg/L (12.81 mM) Formaldehyde.

Formaldehyde has shown very strong competitive inhibition to iso-propanol-utilizing acetogenesis, propionate formation, hydrogen-utilizing methanogenesis and acetate-utilizing methanogenesis.

The competitive inhibition of formaldehyde to acetate utilization was extremely strong, so that propionate formation has ceased from acetate, which was observed by the experimental results. On the other hand, formaldehyde does not exhibit strong substrate inhibition to iso-propanol-utilizing acetogenesis (K_{i4p}=30.67 mM) as well as its own biotransformation (K_{i1f}=30.510 mM). But, due to the competition of microorganisms for the active enzymes and substrates, the iso-propanol and formaldehyde utilization rates decrease.

7.4.   Lipid phosphate analysis

Determining the viable biomass of a microbial community provides an estimate of the amount of active microorganisms in a particular environment and, therefore, the capability for metabolic transformations in that environment.

In biological treatment systems in which support materials such as granular activated carbon, sand, zeolite, etc. are used to maintain the growth of microorganisms on the surface of the material in attached form, the determination of the biomass concentration can be done by measuring the phospholipid content of the microbial community or by using any other specific method like ATP determination that measures specifically the biomass concentration. In suspended-growth biological systems, the growth of the microorganisms is in suspension, the total biomass concentration may be measured by determining the concentration of Volatile Suspended Solids (VSS) of the effluent. This method is mostly used for aerobic biological treatment systems or anaerobic biological treatment systems that treat low strength wastewaters in systems fed mostly with soluble substrates. The VSS concentration of the effluent represents the biomass concentration.

This relatively simple test is not practical in the case of attached-growth biological treatment systems where most of the biomass in the bioreactor is in attached form with the support material.

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Lipid phosphate analysis differs from the VSS analysis by the way of determining the viable biomass content of the biosystem. Determining of the concentration of VSS gives the total biomass concentration of the sample that comprises in both viable and inanimate microorganisms. Therefore, it is impossible to determine the actual capacity of microorganisms in treating a wastewater. In contrast, lipid phosphate analysis gives a specific indication for the viable biomass content, at any given moment, in the bioreactor. In lipid phosphate analysis, there are no certain values to compare the results, which are obtained experimentally, and every case is independent than the other. However, the comparison of the results can be made by evaluating the results for the same biosystem.

In this present study, the viable biomass content of the biosystem was determined by performing the lipid phosphate analysis and the efficacy of the microorganisms was obtained by establishing correlation between the performance of the biosystem and the obtained results of the lipid phosphate analyses.

Continuous lipid phosphate analyses were started to be performed, after the highest concentrations of iso-propanol and formaldehyde were fed into the anaerobic bioreactor and the steady-state conditions were achieved. The related results of experiments are presented in Table 7.10.

The samples were taken from the bottom, middle and top parts of the anaerobic bioreactor in order to determine the more and less effective regions of the column and the distribution of the microorganisms along the bioreactor. Triplicate samples were run for each region. Figure 7.58 shows the variation of the viable biomass concentrations in the bioreactor.

Table 7.10. Lipid phosphate analysis results.

Day	Lipid phosphate (nmol)		
	Bottom	Medium	Top
377	56.43	41.70	42.30
384	55.05	47.14	46.28
419	43.99	41.45	32.48
427	44.34	43.40	44.18
434	55.30	51.38	53.37
440	53.96	52.82	54.90
451	30.14	28.05	28.23
461	53.97	56.30	54.77
480	64.07	64.78	64.72
491	50.08	49.34	53.87
504	55.92	54.70	52.46
505	31.09	34.61	35.88
550	59.29	54.45	54.56
557	57.47	53.47	55.12
564	63.25	61.03	57.89
600	51.50	57.53	56.47
607	35.21	31.34	29.67
614	45.56	42.73	43.67
621	49.61	45.76	41.63
657	52.18	48.53	42.34
664	62.16	59.56	55.23
671	67.45	62.17	60.60
678	64.25	60.17	57.49
685	58.58	57.15	55.28

Bottom: Sample was taken from the bottom part of the anaerobic bioreactor.
Medium: Sample was taken from the medium part of the anaerobic bioreactor.
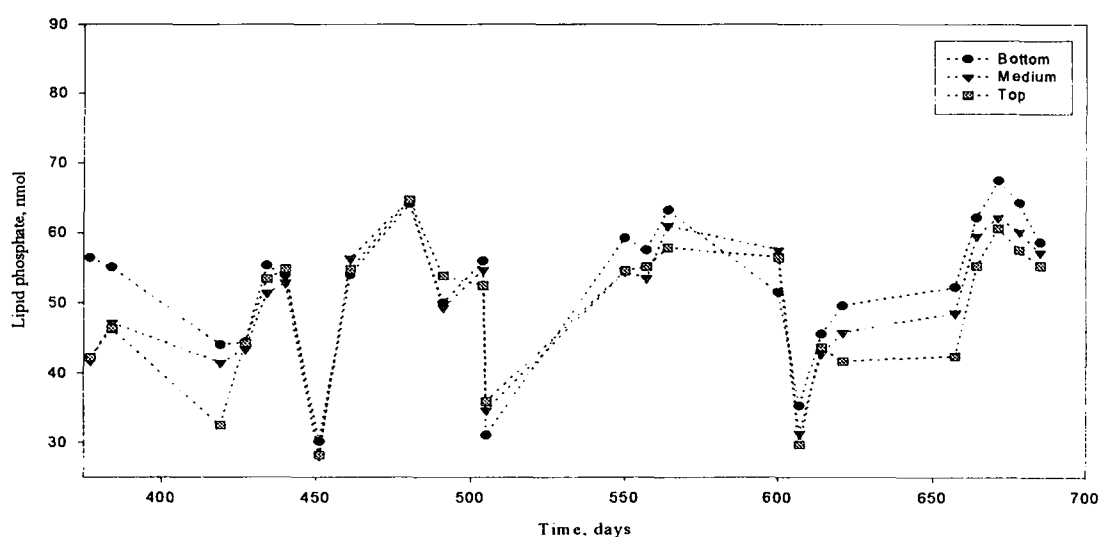Top: Sample was taken from the top part of the anaerobic bioreactor.

Figure 7.58. Variation of viable biomass in the anaerobic bioreactor.

The experimental results depict that the most effective part of the anaerobic bioreactor is the bottom part. In this zone, the viable (active) biomass concentration is higher than the other two zones (Figure 7.58). This conclusion can be well expressed by the following two basic reasons:

1) The bottom part of the bioreactor always encounters the fresh wastewater. Microorganisms that are located in the bottom part of the bioreator receive the highest concentrations of the feedstock substrates and a significant part of the substrates are consumed here with concomitant biomass growth.

2) The distribution of the flow is more effective in the bottom part than the other parts of the bioreactor. The recirculation of the effluent with the fresh wastewater together is introduced to the bioreactor from the bottom and all the substances that are essential for the biologic activity of the microorganisms are effectively delivered from this part of the bioreactor. The middle and top parts of the bioreactor show almost the same lower efficacy regarding the concentration of the viable biomass. Experimentally, it was concluded that the lipid phosphate results vary between 50 nmol and 60 nmol when the anaerobic bioreactor is effective to treat the substrates. As can be seen from Figure 7.58, the lipid phosphate results sometimes exhibit pronounced declines. These declines were obtained particularly, during the times that the biosystem was failed. At these times, the lipid phosphate readings decreased down to 28.05 nmol, which mean that there was almost no biological activity in the biosystem during the periods of failure.

8.   OVERVIEW AND CONCLUDING REMARKS

In this study, an Anaerobic Fluidized Bed Granular Activated Carbon (AFBGAC) bioreactor was used to determine the biotreatability of a synthetically prepared high-strength wastewater containing formaldehyde as an inhibitory and toxic compound and high concentration of iso-propanol. These compounds are typical for the wastewaters from chemical, textile, paper, wood, pharmaceutical, cosmetic, rubber and other industries therefore their biodegradation has a very big environmental importance. Individual and mixed substrate biodegradation experiments were performed by applying different initial concentrations to determine the correlation between substrate concentrations and biodegradability. Kinetic assessments were conducted to determine the mechanism of the biodegradations.

Some conspicuous results from which were extracted by these executed experiments are given in the followings:

√ In treating the high-strength (~ 7.0 g/L of COD) wastewater containing toxic and inhibitory concentrations of formaldehyde and high concentrations of iso-propanol, very high substrate removal efficiencies of over 99.9 % removal of both formaldehyde and iso-propanol were obtained. The highest feed concentrations of iso-propanol and formaldehyde were around 33 g/day and 17 g/day, respectively which were later reduced to around 1 mg/L for both iso-propanol and formaldehyde during the stable operation periods of the anaerobic bioreactor. The organic loading rate was around 10.5 kg COD/m^3.day for the anaerobic bioreactor. Typical organic loading rates have been reported in the literature as 4.81 to 9.61 kg COD/m^3.day for the fluidized bed bioreactors. Although high organic loading was applied to the anaerobic bioreactor, very high removal efficiencies were obtained in substrate and COD reductions.

√ Initially the total COD of the wastewater was around 7000 mg/L and after the treatment the total COD concentration was reduced to around 70 mg/L in the

anaerobic bioreactor. Hence, the total COD reduction for the biosystem was more than 98.9 % during the stable operation periods of the anaerobic bioreactor. In the literature the typical initial COD values for fluidized beds have been reported as 5 to 10 g/L and for this interval the COD removal efficiency was given in range of 80 to 85 %.

√ The operating parameters of the AFBGAC bioreactor showed a large compliance with the operating parameters of which have been given in the literature for anaerobic fluidized bed bioreactors. Particularly, the total COD (%) removal efficiency, COD removal capacity (volumetric) and methane gas efficiency values were impressive during the stable operation periods of the anaerobic bioreactor. The hydraulic retention time of wastewater has been reported to vary in a wide range in the literature and its value changes significantly with the initial organic loading rate. However, the generally accepted hydraulic retention time range can be given to be between 5 and 10 hours for fluidized bed processes. In this present study, the hydraulic retention time was initially 1.5 days and after achieving the steady-state, the value was decreased to 16 hours.

√ Based on the results of acetate concentrations in the effluent stream, it was shown that at the beginning of the bioprocess, even the lowest concentration of iso-propanol exhibited a shock influence on acetate utilization and the response of the biosystem was quite similar to that as if the biosystem was overloaded by the substrate. Subsequently, the acetate utilization increased when the biosystem was underloaded. Because of such a behavior of the biosystem, in the ensuing period, the feed loads were applied in small increments and the loads were not further increased until the acetate was reduced to steady-state levels. Such a treatment prevented total inhibition of the bioprocessThe acetate concentrations produced were obtained as 50-100 mg/L (generally accepted range is 50-300 mg/L) in the anaerobic bioreactor.

During the stable operation periods of the anaerobic bioreactor, the acetate concentrations decreased down to around 10 mg/L indicating that the biological balance between the two major clusters of microorganisms (acetogenic and methanogenic microorganisms) was established very well in the anaerobic bioreactor.

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√ The pH values were almost the same during the entire course of the study and it was around 7.2. This depicts that the concentration of intermediate organic acids never reached to high levels during the stable operation of the bioreactor. During the times of failure, the intermediate products (particularly acetate) exhibited higher concentrations but even under these circumstances, the changes of pH were not in a significant extent.

√ In the present anaerobic bioreactor the total gas production was obtained to be 51.6 liters per day as the mean value (~ 5 L gas per L bioreactor volume per day) by applying the maximum concentrations of the substrates. The produced gas was containing basically the mixture of the 78 % of methane and approximately 21 % of carbon dioxide. Hence, the methane gas efficiency of the anaerobic bioreactor was 0.38 m^3 methane/kg $COD_{removed}$. Based on these results, it can be stated that a considerable amount of gas was produced from the present laboratory-scale anaerobic bioreactor (the expected value is generally 5-6 L gas per L digester) and the gas was highly enriched with methane (the expected value is generally 0.35-0.40 m^3 methane/kg $COD_{removed}$). Consequently, the produced gas can be used effectively for many purposes such as in heating the bioreactor, in producing electricity, in operating some sort of engines or in providing heat for buildings due to the high methane content of the gas.

√ According to the biogas composition results, it can be stated that the anaerobic bioreactor is not stressed because of the proportion of carbon dioxide which exhibited continually very close results in each measurement. This signifies that the methane-forming microorganisms were always active during the stable operation of the anaerobic bioreactor and produced stable concentrations of methane, which was around 78 % of the total gas composition.

Oxygen and nitrogen were obtained in trace concentrations in the biogas indicating that at the moments samples were taken from the bioreactor, air intrusions occurred. However, no any significant influence on process stability and efficiency was observed due to these fleas.

√ The possibility of plugging or channeling due to solids accumulation in anaerobic fluidized bed bioreactors is very small. However, in the present biorector, there was a problem that caused failure in providing an adequate distribution of the influent particularly when the microorganisms were very mature in the environment. When these mature microorganisms were not discarded from the media just on time, the solids accumulation took place particularly in the bottom part of the bioreactor. That is why, the biosystem and operational parameters must be closely monitored specially in continuous flow regime in order to sustain the stability of the bioreactor.

√ The intensively growing microorganisms sometimes clogged the bottom part of the bioreactor and this brought about channeling or some dead-zone problems in the bioreactor. When this happened, the biodegradation efficiency declined in all parameters but it never showed sudden falls.

√ During the experiment that was conducted in the model bioreactor, it was observed that the biomass thickness on the GAC surface area was very thin at the beginning of the bioprocess. Depending on organic and hydraulic rates, the maximum growth of microorganisms on the GAC particles have been achieved mostly within 2 weeks and at that time the maximal substrate reduction and gas production rates were obtained. Removing the inactive biomass from the top of the bioreactor carried out the regeneration of microorganisms.

√ During the stable operation of the bioreactor, it was observed that the bioreactor contents were mixed very well and the effective mixing resulted in a homogenous mixture inside the bioreactor.

√ In full-scale applications for anaerobic fluidized bed bioreactors the organic loading rate are usually selected in the range of 10 to 30 kg COD/m^3.day and the COD removal capacity (volumetric) usually remains below 25 kg $COD_{removed}/m^3$.day. Based on these criteria, it can be considered that the presented biosystem gives reliable and applicable outcomes with respect to kinetic and design parameters especially for full-scale applications.

√ During the entire study, the organic loadings applied to the bioreactor were not very variable. Thereby, probable bioprocess upsets posed by the variation of loadings were

not encountered. However, it is not always possible to ensure a consistent feed to the bioreactor and especially in a full-scale operation, the waste flow will most probably be interrupted due to some operational or strategically reasons. Thus, these transient organic loadings will adversely affect the efficiency of full-scale biosystem. That is why, the effect of organic load transients on a laboratory- or pilot-scale anaerobic fluidized bed biosystem have to be investigated.

√ During the stable operation of the bioreactor, the fluidization was realized perfectly by the effect of the recirculation of the effluent. In this study, the energy consumption considerations have not been done for the recycling. However, this concept must be taken into consideration very sensitively specially for full-scale applications because recycling expenses seem to be the most important drawback of anaerobic bioreactors.

√ During performing the experiments in the respirometer, relatively lower substrate concentrations were applied to the batch reactors. In these experiments, the maximum initial concentrations of iso-propanol and formaldehyde were 1244 mg/L and 387 mg/L as the mean values in feed. It was experimentally determined that after having applied either the higher or even the lower concentrations, the inhibition influence was mostly effective on the biodegradation of the substrates. On the other hand, the feeding concentrations of the substrates were almost three-fold more in the main anaerobic bioreactor.

Although these higher concentrations applied, during the entire course of the work, there was no upset that was encountered because of the high initial substrate concentrations or their toxic or inhibitory effects and actually the removal efficiencies were spectacularly high. This contradiction can be well expressed by the effect of recycle which provided a lot of advantages over the biosystem such as in maintaining effective fluidization, neutralizing the influent by reducing its pollutant concentration and smoothing the effects of the toxicant and inhibitory substrate. Besides the adsorption of the toxic substrate onto activated carbon reduced the immediate toxic shock loads and excess toxins were probably biodegraded more slowly by the formaldehyde acclimated anaerobic culture.

√ During the experiments of biotransformation of formaldehyde, it was found that formaldehyde exhibited inhibition effect on its own biotransformation as well as inhibition of the biodegradation of other substrates up to the concentration of 437.0 mg/L of formaldehyde. At this concentration, formaldehyde exhibited toxic effect on its own biotransformation and no bioconversion of the substrate was obtained. In the literature, the toxic effect of formaldehyde has been reported for similar concentrations when the wastewater was biotreated by an anaerobic filter [57,s 78]. Low concentrations of formaldehyde exhibited low inhibition effects (K_i = 90-130 mM) whereas relatively higher concentrations exhibited moderate inhibitions (K_i = 13-23 mM).

√ No change in lag time periods was detected for formaldehyde acclimated anaerobic culture while treating the different initial concentrations of formaldehyde alone as well as in the double and triple substrate combinations. The obtained lag time was 2.6 hours for all cases. The effect of the acclimation of microorganisms in advance can be observed here clearly. Normally, increasing concentrations result in increasing lag times. In this present study, the microorganisms that were acclimated to formaldehyde in advance and used in the anaerobic bioreactor were manipulated in the respirometer experiments as inoculum as well. Thereby, the microorganisms were already used to biodegrade the high concentrations of formaldehyde and there was no need to expend time to accustom for a new environment.

√ Although acetate was not a basic substrate for the anaerobic bioreactor, due to its role in the mechanism of the anaerobic biodegradation process (as an intermediate product), acetate biodegradation, alone and in association with other substrates, was examined independently in the work programme. According to the experimental results, it was determined that during the biodegradation of acetate alone, even the lowest concentration of acetate used (476.4 mg/L or 7.93 mM) exhibited a strong inhibition on its own biodegradation. Kinetic data showed that the inhibition extent was identical for the different concentrations of acetate used (K_i = 2.07 mM). When these results are compared with the results obtained from the main anaerobic bioreactor, it can be seen that the anaerobic bioreactor never reached even the lowest concentration of acetate which was examined in the respirometer experiments,

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therefore, the biosystem never failed becauseof high acetate concentrations or accumulations. However, the biodegradation of acetate in different concentrations and their kinetic evaluations depict that the anaerobic bioreactor may demonstrate operational upsets posed by the acetate concentrations which were investigated in the respirometer experiments, so it is necessary that some measure of organic and hydraulic loading control be taken to prevent the accumulation of acetate and a further fall in pH.

√ During the biodegradation experiments of iso-propanol, the employed concentrations varied between 440 mg/L and 1240 mg/L and moderate inhibition influences were obtained for concentrations (K_i = 13-42 mM).

√ During the biodegradation experiments of triple substrate combinations, high inhibition effects were obtained for all substrates, especially when the formaldehyde concentrations were increased, the range of inhibition was obtained as 1.7-4.2 mM.

√ The dual biosystem of acetate-formaldehyde showed a strong inhibition effect on both of the substrates (K_i = 2-5 mM) only when formaldehyde was applied in high concentration.

√ The dual biosystem of iso-propanol-formaldehyde showed moderate inhibition effects on both of the substrates, independently of concentration changes of formaldehyde (K_i = 30 mM)

√ During establishing the competitive inhibition model, it was found that the produced acetate from the biodegradation of iso-propanol exhibited a very strong competitive inhibition effect on the biodegradation of propionate (K_i = 2.76 × 10^{-6} mM). It is thought that acetate blocks the active sites of the enzymes, which catalyze the biodegradation of propionate, and reduces their efficacy. On the other hand, formaldehyde exhibited very strong competitive inhibition effects on acetogens, which use the iso-propanol, methanogens that use the acetate and hydrogen, and on acetate for propionate formation. When acetate utilization was inhibited, the propionate formation stopped completely.

√ According to the experimental results, it was found that the biomass activity was in the highest level in the bottom part of the bioreactor. Therefore, it can be stated that a large part of the substrate was biodegraded in that part of the bioreactor.

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CURRICULUM VITAE

Msc. Chemical Engineer Moiz ELNEKAVE was born in İstanbul, 1965. He launched his elementary education in the American Community School, Athens in 1972. After one year education, he fulfilled the rest of his all high school education in the lycee of Şişli Terakki and graduated from the same high school in 1983. The same year, he was entitled to participate to the Chemical Engineering Programme of the İstanbul Technical University. He graduated from the Faculty of Chemical- Metallurgical of İstanbul Technical University in 1988. The same year, he started to work as a research assistant in the İstanbul Technical University, Department of Chemical Engineering, Unit Operations division. His master science education as a secondary degree started at the same time in the Institute of Science and Technology of İstanbul Technical University at the Chemical Engineering Programme. In 1990, he fulfilled the programme and gained the title of Master Science Chemical Engineer. After completing his second degree, he joined to the army and by getting the best grade, he performed his military service as a second lieutenant in the educational Military Hospital of GATA, İstanbul. In 1992, he began to studies for his third degree (PhD.) in the Institute of Science and Technology of İstanbul Technical University at the Chemical Engineering Programme.

In 1996, he continued to his Ph.D. studies in the Department of Civil and Environmental Engineering of University of Cincinnati, Ohio, USA for 1 year.

In 1997, he was granted by the Turkish Council of Higher Education for doctoral study in Israel at Technion-Israel Institute of Technology, Faculty of Environmental and Water Resources Engineering for 1 year.

Until now, he has taken part in many environmental research projects in Türkiye and abroad particularly in the subjects of biological wastewater treatment, physicochemical treatment facilities of domestic and industrial wastewaters, wastewater recycling supplied by renewable energies, reuse of wastewaters, environmental effects on anaerobic digestion such as mixing, and the control of air pollution, stack-gas measurements of various industrial establishments and their evaluations for industrial purposes.

Moiz ELNEKAVE has been working as a research, teaching and demonstrating assistant in the İstanbul Technical University, at the Chemical-Metallurgical Faculty, Chemical Engineering Department for 11 years and still continuing his duty.