PERFORMANCE EVALUATION OF THREE ANAEROBIC BIOREACTORS: ASBR, HAIS, AND UASB

Thesis submitted to the School of Public Health, Isfahan University of Medical Sciences and Health Services in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Ph.D)
In
ENVIRONMENTAL HEALTH ENGINEERING

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November 25, 2004
Isfahan – Iran

In the name of God

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INFLUENCE OF THE ERYTHROMYCIN ON ANAEROBIC TREATMENT OF A PHARMACEUTICAL WASTEWATER IN ANAEROBIC SEQUENCING BATCH REACTOR

PART I
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PERFORMANCE COMPARISON OF HORIZONTAL - FLOW ANAEROBIC IMMOBILIZED SLUDGE (HAIS) AND UASB REACTORS USING SYNTHETIC SUBSTRATE

PART II
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INFLUENCE OF THE ERYTHROMYCIN ON ANAEROBIC TREATMENT OF A PHARMACEUTICAL WASTEWATER IN ANAEROBIC SEQUENCING BATCH REACTOR

ABSTRACT – PART I

A laboratory-scale anaerobic sequencing batch reactor (ASBR) was used to treat a synthetic substrate mixture representing pharmaceutical wastewater. The ASBR was operated with increasing organic loading rates until stable removal efficiencies were reached at a loading rate of 3 gCOD L^{-1} d^{-1}. At that point the reactor was exposed to low (1 mg/l) and subsequently, to high (200 mg/l) concentrations of the antibiotic erythromycin.

The effect of addition of erythromycin on the microbial community and the development of antibiotic resistance were evaluated based on overall reactor performance and using specific methanogenic activity (SMA) tests. It was found that the addition of erythromycin resulted in a reduction of biogas production and COD removal efficiency by 5 to 10% indicating that a substantial fraction of the microbial populations in the ASBR were resistant to the antibiotic.

As expected based on the reactor performance, biogas production in SMA tests using the synthetic substrate mixture was only slightly affected by the presence of erythromycin. However, the conversion of butyric acid was inhibited when erythromycin was present, suggesting that fatty acid-beta oxidizing bacteria were sensitive to the antibiotic. When sludge from ASBR that had been exposed to erythromycin for 47 days was used for SMA tests, the concentration of butyric acid was inhibited to a lesser extent.

The specific methane production rate (SMPR) increased linearly with the specific substrate utilization rate (SSUR) with a slop of 0.9001and indicating that 90% of the COD removed was converted to methane. Calculated sludge yield (Y) was 0.066 gVSS/$gCOD_{rem}$, which was comparable to the reported yield value on mixed VFA (0.054 gVSS/$gCOD_{rem}$).

Maximum specific methane production rate (SMPR) in ASBR at steady state and in batch test without erythromycin are 0.102 and 0.186; and with erythromycin are 0.093 and 0.158 g-CH_4-COD/g VSS.d, respectively, presumably because higher HRT in batch test.

Erythromycin removals were in rang of 42-82 % in batch tests. The removal percents in ASBR were from 51 to 94 % for low (1 mg/l) and 22-89 % for high (200 mg/l) erythromycin concentrations.

Maximum methanogenic activity was reduced from 29.6 to 5.5 mL CH_4/g.VSS.d (81.4 % reduction) after two month storing of granules in cold room (4 °c) confirming other research suggested storage of granules in low temperature is not a convenience method

KEYWORDS

Erythromycin; antibiotic resistance; anaerobic treatment; anaerobic sequencing batch reactor (ASBR); pharmaceutical wastewater; specific methanogenic activity (SMA); volatile fatty acids (VFA).

PERFORMANCE COMPARISON OF HORIZONTAL- FLOW ANAEROBIC IMMOBILIZED SLUDGE (HAIS) AND UPFLOW ANAEROBIC SLUDGE BED (UASB) REACTORS

ABSTRACT – PART II

The performance of fixed-bed and suspended-growth high-rate anaerobic reactors was investigated in this study. Two 2-L and 3.3-L horizontal-flow anaerobic immobilized sludge (HAIS) reactors were filled with polyurethane (PU) and polyethylene (PE) beads with surface to volume ratios of 1100 and 1250 m^{-1}, density of 700 and 12 kgm^{-3}, and bed porosities of 0.42 and 0.28, respectively. The 3.3-L upflow anaerobic sludge blanket (UASB) reactor was filled with granular sludge with a VSS concentration of 45 g/L and both sets of reactors were operated at 32±2 ºC. Synthetic glucose-based substrate was the main carbon source (COD of 1750±250 mg/L) feeding both sets of reactors.

In this study, the concept of quantitative CT number in terms of Hounsfield units (H) that is approximately linearly related to the mass density of the attenuating tissue and materials in objects in Computed Tomography Scanning (CT-Scan) technology was used to characterize of the HAIS reactor contents including biofilm, media, and biogas. Then the obtained CT-images from various sections of HAIS reactors were evaluated with using the concept of Resolution (pixel per inch) in Photoshop to estimate the VSS contents of HAIS reactors.

The results confirmed satisfactory performance of HAIS in providing to provide suitable environmental conditions for biomass growth and retention during the short startup period of 25 days. At the optimum period of operation, the loading rates of reactors were: organic loading rate (OLR) of 13 ± 6 g COD/ L.d and F/M: 1.34±0.82 g COD/g VSS.d in UASB; surface loading rate (SLR) of 28 ± 12 g COD/ m^2 .d and F/M: 1.72±0.77 g COD/g VSS.d for HAIS-PU; and SLR of 23 ± 14 g COD/m^2 .d and F/M of 1.52±0.99 g COD/g VSS.d for HAIS-PE. For these loadings, the observed COD removal efficiencies and biogas productions were: 64.3±15.2 % and 1771±704 mL/d for UASB; 63.5±17.5 % and 1160±400 mL/d for HAIS-PU; and 61.6±18.6 % and 1018±645 mL/d for HAIS-PE.

It was found that UASB operation with granular sludge and more efficient gas-liquid-separator (GLS) has a better performance than the both HAIS reactors containing immobilized sludge, and with two gas collectors in different shapes: perforated tube (HAIS-PU) and sheet (HAIS-PE).

The performance of HAIS-PU was better than PE possibly due to higher bed porosity (ε) of HAIS-PU (ε=0.42) compared to HAIS-PE (ε= 0.28). Channeling effects for low bed porosity reactors can be the main factor responsible for such performance.

KEYWORDS

Anaerobic wastewater treatment, Fixed bed reactor, HAIS reactor, UASB, CT-Scanning, Resolution and Histogram in Photoshop;

تاثیر اریترومایسین بر روی تصفیه بی هوازی پساب صنایع داروسازی در راکتور بی هوازی پر و خالی شونده متوالی (ASBR)

چکیده قسمت اول

یک راکتور بی هوازی پر و خالی شونده متوالی (ASBR) در مقیاس آزمایشگاهی برای تصفیه یک سوبسترہ مخلوط سینتتیک نمایانگر پساب صنایع داروسازی مورد استفاده قرار گرفت. راکتورASBR با افزایش بارگذاری آلی بهره برداری گردید، تا اینکه راندمان حذف پایداری در بارگذاری آلی $3g\ COD.L^{-1}.d^{-1}$ بدست آمد . در این زمان مقادیر کم (۱ mg/l) و سپس غلظت بالایی(۲۰۰ mg/l) از اریترومایسین به راکتور تزریق گردید.

اثر افزایش اریترومایسین بر روی جمعیت میکروبی و ایجاد مقاومت در برابر آنتی بیوتیک، بر اساس عملکرد کلی راکتور و همچنین با استفاده از آزمون فعالیت متان سازی ویژه (SMA) مورد ارزیابی قرار گرفت. مشخص گردید که افزودن اریترومایسین موجب کاهش تولید بیوگاز و راندمان حذفCOD به میزان ۵ تا ۱۰ درصد می شود، و نشان دهنده آن است که بخش عمده ای از جمعیت های میکروبی در ASBR به آنتی بیوتیک مقاوم بوده اند.

همانگونه که بر اساس عملکرد رآکتور انتظار می رفت، تولید بیوگاز در آزمون فعالیت متان سازی ویژه با استفاده از سوبسترہ مخلوط سینتتیک، فقط به مقدار جزیی تحت تاثیر اریترومایسین قرار گرفته است. با وجود این، تجزیه بیولوژیکی اسید بوتیریک در حضور اریترومایسین متوقف شد، که مبین آن است که باکتریهای اکسید کننده اسیدهای چرب (fatty acid-beta oxidizing bacteria) به آنتی بیوتیک حساس بودند. هنگامی که لجن حاصل از رآکتور ASBR که به مدت ۴۷ روز در معرض بود برای انجام آزمون های متان سازی ویژه مورد استفاده قرار گرفت، میزان بازدارندگی برای اسید بوتیریک در حد کمتری بود.

میزان تولید ویژه متان (SMPR) بطور خطی متناسب با میزان برداشت ویژه سوبسترہ (SSUR) با شیب۰/۹۰۰۱ افزایش یافت که نشان دهنده آن است که ۹۰٪ از COD حذف شده به متان تبدیل شده است. میزان محاسبه شده محصول دهی لجن(Y) حدود $0.054\ g\ VSS/\ g\ COD_{rem}$ بود که با مقدار محصول دهی گزارش شده بر روی مخلوط اسیدهای چرب فرار ($0.054\ g\ VSS/\ g\ COD_{rem}$) قابل مقایسه است.

حداکثر میزان تولید ویژه متان (SMPR) در ASBR در حالت پایدار ود ر آزمون متان سازی ویژه بدون اریترومایسین به ترتیب ۰/۱۰۲ و ۰/۱۸۶، و با وجود اریترومایسین به ترتیب ۰/۰۹۳ و ۰/۱۵۸ g-CH_4- COD /g VSS.d بود، که دلیل احتمالی آن زمان ماند هیدرولیکی (HRT) بالاتر در آزمون متان سازی ویژه می باشد.

میزان حذف اریترومایسین در آزمون متان سازی ویژه در محدوده ۴۲ تا ۸۲ ٪ بود. درصد های حذف اریترومایسین در راکتور ASBR به ترتیب از ۵۱ تا ۹۴٪ برای غلظت کم (۱ میلی گرم در لیتر) و ۸۹٪ – ۲۲ برای غلظت بالای (۲۰۰ میلی گرم در لیتر) اریترومایسین تغییر می کرد.

پس از دو ماه نگهداری گرانول ها در یخچال (۴ درجه سانتیگراد)، حداکثر فعالیت متان سازی گرانول ها از ۲۹/۶ به ۵/۵ mL CH_4 /g VSS.d کاهش یافت که موید تحقیق دیگری بود مبنی بر اینکه نگهداری طولانی مدت گرانولها در دمای پایین یک روش صحیح نیست.

کلمات کلیدی: اریترومایسین، مقاومت در برابر آنتی بیوتیک، تصفیه بیهوازی،ASBR ، پساب صنایع داروسازی، آزمون متان سازی ویژه (SMA test)، اسیدهای چرب فرار (VFAs).

مقایسه عملکرد راکتور جریان افقی با بستر ثابت بی هوازی (HAIS)
و راکتور بستر لجن بی هوازی با جریان رو به بالا (UASB)

چکیده قسمت دوم

عملکرد دو نوع راکتور بی هوازی با سرعت بالا(high rate) شامل یک فرایند بستر ثابت (HAIS) و یک فرایند رشد معلق (UASB) در این مطالعه مورد بررسی قرار گرفت. دو راکتور HAIS با حجم ۲ و ۳/۳ لیتر به ترتیب با دانه های پلی یورتان (PU) و پلی اتیلن (PE) با نسبت سطح به حجم ۱۱۰۰ و ۱۲۵۰ m^{-1}، دانسیته ۷۰۰ و ۱۲ kg/m^3، و تخلخل بستر(ε) ۰/۴۲ و ۰/۲۸ پر گردید. راکتور UASB با حجم ۳/۳ لیتر با استفاده از لجن گرانوله با غلظت جامدات معلق فرار (VSS) ۴۵ g/L بذردهی شد. هر سه راکتور در دمای ۲ ± ۳۲ °C مورد بهره برداری قراز گرفت. سوبسترہ ای عمدتا متشکل از گلوکز، بعنوان منبع اصلی کربن (با COD ، ۲۵۰ ± ۱۷۵۰ mg/L) هر سه سیستم را تغذیه می نمود.

در این مطالعه، مفهوم " عدد کمی CT (quantitative Computed Tomography number) " بر حسب واحد Hounsfield (H) که تقریبا بطور خطی با دانسیته جرمی بافت یا ماده مورد نظر در تکنولوژی CT-Scanning مرتبط است، برای تعیین مشخصات محتویات راکتور های HAIS شامل بیوفیلم، بستر نگهدارنده (media)، و بیوگاز مورد استفاده قرار گرفت. سپس تصاویر CT بدست آمده از مقاطع مختلف راکتورهای HAIS با استفاده از مفهوم وضوح تصویر (Resolution; pixel per inch) در Photoshop برای برآورد میزان VSS در داخل راکتورهای مورد ارزیابی قرار گرفت . نتایج این مطالعه عملکرد مطلوب راکتور HAIS برای فراهم آوردن شرایط محیطی مناسب برای رشد و ماند بیومس در طول مدت زمان راه اندازی کوتاه مدت ۲۵ روزه را تایید نمود. در دوره بهره برداری مطلوب، بارگذاری راکتورها عبارت بود از : میزان بار گذاری آلی ۶ ± ۱۳ g COD/L.d و نسبت غذا به میکروارگانیسم (F/M) ۰/۸۲ ± ۱/۳۴ g COD/g VSS.d در راکتور UASB ، میزان بارگذاری سطحی (SLR) ۱۲ ± ۲۸ g COD/m^2.d، و و نسبت غذا به میکروارگانیسم (F/M) ۰/۷۷ ± ۱ /۷۲ g COD/g VSS.d برای راکتور HAIS-PU ، و میزان بارگذاری سطحی (SLR)

g COD/m^2.d ۱۴± ۲۳، و و نسبت غذا به میکروارگانیسم (F/M) g COD/g VSS.d ۰/۹۹ ± ۱/۵۲ برای راکتور HAIS-PE . در این بارگذاریها، راندمان حذف COD و میزان تولید بیوگاز عبارت بود از: ۱۵/۲ ± ۶۴/۳ ٪ و ml/d ۷۰۴ ± ۱۷۷۱ برای UASB، ۱۷/۵ ± ۶۳/۵ ٪ و ml/d ۴۰۰ ± ۱۱۶۰ برای HAIS-PU ، و ۱۸/۶ ± ۶۱/۶ ٪ و ml/d ۶۴۵ ± ۱۰۱۸ برای HAIS-PE. نتایج این بررسی نشان دادکه راکتور UASB با حفظ لجن گرانوله در طول مدت زمان راهبری و همچنین با دارا بودن قسمت کارآمد جداکننده گاز-مایع (GLS) ، دارای عملکرد بهتری از راکتورهای HAIS حاوی لجن تثبیت شده (immobilized sludge) و با بهره گیری از دو جمع آوری کننده بیوگاز با دو شکل مختلف: لوله سوراخدار (HAIS-PU) و صفحه شیاردار (HAIS-PE) می باشد. در بین دو راکتور HAIS، عملکرد راکتور HAIS-PU بهتر از راکتور HAIS-PE بود. این برتری می تواند با تخلخل بیشتر بستر (ε) در راکتور HAIS-PU (ε = ۰/۴۲) نسبت به راکتور HAIS-PE (ε = ۰/۲۸) در ارتباط باشد. اتصال کوتاه ناشی از تخلخل کم بستر می تواند علت اصلی این عملکرد باشد.

کلمات کلیدی: تصفیه بیهوازی فاضلاب، راکتور بستر ثابت، راکتور HAIS ، راکتور UASB، CT-Scanning ، وضوح تصویر(Resolution) و هیستوگرام در Photoshop .

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TO MY WIFE AND MY PARENTS

AND

TO MY TEACHERS AND MY PROFESSORS

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ABBREVIATIONS

AHR	Anaerobic Hybrid Reactor
AMA	Aceticlastic Methanogenic Activity
AMP	Actual Methane Production
ASBR	Anaerobic Sequencing Batch Reactor
BMP	Biochemical Methane Potential
BOD	Biochemical Oxygen Demand
CMFR	Completely Mixed Flow Reactor
COD	Chemical Oxygen Demand
CT-Scan	Computed Tomography Scan
DDW	Distilled Deionized Water
EGSB	Expanded Granular Sludge Bed
EPA	Environmental Protection Agency
FID	Flame Ionization Detector
F/M	Food to Microorganism Ratio
GAC/PAC	Granular/ Powdered Activated Carbon
GC	Gas Chromatography
HAIB	Horizontal-flow Anaerobic Immobilized Biomass
HAIS	Horizontal-flow Anaerobic Immobilized Sludge
HOMs	Hydrogen Oxidizing Methanogens
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention time
HU	Hounsfield Unit
MPN	Most Probable Number
NHOMs	Non-Hydrogen Oxidizing Methanogens
OLR	Organic Loading Rate
PCBs	Poly-Chlorinated Biphenyls
PFR	Plug Flow Reactor
PMP	Potential Methane Production
RBC	Rotating Biological Contactors
SBR	Sequencing Batch Reactor
SEM/TEM	Scanning/Transmission Electron Microscopy

SLR	Sludge Loading Rate
SLR	Surface Loading Rate
SMA	Specific Methanogenic Activity
SMPR	Specific Methane Production Rate
SRT	Solids Retention Time
SSUR	Specific Substrate Utilization Rate
TMA	Total Methanogenic Activity
UAF	Upflow Anaerobic Filter
UASB	Upflow Anaerobic Sludge Reactor
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solid
WHO	World Health Organization
WWTP (WTP)	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Introduction

The occurrence of the pharmaceuticals in the aquatic and the terrestrial environment can be linked to different sources such as emission from production sites (industrial wastewater), direct disposal of surpluses drugs in households, excretion after application for human and animal medical care, therapeutic treatment of livestock on fields, and finally effluent of fish farms. Therefore, we have to consider both point sources such as production effluents and waste disposals as well as diffuse sources such as runoff from fields and anthropogenic effluents as possible routes of the pharmaceutical compounds to the environment. Mass balances of the input and output of pharmaceuticals in sewage treatment plants reveal that during sewage treatment not all pharmaceuticals are totally removed. Consequently they are discharged into surface waters. This is of special importance, since surface water is a possible source for drinking waters. In drinking water treatment the pharmaceuticals can be removed by several technical processes such as flocculation, filtration, adsorption, or oxidation. In case of river water treatment, bank filtration is often used as the first treatment step. The compounds, which are not removed by the drinking water processes, reach the consumer. Even though the

resulting concentrations of pharmaceuticals in the aquatic system can be expected to be fairly low and the toxicity of the medical compounds is within the approved application limits, there has been no reliable information on the long-term effect on human.

The presence of antibiotics and synthetic antimicrobial drugs can impart an adverse short-term impact on the biological treatment of pharmaceutical effluents. However, long-term exposure to antimicrobials can result in the development of antimicrobial resistance making it possible to efficiently treat pharmaceutical wastewaters in biological treatment processes.

The current study is focused on evaluating the effect of erythromycin on anaerobic biological treatment of pharmaceutical wastewater. Erythromycin belongs to a class of antimicrobials known as 'macrolide' which have in common a macrocyclic lactone ring. Macrolides are relatively broad-spectrum antimicrobials, affecting Gram-positive and Gram-negative bacteria, although Gram-positive organisms are generally more susceptible (Oleinick, 1975).The activity of Erythromycin is pH dependent, increasing with pH up to about 8.5 (Hugo et al., 1998). Erythromycin has a 14-membered lactone ring (Kucers et al., 1997), which prevents bacterial growth by inhibiting protein synthesis through binding to the 23S ribosomal RNA. Mechanisms of resistance to macrolides include efflux of the antibiotic and alteration of the target site (through methylation of a nucleotide in the 23S ribosomal RNA) so that it can no longer bind the antibiotic.

Recent studies have determined the presence of a variety of antibiotics surface and groundwaters in many countries as well as throughout the United States (Adams et al., 2002). This occurs, in part, from the discharge or disposal of antibiotics from medical (human and veterinary), municipal, agriculture and industrial sources. The published data by United State Geological Survey (USGS) indicate that the erythromycin is one of several representative pharmaceuticals in wastewater with potential of impact on surface water. Erythromycin has a detection frequency (number/sample sites) of 22/104, with maximum concentration 1.7 µg/L, mass/medical dose (mass per tablet or prescribed dose) of 250- 500 mg and $LC_{50.}$ min of 665000 µg/L (Amold, 2002). The maximum estimated amount of erythromycin in Germany as applied mass (ton/yr), concentration in sewage and surface water (µg/L) has been 3.9-19.8; 6.00 and 1.70 respectively (Kummerer and Heberer, after 1999). In 1997, approximately 90 t of antibiotics were used in Switzerland- 38% in humane medicine and 62% in veterinary medicine. The private consumption of erythromycin in year 1999 in Switzerland amounted to 0.17 t and the annual average use per person of erythromycin was 24 mg for erythromycin. However, these values are based only on private consumption and do not include use in hospitals (Giger et al. 2003). These finding have raised concern regarding potential human health effects caused by low levels of antibiotics in drinking waters (Adams et al. 2002). Kim et al. (2004) reported that curing food processing animals with antimicrobial agents might increase public health risk by transfer of antibiotic resistance zoonotic pathogen or the resistance genes from animals to human (World Health Organization, 1998).

Pharmaceutical wastewaters are comprised of substrates, which are difficult to treat in biological systems. Based on the production processes, the pharmaceutical industry can be divided into five categories, namely fermentation, natural product extraction, chemical synthesis, formulation, and research and development. A wide range of natural and xenophobic organic chemical in pharmaceutical wastewaters are recalcitrant and non-biodegradable to the microbial mass within the conventional treatment systems. However, anaerobic microorganisms, especially methanogenic archaea, can adapt to levels many times those that inhibit unadapted methanogens. The anaerobic systems most usually employed for pharmaceutical wastewater treatment facilities include biomembrane reactors, completely stirred tank reactors (Kasapgil Ince et al., 2002), upflow anaerobic filters (Chua et al. 1996), fluidized bed reactor (Stronach et al. 1987), anaerobic hybrid (combined fixed-bed and sludge blanket) reactor (Henry et al. 1986), anaerobic biofilter, packed with expanded clay pellets (Yap et al. 1992), and sequencing batch reactors (Ng et al. 1989).

The utility of the SBR in the treatment of wastewaters containing toxic substances suggests that batch processes may also be suitable for the treatment of pharmaceutical wastewaters (Ng et al. 1989). Like the aerobic SBR, the ASBR involves repetition of a cycle including four discrete steps: fill (feed), react, settle, and draw off and decant (Ruiz et al. 2002).

The process of sequencing batch reactor (SBR) was introduced for the first time by Irvine et al. (1971) and then anaerobic SBR (ASBR) as a new high rate anaerobic process (US. Pat. No.5, 185,079) was introduced by Dague, Sung and coworkers (1992), issued to the Iowa State University Research Foundation in February 1993.

The Specific Methanogenic activity (SMA) test has been used to evaluate inhibitory effects of different compounds, but a few researches have been done on SMA test with using antibiotics and pharmaceutical wastes. Lallai et al (2002) have evaluated inhibitory effects of three antibiotics on methanogen bacteria by SMA test. Zabranska et al (1994) have used SMA test for the concentrated waste biomass from threonine production, mycelium after penicilium extraction and excess activated sludge used for treatment of other pharmaceutical wastewaters.

The impact of antibiotics on anaerobic microbial communities that contain bacteria as well as methanogenic archaea and on the performance of anaerobic treatment systems depending on these communities are not well understood. The overall purpose of this study is to evaluate the influence of different concentrations of erythromycin on the anaerobic treatment of a synthetic pharmaceutical wastewater in an Anaerobic Sequencing Batch Reactor (ASBR). Inhibition of the anaerobic digestion process was evaluated in short-term batch experiments seeded with biomass from the ASBR and through the long-term operation of the ASBR with increasing amounts of erythromycin in the influent. The inhibitory effect of erythromycin was evaluated by monitoring biogas production, COD removal and accumulation of individual volatile fatty acids

Objectives

The objectives of this research project were:

(1) To evaluate short- and long-term effects of low and high erythromycin concentrations on COD removal and biogas production in steady-state anaerobic treatment of synthetic pharmaceutical effluents;
(2) To develop the relationships between depletion or accumulation of VFAs using pure and mixed substrates;
(3) To compare removal efficiencies of erythromycin in ASBR and SMA test;

Organization of the report

The literature review on subjects of this study, such as antibiotics mainly erythromycin, pharmaceutical wastewaters, anaerobic treatment, ASBR and SMA are presented in Chapter 2. The materials and methods are described in Chapter 3, results and discussion in chapter 4, and finally conclusions of this study in chapter 5.

CHAPTER 2

LITERATURE REVIEW

Introduction

The current state of researches and knowledge in regard to topics of this study including: antibiotics, erythromycin, pharmaceutical wastewater, anaerobic treatment, anaerobic sequencing batch reactor (ASBR), and specific methanogenic activity (SMA) test, respectively, is described in this chapter.

Antibiotics

In recent years the emphasis was on so-called emerging contaminants including pharmaceuticals such as antibiotics, endocrine disrupters (*e.g.* steroid hormones, nonylphenol, bisphenol A) and on various additives such as hardeners in foundry sands or concrete admixtures, anticorrosive, and flame-retardants. In all these studies the analytical methods were applied in elaborate field studies in wastewaters and natural waters (Giger et al., 2003).

Pharmaceuticals belong to the environmentally relevant compounds. They are produced and administered for human and animal medical care. Due to amount and types of application pharmaceuticals can reach the environment, in particular the aquatic systems. Being produced and applied with the aim of causing a biological effect, their occurrence in the environment is of ecotoxicological interest. In particular this is of important for antibiotics, but also for antineoplastics, hormones (compounds with endocrinic effects) and various compounds and methabolites that have already been detected in sewage plant effluents and surface waters in considerable concentrations (Kummerer, after 1999).

Endocrine disrupting compounds are an important subgroup of pharmaceutically active compounds. Since these substances are present in wastewater effluent in extremely small quantities, the significance of exposure at those levels is exceptionally important.

The endocrine system consists of glands that produce and excrete hormones. Our cells respond to hormones at extremely low systemic levels- on the order of picomoles to nanomoles per liter. It follows that presence of the hormones and hormone mimics at such levels in drinking water is potentially troublesome (Amold, 2002).

Attention to the endocrine disrupting compounds has been focused for the most part on estrogens and synthetic organic compounds that are estrogen-like. Anthropogenic compounds with estrogen-like qualities include PCBs, DDT, and alkylphenols (e.g., octylphenol and nonylphenol) and others. Domestic wastewater effluents also contain suggestively high levels of 17β-estradiol (human estrogen, E2), 17α-ethinyl estradiol (EE2, an active ingeredient in birth control pills) and estrone (an E2 metabolite). Some mail fish respond to E2 and EE2 at aqueous-phase concentrations approaching 1 ng/L by inappropriately producing vitellogenin, an egg yolk protein, or developing other signs of fermentation. The finding underscores the apparent similarities in endocrine system structure and function across divergent taxonomic lines, although fish are considerably more sensitive to waterborne estrogens than are human and other mammals.

Human health effects from exposure to estrogenic compounds are entirely at this point. Nevertheless, effects about which there have been speculation-rising rates of breast cancer and broadly declining sperm counts-are potentially calamitous (Amold, 2002).

Pharmaceutical industries, mainly antibiotic and synthetic drug based industries suffer from inadequate treatment due to the presence of toxic substances and complex biomass formed during the secondary treatment process. Aromatic compounds such as phenols and methylated phenyls are commonly encountered pollutants, originally typically from modern industrial activities. Because of their bacterial toxicity and recalcitrance, when present in wastewaters, they play a key role in decreasing COD removal rates in treatment systems. As a result of their toxicity and despite their usually relatively low concentration, their removal from contaminated effluents is as important as removal of the COD fraction. Anaerobic degradation of different constituents of the antibiotics and synthetic drug based effluent has recently been proved, under methanogenic, sulphate reducing, nitrate reducing and iron reducing conditions (Saravanane et al., 2001).

In wastewater treatment plants the antibiotics are only partially eliminated and residual amounts can reach ambient waters or groundwater (Giger et al., 2003). Adams et al. (2002) reported that biological treatment processes have been shown to be ineffective in the removal of antibiotics. For example, Ingerslev and Halling-Sorensen (2000) found that 12 different sulfonamides were not readily biodegradable in activated sludge. Belter et al. (1973) patented an ion exchange process that used a weak –base anion exchange resin to absorb streptomycin, which could subsequently be eluted with a dilute acid solution. In a later study, Belter (1983) again used ion exchange to remove and recover the antibiotics from pharmaceutical water (Adams et al., 2002).

Most pharmaceuticals are found in natural waters in only very low concentrations. Despite of this general finding, the question arises what risks these traces of pharmaceuticals pose for aquatic ecosystems. Antibiotics are of particular interest because we don't currently know whether their presence in natural waters contributes to the spread of antibiotic resistance of microorganisms (Giger et al., 2003). Adams et al. (2002) in their literature review of studies on pharmaceutical compounds in the environment, virtually did not find no studies on potential health effects of chronic low –level exposure to pharmaceuticals.

World Health Organization (WHO, 1998) reported that curing food-processing animals with antimicrobial agents might increase public health risk by transfer of antibiotic resistance zoonotic pathogen or the resistance genes from animals to human (Kim et al., 2004).

Through better analytical chemistry, we know beyond doubt that a great number of pharmaceuticals are present in domestic wastewater and routinely survive conventional wastewater treatments, although generally in minute amounts. This information has caught the eye of the public and the environmental community in a big way (Amold, 2002).

The sensitivity of Archaebacteria to antibiotics. Methanogens are resistant to macrolides (Hummel et al., 1985). The impact of antimicrobials on anaerobic microbial communities that contain bacteria as well as methanogenic archaea and on the performance of anaerobic treatment systems depending on these communities are not well understood.

Most of the species of the two largest groups of the Archaebacteria- halobacteria (9 strains) methanogens (10 strains)- were tested for their sensitivity to 28 well-known antibiotics including Erythromycin by Hilpert et al. (1981). They found Archaebacteria are insensitive to many antibiotics acting against eubacteria and eucaryotes, e.g. those inhibiting the synthesis or cross-linkage of the peptide subunit of murein or the synthesis of RNA. Among the macrolid antibiotics only virginiamycin inhibits almost all strains of the archeabacteria tested, whereas oleandomycin and erythromycin are ineffective with the exception of a slight inhibition of *Mc. Vannielii* by erythromycin. These findings are helpful in designing selective media for isolation of new types of archebacteria or in controlling of mixed populations of eubacteria, archeabacteria, and eucaryoets, e.g. in the rumen and in biogas plants.

In the other study, effects of three antibiotics on biogas production in anaerobic digestion of pig waste slurry with using 160 ml serum bottles on batch wise, the same as SMA test, have been evaluated (Lallai et al, 2002). They concluded that: thiamphenicol (80, 160 mg/l) added to anaerobic culture had a considerable effect on methane production when compared to the control culture, amoxicillin trihydrate (60, 120 mg/l), had a lower but significant inhibitory effect on the methane production related to the concentration of the antibiotic. Oxytetracycline hydrochloride (125, 250 mg/l) seemed not to exert any effect on the methane production. Moreover, the results obtained with antibiotics that cause inhibition did not allow them to state which part of the anaerobic community (methane or acid-producing bacteria) was affected by antibiotics. For this reason, further experimentation with measurement of volatile fatty acids is needed.

Archaebacteria differ from the classical bacteria and the eucaryotic organisms with respect to many basic biochemical characteristics, e.g. the sequence of the ribosomal 16s RNA cell wall polymers, membrance lipids, DNA-dependent RNA polymerase, etc. The biosyntheses or functions of compounds and structures unique to archeabacteria might also serve as targets for antibiotics, whereas typical targets for antibiotics present in eubacteria and eucaryoets, respectively, may be absent. Hence, it is to be expected that the sensitivity of archeabacteria towards antibiotics is quite different from that of the members of the other two kingdoms, as already exemplified in at least two distinct cases. Firstly, the inhibitors of murein synthesis, cycloserine, penicillin and vancomycin do not inhibit methane bacteria, thus reflecting the absence of murein in these organisms and, secondly, rifampicin and streptolydigin, potent inhibitors of the DNA-dependent RNA polymerase of eubacteria, do not inhibit the corresponding enzyme of archaebacteria (Hilpert et al., 1981).

A comparative study of the sensitivity of a wider range of archaebacteria towards antibiotics may help to characterize other biochemical differences between the 3 kingdoms of organisms, although it must be emphasized that insensitivity to a particular antibiotic does not necessarily indicate the absence of the respective target but may merely be due to the inability of the organism to transport, or the ability to inactivate the particular antibiotic.

It is pointed out that the very characteristic sensitivity pattern of archaebacteria may serve as a guideline in the search for further biochemical and molecular difference between archaebacteria, eubacteria, and eucaryotes, and that it may also be helpful in designing selective media for the enrichment and isolation of new types of archaebacteria or in controlling mixed populations of eubacteria, archaebacteria, and eucaryotes, e.g. in the rumen and in biogas plants (Hilpert et al., 1981).

Removal of antibiotics in water treatment plant and river water. The removal of antibiotics: carbadox, sulfachlorpyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfathiazole, and trimethoprim, were evaluated by Adams et al. (2002) in eight common water treatment processes with using distilled-deionized (DD) water and river water. Sorption, on powdered activated carbon (PAC), reverse osmosis, oxidation with chlorine and ozone under typical plant conditions were all shown to be effective in removing the studied antibiotics. Conversely, coagulation-flocculation-sedimentation with alum and iron salts, excess lime-soda ash softening, ultraviolet irradiation at disinfection dosage, and ion exchange were all relatively ineffective methods of antibiotic removal. With a PAC dosage of 50 mg/l in both DD water and river water, the percent removal was greater than 90% for all compounds. Based on the results for PAC, one would expect that similar granular activated carbon (GACs) would also be highly effective at removing the study antibiotics.

Cephradine. Two methanogenic cultures were grown in the presence of Cephradine (a main constituent of anti-osmotic drug) by Saravanane et al. (2001). Inhibition concentrations against methane production were similar in both cases in a first experiment, despite the fact that only one of cultures was able to degrade the compound. After several transfers the adapted culture (Culture A) tolerated higher concentration of cephradine. Culture B (non-adapted) tolerated the presence of cephradine though it did not degrade it, meaning that continuous methanogenic wastewater treatment system (fluidized bed reactor) could very well show a higher methanogenic activity and COD reduction, despite their inability to degrade the refractory organic fraction, in case of toxic compound, in anti-osmotic pharmaceutical effluents.

Antibiotics in animal medical care. Antibiotics are widely used in pig farms as feed additives, to promote growth and to prevent infection, and at therapeutic level to treat animal that are ill. Certain antibiotics contain carriers that allow them to target specific parts of the animal organism (the intestine, the respiratory system, etc.) while others act at a general level. Some antibiotics become active when metabolized, whereas others operate in an initial active form. In either case, a fraction of the ingested antibiotics may be excreted in an active form (Gamel-El-Din, 1986) and is then found in the wastewater from the farm (Lallai et al. 2002).

The overuse of antibiotics is the primary cause of high antibiotic concentration in the slurry sent to treatment plants. A second factor is the quantity of water and the methods used to clean out pig houses. Since the most frequently used biological process in wastewater treatment plants is the anaerobic digester, it is possible that these antibiotic concentration could have negative effects on the mixed population of anaerobic bacteria: they could determine the selection of cultures or reduce their rate of growth, and therefore might have a significant on both the degree of degradation of the organic load of the waste and on the production of biogas. Problems of this nature have often been noted by those working in the field and have sometimes been reported in the literature (Hobson and Shaw, 1976, Poels et al., 1984). Unfortunately, there are not many experimental studies on the effects of antibiotics on the performance of anaerobic digestion. The biological activity of the anaerobic digestion of pig slurry was found to be reduced by the presence of lincomycin (Fisher et al., 1981). In studying the anaerobic digestion of cattle manure, Blotevogel and Jannsen (1988) found that carbadox, lasalocid and monesin inhibited biogas production, while avoparcin did not (Lallai et al. 2002).

Lallai et al. (2002) added three commonly used antibiotics in the treatment of pigs-amoxicillin trihydrate, oxytetracycline hydrochloride and thiamphenicol were added at different concentrations to aliquots of pig waste slurry plus anaerobic sludge in serum bottles. The biogas production and methane concentration in the headspace were monitored to determine the effect of the antibiotics on the anaerobic process. With thiamphenicol significant difference in methane production were found for concentration of 80 and 160-mg/l slurry. Compared to the control, only minor differences in methane production were noted in the bottles to which amoxicillin (60 and 120 mg/l) had been added. Methane production was about the same for the bottles with deferent oxytetracycline concentration (125 and 250 mg/l) and for the control.

Antibiotic and Erythromycin Data in Switzerland. Giger et al. (2003) collected use figures for antibiotics in Switzerland to assess the potential inputs of these chemicals into wastewater. In 1997, approximately 90 t of antibiotics were used in Switzerland- 38% in humane medicine and 62% in veterinary medicine. The amount of antibiotics applied annually for human medicinal purposes is around 34 t and has remained constant since 1992. β-Lactam antibiotics including penicillins and cephalosporins are the largest fraction of human use antibiotics, accounting for approximately 18 t. Following are sulfonamides (5.5 t), macrolides (4.3 t), and fluoroquinolones (4.8 t). The major human-use fluoroquinolones (FGs) consumed in are ciprofloxacin and norfloxacin. The private consumption of macrolides in year 1999 amounted to 1.7 t of clarithromycin, 0.32 t of azithromycin, 0.26 t of spiramycin, 0.17 t of

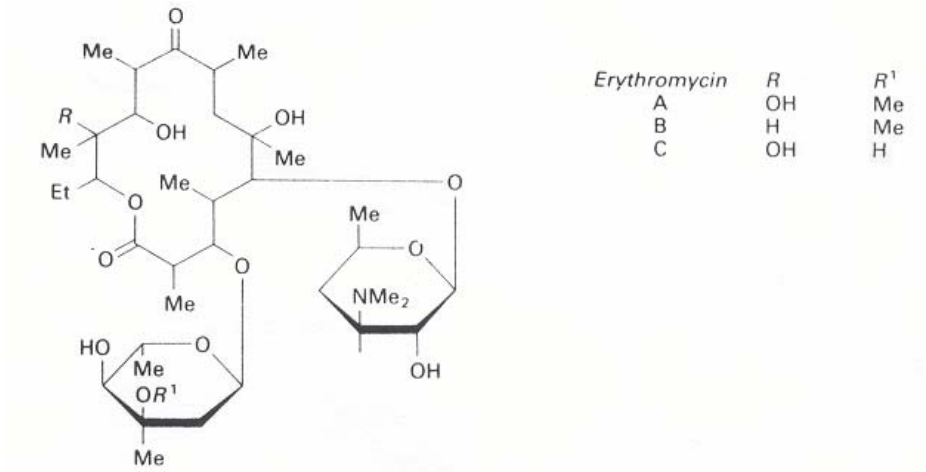
erythromycin and 0.15 t of roxithromycin. Additionally, unknown amounts of 20-40 % are employed in hospitals. The annual average uses per person in Switzerland in 1999 were 264, 24, and 21 mg for claritomycin, erythromycin, and roxithromycin, respectively. However, these numbers are based only on private consumption and do not include use in hospitals.

Erythromycin

Description. Erythromycin was isolated from a strain of *Streptomyces erythreus* from the lactone precursors, propionate and 2-methylmalonate, with subsequent attachment of the sugars. It belongs to a group of antibiotics known as the ‘ macrolides’, which have in common a macrocyclic lactone ring. The discovery of the erythromycin was reported in 1952 (McGuire et al., 1952). Erythromycin has a 14-memberd lactone ring (one oxygen and 13 carbon atoms). These antibiotics are all weak bases, only slightly soluble in water. Erythromycin base is very bitter, insoluble in water and inactivated by acid. Various erythromycin salts and esters subsequently became available for clinical use, which are more acid-resistant. Four of these have been used clinically: 1) Erythromycin stearate (a salt); 2) Erythromycin ethyl succinate (an ester); these two preparations are still susceptible to acid inactivation. 3) Propinyle erythromycin ester lauryl sulfate (erythromycin estolate) (the salt of an ester); and 4) Stearate salt of 2-acetyl ester of erythromycin (erythromycin ascitrate). These last two formulations are more resistant to acid inactivation (Kucers et al. 1997). Erythromycin A is the most common and widely used of the erythromycins both in research and in clinical practice. Unless otherwise noted, the simple term erythromycin hereafter will refer to the A form (Oleinick, 1975; Kucers et al. 1997).

Wild-type strains of *S. erythreus* produce all three erythromycins: A ($C_{37}H_{67}O_{13}N$) and two other closely related compounds erythromycin B ($C_{37}H_{71}O_{12}N$) and erythromycin C ($C_{36}H_{65}O_{13}N$). The structure of the erythromycin is shown in Figure 2.1. The amino sugar of all erythromycin is desosamine and the neutral sugars are cladinose in erythromycin A and B and mycarose in erythromycin C (Vanek and Majer, 1967; Oleinick, 1975).

Sensitive Organisms. Erythromycin is normally highly active against organisms such as streptococcus and coagulase-negative staphylococci (Camprubi et al., 1988). Erythromycin inhibits the growth of many Gram-positive and some Gram-negative organisms. The bacteriostatic action of erythromycin is a result of the antibiotic’s ability to selectively inhibit protein synthesis in the susceptible



Erythromycin	*R*	*R*¹
A	OH	Me
B	H	Me
C	OH	H

bacteria, but not in host mammalian tissue (Oleinick, 1975).

Figure 2.1. Structure of Erythromycin (Russell, 1998)

Excretion. Erythromycin is partly excereted in urine and only about 2.5% of an orally administered dose and 15% of a parentally administered dose is recoverable from the urine in the active form (Abbott Laboratories, 1966). Urinary concentrations of the active drug are usually low and variable. As renal excretion is not the main method of erythromycin elimination from the body, there is no significant accumulation of the drug in uremic patients.

A considerable proportion of erythromycin is excreted in the bile, where high levels of the active drug are attained. Some erythromycin excreted in this way is reabsorbed from intestine.

A large proportion of administered erythromycin cannot be accounted for by combined renal and biliary exertion, and so a considerable amount appears to be inactivated in the body, probably in the liver (Kucers et al. 1997).

Mode of Action. Erythromycin interferes with bacterial protein synthesis at the ribosomes. This drug, similar to other macrolide antibiotics, becomes bound to the 50 S subunit of the ribosome. The exact stage of bacterial protein synthesis affected by erythromycin is not known with certainty but it was suggested that it might interfere with the 'translocation reaction', which is catalyzed by an enzyme, translocase. During this reaction the growing peptide chain with its t-RNA moves from the 'acceptor site' to the 'donor site' on the ribosome. Erythromycin probably binds to the donor site, and by competing for this site of attachment, prevents translocation of the peptide chain from the acceptor to the donor site. More recently it has been suggested that erythromycin stimulates dissociation of peptidyl-tRNA from the ribosomes during the elongation phase, leading to inhibition of protein synthesis (Kucers et al. 1997).

It is possible that one reason why Gram-negative bacilli are resistant to erythromycin is because it cannot penetrate their cell walls. Stable L-forms of *Pr. mirabilis*, which have cell walls, are very susceptible to erythromycin.

Erythromycin has been shown to produce other effects in addition to its direct antimicrobial activity. It appears to act as immunomodulator or bacterial virulence suppressing agent, especially against *Ps. aeruginosa* infections (Kucers et al. 1997).

Concentration. Erythromycin is normally bacteriostatic; however, at concentration 25-1000 folds greater than that necessary to produce bacteriostsis, the antibiotic can become bactericidal (Oleinick, 1975).

Erythromycin Resistance. Bacteria may mutate to resistance to erythromycin *in vivo* or upon serial transfer in culture in the presence of the drug. Resistant mutants are less able to accumulate extracellular erythromycin and form less stable antibiotic-ribosome complexes. Bacterial destruction of erythromycin has not been reported.

Erythromycin-resistant mutants are often found which are also resistant to other macrolide antibiotics, to lincomycin and chloramphenicol. However, such cross-resistance is not universal, since some erythromycin-resistant mutants remain sensitive to one or more of these other antibiotics. Both synergism and antagonism between erythromycin and other antibiotics have been observed.

The inhibition of bacterial growth by erythromycin is dependent upon environmental conditions. The minimum inhibitory concentration increase at the pH of the medium is lowered from 8.5 to 5.5 observed that inhibition by erythromycin.

The genetic locus that codes for resistance to erythromycin (the erythromycin locus) is liked to a gene for 50S ribosomal proteins in *Bacillus subtilis*, and is clustered with other antibiotic markers for ribosomal proteins near the major group of genes for rRNA (Oleinick, 1975).

Inhibition of Protein Synthesis. In intact bacteria erythromycin acts by inhibiting protein synthesis without any direct effect on nucleic acid synthesis. Amino acid incorporation into acid-insoluble material is susceptible to inhibition by erythromycin in cell-free preparations from *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus.* The inhibition is exerted on a step in protein synthesis, which comes after the activation of the amino acid and its transfer to tRNA (Oleinick, 1975).

Resistance of Macrolides and Erythromycin in Anaerobes. The basic resistance mechanisms described for anaerobic bacteria can be categorized at three levels: (a) decreased permeability of the anti-microbial agent through the wall of the organism; (b) hydrolysis of the anti-microbial drug by several enzymes before reaching the site of action; and (c) modifications in the site of action of anti-microbial agent (Garcia-Rodriguez et al., 1995).

The mechanisms of resistance to macrolides include efflux of the antibiotic and alteration of the target site (through methylation of a nucleotide in the 23S ribosomal RNA) so that it can no longer bind the antibiotic. Macrolides are relatively broad-spectrum antibiotics, affecting gram-positive and some gram-negative bacteria (Oleinick, 1975)., but not methanogenic archaea.

Resistance to macrolides in anaerobes is usually plasmid – or transposon – mediated and related to lincosamides resistance. That resistance is usually due to a N6 dimethylation of an adenine residue in the 23S ribosomal RNA of the 50S ribosomal subunit. The most frequent of these genes in anaerobic bacteria appears to be the ermF, on the Tn4351 and Tn4351 and Tn4400 in *B. fragilis*. Other genes very similar to the ermF are ermFS, on the Tn4551 in *B.ovatus* and the ermFU, on the Tn5030 in *B. vulgatus*. The resistance mediated by these genes is characteristically inducible by the presence of macrolides. Nevertheless, mutations in the genes that regulate that induction can lead to constitutive production of the RNA methylase, with a phenotype of resistance MLS_B (Garcia-Rodriguez et al., 1995).

The mechanism involved in macrolide resistance in Gram-positive anaerobes is similar. Erythromycin resistance methylase (erm) genes related to the erm AM, originally described in *Streptococcus sanguis*, have been described in *C. perfringens* (ermP) and *C.difficile* (ermZ). Recently, Regi et al. (1992) have described resistance phenotypes in *Peptostreptococcus* similar to phenotype characteristic of the presence of a RNA methylase, but the genes involved are not know so far (Garcia-Rodriguez et al., 1995).

The sensitivity of Archaebacteria to Erythromycin. Among the macrolide antibiotics only virginiamycin inhibits almost all strains of archaebacteria tested, whereas oleandomycin and erythromycin are ineffective with the exception of a slight inhibition of *Mc. vannielii* by erythromycin (Hilpert et al., 1981).

Erythromycin in groundwater wells, streams, and WWTP effluents. Sacher et al. (2001) analyzed 105 groundwater wells in Germany. Among 60 pharmaceuticals, they found erythromycin-H_2O and sulfomethoxazole, which were the only antibiotics out of eight compounds detected in at least three groundwater samples. The study of Kolpin et al. (2002) shows the occurrence of 95 organic wastewater contaminants including pharmaceuticals in 139 streams across the USA. Among 31 antibiotics from the groups of tetracyclines, macrolides, sulfonamides, and fluoroquinolones, erythromycin-H_2O and sulfametoxazole were found in concentrations of up to 1.7 and 1.9 μg/l, respectively. Hirsch et al. (1999) investigated the occurrence of several representatives from the main groups of antibiotics in wastewater treatment plant effluents and in river water. They described the analysis of

various water samples for 18 antibiotics substances from the antibiotic classes of macrolides, sulfonamides, penicillins and tetracyclines. They observed the frequent occurrence of erythromycin-H_2O, roxithromycin, and sulfamethoxazole with concentration up to 6 μg/l in wastewater treatment plant (WWTP) effluents. Neither tetracyclines nor penicillins could be detected at concentration levels above 50 and 20 ng/l, respectively. Penicillins are not very likely to occur in the aquatic environment due to the chemically unstable β-Lactam ring, which is readily susceptible to hydrolytic cleavage (Giger et al., 2003).

Seasonal variations of macrolides in WWTP. The results of two sampling campaigns by McArdell et al. (2003) at the WWTP Kloten-Opfikon showed that during the winter season two-time higher loads of macrolide antibiotics were observed than in summer. This difference can best be explained by the seasonal variation of the use figures of theses pharmaceuticals, as documented by the monthly sales data showing that macrolides are sold in two times higher amounts in January/February than in summer. This seasonal effect arises because the macrolide antibiotics are mainly used to cure infections of the respiratory tract. Based on detected seasonal and regional differences of the macrolide wastewater loads and eventual varying concentrations in ambient waters, it can be postulated that such variations must be considered for environmental exposure assessments (Giger et al., 2003).

Erythromycin removal by clays. In batch experiments with homoionic clays (montmorillonites and kaolinites), by Kim et al. (2004), the Ferundlich isotherms were determined at 10 and 25 °C. The adsorption of erythromycin A was strongly influenced by clay type, exchanged cations, the pH of bulk solutions, and the acidity of clay surfaces. The formation of clay-erythromycin A complexes was thermodynamically favorable except for K^+- and Fe^{3+}-exchenged montmorillonites, since the reactions were exothermic ($\Delta H° > 0$) and the systems became stable ($\Delta S° > 0$). Clays catalyzed the erythromycin A degradation by the hydrolysis of the neutral sugar and the multiple dehydrations. The surface acidity of clay surface enhanced the rate of clay-catalyzed degradation of erythromycin A. In addition, the Fe^{3+} -exchanged clay mineral seemed to have an electrostatic interaction with the erythromycin A molecule, by which the hydrolysis of the neutral sugar was influenced.

As a conclusion, the formation of clay-erythromycin A complexes is an important process to mitigate the biological activity of erythromycin A effluent from the use in humans and animals. Since the *tert*-amino sugar moiety of erythromycin A seems to be intact against the acid- and clay-catalysis under ambient conditions, soil-sorbed erythromycin A might persist for a prolonged time, similarly as tylosin with a low mobility in soil (Rabolle and Spliid, 2000). However, clay-sorbed erythromycin A loses the activity rapidly by the acid- and clay-catalyzed degradation. The adsorption processes using clays may be simply applicable in the agricultural, municipal, and industrial wastewater treatments to reduce the biological activity of erythromycin A effluent and to facilitate the clay-catalyzed degradation (Kim et al., 2004).

Erythromycin in animal medical care. Erythromycin A is widely used for the treatment and prevention of infectious bacterial diseases in livestock, and poultry and is also used under Investigative New Animal Drug (INAD) permits in the USA to treat some fish diseases in food fish. Erythromycin A can reach streams, soils and sediments through manure and other farm wastes. Thus, not only the intestinal microflora of animals exposed to antimicrobial agent, but also the accumulation of antibiotics in soils and sediments can serve as potential reservoirs of antibiotic-resistant microorganisms (Kim et al., 2004).

Effects of Erythromycin on anaerobic treatment of in piggery wastes. In modern swine production system, feed additives and antibiotics are added to pig to promote growth and control diseases. Historical developments leading to the use of antibacterial agents in animal feeds and the generally accepted concepts about their growth promotion action were reviewed by Visek (1978). However, there is limited information about the performance of organic matter removal and methane production by anaerobic fermentation of piggery wastes which include those agents (Camprubi et al., 1988).

Cleaning and disinfection of the big houses occurs normally twice a year and is, depending on the particular farm routine, completed with intermediate cleanings. Furthermore, the addition of antibiotics in the drinking water during several days to combat bacterial infective diseases is commonly practiced on pig farms. The use of disinfectants and antibiotics can disturb anaerobic digesters treating piggery wastes (Poels et al., 1984).

Brumm et al. (1979, 1980) reportad that the presence of dietary arsonic acids in swine diets did not increase methane production but increased dry matter and volatile solids degradation in anaerobic pits and digesters, because of a higher conversion of complex organic matter to volatile fatty acids. On the other hand, Brumm and Sutton (1979) concluded that copper sulfate content higher than 50mg/l (as Cu) decreased the piggery waste decomposition in anaerobic pits, without affecting fresh waste composition (Camprubi et al., 1988).

Varel and Hashimoto (1982) and Poels et al. (1984) studied the short-term effects of some antibiotics, feed additives and disinfectants on anaerobic fermentation of swine manure at 35°C. They concluded that most of these agents did not affect biogas production when used in normal doses, but reduced it at higher concentrations. Furthermore, Fisher (1981), studying the effect of pig diets on waste anaerobic digestion, found that the antibiotic lincomycin reduced biological activity (Camprubi et al., 1988).

Poels et al. (1984) investigated the effects of disinfectants: Tego 51, Dettol, NaOcl, and Creolin (in two different dose) and antibiotics: chlortetracyclin, tylosin, erythromycin, chloramphenicol, bacteriacin and virginiamycin (in three different dose) on the anaerobic digestion of piggery waste. The first concentration of each agent corresponded with the one used in practice on the basis

of washing down piggery once with an appropriate concentration of disinfectant. To calculate this concentration, the pig house was assumed, to contain 180 fattening pigs on fully slatted floors and to have a 120 m^3 manure storage pit half full. The second concentration of the disinfectants corresponded with a dose ten times higher than the usual one. This dose should reveal effects on the mechanization process under extreme conditions (for example, very intensive disinfection). For the experiments with antibiotics the concentrations were calculated on the basis of one veterinary prescribed dose in the drinking water on one day (does 1) all passing through the pigs and collecting in the manure pit. The second and third concentrations of antibiotics corresponded to those quantities present in the piggery manure storage pit after a consecutive daily treatment during a period of 5 (dose 2) and 10 days (dose 3), respectively. The erythromycin concentration in does 1,2 and 3 were: 0.4, 2, and 4 mg/l respectively. The effluent COD from dose 1 to 2 was increased around 5% and from dose 2 to 3 was increased around 13% (Poels et al., 1984).

Camprubi et al. (1988) studied the effect of some antimicrobial agents and feed additives on performance of batch and semi-continuous piggery wastes anaerobic digesters. Their research was carried out to elucidate that potential inhibitory effect in the case of furazolidone. chloramphenicol, chlortetracyclin, tylosin, erythromycin, carbadox and copper sulfate. Results showed that chlortetracyclin, tylosin and erythromycin do not inhibit methanogenic activity at concentrations up to 225, 200 and 50 mg/l, respectively. The same behavior was observed with furazolidone in both batch and semi-continuous runs, with a maximum inhibition of 10% at a concentration of 150 mg/l. The continuous addition of carbadox increased the inhibition to 30%, being 80% in the case of copper sulfate at 94 mg/l (as Cu). A strong inhibition by chloramphenicol was found. After an apparent recovery of the digester performance, methane production was reduced to 5% of the control value, thus showing the flora to be unable to acclimatise to such an inhibitor.

Activated sludge kinetics for Erythromycin biodegradation. The kinetics of continuous flow completely mixed activated sludge process for biodegradability of pharmaceutical wastewater with mixture of three antibiotics: erythromycin, furazolidone and furadantin were achieved by Hu et al. (1986).

Anaerobic Treatment

Introduction. Wastewater engineering, as a part of environmental engineering uses the basic principles of science and technology together with engineering principles to carry out the ultimate goal- wastewater management – which in fact ensures the protection of the environment in a manner commensurate with public health, economic, social, and political concern.

Wastewater is a combination of the liquid- or water- carried wastes removed from residences, institutions, commercial, and industrial places together with such ground water, surface water, and storm-water.

Methods for wastewater treatment were first developed in response to the concern for public health and the adverse conditions caused by the discharge of the wastewater in the environment. If untreated wastewater is allowed to accumulate, the decomposition of the organic materials it contains can lead to the production of large of large quantities of malodorous gases. In addition, the pathogenic or diseases-causing microorganisms living in wastewaters lead to human diseases and infections. Also the nutrients present in industrial wastewater can simulate the growth of aquatic plants, which is eutrophication.

Almost all wastewaters can be treated biologically if proper analysis and environmental control are carried out. There is a growing interest to depollute high-strength industrial wastewaters, which more often does not proceed optimally because the composition of these effluents is typically time-variable and nutritionally imbalanced. In these circumstances it is essential that the environmental engineer understand the characteristics of the each biological process to ensure the proper environment is produced and controlled effectively. The treatment of wastewater can be performed so that changes are brought about by means of biochemical reactions produced as a result of the presence of microorganisms, under aerobic or anaerobic conditions. Those processes are known as biological unit processes and they are designed to coagulate and remove the non-settable colloidal solids and to transform the hazardous organic matter as well as stabilize it. Anaerobic decomposition of organic matter occurs in the absence of oxygen. An anaerobic process upon as a variable alternative to the aerobic system for treatment of medium strength wastes. The anaerobic process can be considered as one of the oldest technologies for stabilizing wastewaters. It has been applied since the end of the 19th century, mainly for the treatment of household waste (water) s in septic tanks, treatment of slurries in digesters and for the treatment of sewage sludge in municipal treatment plants. The request for more cost-effective treatment systems for the growing food industry, combined with the occurrence of an international oil crises, was the driving force that simulated the most important research achievements of the seventies in the field of anaerobic treatment. Particularly, the introduction of the modern "high rate" reactors, in which hydraulic retention times (HRTs) are uncoupled from the solids retention time (SRT), led to word-wide acceptance of the anaerobic technology as a cost-effective alternative for conventional wastewater treatment systems. A comparison of anaerobic and aerobic treatment processes (Table 2.1) shows that the anaerobic process offers some advantages (Gavrillescu, 2002).

It is not our aim to give a comprehensive review of these topics, but to give a feel for the types of processes involved in biological anaerobic wastewater treatment, and to introduce a new high rate reactor, i.e. anaerobic sequencing batch reactor (ASBR).

Anaerobic microbiology. Anaerobic treatment converts the organic pollutants (COD, BOD) in the wastewater into small amount of sludge and a large amount of biogas (methane, carbon dioxide), while leaving some pollution unremoved. Aerobic processes, in contrast produce a lot of excess sludge, and no biogas, while also leaving some pollution, though less than after anaerobic treatment.

In the absence of oxygen, many different groups of anaerobic bacteria 'work' together to degrade complex organic pollutants to methane and carbon dioxide. The microbiology is more complex and delicate than in the case of aerobic processes, where most bacteria 'work' individually. As a result, anaerobic systems require more control and monitoring systems to operate successfully (Gavrillescu, 2002).

Table 2.1. Comparison of aerobic and anaerobic systems (Gavrillescu, 2002)

Aerobic treatment	Anaerobic treatment
Conditions	
Preferably after preclarification	Also without presettling
Best for waste-water with lower concentration	Only for medium and high concentration concentrated wastewater
Also for rather cold waste-water	Only for warm waste-water (>20 °c)
Toxic components often acceptable	Few toxic compounds allowed
Neutralization for alkaline waste-water required	Treatment of alkaline wastewater without neutralization
Process	
Only continuous, no long shut-downs allowed	Seasonal operation possible
Low effluent values can be attained through multi-stage or cascade design	Low effluent values only with additional aerobic post - treatment (polishing)
Simultaneous N and P removal possible	No significant N or P removal
High excess sludge production	Very small excess sludge production
Clogging danger, when using carrier material	No clogging danger from sludge growth
Low volumetric loading rates	High volumetric loading rates possible
High maintenance (equipment)	Low maintenance costs
Possible odour problems, or high volumes of waste air to be treated	No odour problems and waste air in case of system using closed tanks
Byproducts	
A lot of excess sludge	Valuable biogas
Costs	
Relatively low investment costs	Often higher investment costs
High operational costs for:	Low operational costs for :
Aerobic (power)	*Power consumption*
Nutrients (N, P)	*No or little nutrients*
Sludge disposal	*No or little excess sludge*
Small plants also feasible	*Small plants less economical*

The biological degradation of complex organic compounds takes place in several consecutive biochemical steps (chain reaction), each performed by different groups of specialized bacteria, which have been studied in detail. These steps can take place simultaneously in one bioreactor (one phase system), or partially separated in two consecutive tanks (two phase system). Several intermediate products are continuously generated and immediate processed further. Simplifying the degradation process, four major steps can be distinguished, as shown hereby (Figure 2.2). In practice it is important to realize that all steps have to occur at matching rates, in order to avoid a build-up of intermediate products. Without good "teamwork" of all the microbial communities involved, no complete degradation is possible (Gavrillescu, 2002).

The consortium of anaerobic organisms that work together to bring about the conversion of organic sludges and wastes can be grouped as follows:

- Organism responsible for hydrolyzing organic polymers and lipids to basic structural building blocks, such as monosaccharides, amino acids, and related compounds. This step is carried out by extracellular enzymes of facultative or obligate anaerobic bacteria, e.g. *Clostridium* (degrading compounds which contain cellulose and starch) and *Bacillus* (degrading protein and fats).
- Anaerobic bacteria, which ferment the breakdown products to simple organic acids, the most common of which in an anaerobic digester is acetic acid (acidogens or acid formers). These bacteria are described as non-methanogenic and can be *Clostridium spp.*, *Peptoccocus anaerobus, Bifidobacterium spp., Desulphovibrio spp., Corynebacterium spp., Lactobacillus, Actinomyces, Staphylococcus, and Eschrichia coli.*
- Organisms, which convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide, designated as methanogens or methane fromers. The most important microorganisms that have been identified include the rods (*Methanobacterium, Methanobacillus*) and spheres (*Methanococcus, Methanosarsina*). (Figure 2.2)

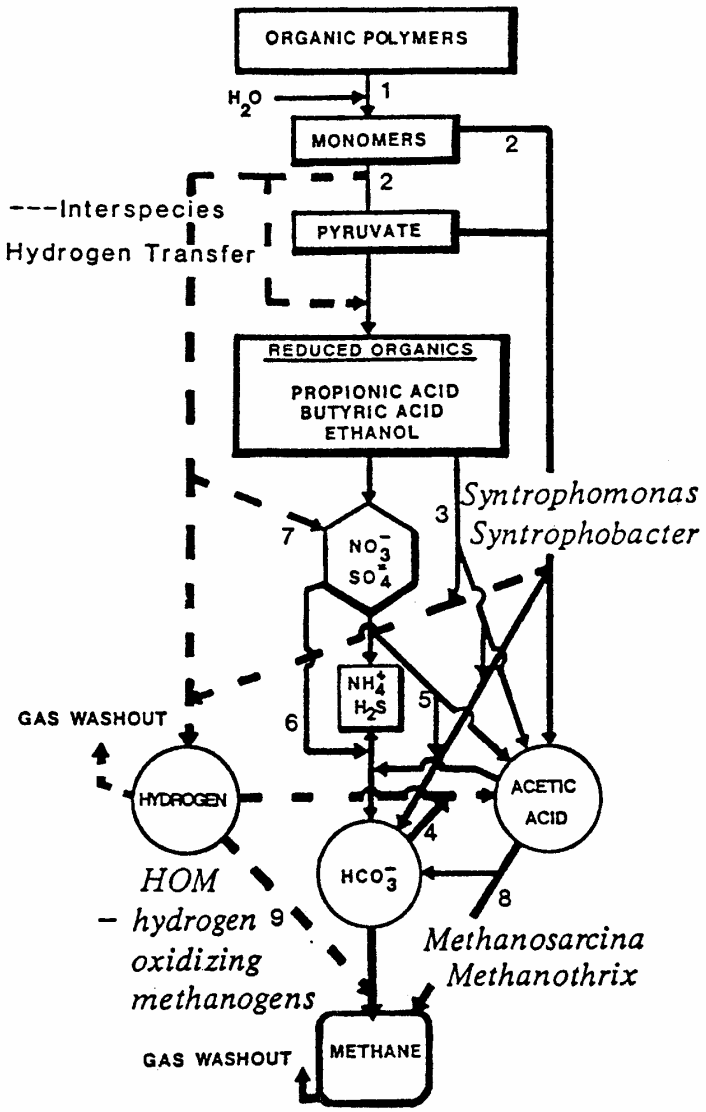

ORGANIC POLYMERS
H_2O
1
MONOMERS
2
2
---Interspecies
Hydrogen Transfer
PYRUVATE
REDUCED ORGANICS
PROPIONIC ACID
BUTYRIC ACID
ETHANOL
3
Syntrophomonas
Syntrophobacter
7
NO_3^-
$SO_4^=$
NH_4^+
H_2S
5
6
GAS WASHOUT
HYDROGEN
ACETIC
ACID
4
HCO_3^-
8
HOM
– hydrogen
oxidizing
methanogens
9
Methanosarcina
Methanothrix
GAS WASHOUT
METHANE

Figure 2.2. Substrate conversion patterns associated with the anaerobic treatment of industrial, municipal, and agricultural wastewaters. Legend: (1) Hydrolysis of organic polymers; (2) Fermentation of organic monomers; (3) Oxidation of propionic and butyric acids and alcohols by OHPA (obligate hydrogen-producing acetogens); (4) Acetogenic respiration of bicarbonate by homoacetogens (HA); (5) Oxidation of propionic and butyric acids and alcohols by nitrate-reducing bacteria (NRB) and sulfate-reducing bacteria (SRB); (6) Oxidation of acetic acid by SRB and NRB; (7) Oxidation of hydrogen by SRB and NRB; (8) Aceticlastic methane fermentation; and (9) Methanogenic respiration of bicarbonate (Malina and Pohland 1992).

Engineered anaerobic consortia are needed to expand the catabolic diversity of sludge and shorten the period of adaptation to recalcitrant and toxic substrates. The specialized microbial consortia can be "biochemically rerouted" by the induction of a desirable biochemical pathway and the repression of an undesirable pathway (Gavrillescu, 2002).

A narrow thermodynamic window must therefore be maintained with respect to the hydrogen concentration in order to permit methane formation but not inhibit propionic acid degradation. This bottleneck can best be prevented by use of a microorganism population particularly active with respect to hydrogen utilization. Measurements of the hydrogen concentration can provide important information on the interaction of the various types of microorganisms and may be used for process control.

A number of contributions report studies of the startup and transition behavior of anaerobic reactors with the aid of mathematical models for the essential degradation step of acid hydrolysis and methane formation.

Most models developed for this reaction type considered the four overall steps of the process (Figure 2.3):

1. Enzyme –mediated hydrolysis of carbohydrates, proteins, and/or lipids, resulting in monomer compounds such as amino acids, sugars, and fatty acids;
2. Fermentation of organic acids (bacterially assisted), through which organic acids (acetic, propionic, and butyric) can be obtained (acidogenic step);
3. Conversion (bacterially assisted) of organic acids containing more than three carbon atoms to acetic acid and hydrogen (acetogenic step);
4. Conversion of acetic acid to methane and reduction of carbon dioxide with hydrogen to methane (methanogenic step);

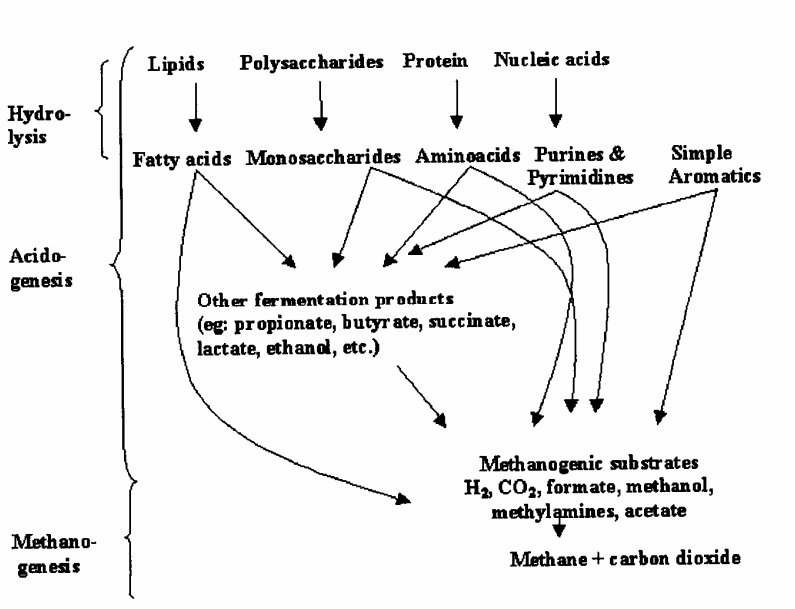


Figure 2.3. Schematic diagram of the patterns of the carbon cycle in anaerobic processes (Gavrillescu, 2002)

The necessity of an interlinkage of sequential degradation steps in the anaerobic process by various microorganisms means that the various steps must proceed at the same speed in order to avoid disturbances. This explains the higher sensitivity of the anaerobic processes to disturbances compared to the aerobic processes.

Anaerobic reactor technology

In the past decades, the number of different anaerobic processes and the range of waste type that can or are being treated via anaerobic processes have been expanded, mainly due to the improvement of the classical reactors and new reactor designs. Also, aerobic treatment, such as activated sludge or trickling filters showed high costs (U.S.$50 per inhabitant equivalent per year), while

treatment costs are halved when anaerobic treatment is used. The anaerobic treatment processes can occur in suspended and attached-growth systems, in anaerobic filters and expanded-bed bioreactors, applied for the treatment of carbonaceous organic wastes, as well as for denitrification.

The more common processes and reactors now in use can be grouped as follows (Figure 2.4):

- Completely mixed anaerobic digestion
- Anaerobic contact processes
- Upflow packed bed
- Downflow packed bed
- Fluidized bed
- Expanded bed
- Upflow anaerobic sludge blanket (UASB)
- UASB incorporating separate settler
- Baffled reactor
- Two-stage leaching separate settler
- Baffled reactor
- Two-stage leaching-bed leachate filter
- Membrane solids separation
- Anaerobic contact coupled with aerobic polishing

And the new reactor:

- Anaerobic sequencing batch reactor (ASBR)

The advantages and disadvantages of the anaerobic treatment of an organic waste, as compared to aerobic treatment (Table 2.1), stem directly from the slow growth rate of the methanogenic bacteria. Slow growth rates require a relatively long detention time in the system to perform an adequate waste stabilization. An extensive study on anaerobic reactions can lead to these disadvantages being overcome by advanced reactor design and control of feed rate.

Because of the low rates of growth and low specific rates of anaerobic organisms, it is important for the design of efficient treatment processes to achieve high biomass concentration and to retain the active biomass within the reactor. Also, the range of waste types that can or is being treated via anaerobic treatment has been expanding due to new reactor desings (Gavrillescu, 2002).

Low -rate anaerobic processes

Anaerobic lagoon. The anaerobic lagoon is the oldest low rate anaerobic treatment process. Due to their limitations, anaerobic lagoons are limited to the digestion of municipal sludge. Some technologies are especially applied on particular waste streams. An example is the low-rate anaerobic pretreatment. This technology is very successful at treating dairy and dairy –type waste – waters that contain high quantities of fat, oil, grease (FOG)**.**

*ADI bulk volume fomenter (BVF) reactor***.** This patented low-rate anaerobic system combines features of the upflow anaerobic sludge blanket and anaerobic contact processes. It seems to be the leading low rate anaerobic technology in the world (Brinkman and Schultz 1994; Paques ADI 2001). This system is well suited to treat difficult industrial wastewaters including pharmaceutical, brewery, distillery, dairy, potato and other food processing wastes. It can also designed to handle streams that are very high in TSS and FOG, such as DAF floats and sludges. Two industries that have benefited from the patented low-rate anaerobic ADI-BVF technology are: a candy bar manufacturing facility in Canada and another successful installation is a proprietary ADI-SBR (sequencing batch reactor) in Virginia USA (Paques ADI 2001).

High-rate anaerobic

The sensitivity and the low growth rate of the anaerobic bacteria were seen as an important risk factor of anaerobic systems. Important factors for high rate anaerobic processes are solids retention, sludge activity, temperature, and reactor design (Gavrillescu, 2002).

Anaerobic contact process. On of the simplest ways to retain biomass is to settle the reactor effluent and recycle the sludge, as in the aerobic activated sludge process. The equivalent anaerobic system is referred as the anaerobic contact process, developed in the 1950s, and was the first high rate anaerobic treatment system. This system is suitable for treating effluents containing a high concentration of suspended solids.

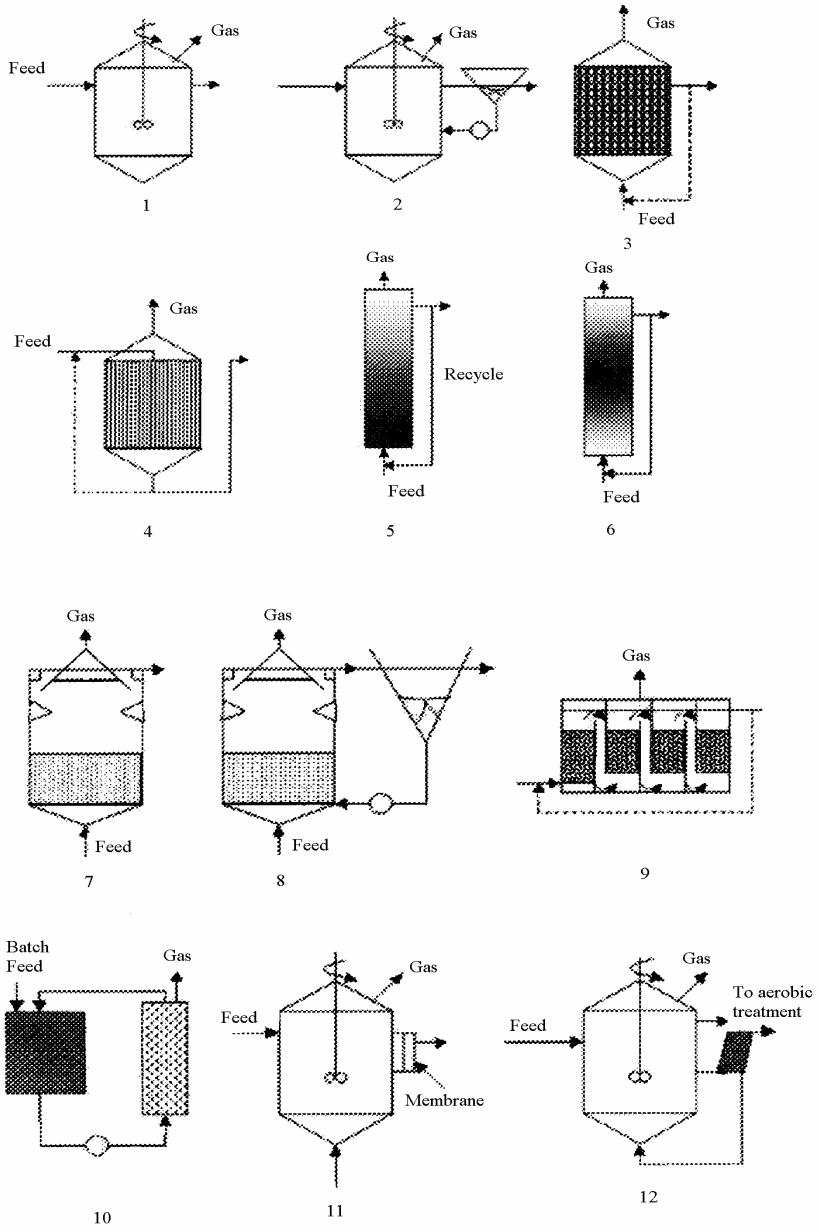


Figure 2.4. Typical process in process for anaerobic waste treatment: 1) Anaerobic digestion, 2) Anaerobic contact process, 3) Upflow packed bed, 4) Downflow packed bed, 5) Fluidized bed, 6) Expanded bed, 7) Upflow anaerobic sludge blanket (UASB), 8) UASB incorporating separate settler, 9) Baffled reactor, 10) Two stage leachate bed-leachate filter, 11) Membrane solids separation, 12) Anaerobic contact coupled with aerobic polishing (Gavrillescu, 2002).

In the anaerobic contact process, untreated wastes are mixed with recycled sludge solids and then processed in a reactor sealed off from the entry of air. After digestion, the mixture is separated in a clarifier and the supernatant is discharged as effluent and sent for further treatment. The settled anaerobic sludge is recycled to seed the incoming wastewater. Because of the low synthesis rate of anaerobic microorganisms, the excess sludge that must be disposed of is minimal. Its efficiency is limited by the difficulty in achieving sludge concentrations in the sedimentation tank, owing to the nature of the anaerobic sludge.

Biobulk is a conventional anaerobic contact process (Biothane Corporation 2001) that is applicable for waste streams containing high strength COD/BOD concentration and FOG concentration greater than 150 mg/l. Biobulk is a " medium loaded " system with volumetric loading of 2-5 kg COD/ m^3 .day.

Upflow anaerobic sludge-blanket process. The treatment of wastewater with a high degree of operational reliability is now in demand all over the world. At the same time, preconditions are becoming more important. These apply to the limitation of sludge yield, energy consumption and space need. Through fundamental and applied research, the following hypothesis was confirmed: as a result of carefully controlled processing conditions, anaerobic bacteria can effectively remove organic compounds from industrial wastewater. In addition, the phenomenon of "granule formation ", which has since become an essential part of the process, was also formulated. This granule formation, the result of the aggregation of anaerobic bacteria, makes possible a high degree of sludge retention, particularly useful in the treatment of industrial wastewater (Gavrillescu, 2002).

In these circumstances, the UASB reactor was invented in the mid-1970s at the University of Wageningen (Lettinga et al. 1979) and was applied at full scale in the Dutch sugar industry. Over several years, it was continually improved in order to treat high amounts of wastewater flow and load with important daily fluctuations. Some operating data and recommendations resulted from the experience, which enables the reactor to handle these very different situations.

The UASB reactor is a high-rate system that operates entirely as a suspended growth system. The high biomass concentration renders UASB more tolerable of toxicants. It is essentially an open column through which liquid waste is passed with a very low upflow velocity. The sludge blanket is composed of biological formed granules or particles and the wastewater, introduced in the bottom of the reactor, flows upwards through them (Biothane corporation 2001). The granular sludge innocula necessary for the startup of a UASB are already commercially available throughout the world. Treatment occurs at the contact with the granules. The gases produced under anaerobic conditions cause internal circulation, which helps in the formation and maintenance of the biological particles, on which some gas adheres. The free gas and the gas released from the granules are retained in the gas collection zone at the reactor top. Liquid containing some residual solids and biological granules passes in to the settling device where the residual solids are separated from the liquid. The solids fall back through the baffle system to the top of the sludge blanket. Proper operation requires the formation of a flocculating biomass, and startup may require inoculation of large amounts of sludge from another operating system (Gavrillescu, 2002).

Anaerobic filter. The anaerobic filter, also known as fixed bed or fixed film, is suitable for the treatment of only effluent containing low concentrations of suspended solids. It is similar to the trickle filter in that a microbial film grows of an inert solid support. This contactor is a column filled with various solid media (Figure 2.2). The waste flows upwards through the column, contacting the media on which anaerobic bacteria grow. The mean cell-residence times are of 100 days. In this bioreactor, a large value of the

detention time can be achieved with short hydraulic retention times. In these conditions the anaerobic filter can be used for the treatment of low-strength waste at ambient temperature. The advantage of this system is that it produces high concentrations of active biomass without the use of a settler. The disadvantage is that plugging may develop with high load conditions or if suspended solids are present. Modification of this reactor have given rise to many other reactor configurations (Gavrillescu, 2002).

Fluidized-bed system. The reactor is a column with fluidized suspended particles, which serve as a support for biomass immobilization (Figure 2.4). The influent flows upward through a medium, usually sand, at a velocity sufficient to keep the medium in suspension. The process usually requires recycling of the effluent from the reactor to keep the medium fluidized. Operating costs are elevated since recycling of effluent inside reactor consumes a large amount of power.

Expanded-bed process. The wastewater to be treated in expanded-bed process (Figure 2.4) is pumped upward through a bed of an appropriate solid support made of sand, coal, or expanded aggregates, on which the biological growth occurs. This expansion has the advantage that channeling is reduced, liquid mixing is improved, and the blockage by the solids is eliminated. Effluent is recycled to dilute the incoming waste and to provide an adequate flow to maintain the bed in an expanded condition. As the quantity of sludge produced in the expanded-bed process is considerably less than that produced in aerobic systems, it is in a greater use for the treatment of municipal wastewater. The usable gases recovered from this process, such as methane, are another important advantage of this anaerobic process.

As an out growth of successful operating experience with UASB technology, an Expanded Granular Sludge Bed (EGSB) process that incorporates the best feature of two technologies: growth of granular sludge and three-phase fluidized bed have been combined resulting in expanded granular sludge bed (EGSB). The ultrahigh loading rates of the fluidized bed process are achieved with granular biomass, while no carrier material is required for start up or operation. This process can therefore be perceived either as an ultrahigh rate UASB or a modified conventional fluidized bed. The process is especially suitable to treat wastewater that contains compounds that are toxic in high concentrations (such as those in chemical industry). It is also possible to operate the reactor as an ultrahigh loaded reactor (to 30 kgCOD/m^3 .day) for application in other sectors of industry (Gavrillescu, 2002).

Control of anaerobic reactors

The primary objectives of control systems are to provide quality assurance and economic incentives. Some experience was gathered on the feedback control on the following variables: pH, Propionic and acetic acid, product gas flowrate, methane and carbon dioxide content in biogas, and dissolved hydrogen concentration.

The last decades saw significant cost reductions and improvements in the speed and reliability of computer hardware and increased availability of software. These factors have resulted in an increase in the use of computers for control and optimization of processes. However, the extent of computer application depends on the development of better on-line instrumentation that can monitor cellular physiology, as well as on reliable mathematical models that can describe various cellular dynamics (Gavrillescu, 2002).

Anaerobic sequencing batch reactor (ASBR)

The process of sequencing batch reactor (SBR) was introduced for the first time by Irvine et al. in 1971 (Wilderer et al., 2001), and then anaerobic SBR (ASBR) as a new high rate anaerobic process (US. Pat. No.5, 185,079) was introduced by Dague, Sung and coworkers (1992), issued to the Iowa State University Research Foundation in February 1993.

Several high rates anaerobic processes have been developed since early 1950s (Dague et al., 1998). The first process was analogous to aerobic activated sludge and was called the " anaerobic contact process" (Fullen et al. 1953). Several other include the anaerobic filter (McCarty, 1966; Young, 1968), the upflow anaerobic sludge blanket (Lettinga et al., 1979), and the fluidized bed process (Switzenbaum and Jewell, 1980) (Sung et al., 1995). Finally, another anaerobic process, the anaerobic sequencing batch (fill-and-draw) reactor (ASBR) was developed at the beginning of the 90s (Kennedy et al., 1991; Suthaker et al., 1991; Dague et al., 1992) and (Kennedy et al., 1991; Sung et al., 1995; and Ruiz et al., 2002).

In the 1960s, Dague (1967) conducted laboratory studies on methods of increasing the microbial population in anaerobic reactors. The studies involved batch feeding, internal solid separation, and supernatant wasting. The process was called "anaerobic activated sludge"(Dague et al., 1966; 1970). This process was capable of achieving long solids retention times (SRT) with relatively short hydraulic retention times (HRT) as a result of bioflocculation and efficient solid separation within the reactor. Although not recognized at the time (1966), what is now called "granulation" of the biomass was occurring (Sung et al., 1995). After that; this process was called ASBR (1992).

Process Description

The SBR process is characterized by a series of process phases (for example fill, react, settle, decant and idle), each lasting for a defined period. Sludge wasting normally take place after settle but can take place near the end of react or during settle and can take place weekly, daily, during each cycle, or longer time for ASBR. The different phases of SBR operation are presented in Figure 2.5. The idle phase can be eliminated when equalization or holding tank or some other method of handling excess inflow is available (Wilderer et al., 2001).

The volume of wastewater introduced into the reactor is ΔV_f. It is added to the volume of water and sludge that remains in the reactor at the end of past cycle (V_0). At the end of fill phase the reactor contains $V_{max} = V_0+\Delta V_f$. Once the reactor phase has been completed and the mixing energy has been dissipated, the sludge starts coagulating and settling. After wasting of excess sludge (ΔV_w) and discharge of the treated supernatant (ΔV_d) the reactor is available to receive a new supply of wastewater. Thus, an SBR process is basically characterized by following set of parameters (Wilderer et al., 2001):

t_i time for the *i*th phase
t_c total time of one cycle ($t_c = \sum t_i$)
FTR fill time ratio, t_f / t_c, where t_f is the time for fill
VER volumetric exchange ratio, $\Delta V_f / V_{max}$
HRT hydraulic residence time, $nV_{max}Q^{-1}$, where n is number of tanks. V_{max} is the total liquid volume of the reactor and Q is the volumetric flow rate of the influent to the treatment plant. For each of the tanks, $HRT_i = t_c\ VER^{-1}$

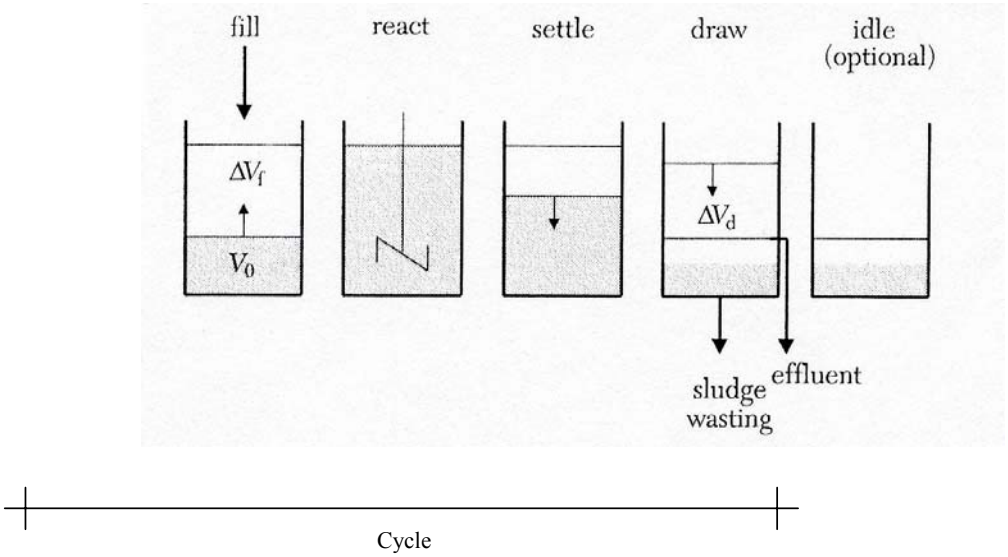


Figure 2.5. Operation Phases following each other during one cycle of the generic SBR process (Wilderer et al., 2001)

In addition, processes parameters apply that are typical for suspended or attached (biofilm) growth. Because the fill phase is usually only a fraction of the cycle time, it is necessary to provide more than one SBR to handle a continuous influx or wastewater if some temporary influent storage is not available.

SBR plants typically consist of a number (n) of biological reactors. A general diagram of an SBR plant is shown in Figure 2.6. The system consists of primary treatment, an influent holding tank (optional), several SBRs, a chlorine contact chamber (required in some countries) and an effluent buffer tank (optional). Independently of the inclusion of a holding tank, the numbers of SBRs available determines the time for fill and total cycle time, the fill time ratio (FTR). An effluent buffer tank can be used to smooth the variations in the effluent flow rate, which might be necessary for large SBR systems discharging into small receiving water bodies.

The number of tanks to be chosen depends on the overall treatment objectives and on the result of a cost analysis. In principle, it can be stated that the flexibility to handle variable influent conditions increases with the number of tanks available. For maintenance reasons at least two SBR tanks should be available at an SBR plant (Wilderer et al., 2001).

Like the aerobic SBR, the ASBR involve repetition of a cycle including four discrete steps: fill (feed), react, settle, and draw off. The cycles should be as frequent as possible while allowing for completion of each of the four stages without any intervening idle time (Ruiz et al., 2002).

The feed (fill) step involves the addition of substrate to the reactor. With continuous mixing during feeding, the substrate concentration increases rapidly, and metabolic rates increase to their highest values. The feed volume is determined on the basis of a number of factors, including the desired HRT, organic loading, and expected settling characteristics of the sludge (Sung et al., 1995).

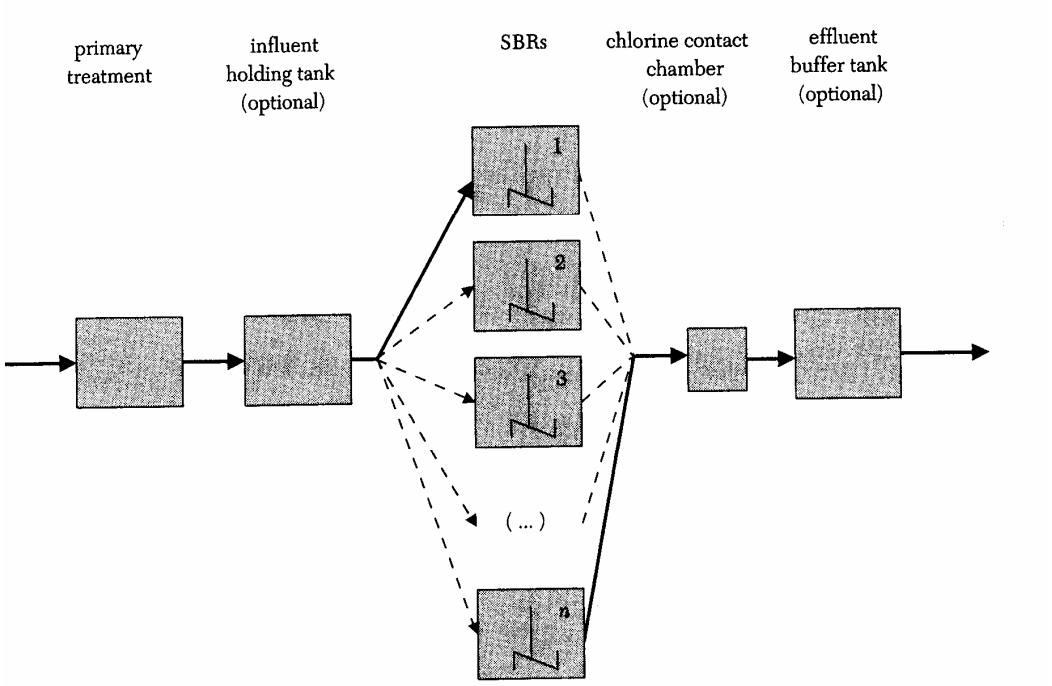


Figure 2.6. Diagram of an SBR plant (Wilderer et al., 2001)

The react step is most important in the conversion of organic substrate to biogas. The time required for the react step depends on several parameters, including substrate characteristics and strength, required effluent quality, biomass concentration, and waste temperature (Sung et al., 1995).

During the settle step, mixing is shut off to allow biomass-solids separation. The reactor itself acts as the clarifier. The time required for clarification will vary, depending on biomass settleability, but typically ranges from 10 to 30 minutes. The concentration of mixed liquor suspended solids (MLSS) in the reactor is an important variable affecting the settling velocity of the

biomass and also the ability to achieve a clear supernatant for discharge as effluent. An important related variable is the specific process-loading rate (food: microorganism ratio, F: M).

The decant step takes place after sufficient solids separation has occurred. The decant volume is normally equal to the volume fed during the previous feed step. The time required for the decant step is governed by the total volume to be decanted during each cycle and the decanting rate. Once the decant step is completed, the reactor is ready to be fed another batch of substrate. Then the influent is introduced to the reactor at the highest possible rate, and mixing is begun. No discharge from the reactor occurs until the react and settling cycles have been completed. Of course, to accomplish intermittent feeding and decanting, the reactor must be equipped with a flexible membrane or rigid floating cover (Sung et al., 1995).

In ASBR, no liquid or solid recycle is involved. In addition, batch feeding offers some significant kinetic advantages over continuous-flow processes. The altering feast and famine conditions in the reactor result in high rates of substrate removal during the react the react phase but also result in low levels of intermediate soluble organics in the reactor decant. The ASBR provides a competitive advantage to methanogens that are capable of growing at low-volatile fatty acids (VFA) concentrations. This is a possible explanation for the low concentrations of VFA observed in the ASBR effluent (Sung et al., 1995).

Design parameters. The simple expressions to calculate main design parameters such as: organic loading rate (OLR), g COD/l.d; sludge loading rate (SLR or F/M), g COD/g VSS.d; hydraulic retention time (HRT), day; and soiled retention time (SRT), day; are defined as below:

$$OLR = \frac{(\text{Cycle/day; n})\,(\text{Fill Time; } t_{fill})(\text{Influent Flow rate; } Q_{fill})\,(\text{Influent COD; } COD_{in})}{\text{Volume of Reactor}}$$

$$SLR = \frac{(\text{Cycle/day; n})\,(\text{Fill Time; } t_{fill})(\text{Influent Flow rate; } Q_{fill})\,(\text{Influent COD; } COD_{in})}{\text{Total VSS of Reactor}}$$

$$HRT = \frac{\text{Reactor Volume}}{(\text{Volume decanted per cycle})\,(\text{Cycle per day})}$$

$$(\text{VSS concentration in reactor})\,(\text{Reactor Volume})$$

$$SRT = \frac{}{(\text{Effluent VSS})(\text{Volume decanted per cycle})(\text{Cycle per day})}$$

Organic loading rate in ASBR. One possible disadvantage of the ASBR, is loading. The highest reported loading treatable by ASBRs receiving sucrose was only 19 kg COD/ m^3.d, much less than the 100 kg COD/m^3.d reported for an upflow anaerobic sludge blanket (UASB) reactor fed milk powder and sucrose. Although different substrate will lead to loading differences, the operational strategy of the ASBR may also inadvertently depress the maximum achievable loading (Shizas et al., 2002).

However, granulation as an important factor in the ability of UASB process to achieve high organic loadings (Lettinga and Hulshoff Pol, 1991) is also true of the ASBR (Sung et al., 1995).

The start-up of ASBR systems, like other high-rate anaerobic processes, generally takes a long time. However, ASBRs seeded with granules require less time for start-up, compared to reactors started with flocculent seed (e.g., biomass from a conventional anaerobic digester) (Banik et al., 1997).

Specific loading rates(F/M) in ASBR. The reported typical values of specific loading rates for ASBR work range from 0.11-0.28 kg COD/kg VSS.d up to 0.47-0.63 kg COD/kg VSS.d. Shizas et al. (2002) operated the ASBR with specific loading rates in range of 0.27 kg COD/kg VSS.d.

One of the key characteristics of an ASBR process is that the food-to-microorganism (F/M) ratio is at its highest-level right at the end of feed phase and then declines thereafter. This provides a high driving force for methabolic activity and high overall rates of waste conversion to biogas. Near the end of react, substrate concentration is at its lowest level, providing ideal conditions (low F/M ratio and hence low gasification) for biomass flocculation and separation during the settle phase (Ong et al., 2002).

Intermittent mixing instead of continuous mixing in ASBR. Sung et al. (1995) in their laboratory studies on the ASBR found that intermittent mixing such as 5 min/hr; 2.5 min/30 min; and 100sec/20 min; is preferable to continuous mixing from both COD removal and methane production standpoints.

The results of another study on mixing intensity on performance and microbial community structure in ASBR by Angenet et al. (2001) indicated that continuous mixing is not necessary for digestion of animal wastes. The intermittent mixing was performed by biogas recycling: 1 min of biogas recycling every hour at a flowrate of 26 liter/hr.

Comparison between continuous flow and SBR systems

The advantages of SBRs over continuous flow systems, cited in the literature, include operational flexibility, flow equalization, non-ideal settling, simple operation, and compact layout of system (Kennedy, 1991).

The application of SBR technology, to anaerobic treatment is of interest because of its inherent operational flexibility, including a high degree of process flexibility in terms of cycle time and sequence and no separate clarifiers required (Kennedy et al., 1991; and Ruiz et al., 2002).

As another attractive advantage of ASBR over current anaerobic technologies, because the reactor is batch-fed, there is no short-circuiting and there is no need for an extensive feed distribution system in the bottom of the reactor, as for the UASB reactor and upflow anaerobic biofilters (Sung et al., 1995).

Due to the ease of instrumentation and control, these reactors can be employed in fundamental research in order to elucidate certain aspects of anaerobic digestion. In this way, knowledge of the optimal condition can be applied on an industrial scale without need of complex instrumentation and control (Ruiz et al., 2002).

Depending on the mode of operation, an SBR system can be compared with a plug flow reactor (PFR) or a completely mixed flow reactor (CMFR). Dump fill, the instantaneous addition of wastewater into the reactor, is rarely, if ever, used in the field but is implemented practically by including a static fill in the operating strategy. In Figure 2.7., two SBR cycles are shown, each with its corresponding continuous flow system. The SBR with dump fill is the same as that for the plug flow reactor at steady state, where the hydraulic residence time
or space time in the PFR compares to ‘clock’ time in a completely mixed batch reactor (CMBR) (in other words the time for react in the SBR) (Wilderer et al., 2001).

The primary difference between the PFR and the SBR during react is that the equivalent to true plug flow conditions can be established in an SBR but cannot be achieved in a single activated sludge tank because of the dispersion resulting from the aeration system. However, a cascade of CMFRs consisting of three or four tanks in series can be considered as a suitable approximation.

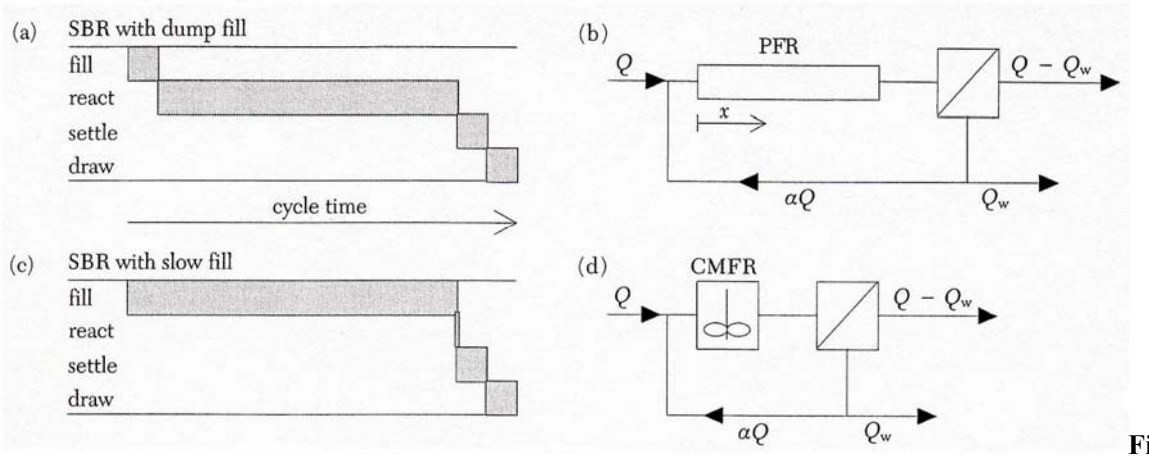


Figure 2.7. SBRs with dump and slow fill are comparable to a PFR and a completely mixed flow reactor (CMFR) (Wilderer et al., 2001)

During the past decade, SBR technology has received worldwide attention. Several thousands SBR facilities have been designed, built and put into operation. In most cases the SBRs worked well; in some cases, however, the operators' lack of understanding and experience resulted in a facility' failure to meet the required effluent standards.

SBR technology has reached an advanced state of development. However, in comparison with continuous flow systems, the knowledge base for SBR performance during practical situations remains limited (Wilderer et al., 2001). .

Microbial ecology and population dynamics

The term "ecology" applies to interdependences that occur between environmental factors and the microbial community. In a biological wastewater treatment plant, process conditions prevailing in the biological reactor are the major environmental factors and are described by parameters that include the hydraulic retention time, solid retention time or sludge age, substrate, co-substrate, oxygen, pH, temperature and salinity. Although treatment facilities can be engineered to control most of these parameters, factors such as temperature can be subjected to only limited control.

The art of biological wastewater engineering, involves the development of treatment system that controls process conditions, selects for the most suitable microbial community, and achieves the desired treatment both reliably and economically. The physiological state of the microbial consortium selected must have a collective metabolic activity that meets these goals while producing a suitable biomass structure (e.g. floc or granule) that permits easy separation from the treated effluent (Wilderer et al., 2001).

Seeding ASBR with granular sludge instead of flocculent sludge. The start up of ASBR systems, like other high rate anaerobic processes, generally takes a long time. However, ASBRs seeded with mature granules require less time for start-up, compared to reactors started with flocculent seed (e.g., biomass from a conventional anaerobic digester). The phenomenon of granulation is the process in which a nondiscrete flocculent biomass forms discrete well-defined pellets, or granules. Granular biomass has several important advantages over flocculent biomass, two of which are that granular biomass has a higher settling velocity than flocculent biomass, which enables it to be retained in a reactor more efficiently, and granular biomass has a higher specific activity than flocculent biomass, which enables it to biodegrade waste at higher rate (Wirtz and Dague, 1996).

The first advantage of granular biomass is easily observed, measured, and understood. Discrete large particles of given density have higher settling velocities than do flocculent small particles. The obvious advantage of faster settling biosolids is that they are more easily retained within the treatment unit, and therefore, fewer solids are lost and a higher degree of treatment can be attained.

The higher activity of granular biomass, although readily measured, is not so readily understood. The following explanation has been put forth by several authors. Anaerobic degradation of complex organic compounds to methane and carbon dioxide, in its most basic form, is a three-step process involving at least four groups of anaerobic bacteria. The four bacterial groups involved are responsible for: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The interaction of all four groups of bacteria is essential for efficient anaerobic digestion. Especially important is the maintenance of low H_2 partial pressures. Thermodynamically, oxidation of longer chained volatile fatty acids to acetate by acetogenic bacteria is impossible except at H_2 partial pressures below approximately 10^{-5} atm. This implies that methanogenesis from CO_2 and H_2 is a necessary requirement for efficient acetogenesis. A close spatial association between acetogenic and methanogenic bacteria, therefore would seem beneficial to the overall digestion process in that H_2 and other intermediates could be efficiently transferred among the respective bacterial groups. This is precisely the benefit that granular biomass is theorized to provide. Although, the transfer of H_2 and other intermediates also occurs in flocculent biomass systems, the distance that each intermediates product must travel is minimized in a system in which the bacteria are fixed in a position close to other bacteria, such as occurs within each individual granules (Wirtz and Dague, 1996).

Several mechanisms could be involved in the formation of different types of granules depending on waste stream, reactor configuration, and operating conditions. Granule formation and retention is generally thought to be result of environmental pressure

or selection, i.e., any non-granular material is washed out of the reactor. Other mechanisms, however, have been suggested as being associated with the granulation process and subsequently with granule structure and stability. The granulation process may be inhibited by bacterial adhesion to inert matter or inorganic precipitants. Bacterial agglomeration may also result from physiochemical interactions.

It has been suggested that filamentous bacterium *Methanotrix* plays an important role in binding granule components of nuclei together. These loosely adhered bacterial aggregates are strengthened by extracellular polymers secreted by bacteria to form firmly attached granules (Banik et al., 1997).

Enhancement of granulation and Start-up in ASBR. Anaerobic treatment systems in spite of several advantages than aerobic systems, have not gained widespread acceptance, perhaps because of the long start-up times typically associated with high-rate anaerobic treatment systems. For a typical high-rate application, the time required to reach full-design COD and hydraulic loading rates may take as long as several months to more than a year. This can be a major deterrent to the use of anaerobic treatment systems because industry generally prefers immediate responses to their problems and may not be willing to wait for a long period of time for an anaerobic system to start treating their wastewater at the rate it was intended.

Wirtz and Dague (1996) in their research addressed this concern. By adding a cationic polymer to the ASBR, the start-up period was reduced from 4 or 5 months to 1 or 2 months. GAC had similar, although somewhat less significant, effect on reducing start-up times. PAC had a beneficial effect, but probably not significant enough to warrant the extra cost of purchasing PAC for enhancing start-up.

Attachment matrices, including PAC, GAC, and garnet were added to separate ASBRs immediately after the respective ASBRs were seeded. In other experiments, coagulants, including cationic polymer, polyquanternary amine polymer, and ferric chloride, were added once per cycle throughout the first several weeks after start-up. Coagulants were added to the ASBR during the final mixing phase, just before the settling phase.

It was hypothesized that the attachment matrices would serve as a growth surface for the anaerobic bacteria, thereby artificially producing granules, and that the coagulants would enhance flocculation of the biomass during the critical start-up period, resulting in granulation more quickly than could otherwise be obtained.

The cationic polymer had the most beneficial effects on granulation enhancement, reducing the time required to form granules by approximately 75% compared to an unenhanced control ASBR (Wirtz and Dague, 1996).

Methanogenic population in ASBR. Angenet et al. (2002) followed changes in methanogenic population levels in base of 16S ribosomal RNA (rRNA) during startup of a full scale, farm-based ASBR. They linked these changes to operational and performance data. Their results indicated, it is likely that during operation with high ammonia levels, the major route of methane production is through a syntrophic relationship between acetate-oxidizing bacteria and hydrogen-utilizing methanogens.

ASBR treating different substrates and wastewaters. The literature review on ASBR indicated a broad performance of this process. The ASBR treating different substrates and wastewaters are included: dairy, slaughterhouse and winery effluents (Ruiz et al. 2001&2002); swine wastes (Zhang et al. 1997); cheese whey and dairy manure (Lo et al. 1989); landfill leachate (Timur et al. 1999); digestion of municipal sludge and night soil (Change et al. 1994; Lee et al. 2001).

ASBR with different operational conditions. Other researches suggested applications of ASBR with different operational conditions, such as: termophilic versus mesophilic and also psychrophilic operation (Banik et al. 1997); temperature phased operation (Welper et al, 1997); modifying fill time to cycle time (F/C) ratio (Shizas et al. 2002), and effects of HRT with low strength wastewater (Ndon et al. 1997).

Pharmaceutical wastewaters

Introduction. Increased industrial activity, over the past 40-50 years, has resulted in the generation of increasing quantities of wastes and wastewaters containing high levels of organic pollutants. Production processes used in the pharmaceutical/fine chemical, cosmetic, textile, rubber and other industries give rise to wastewaters containing significant levels of aliphatic organic solvents (Henry et al., 1996).

Wastewaters from pharmaceutical plants are characterized by a high chemical oxygen demand (COD) and variable concentrations of salts. Pharmaceutical manufacturers generate wastewaters containing a variety of organic and inorganic constituents but the most important ones are priority pollutants such as methylen chloride, most of which are generated during chemical synthesis operation. A large portion of the COD may be made up of relatively biodegradable solvents, but they also contain specific compounds that are difficult to biodegrade or inhibitory to biological systems (Kasapgil Ince et al., 2002).

A Pharmaceutical/fine chemical wastewaters present difficult substrates for biological treatment due to their varying content of a wide range of organic chemicals, both natural and xenophobic, which may not be readily metabolized by the microbial associations present in the bioreactors. Recent investigations of the biochemistry, genetics and thermodynamics of bacterial catabolism have considerably improved our understanding of microbial biodegradation processes. This has facilitated the successful full-scale biological treatment of pharmaceutical/fine chemical wastewaters (Henry et al., 1996).

The literature review on used processes for evaluation of treatability of pharmaceutical wastewater indicated anaerobic and aerobic processes have both been used.

Aerobic treatment of pharmaceutical wastes. Aerobic treatment systems have traditionally been employed, including activated sludge systems, with or without the addition of granular activated carbon, or natural or genetically- engineered microorganisms, and aerobic fixed growth systems, such as trickling filters, rotating biological contactors (RBC) (Henry et al., 1996), and aerobic sequencing batch reactors (SBR). The most popular aerobic systems employed for treating pharmaceutical wastewaters are activated sludge systems (Kasapgil Ince et al., 2002).

Hu, et al (1986) have studied the kinetics of continuous flow completely mixed activated sludge process for biodegradability of pharmaceutical wastewater with mixture of three antibiotic: erythromycin (COD=39-48 g/l), furazolidone (COD=23-28 g/l) and

furadantin (COD=149-187 g/l) with ratio 1:0.53:0.14 and diluted COD 500-1000 mg/l. From the experiment, the kinetic constants for these three antibiotic at 20 °c has been achieved as: V_{max}=1.5 d^{-1}, K_s=35.2 mg/l, Y=0.368, A_d=0.054 d^{-1}.

In the past few years the treatment of wastewaters by a modified activated sludge process, the sequencing batch reactor (SBR), has gained recognition. Of interest has been the use of the system to treat wastewaters containing toxic substances. The biomass in the reactors adapted to the wastewater and successfully provided treatment. In part, the rapid adoption of the biomass could be due to the population selection pressures brought to bear on the biomass as a result of the manner in which an SBR was operated. The development of the system along the lines of SBR concept seemed likely to produce a viable alternative to the existing treatment of the pharmaceutical wastewater (Ng et al. 1989).

SBR treating Pharmaceutical Wastewater. Ng et al. (1989) investigated biological treatment of pharmaceutical wastewater including 2-ethylhexanoic acid (2-EHA), methylen dichloride, triethylamine, methyl isobutyl ketone, and aceton by two stage aerobic SBR. They initially thought that the 2-EHA present in the wastewater at the concentration of 2500 mg/l might prove inhibitory to the biological process and adversely affect treatment performance. The batch process removed the 2-EHA to non-detectable levels. The system consisted of two reactors operated in a batchwise mode with the effluent of reactor A becoming the feed for reactor B. During a cycle of operation each reactor served, in turn, as the aeration basin and then clarifier. Reactor A was supplied with sufficient air to maintain good mixing of the reactor's mixed liquor. Attempts were made to keep the dissolved oxygen (DO) below 0.3 mg/l, and at hydraulic retention times (HRT) below 6.6 days the DO was 0 mg/l. Reactor B was operated under aerobic conditions during the react phase but DO levels dropped to 0 mg/l during settle when the reactor was most heavily loaded. Results indicated that a wastewater with a chemical oxygen demand (COD) and 5-day biological oxygen demand (BOD_5) of about 26,500 mg/l and 12,500 mg/l, respectively could be treated with the system. COD removal by reactor A ranged from 96 to 63% while system COD removal ranged from 99 to 86% as various loading conditions were investigated. It was initially thought that the 2-ethylhexanoic acid present in the wastewater at a concentration of 2500 mg/l might prove inhibitory to the biological process and adversely affect treatment performance. The system, however, adapted well and the 2-ethythexanoic acid was effectively removed. Gas chromatographic analysis of reactor A's effluent suggested the occurrence of fermentation. Ethanoic, propanoic, butanoic and, on one occasion, pentanoic acids were detected. These were originally absent in the wastewater. The results suggested that the system might be further developed on the lines of the sequencing batch reactor (SBR) concept. In comparison to the conventional effluent treatment plant configuration comprising of trickling biofilters, activated sludge basins, secondary clarifiers and sludge return, the two-stage batch system would probably be simpler in terms of construction, operation and maintenance.

Anaerobic treatment of pharmaceutical wastewaters. Anaerobic systems utilized for pharmaceutical wastewater treatment include membrane reactors, continuously stirred tank reactors, upflow filters, and fluidized bed reactors (Henry et al., 1996). The selection of the most suitable equipment to be employed in the anaerobic treatment of a particular substrate strongly depends on the substrate nature and consequently on the limiting steps of the process (Zabranska et al., 1994). The treatment of high strength soluble organic wastewater by anaerobic processes has recently attracted considerable attention, as potentially high methane yields may be obtained. The advanced high rate anaerobic treatment systems provide more efficient and economic treatment than conventional aerobic biological processes, especially where highly concentrated organic wastes are involved. Anaerobic systems are not subject to oxygen transfer limitations and their production of biological solids is low (Stronach et al., 1987).

Application of anaerobic treatment to pharmaceutical/fine chemical wastewaters has been hindered by the lack of knowledge of both the biodegradability and the potential toxicity of the constituents of these wastewaters under anaerobic conditions. Most of the published work has focused on the biodegradation and toxicity of halogenated aromatic and aliphatic compounds of both natural and xenobiotic origin. By contrast, little has been published on the amenability of aliphatic solvents to anaerobic digestion. The aliphatic solvents constitute a significant proportion of the BOD/COD content of pharmaceutical plant effluents. Organic solvents are flammable, malodorous and potentially toxic to aquatic organisms and thus require complete elimination by wastewater treatment systems (Henry et al., 1996).

Anaerobic Fluidized bed treating Pharmaceutical Wastes. An example of high-rate anaerobic systems is the anaerobic fluidized bed reactor. This unit provides a large surface area for microbial growth as attached biofilm whilst the recycle mode of operation ameliorates the effects of toxic waste components by providing a dilution factor. This ensures short exposure times to damaging substances, as well as allowing efficient substrate breakdown.

Organic wastes with high oxygen demand are generated by a large number of industries. Many waste streams, however, especially those from chemical or related manufacturing operations, can contain varying levels of potentially toxic materials, which may inhibit the anaerobic digestion process. In some instances the effects of introducing such a stream into a biological system of unacclimated microorganisms may be highly deleterious, even if the waste is known to be biodegradable. However, with acclimation, the toxic effects may be greatly reduced or eliminated (Stronach et al., 1987).

The occurrence of toxic waste compounds is a common feature of the pharmaceutical industry and many hazardous aqueous streams produced must be removed in drums for off-site disposal. The processing of organic pharmaceutical wastes using biological systems may prove to be an effective and economical alternative, if microbial population can be successfully acclimated

to particular effluents. Pharmaceutical wastes have been successfully treated using the anaerobic filter reactor and acclimation to particular organic compounds has been demonstrated.

The purpose of Stronach et al. (1987) in their study was to apply two pharmaceutical wastes from therapeutic chemicals manufacture to anaerobic fluidized bed reactors as part of the influent feed, at gradually increasing proportion, in order to evaluate: (a) the potential of the wastes for degradation by anaerobic fluidized bed treatment without nutrient supplementation; and (b) the effects on microbial performance of two highly concentrated organic pharmaceutical waste streams. The principal constituent of the first waste was n – propanol, which can be anaerobically degraded after acclimation, while the second contained 30% dimethylformamide (DMF), a potentially carcinogenic solvent that has been reported to degrade aerobically.

The acclimation of anaerobic fluidized beds to two pharmaceutical wastes gradually introduced with the influent feed was applied at a COD concentration of 2500 mg/l, at an organic loading rate of 4.5 kg COD/m^3.d and with an HRT of 0.53d, and operation continued until the feed comprised 100% industrial waste. Final COD removals reached 54% and 45% for the propanol and the dimethylformamide containing wastes respectively, without nutrient or trace element supplementation. Analytical results suggested that propanol was nutrient limited and caused inhibition of methanogenesis, whereas dimethylformamide appeared to contain a non-biodegradable or toxic fraction which did not inhibit methanogenesis but caused a reduction in COD removal and erratic volatile acids production.

At inputs propanol, biomass was not lost but the acids produced in the system increased and CH_4 production decreased, probably as a result of depressed methanogenic activity. The dimethylformamide contained a fraction, which appeared to be non-biodegradable or toxic under the test conditions used, but the CH_4 generated exceeded that of the control, indicating that the substrate was amenable to methanogenic metabolism (Stronach et al., 1987).

Anaerobic Hybrid Reactor treating pharmaceutical wastewater. The anaerobic hybrid reactor (AHR) combine the advantages of both fixed bed and sludge blanket reactors in having a support matrix restricted to the upper third or quarter of the reactor bed and a matrix free section underneath within which a granular or flocculant sludge bed may be developed. Henry et al. (1996) evaluated the performance of anaerobic hybrid reactors (AHR) treating an organic solvent–containing synthetic pharmaceutical wastewater under various wastewater volumetric loading rates and influent compositional changes. The biodegradation, toxicity and treatability of the target C_3 and C_4 solvents, *tert*-butanol, isopropanol, isobutanol, *sec*-butanol and ethyl acetate, were examined. Anaerobic hybrid reactors started up on C_1 and C_2 alcohols and VFA adapted readily (within 11 days) to the successive introduction of a range of C_3 and C_4 aliphatic solvents characteristic of many pharmaceutical wastewaters. At a hydraulic retention time (HRT) of 2 days and volumetric loading rates ranging from 3.5 to 4.5 kg COD/m^3.d, the reactors achieved total and soluble COD removal

efficiencies of 97-99% in less than five times the HRT. These removal rates were achieved following the introduction of target solvents not previously supplied to the reactors: However, inadequate removal of *tert*-butanol resulted in a decrease in the soluble COD removal efficiency to 58%. Thus, of the solvents tested, only *tert*–butanol was shown to be recalcitrant to anaerobic digestion and bacterial enrichments from the reactor biomass using *tert*-butanol as the sole substrate proved unsuccessful, confirming that *tert*-butanol is poorly degradable anaerobically. Aerobic post-treatment resulted in complete removal of *tert*-butanol, highlighting the advantages of aerobic polishing of anaerobically treated pharmaceutical wastewaters prior to discharge. The inclusion of a trace metal cocktail in the feed did not affect steady state reactor performance at the loading rates tested, although it appeared to decrease the time needed to adapt to influent compositional changes. The AHRs exhibited very stable performance with the feed mixtures used and at the loading rates applied (1.0-4.5 kg COD/m^3.d) for the duration of the trial (405 days). After 405 days of operation, the matrix–associated biomass contributed only a minor fraction (2-4%) of the total biomass present in both reactors. On takedown, the retained biomass present in the matrix–free section of both reactors was found to be granular in nature, despite the omission of trace elements from the influent to one of the AHRs. The specific methanogenic activity profile of the granular sludge from the trace element limited AHR was, however, significantly lower ($\alpha = 0.05$) than that of the reference AHR.

Shedding of biofilm in anaerobic biofilter treating 2-ethylhexanoic acid. Although 2-ethythexanoic acid was effectively removed by aerobic SBR process, this acid, however, was caused shedding of the biofilm in the biofilters of the existing plant and leakage of the acid in the effluent (Yap et al., 1992).

*UAF treating pharmaceutical wastewater***.** Kasapgil Ince et al. (2002) investigated the treatment of chemical synthesis-based pharmaceutical industry wastewater by an upflow anaerobic filter (UAF). The UAF had a maximum 70% COD removal efficiency at an organic loading rate of approximately 7.5 kg COD/m^3.d with a methane yield of 0.20 dm^3 CH_4/g $COD_{removed}$. This process yields raw materials used in the production of various pharmaceutical products including bacampicilline and sultamicilline tosylate. These production processes generate several different waste streams from process steps such as chemical reaction, extraction, crystallization, filtration, quality control, packaging and solvent recovery. Examples of the main effluent compounds produced from these steps are *n*-butyl acetate, dimethyl formamide, isopropyl alcohol, ethyl acetate and methylene chloride. Accumulation of biomass on the media was not operated to be sufficient because the UAF was not operated at high shear stresses, e.g. maximum upflow velocity of 12 m/day.

Methanogenic experiments using iso-propanol as substrate. Iso-propanol is a used solvent and easily found in an industrial effluent. The presence of organic solvents is undesirable in the sewerage system and so must be removed from the industrial effluent. Anaerobic treatment of many of these organic solvents is possible, in which the organic material is converted ~90% to volatile substances carbon dioxide and methane gas- and ~10% to new bacterial cells (solids). Industry will be using less water in

the future. Increased water charges will lead to more precise control and integrated processes will reduce wastage. The smaller volumes of more concentrated waste will be ideal for anaerobic digestion.

In order to evaluate the optimum conditions for the anaerobic digestion of propan-2-ol (*iso*-propanol) the kinetic parameters of the Monod rate model, namely, maximum growth rate (μ_m), yield (Y), half velocity constant (K_s) and endogenous decay coefficient (K_d) were determined at the temperature range 25-40°C , inclusively. The regulatory role of molecular hydrogen was investigated, and also its possible use as a monitor feature in the anaerobic digestion (Terzis, 1994).

Herbal-based pharmaceutical wastewater treatment using fixed film reactor. Herbal pharmaceutical wastewater distinguishes itself because of its high content of organic pollutant and its high acidic nature. The anaerobic fixed-film fixed-bed reactor with random support is an appropriate option for pre-treatment of such wastewater. It provides efficient organic removal efficiencies, even when operated at high loading rates and under intermittent operation (Nandy and Kaul, 2001).

Specific Methanogenic Activity (SMA) test

Maintenance of sufficient methanogenic population is critical for stable performance of anaerobic systems. The usually monitored parameters like COD removal, VFA levels, quantity and composition of biogas produced ect. do not reflect the composition of biomass under varied operational /environmental conditions. The change in biomass composition in terms of population levels of methanogens has been indirectly assessed through methanogenic activity tests.

Several anaerobic process variants having specific biomass retention mechanisms are available for field application. Laboratory pilot- and full-scale studies have made varied claims regarding applicability and performance of these process variants. Maintenance of sufficient methanogenic population in the system is critical for stable performance. Methanogenic species types and their relative population levels in reactor biomass depend on wastewater characteristics as well as operational/ environmental conditions maintained. Any imposed stress (intentional or otherwise) may lead to a change in species types and their relative population levels which is ultimately reflected in the reactor performance. The reactor performance is usually evaluated in terms of process efficiency and stability through estimation of organic matter removal, VFA levels, quantity and composition of biogas produced, etc. However, little effort has been made to assess reactor biomass in terms of relative population levels of methanogenic species under varied operational environmental conditions.

Several investigators have made counts of methanogens and non-methanogens in reactor biomass. These efforts led to the development of well-established laboratory techniques. However, these techniques require a high level of skill, advanced equipment, and costly and specific growth media, which restrict its application at the plant site. SMA test on anaerobic sludge

(biomass) have been gaining importance. Initially, these tests were mainly used to select sludge as inoculum but now these tests can also be used for many other purposes such as to:

- Evaluate the behavior of sludge under the effect of potentially inhibitory compounds;
- Establish the degree of degradability of various substances;
- Follow the changes in sludge activities due to a possible buildup of inert materials;
- Estimate maximum applicable loading rate to a certain sludge;
- Evaluate batch kinetic parameters, etc.

A number of methods have been proposed for the estimation of maximum methanogenic activity. Some of these methods are quite simple but the simple volume needed is too high (500ml or larger). Several solutions were proposed to reduce the working volume and to automate the monitoring process. Very small working volumes (30 to 125 ml) lead to smaller amounts of methane gas production. It necessitates careful measurements of methane gas with sophisticated techniques and hence its application at the plant site becomes even more restricted (Jawed and Tare, 1999).

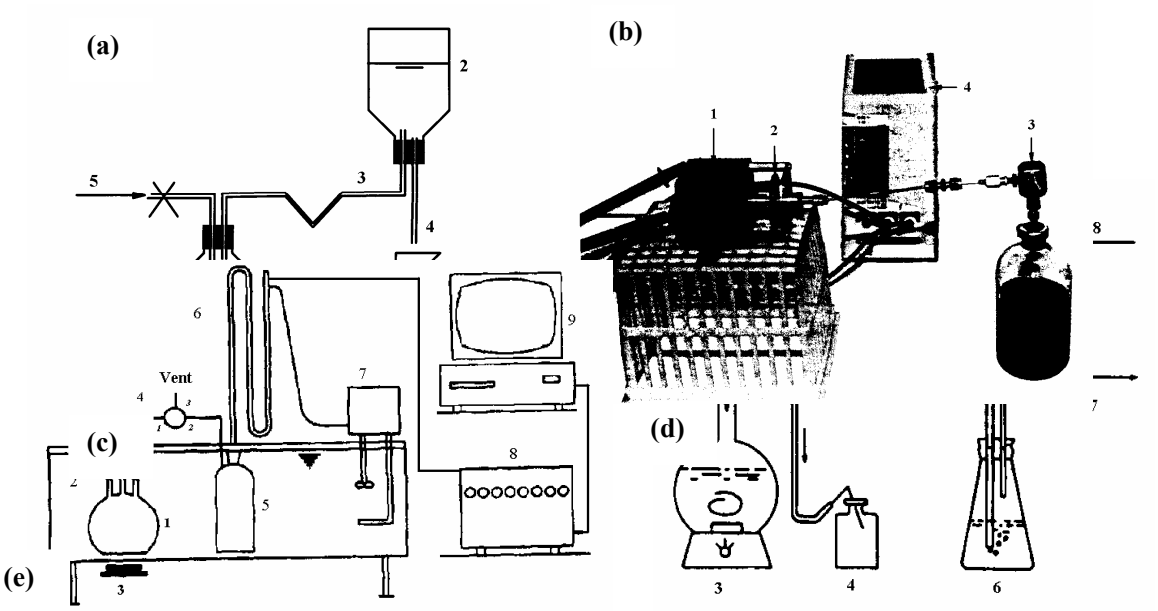


Figure 2.8. Schematics of several simple and instrumental SMA set-up:

(a) 1- Bottle I: wastewater + sludge, 2- Bottle II: alkaline or acid solution, 3- Biogas, 4- Solution, 5- Methane or biogas before test (Van-Haandel and Lettinga, 1994);
(b) 1- Pressure transducer, 2- P-8 adapter, 3) Hamilton three-way valve, 4) Multimeter (Shelton and Tiedje 1984);
(c) 1- Digestion flask (V=1000 ml), 2- Port with septum for gas sampling, 3- Magnetic stirrer, 4- Three-way solenoid valve, 5- Gas bulb (V= 80 ml), 6- Manometer with electric contact points, 7- Water batch heater, 8- Solenoid valve controller board, 9- Micro Computer with data acquisition interface (Ince et al., 1995; and Kasapgil Ince et al., 2002);
(d) 1- Graduated open-tipped 100 ml volumetric pipet, 2- Defined media, 3- Magnetic stirrer, 4- Serum bottle, 5- Flushing needles, 6- Water seal, 7- Suction, 8- 70% N_2 + 30% CO_2 (EPA, 1998);
(e) Rotary cell culture device used in SMA test (Lay et al. 1998);

Proposed SMAs Set-up. Of the proposed methods for design SMA test, simple methods use displacement liquid to measure methane production. The experimental setup of such an anaerobic biodegradability test by Van-Haandel and Lettinga (1994) is shown in Figure 2.8.a. Jawed and Tare (1999) have almost used the same SMA set-up. Shelton and Tiedje (1984) and Coates et al. (1996) measured total gas production in SMA test by a pressure transducer equipped with a P-8 adapter (bellows) capable of measuring up to 8 lb/in^2 of gas pressure (Figure 2.8b). The needle **was** inserted through the stoppers of the serum bottles and the signal (in milliohms) from the transducer was quantified with a Fluke Multimeter. Serum bottles were shaken vigorously before pressure measurements were taken, and excess gas pressure was vented afterwards through the three-way valve to avoid cumulative gas pressures beyond the response rang of the P-8 adapter. The milliohm response was related to milliliters of gas produced by a standard curve constructed by adding known quantities of gas to serum bottles by syringe: the r^2 was > 0.999%.

In the SMA test equipment (Figure 2.8c) used by Ince et al. (1995) and Kasapgil Ince et al. (2002), the gas metering system was consisted of a three-way solenoid valve controlled by a pressure measurement device (manometer), a gas bulb for temporary storage of the gases and the necessary tubing for the interconnection of the anaerobic reactor and the units of the system. The solenoid valve was set so that the two normally open ports (1 and 2) communicate with the pressure measurement device and the gas bulb. When the third port was closed, the pressure in the reactor and in the bulb increased progressively. As the pressure inside the system reached a set value, the control system sent an electric signal to a control interface that activated the three-way solenoid valve, simultaneously closing the second port (to maintain the pressure inside the reactor) and opened the third port to the atmosphere. This made the connection of the bulb to the atmosphere, releasing excess gas accumulated during the build-up in pressure. The valve was deactivated after an interval of time (3 s for the complete release of the gases) and a new cycle was initiated.

EPA guideline. The guideline: EPA 712-C-98-090 was developed for anaerobic biodegradability of organic chemicals by US-Environmental Protection Agency (January 1998). The purpose of harmonizing these guidelines into a single set of guideline is to minimize variations among the testing procedures that must be performed to meet the data requirements of the EPA under the Toxic Substance Control Act (Figure 2.8d).

Rotary cell culture device used in SMA test. Lay et al. (1998) evaluated the dynamics of methanogenic activities in a landfill bioreactor treating the organic fraction of municipal solid wastes. They performed SMA test with 120 ml vial at 41 °C. Glucose, peptone, and linseed oil were used as the typical substrates of the carbohydrates, proteins and lipids, respectively. The vials were incubated in a rotary cell culture (Figure 2.8e) rotated at 1.5 rpm for providing better contact among samples, nutrients and microorganisms. The volume of biogas was measured with glass syringes according to the approach reported by Owen et al. (1979).

Biochemical Methane Potential and Anaerobic Toxicity Assay. The methane potential of a waste is related to the concentration of organic (COD) in it and the efficiency of treatment. The 5-d biochemical oxygen demand (BOD_5) is also a common parameter used to measure waste strength. A BOD_5 value can be conservatively converted to a COD value by multiplying BOD_5 by 1.5. The maximum theoretical yield of methane, M, is 0.35 m^3CH_4/kgCOD (5.6 ft^3/lb) removed.

The maximum methane potential of a waste may not be realized in a treatment process for reasons such as toxicity or the refractory nature of some of the organics. In 1979 a procedure named the biochemical methane potential (BMP) test, analogous to the BOD test, was defined to assess the methane potential of a waste. The procedure is readily modified to a toxicity assay (Owen et al., 1979). Although these procedures have not been incorporated in to *Standard Methods* (1992), they are widely used in the field (Droste, 1997).

In the BMP test a sample is inoculated with an active culture and supplemented with growth requirements to provide optimal conditions for anaerobic metabolism. Stock solutions containing minerals, nutrients, vitamins, and other growth factors are prepared. The appropriate sample volume is anaerobically transferred to a serum bottle. The prepared solutions are combined, inoculum is added, and the mixture is transferred to the serum bottle. Anaerobic transfers prevent oxygen toxicity. The tubing and

flasks used to transfer the sample, medium, and inoculum are flushed with 70:30 nitrogen: carbon dioxide gas before a transfer is made. One of the prepared solutions contains sodium sulfide to provide a reducing environment and another solution contains resazurin, an indicator that turns pink when it is oxidized, which indicates the presence of oxygen (Droste, 1997).

The serum bottle is capped after all solutions are added and incubated at the desired temperature, which is usually 35 ºC. Gas production and composition are monitored over time. The incubation period is typically 30 d or the time for gas production to cease. Gas volume produced is monitored with a glass syringe that is allowed to equilibrate with atmospheric pressure after the needle is inserted in to the serum bottle. Samples for analysis of gas content are also taken with a syringe.

The anaerobic toxicity assay used the procedure for BMP except that an acetate propionate spike is added to the serum bottle to provide a readily degradable substrate. Methane production from various sample sizes (and dilutions in the serum bottle) is compared to gas production from a control to assess the toxicity of the sample (Droste, 1997).

SMA test on concentrated pharmaceutical wastes. Methanogenic activity tests were used for the determination of anaerobic biodegradability of some concentrated wastes from the pharmaceutical industry – waste biomass from threonine production, mycelium after penicilium extraction, and excess activated sludge from the treatment of other pharmaceutical wastewaters. The tests were performed in 120 ml vials for each of the wastes and for a mixture of the wastes in real ratio. Specific methane yields for each waste and for the mixture were determined, for the threonine biomass, the mycelium and the activated sludge as 0.28. 0.33 and 0.19 L CH_4/g COD respectively and for the waste mixture as 0.31 L CH_4/g COD (Zabranska et al., 1994).

Microbial composition assessment by SMA test. Jawed and Tare (1999) presented the application of a simple methanogenic activity test procedure to monitor reactor biomass in terms of relative population levels of methanogenic species by using two different test substrates. There are essentially two classes of methanogens namely hydrogen oxidizing methanogens (HOMs) and non-hydrogen oxidizing methanogens (NHOMs). The later (NHOMs) are substrate-specific and cleave the acetic acid molecule to produce methane. As such, the methanogenic activity test with neutralized acetic acid or acetate as sole substrate reflects activity of NHOMs, also known as aceticlastic methanogens, and it has been as the aceticlastic methanogenic activity (AMA) test. With using mixed substrate (carbon source), both HOMs and NHOMs contribute to methane production and therefore it is referred to as the total methanogenic activity (TMA) test. The activities so obtained correlate well with the performance of the upflow anaerobic filter (UAF) and clearly demonstrate the change in relative levels of methanogenic species (mainly HOM and NHOM) with

changing operational conditions. Therefore, the activity test usual can be used to monitor the biomass composition along with usual reactor performance evaluation parameters for giving a better insight in to the reactor stability and performance.

Dolfing and Bloemen (1985) proposed activity measurements as a tool to monitor the microbial composition of methanogenic environments using H_2, formate, acetate and propionate as test substrates. The anaerobic biomass was obtained from a digester and maintained for three months on synthetic growth substrates comprising a mixture of acetate and propionate (50% each on COD basis) and mainly sucrose (95% on COD basis and the rest 5% on COD basis augmented by acetate and propionate). Dollfing and Bloemen (1985) observed 30 to 70% reduction in methanogenic activity of biomass maintained mainly on sucrose compared to that maintained on acetate and propionate. It is not clear from this study whether these reductions were due to experimental limitations (such as lack of acclimatization of test biomass, short monitoring period for methane production, ect) or otherwise (Jawed and Tare, 1999).

Control of Organic Loading Rate (OLR) using SMA test. It is important to recognize that the amount of active methanogenic population in an anaerobic reactor is the critical factor in achieving efficient wastewater treatment. A long sludge retention time, as reflected by the traditional volatile suspended solid (VSS) measurements do not distinguish between microbial biomass and any other particulate organic material, which may be present in the reactor, nor does it give any indication of the potential methanogenic activity of the microbial biomass present (Reynolds, 1986). From the standpoint of the design and operation of anaerobic processes, methanogenic 'activity' is of great importance. Ince et al. (1995) used the specific methanogenic activity test (SMA) to determine a suitable organic loading rate during the start-up phase of a crossflow ultrafiltration membrane anaerobic reactor system (CUMAR).

Results obtained from this study showed that the use of the SMA test compared to conventional parameters is its ability to predict OLRs, which could be applied to the anaerobic digestion reactors.

During start-up period the AMP/PMP ratio (the ratio of actual methane production rate to potential methane production rate) of less than 0.6 was found to be satisfactory in order to run the system efficiency in terms of COD removal and methane yield. The maximum SMA of the reactor sludge from the CUMAR system was 50 ml CH4/g. VSS.d at an AMP/PMP ratio of 0.45 at which point the system performed very well with over 98% COD and almost 100% BOD removal efficiencies. On the other hand, an AMP rate of between 60-and100% of the PMP rate resulted in low COD removal efficiencies with high TVFA concentrations in the effluent (Ince et al., 1995).

The SMA tests were also carried out in the membrane permeate and the results showed that there was almost no acetoclastic methanogenic activity loss in the effluent of the CUMAR system which resulted in an effluent in the stability of the system during the operation. As a result, the SMA test can be used as a reliable method for the determination of potential loading capacity of anaerobic digestion reactors.

De Zeeuv (1984) stated that the beginning of the start-up of a new reactor, the specific activity of the seed sludge together with the amount of sludge present determines the permissible initial organic loading rate, subsequent measurements of SMA and total volatile suspended solids would provide a safe guideline for further increases in organic loading rate during the start-up period. McCarty (1964) stated that approximately 70% of the methane formed during the anaerobic digestion of a complex substrate results from acetic acid (Ince et al., 1995).

SMA test to determine optimum operating condition. The SMA test can also be used for the determination of optimum operating conditions of anaerobic reactors. Three fundamental operating conditions were defined by Monteggia (1991) in a study of laboratory –scale upflow anaerobic sludge blanket reactors. Operating condition one corresponded to an actual methane production (AMP) rate in the digester of 60% of the potential methane production (PMP) rate of the sludge using the SMA test, thus resulting in high operating stability and an excellent COD removal. Operating condition two was identified as being from approximately 60-100% of the PMP rate, resulting in a lower COD removal and stability dependent on the available alkalinity. Operating condition three took place at excessive organic loading rates (i.e. where the AMP rate in the digester is greater than the PMP rate) resulting in an irreversible imbalance in the sequential stages of anaerobic biodegradation.

Although it has been well established that stable operation of anaerobic processes requires the regular measurement of parameters such as pH, alkalinity, gas production and gas composition, removal of organic matter, as well as the maintenance of a stable temperature, preferably at mesophilic or termophilic conditions these parameters only provide information concerning the current conditions inside the reactors. Therefore the use of SMA test as a control parameter could provide safer operation under field conditions, i.e. under varied influent flows and variable concentrations of organic matter (Ince et al., 1995).

Effects of storage on methanogenic activity of granules. Quantitative effects of storing period and temperature on the preservation and the re-activation of granular sludges are needed for industrial scale of reactors operating seasonally or intermittently.

Re-activation characteristics, specific biomass activities, and microbial and the morphological changes of anaerobic granular sludge starved for 10 months were studied by Bae et al. (1995).

In order to investigate the re-activating capability of granular sludges starved for 10 months, the recovery of microbial activity was observed in a reactor re-feeding test as well as in a serum bottle test (SBT). During 10 months of storage without feeding, specific acidogenic activity (SAA) reduced to about 30% of an initial value, regardless of the storage condition. The specific methanogenic activity (SMA) at room temperature conditions decreased to 60% whereas 80% of SMA was decreased at 4 ºC. In spite of being starved for 10months, anaerobic granular sludge recovered their activities very quickly. It took only 12 days to reach the organic loading rate of up to 11.2 kgCOD/m^3.day with a COD removal rate of about 80%. Room temperature was more beneficial than 4 ºC in maintaining the methanogenic activity and also on re-activation characteristics of granular sludge. In 8-month storage of granular sludge, no remark able change was observed in their shape. However, granular sludge had a tendency to float, when they were exposed to continuous re-feeding (Bae et al., 1995).

Psychrophilic activity of granules. With the increased use of high rate anaerobic processes, the transfer of granules of seed sludge from one treatment plant to another plant may become more common. It is therefore great technical and commercial interest to examine the changes in granular sludges at different operational and environmental conditions.

The specific methanogenic activity of granules was tested by Banik et al. (1997). The SMA test were carried out at 35 ºC in duplicate for granules grown at temperatures of 25, 15, 10, and 5ºC using 250 ml bottles containing the batch medium. The amount of granules was sufficient to ensure that a measurable amount of methane would be produced within 2 to 4 hours. They results revealed that even after long periods of operation at psychrophilic temperatures (5 and 15 ºC), mesophilic bacteria are active and have the ability to rapidly degrade acetate and mixtures of acetate and propionate. The SMA of granules grown at psychrophilic temperature was slightly less than that of granules grown at psychrophilic temperatures indicated the existence of mesophilic microorganisms and/or psychrophilic microorganisms that could grow at mesophilic temperatures. The existence of obligate psychrophilic microorganisms was not confirmed. The specific methanogenic activity for granules biodegrading mixtures of acetate and propionate was significantly lower than that for acetate alone.

Reference chemicals to confirm SMA test. Shelton and Tiedje (1984) refined and validated a simple, generalized method to test whether an organic chemical was susceptible to anaerobic degradation to methane and CO_2. In base of their results, the sludge

could be stored anaerobically at 4°c for up to 4 weeks with satisfactory test result. They suggested *p*-cresol, phthalic acid, and ethanol as reference chemicals to confirm sludge activity and method reliability with using sewage sludge from municipal digesters.

SMA test on 13 chemicals. The results of biodegradability test by Kawahara et al. (1999) showed that chemicals with –OH and –CH_2OH radicals were readily biodegraded and those with –Cl, -NO_2, -NH_2, -SO_3H, and –CH_3 had inhibited degradation responses. m-nitrophenol and 2,4,6-trichlorophenol were highly toxic to methanogenic bacteria, with m-nitrophenol completely inhibiting methane fermentation as low as 20 mg/l.

Activities of attached and occlude biomass. The attached biomass was always less active than the occluded mass, which agrees with previous reports by several authors. However, Hanaki et al (1994) have reported that the attached biomass from upflow anaerobic filters treating different types of substrates gave higher specific methanogenic activity than did the occluded biomass (Punal et al., 1999).

SMA test on different seeds, substrates, and processes. The SMA test has been used for evaluation of methanogenic activity of different seeds, substrates, and processes such as: the inhibition of methanogenic activity of starch-degrading granules by nine common aromatic pollutants from chemical industries (Herbert et al., 1997), SMA test to evaluate performance of UASB, DSFF and USFF (upflow and downflow stationary fixed film) reactors (Jawed and Tare, 1996) and anaerobic digestion of municipal solid waste (Nopharatana et al., 1998).

Volatile Fatty Acids (VFAs)

VFAs include components such as acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric acid. This group of components is described as volatile since they can be seam distilled under acidic condition, but they are also classified as short chain fatty acids due to their low molecular weights. Some of theses compounds are produced during normal methabolic processes, while all of them are produced via fermentation pathways (Traitler et al., 1988).

VFAs in the methanogenesis model. The inhibitory effects of longer-chain (C_4-C_5) volatile fatty acids (VFAs) have been monitored in the methanogenesis model (Hill, 1982; Hill and Bolte, 1987).

These changes to the model are based upon the other work, which demonstrated that interconversion of $C_2 - C_5$ VFAs plays a significant role in the operation of anaerobic fermenters. These works also demonstrated that monitoring the levels of long-chain VFAs can be a valuable tool in predicting and preventing process failure in the operation of anaerobic fermenters.

The previously validated model has been revised to include the longer-chain VFAs so that the fermentation process can be more an accurately predicated. Specifically, the model has been improved by: 1) modification of the model stoichiometry to include valeric acid production via acetogenesis and valeric acid hydrogenogenesis: 2) addition of triple-use substrate kinetics for hydrogenogenesis: 3) incorporation of fractionation functions for butyrate and valerate which divide these acids in to their iso-and n-forms to make the model useful for predicting future stress and failure of fermentation system.

Although the incorporation of valerate hydrogenogenesis and the prediction of digester failure through isobutyric and isovaleric acids levels was the primary objective, at least two additional improvements resulted form this modification of the model; first, a better representation of fermenters performance parameters (particularly gas and VS relationships) was observed; and second, the model provides an improved representation of the complex mechanism of fermenter failure. The current model demonstrates that complete fermenter failure is actually a sequence of failures due to the inhibition of the individual population of microorganisms (i.e., methanogens, homoacetogens, hydro-genogens, and acetogens). Due to the improvements noted, the newly modified model can be used to provide better predictions of fermenter performance and improved analysis and a better understanding of the fermentation process.

More research in to the microbiology and chemistry of VFA consumption and interaction is needed to enable the further development and refinement mathematical modeling of anaerobic fermentation system (Hill and Cobb, 1993).

Potentialities of a methanogenic microbial ecosystem to degrade VFAs. The degradation potentiality of VFAs the main metabolic intermediates during methanization of complex organic substrates has been evaluated. The kinetics of acetic, propionic, butyric and valeric acid degradation by the particular microbial ecosystem are of zero order in relation to the substrate, and acid degradation rate decreases with increasing length of the acid carbon chain.

The process of methane production involves various biochemical and microbiological mechanisms in which VFAs (acetic, propionic and butyric) are the principal intermediates. It is frequently reported that those VFA tend to accumulate in digesters stressed, for example, by a substrate overload. Such an accumulation, resulting from a VFA production rate higher than the VFA consumption rate, can cause a severe inhibition of methane production. Except for acetic acid, which is directly degraded to methane by methanogen acetoclastic bacteria, the VFA (e.g. propionic and butyric) must first be degraded by obligate hydrogen producing acetogenic bacteria (OHPAB) to acetate, hydrogen and carbon dioxide, which are utilized by methanogenic bacteria. The stability of the microbial ecosystem is very dependent on acetogenic activity, this activity being characterized by slow growth rates of microorganisms and great sensitivity to inhibition processes (Segretain and Moletta, 1987).

For the same VFAs, during glucose methanization, a degradation rate varying from 0.07 to 0.092 mMh^{-1}. Acetate inhibits propionate degradation, as does hydrogen. A similar diversity of microbial methanogenic ecosystems responses is also found in metabolic flows resulting from acetogenesis.

If the acetogenic microorganisms are not active, increasing initial concentration of propionate or butyrate causes a decrease in the acid degradation rates. On the contrary, if the ecosystem is already degrading one of these two acids (that is acetogenic microorganisms are active) an external addition if the same acid (that is an increase of acid concentration) leads to increased acetogenic activity. So, the kinetic acetogenic responses of this ecosystem to an increase of acid concentration (propionic or butyric) differ according to whether these reactions are already taking place or not. Additional work is needed to find an explanation of this.

Acetogenic activities on propionate or butyrate are inhibited when other VFAs are added in the fermentor. Inhibition by acetate has already been reported by several authors and may be explained by the fact that this acid is a product of propionic and butyric acid degradation. The inhibition of propionate acetogenic activity by butyrate and vice versa has been noted. The acetogenesis of an

ecosystem coming from an UASB adapted to soured beet – sugar-processing wastewaters, revealed that butyric acid degradation was slightly inhibited by propionic acid. A comparison of the results of these two works is difficult because the concentrations studied were very different. The UASB worked generally with acid concentrations lower than 1 mM while the present once were around 50mM. For UASB ecosystem the results suggested that the inhibiting effect of butyrate on propionate degradation is indirect and comes from acetic acid produced as butyrate is degraded. In the other studied case, this process cannot be considered because during propionate degradation with butyrate addition, acetic acids concentration stayed very low (below 3mM) as in the control fermentor (Segretain and Moletta, 1987).

Results obtained suggest that inhibition processes must come from the nature of the acid and not from the carboxyl group. In the case of propionate acetogenic activity a 45mM propionate addition increasing this acid concentration to 80mM, brings the propionate degradation rate to $1.06 mMh^{-1}$. On the other hand, 45mM butyrate addition, giving a propionate plus butyrate concentration of 75mM, produces a propionate degradation rate of $0.37 mMh^{-1}$. The same effects were observed in the case of butyrate degradation, though they were less apparent.

Degradation of propionate at high concentration shows a transient accumulation of butyrate. It is interesting to notice that very different ecosystems, like the one obtained on vegetable cannery wastewater show a similar reaction, but of different range.

Raising the organic load is expressed by increase of VFA residual concentration and particularly of propionate. In fact, this acid is the main intermediate formed during the vinase fermentation and its accumulation (80%-90% of total VFA) shows that its degradation is the limiting step of the overall reaction.

Other types of microbial methanogenic ecosystems are also limited by propionate acetogenesis, but it seems less frequent than a limitation by acetate methanization. This apparent contradiction is the reflection differences existing in the substrate adaptation of reactor microbial population (Segretain and Moletta, 1987).

Anaerobic reactor stress as related to iso-butyric and iso-valeric acids. The results of published studies have proposed using *iso*-butyric and *iso*-valeric acid levels as indicators of impending digester failure. The data suggested that individual levels of these acids below 5 mg/l signified good, healthy digestion. Levels between 5 and 15 mg/l suggested that problems were developing and failure was imminent, while a level grater than 15 mg/l indicated failure had already occurred to the extent that a long recovery period would be necessary. When *iso*-butyric and *iso*-valeric acids were in the 5-15 mg/l range, the study suggested that this was a signal that provided as much as weeks advance notice of the failure (Hill, 1989).

Treatment of wastewater with concentrated mixed VFA. The VFAs, which are mainly composed of butyrate, propionate, and acetate, become the substrates to the acetogenic and methanogenic bacteria. However, degradation of propionate and butyrate to acetate is thermodynamically unfavorable unless the other product, hydrogen, can be readily removed. In the case of propionate degradation, the hydrogen partial pressure must be kept under 10^{-4} atmosphere. Thus, degradation of propionate, and to a lesser degree, butyrate, requires syntrophic associations of hydrogen-producing acetogens in juxtaposition with the hydrogen-consuming methanogens. Furthermore, propionate is known to have the lowest tolerance level among the VFA for the anaerobic bacteria. It is easily accumulated in anaerobic digesters during overloading and is difficult to remove during recovery (Fang et al., 1995).

CHAPTER 3

MATERIALS AND METHODS

Described in this chapter are the materials and methods used during this research, including the specifications of reactor set-up, start-up and operation, two different HPLC methods for VFA and erythromycin analysis, GC method to determine biogas composition, and finally, a procedure used to perform SMA test.

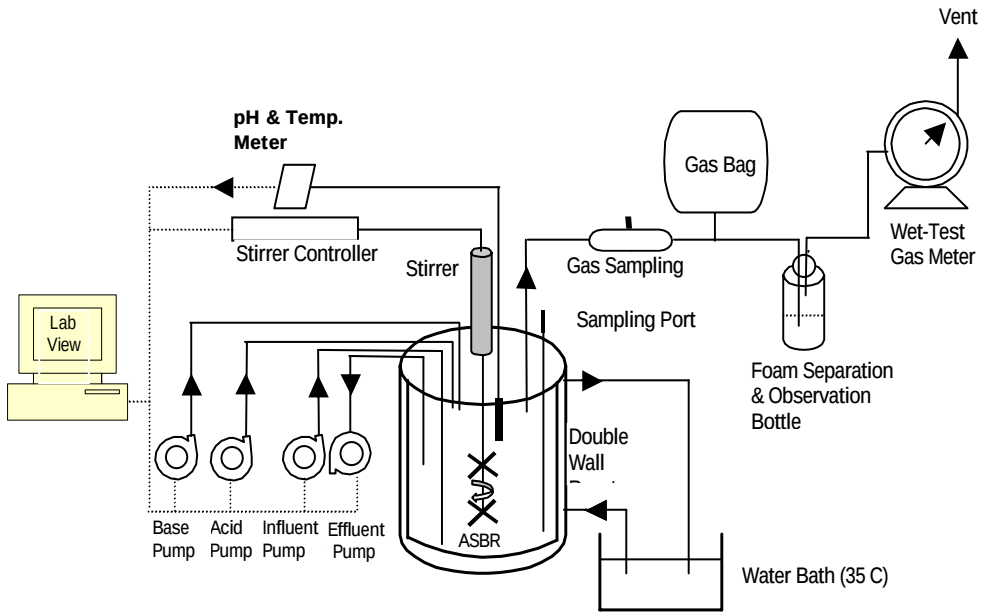
Reactor Design

The experiments were conducted using a double wall cylindrical 7-L glass laboratory-scale ASBR (Figure 3.1). The jacketed bioreactor (Applikon instruments Co, Netherlands) had an effective height of 35 cm (H/D 2.2) with liquid volume of 5-L and a 2-L headspace for collection of biogas and foam. The reactor was maintained at 35º C by a thermostatically regulated recirculating water bath. Intermittent 5-min mixing at 250 rotations per minute (rpm) was done every 30 min by rushton type mixer. In biogas collection system, a 4-L gas-bag attached to the headspace ensured availability of gas was during decanting. Overall gas production was monitored using a 1-L wet-test gas meter (Schlumberger Industries, Dordrecht, The Netherlands). Provisions were made for

gas sampling port, and a bottle both for preventing leakage of foam to gas meter and for observation of biogas production. The cycle time was set at 24 hour (1 cycle/day) as follows: fill, 10 min; react, 22hour 40 min; settle: 1 hour; and decant, 10 min. Two peristaltic pumps that were used to fill the reactor and draw off the effluent after settling. Continuous monitoring of pH-temperature meter and mixer was done by Lab-View®.

Seeding

The reactor was inoculated with mesophilic granular methanogenic sludge obtained from a full-scale UASB reactor treating brewery wastewater (Anheuser Busch Brewery; St. Louis, Missouri, USA). The granules were stored at 4º C for a period of two weeks, then washed with N_2-sparged water. Washed granules mixed with VSS 80 g/L (VSS/SS = 0.79) were used to supply a seed with a 30 g-VS of granulated sludge per liter of reactor.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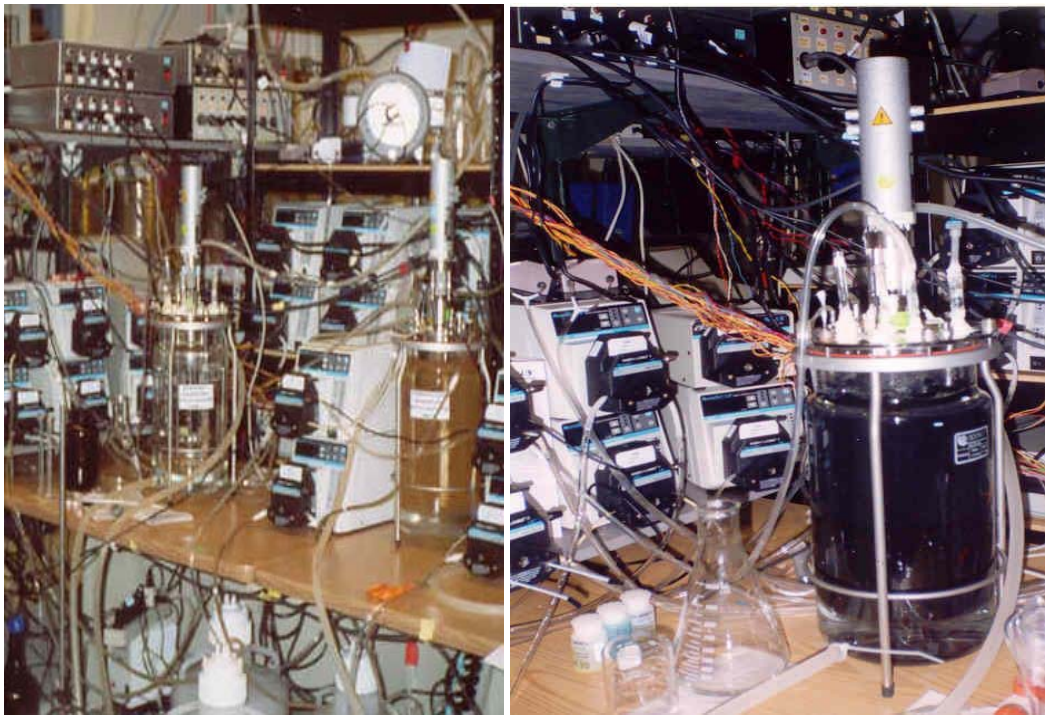

Figure 3.1. Experimental Setup for the Anaerobic Sequencing Batch Reactor (ASBR)

Substrate

The ASBR was fed with a synthetic wastewater with a total chemical oxygen demand (COD) of 16,250 mg/l that resembled the high strength fermentation wastewater at Abbott pharmaceutical plant (Abbott WTP data, 2003; Chicago, IL, USA)(Table 3.1). Inorganic nutrients (NH_4Cl, 76.45 mg/g COD and KH_2PO_4, 10.00 mg/g COD); trace elements as mg/g COD (K_2HPO_4, 25.32; $FeCl_3$, 1.021; $CaCl_2.2H_2O$, 2.06; $MgSO_4.7H_2O$, 2.14; $MnCl_2.2H_2O$, 0.34; $CoCl_2.6H2O$, 0.092; $NiSO_4.6H_2O$, 0.0763; $ZnSO_4$, 0.0592; $Na_2MoO_4.2H_2O$, 0.0822; $CuCl_2.2H_2O$, 0.016; H_3BO_3, 0.020), yeast extract, 36 mg/L; peptone, 36 mg/L were also added. Operating pH was maintained at 7 through addition of NaOH and KOH (1:1 v/v).

Operation

Organic loading rate of the reactor was increased in a stepwise manner from 0.4 to 3 g COD/(l d), while ensuring that VFA accumulation did not occur. Starting on day 88, 1 mg/l of erythromycin was added to the influent of the reactor; on day 120, it was increased to 200 mg/l.
The system was started with a hydraulic retention time (HRT) of 42 days for low organic loading rate; HRT was reduced to 6 days for the highest organic loading rate. The solids retention time was significantly longer, but not well defined because biomass was only wasted through sampling.

Table 3.3. Composition of the synthetic substrate mixture

Components	Concentration, g/L	Percent of total COD	COD, mg/l
Ethanol	3.4	43 %	7000
Acetic acid	5.2	34 %	5500
Propionic acid	1.3	12 %	2000
Butyric acid	0.84	5 %	750
Valeric acid	0.25	3 %	500
Isopropanol	0.22	3 %	500

Sampling and analysis

Performance of the reactor was monitored through daily measurement of volumetric gas production and automated control of pH, temperature, and flow rate, and mixing. Soluble chemical oxygen demand (COD) and VFA in influent and effluent were determined on a daily basis. The COD and VFA samples were centrifuged and then were filtered with using Fisher-brand filter with a nominal pore size 0.45 µm. Suspended solids (SS) and volatile suspended solids (VSS) in reactor and effluent were measured once every 10 days. Gas analysis was performed once per month. Routine analyses were conducted according to *Standard Methods* (APHA et al., 1998).

VFA levels were determined with a high pressure liquid chromatograph (Waters 486 using a UV detector and a 712 WISP autosampler, Waters, Milford, MA, USA) and an Aminex HPX-87H Ion Exclusion column of 300 mm*7.8 mm (Bio-Rad, city, CA, USA), using 0.005-N H_2SO_4 as mobile phase and a flowrate of 0.7 mL/min.

The methane content in the biogas was measured using a gas chromatograph (AutoSystem, Perkin Elmer, Norwalk, CT, USA) equipped with a GS-Q column and a flame ionization detector.
In addition, the CH_4 was analyzed with another GC: Super GC, Model- HP 6891 with FID detector and column size: length =30 m, diameter=0.25 mm, film thickness= 0.5 μm, and temperature range: -60 to 325/350 °C.

Erythromycin samples were analyzed (in Abbott Plant) with a HPLC/MS with electrospray interface in the positive mode, using 35:65 acetonitrile: 0.5 % formic acid as mobile phase, and with column specifications: supelco ABZ, 25 cm * 4.4 mm, 5 μm particle size. For Sample treatment: 13 ml wastewater was mixed with 7 ml acetonitrile and 65 μl Formic acid (same ratio as mobile phase).

Specific Methanogenic Activity (SMA) Test

Batch tests to determine the specific methanogenic activity (SMA) were performed using 120-mL serum vials. The vials were filled with 10 % (v/v) of washed seed sludge, 75 % (v/v) substrate and 15 % (v/v) open space of vial for gas accumulation. An EPA-approved modified simple method (Van-Haandel and Lettinga, 1994; Jawed and Tare, 1999) was used for SMA test in this study. The granular sludge was taken from ASBR and after washing with N_2- sparged water with VSS 70 g/L used as seed sludge. In parallel with the assay bottle, two blank tests (with and without erythromycin) were conducted using the same amount of anaerobic sludge, but with water instead of substrate. Methane production was measured by displacement of liquid by 2N KOH solution as CO_2 scrubber and Bromothymol blue as indicator. When an alkaline solution was used as the displacement liquid, CO_2 was scrubbed from the biogas and only methane was collected. Tests were performed with the synthetic mixture fed to the ASBR and individual VFAs, and different amounts of erythromycin were added to these tests.

Experimental setup of SMA test is shown in Figure 3.2. The substrate sample was placed in a serum bottle together with a granulated anaerobic sludge. The serum bottle was capped and crimped after all solutions and seed were added and incubated at the desired temperature, which was usually 35 ºC. Gas production was monitored over time. The incubation period was typically 10 d or the time for gas production to cease. The septum of the serum bottle was perforated by a needle, reaching into the headspace. Contents of serum bottles was mixed by magnetic stirrer continuously.

The headspace of the bottle then was connected by another needle to the second serum bottle, which hangs upside down and contains a basic (KOH) solution. The biogas formed in the sample displaced liquid from the second bottle, which was discharged by third needle and was collected in a measuring cylinder. The CO_2 was absorbed by KOH; hence, the collected volume of the solution was then equal to the volume of methane produced.

The specific methanogenic activity (SMA) test is more rapid and reliable to monitor any changes in the numbers or activities of the methanogen bacteria, than other tests such as coenzyme $F_{420,}$ ATP, and most probable number (MPN) (Ince et al. 1995). The BOD is an adequate parameter for aerobic biodegradability, but not for anaerobic biodegradability (Van-Haandel & Lettinga, 1994). In the anaerobic condition, the specific methanogenic activity (SMA) test can be used to measure the amount of methane generated from the organic matter present in a wastewater sample when exposed to an active anaerobic sludge under well-defined condition.

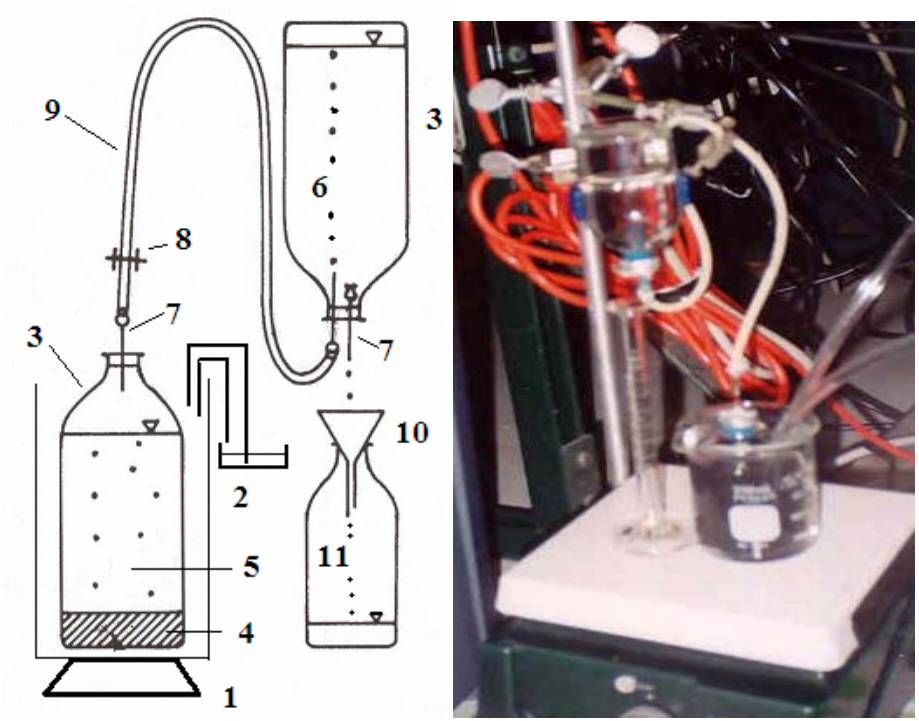

Figure 3.2. Specific methanogenic activity (SMA) test set-up: 1) Magnetic stirrer 2) Water bath 3) Serum bottles 4) Test biomass 5) Reaction mixture (substrate) 6) CO_2 scrubber: 2N KOH solution and Bromothymol blue indicator 7) Hypodermic needles 8) Pinchcock 9) Rubber tubing 10) Conical funnel 11) Displaced liquid

Erythromycin by-products at Abbott plant

Several Erythromycin by-products such as: Erythromycin ethyl succinate, Erythromycin stearate, Erythromycin phosphate etc. was developed for Erythromycin Base**.**

Operational and performance parameters

Operational and performance parameters include organic loading rate, sludge loading rate, elimination capacity, and detention time. Loading rates can be looked at from the pollution indicator, reactor volume, and microbial mass. Organic loading rate (OLR) takes into account the liquid flow rate and contaminant concentration and is defined as the mass of pollutant introduced in a unit volume of ASBR reactor per unit time (e.g. g COD/L.d). As such, this parameter integrates reactor characteristics, operational characteristics, and bacterial mass and activity into the volume of media. Sludge loading rate (SLR) or food to microorganism ratio (F/M) integrates contaminant concentration and microbial mass and is the mass of pollutant applied to a unit mass of microbial mass per unit time (e.g. g COD/ g VSS. d).

Elimination capacity is related to organic loading rate and sludge loading rate in that it is defined as the fraction of the organic load biodegraded in a unit volume of the ASBR reactor or a unit mass of microbial mass. This parameter can be expressed either volumetrically (EC_V, g pollutant removed per unit volume of reactor per day) or on the basis of microbial mass (EC_m, g pollutant removed per unit mass of microorganisms in the reactor per day).

Methanogenic activity (MA) can be expressed on the basis of pollutant (Liter biogas produced per unit mass of pollutant removed, MA_{scod}) or on the basis of microbial mass (L biogas produced per unit mass of microbial population, MA_{vss}).

Mass loading rate (g/L.d), sludge loading rate (g/g.d), and elimination capacity (g/L.d or g/g VSS.d) were determined using the relationships between influent and effluent contaminant concentration, effluent flow rate, the effective volume of ASBR reactor, and applying appropriate conversion factors as follows:

$$OLR = \left(\frac{Q}{V_r}\right) C_{in}.n.t_{fill} \quad (1)$$

$$SLR = Q\left(\frac{C_{in}}{VSS}\right) n.t_{fill} \quad (2)$$

$$EC_V = \left(\frac{Q}{V_r}\right)\left(C_{in} - C_{out}\right)\left(n.t_{fill}\right) \quad (3)$$

$$EC_m = \frac{EC_V}{VSS} \quad (4)$$

$$MA_{scod} = \frac{V_{CH_4}}{Q(C_{in} - C_{out})} . \frac{1}{n.t_{fill}} \quad (5)$$

$$MA_{vss} = \frac{V_{CH_4}}{Q(VSS)} . \frac{1}{n.t_{fill}} \quad (6)$$

$$OLR = \left(\frac{Q}{V_r}\right) C_{in}.n.t_{fill} \quad (7)$$

$$SLR = Q\left(\frac{C_{in}}{VSS}\right) n.t_{fill}$$

(8)

$$SRT = \frac{\left(VSS_r\right)}{\left(VSS_{effl}\right) \times \left(V_{decanted}\right) \times n} \quad (9)$$

$$HRT = \frac{\left(V_r\right)}{\left(V_{decanted}\right) \times n} \quad (10)$$

where Q is the effluent flow rate (Lit/day), V_r is the effective volume of reactor (Lit), n is the number of cycles per day, t_{fill} is the fill time in ASBR feeding, VSS is the microbial concentration of the reactor (mg VSS L^{-1}), V_{CH4} is the volume of biogas produced per day (L/d), and C_{in} and C_{out} are the contaminant concentrations (mg SCOD L^{-1}) in the influent and effluent stream, respectively.

CHAPTER 4

RESULTS AND DISCUSSION

The effect of addition of erythromycin on the microbial community and the development of antibiotic resistance were evaluated based on overall performance of a laboratory-scale anaerobic sequencing batch reactor (ASBR) and using specific methanogenic activity (SMA) tests. The inhibitory effect of erythromycin was evaluated by monitoring biogas production, methane content of the biogas, and accumulation of individual volatile fatty acids (VFAs).

The goal was to study the inhibition of the anaerobic digestion process after adding erythromycin in the influent that could be used in the desing and operation of pharmaceutical wastewater plants.

Reactor start-up

The ASBR was started up by using an inoculum of mesophilic granular sludge from a full-scale UASB reactor treating brewery wastewater. Synthetic pharmaceutical wastewater (Table 3.1) was used as the influent to the system for 90 days without any erythromycin addition. Both COD removal efficiency and biogas production increased steadily with increasing organic loading rates. Influent COD was maintained at 16.25_± 0.25 g/L during the start up period. The pH value in the reactor remained in the range of 7.2-8.5 without a need for further adjustment.

The use of high-density granules of 30 gVSS/l resulted in substantial reduction of the adaptation period (see Figure 4.1). As shown in the Figure, removal efficiencies were around 80% from the very beginning and reached to above 90% during the first 20 days. The results are consistent with the findings of substantial reduction in adaptation period for biofilm anaerobic reactors due to use of granular sludge as seed material (Van Lier et al. 1992) with bacterial densities in excess of 10^{12} per mL (Ferry, 1993). In suspended-growth biomass systems, where potential for wastewater treatment is primarily dictated by dual parameters of retained biomass quantity and the specific activity of biomass, an inoculum of about 30% reactor volume of active biomass results in similar performance. Recommended concentration of biomass is in the range of 10-40 g VSS/l (Stronach et al. 1986).

Reactor operation

Overall performance of ASBR is summarized in Table 4.1 and Figure 4.1. Three distinct phases are noted: zero, low (1 mg/L), and high (200 mg/L) concentration of erythromycin. In the first phase, the organic loading rate (OLR) was increased from 0.4 to 3 g COD/L.d by increasing influent volume from 120 to 900 mL per day, thus reducing a reduction of HRT from 42 to 6 days. The main parameter to change as a result of OLR variation was the removal efficiency of C_2-C_4 VFAs.

For OLR of 0.4 g COD/l.d (days 1- 11), the removal efficiency of acetic, propionic, butyric acids were in the range of 78-93.5 percent. However, COD removal efficiency was 83 percent because the concentration of valeric and caproic acid in the effluent was 2500 and 7200 mg/L. Table 4.2 summarizes the concentration of butyric, valeric, and caproic acid in influent (start of cycle; run time: 00:10) and effluent (end of cycle; run time: 24:00).

Full potential of the anaerobic biomass' adaptation to many toxicants can be realized if biomass is initially exposed to relatively low concentrations with subsequent gradual increase (Speece et al., 1996).

At OLR of 1 gCOD/l.d (days 12- 57), COD removal efficiency was 92.5% with continued presence of considerable valeric and caproic acid in the effluent; 807 and 3253 mg/L, respectively (Table 4.2). At OLR of 2 g COD/l.d (days 58- 71), the removal efficiency of COD was 96.7% with effluent concentration of 314 and 284 mg/l for valeric and caproic acids, respectively. At OLR of 3 g COD/L.d (days 72- 87) with 99.3% removal for COD and 0.35 L CH_4/g CODrem., the removal of acetic, propionic, butyric acids were in the range of 98-100 percent; residual of valeric and caproic acids in the effluent were as low as 27 and 23 mg/L, respectively.

In the second phase, soon after the target organic loading of 3 g COD/(l d) was reached, stable performance with almost complete COD removal and a biogas production of 7 l/d was observed. Starting on day 88, 1 mg/l of erythromycin was added to the influent to evaluate the impact of a low concentration on the overall performance. Following this addition, COD removal and biogas production decreased to about 94% and 6 l/d, respectively. At the same time, it was observed that butyric and valeric acids increased in the reactor effluent (Figure 4.2). However, butyric and valeric acids were still degraded in the system but at a lower rate (Table 4.1 and Figure 4.7).

The increase in the influent erythromycin concentration from 1 to 200 mg/l in the third phase (days 123-135) resulted in a significant decrease of the COD removal efficiency while the reduction of the biogas production is not apparent (Figure 4.1).

Methane is produced by methanogenic archaea, which are not affected by erythromycin because of differences in their ribosome structure (Hummel et al., 1985; Leffers et al., 1987). Therefore, it was anticipated that processes only involving methanogens, such as the utilization of acetate by aceticlastic methanogens, would continue after erythromycin addition, and this was supported by continued COD removal and biogas production. However, utilization of other VFAs could be inhibited, since these processes involve both bacteria and methanogens. Although some inhibition was observed, the substantial COD removal (not just COD associated with acetate) and biogas production after introduction of erythromycin suggests that at least some of the bacteria in the microbial community were also not affected by the presence of the antibiotic. Information on the effects of antimicrobials on non-pathogenic organisms is scarce, but the general trend of reduced sensitivity to erythromycin among Gram-negative organisms is probably also applicable to the bacteria in this study and was consistent with observed results. Specifically, propionic acid can be utilized by Gram-negative Syntrophobacter species, and propionic acid did not accumulate in the effluent. Conversely, butyric and valeric acids can be used by Gram-positive saturated fatty acid-beta oxidizing bacteria such as Syntrophomonas species, and the expected sensitivity of these organisms to erythromycin was supported by the accumulation of butyric and valeric acids in the reactor effluent following erythromycin addition.

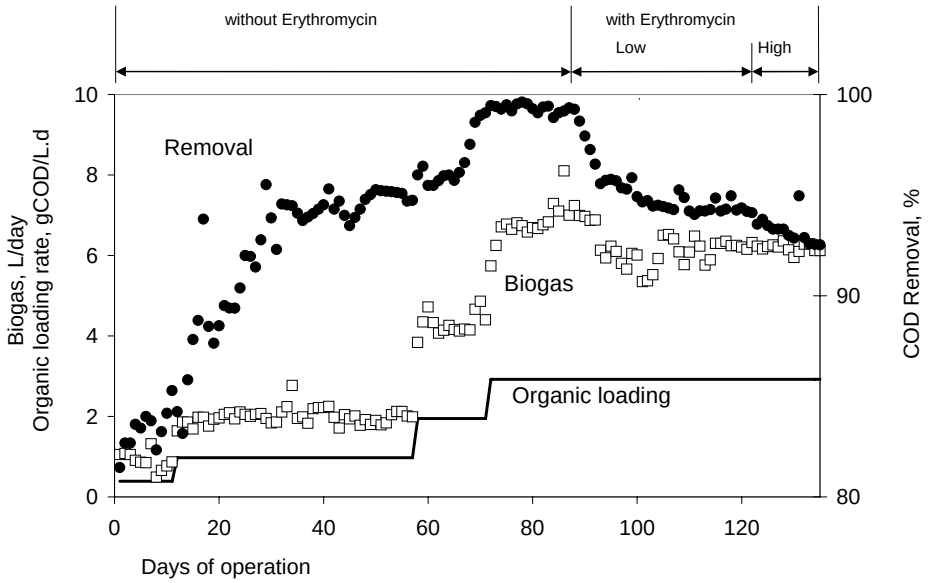

Figure 4.3. Influence of erythromycin on COD removal and biogas production without erythromycin, with low influent erythromycin concentrations (1 mg/l) and high influent erythromycin concentrations (200 mg/l).

Table 4.1. ASBR Performance (Attached file)

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Days Of Operation	Loading Rate		Ret. Time	COD Removal		Biogas Production			Volatile Fatty Acids (VFAs) removal, % & mM/hr				
	OLR gCOD/l.d	F/M gCOD/gVSS.d	HRT day	%	gCODrem/ gVSS	Lit./d	LCH4 / gCODrem	VMPR* LCH4/L .d	Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Caproic Acid
Without Erythromycin													
1 – 11	0.4	0.01	42	83.3±1.0	0.008±0.003	0.84±0.18	0.52±0.14	0.13±0.03	82.2±5.6 0.065±.013	93.6±6.8 0.016±.002	78.2±15 0.007±.002	-37.8±49 -0.23±.304	-4.1±20.5 -0.072±.44
12 – 57	1	0.04	17	92.5±3.2	0.032±0.010	1.97±0.25	0.40±0.11	0.32±0.04	78.7±19 0.163±.037	86.2±13.40 0.046±.015	85.8±9.1 0.016±.003	-32.9±86 0.002±.212	-1.6±10.9 -0.007±.16

58 – 71	2	0.07	8	96.7±1.3	0.067±0.008	4.30±0.28	0.38±.0.04	0.69±0.04	97.4±0.4 0.305±.027	99.8±0.6 0.086±.006	96.3±2.8 0.039±.004	14.9±90 0.013±.044	13.9±17.9 0.011±.009
72 – 87	3	0.1	6	99.3 ±0.2	0.105±0.008	6.79±0.49	0.35±0.04	1.09±0.08	98.3±0.9 0.46±..107	100±0.0 0.125±.023	100±0.0 0.061±.013	55.0±20 0.033±.013	5.9±31.8 0.001±.003
With Erythromycin- Low Concentration													
88 – 122	3	0.1	6	95.2±1.3	0.097±0.012	6.20±0.44	0.34±0.02	0.99±0.07	98.2±0.3 0.463±.048	100±0.0 0.123±.020	96.0±5.8 0.056±.013	28.9±56 0.028±.027	-35.3±83.3 -.001±.004
With Erythromycin- High Concentration													
123 - 135	3	0.13	6	93.2±0.7	0.093±0.009	6.19±0.11	0.36±0.04	0.99±0.02	97.6±1.0 0.475±.016	99.20±1.97 0.146±.018	95.78±1.14 0.062±.010	54.7±12.1 0.037±.011	4.90±46.0 0.001±.004

Table 4.2. ASBR low efficiencies in start-up and after adding Erythromycin

Day Of Operation	OLR (g COD/ L.d)	Butyric acid (mg/l)		Valeric acid (mg/L)		Caproic acid (mg/L)	
		Start of run (00:10)	End of run (24:00)	Start of run (00:10)	End of run (24:00)	Start of run (00:10)	End of run (24:00)
Without Erythromycin							
1 - 11	0.4	18.7±4.6	4±2.8	2005±690	2514±472	7109±1086	7218±1265
12 - 57	1	38.6±6.3	5.5±3.8	811.6±735	807.4±663	3234±1617	3253±1622
58 - 71	2	85.2±8. 8	3.2±2.4	129.6±28.2	101.6±87.3	314.6±272	284.2±270.3
72 - 87	3	128.8±26.5	0.00±0.0	127.1±33.9	53.7±21.3	26.7±13.35	23.1±9.9
With Erythromycin – Low concentration							
88 - 122	3	121.3±25.1	4.1±4.9	136.7±56.6	75±35.8	20.7±9.8	24.32±12.7
With Erythromycin – High concentration							
123 - 135	3	135.9±21.9	5.7±1.3	148.0±15.7	65.8±14.5	27.4±8.2	23.8±8

The VFA degradation rate decreases as the length of acid carbon chain increases. The acetic, propionic, butyric, valeric and caproic acids degradation rates in this study were in range of 0.065–0.475; 0.016–0.146; 0.007–0.062; -0.230-0.037; and -0.072-0.001 mmol/hr (mM/h) respectively (Table 4.1). As it is indicted in Table 4.2, during days 1-11 the concentration of valeric and caproic acids and during days of 12-57, only the concentration of caproic acid in the effluent was more than influent. In the other word, the valeric and caproic acids were produced during run time. Thus, the degradation rates of these two acids were negative. This may be partly due to the influence of varying substrate composition and hydraulic retention time on the kinetics of acetogenesis. However, the degradation rate of butyric and propionic acids were in line with findings by Mackie & Bryant (1981), who demonstrated that propionate and butyrate degradation rate varied from 0.09 to 0.27 and from 0.042 to 0.072 mM/h, respectively during cattle-waste treatment. The reported degradation rate of 0.03 mM propionate /h for urban wastes is much lower, (Segretain et.al, 1987). Aguilar et.al (1995) reported that acetate degradation rate had a range of 0.67-1.22 and 0.82–1.37 mM/h for $V_{max.}$ for acetate and glucose pre-grown innocula, respectively.

Specific methanogenic activity for individual VFAs

To study the influence of erythromycin on the specific metabolic products, a range of SMA tests were performed. The results are shown in Table 4.3 and Figure 4.2. High concentrations of erythromycin (200 and 500 mg/l) had a significant impact on methane production capacity of ASBR when a mixed-substrate influent was fed into the reactor (Figure 4.2a). When acetic acid was used as the sole substrate, the impact of erythromycin addition on the specific methane production rate was substantial (Figure 4.2b). These results suggest that an apparent utilization of acetate was made by homo-acetogenic bacteria, which can produce hydrogen and CO_2 from acetate. This is in addition to the normal utilization of this metabolite by aceticlastic methanogens, a pathway that not affected by erythromycin. The hydrogen produced by these bacteria can be used as a substrate for hydrogenotrophic methanogens. The reduced biogas production in the presence of erythromycin thus suggests that the homo-acetogenic bacteria (Gram-positive) were sensitive to this antibiotic. Reduced methane generation rate when propionic acid was used as the only substrate (Figure 4.2c) was unexpected. As discussed above, it was found that syntrophic propionate oxidizing bacteria were apparently not affected by the addition of erythromycin to the influent of the ASBR at concentrations of 1 mg/l or 200 mg/l. The results of SMA tests indicated substantial adverse impact on these microorganisms at high erythromycin concentration of 500 mg/l. Finally, methane production was most affected when butyric or valeric acids were used as the substrate (Figure 4.2d,e). This result is consistent with the observations and discussion provided for the ASBR results.

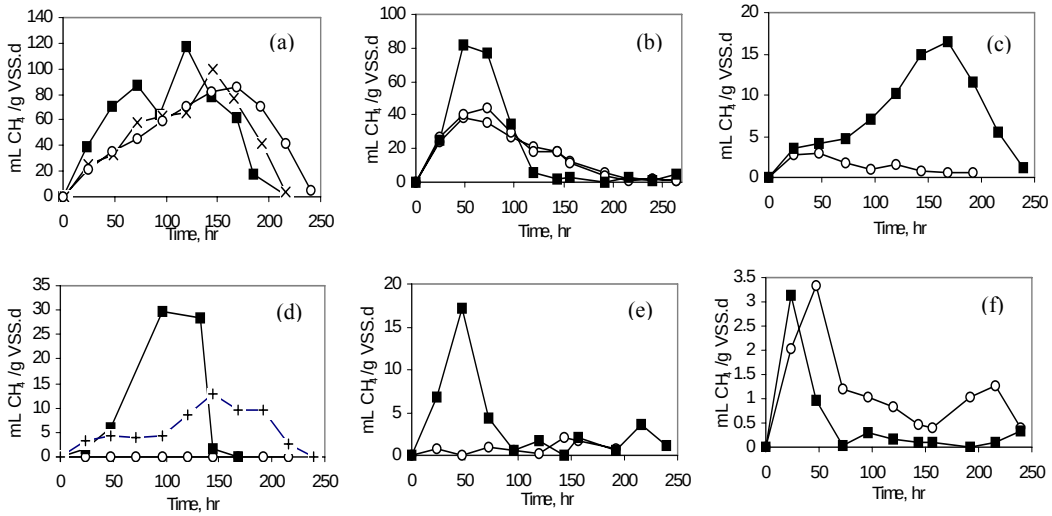


Figure 4.2. Specific methanogenic activity (SMA) using (a) mixed reactor influent, (b) acetic acid, (c) propionic acid, (d) butyric acid, (e) valeric acid as the sole substrate and (f) blank. SMA tests were performed without (■), with 10 mg/l (+), with 200 mg/l (×) or with 500 mg/l (o) of erythromycin.

The results in Table 4.3 suggested that erythromycin with concentration of 500 mg/l caused to decrease the maximum methane production rate (ml CH_4/g VSS.d) of propionic acid from 16.4 to 2.9 (82 % reduction) and for butyric acid from 29.6 to 0 (100 % reduction).

Table 4.3. SMA of different concentration of mixed VFAs in base of concentrated ASBR Substrate

Erythromycin Concentration (mg/L)	Maximum Specific Methanogenic Activity* (mL CH_4/ g VSS.d)					
	Acetic acid (5100 mg/l)	Propionic Acid (1290 mg/l)	Butyric Acid (834 mg/l)	Valeric acid (248 mg/L)	Mixed VFAs and Alcohols (11140 mg/l)	Blank 0
0	82.2	16.4	29.6	17.1	117	3.1
500	44.6	2.9	0	2	72	3.3

* All of tests was done with using fresh granules at OLR= 3 g COD/L.d in ASBR unexposed with Erythromycin

- The individual and mixed VFAs concentration is in base of Table 3.1

The analysis of VFAs after SMA test on ASBR influent substrate with using 0, 200 and 500-mg/L erythromycin indicated that the accumulated butyric acid after tests were: 1, 724, and 828 mg/l respectively (Table 4-4). The trend in the data provided the incentive to continue other SMA tests on butyric acid.

Table 4.4. VFAs concentrations (mg/l) before and after SMA Test with and without Erythromycin using mixed ASBR substrate

	Acetic Acid		Propionic Acid		Butyric Acid		ValericAcid	
	Before	After	Before	After	Before	After	Before	After
Substrate Without Erythromycin	4120	29	1525	1245	901	1	1637	1458
Substrate With 200 mg/L Erythromycin	3968	123	1403	1413	849	724	444	1094
Substrate With 500 mg/L Erythromycin	4278	40	1443	1326	973	828	585	912

Development of antibiotic resistance

Possibility of development of erythromycin resistance during ASBR operation was evaluated using butyric acid as the sole substrate in SMA tests. The results are shown in Figure 4.3. SMA tests using sludge from the ASBR before the addition of erythromycin (sludges sampled on days 50 and 58) are compared with the results for sludges exposed to erythromycin for 47 days (sludge was sampled on day 135). SMA tests for both sludges and without any addition of erythromycin showed significant methane production. Saturated fatty acid-beta oxidizing bacteria responsible for converting butyric acid were still present after the ASBR had been fed erythromycin for 47 days but the maximum specific rate of methane production was significantly reduced suggesting a reduction in the bacteria population. The sludges collected from the ASBR before and after addition of erythromycin responded differently to the addition of erythromycin in the SMA test. The specific rate of methane production for the sludge previously exposed to erythromycin was much higher than the no exposure case (this sludge did not produce any methane). Thus, at least for the saturated fatty acid-beta oxidizing bacteria, it can be concluded that long term exposure to erythromycin resulted in the development of resistance, or that resistant populations that had been present at very low levels were able to become more competitive in the presence of erythromycin. While some of bacteria likely developed resistance during the period of 47 days of exposure, others did not. This is substantiated by lack of improvement in the COD removal efficiency and biogas production in the ASBR.

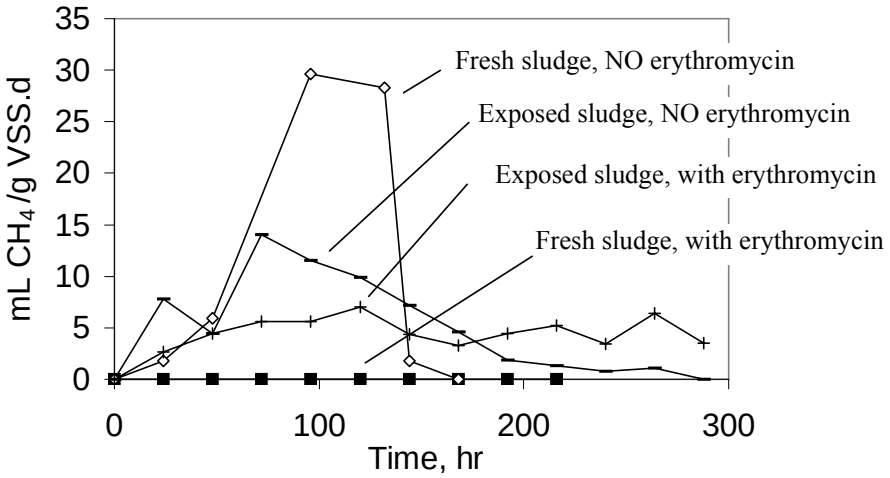
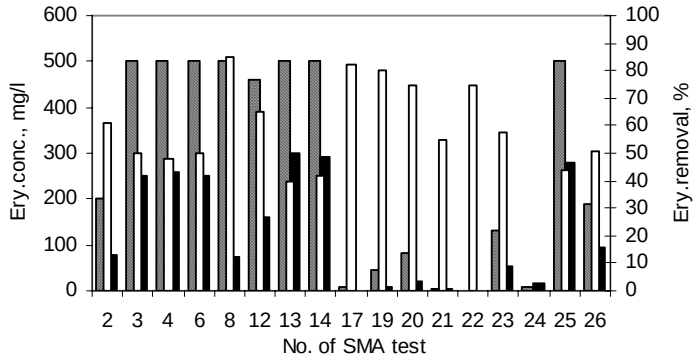


Figure 4.3. Specific methanogenic activity (SMA) using butyric acid for sludges that had either not been exposed to erythromycin or that has been exposed for 47days to erythromycin in the ASBR operation. No erythromycin was added to two of the tests while the other two tests had 500 mg/l of erythromycin.

Erythromycin Removal in ASBR and SMA Tests

In this study, the erythromycin analyses before and after 16 SMA tests revealed a removal of 42 to 82% (Figure 4.4). However, as seen in Figure 4.5, erythromycin removal in ASBR after adding 1 mg/l erythromycin to substrate for during one month were in range of 51-94 % and then 200 mg/l (next one month) were from 22 to 89 % (Table A.4. Appendix).



SMA, No.	Substrate	Inoculums (granule)
2 &3	ASBR substrate (concentrated)	Fresh **unexposed** with Ery.
4	Propionic acid	**Fresh** unexposed with Ery.
6	Butyric acid	Fresh unexposed with Ery.
8	Valeric acid	Fresh unexposed with Ery.
12	Blank	Fresh unexposed with Ery.
13&14	Acetic acid	Fresh unexposed with Ery.
17, 19 &20	ASBR substrate (Diluted)	**Stored** unexposed with Ery.
22, 23&24	Butyric acid	Stored unexposed with Ery.
25&26	Butyric acid	Fresh **exposed** with Ery.

Figure 4.4. Concentrations and removal of Erythromycin in SMA tests: (▨) before; (■) after SMA test, and erythromycin removal, % (□)

Two possibities exist for the removal of erythromycin: adsorption and/or biodegradation. Adams et al. (2002) investigated the removal of seven antibiotics from surface and distilled water in eight water treatment processes. Sorption on Calgon WPH powdered activated carbon, reverse osmosis, and oxidation with chlorine and ozone under typical plant conditions were all shown to be effective in removing the studied antibiotics. With PAC dosage 50 mg/l the percent removal was greater than 90 % for all compounds. However, they did not use granular activated carbon (GAC) to treat antibiotics.

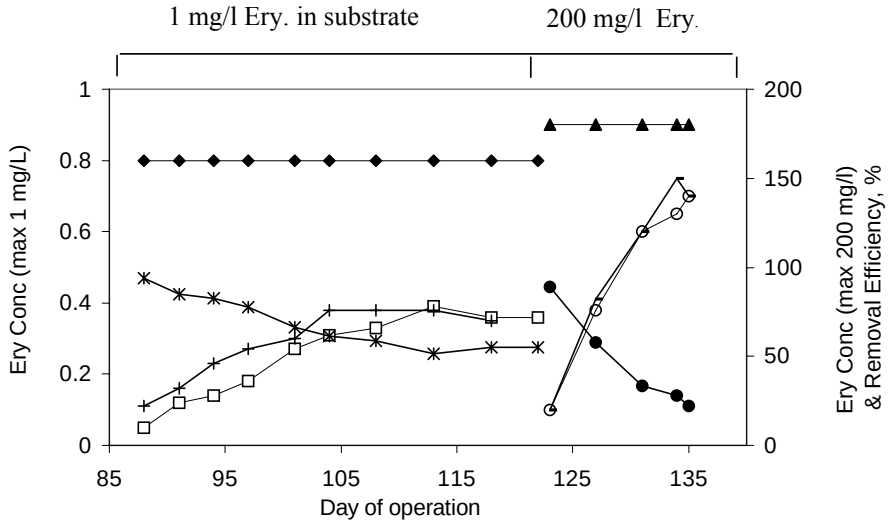


Figure 4.5. Erythromycin in ASBR: In Substrate: 1 (♦) and 200 (▲) mg/l erythromycin; Fill time: 1 (+) and 200 (-) mg/l erythromycin; Decant time: 1 (□) and 200 (o) mg/l erythromycin; Removal Efficiency: 1 (∗) and 200 (●)mg/l erythromycin ;

Giger et al. (2003) in their study on fate of antibiotics (including macrolids: erythromycin) reported elimination (80-90%) of fluoroquinolones ciprofloxacin and norfloxacin in wastewater treatment by sorption transfer to sewage sludge. They also found the erythromycin concentration in secondary WWTP effluent (i.e. mechanically and biologically treated wastewaters) from nondetectable to 287 ng/l.

Kim et. al (2004) in their research on adsorption and clay-catalyzed degradation of erythromycin-A on homoionic clays, indicted that the adsorption of erythromycin on clay particles became easier as concentration increases. This implies a tendency of association between the adsorbed molecules to hold them on the surface. Adsorption process of erythromycin-A depends on types of clays and exchanged cation. The catalytitic activity of montmorillonites for the erythromycin –A degradation was much greater that of kaolinites. Although, the adsorption of erythromycin on clay particles is not comparable with sludge particles, however, in current research the erythromycin concentration was not a main factor influencing erythromycin adsorption on flocculent (in SMA test) and granular (in ASBR) sludge.

Since erythromycin is a weak base, the pH of bulk solution is important for the protonation of a weakly basic organic compound, and plays a role in exchange of cations with particles. The difference between pH of bulk solution in SMA test and ASBR was less than 0.5 unit of pH, so there does not seem to be a correlation between pH and difference in erythromycin removal in ASBR and SMA tests.

Methane Production, COD Balance, and yield Coefficient

The biogas collected throughout this study was composed of around 80% methane; the balance was made up with other gases including CO_2. Specific methane production rate (SMPR) was calculated from the COD equivalent of daily methane production and the total biomass (VSS) inside the reactor. At standard condition (25 °c, 1 atm.) 1 m^3 CH_4 weighs 0.667 kg and each gram of methane is equivalent to four grams of COD.

Figure 4.6 illustrates a linear relationship between SMPR with specific substrate utilization rate (SSUR) with a slope of 0.9001. In average, 90% of the COD removed was converted to methane and the remaining 10% was apparently converted to biomass. This ASBR result was comparable to SMPR of 0.926 for VFA mixture (Fang et al. 1995), and 0.94 for formate (Chui et al. 1994), both of them on the UASB treatment, and SMPR of 0.83 for landfill leachate on the ASBR (Timur and Ozturk, 1999). The relation between microbial growth and substrate utilization can be formulated in a basic expression ($r_x = 1/Y.\ 1/\theta_x + b/Y$). In order to calculate Y (biomass yield coefficient, $gVSS/gCOD_{rem}$), specific COD utilization rate (r_x) was plotted vs. the reciprocal of solids retention time ($1/\theta_x$) (Figure 4.7). According to the equation, slope of the curve give 1/Y. Calculated sludge yield (Y) was 0.066 $gVSS/gCOD_{rem}$, which was comparable to the yield value on mixed VFA (0.054 $gVSS/gCOD_{rem}$) as reported by Fang et al. (1995), and individual VFA, acetate (0.04-0.054 $gVSS/gCOD_{rem}$), propionate (0.042-0.051 $gVSS/gCOD_{rem}$), and butyrate (0.047 $gVSS/gCOD_{rem}$) as reported by Lawrence and McCarty (1969), and also 0.05 $gVSS/gCOD_{rem}$ on formate (Chui et al. 1994), 0.1 $gVSS/gCOD_{rem}$ on landfill leachate (Timur and Ozturk, 1999).

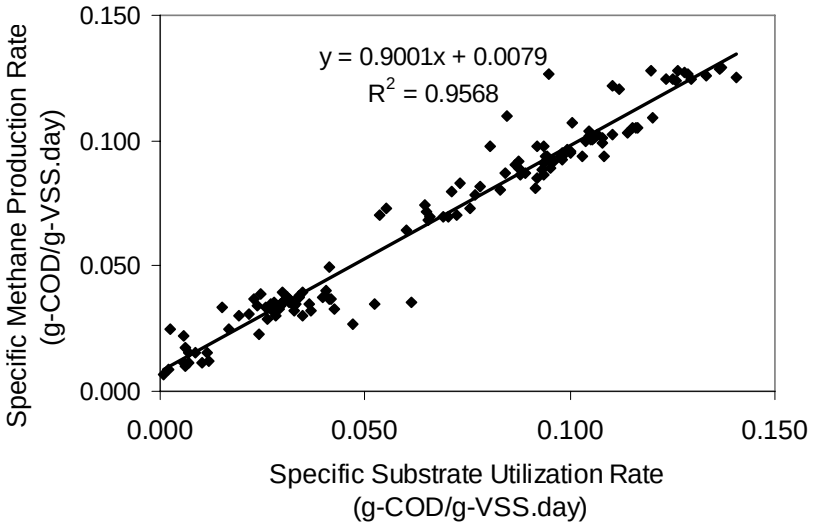


Figure 4.6. Specific Methane Production Rate at Various Specific Substrate Utilization Rate

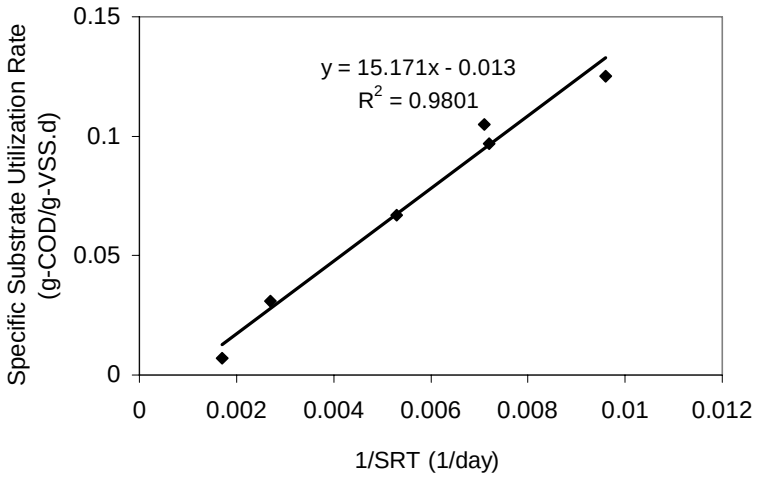


Figure 4.7. Determination of biomass yield coefficient

Low organic loading in SMA test

The effect of lower organic loading rate (diluted substrate, 1/5 dilution of concentrated) compared to main ASBR substrate (concentrated, 16.25±200 g COD/l) on SMA tests was evaluated in presence of different concentrations of erythromycin (Figure 4.6). As shown in Figure 4.8, erythromycin behavior on diluted and concentrated substrates with regards to used seed is not significantly different, but gas production rate in SMA tests on diluted substrate in lower than concentrated substrate.

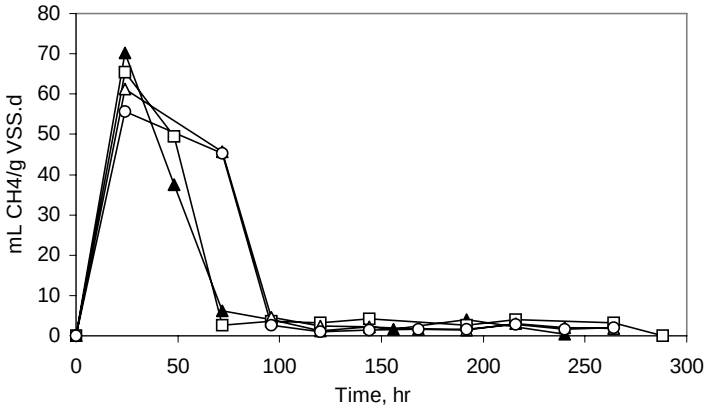


Figure 4.8. SMA test on Diluted ASBR substrate with using: 0 (□); 10 (▲); 50 (Δ); and 100 (o) mg/l erythromycin on unexposed granules with Ery stored in 4 °c;

Figure 4.9 reveal that the erythromycin in concentrations as low as 1 mg/l has considerable effects to decrease methane production in batch test. This result is correspondence with effects of continuous erythromycin injections in ASBR (Figure 4.1). This result also is accordance with the results of Poels et al. (1984) who investigated the effects of three does (No: 1, 2 and 3) : 0.4, 2, and 4 mg/l of erythromycin on methane production from piggery waste in completely mixed laboratory fermentors with HRT 20 days and loading rate 2-4 gCOD/L.d (semi-continuous feedings at 30-33°c). In this experiment, effluent COD and acetic acid were increased from 6500 and 190 mg/l in does-1 to 6800 and 300 mg/l in does-2 and to 7800 and 350 mg/l in does-3, respectively. The concentrations of COD, acetic acid, propionic acid and $\sum C_4$-C_5-C_6-VFA in influent were: 26400, 8800, 3700, and 1650 mg/l, respectively. However, the result of this study doesn't completely support the findings of Campurbi et al. (1988) who found in their batch and semi-continuous experiments, that erythromycin do not inhibit methanogenic activity at concentrations up to 50 mg/l.

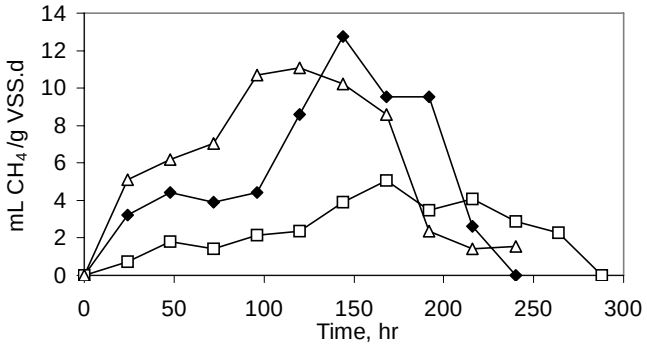


Figure 4.9. SMA test on butyric acid with using: 1 (Δ); 10(♦); and 200 (□) mg/l Erythromycin. on unexposed granules to Erythromycin stored in 4 °c

Effects of storage on activity of granules

To illustrate the role of storing granules in cold room (4 °c) for two months, two other SMA test was done without erythromycin and on two different sample of unexposed granules to erythromycin: fresh and stored to cold room. The difference of the maximum methanogenic activity was also considerable: 29.6 and 5.5 mL CH_4/g.VSS.d (81.4 % reduction) for fresh and stored granules, respectively (Figure 4.10).

Bae et al. (1995) found that during 10 months storage of granules without feeding at room temperature condition and also at 4 °c, the specific methanogenic activity (SMA) decreased 60% and 80%, respectively.

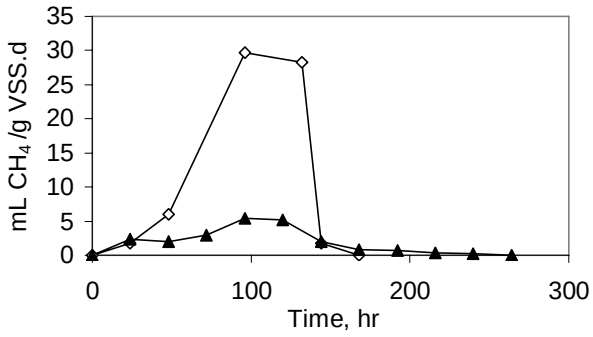


Figure 4.10. SMA test on butyric acid without erythromycin: on unexposed granules to Erythromycin stored in 4°c (▲); and on fresh granules unexposed to erythromycin. in ASBR (o)

ASBR Profiles

For every change to operation condition, in base of loading rate without and with erythromycin in one cycle, samplings were regularly withdrawn from reactor and measured for concentration of individual VFAs, COD, biogas and pH to study evolution of these parameters during a cycle. The profiles obtained for typical cycles with simulated pharmaceutical wastewater are presented in Figure 4.11.

Since, the cycle time in this study was 24 hr, so to prepare each profile, the samples in the first 7 hours of cycle were taken with interval of 30 min, during next 5 hr with interval of 60 min, next 8 hr, every 120 min. or more, and during 4 hours before end of cycle, every 1 hr. In the condition prevailing in this study, a treatment cycle can be divided into three parts for VFAs and pH, and two part for COD and biogas. In the first part, corresponding to the around 10 hours following feed input, gas production with a sharp rising was maximum, and the same time COD and VFAs with a sharp drop was minimum. In the second part, COD and VFAs decreased at a lower rate, compared to the first part. In the third part, there was a little increase to certain VFAs such as valeric and caproic acids.

Since, there was individual VFAs in substrate (Table 3.1), so the acidification phase was restricted to anaerobic oxidation of ethanol, 2-propanol, and also biodegradation of caproic to butyric and acetic acids, dissociation of valeric acid to propionic and acetic acids, and finally conversion of butyric and propionic acids to acetic acid and hydrogen. Figure 4.11 shows the occurrence of one peak in concentrations of propionic and acetic acids (during all of OLRs) in cycle time 2:40, another peak in graph of propionic acid in OLR, 1&2 gCOD/l.d, in cycle time 5:00, and one peak in valeric acid curve in OLR, 1 gCOD/l.d. The first peak (cycle time 2:40) was corresponding with sharp loss in pH curve at cycle time 2:40.

The odd-numbered C fatty acids such as valerate are converted to propionic and acetic acids and H_2. (Drake, after 1994). However, no propionic acid is formed when long chain even-numbered carbon fatty acids are fermented. Each of the longer-chained organic acids will be degraded microbiologically to either butyric or propionic acid and then to acetic acid and methane (Malina et al. 1992).

The production of caproic acid during all of OLRs, in spite of the absence of this acid in influent substrate and also valeric acid in certain OLRs (Table 4.1) occurred in ASBR. In base of third part of graphs in Figure 4.11, presumably the interaction of C_2-C_6 caused the reproduction of some VFAs in the end of cycle. In OLR 3 gCOD/l.d without erythromycin (steady state in ASBR), in run time 12-13 hr, the concentrations of all of VFAs have received to zero, and then production of valeric and caproic acids have been started. These VFAs re-production was more evidenced in stages of start-up and after adding Erythromycin.

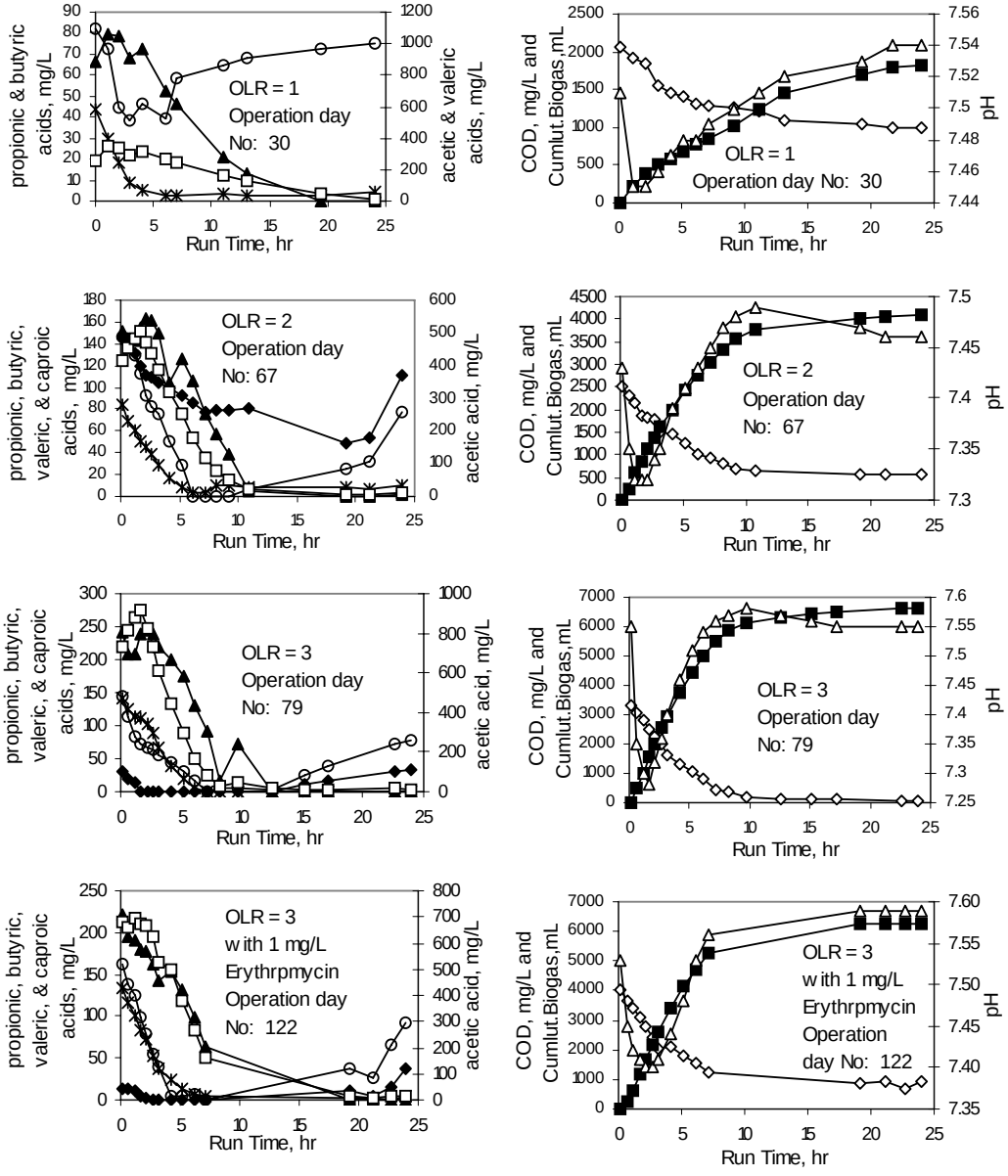


Figure 4.11. Profiles of COD (◊); Biogas (■); pH (Δ); and individual VFAs including: Acetic (□); Propionic (▲); Butyric (×); Valeric (○); and Caproic (♦) acids; at different OLR and with 1 mg/ Erythromycin, during a cycle in ASBR treating simulated Pharmaceutical effluent

VFAs in ASBR

The concentration of acetic, propionic, butyric, and valeric acids in influent substrate were 5.2, 1.3, 0.84, and 0.25 g/l, with COD equivalent 5.5, 2, 0.75, and 0.5 g/l respectively (Abbott WTP data 2003). The results obtained in Figure 4.12, reveal that C_2-C_4 VFAs are amenable to anaerobic treatment using ASBR, but C_5 and C_6 VFAs were two compounds found to be poorly degraded or even produced during a run, especially in stages of start-up and after adding erythromycin. High levels of valeric and caproic acids in the ASBR effluent evidenced this (Figure 4.12, and Tables 4.1 & 4.2).

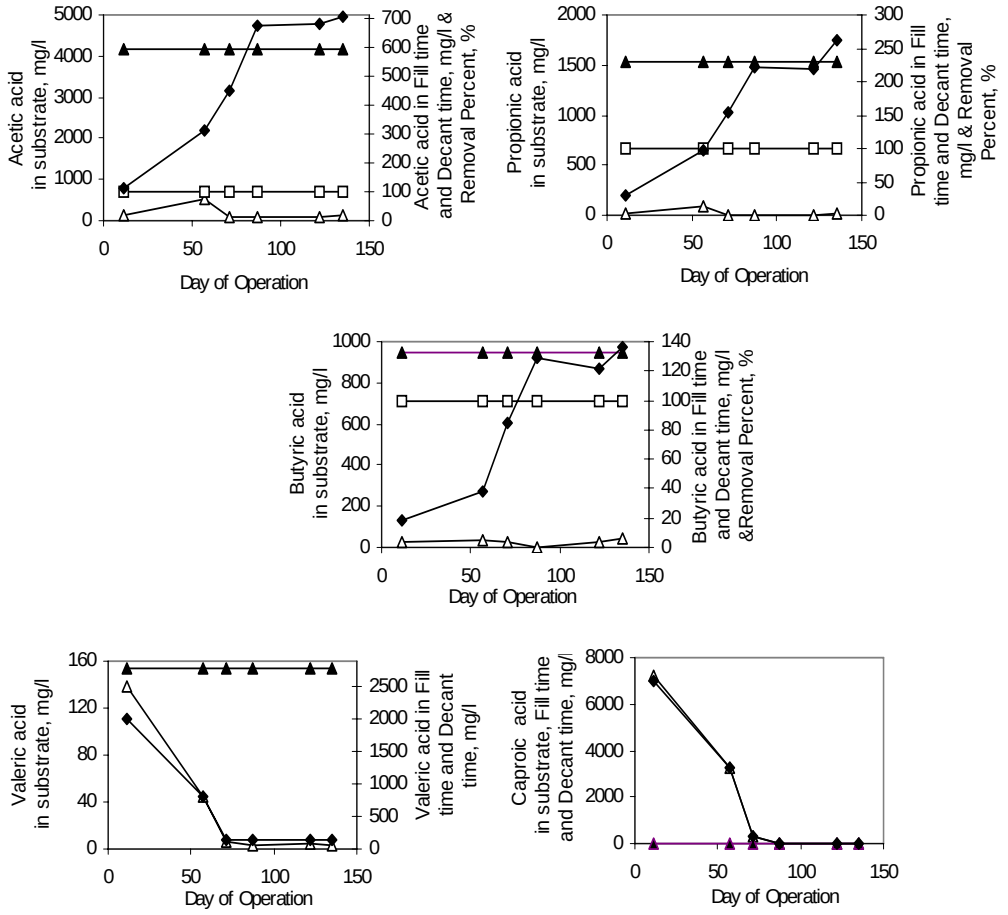


Figure 4.12. VFAs in ASBR including acetic, propionic, butyric, valeric and caproic acids: Influent: in substrate (▲); Influent: cycle time 00:10 (♦); Effluent: Decant time (or cycle time 24:00) (Δ), Removal efficiency, % (□);

SMPR in ASBR and Batch tests

Maximum methane production rate (SMPR) in ASBR at optimum operation conditions, without erythromycin is 0.102 g-CH_4-COD/g VSS.d, a little lower than 0.186 for batch test with using the same substrate composition and concentration. The higher SMPR in batch test can be related to higher HRT. (Table 4.5).

Table 4.5. Maximum SMPR in ASBR and Batch tests

	ASBR						Batch Test*			
Organic Loading Rate, g COD/l.d	0.4	1	2	3	3	3				
COD in start of run, g/l				3.25	3.25	3.25	3.25	3.25	3.25	3.25
Erythromycin concentration, mg/l	0	0	0	0	1	200	0	10	50	100
Maximum Methane Production Rate (SMPR), g-CH_4-COD/g VSS.d	0.012 ± 0.003	0.034 ±0.006	0.072 ±0.005	0.102 ±0.007	0.093 ±0.007	0.093 ±0.007	0.186	0.199	0.174	0.158
Hydraulic Retention Time, day	42	17	8	6	6	6	10	10	10	10

*With using diluted ASBR substrate

Specifications of SMA tests

In this study, 28 SMA tests were performed. Some of samples were duplicated. The Table A-1 (Appendix) reveals the specifications of SMA tests such as: COD, erythromycin and individual VFAs concentrations before and after test, and also used substrate in each test. The Table A-2 (Appendix) shows the results of maximum specific methanogenic activity in several SMA tests.

CHAPTER 5

CONCLUSIONS

1. The influence of erythromycin on anaerobic treatment performance and on the utilization of specific compounds in a synthetic pharmaceutical wastewater was evaluated using batch tests and long-term reactor operation of an ASBR. It was found that biogas production and COD removal in the ASBR reduced by 5 to 10 % when erythromycin was added to influent. Based on SMA tests with individual compounds it was shown that certain pathways of anaerobic food web were completely inhibited (e.g., syntrophic conversion of butyric acid) while others were only slightly affected (e.g., aceticlastic methanogenesis). Long-term exposure to erythromycin in the ASBR resulted in increased degradation of butyric acid in the presence of erythromycin presumably due to the development of an antibiotic resistance among the syntrophic bacteria degrading butyric acid (fatty acid-beta oxidizing bacteria).
2. The specific methane production rate (SMPR) increased linearly with the specific substrate utilization rate (SSUR) with a slop of 0.9001and revealed that 90% of the COD removed was converted to methane.
3. Maximum specific methane production rate (SMPR) in ASBR at steady state without erythromycin is 0.102 g-CH_4-COD/g VSS.d; lower than 0.186 for batch test. In presence of erythromycin the maximum SMPRs in ASBR and Batch test are 0.093 and 0.158 g-CH_4-COD/g VSS.d, respectively. The higher SMPRs in batch test can be related to higher HRT.
4. Calculated sludge yield (Y) was 0.066 gVSS/gCOD_{rem}, which was comparable to the reported yield value on mixed VFA (0.054 gVSS/gCOD_{rem}).
5. Erythromycin removals were in rang of 42-82 % in batch tests. The removal percents in ASBR were from 51 to 94 % for low (1 mg/l) and 22-89 % for high (200 mg/l) erythromycin concentrations.
6. Maximum methanogenic activity was reduced from 29.6 to 5.5 mL CH_4/g.VSS.d (81.4 % reduction) after two month storing of granules in cold room (4 °c) confirming other research suggested storage of granules in low temperature is not a convenience method.

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In the Name of Allah

PERFORMANCE COMPARISON OF HORIZONTAL - FLOW ANAEROBIC IMMOBILIZED SLUDGE (HAIS) AND UASB REACTORS USING SYNTHETIC SUBSTRATE

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ABSTRACT – PART II

The performance of fixed-bed and suspended-growth high-rate anaerobic reactors was investigated in this study. Two 2-L and 3.3-L horizontal-flow anaerobic immobilized sludge (HAIS) reactors were filled with polyurethane (PU) and polyethylene (PE) beads with surface to volume ratios of 1100 and 1250 m^{-1}, density of 700 and 12 kgm^{-3}, and bed porosities of 0.42 and 0.28, respectively. The 3.3-L upflow anaerobic sludge blanket (UASB) reactor was filled with granular sludge with a VSS concentration of 45 g/L and both sets of reactors were operated at 32±2 ºC. Synthetic glucose-based substrate was the main carbon source (COD of 1750±250 mg/L) feeding both sets of reactors.

In this study, the concept of quantitative CT number in terms of Hounsfield units (H) that is approximately linearly related to the mass density of the attenuating tissue and materials in objects in Computed Tomography Scanning (CT-Scan) technology was used to characterize of the HAIS reactor contents including biofilm, media, and biogas. Then the obtained CT-images from various sections of HAIS reactors were evaluated with using the concept of Resolution (pixel per inch) in Photoshop to estimate the VSS contents of HAIS reactors.

The results confirmed satisfactory performance of HAIS in providing to provide suitable environmental conditions for biomass growth and retention during the short startup period of 25 days. At the optimum period of operation, the loading rates of reactors were: organic loading rate (OLR) of 13 ± 6 g COD/ L.d and F/M: 1.34±0.82 g COD/g VSS.d in UASB; surface loading rate (SLR) of 28 ± 12 g COD/ m^2 .d and F/M: 1.72±0.77 g COD/g VSS.d for HAIS-PU; and SLR of 23 ± 14 g COD/m^2 .d and F/M of 1.52±0.99 g COD/g VSS.d for HAIS-PE. For these loadings, the observed COD removal efficiencies and biogas productions were: 64.3±15.2 % and 1771±704 mL/d for UASB; 63.5±17.5 % and 1160±400 mL/d for HAIS-PU; and 61.6±18.6 % and 1018±645 mL/d for HAIS-PE.

It was found that UASB operation with granular sludge and more efficient gas-liquid-separator (GLS) has a better performance than the both HAIS reactors containing immobilized sludge, and with two gas collectors in different shapes: perforated tube (HAIS-PU) and sheet (HAIS-PE).

The performance of HAIS-PU was better than PE possibly due to higher bed porosity (ε) of HAIS-PU (ε=0.42) compared to HAIS-PE (ε= 0.28). Channeling effects for low bed porosity reactors can be the main factor responsible for such performance

KEYWORDS

Anaerobic wastewater treatment, Fixed bed reactor, HAIS reactor, UASB, CT-Scanning, Resolution and Histogram in Photoshop;

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

Introduction

The science and 'art' of wastewater engineering stretches only slightly beyond one hundred years. Within this period, the applied technology has certainly made significant strides in promoting disease control and environmental protection.

High concentration of biodegradable organic matter renders aerobic treatment inefficient because of high-energy requirements for aeration, limitations in liquid-phase oxygen transfer rates, and the high sludge production. Traditional anaerobic processes are also limited by low rates of organic matter removal, long hydraulic retention times, accumulation of excessive residual organic matter and intermediate products, and large reactor volume requirements. Recent developments in anaerobic treatment processes, especially high retention of biomass in the reactor, has made the decoupling of solids retention time and hydraulic residence time in the high-rate anaerobic reactors possible.

Fixed-film treatment plays an important role in wastewater engineering history, particularly since it represented the original biological mechanism. Beginning with options like the trickling filter, intermittent filter and contact bed, fixed-film systems dominated the technology of wastewater treatment for several decades. And although this status has subsequently been assumed by suspended growth process, there is unquestionably a resurgence of interest in fixed-film applications (Alleman, 1985).

Fixed-film biomass accounts for almost all of the activity observed and the role of suspended growth is minimal and significant only for enhancing biofilm development on the support media (Ware, 1989).

The upflow anaerobic sludge blanket (UASB) and horizontal-flow anaerobic immobilized sludge (HAIS) processes are the primary suspended and fixed-growth variations of these systems. UASB systems have been widely adopted for treatment of medium to high-strength industrial wastewaters (Lettinga and Hulshoff Pol, 1991, Fang et al., 1995). HAIS system was introduced by Zaiat et al.(1994) and has shown promising results for treating various types of municipal and industrial wastewaters (Zaiat et al., 1997 and 2000 ; Varesche et al., 1997 ; Cadavid et al., 1999 ; Sarti et al., 2001 ; and Oliveira et al., 2004).

Oliveira et al. (2004) investigated the degradation and toxicity of formaldehyde in a horizontal-flow anaerobic, immobilized-biomass (HAIB) reactor. The results obtained in this study led to this conclusion that the HAIB reactor was suitable for the treatment of formaldehyde-containing wastewater, presenting operating stability throughout the experiment. The probable polymerization of formaldehyde, the use of immobilized cells

on inert and macro-porous support, high biomass concentrations and a hydrodynamic flow pattern similar to plug flow were believed to be the main factors responsible for the excellent performance of the HAIB reactor in the degradation of formaldehyde.

The influence of the liquid- phase mass transfer on the performance of a HAIB reactor treating low-strength wastewater was reported by Sarti et al., (2001). It was concluded that the performance of the HAIB reactor was improved significantly by increasing liquid superficial velocities (v_s), thus decreasing the liquid-phase mass transfer resistance.

The development and the preliminary evaluation of a mathematical model used to design a horizontal-flow anaerobic immobilized biomass (HAIB) reactor were applied to domestic sewage treatment. Based on these data obtained from the mathematical model, a pilot-scale HAIB reactor was constructed adopting some design parameters and following the design criteria established. The HAIB reactor was comprised a 14.5 cm internal diameter PVC tube with length of 14.4 m divided in to five stages of 2.88 m each. A preliminary evaluation of the mathematical model proposed to design the HAIB reactor was performed based on data of chemical oxygen demand (COD) profiles along the reactor length. Although deviations were observed between experimental and theoretical data, the proposed model was found to be suitable for predicting the overall behavior of the reactor (Zaiat et al., 2000).

Different operational parameters influencing reactor performance have been studied including organic and hydraulic loading rates for UASB (Van-Haandle and Lettinga, 1994; and Metcalf & Eddy, 2003), and for HAIS (Zaiat et al., 1997,2000, 2001; Sarti et al., 2001); cell washout in HAIS (Zaiat et al., 1996) and in UASB (Hulshoff pol, 1989); granulation in UASB (Bae et al., 1999; Tay et al., 2000; and Torkian et al., 2002); the use of polyurethan as microbial carrier (Varesche et al., 1997); and the comparison of the immobilized cell (Varesche et al., 1997) to elucidate the behavior and thus the performance of these processes.

In this study, performance of HAIS and UASB systems in treating synthetic glucose-based substrate was studied in laboratory reactors. Particular attention was paid to system stability under different organic and hydraulic loading rates. The results for spatial variation in performance of HAIS system is also presented.

Objectives

The objectives of this research project were:

(1) To evaluate the optimum organic loading rate and upflow velocity in UASB and surface loading rate and superficial velocity in HAIS reactors; and also optimum retention time in both set of reactors;

(2) To develop a method for estimation of biomass content in a packed bed horizontal flow bioreactor through CT-Scan Technology and image analysis by software of Photoshop;

(3) To compare removal efficiencies of COD and biogas production in both sets of reactors;

(4) To determine the effect of bed porosity on performance of fixed bed reactors;

Organization of the report

The literature review on subjects of this study, such as immobilized cell horizontal flow packed bed systems or HAIS bioreactors, and granulation in UASB systems, the concepts of Computed Tomography scanning (CT-Scan), image analysis of biofilm in fixed film reactors through Photoshop are presented in Chapter 2. The materials and methods are described in Chapter 3, results and discussion in chapter 4, and finally conclusions of this study in chapter 5.

CHAPTER 2

LITERATURE REVIEW

The current state of researches and knowledge in regard to topics of this study including: fixed bed, Horizontal flow (HAIS) and UASB reactors; Computed Tomography Scanning (CT-Scan) Technology, and concepts of image analysis through Photoshop software, is described in this chapter.

Immobilized Bioreactors

The first generation of anaerobic bioreactors was composed of mixed units without sludge recycle in which the biomass retention time (θ_c) was similar to the hydraulic retention times (θ_h). The better understanding of the biological processes resulted in the development of reactors with (θ_c) higher than (θ_h) that utilized secondary settlers for sludge recycle. Immobilized sludge reactors, in which the biomass is attached to an inert support or self-immobilized, permit the attainment of high (θ_c), even when low values of (θ_h) are applied. For most of the immobilized biomass reactor configurations, sludge recycle can be suppressed. Upflow anaerobic sludge blanket (UASB) reactors, anaerobic filters and expanded- or fluidized-bed anaerobic reactors are examples of immobilized-cell systems.

The design and scale-up of anaerobic immobilized biomass reactors has usually been carried out utilizing empirical criteria. The utilization of such criteria makes the system optimization very difficult, since the "black-box" approach has application strictly limited to the boundaries within which the model was developed. The adoption of fundamental criteria is essential for permitting the design of heterogeneous units at desired organic matter degradation rates (Zaiat, Passig, and Foresti, 2000).

The development of immobilized cell bioreactors led to increasing use of anaerobic technology for wastewater treatment. In fact, anaerobic filters (AF), anaerobic fluidized bed reactors (AFBR) and up-flow anaerobic sludge blanket (UASB) reactors have their performance closely associated with the biomass self-immobilization processes occuring on the surface of a packed media (AF), on suspended inert particles (AFBR), or in the sludge granules (UASB), which retain the biomass within the reactors, allowing their operation at very high cellular retention times (CRT), even at very short hydraulic detention times (HDT) . On the other hand, unexpected failures in biofilm and granular sludge development may happen due to several factors such as the wastewater characteristics, the nature of the support media, and reactor design and operation. Therefore, a variable degree of uncertainty is present in designing and operating such full-scale anaerobic reactors since the present knowledge of the self-immobilization process is not sufficient to guarantee its occurrence and control in adverse conditions (Zaiat, Cabral, and Foresti, 1996).

Attempting to assume the biomass immobilization control, Karube et al. (1980) succeeded in entrapping active methanogenic bacteria in agar gel, polyacrylamid and collagen membrane, in short duration experiments. Huysman et al.(1983) studied sepiolite and

polyurethane foam for methanogenic bacteria immobilization, concluding that polyurethane foam is an excellent biomass carrier. Their findings were confirmed by Fynn and Whitmore (1984), Calzada et al. (1984) and Gijzen et al.(1988) . However, high liquid superficial velocities were found to cause severe cell washout from bio-particles. Other carriers for biomass immobilization, such as polytetrafluorethylene (PTFE), polypropylene (pp), polyethylene (PE), polyvinyl chlorine (PVC), polyacetal (PHC) and polyamide (PAM) were also assayed by Verrier et al. (1987), while Zellner et al. (1987) and Kawase et al (1989) made use of porous ceramic.

Although the utilization of carriers for biomass (cell or sludge) entrapped immobilization offer an alternative for increasing the CRT, several aspects related to performance optimization and operational stability have to be solved to permit their use in full-scale anaerobic reactors. One of the key factors is to find the reactor configuration adequate to the type and dynamics of the carrier within it. In fact, during the operation of a bench-scale UASB reactor seeded with methanogenic and acidogenic sludge immobilized in agar gel, Del Nery (1993) reported an unexpected accumulation of gel matrices in the gas/solid/liquid separation device. Moreover, although the reactor performed well, the results indicated mass transfer limitations associated with the use of agar gel matrices. While internal diffusion limitations can be partially overcome by decreasing the particle size, external diffusion can sometimes be improved by increasing the liquid superficial velocity. However, this would imply increasing the forces, which cause biomass washout, and thus it should be carefully studied in anaerobic reactors.

Based on these pervious works, Zaiat et al. (1994) proposed the horizontal-flow anaerobic immobilized sludge (HAIS) reactor, using polyurethane foam for biomass immobilization. A bench-scale HAIS reactor was first assayed for the treatment of Kraft paper industry wastewater exhibiting a performance comparable to the full-scale UASB reactor installed at the paper industry (Zaiat, Cabral, and Foresti, 1996).

HAIS reactors

HAIS or HAIB definition. A new configuration of anaerobic fixed- bed bioreactor, the horizontal-flow anaerobic immobilized sludge (HAIS) or biomass (HAIB) reactor was conceived to be developed under rational basis. A HAIS reactor containing immobilized sludge in polyurethane foam matrices (Zaiat, Vieira, and Foresti, 1997). In such reactor configuration, the liquid has horizontal flow while the gas moves vertically, thus favoring the predominance of a plug-flow hydrodynamic regime (Zaiat, Cabral, and Foresti, 1996). A very short start-up period was observed and the reactor achieved stable operation by the eighth day (Zaiat, Vieira, and Foresti, 1997).

*Conception of the bench-scale HAIS reactor***.** The horizontal-flow anaerobic immobilized sludge (HAIS) bench-scale bioreactor is comprised of a 1 m long glass tube of 0.05 m diameter provided with a perforated tube of 0.9 cm diameter installed at its upper part for gas separation and collection. The reactor's total volume is 2 liters. The HAIS scheme is shown in Figure 2.1.

The HAIS reactor was filled with polyurethane foam cubic matrices (side of 3-5 mm) containing anaerobic immobilized sludge and it is meant to be a predominantly plug-flow

regime reactor. The axial mixing due to the formation and vertical rise of gas across the horizontal liquid flow, as well as the tubular form of the reactor, is expected to promote a plug-flow like regime behaving as N-mixed reactors in series. Moreover, the gas tube collector along the reactor permits the minimization of dead volume for gas separation (Zaiat et al., 1996)

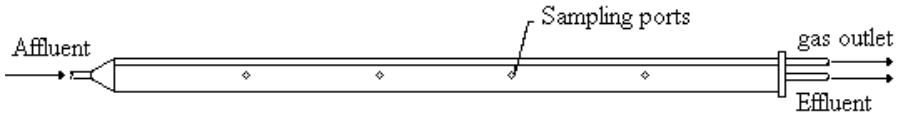

Figure 2.1. Scheme of the bench-scale horizontal-flow anaerobic immobilized sludge (HAIS) reactor (Zaiat and Vieira et al., 1996)

Further development of the HAIS reactor. It depends on knowledge of the fundamental aspects relating to the limiting liquid velocities, internal and external mass transfer resistance, hydrodynamic characteristics and biological growth and substrate utilization kinetics (Zaiat, Cabral, and Foresti, 1996).

Degradation of toxic substances in bench scale HAIB. Oliveira et al. (2004) evaluated the performance of an anaerobic fixed-bed reactor having polyurethane foam as support material for biomass immobilization in the treatment of formaldehyde based substrate (Figure 2.2). According to the ranking of environmental impacts generated by 45 chemical products formaldehyde ranks in first place. Many studies have demonstrated the toxicity and the carcinogenicity of this substance. As a disinfectant, a 0.5% formaldehyde solution (~5.4 g/L) destroys all species of microorganisms in a period of 6–12 h.

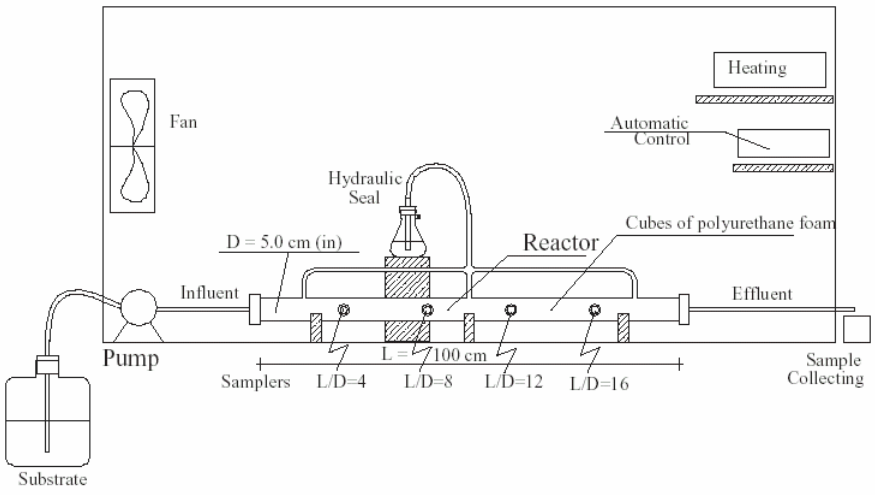

Figure 2.2. Scheme of the bench-scale HAIB reactor (Oliveira et al., 2004)

The literature contains little information about the anaerobic degradation and toxicity of formaldehyde. The pathway of anaerobic degradation of formaldehyde and the microorganisms involved in this process are not yet well defined. It is not clear if formaldehyde is converted directly into methane or if intermediate products act as substrates for methanogenic microorganisms. Similarly, there is no consensus about the concentration of formaldehyde that causes inhibition of the anaerobic biomass activity or about the most appropriate system to treat formaldehyde-containing wastewaters, the intermediate products of degradation, the microorganisms responsible for each degradation pathway and the importance of co-substrates.

The full potential of the anaerobic biomass's adaptation to many toxicants could be achieved if common sense and patience were used in exposing the biomass initially to relatively low concentrations and gradually increasing the concentrations up to a maximum value. The formaldehyde is a good example of a compound that is highly toxic in high concentrations, but rapidly biodegradable in lower concentrations. Many reactors have been applied for the evaluation of the anaerobic degradation of formaldehyde, among them are batch reactors, continuous stirred tank reactors, anaerobic filters, upflow anaerobic sludge blanket (UASB) reactors; chemostats and expanded granular sludge bed (EGSB) reactors (Table 2.1).

In those studies, the wastewaters contained formaldehyde concentrations varying from 30 to 3000 mg/L, with or without co-substrate, and the results of inhibition of the microbial activity were quite different. The feeding strategy and the way the formaldehyde concentrations were increased also varied. However, the available literature on the subject was difficult to interpret as well as insufficient for design purposes. Most of the reports found in the literature indicate that high cellular retention times are indispensable to obtain the best performance of the reactor. Continuous- flow reactors were also reported to achieve better results than batch reactors (Oliveira et al., 2004).

Table 2.1. Some results from the literature obtained in continuous systems treating formaldehyde-containing wastewaters (Oliveira et al., 2004)

Reactor	C_{Fin} (mg HCHO/L)	HDT (day)	Limiting dose (mg HCHO/L)	Temperature (°C)	Formaldehyde removal efficiency (%)
Anaerobic filter	100–400	1	400	35	—
Continuous stirring tank	—	10	125	35	85–88
Continuous stirring tank with immobilized biomass	—	10	375	35	95–98
Chemostat	100–1110	14	1110	35	99.9
EGSB	333	1.25	—	—	>93
EGSB	200/400/600	—	—	—	High
UASB	50–2000	0.62	1000	37	98 (of COD)
UASB	95–950	0.62	380	37	95
HAIB	26.2–1158.6	0.5	No limiting dose was observed	35	>95

The results obtained concluded that the HAIB reactor was suitable for the treatment of formaldehyde-containing wastewater, presenting operating stability throughout the experiment. The average formaldehyde and COD removal efficiencies were 92% and 95%, respectively. Efficient formaldehyde degradation was achieved by applying an HDT equal to or less than 4.8 h for influent formaldehyde concentrations ranging from 26.2 to 1158.6 mg HCOH/L (Oliveira et al., 2004).

The polyurethane foam favored the biomass retention in the reactor, with the average biomass reaching the concentration of 26.88 g VSS/L, which allowed for specific formaldehyde loads of over 0.086 gHCHO/g VSS day to be attained for a formaldehyde load of 2.316 gHCHO/Lday. The presence of organic acids with 2–5 carbons along the reactor's length and in the effluent stream indicated the probable polymerization of formaldehyde in the absence of methanol, which may have been rapidly consumed in the anaerobic reactor. The condensation of formaldehyde, with the formation of six-carbon carbohydrates, may also explain the detection of intermediate products with up to five carbons.

The probable polymerization of formaldehyde, the use of immobilized cells on inert and macro-porous support, high biomass concentrations and a hydrodynamic flow pattern similar to plug flow were believed to be the main factors responsible for the excellent performance of the HAIB reactor in the degradation of formaldehyde.

Microscopic exams revealed a biomass containing multiple morphologies. This diversification likely contributed to the assimilation of the formaldehyde and to the intermediate products of degradation. *Methanosaeta*-like organisms probably used acetate preferentially, while *Methanosarcina* fed preferentially on methanol.

The fast adaptation of the biomass to formaldehyde was attributed to its acclimatization to phenol in a previous experiment. The Monod kinetic model provided a suitable representation of the anaerobic degradation of formaldehyde with $r_{max} = 2.79 * 10^{-3}$ ($\pm 3.7*10^{-4}$) mg HCHO/mg VSS. h and $K_S = 242{:}8$ (± 114.1) mg HCHO/L. No inhibition of cellular activity occurred with formaldehyde concentrations of up to 1416.8mg HCHO/L (Oliveira et al., 2004).

Pilot-scale HAIB reactor. A pilot-scale HAIB reactor was constructed in order to evaluate the proposed model and the assumed criteria. The dimensions of the reactor obtained by the theoretical model were slightly changed in order to make construction using commercially available PVC tubes. The reactor was filled with 1 cm side cubic particles of polyurethane foam (density of 23 Kg m‹3), resulting in a bed porosity of 0.4 according to the design criteria adopted. The characteristics of the designed and constructed reactor are presented in Table 2.2. The reactor was composed of five 2.88 m PVC tube stages in series (Figure. 2.3). Intermediate sampling ports were allocated along the reactor length in order to obtain profiles of some monitoring parameters (Zaiat, Passig, and Foresti, 2000).

Table 2.2. Characteristics of HAIB reactor as design based on rational based and the actual characteristics of the constructed reactor (Zaiat, Passig, and Foresti, 2000).

Characteristic	Designed	Constructed
Diameter of the reactor, D (cm)	14.0	14.5
Length of the reactor, L (m)	13.7	14.4
Diameter of the tube for gas separation, D_{TS} (cm)	2.1	2.0
Total volume, V_T (L)	210.0	237.5
Useful volume, V_u (L)	190.0	217.2
Hydraulic retention time, (θ_h) (h)	3.8	4.3
Liquid superficial velocity, v_s (cm s^{-1})	0.1	0.09
Expected COD removal efficiency, E_{COD} (%)	90	94
Length to diameter ratio, L/D	98	99

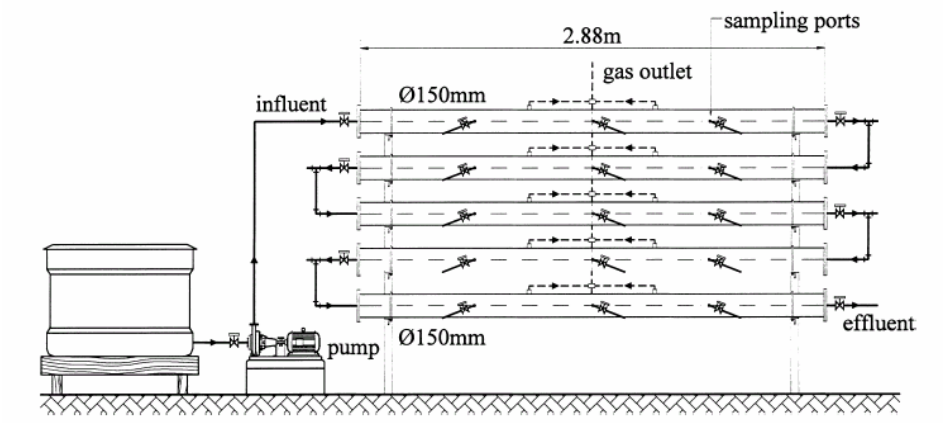

Figure 2.3. Sketch of the pilot-scale Horizontal flow anaerobic immobilized biomass (HAIB) reactor (Zaiat, Passig, and Foresti, 2000).

Mass Transfer in HAIS reactor. Mass-transfer rates are very important in heterogeneous systems, since the materials may have to move from one phase to another. In such systems mass transfer and the relative quantities of the various phases can play an important role in determining the overall reaction rate. Both mass-transfer and biochemical reaction rates must be evaluated for identification of the slowest step. The quantification of the mass-transfer limitations becomes important for the design of high-performance, immobilized cell systems. This performance is directly related to the minimization of such limitations, since the overall reaction rate in these systems can be reduced because of liquid and solid mass-transfer resistance (Zaiat, Passig, and Foresti, 2000).

The liquid-phase (external) mass-transfer resistance decreases as the liquid superficial velocity is increased, due to the decrease of the boundary liquid layer. According to Atkinson (1974), the influence of the fluid velocity on the overall rate of reaction is such

that at high values the overall rate becomes essentially constant. However, this is true only if the external mass-transfer is the limiting phenomenon of the overall process. In fact, a global analysis, including external and internal mass-transfer besides the biochemical reactions, has to be done to relate the sensitivity of the overall reaction rate to the physical and biochemical process parameters.

The solid-phase mass-transfer resistance is influenced by the size of the support particle, besides the characteristics of the inert support and biomass concentration. The diffusion velocity increases as the particle size decreases. Therefore, an operating parameter (Vs) and a design parameter (the size of the support for biomass immobilization) are intrinsically related and affect the overall reaction rate and, consequently, the reactor performance.

A mathematical model, that includes mass-transfer and biochemical reaction rates, was proposed, and used for designing a pilot-scale biological heterogeneous reactor, the horizontal-flow anaerobic immobilized biomass (HAIB) reactor, to be applied for treatment of prescreened domestic sewage. Afterwards, the designed reactor was constructed and assayed in order to evaluate the applicability of the model and the design criteria adopted (Zaiat, Passig, and Foresti, 2000).

Mathematical model proposed to aid in design of HAIB reactor. A bench-scale HAIB reactor was first built to treat recycled paper wastewater (Foresti et al., 1995) and, afterwards, it was applied for kinetic, mass-transfer and hydrodynamic studies (Zaiat et al., 1997a). The HAIB bench-scale bioreactor used in all these previous experiments comprised a 1 m long glass tube of 0.05 m diameter with total volume of 2 l. It was provided with a perforated tube of 0.9 cm diameter installed at its upper part for gas separation and collection. Polyurethane foam was used as support for biomass immobilization in all previous work and this material was chosen as support in the pilot-scale unit.

The studies performed in the bench-scale HAIB reactor permitted the estimation of some fundamental parameters that were applied in the present work to develop a mathematical model for design purposes.

Initially, the flow pattern was considered to be properly described by a plug-flow model. This assumption was based on previous hydrodynamic studies carried out by de Nardi et al. (1999) in the bench-scale HAIB reactor. Afterwards, substrate consumption was considered to follow a first-order kinetic model in the specific case of domestic sewage. This assumption was based on kinetic data obtained for anaerobic degradation of domestic sewage by Vieira (1996). The intrinsic characteristics of this wastewater and the low concentrations of organic matter permitted the representation of the biochemical reaction rate by a first-order model. In this case, the overall anaerobic degradation process can be reduced to only one step. This approach simplifies the model since only one kinetic parameter is necessary to describe the biochemical reaction rate. This parameter refers to the slowest step of the anaerobic conversion. If several anaerobic degradation steps are assumed, kinetic parameters of each step considered must be supplied to the model. In this case, the experimental effort can be difficult and the results unsatisfactory, since the experimental identification of each step can be complicated. Moreover, the resultant model can become complicated, making its practical application difficult. However, the adoption

of one-step anaerobic degradation kinetic is an assumption to be proved (Zaiat, Passig, and Foresti, 2000).

The mathematical model proposed for design purposes was found to be suitable though some deviations between experimental and theoretical data were observed. The model assumed that the influent COD could be completely converted to methane and carbon dioxide. Thus, any reactor efficiency could be achieved by adopting a proper L/D ratio, a single biochemical kinetic parameter, a proper biomass concentration, and by calculating an effectiveness factor based on the operating conditions. In fact, the model did not consider the volatile suspended solids production resulting from COD conversion nor the changing in the kinetic parameter along the reactor as the substrate composition and the characteristics of the biocatalyst changed. Also, it did not consider the fraction of non-biodegradable COD resulting from anaerobic processes. However, the model can be However, the model can be successfully applied, even assuming some simplifications, and a safety factor can be adopted by the assumption of a high COD removal efficiency as a criterion in the design stage. The improvement of the model, without it becoming too complicated and impracticable or practical applications, is a challenge to be confronted in future researches.

The estimation of biomass. The value of the mean biomass concentration (X) can be derived from the kinetic experiments. It can also be evaluated from specific experiments carried out to provide data about the maximum ability of the support to retain biomass. It was observed experimentally that polyurethane foam matrices were able to retain 23 mg of volatile suspended solids m L‹1 of foam. However, this value is based on a small number of experimental observations and a high degree of uncertainty can be associated with it (Zaiat, Passig, and Foresti, 2000).

Hydrodynamic characterization. Hydrodynamic studies demonstrate that the HAIS reactor can be simulated, approximately, as 30 continuous stirred tank reactors (CSTR) in series. This behavior permits us to consider the HAIS reactor a “plug-flow” reactor for design purposes. Therefore, the observation of spatial variations of some monitoring parameters in HAIS reactor is very important to inform about substrate conversion and intermediate products generation/degradation along the reactors length (Zaiat, Vieira, and Foresti, 1997).

Cabral (1995) reported on stimulus-response experiments on a bench-scale HAIS reactor, aiming to verify its hydrodynamic characteristics. An additional method was developed to neglect the effect of the tracer-effective diffusion in the porous media on residence time distribution (RTD) curves.

The results from these experiments, shown in Figure 2.4, lead to the conclusion that the N-CSTR in series and the low and high dispersion theoretical models were well adjusted to the experimental data. It was demonstrated that the HAIS reactor can be simulated by approximately 30 continuous stirred tank reactors (CSTR) in series. This behavior suggests that the HAIS reactor is a "plug-flow" reactor for designing purposes. It was also found that the tracer effective diffusion in the particles' bed interferes strongly in the RTD curves and, consequently, in the parameters of the theoretical models. This may cause misinterpretation of the experimental data, resulting in design and scale-up errors (Zaiat et al., 1996).

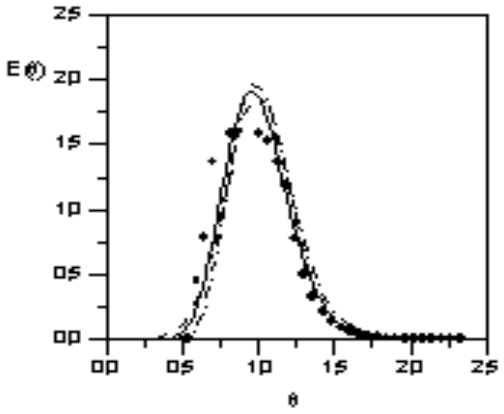

Figure 2.4. RTD curve obtained from hydrodynamic experiment in HAIS reactor operating with mean detention time of 2.4 hr and gas production of 1136 ml/day. Theoretical models adjusted to experimental data (•): N-CSTR in series (—), low dispersion (---) and high dispersion (- • -), (Zaiat et al., 1996).

Microbial colonization of polyurethane foam matrices in HAIS. Several cell-immobilization supports have been tested and used in different reactors for anaerobic wastewater treatment. Among them, one can mention: agar gel, acrylamide, porous ceramic material and polyurethane foam. It is very important to know how the biomass attaches to the support to prevent cell washout from reactors. Which could result in process failures. Huysman et al. (1983) tested different materials for their ability to immobilize methanogenic bacteria and found polyurethane foam to be an excellent colonization matrix. Moreover, Zaiat et al. (1996) concluded that polyurethane foam matrices are completely adequate for anaerobic sludge immobilization (Varesche et al., 1997).

Aiming to develop a new anaerobic reactor configuration, Zaiat et al. (1994) conceived the horizontal – flow anaerobic immobilized – sludge (HAIS) reactor utilizing polyurethane foam matrices for biomass immobilization. Experiments using wastewater from the paper substrate confirmed the technical viability of the HAIS reactor, which performed well under different operational conditions. In both sets of experiments, the start-up period was very short (less than 10 days), and polyurethane foam matrices were found to provide an adequate environment for biomass growth and retention.

In spite of several investigations on the suitability of polyurethane foam as a biomass immobilization support, the conformation of anaerobic bacteria inside the matrices remains unknown. There is a lack of information on the framework of the different bacterial groups responsible for the stepwise anaerobic degradation of wastewater in a fixed bed of this type. On the other hand, it is well know that the granulation process inside upflow anaerobic sludge- blanket (UASB) reactors is responsible for the formation of well – structured pellets, in which the fluxes of substrate and products are optimized. Although the start-up period of UASB reactors are know to be long, the presence of granular sludge has been responsible for the reactor capacity accommodating high organic and hydraulic loads.

Knowledge of sludge granulation and biofilm formation has been improved with studies on the structure and biological composition of cell aggregates that make use of optic, fluorescence and scanning electron microscopy (SEM). In fact, the morphological and physiological characteristics of anaerobic species have been used to clarify the main mechanism of the anaerobic process. Methanogenic species have been easily identified by their fluorescence at 420 nm. Even some species that do not fluoresce (methanogenic and non-methanogenic bacteria) have been identified on the basis of their morphological characteristics (Varesche et al., 1997).

Evaluation of biofilm image

Yang et al. (2001) compared the results of the manual thresholding of biofilm images with those obtained via application of a number of automatic algorithms. They conclude that the histogram-based iterative selection algorithm of Ridler and Calvard (1978) commonly known as the ‘‘intermeans’’ algorithm, is superior to the other automatic methods used, and that it is sufficiently precise to be considered as a potential replacement for manual thresholding. For several reasons, he feels that these conclusions are misleading. He also question whether a software package based on the intermeans algorithm would be an appropriate tool to study the structure and morphology of aggregated microorganisms and their associated byproducts.

Implicitly, the application of segmentation (including thresholding) algorithms to images is predicated on the premise that these images contain two or more regions or categories that correspond to different objects or parts of objects. Images with such distinct regions or categories typically have bi- or multimodal histograms. The biofilm images used by Yang et al. (2001), on the other hand, have unimodal histograms, indicative of an inadequate separation of the gray-scale intensities of the foreground (microbial aggregates) and background (interstitial spaces). This problem can be alleviated to some extent by the use of computational filters. An example is the unsharp mask filter, available in image analysis software like NIH Image or Adobe Photoshop, and which sharpens gray-scale intensity gradients. Nevertheless, as powerful as they are, these software tools can do little when the starting images are very poorly contrasted.

A better option probably would have been for Yang et al. (2002) to retain the color content of the images produced by the different microscopes used, and to manipulate these images in such a way as to obtain suitably contrasted gray-scale images. Color images can be saved under a number of color encoding systems (e.g., RGB, CMYK, Lab), and individual color channels in each of these formats can be analyzed separately. In some cases, one of these channels may be significantly more contrasted than the others and be far more amenable to segmentation. Perhaps of even more significance for the study of the structure and morphology of microbial aggregates would be the use of compounds that would positively or negatively stain the biomass and its associated exopolymers, and would thereby physically enhance image contrast. These stains may be fluorescent but need not be. A procedure involving a non-fluorescent negative stain (China ink) has been used for decades, and appears to work well, to determine the geometrical extent of extracellular polymers around individual cells (Vandevivere and Baveye, 1992). If adapted to the more complex situation envisaged by Yang et al. (2002), this procedure might lead to similar biologically meaningful segmentations of images (Baveye, 2002).

A collateral problem with the analysis of Yang et al. (2002) is the authors’ conviction that the opinion of a panel of human experts is a reliable reference against which to compare the performance of automatic thresholding methods. Confronted with poorly contrasted images and lacking any biologically-based criterion to decide where thresholds should be located in the image histograms, each individual ‘‘expert’’ proposes what amounts to little more than guesses as to the appropriate thresholds. These may be partially educated guesses but, more likely than not, they are largely the result of other influences, unrelated to the expert’s acquaintance with the geometry of microbial aggregates and with the compactness or fluffiness of microbial exopolymers in specific situations. My own experience with images of soil profiles and bacterial aggregates leads me to think that

esthetic considerations likely made the experts shy away from extreme threshold values, and encouraged them to gravitate instead toward a happy compromise, corresponding to thresholds that provide a reasonable balance between background and foreground. These thresholds may or may not make sense biologically. In other words, the experts consulted by Yang et al. (2002) may or may not have been right, in spite of the fact that they may have generally concurred in their recommendations! (Baveye, 2002).

Given this context, one can easily explain the correlation found by Yang et al. (2002) between expert opinions and the thresholds produced by the intermeans algorithm. Indeed, one of the common criticisms of this algorithm is that, arguably like the ''experts'', it has a tendency to find a threshold that divides the histogram in two, so that there are approximately equal numbers of pixels in the two categories. In some applications, this tendency produces thresholds that appear plausible, but in many other cases, the resulting thresholds are not physically or biologically sound. To circumvent this key limitation of the intermeans algorithm, other iterative thresholding algorithms have been developed and ought to be used instead, such as the minimum-error algorithm of Kittler and Illingworth (1986), or Besag's Iterated Conditional Mode (ICM) algorithm (1986). It remains to be seen whether, *with suitably- contrasted images* of microbial aggregates, these histogram-based thresholding methods would have competitive advantages over the algorithms considered by Yang et al. (2002), or over alternative segmentation techniques, either edge-based or region-based (Baveye, 2002).

Technical Considerations of *CT*

The technical advances of Computed tomography (CT) achieved during the 1980s provide the basis for rapid, efficient, and reliable performance, and now CT is used routinely and universally in a broad array of applications.

The advantages of CT over conventional radiography are the capabilities for three-dimensional imaging, good contrast resolution, accurate measurement of tissue attenuation coefficient, noninvasive nature, and in many instances, reduced radiation exposure. All of these factors combine to make CT a powerful assessment tool. CT scanners have the capability for submilimeter resolving power in high contrast tissue such as bone. This resolution, coupled with the use of thin sections (1 to 3 mm), means that high-quality images can be readily obtained. The contrast resolution of CT is recognized as superior to that of conventional films. Motion artifacts are minimized with average scanning time of less than 5 sec. Rapid-sequence scanning in the dynamic mode facilitates the performance of studies requiring 40 to 50 contiguous scans (Moss et al., 1992).

Basic Principles of Computed Tomography. When a beam of monochromatic x-rays passes through a homogeneous medium, it diminishes in intensity because of interactions with the medium. In the diagnostic energy range, these interactions are primarily molecular ionizations resulting from Compton scattering and photoelectric absorption. In traversing a very small thickness of the medium, the decrease in the beam (i.e., the number of x-ray photons removed from the beam) is proportional to the initial number of photons and the thickness traversed (Moss et al., 1992).

The most simple visualization of the geometry of making the projection measurements along line can be seen in Figure 2.5. In the original scanner, a single pencil x-ray beam traverses the slice to be examined at multiple view angles. An x-ray detector records the transmitted x-ray intensity during each crossing, as shown in the figure. This set of measurements is referred to as a *parallel ray projection* of the section being examined. It is also often referred to as a *profile* or *parallel ray view.* Each point in the profile represents the transmitted intensity at each point along the traverse; a separate profile is obtained for each angle (Moss et al., 1992).

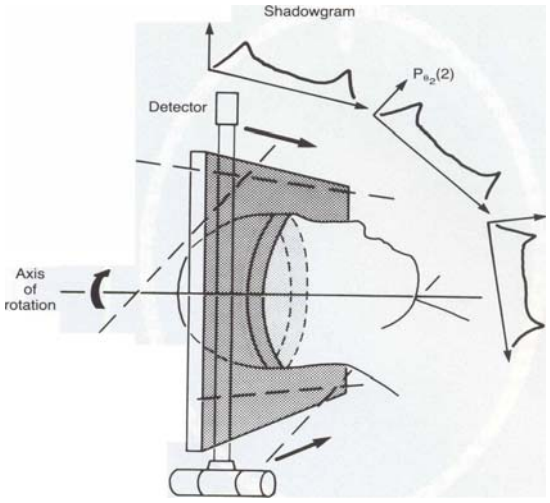

Figure 2. 5. CT scanning pencil beam. Translaterotate two-motion scanning of head with a single x-ray beam (Moss et al., 1992).

Practical Implementation of Computed Tomography. The ideal implementation of computed Tomography would reconstruct a continuous distribution of attenuation coefficient measurements within a plane or slice through the object of interest. The distribution would be known accurately and precisely at all points within the two-dimensional object plane. The reconstruction of a continuous distribution requires that measurements be made of all possible projections, along lines of negligible width, through the object within the imaging plane. Because the lines are of negligible width, this requires a very large (infinite) number of projection measurements. The negligible width requirement also means that the x-ray source and detector elements must all have negligible width. Thus, the reconstruction of a continuous distribution (at very high resolution) is impractical because of the large amount of measurements required, as well as the constraints on smallness of the x-ray source and detectors.

The reconstructed CT image is represented by a two-dimensional matrix of CT numbers. Because of the practical limitations of finite beam width and the finite number of projection measurements, the spatial resolution possible in the reconstructed image is compromised. Intuitively, this is seen to happen because of the blurring effect of scanning very fine details by a rather coarse beam. Typically the effective width of the beam created by the geometry of the source, detector, and associated collimators varies from about 3 mm down to less than 0.5 mm among various commercially available CT scanners. The number of individual projection measurements varies between about 65,000 and 1 million.

It is generally true that the number of elements in the final display matrix should not be greater than the number of individual projection measurements, which gives the number of resolution elements that can be determined. This would mean that the final reconstruction matrix should be between 256 × 256 and 1000 × 1000. The matrix size of commercial CT scanners is found to vary between 256 × 256 and 1024 × 1024 picture elements (pixels). The maximum matrix size is typically restricted to 512 pixels × 512 pixels. This restriction is partially a result of the fact that a substantial increase in x-ray dose is required in CT if it is desired to increase spatial resolution while maintaining contrast resolution for the smaller pixels. Indeed, it is found that the pixel variance varies inversely with the cube of the linear dimension of the in-plane resolution element. Because of the long-range negative correlation of noise in CT images, the dose required for the more common task of perceiving contrast differences in large (many pixel) areas is relatively unaffected by decreasing pixel dimensions. This conclusion is substantiated by the experimental observation of Cohen and DiBianca that the minimum perceptible area contrast was the same for the GE 7800 and 8800 CT scanners, where the only difference was spatial resolution. It should also be noted that higher resolution reconstructions of local regions (target reconstructions) within the full image can generally be obtained to utilize the full resolution capabilities of the scanner without increasing the dose when the number of display elements is significantly less than the number of measurements.

Another geometric limitation in CT scanners is the fact that the beam does not have negligible width in the direction perpendicular to the scan plane. To obtain sufficient x-ray signals, this width is generally between 2 and 20 mm and can often be varied under operator control. Thus the CT number corresponds to the mean value of density or, more accurately, the linear attenuation coefficient within the volume of tissue denoted by a particular pixel. This volume element has a depth, L, which is the CT section thickness or slice thickness as indicated in Figure 2.6 (Moss et al., 1992).

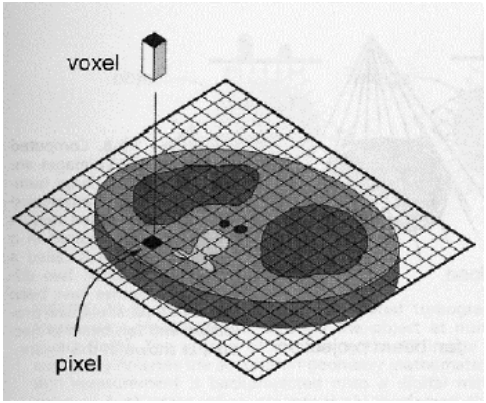

Figure 2.6. A pixel (picture element) is the basic two-dimensional element of a digital image. Computed tomographic (CT) images are typically square arrays containing 512 ×512 pixels, each pixel representing 4,096 possible shades of gray (12 bits). Each pixel in the CT image corresponds to a voxel (volume element) in the patient. The voxel has two dimensions equal two the pixel in the plane of the image, and the third dimension represents the slice thickness of the CT scan (Moss et al., 2000).

Limitations Inherent in Computed Tomography. In addition to geometric considerations, there are other limitations that are directly related to the number and energy distribution of the photons within the beam. These affect the accuracy and precision of the measurements and lead to artifacts.

The number of photons that are detected directly affects the precision with which the attenuation coefficient can be measured. For example, the total attenuation of an x-ray beam that passes through the thicker parts of the body is considerably greater than that of beams used in head scanning. The practical implementation of whole-body CT is thus much more difficult than that of head CT because of this greatly increased constraint on the dynamic range that is observed by the detection system. Also, because the body is larger, either more detectors or longer scan times (or both) are required to produce images of contrast and spatial resolution comparable to those of head CT. Finally, internal motion of organs (lung, heart, digestive tract) is much more problematic in body CT than in head CT (Moss et al., 1992).

CT numbers or Hounsfield Units. After CT re-construction, each pixel in the image is represented by a high-precision floating point number that is useful for computation but less useful for display. Most computer display hardware makes use of integer images. Consequently, after CT re-construction, but before storing and displaying, CT images are normalized and truncated to integer value. The number CT (x, y) in each pixel, (x, y), of the image is converted using the following expressions:

$$CT(x,y) = 1000\frac{\mu(x,y) - \mu_{water}}{\mu_{water}}$$

where $\mu(x,y)$ is the floating point number of the (x,y) pixel before conversion, μ_{water} is the attenuation coefficient of water, and CT(x,y) is the CT number (or Hounsfield unit) that ends up in the final clinical CT image. The value of μ_{water} is about 0.195 for the x-ray beam energies typically used in CT scanning (Moss et al., 2000).

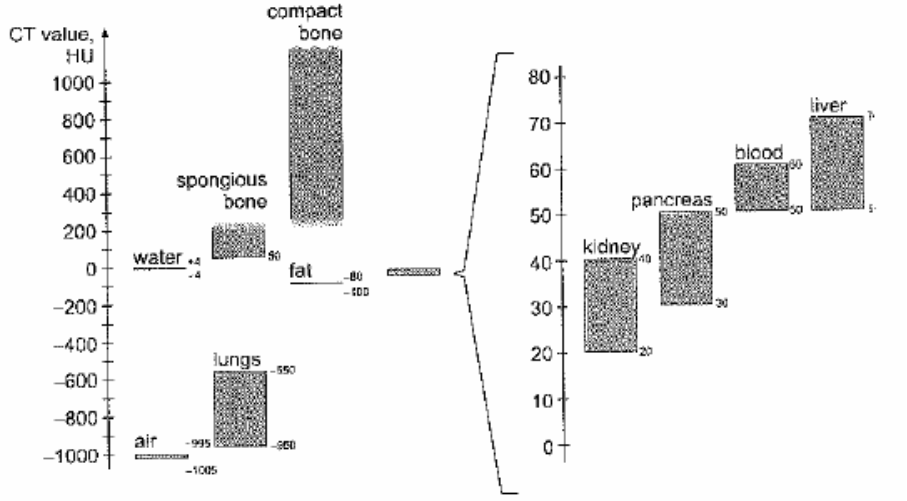

Figure 2.7. The Hounsfield scale. CT values characterize the linear attenuation coefficient of the tissue in each volume element relative to the μ-value of water. The CT values of different tissues are therefore defined to be relatively stable and to a high degree independent of the x-ray spectrum (Moss et al., 2000).

Numeric Illustration of *the Partial Volume Effect.* As has been mentioned earlier, the fact that the CT scanner slice thickness is finite in dimension can lead to an error in the measured CT numbers. This error is referred to as the *partial volume effect* and occurs whenever more than one tissue is present within a volume element or voxel. Indeed, the CT number within a voxel is equal to the sum of the CT numbers of the constituents within the voxel weighted by their volume fractions; For example, if a voxel within a bone marrow cavity contains bone and fat with volume fractions of 0.15 and 0.85, respectively, the CT number within that voxel would be:

$$\text{CT Number}_{\text{bone}} \times V_{\text{bone}} + \text{CT Number}_{\text{fat}} \times V_{\text{fat}} =$$
$$1000 \times 0.15 + -90 \times 0.85 = 65,$$

which is approximately equal to the CT number of muscle (Moss et al., 1992).

Post-processing Techniques. Post-processing techniques are applied to the final reconstructed images rather than the raw projection data. They are available in two types: single-energy and dual-energy.

Post-processing Single-Energy QCT (SEQCT). The post-processing QCT method that is employed most frequently is the so-called *"Cann-Genant"* technique. This is a single-energy (SE) technique in

which the bone mineral content of a patient's vertebra is determined from the mean CT number (N) of the patient's vertebra and the mean CT numbers of a set of calibration standards. The latter are employed to derive a calibration line, and the mineral content of the vertebra is calculated using the equation:

$$BMD_{vertebra} = \frac{N_{vertebra} - b}{m}$$

where $BMD_{vertebra}$ is the average bone mineral density (BMD) within a user-selected region of interest that is positioned within the trabecular region of the vertebra, CT $Number_{vertebra}$ is the mean CT number in that region of interest, and m and b are the slope and intercept, respectively, of the calibration line. The m and b parameters are determined by applying a linear regression routine to the mean CT number and concentration data for the calibration standards. The BMD calculation is illustrated graphically in Figure 2.8.

The standards that are employed conventionally are five cylinders filled with Solutions of 0, 50, l00, and 200 mg/mL K_2HPO_4 in water. K_2HPO_4 was selected as the calibration mineral because it attenuates x-rays, similar to actual bone mineral (calcium hydroxyapatite), and unlike that mineral, it is soluble in water and can readily be made into solutions having known concentrations.

To determine the status of a patient, the average density of the patient's four analyzed vertebrae (in mg/mL K_2HPO_4) is computed and compared with age-matched normal data (Moss et al., 1992).

An advantage of the post-processing SEQCT technique is that it is fairly precise (one to three percent). However, the accuracy of SEQCT is degraded when fat is present in the marrow cavity. The CT number within the selected region of interest is the volume average of the CT numbers of the trabecular bone, red marrow, and fat within that region. Fat is characterized by a relatively large negative CT number (e.g., about -100). Thus when fat is present, the CT number within the region is decreased, resulting in an underestimate of the true bone mineral content. This underestimate can be as large as -12 mg/mL K_2HPO_4 for each ten per cent fat by volume. Age-related fat corrections for SEQCT have been proposed and utilized with some success. However, when maximum accuracy is desired, researchers turn to dual-energy QCT, which is much less influenced by fat (Moss et al., 1992).

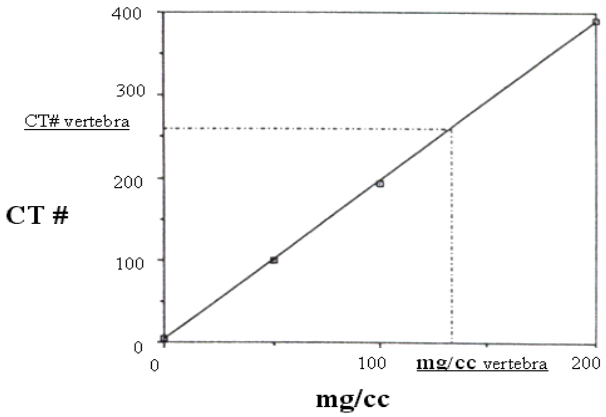

Figure 2.8. A typical calibration line obtained using the CannGenant technique. The line is used to convert the measured mean CT number of the vertebra to an equivalent concentration of bone mineral in unit, of mg/mL (Moss et al., 1992).

Upflow anaerobic sludge-blanket process (UASB)

Some of the UASB specifications was reported in the first part of this text (see page 23). The UASB concept can be applied to a wide variety of industrial wastewaters.

UASB system treating slaughterhouse wastewater. Direct discharge of high-strength industrial effluent often upsets municipal wastewater treatment plant processes. This pilot study was undertaken to evaluate the performance of a UASB system in pre-treating effluent from a medium-size traditional slaughterhouse. Experiments were conducted in a continuous flow 500-L pilot plant initially inoculated with 200 L municipal anaerobic digested sludge. With an influent COD concentration of 3000-5000 mg/L, the system was started with a loading of 1.8 kg COD/m^3.d (F/M of 0.24 kg COD/kg VSS.d) at 25 ºC. Upflow velocity was gradually increased to 0.8-1 m/hr (HRT of 2.5 hr) and VSS concentration reached 25 g/L once granules were formed. It was possible to increase the loading up to 14 kg COD/m^3.d (F/M of 1.4 kg COD/VSS.d) at 29 ºC with total COD removal efficiencies of 85-90%. At these conditions 250-350 L gas (75% methane) was generated for each kg COD removed (Figure 2.9) (Torkian et al., 2002).

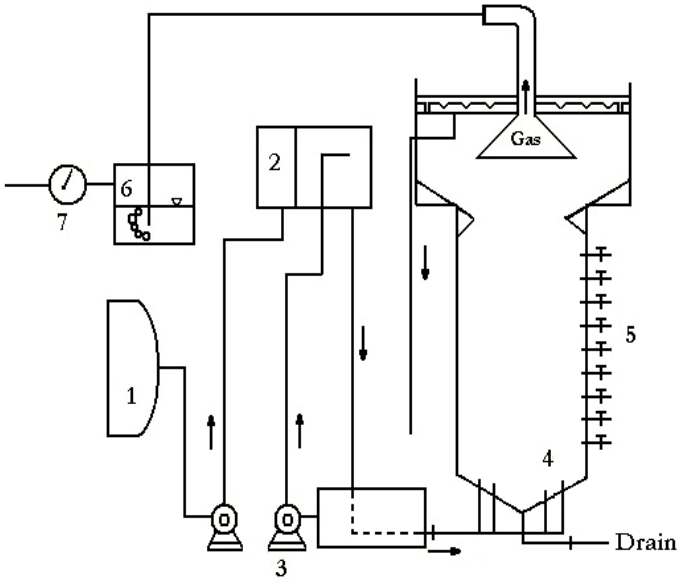

Figure 2. 9. Schematic of UASB system receiving effluent from slaughterhouse (1 – Feed tank, 2 – Flow control weir, 3 – Recycle pump, 4 – Influent distribution, 5 – Sampling taps, 6 – Water seal, 7 – Gas meter) (Torkian et al., 2003).

The high-rate UASB technology, which relies on the growth of granular sludge and three phase separator (biogas-liquids-solids), has been a commercial success with over 300 installations (before 1997) from various suppliers operating throughout the world (Gavrillescu, 2002).

*Granulation in UASB system treating slaughterhouse wastewater***.** Granulation is an important aspect of high-rate UASB reactors treating industrial effluents. Torkian et al.(2003) have experienced granulation of sludge, six months after start-up of a pilot-scale UASB reactor inoculated with mesophilic municipal anaerobic digested sludge and fed with slaughterhouse wastewater without blood recovery. At an upflow velocity of 0.8-1 m/h superior COD removal efficiencies of up to 85% was achieved with reduced potential of sludge washout. These granules were dark brownish with a diameter of 1-4 mm and a settling velocity of 20 m/hr. SS and VSS of the granules' layer were 55-68 and 45-57 g/L, respectively with a specific gravity of 1.3. SEM and TEM analyses indicated rod-shaped and filamentous Methanothrix bacteria as well as Methanosarcina cocci aggregates. Microstructures were an intertwined syntrophic association between acetogenic and methanogenic microbial populations with diverse morphologies. X-ray analysis of mineral contents showed a high calcium phosphate apparently related to exracellular polymers of the shell structure (Torkian et al., 2003).

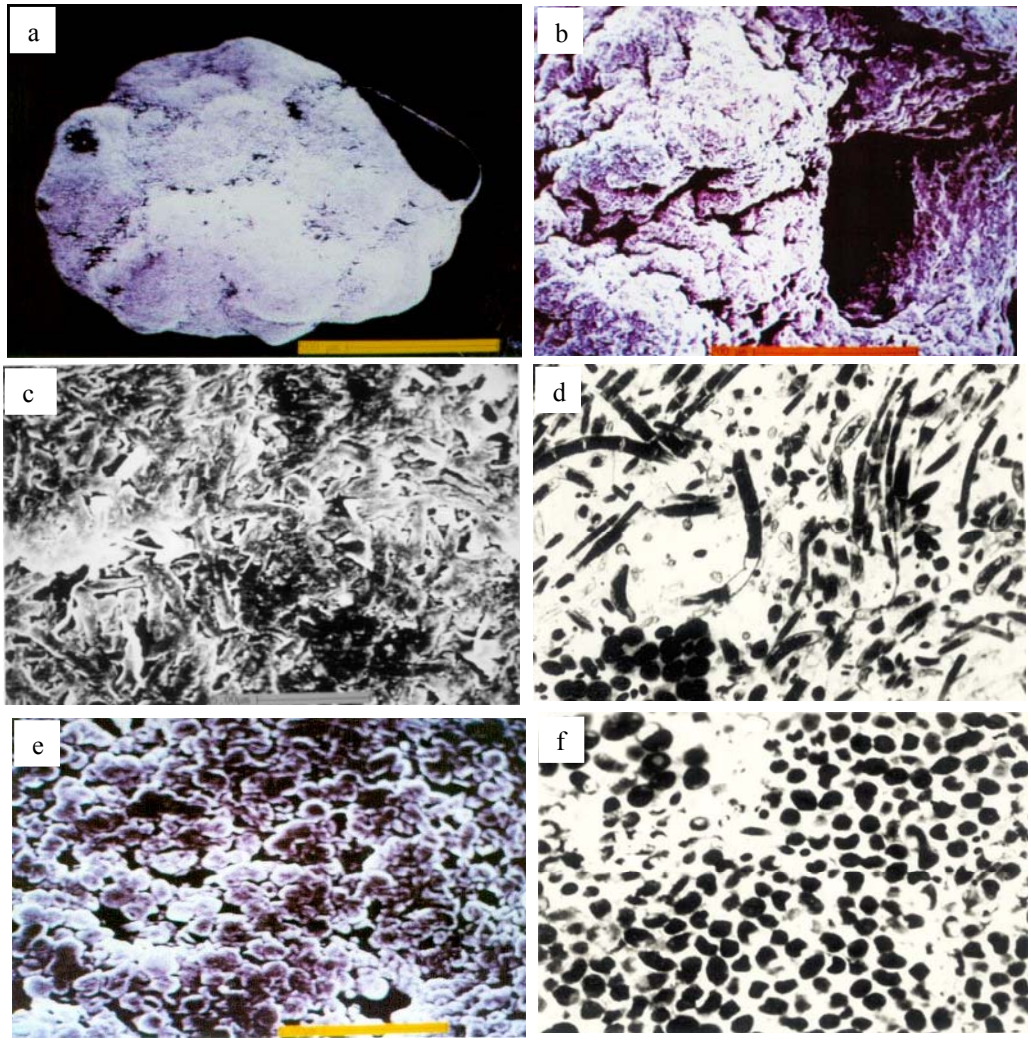

Figure 2. 10. SEM and TEM pictures of obtained granules in UASB system receiving effluent from slaughterhouse;
(a) SEM picture of whole granule; (b) SEM micrograph showing smooth surface of granule with a large opening likely for biogas escape (170X, scale 200 μm); (c) SEM micrograph of acetotrophic Methanothrix (3700X, scale 5 μm); (d) TEM micrograph of syntrophic association between microcolonies of bacteria in the granule matrix (4000X), Note streptobacili at the left top and cocci at the left bottom; (e) SEM of Cocci shape bacteria with array of sarcina – Methanosarcina (5000 X scale, 5 μm); (f) TEM micrograph showing tetrad and diplococci configurations of Methanosarcina (6000X) (Torkian et al., 2003).

CHAPTER 3

MATERIALS AND METHODS

Described in this chapter are the materials and methods used during this research, including the specifications of reactors set-up, start-up and operation, and GC method to determine biogas composition. The develepment of a method for analysis of provided CT-scan images through Photoshop make up a experimental method to estimate VSS contents in packed (HAIS) bioreactors.

The Apparatus

Two 2-L HAIS glass tube reactors had a diameter of 5 cm and length of 1 m. One of the reactors contained 1400 mL of granular polyurethane(PU) with a size range of 2-4 mm and a density of 700 kgm^{-3}. Surface to volume ratio was 1100 m-1 with porosity of 0.28. The total surface area available for biomass immobilization was 1.5 m^2. The other reactor was filled with 1900 mL of polyethylene (PE) beads with a density of 12 kgm^{-3}. Porosity was 0.42 with a Surface/Volume (S/V) value of 1250 and L/D of 15.5 giving it the overall biofilm surface availability of 2.6 m^2. Circular UASB reactor consisted of a 1-m long Plexiglas with a diameter of 65 mm. The effective volume was 2.2 L and provisions were made for gas-solids separation at the top. The reactors are shown in Figure 3.1 and the composition of synthetic feed is provided in Table 3.1.

Constant substrate flow to the reactors was provided by Peristaltic (Masterflex L/S, Cole-Parmer Instrument Co., USA) and Infusion pumps (model OT-601 JMS Co. Hiroshima, Japan). Temperature control was achieved through thermostatically controlled heated glass water baths constructed around the reactors and substrate stock solution. With provision of heaters and thermostats, it was possible to maintain confine temperature variations to within 2 ºC.

Table 3.1. Composition of the synthetic influent to both sets of reactors

Constituent	Concentration, mg L^{-1}
$C_6H_{12}O_6$	1750
$CaCl_2$	44.5
$CoCl_2$	0.08
$FeCl_3, 6H_2O$	0.5
$Na_2HPO_4.7H_2O$	33.4
K_2HPO_4	21.75
KH_2PO_4	8.5
$CH\text{-}COONH_4$	400
$NaHCO_3$	2000
$FeSO_4.7H_2O$	50
$MgSO_4.7H_2O$	25.5

Operation

The UASB reactor contained granulated sludge formed previously in another study (Torkian et al., 2002). The seed sludge was adapted with synthetic wastewater for a month at low OLR value of 1 kgCOD $m^{-3}d^{-1}$. After this period, a volume amounting to 20% of the effective HAIS reactors' volume was placed in there and the systems were started at surface loading rate of 0.01 kg $CODm^{-2}d^{-1}$. This is equivalent to volumetric loading rate of 2 $kgCODm^{-3}d^{-1}$ for these reactors since the sludge had previously already been adapted to the constituents in the UASB reactor.

After the start up period, both sets of reactors were subjected to increasing organic loading rates in a step mode by increasing inflow of stock solution. Sufficient time, defined as less than 5% variation in 3 consecutive effluent sample COD concentration, was allowed for the systems to reach stable conditions prior to any increase in loadings. The upper limit for loading rate increases was the indication of COD removal efficiency deterioration to below 70%. The feed reservoir was filled once a week. Temperature of the wastewater was the same throughout the study. Other operational conditions and performance indicators are presented in Table 3.2.

Table 3.2. **Summary of operational conditions for the UASB and HAIS reactors**

Phase	Reactor	Period days	HRT hr	COD mg/L	COD removal, %	HLR $m^3.m^{-2}.h^{-1}$	SLR Kg $COD.m^{-2}.d^{-1}$
1	HAIS-PU	1-120	3.08 ±2.74	1000±100	51.8 ±2.2	1.02 ± 0.23	0.013 ± 0.005
2		121-220	2.49 ±1.38	1750±250	63.5±17.5	1.28 ± 0.51	0.028 ± 0.012
3		221-320	1.21 ±0.78	1750±250	51.6 ±2.2	2.27 ± 0.78	0.050 ± 0.016
1	HAIS-PE	-	-	-	-	-	-
2		-	-	-	-	-	-
3		250-320	0.32 ±0.08	1750±250	61.6±18.6	1.00 ± 0.65	0.023 ± 0.014
						Upflow velocity, $m.h^{-1}$	OLR Kg $COD.m^{-3}.d^{-1}$
1	UASB	1-120	9.54 ±7.68	1000±100	41.9 ±1.6	0.14 ± 0.08	4.02 ± 1.71
2		121-220	4.71± 2.63	1750±250	64.3±15.2	0.25 ± 0.13	12.8 ± 6.26
3		221-320	1.15± 1.16	1750±250	57.8±20.3	0.43 ± 0.14	21.27 ± 6.83

1 = Start-up period; 2 = Optimum performance period; 3 = Maximum performance period;
SLR: surface loading rate; OLR: organic loading rate; F/M: food to microorganism ratio;

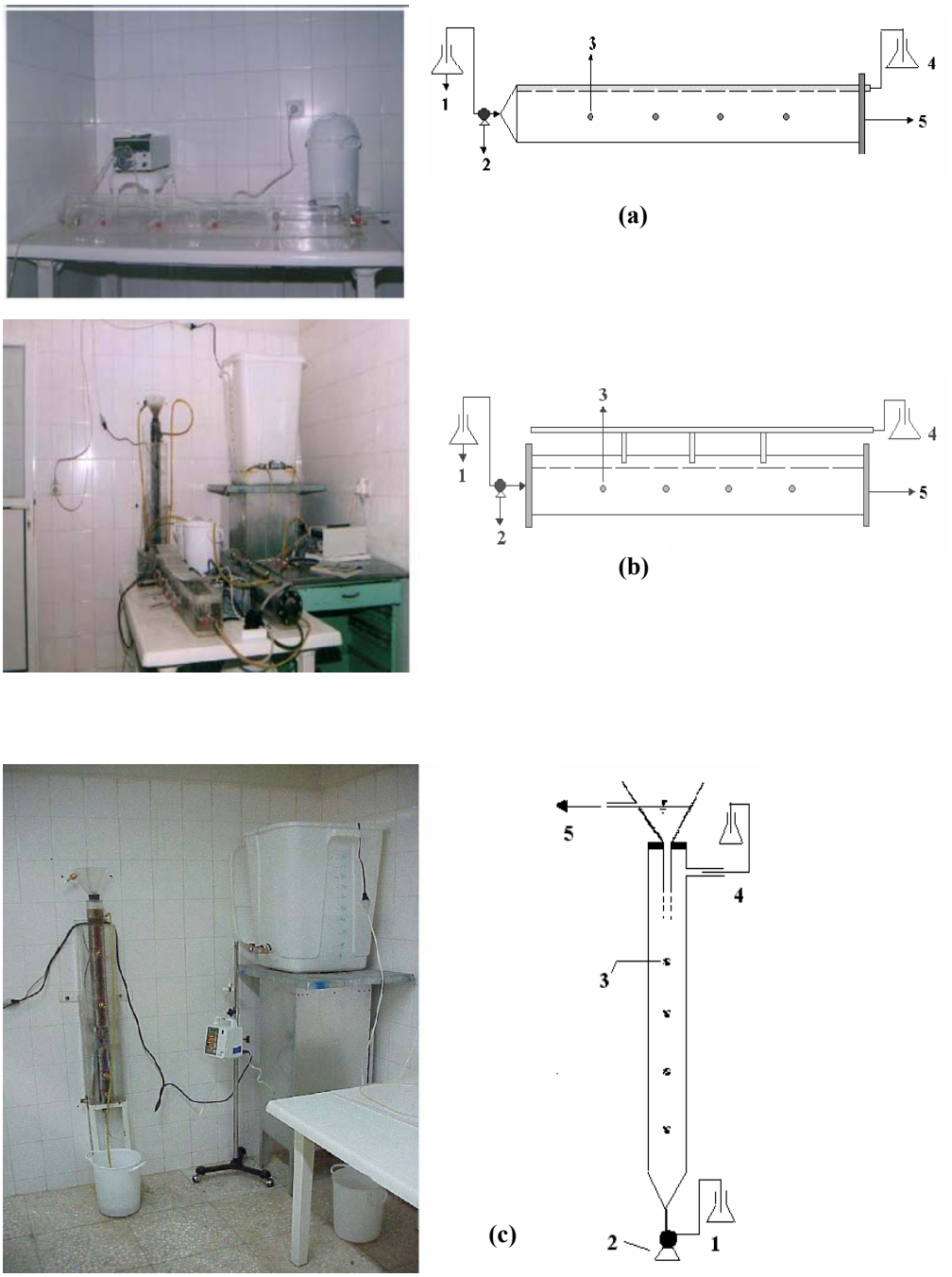

Figure 3.1. Experimental Set-up of HAIS and UASB reactors: (1) Influent synthetic substrate; (2) Peristaltic and Infusion pumps; (3) Sampling port; (4) Biogas collection system; and (5) Effluent. (a) HAIS-PU; (b) HAIS-PE; and (c) UASB;

Analytical Procedures

The substrate concentration was measured as COD and VSS served as a surrogate indicator of reactors' biomass content. Grab samples of influent, effluent, and different sections of the reactors were collected in containers and stored in fridge at 4 ºC and analyzed within 24 hours after collection. Attached biomass content was measured by random removal of biofilm support media from the respective section of the reactor under consideration and subjecting it to routine drying procedures for VSS analysis. Routine analyses including soluble (filtered sample with a 0.45 μm pore size glass micro fiber filter) and total BOD_5 and COD, and solids content were performed using procedures outlined in the Standard Methods (APHA, 1989).

Evolved gas volume was measured using a simple gas meter. Gas composition was determined by using gas chromatography methods (Shimadzo, model 9A, Japan) equipped with FID and TCD detectors.

Computed Tomography Scanning (CT-Scan) images

Spatial biofilm development and distribution was determined by CT-Scan imaging (SCT-2500) in Hospital of Ayat-Allah Kashani in Isfahan. The fixed growth HAIS reactors were drained prior to analysis and immediately inserted into the imaging train of the instrument to avoid any transformation of the biofilm.

The primary purpose of CT is to produce a two dimensional representation of the linear x-ray attenuation coefficient distribution through a narrow planar cross section of any general object. Because tissues of different structures within the object are of different elemental compositions, they also tend to exhibit different x-ray attenuation qualities. Thus in generating cross-sectional images of the x-ray attenuation coefficients, an image is formed that delineates various structures within the object, showing the relative anatomic relationships. The mathematic and physical processes is used to generate the image (Moss et al., 1992).

Definition of Numbers in *Computed Tomography***.** As mentioned, the linear attenuation coefficient of any medium is a strong function of x-ray energy. To determine a tissue characteristic (such as density) using the values of the reconstructed linear attenuation coefficient, it is necessary that the energy spectrum of the x-ray beam be known. Because the spectrum varies from scanner to scanner, as well as for various points along the penetrating beam path of each measurement of a given scanner, an approximation based on the use of CT numbers is commonly used. In this method the measured linear attenuation coefficient for water for a specific scanner is used to transform the measured attenuation coefficient of the object into a standard relative unit called the *CT number***.** The CT number is generally defined by this relationship:

$$\text{CT number} = K\,\frac{\mu_{\text{object}} - \mu_{\text{water}}}{\mu_{\text{water}}}$$

where K is a constant. This equation assigns water the CT number of 0. Because μ_{air} is negligible relative to μ_{water}, the CT number of air is essentially equal to - K. At the present time, nearly all manufacturers have adopted the convention that assigns K the value 1000. The resulting CT numbers are given in terms of Hounsfield units (H). For this case, air has the value -1000 H; dense (cortical) bone is between 1000 H and 2000 H, and soft tissue is in the range of + 40 H to +60 H. Thus CT numbers have a range of about -1000 to + 2000 on the Hounsfield scale. It is evident that a change of one Hounsfield unit corresponds to a change of 0.1 per cent in linear attenuation coefficient relative to water.

It is interesting to note that for the x-ray energies employed in CT (typically those corresponding to a highly filtered 120 kV(p) x-ray beam), the dominant x-ray attenuation mechanism is the Compton effect for which μ: is proportional to mass density (p). Introducing this relationship into the previous equation and setting K equal to 1000, we have the expression:

$$\text{CT number} \simeq 1000 \frac{\rho_{\text{object}} - \rho_{\text{water}}}{\rho_{\text{water}}} = 1000 (\rho_{\text{object}} - 1)$$

The CT number is approximately linearly related to the mass density of the attenuating tissue. CT numbers calculated using this approximation for a variety of tissues are listed in Table 3.3. The CT values in the table are in fairly good agreement with those that are actually measured. However, the linear attenuation coefficient of a tissue, and hence its CT number, is dependent on the x-ray energy, which is not reflected in this table. Thus the CT numbers of fat, muscle, and bone that are measured using an 80 kV(p) x-ray beam are different than those that are measured using a 120 kV(p) beam. The approximation also tends to be less accurate for high-atomic number materials such as bone and iodine for which there is substantial attenuation as a result of the photoelectric effect (Moss et al., 1992).

Table 3.3. Mass densities and approximate CT- Numbers of a variety of tissues (Moss et al., 1992).

BODY TISSUE	MASS DENSITY (g/cm^3)	APPROXIMATE **CT** NUMBER (H)
Air	1.18×10^{-3}	-999
Fat	0.91	-90
Water	1.0	0
Muscle	1.05	+50
Bone	1.92	+920

In this study, the CT numbers of biofilm, biogas and other materials was measured using a 120 kV(p) x-ray beam.

Image analysis with using Photoshop

Adobe Photoshop 7.0, is a professional image-editing standard. Photoshop delivers a comprehensive environment for professional designers and graphics producers to create sophisticated images for print, the Web, and other media.

Checking scan quality and tonal range (Photoshop). A *histogram* illustrates how pixels in an image are distributed by graphing the number of pixels at each color intensity level. This can show you whether the image contains enough detail in the shadows (shown in the left part of the histogram), midtones (shown in the middle), and highlights (shown in the right part) to make a good correction.

The histogram also gives a quick picture of the tonal range of the image, or the image *key type*. A low-key image has detail concentrated in the shadows; a high-key image has detail concentrated in the highlights; and an average-key image has detail concentrated in the midtones. An image with full tonal range has a high number of pixels in all areas. Identifying the tonal range helps determine appropriate tonal corrections.

The horizontal axis of the histogram represents the intensity values, or levels, from darkest (0) at the far left to brightest (255) at the far right; the vertical axis represents the total number of pixels with a given value.

Statistical information about the intensity values of the pixels appears below the histogram:

Mean: Represents the average intensity value. *Standard deviation (Std Dev)*: Represents how widely intensity values vary. *Median*: Shows the middle value in the range of intensity values. *Pixels*: Represents the total number of pixels used to calculate the histogram. *Level*: Displays the intensity level of the area underneath the pointer. *Count*: Shows the total number of pixels corresponding to the intensity level underneath the pointer. *Percentile*: Displays the cumulative number of pixels at or below the level underneath the pointer. This value is expressed as a percentage of all the pixels in the image, from 0% at the far left to 100% at the far right. *Cache Level*: Shows the setting for the image cache. If the Use Cache for Histograms option is selected in the Memory and Image Cache (Windows) or Image Cache (Mac OS) preferences, the histogram displays more quickly and is based on a representative sampling of pixels in the image (based on the magnification), rather than on all of the pixels (equivalent to a cache level of 1). Deselect this option if you want to check for posterization in the image. You can press Shift while choosing Image > Histogram to generate the histogram using all pixels in the image.

Image size and resolution. In order to produce high-quality images, it is important to understand how the pixel data of images is measured and displayed.

Pixel dimensions. The number of pixels along the height and width of a bitmap image. The display size of an image on-screen is determined by the pixel dimensions of the image plus the size and setting of the monitor.

Image resolution. The number of pixels displayed per unit of printed length in an image, usually measured in pixels per inch (ppi). In Photoshop, you can change the resolution of an image; in ImageReady, the resolution of an image is always 72 ppi. This is because the ImageReady application is tailored to creating images for online media, not print media.

In Photoshop, image resolution and pixel dimensions are interdependent. The amount of detail in an image depends on its pixel dimensions; while the image resolution controls how much space the pixels are printed over. For example, you can modify an image's resolution without changing the actual pixel data in the image--all you change is the printed size of the image. However, if you want to maintain the same output dimensions, changing the image's resolution requires a change in the total number of pixels.

When printed, an image with a high resolution contains more, and therefore smaller, pixels than an image with a low resolution. For example, a 1-by-1-inch image with a resolution of 72 ppi contains a total of 5184 pixels (72 pixels wide x 72 pixels high = 5184). The same 1-by-1-inch image with a resolution of 300 ppi contains a total of 90,000 pixels. Higher-resolution images usually reproduce more detail and subtler color transitions than lower-resolution images. However, increasing the resolution of a low-resolution image only spreads the original pixel information across a greater number of pixels; it rarely improves image quality (Adobe Photoshop 7.0).

Operational and performance parameters

Operational and performance parameters include organic and hydraulic loading rate, removal efficiency, elimination capacity, and hydraulic detention time. Loading rates can be looked at from the pollution indicator, empty reactor bed volume, and microbial mass. Organic loading rate (OLR) used here takes into account the liquid flow rate and contaminant concentration and is defined as the mass of pollutant applied onto a unit area of biomass carrier in the reactor per unit time (e.g. g COD m^{-2} carrier. day). As such, this parameter integrates reactor characteristics, operational characteristics, and biofilm mass and activity into the surface area of beads. Hydraulic loading rate (HLR) is a measure of the amount of time a given parcel of liquid is "seen" by a unit surface area (e.g. m^3 wastewater m^{-2} carrier. d^{-1}). This parameter is more indicative of the effective time for mass transfer of pollutants to the biomass than the hydraulic residence time (HRT) because the majority of biodegradation takes place by the biomass attached on the carrier.

Elimination capacity (EC) can be used as a performance indicator. Elimination capacity is related to organic loading rate in that it is defined as the fraction of the organic load biodegraded. It differs from removal efficiency (η), an operational parameter, which is a measure of the effectiveness of the reactor in degrading a contaminant. Elimination capacity is a useful parameter for design purposes and removal efficiency helps the operator determine if his system is complying with regulatory effluent requirements.

Hydraulic residence time (HRT) is the time the contaminant spends in the effective volume of the RBC reactor. This volume may be less than the total reactor volume because of empty spaces at the inlet and outlet and sludge collection zone or the volume occupied by biomass immobilization carrier.

Mass loading rate (g m^{-2} d^{-1}), elimination capacity (g m^{-2} d^{-1}), Hydraulic loading rate (m^3 m^{-2} d^{-1}), HRT (h), and removal efficiency (%) were determined using the relationships between influent and effluent contaminant concentration, effluent flow rate, the effective volume of the reactor, and applying appropriate conversion factors as follows:

$$HRT = \frac{V_r}{Q} \quad (1)$$

$$HLR = \frac{Q}{A_d} \quad (2)$$

$$OLR = \left(\frac{Q}{V}\right) C_{in} \quad (3)$$

$$\eta = \left(\frac{C_{in} - C_{out}}{C_{in}}\right) 100 \quad (4)$$

$$\varepsilon = \frac{V_V}{V_T} \quad (5)$$

where Q is the effluent flow rate (m^3 h^{-1}), A_d is the surface area of biomass carrier (m^2), V_r is the effective volume of reactor (m^3); ε is bed porosity; V_V is volume of voids in bed, V_T is total volume of reactor, and C_{in} and C_{out} are the contaminant concentrations (mg L^{-1}) in the influent and effluent stream of the whole or each stage of the reactor, respectively.

CHAPTER 4

RESULTS AND DISCUSSION

The performance of two packed bed bioreactors with horizontal flow (HAIS) and a suspended-growth (UASB) system in treating synthetic glucose-based substrate was studied in laboratory reactors. An image analysis method was developed in which the CT-Scan technology was used to characterize of the HAIS reactor, and the concept of resolution in Photoshop was used to estimate the VSS contents of fixed bed reactors. The goal was the performance comparsion of two category of anaerobic bioreactors and to advance understandding about desing and operation parameters of these two kinds of systems.

Using CT-Scan images for characterization of HAIS contents

The contents of HAIS reactors differ in their mass densities and atomic numbers, both of which affect their imaged CT numbers. The term quantitative CT (QCT) has been coined to describe this research speciality. The primary purpose of QCT application in this study is to quantify the amount of specific types of material or chemicals within a region of interest. Applications include measuring (1) the total biomass content: biofilm or suspended biomass within the reactor; (2) the media content for the determination of media state and layout; (3) the biogas content for the optimization in design of biogas collection device; and (4) the bed porosity to monitor channeling due to low void ratio (Figure 4.1).

The CT number in terms of Hounsfield units (H) (see chapter 3) is approximately linearly related to the mass density of the attenuating tissue. CT numbers for a variety of materials in HAIS reactors are listed in Table 4.1. In this study, the CT numbers of biofilm, biogas and other materials was measured using a 120 kV(p) x-ray beam.

Table 4.1. Mass densities and approximate CT- Numbers
of a variety of tissues in human body and materials in HAIS reactors

BODY TISSUE	MASS DENSITY (g/cm^3)	APPROXIMATE **CT** NUMBER (H)	HAIS REACTORS TISSUE
Air	1.18×10^{-3}	-999	Biogas
Fat	0.91	-90	Loose (suspended) Biomass
Water	1.0	0	Water
Muscle	1.05	+50	Dense (attached) Biofilm
Bone	1.92	+920	Media

The CT numbers of 74 in Figure 4.1-a-2 and 66 in Figure 4.1-b-1 are near to the CT number of muscle substance and show dense biofilm formed on surface of polyuorethane granules that was used as media in HAIS-PU. The CT number of 39 in Figure 4.1-a-1 is also in the same range, indicating loose biofilm with lower mass density. The CT number of –367 in Figure 4.1.a-3 presumably reveals the porous suspended floculent sludge without water with lower density than fat tissue. The CT numbers of –556, -724, and –836 in Figures 4.1.b-2,3,4 are approximately near to CT number of air and suggest the biogas or biogas with a bit particles suspended sludge or water.

However, the quantitative information provided by CT scanners is not as accurate or precise as desired. This primarily because of deviations from the ideal monoenergetic x-ray beam, and the scatter-free detection situation. Specifically, the polyenergetic x-ray beams employed in all commercial CT scanners have an associated beam-hardening artifact that results in understimates of the true CT numbers. The magnitude of these understimates is directly related to the amount and the densities of the materials surronding the region of interest.

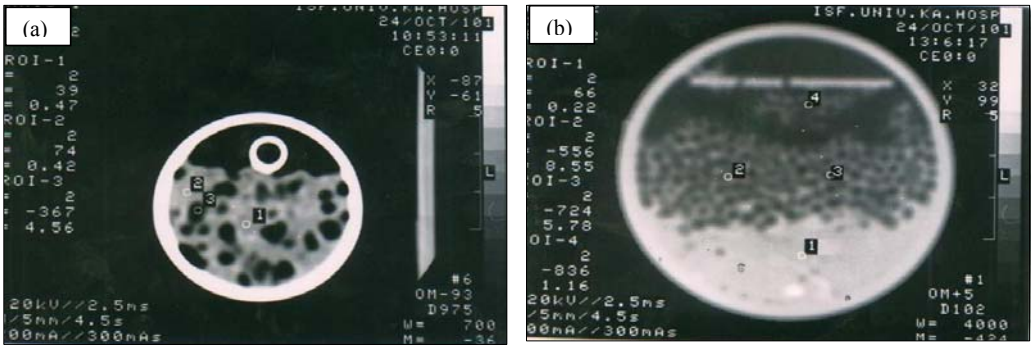

Figure 4.1. CT-Scan of HAIS reactors: (a) HAIS-PU: No.1. Density=39; No.2. Density=74; No.3. Density= -367; (b) HAIS-PE: No.1. Density=66; No.2. Density= -556; No.3. Density= -724; No.4. Density= -836;

In this study, the substance of surounding the reactors were uniform materials such as glass in HIAS-PU and plexiglass in HIAS-PE. It probably is a reason to decrease the error in this regard.

In the other hand, the acceptance of x-ray scatter at the detectors causes the measured attenuation values and CT numbers to be smaller than their true values, with greater errors occurring where more tissue is present. This error also is lower in HAIS reactors, because there is not various materials and tissu in HAIS reactors, including: reactor wall (glass or plexiglass), sampling ports (glass or metal), biofilm, substrate, media, and biogas.

Estimation of biomass in HAIS reactors through CT-Scan image analysis

Figures 4.2 to 4.4 show the CT images and related histograms that were made from different sections of HAIS-PU reactor including, influent port, sampling ports: 1, 2, 3, 4, and effluent port. The CT images of HAIS-PE including influent and effluent ports, only sample port-1, and also several control samples has been indicated in Figures 4.5 and 4.6. It was impassible making CT image from the sample ports of 2, 3, and 4 in HAIS-PE, because the metal sampling valve was caused scatering of X-ray beam with very low quality images. In all of figures, pixels represent the total number of pixels used to calculate the histogram. Table 4.2 reveals all of the pixels data in histograms in three distinct area in images including: "Black part" showing biogas and hollow spaces in that section; "White part" indicating media, biofilm and probably suspended solieds, and "Total" indicating whole of reactor contents in that section.

The calculated VSS in two fixed bed reactors in this study through CT image analysis are 51 (PE) and 28 g/L (PU) (Table 4.2).

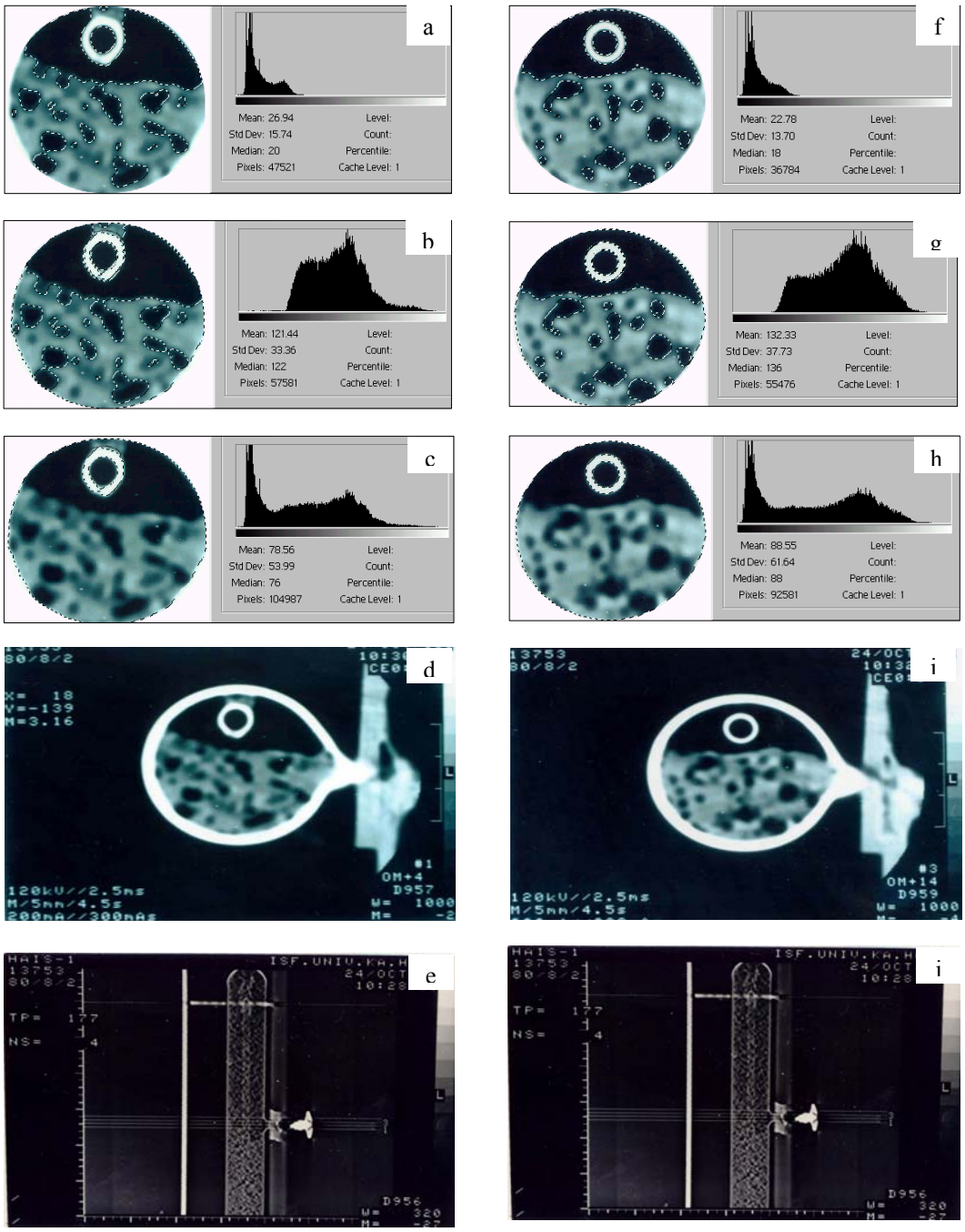

Figure 4.2. CT image: (Left) HAIS-PU-Influent port; (Right) HAIS-PU-Sample port-1:
(a & f) Black: Biogas and hollow space; (b & g) White: Biofilm and Media; (c & h) Total content of reactor; (d & i) Whole shape including reactor wall and gas collector tube; (e & j) CT site on reactor;

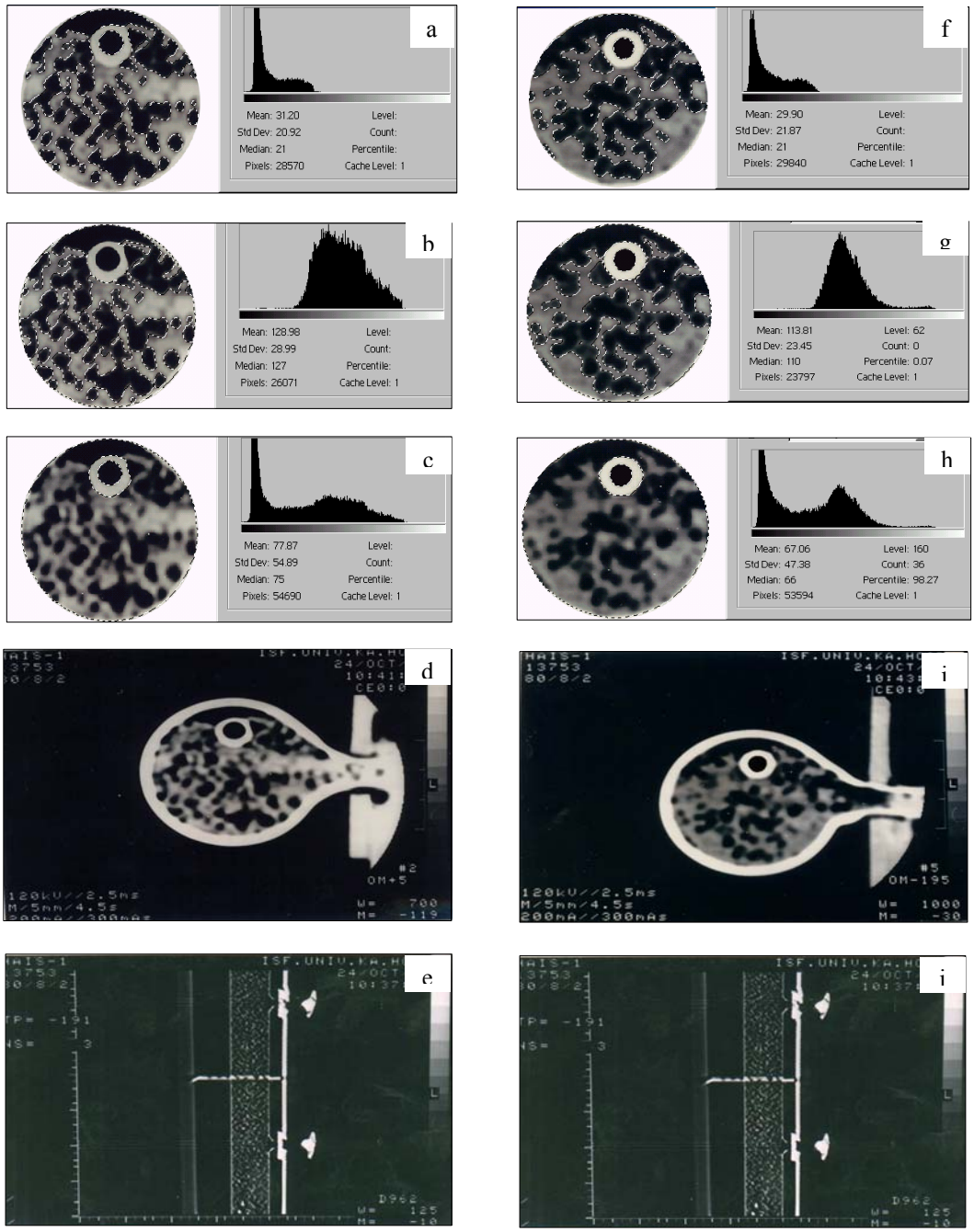

Figure 4.3. CT image: (Left) HAIS-PU-Sample port-2; (Right) HAIS-PU-Sample port-3:
(a & f) Black: Biogas and hollow space; (b & g) White: Biofilm and Media; (c & h) Total content of reactor; (d & i) Whole shape including reactor wall and gas collector tube; (e & j) CT site on reactor;

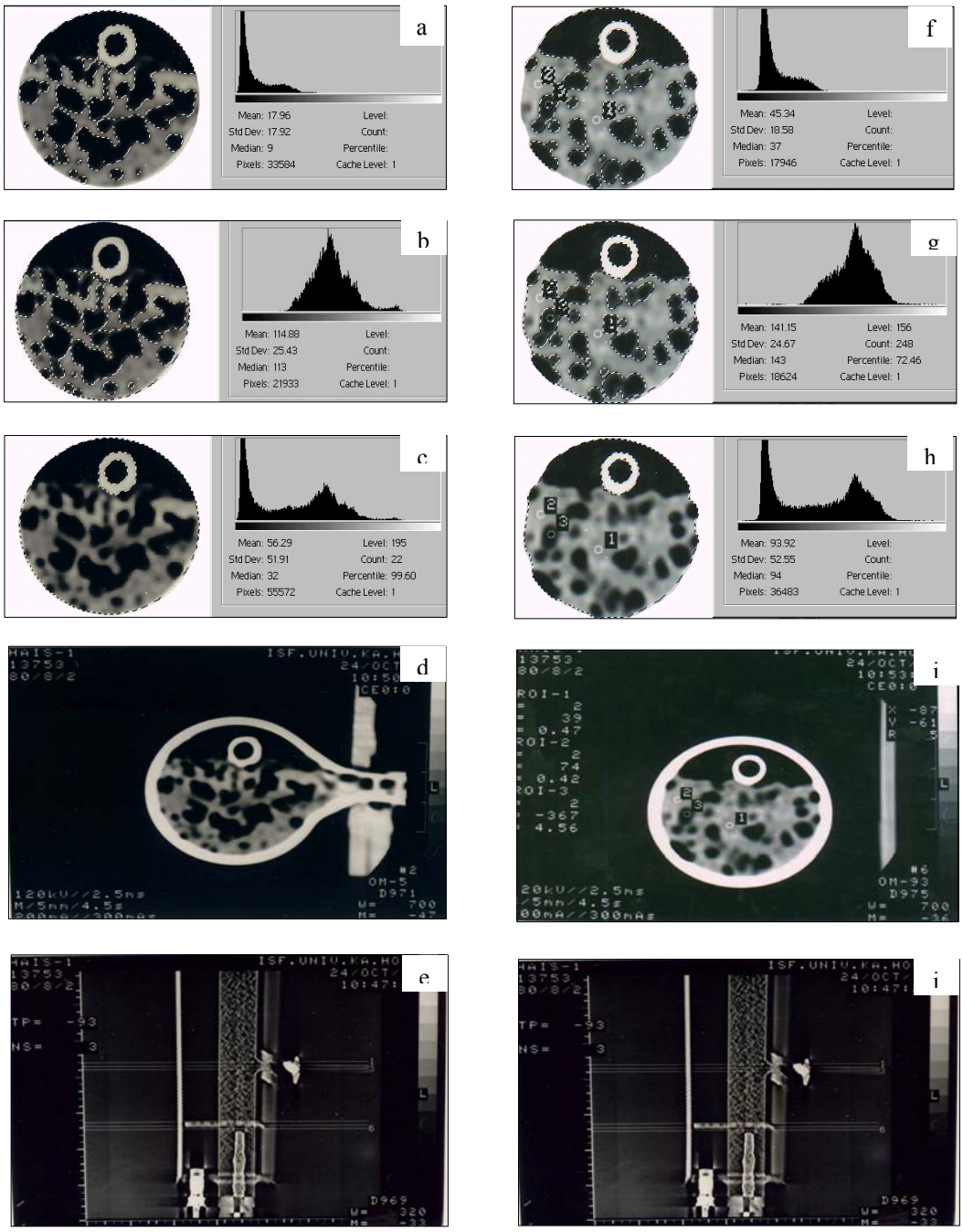

Figure 4.4. CT image: (Left) HAIS-PU-Sample port-4; (Right) HAIS-PU-Effluent port: (a & f) Black: Biogas and hollow space; (b & g) White: Biofilm and Media; (c & h) Total content of reactor; (d & i) Whole shape including reactor wall and gas collector tube; (e & j) CT site on reactor;

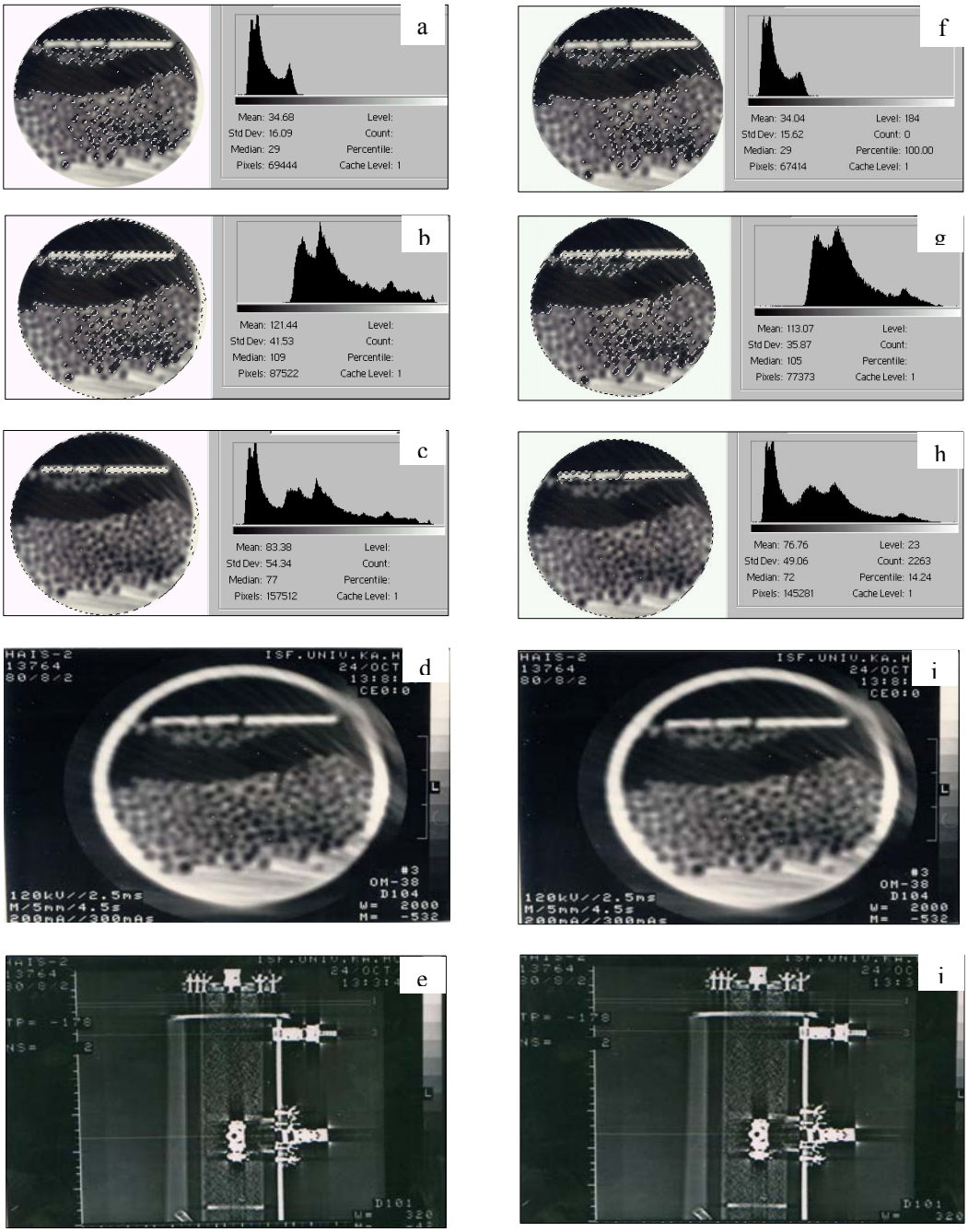

Figure 4.5. CT image: (Left) HAIS-PE-Influent port; (Right) HAIS-PE-Sample port-1:
(a & f) Black: Biogas and hollow space; (b & g) White: Biofilm and Media; (c & h) Total content of reactor; (d & i) Whole shape including reactor wall and gas collector tube; (e & j) CT site on reactor;

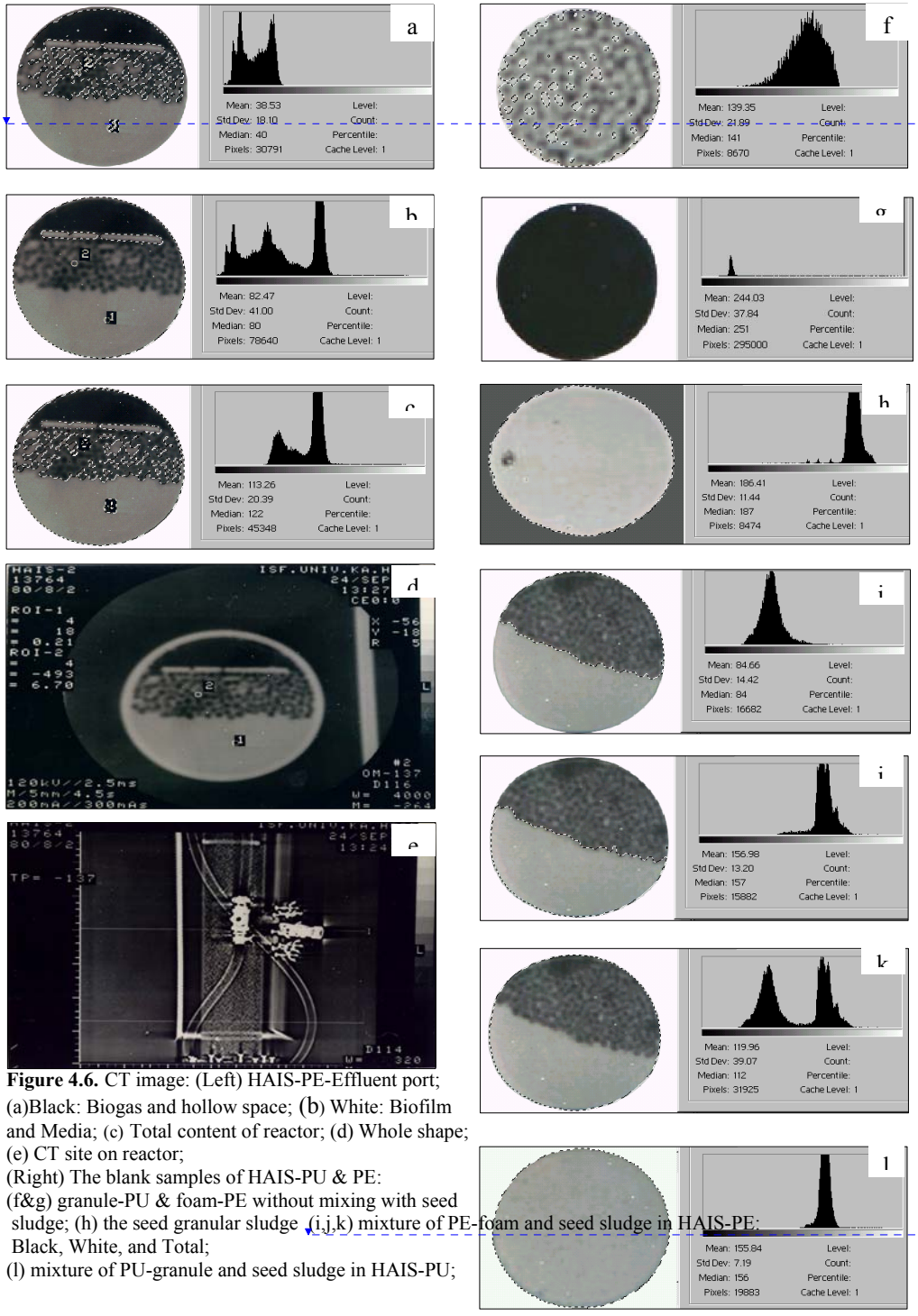

Figure 4.6. CT image: (Left) HAIS-PE-Effluent port; (a)Black: Biogas and hollow space; (b) White: Biofilm and Media; (c) Total content of reactor; (d) Whole shape; (e) CT site on reactor;
(Right) The blank samples of HAIS-PU & PE:
(f&g) granule-PU & foam-PE without mixing with seed sludge; (h) the seed granular sludge (i,j,k) mixture of PE-foam and seed sludge in HAIS-PE: Black, White, and Total;
(l) mixture of PU-granule and seed sludge in HAIS-PU;

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Table 4.2. The results of image analysis of CT-scans by Photoshop to estimate the biomass in HAIS reactors

Using the concepts of CT number and resolution to calculate VSS in HAIS reactors

As seen in Table 4.2, the resolution (column-5) of 200 ppi (Figure 4.7.a) and 150 ppi (Figure 4.7.b) was used to calculate the image surface areas (columns 10-12). Resolution is the number of units that occupy a linear inch in an image. It is measured in terms of ppi, or "pixels per inch" when viewed in an image. A pixel (picture element) is the basic two-dimensional element of a digital image.

The white part of Image surface area (cell: 11,4) in Table 4.2 with using the number of pixels in this part for the Influent port of HAIS-PE, shown in Figure 4.5.b and cell:(7,4) that was 87522 pixels, was estimated as:

$$[87522 \text{ pixel (cell: 7,4)}] / [(150 \text{ ppi})^2 \text{ (cell: 5,4)}] = 3.89 \text{ in}^2 \text{ (cell: 11,4)}$$

Then the occupied surface area by white part of HAIS reactors (biofilm and media), the cell (14,4) in Table 4.2 was calculated as:

$$\{[3.89 \text{ in}^2 \text{ (cell: 11,4; White part of image surface area)}] \times [5.14 \text{ in}^2 \text{ (cell: 12,4; Total surface area of HAIS-PE reactor)}]\} / [7.00 \text{ in}^2 \text{ (Total image surface area)}] = 2.86 \text{ in}^2 \text{ (cell: 14,4; White part of reactor surface area)}$$

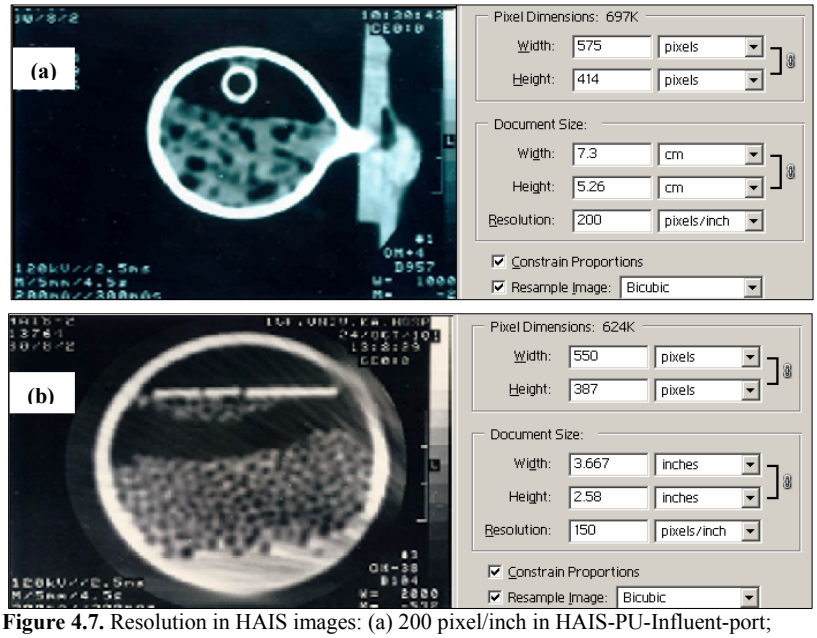

Figure 4.7. Resolution in HAIS images: (a) 200 pixel/inch in HAIS-PU-Influent-port; and (b) 150 pixel/inch in HAIS-PE-Influent-port;

Reactors performance

Performance of the reactors during the study can be subdivided into three different phases according to applied organic and surface loading rates in UASB and Fixed bed (HAIS) reactors respectively. The results of operational conditions and performance of the reactors are presented in Table 4.3.

The obtained data in Table 4.2 was used as basic VSS concentrations in HAIS reactors to claculate the F/M and specific methane production.

Table 4.3. **UASB and HAIS reactors Performance**

Phase	Reactor	Period days	HRT Hr	F/M g COD/g VSS.d	SLR g $COD.m^{-2}.d^{-1}$	COD removal, %	Biogas Production, mL/d
1	HAIS-PU	1-120	3.08 ±2.74	0.84±0.3	13 ± 5	51.8 ±2.2	
2		121-220	2.49 ±1.38	1.72±0.77	28 ± 12	63.5±17.5	1160±400
3		221-320	1.21 ±0.78	3.14±1.02	50 ± 16	51.6 ±2.2	
1	HAIS-PE	-	-	-	-	-	
2		-	-	-	-	-	
3		250-320	0.32 ±0.08	1.52±0.99	23 ± 14	61.6±18.6	1018±645
					OLR g COD. $L^{-1}.d^{-1}$		
1	UASB	1-120	9.54 ±7.68	0.235±0.103	4.02 ± 1.71	41.9 ±1.6	
2		121-220	4.71± 2.63	1.34±0.82	12.8 ± 6.26	64.3±15.2	1771±704
3		221-320	1.15± 1.16	4.85±1.61	21.27 ± 6.83	57.8±20.3	

1 = Start-up period; 2 = Optimum performance period; 3 = Maximum performance period;
SLR: surface loading rate; OLR: organic loading rate; F/M: food to microorganism ratio;

Start-up

During start-up, the UASB reactor was inoculated with granulated sludge developed in an earlier study using sludge from an activated sludge unit of a slaughterhouse wastewater treatment plant. Feeding of the synthetic wastewater (Table 3.1) was started at a hydraulic residence time of 28 hours. Thirty days after sludge adaptation to the new environmental and chemical conditions, a portion of mixed granulated and floculated sludge were transferred to HAIS reactors and feeding was started at HRT value of 16 hours. After one year, a thin biofilm was observed on the biomass carrier surface.

Filter Packing

Surface-to-Volume Ratio, S/V. The S/V ratio in HAIS-PU and PE reactors was 1100 and 1350 m2/m3, respectively. The ideal filter packing is a material that has a high surface per unit of volume. The S/V ratio in "conventional" and "high specific surface area"- plastic random packing is 98 and 150 m2/m3, respectively (Tchobanoglous et al., 2003). In the upflow attached growth anaerobic expanded-bed reactor (AEBR) process, the specific surface area is about 10,000 m2/m3 of reactor volume (Tchobanoglous et al., 2003), which is greater than any other packing. Therefore the arrange of S/V for three anaerobic fixed bed reactors is:

[S/V AEBR: 10,000 m2/m3] > [S/V HAIS: 1100-1350 m2/m3] > [S/V Fixed bed: 98-150 m2/m3]

and this arrange for size of packing material is:

[AEBR: 0.2-0.5 mm] < [HAIS: 3-5 mm] < [Fixed bed: 2.5-13 cm]

Reduction of bed porosity (ε) during operation of HAIS reactors

In operation day 330, following to increase around 1 mm to the average of biofilm thickness in HAIS-PU, the void ratio was decreased around 49 %. If it is assumed that there is a uniform and same growth in whole volume of reactor, the estimated reduction in initial bed porosity will be around 11%. In the other studies, the reduced initial void ratio due to accumulation of biological solids have been 35 and 80 %, one and two years after start-up of fixed bed reactors, respectively.

Bed porosity (ε) was found to be an important factor related to process stability and performance.

Loading rates and biogas production in both sets of reactors

As seen in Figure 4.8, in the SLR range of 8-70 g COD/m^2.d for both reactors, the maximum biogas production in HAIS-PU (around 3100 ml/d) is greater than HAIS-PE (less than 2400 ml/d). In spite of earlier start-up in HAIS-PU that resulted in a very good biofilm formation, however, the performance of gas separation device in HAIS-PE, using a perforated sheet (the same as a ruler) in upper part of reactor with several exhaust ports, was better than HAIS-PU, utilizing a perforated pipe with one exhaust port. Therefore, the average gas production for both reactors were almost the same.

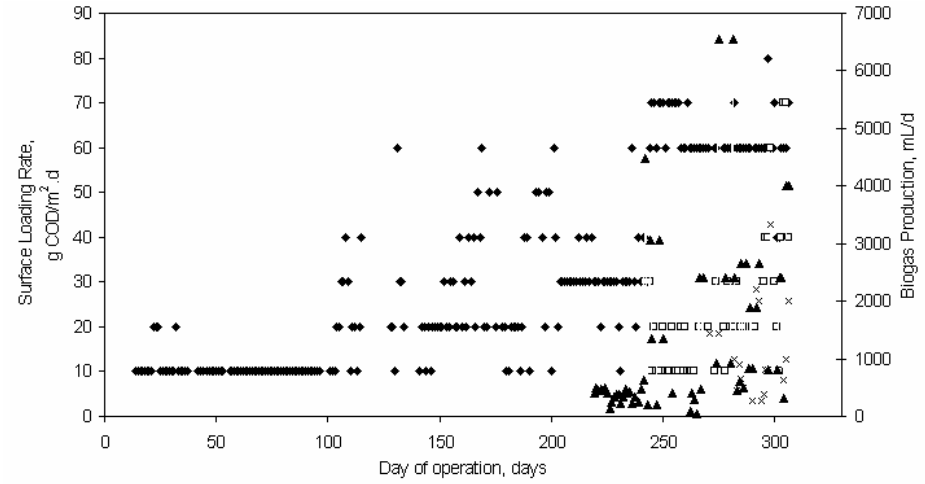

Figure 4. 8. Surface Loading Rate in HAIS-PU (♦) ; PE (□); and Biogas Production in HAIS-PU (▲) , PE (×);

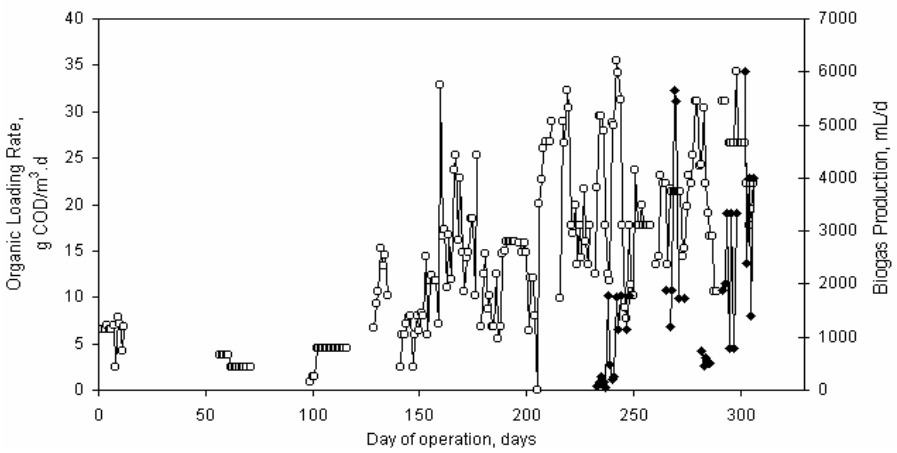

Figure 4. 9. Organic Loading Rate (○) and Biogas Production (♦) in UASB

The UASB system in Figure 4.9 with keeping the granular sludge throughout of operation and also more efficient gas-liquid-separator (GLS) revealed a better performance than the both HAIS reactors in Figure 4.8 holding immobilized sludge, and with two gas collectors in different shapes: perforated tube (HAIS-PU) and sheet (HAIS-PE).

The biomass immobilized as granular sludge obtained from UASB reactors has shown a well-structured conformation that optimizes substrate conversion and metabolic product generation/degradation. However, the attainment of such a conformation is slow, resulting in long start-up periods for UASB reactors. The spatial conformation of the microorganisms inside the polyurethane matrices clearly differs from those observed in granules obtained from UASB reactors, where the biomass is well structured in different layers. Although the spatial conformation of the biomass inside the polyurethane matrices presents no well-defined structure, such as conformation seems to optimize the flux of the primary substrate and intermediate products between species inside the matrices. Moreover, this conformation is attained quickly because of the low level of microbial organization required in such a support material.

The performance of HAIS-PU was better than PE. It can be related to more bed porosity (ε) of HAIS-PU (ε=0.42) than HAIS-PE (ε= 0.28). Channeling due to low bed porosity can be the main factor responsible for such performance.

Horizontal and Upflow Velocity in three reactors

The upflow velocity in UASB and horizontal velocity in HAIS-PU and PE with considering the void ratio of media were in ranges of: 0.03-0.76; 0.15-2.6; and 0.9-1.8 m/h, respectively. The optimum upflow velocity in UASB reactors and velocity in anaerobic fixed film reactors has been reported that to be 0.5 and 1 m/h respectively (Figure 4.10).

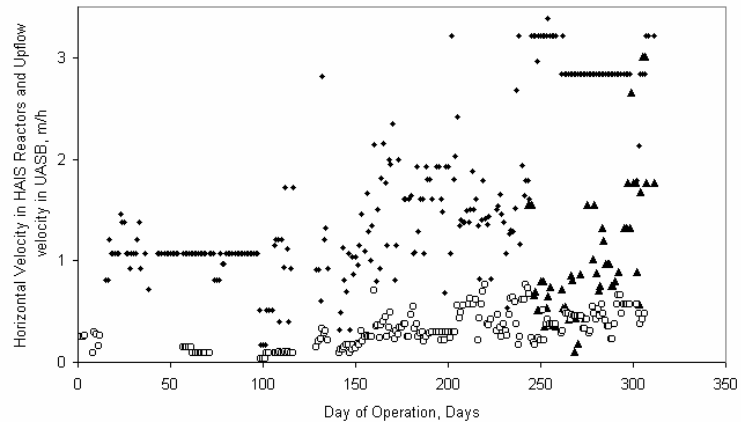

Figure 4. 10. Upflow velocity in UASB (○) and Horizontal velocity in HAIS-PU (♦); and HAIS-PE (▲) ;

Increasing height to diameter (H/D) ratio in upflow and downflow reactors, and length to diameter (L/D) ratio in horizontal reactors cause the greater actual velocities in reactors. In fixed film reactors, with H/D: 3-5, it is not necessary effluent recycling to keep optimum velocity around 1 m/h. In H/D ratio of 1 to 3, the recycle rate is less than 1 to less than 10 %, respectively. The recycle rate in Expanded bed reactors with optimum velocity of 7.5 m/h is 1-20% and for Fluidized bed with optimum velocity of 13.5 m/h is 10-80 %.

In this study with H/D: 15 for UASB, L/D: 20 in HAIS-PU; and L/D: 15 in HAIS-PE, it was not required for effluent recycle.

Hydraulic Retention Time (HRT) in three reactors

Figure 4.11 shows HRT of UASB, HAIS-PU and PE in range of: 28-1; 15.5-1; and less than 1 hr, respectively. In this study, there is a few operation days with HRT of less than 2.5 hr. It should be considered that if the amounts of HRT go down less than 2.5 hr, the biofilm formation will decrease and then the suspended growth will be a predominant in reactor. The result is the presence of high concentrations of suspended solids in effluent.

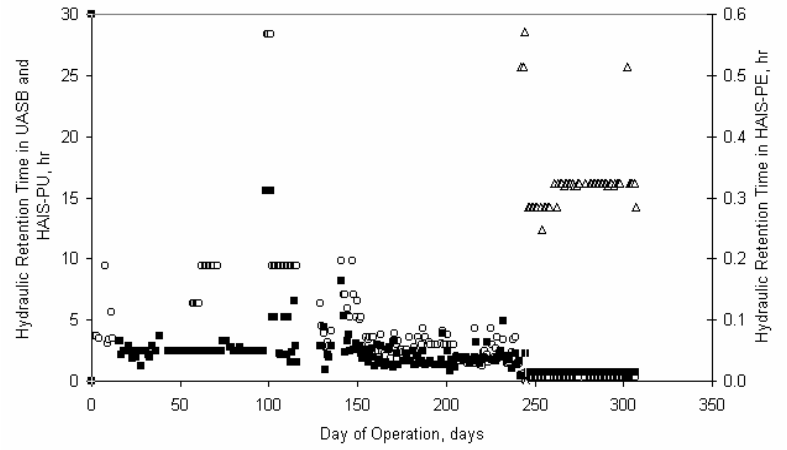

Figure 4. 11. Hydraulic Retention Time (HRT) in UASB (○); HAIS-PU (■); and HAIS-PE (△);

Organic loading rate, removal efficiency, and biogas production

The influence of organic loading rate on process efficiency and biogas production was studied by applying OLR values of 1-27 g COD/L.d for the UASB reactor, 8-66 and 9-37 g COD/m^2.d for HAIS-PU and HAIS-PE reactors, respectively. The results shown in Figure 4.12 reveals a general trend of decreased removal efficiencies and biogas production with increasing organic loading rate even though at different rates for different reactors. The overall COD removal efficiencies at different OLR values were as follows:

- UASB: from: 41.9 ± 1.6 to 57.8 ± 20.3 %

- HAIS-PU: from 51.8 ± 2.2 to 63.5 ± 17.5 %
- HAIS-PE: 61.6 ± 18.6 %

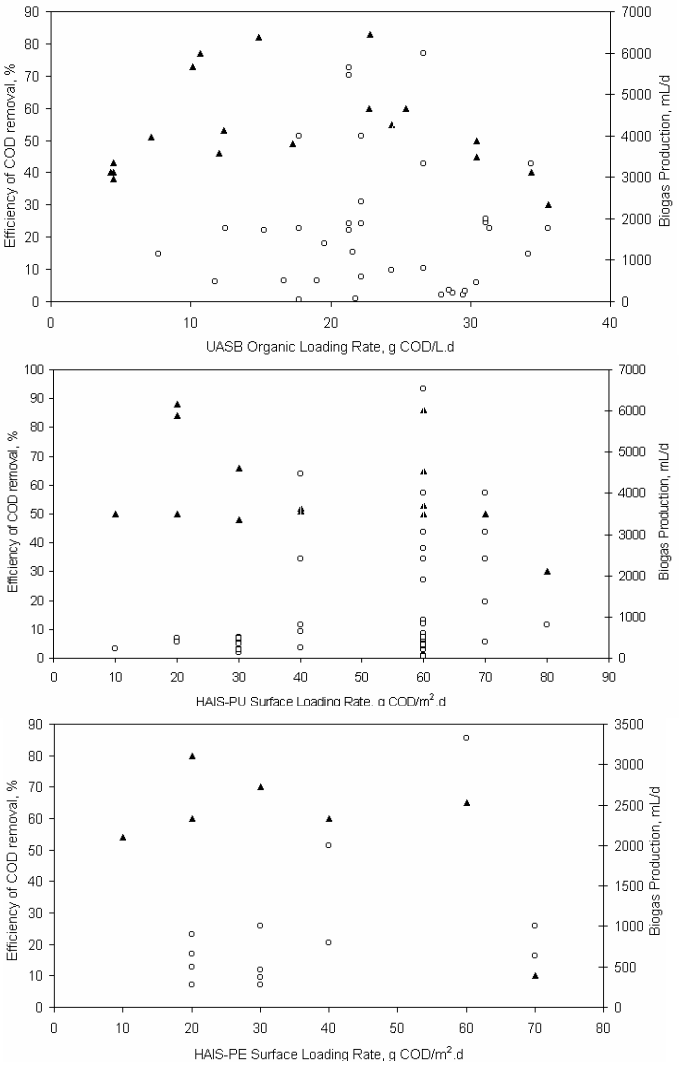

Figure 4. 12. Efficiency of COD Removal (▲) ; and Biogas Production (○); in three reactors;

CHAPTER 5

CONCLUSIONS

1. The performance of fixed-bed and suspended-growth high-rate anaerobic reactors was investigated in this study. The fixed bed reactors including two horizontal-flow anaerobic immobilized sludge (HAIS) reactors were filled with polyurethane (PU) and polyethylene (PE) beads. An inoculated UASB with granular sludge was used as suspended growth reactor. It was found that UASB with keeping the granular sludge throughout of operation and also more efficient gas-liquid-separator (GLS) has a better performance than the both HAIS reactors holder immobilized sludge, and with two gas collectors in different shapes: perforated tube (HAIS-PU) and sheet (HAIS-PE).
   The biomass immobilized as granular sludge obtained from UASB reactors has shown a well-structured conformation that optimizes substrate conversion and metabolic product generation/degradation. However, the attainment of such a conformation is slow, resulting in long start-up periods for UASB reactors. The spatial conformation of the microorganisms inside the polyurethane matrices clearly differs from those observed in granules obtained from UASB reactors, where the biomass is well structured in different layers. Although the spatial conformation of the biomass inside the polyurethane matrices presents no well-defined structure, such as conformation seems to optimize the flux of the primary substrate and intermediate products between species inside the matrices. Moreover, this conformation is attained quickly because of the low level of microbial organization required in such a support material.
2. The performance of HAIS-PU was better than PE. It can be related to more bed porosity (ε) of HAIS-PU (ε=0.42) than HAIS-PE (ε= 0.28). Channeling due to low bed porosity can be the main factor responsible for such performance.
3. In this study, the concept of quantitative CT number in terms of Hounsfield units (H) that is approximately linearly related to the mass density of the attenuating tissue and materials in objects in Computed Tomography Scanning (CT-Scan) technology was used to characterize of the HAIS reactor contents including biofilm, media, and biogas. Then the obtained CT-images from various sections of HAIS reactors was evaluated with using the concept of Resolution (pixel per inch) in Photoshop to estimate the VSS content of HAIS reactors.

   Providing CT-images from Fixed Film reactors can be a convenience quantitative method to determine the biofilm contents of this kind of reactor.
4. The results confirmed satisfactory performance of HAIS to provide suitable environmental conditions for biomass growth and retention during the short startup period of 25days. In the optimum period of operation the loading rates of reactors

were: organic loading rate (OLR) of 13 ± 6 g COD. L^{-1}. d^{-1} and F/M: 1.34±0.82 g COD/g VSS.d in UASB; surface loading rate (SLR) of 28 ± 12 g COD. m^{-2} .d^{-1} and F/M: 1.72±0.77 for HAIS-PU; and SLR of 23 ± 14 g COD.m^{-2} .d^{-1} and F/M of 1.52±0.99 g COD/g VSS.d for HAIS-PE.
In these loadings, the observed COD removal efficiencies and biogas productions were: 64.3±15.2 % and 1771±704 mL/d for UASB; 63.5±17.5 % and 1160±400 mL/d for HAIS-PU; and 61.6±18.6 % and 1018±645 mL/d for HAIS-PE.

5. With considering the following arrange for size of packing material:
   [AEBR: 0.2-0.5 mm] < [HAIS: 3-5 mm] < [Fixed bed: 2.5-13 cm]
   the arrange of Surface-to-Volume Ratio, S/V for three anaerobic fixed bed reactors as:
   [S/V AEBR: 10,000 m^2/m^3] > [S/V HAIS: 1100-1350 m^2/m^3] > [S/V Fixed bed: 98- 150 m^2/m^3]

6. Following to increase around 1 mm to the average of biofilm thickness in HAIS-PU, the void ratio was decreased around 49 %. With assumption of uniform and same growth in whole volume of reactor, the estimated reduction in initial bed porosity will be around 11%.

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APPENDIX

Table A.1. The specifications of SMA test

Table A.2. SMA tests on exposed and unexposed granules with erythromycin

Substrate (Individual& Mixed VFAs)	Erythromycin Concentration, mg/L						
	0	1	10	50	100	200	500
	Maximum Specific Methanogenic Activity (SMA) test, mL CH_4/g.VSS.d						
Butyric acid[(1)]	29.6						0
Butyric acid[(2)]	14		32.4			19	7
Butyric acid[(3)]	5.5	11	12.7			5	
mixed VFAs[(3)(4)]	65.5		70	61.3	55.6		
Blank	3.1						3.3

(1) With using fresh granules at OLR= 3 g COD/L.d in ASBR unexposed with Erythromycin
(2) With using fresh granules at OLR= 3 g COD/L.d in ASBR exposed with 1& 200 mg/L Erythromycin (2 months)
(3) With using stored granules in cold room (2 months) unexposed with Erythromycin
(4) Diluted ASBR substrate with ratio 1/5(the same as concentration of substrate internal of reactor after fill time)

Table A.3. Solid Retention Time (SRT) in ASBR

OLR, gCOD/l.d	0.4	1	2	3	3 (1mg/l Ery.)	3 (200 mg/l Ery.)
SRT, day	570	378	189	141	141	141

Table A.4. Erythromycin removal in ASBR

VITA

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