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University of Stavanger

Biological treatment of industrial wastewater for biogas production

Written by:

Erica Milagros Gómez Graterol

Abstract

Anaerobic biodegradation is a method of degradation that converts approximately 90% of the available chemical energy (in the form of organic material), into gas methane. Apart from the economic value of the methane gas produced, anaerobic treatment has many advantages over traditional aerobic treatment processes, such as less biomass produced per unit of substrate utilized; higher organic loadings are possible as anaerobic processes are not limited by oxygen transfer rates, and the lower constructional and operational costs compared with aerobic processes.

Since anaerobic biodegradation is an attractive waste treatment practice, industrial wastewaters from the oil and gas industry, typically known as "produced water" are ideal candidates for anaerobic digestion if they contain high levels of easily biodegradable materials. That is the case of the produced water from the gas field "Sleipner", which contains high concentration of methanol and glycol, substrates in theory easily biodegradable. However, process instability can be produced in anaerobic system, since it is a system where various groups of microorganisms operate and have different physiology, nutritional needs, growth kinetics, and sensivity to environmental conditions.

Through the installation and follow-up of a bioreactor in Laboratory, it was possible to demonstrate that it is possible to treat the produced water from Sleipner anaerobically. As evidence of this, some COD removal and produced gas were reported.

Difficulties on keeping the stability of the system occurred during the experimentation, specially when increasing organic loading rate. Some important parameters that required a better control during the experimentation were: buffering system capacity and residence time.

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God for giving me the strength to reach this goal.

My family for all the support they gave me during this time, specially my husband and daughter, from whom I stole part of our time as family.

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University of Stavanger.

TABLE OF CONTENTS

Pagina

LIST OF ABREVIATIONS

BTEX: Benzene, Toluene, Ethylbenzene, and Xylenes

PAH: Poly-Aromatic Hydrocarbons or Poly-nuclear Aromatic Hydrocarbons

LNG: Liquefied natural gas

MEG: Monoethylene glycol

DEG: Diethylene glycol

TEG: Triethylene glycol

MCRT: Mean Cell Residence Time

FA: Free ammonia

CSTR: Completely Stirred Tank Reactor

AF: Anaerobic filter

VFA: Volatile fatty acids

HRT: Hydraulic Retention Time

LIST OF FIGURES

INTRODUCTION

Nature Technology Solutions (NTS) is a company that provides environmental friendly, cost effective treatment of wastewater to the oil, shipping and offshore industries. But also this company provides physical, chemical and biological treatment methods for onshore industrial wastewater treatment.

Biological treatment is one of the most important treatments to be performed, since it will reduce the concentration of dissolved organic compounds contained in industrial wastewaters. In the case of the oil industry, dissolved hydrocarbons are found naturally in formation water and can be both toxic and bio-accumulative. Such water-soluble components, which in produced water are mainly BTEX, PAH and alkyl-phenols are together with heavy metals considered the most harmful contaminants in produced water.

At the present, NTS receives and treats biologically wastewaters derived from gas and hydrocarbon processing plants (like the Refinery at Kårstø, LNG plant at Melkøya), and from drilling operations and platforms.

Typically these wastewaters are treated with *aerobic* biological treatment, where they are decomposed in carbon dioxide and biomass (bio sludge). However, this type of biological treatment has some disadvantages such as: energy must be expended to supply oxygen to the system; there is no recovery of energy; produces considerable residual sludge and, it is usually required to add nutrients (phosphorus and nitrogen) to achieve biological degradation.

One possible better alternative for biological treatment is the *anaerobic* process. As its name indicates, it is a process where the microorganisms perform their metabolism in the absence of oxygen and they may be net *energy producers* instead of *energy users*, as is the case for aerobic processes. Summarizing, the principal advantages of this type of treatment are: less energy required, less biological sludge production, fewer nutrients required, methane production (potential energy source), smaller reactor volume required, with acclimation most organic compounds can be transformed, and rapid response to substrate addition after long periods without feeding.

Despite of anaerobic treatment seems to be the alternative to be chosen for the treatment of these industrial wastewaters, it is a process that has very sensible operational stability. In anaerobic digestion, the acid-forming and the methane-forming micro organisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. Inhibitory substances are often found to be leading cause of anaerobic reactor upset and failure since they are present in substantial concentrations in industrial wastewaters. A wide variety of substances have been reported to be inhibitory (sulphide, high metal ions, organics, etc) causing an adverse shift or inhibition in the microbial population.

The aim of this work is to evaluate the feasibility of the anaerobic biological treatment of one the most important industrial wastewater received and treated aerobically so far: Sleipner, from Karstø Refinery, which contains appreciable concentrations of mono-ethylene-glycol and methanol, among other compounds.

According to some researches done previously, it has been established that these compounds (methanol and glycols) are biodegradable in anaerobic conditions; however, taking into

account that these wastewater contains other compounds that could be inhibitory of the anaerobic process, it might be a challenge to demonstrate the anaerobic biodegradability of such wastewater.

It is believed that controlling the adequate conditions, acclimating the micro organisms to the prevailing “hard conditions” in this wastewater and avoiding possible activity of nuisance organisms (like SRB), it might possible to achieve the biodegradability of pollutants presents in the wastewater, especially methanol and glycols, which are present in appreciable concentrations and to get biogas as main targets.

I. THEORETICAL BASIS FOR THE RESEARCH

1. COMPOSITION OF PRODUCED WATER

In the oil and gas industry, the term used to describe the "industrial wastewaters" produced during extraction and processing of oil and gas is *produced water*. It implies formation water (natural water layer in the gas and oil reservoirs), injection water (if any water has been injected to the formation) and any chemicals added during the production and treatment processes.

Hydrocarbon production generates aqueous effluents that may contain traces of either hydrocarbon or chemical products, which have to be eliminated according to regulations and to operator's policy, to reduce the potential impact of their discharge on the environment or to meet re-injection specifications. Levels of dissolved components will be more and more present in such regulations.

Produced water composition is qualitatively similar to oil and gas production. The major compounds of produced water include: dissolved and dispersed oil compounds, dissolved formation minerals, production chemical compounds, production solids (including formation solids, corrosion and scale products, waxes and asphaltenes) and dissolved gases. The major compounds of produced water include [1]:

1.1. Dissolved and dispersed oil compounds

The amount of dissolved and suspended oil present in produced water (prior treatment) are related to the following factors: oil composition, pH, salinity, TDS, temperature, oil/water ratio, type and quality of oilfield chemicals, type and quantity of various stability compounds (waxes, asphaltenes and fine solids).

BTEX and phenols are the most soluble compounds in produced water. Aliphatic hydrocarbons, phenols, carboxylic acid, and low-molecular weight aromatic compounds are included as soluble compounds in produced water.

PAHs and some of the heavier alkyl phenols are less soluble in produced water and are present as dispersed oil. The concentration of PAHs and C_6-C_9 alkylated phenol are strongly correlated to dispersed oil content of produced water.

1.2. Dissolved formation minerals

Dissolved formation minerals or inorganic dissolved compounds in produced water include anions and cations, heavy metals and radioactive materials. Cations such as Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Fe^{2+} and anion such as Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^- affect produced water chemistry in terms of buffering capacity, salinity, and scale potential.

Salinity is due to dissolved sodium and chloride and is less contributed by calcium, magnesium and potassium. Salt concentration of produced water may vary from about 1000-300,000 ppm. Some researches believe that when salinity increases more than 100,000 ppm the biodegradation rate fell dramatically because high concentration of sodium chloride causes environmental stress, microbial lysing effects and promotes loss of biomass. Under high salinity conditions, halotolerant or halophilic species have been involved in the produced water aerobic biodegradation process. However, biological kinetics seems to be lower.

Species identified are Gram-positive bacillus (Halobacillus mainly, but also Halomonas and Virgibacillus). Sulphate concentration in produced water is lower than seawater.

Heavy metals concentrations in produced water depend on age of the wells and formation geology. Produced water contains traces quantities of cadmium, chromium, copper, lead, mercury, nickel, silver and zinc.

The source of radioactivity in scale is from radioactive ion, primarily radium that is co-precipitated from produced water along with other types of scales. Barium sulphate is the most common scale co-precipitated. ^{226}Radium and ^{228}Radium are the most abundant NORM (naturally occurring radioactive material) in produced water. There is a strong correlation between concentrations of barium and radium isotopes.

1.3. Production chemical compounds

Treatment chemicals (production treating, gas processing and stimulation) and production treating chemicals (scale and corrosion inhibitors, biocides, emulsion breakers, antifoam and water treatment chemicals) are used in these processes.

A wide range of gas treatment chemicals is used in gas fields and gas treatment including methanol, ethylene glycol, and tri-ethylene glycol. About one-third of these chemicals are discharge in produced water.

1.4. Production solids

Production solids are a wide range of materials including formation solids, corrosion and scale products, waxes and asphaltenes. In anoxic produced water, sulphides (poly-sulphides and hydrogen sulphide) are generated by bacterial reduction of sulphate.
Because of different toxic chemicals in produced water, few micro organisms can survive (mainly aerobic Gram-positive bacteria). Bacterial can clog or cause corrosion of equipment and pipelines. Some inorganic crystalline substances such as SiO_2, Fe_2O_3, Fe_3O_4, $BaSO_4$ are found in the suspended solids (SS) in produced water.

1.5. Dissolved gases

CO_2, O_2 and H_2S are common gases included in produced water

2. GENERAL ASPECTS OF ANAEROBIC OXIDATION

2.1. Basis steps of anaerobic oxidation

Unlike aerobic operations, which contain diverse microbial communities and complex food chains, anaerobic operations contain communities which are essentially totally bacterial [12]. In spite of this apparent simplicity, interactions among the bacterial species have a severe effect upon system performance. That is why it is important to know how the interactions in this ecosystem work.

Three basic steps are involved in the overall anaerobic oxidation of a waste: Hydrolysis, Fermentation (acidogenesis), and Methanogenesis. (Fig. Nr. 01) [12, 13]

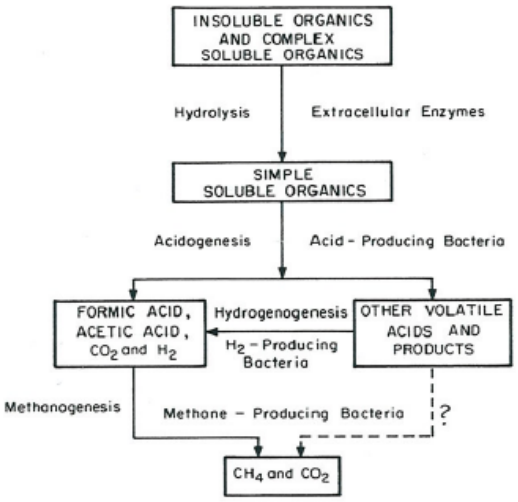


Fig. Nr .01: Multistep nature of anaerobic operations

2.1.1. Hydrolysis

It is the process where insoluble organics, before can be consumed, are converted to soluble compounds that can be hydrolyzed further to simple monomers that are used by bacteria that perform fermentation. In addition, large soluble organic molecules must be reduced in size to facilitate transport across the cell membrane.
These reactions responsible for solubilisation and size reduction are catalyzed by enzymes which have been released to the medium by the bacteria.
For some industrial wastewaters, fermentation may be the first step in the anaerobic process if all substrates are soluble.

2.1.2. Fermentation or Acidogenesis

The small molecules resulting from hydrolysis (amino-acids, sugars, and some fatty acids) are used as carbon and energy sources by bacteria which carry out fermentations. The oxidized end products of those fermentations are primarily short-chain volatile acids such as acetic, propionic, butyric, valeric and caproic. Their production is referred to as *acidogenesis* and the responsible organisms are called *acid-producing bacteria*.

The reduced end products of the fermentation depend upon the nature of the culture and the environmental conditions in the reactor. Some of the acid-producing bacteria possess a specialized enzyme system which allows them to oxidize reduced coenzymes without passing the electrons to an organic acceptor, thereby releasing hydrogen gas, H_2, to the medium. As a result, these bacteria produce few organic end products.

The following reaction **(1)** shows the products of the fermentation or acidogenesis of glucose as substrate [11]:

$$C_6H_{12}O_6 \rightarrow 1{,}14\ H_2 + 1{,}14\ CO_2 + 0{,}34\ C_2H_5OH + 1{,}31\ CH_3COOH + 0{,}2\ CH_3CH_2COOH + 0{,}19\ CH_3CH_2CH_2COOH \quad \textbf{(1)}$$

Products are respectively hydrogen, carbon dioxide, ethanol, acetic acid, propionic acid and butyric acid.

In addition, some of them utilize volatile acids larger than acetic (propionate, butyrate), as well as reduced organic compounds released by other bacteria, to produce acetic acid, CO_2 and H_2 (precursors of methane formation). The process is generally known as *acetogenesis*. The reactions are [11]:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad \textbf{(2)}$$

$$CH_3CH_2\ CH_2COOH + 2H_2O \rightarrow 2\ CH_3COOH + CO_2 + 2H_2 \quad \textbf{(3)}$$

The free energy change associated with the conversion of propionate and butyrate to acetate and hydrogen requires that hydrogen be at low concentrations in the system ($p_{H2} < 10^{-4}$ atm), or the reaction will not proceed. The collective activity of these hydrogen-producing bacteria is called *hydrogenesis*.

Actually, the distinction between acid-producing and hydrogen-producing bacteria is not clear. Since hydrogen-producing bacteria usually produce acids, but acid-producing bacteria do not all produce hydrogen, it is probably best to think of the hydrogen-producing bacteria as a subset of the acid-producing group.

The combined groups of acid- and hydrogen- producing bacteria are generally referred to as non-methanogenic bacteria and their integrated metabolism results primarily in formic acid, acetic acid, CO_2 and H_2.

If no hydrogen is formed, the non-methanogenic phase results in insignificant reductions in COD because all electrons released in the oxidation of organic compounds are passed to organic acceptors which remain in the losses due to microbial inefficiency. When hydrogen is formed, however, it represents a gaseous product which might escape from the medium thereby causing a reduction in the energy content, and thus the COD, of the liquid. However, hydrogen is one of the products of the non-methanogenic phase that can be used for methanogenic bacteria to produce methane gas, as it is indicated in the following part:

2.1.3. Methanogenesis

The products of the non-methanogenic phase (i.e., formic acid, acetic acid, CO_2 and H_2) are utilized by a group of microorganisms known collectively as *methanogens*.
With the exception of losses due to microbial inefficiency, almost all of the energy removed from the liquid is recovered into methane. 1 mole of methane requires 2 moles of oxygen to oxidize it to CO_2 and water, consequently each 16 grams of methane produced and lost to the atmosphere correspond to the removal of 64 grams of COD from the liquid.
At standard conditions, this corresponds to approx. to 0,35 $m^3\ CH_{4\ produced}$ /kg $COD_{\ removed}$.

Two groups of methanogenic organisms are involved in methane production. One group, termed *aceticlastic methanogens*, split acetate into methane and carbon dioxide, according to the reaction (4):

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \textbf{(4)}$$

The second group, termed *hydrogen-utilizing methanogens*, use hydrogen as the electron donor and CO_2 as the electron acceptor to produce methane.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \textbf{(5)}$$

Bacteria within anaerobic processes, termed acetogens, are also able to use CO_2 to oxidize hydrogen and form acetic acid. However, the acetic acid will be converted to methane, so the impact of this reaction is minor. About 72% of the methane produced in anaerobic digestion is from acetate formation.

The methanogens and the acidogens form a *synthrophic* (mutually beneficial) relationship in which the methanogens convert fermentation end products such as hydrogen, formate, and acetate to methane and carbon dioxide. Because the methanogens are able to maintain an extremely low partial pressure of H_2, the equilibrium of the fermentation reactions is shifted toward the formation of more oxidized end products (e.g. formate and acetate). The utilization of the hydrogen produced by the acidogens and other anaerobes by the methanogens is termed *interspecies hydrogen transfer*. In effect, the methanogenic organisms serve as a hydrogen sink that allows the fermentation reactions to proceed. If process upsets occur and the methanogenic organisms do not utilize the hydrogen produced fast enough, the propionate and butyrate fermentation will be slowed with the accumulation of volatile fatty acids in the anaerobic reactor and a possible reduction in pH.

As example, the anaerobic degradation pathway of glucose is shown in the following figure (Fig. Nr. 02)[17]

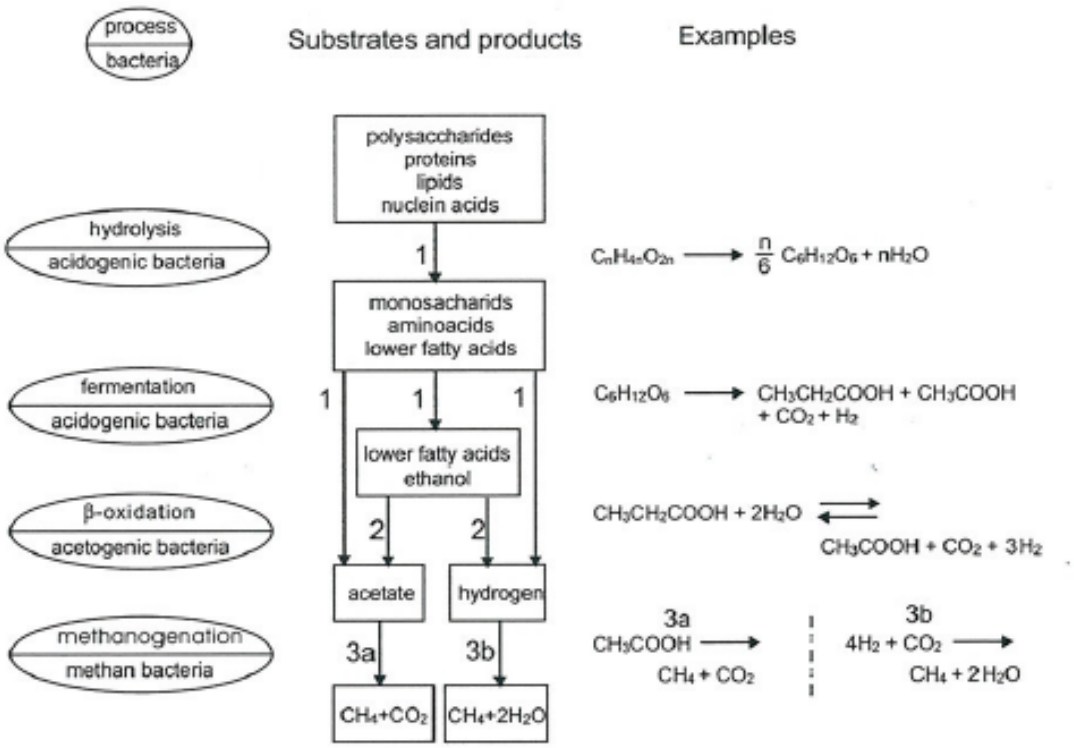


Fig. Nr. 02: Anaerobic metabolism of glucose as example

2.2. Main microorganisms[13]

1.2.1. Hydrolysis and Fermentation (Acidogenesis)

Non-methanogenic microorganisms, responsible for hydrolysis and fermentation, consist of ***facultative and obligate anaerobic bacteria***. Nevertheless, it does appear that the most important hydrolytic and fermentative reactions are carried out by strict anaerobes.

Organisms isolated from anaerobic digesters include *Clostridium spp*., *Peptococus anerobus*, *Bifidobacterium spp*., *Desulphovibrio spp*., *Corynebacterium spp*., *Lactobacillus, Actinomyces*, S*taphylococcus*, and *Escherichia coli*. Other physiological groups present include those producing proteolytic, lipolytic, ureolytic, or cellulytic enzymes.

1.2.2. Methanogenesis

Methanogenic microorganisms, classified as *archaea*, are ***strict obligate anaerobes***. Many of the methanogenic organisms identified in anaerobic digesters are similar to those found in the stomachs of ruminant animals and in organic sediments taken from lakes and rivers. The principal genera of microorganisms that have been identified at mesophilic conditions include the rods (*Methanobacterium*, *Methanobacillus*) and spheres (*Methanococcus*, *Methanothrix*, and *Methanosarcina*).

Methanosarcina and *Methanothrix* (also termed *Methanosaeta*) are the only organisms able to use acetate to produce methane and carbon dioxide. The other organisms oxidize hydrogen with carbon dioxide as the electron acceptor to produce methane. The acetate-utilizing methanogens were also observed in thermophilic reactors. Some species of *Methanosarcina* were inhibited by temperature at 65 °C, while others were not, but no inhibition of *Methanothrix* was shown. For hydrogen-utilizing methanogens at temperatures above 60 °C, *Methanobacterium* was found to be very abundant.

2.3. Stoichiometry and kinetics of anaerobic degradation of organic compounds[10]

In anaerobic processes two rate-limiting concepts are important:
(1) Hydrolysis conversion rate.
(2) Soluble substrate utilization rate for fermentation and methanogenesis.

Hydrolysis

The hydrolysis of colloidal and solid particles does not affect the process operation and stability but does affect the total amount of solids converted. In anaerobic digestion processes used for municipal waste sludges, greater than 30 days detention time is needed to approach full conversion of solids. The soluble substrate utilization kinetics is of great concern to develop a stable anaerobic process.

With substrate mainly as particulate compounds, the hydrolysis process or methane generation is slowest and controls the overall rate:

$$r_{acid} > r_{acet} > r_{meth} \text{ or } r_{hydr}$$

When substrate is dissolved (monomers or simple compounds) hydrolysis is not necessary (or is fast) methanogenesis is the slowest process and control overall rate. If acid production is too fast, acids accumulate and pH drops resulting in inhibition of the reactions and the process fails.

$$r_{acid} > r_{acet} > r_{meth}$$

Hydrolysis is carried out by extracellular enzymes, generated by the acidogenic organisms that feed on the products of hydrolysis. Hydrolysis of particulates are modelled as a first order reaction with respect to hydrolysable compounds:

$r_{hydr} = k_h \cdot X_S$ k_h: 0.3 – 0.7 d^{-1}

The products of hydrolysis, monomers, serve as substrates for the acidogenic organisms.

Acidogenesis

- The acid-phase process is rapid and the growth rate of acidogenic organisms is comparable to aerobic rates with $\mu_m \sim 2 - 7\ d^{-1}$. The growth is described according to the Monod equation.
- The products from acidogenesis are mainly short chain fatty acids (SCFA) such as acetic, propionic and butyric acids. Alcohols such as ethanol, propanol and butanol may also be produced in addition to lactic acid and formic acid.
- Due to no external electron acceptor the electrons from the substrate is captured in reduced organic compounds or H_2, originating from the substrate and is excreted from the cells as fermentation products.
- The large fraction of energy associated with the excreted fermentation products cause the remaining energy for growth to be limited and thus the growth yield is low: Y ~ 0.1 – 0.2 gVSS/gCOD.
- Reactions in generation of fermentation products:

Acetic acid: Glucose → Acetic acid + Hydrogen-gas

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

Propionic acid: Glucose + Hydrogen-gas → Propionic acid

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$

Butyric acid: Glucose → Propionic acid + Hydrogen-gas

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$

Acetogenesis

- The fermentation products other than acetic acid must be converted to acetic acid for being utilised by the methane generating organisms.
- Acetogenic organisms do this, where the substrates are the products in acidogenesis and the products, acetic acid and H_2, serve as substrates for methane producing organisms.
- The growth rate of acetogenic organisms is slightly higher than methane generating organisms, $\mu_m \sim 0.5 - 0.8\ d^{-1}$, but lower than the acidogenic organisms.
- Acetogenesis may be inhibited by high H_2, requiring continuous removal of H_2 in order to function.
- Reactions in acetogenesis:

Propionic acid: Propionic acid → Acetic acid + Hydrogen-gas

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$

Butyric acid: $\underset{\text{Butyric acid}}{CH_3CH_2CH_2COOH} + 2H_2O \rightarrow 2\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Hydrogen-gas}}{2H_2}$

Ethanol: $\underset{\text{Ethanol}}{C_2H_5OH} + H_2O \rightarrow 2\underset{\text{Propionic acid}}{CH_3COOH} + \underset{\text{Hydrogen-gas}}{2H_2}$

Methanogenesis

Methane is produced by two groups of organisms:

- Acetoclastic methanogens (1) using acetic acid as substrate and hydrogen utilising methanogens reducing CO_2 (2):

1. $CH_3COOH \rightarrow CO_2 + CH_4$
2. $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

- The growth rate of methanogenic organisms is low; $\mu_m \sim 0.3 - 0.5\ d^{-1}$, and long retention time is required for methane producing processes.
- The growth yield is also very low, as the majority of the energy in the substrate is converted into methane gas with a growth yield of Y ~ 0.05 – 0.1 gVSS/gCOD.

Because of the relatively low free energy change for anaerobic reactions, growth yield coefficients are considerably lower than the corresponding values for aerobic oxidation. Typical synthesis yield and endogenous decay coefficients for fermentation and methanogenic anaerobic reactions are Y=0.10 and 0.04 g VSS/g COD and Kd=0.04 and 0.02 gVSS/gVSS*d, respectively.

3. DESIGN OF ANAEROBIC SYSTEMS [12]

The primary decision which must be made during the design of the anaerobic reactor is the SRT to be used. SRT (Solid Retention Time, in past time called MCRT: Mean Cell Residence Time) represents the average period of time during which the sludge has remained in the system [13]. In case of solid substrate is being treated, two objectives are foremost in importance: the stabilization of the waste and improvement of its dewatering characteristics. If a soluble substrate is being treated the major emphasis will be upon its removal and the settling characteristics of the resulting sludge.

In addition, the mixing and the heating requirements must also be estimated in order to complete the process design.

3.1. Process design from lab data[12]

Process design data can be obtained from well mixed lab-scale anaerobic CSTR's operated at a variety of space times (MCRT's) between 5 and 30 days. Ideally, feeding should be continuous but this is often difficult when sludge is being digested so it is common practice to add feed and withdraw effluent once or twice a day. The frequency of feeding will be determined by the space time of the reactor since it is best to remove not more than 5% of the reactor contents at any one time. The reactors must be kept free of oxygen and provision should be made for collection and measurement of the gas produced. Each reactor should run

for several MCRT's to ensure stability and samples should be taken over a period of at least one MCRT's for determination of the concentrations of suspended solids, volatile suspended solids, volatile solids, soluble COD, total COD, alkalinity, volatile acids, and ammonia nitrogen in both the effluent and the influent. In addition the pH of the reactor should be monitored and the effluent solids should be evaluated for their settle-ability and dewater-ability.

3.1.1. Solid substrate (anaerobic digestion)

The main objective in the anaerobic digestion is the stabilization of the volatile solids. Often when the percent volatile solids destruction is plotted as a function of the log of MCRT the result is a straight line (Fig. Nr. 03). Another variable of interest is the methane production per unit of volatile solids destroyed, apparently as a function of the MCRT too (Fig. Nr. 04).

Data on pH, concentration of soluble COD, volatile acids, ammonia and alkalinity will aid in estimating the stability of the digestion process and in determining the best technique for disposal of supernatant which will normally be too high in COD and ammonia for discharge directly to the environment. The chosen MCRT will give the grade of volatile solids destruction.

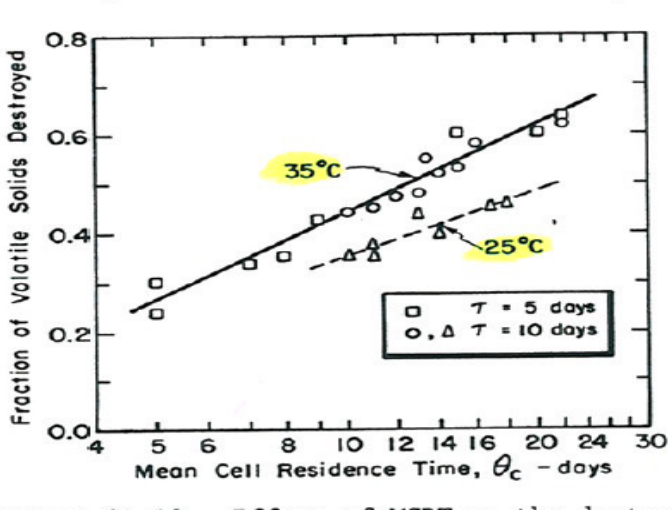


Fig. Nr 03: Effect of MCRT on the destruction volatile solids during anaerobic digestion of primary and waste activated sludge.

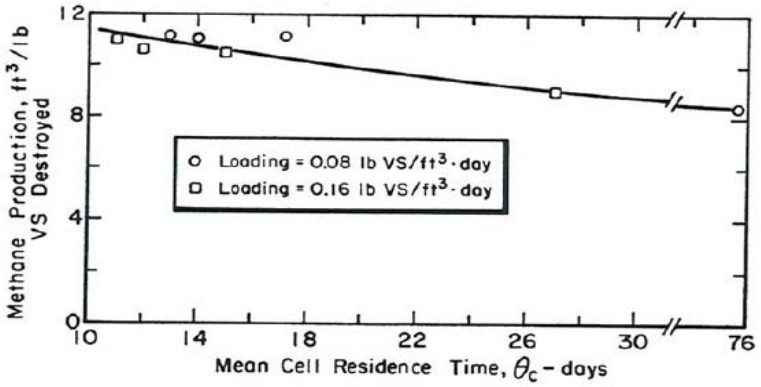


Fig. Nr. 04. Effect of MCRT on methane production

3.1.2. Soluble substrate (anaerobic contact)

Anaerobic contact is used to reduce the concentration of a soluble substrate, generally before discharge to an aerobic treatment process. Soluble substrate concentration and concentration of suspended solids in the effluent are necessary to predict the overall treatment efficiency. Using the data collected on the lab-scale CSRT's, plots should be made of the effluent soluble COD, c, the observed yield, Y, and the settleability of the sludge as a function of the MCRT. In addition, a plot should be made of either the volume of methane produced per unit mass of COD destroyed, the oxygen demand coefficient of the cells formed, as a function of MCRT.

3.2. Factors influencing the design [12,13]

3.2.1. Characteristics of the wastewater

Anaerobic processes are attractive for high strength and warm temperature wastewaters

3.2.2. Flow and load variations

Wide variations in influent flow and organic loads can upset the balance between acid fermentation and methanogenesis in anaerobic processes. For soluble, easily degradable substrates, such sugars and soluble starches, the acidogenic reactions can be much faster at high loadings and may increase the reactor volatile fatty acids (VFA) and hydrogen concentrations and depress the pH. High hydrogen concentrations can inhibit propionic and butyric acid conversion. Flow equalization or additional capacity must be provided to meet peak flow and loading conditions.

Organic loading rates of 3.2 to 32 kg COD/m3·d may be used (higher than in aerobic processes, so smaller reactor volumes and less space may be required for treatment.) [13]

3.2.3. Organic concentrations and temperatures

Reactor temperatures of 25-35 °C are generally preferred to support optimal biological reaction rates and to provide more stable treatment. Generally, COD concentrations greater than 1500 to 2000 mg/L are needed to produce sufficient quantities of methane to heat the wastewater without an external fuel source. At 1300 mg/L COD or less, aerobic treatment may be the preferred selection.

Anaerobic treatment can be supplied at lower temperatures and has been sustained at 10-20 °C in suspended and attached growth reactors. At lower temperatures, slower reaction rates occur and SRTs, larger reactor volumes, and lower organic COD loadings are needed. Further, at temperatures in the range 10-20 °C, the degradation of long chain fatty acid is often rate limiting. If load fatty acids accumulate, foaming may occur in the reactor. When higher SRTs are needed, the solids loss in the anaerobic reactor can become a critical limiting factor. Anaerobic reactors generally produce more dispersed, less flocculent solids than aerobic systems, with effluent TSS concentrations for suspended growth in the 100-200 mg/L range. For dilute wastewaters, the effluents TSS concentration will limit the possible SRT of the process and treatment potential. Either a lower treatment performance occurs or it is necessary to operate the reactor at a higher temperature. Thus, the method used to retain solids in the anaerobic reactor is important in the overall process design and performance.

3.2.4. Fraction of nondissolved organic material

Wastewaters with high solids concentrations are treated more appropriately in suspended growth reactors than by upflow or downflow attached growth processes. Where greater conversion of particulate organic matter is required, longer SRT values may be needed if solids hydrolysis is the rate-limiting step as compared to acid fermentation or methanogenesis in anaerobic treatment.

3.2.5. Mixing

Adequate mixing is particularly important because it prevents the development of microenvironments unfavourable to the methanogenic population (e.g. regions of low pH) which could reduce process efficiency.

Mixing also maintains a uniform temperature in the reactor and can help to break apart sludge particles, thereby exposing a greater surface area to biological attack. Finally, mixing helps to prevent the formation of a scum layer on the reactor surface.

3.2.6. Alkalinity

The alkalinity of the reactor will be indicative of its buffering capacity and the higher the alkalinity the more stable the pH is likely to be.

The primary buffering system in an anaerobic reactor is the carbonic acid- carbon dioxide system. If the concentration of the waste being treated is low, the amount of carbon dioxide produced will be small, which will result in lower alkalinity, thereby giving a reaction system which may experience stability problems. This can be compensated for by concentrating the waste prior to treatment or by adding additional alkalinity in the form of a bicarbonate salt.

With high CO_2 content (typically in the range from 30 to 50 percent) in the gas produced in anaerobic treatment, alkalinity concentrations in the range from 2000 to 4000 mg/L as $CaCO_3$ are typically required to maintain the pH near neutral.

3.2.7. Nutrients

The chemical composition of anaerobic cells is quite similar to that of the aerobic cells, and consequently the amounts of nitrogen and phosphorus required per unit mass of cells formed are the same.

Much of the energy in the original substrate is lost from the liquid as methane, however, so that the mass of cells formed per unit of mass of COD removed an anaerobically is much lower than it is aerobically. Consequently, the amount of nitrogen and phosphorous needed per unit mass of COD removed will also be much smaller.

In case of industrial waste, it may be necessary to add nutrients like phosphorus and nitrogen, but also trace of inorganic nutrients as well as organic nutrients like vitamins. This need should be investigated during lab-scale treatability studies because the provision of proper nutrients may allow a reduction in the MCRT. In general:

Macronutrients typical requirements for N, P and S: 10-13, 2-2.6 and 1-2 mg/100 mg of biomass, respectively.

Micronutrients typical requirements for Fe, Co, Ni and Zn: 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced, respectively.

3.2.8. Solids Retention time

In general, SRT values greater than 20 days are needed for anaerobic processes at 30 °C for effective treatment performance, with much higher SRT values at lower temperatures.

3.2.9. Treatment efficiency needed

Anaerobic treatment processes are capable of high COD conversion efficiency to methane with minimal biomass production. At SRT values greater than 20-50 days, maximum conversion of solids may occur at temperatures above 25 °C. Some other form of aerobic treatment would be necessary to provide effluent polishing. Pilot studies may be required.

3.2.10. Others

Proper analysis and treatability studies of inorganic an organic compounds, expected methane gas production, sulphide production, liquid-solids separation

3.3. Anaerobic reactor systems

The implementation and successful applications of the anaerobic systems was mainly due to the development of high-rate reactors. One of the main characteristics of the high-rate reactors is the uncoupling of solids retention time (SRT, defined in part 3) and hydraulic retention time (HRT, which it is a measure of the average length of time that a soluble compound remains in a bioreactor), resulting in high retention of active biomass. This uncoupling can be achieved by various means of sludge retention, such as sedimentation, immobilization on a fixed matrix or moving carrier material, and granulation.

Summarizing, most common anaerobic treatment systems (ATS), based on loading rate are [13, 18].

3.3.1. Low rate ATS (volumetric loading rate < 8 kg COD/m^3*d)

3.3.1.1. Completely stirred tank reactor (CSTR).

- Volumetric loading rate: 1.0-5.0 kg COD/m^3*d
- Without sludge recycle
- Suitable for sewage sludges and manure digestion
- Suitable for wastes with high concentrations of solids or extremely high dissolved organic concentrations
- No sludge retention (HRT= SRT)
- Long hydraulic retention time (15-30 days)

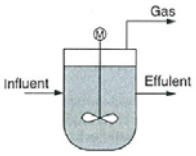


Fig. Nr. 05: Completely Stirred Tank Reactor (CSTR)

3.3.1.2. Anaerobic Contact Process

- Volumetric loading rate: 1.0-8.0 kg COD/m^3*d

- Wastewaters with lower solids concentrations than in CSTR are treated anaerobically by contact systems

- Sludge retention by external sedimentation and sludge recycling, so SRT>HRT

- Hydraulic retention time: 0.5-5 days

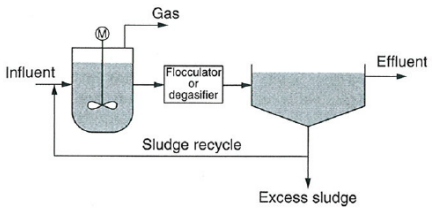


Fig. Nr. 06: Anaerobic contact process

3.3.1.3. Covered Anaerobic Lagoon

- Suitable for wastewaters rich in suspended solids

- Sludge retention by sedimentation

- Large reactor with large footprint

- Long hydraulic retention time

- It is estimated SRT >HRT because large fraction of influent solids will settle and undergo long-term degradation.

- SRT is estimated varies from about 50 to 100 days

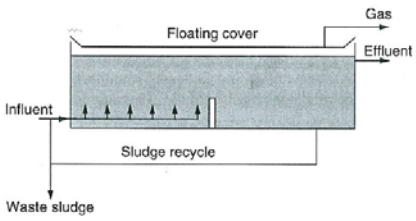


Fig. Nr. 07: Covered anaerobic lagoon

3.1.3.4 Anaerobic Sequencing Batch Reactor (ASBR)

- Volumetric loading rate: 1.2-2.4 kg COD/m^3*d

- Considered as a suspended growth process with reaction and solid-liquid separation in the same vessel

- Suitable for wastewaters rich in suspended solids

- Operation in 4 steps: feed, react, settle and decant/effluent withdrawal

- Sludge retention by sedimentation (settling times used about 30 min)

- Hydraulic retention time: 0.25-0.50 days

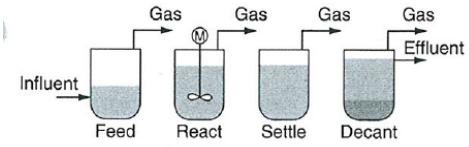


Fig. Nr. 08: Anaerobic Sequencing Bacth Reactor (ASBR)

3.3.2. High rate ATS (volumetric loading rate < 3-20 kg COD/m^3*d)

3.3.2.1. Anaerobic Filter (AF).

- Also known as *upflow packed-bed reactor*. Packing material typically is synthetic plastic packing (Pall rings, corrugated cross-flow or tubular modules)

- Sludge retention by attachment on filter material and entrapment in voids in the filter

- Effluent recycle is used just for high-strength wastewaters

- Can be operated upward and downward

- The process is best suited for wastewaters with low suspended solids concentrations

- At loadings of 1-6 kg COD/m3d, processes efficiencies up to 90% are shown for high-strength wastewaters.

- Advantages: high COD loadings, relatively small reactor volumes, and operational simplicity.

- Limitations: Cost of packing material, operational problems and maintenance due to solids accumulation and possible packing plugging.

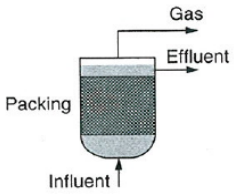


Fig. Nr. 09: Anaerobic filter (AF)

3.3.2.2. Upflow Anaerobic Sludge Bed (UASB) Reactor

- Sludge retention by granulation and settling

- Internal three-phase separator to separate gas, sludge and water

- Optimal contact of water with biomass by gas mixing

- Removal efficiencies of 90-95% for COD have been achieved at COD loadings ranging from 12 to 20 kg COD/m^3·d.

- Values for HRT for high-strength wastewater have been as low as 4-8 hours at these loadings.

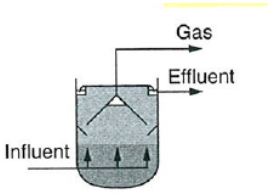


Fig. Nr. 10: Upflow Anaerobic Sludge Bed (UASB) Reactor

3.3.3. Super high rate ATS (volumetric loading rate: 10-30 kg COD/m^3*d)

3.3.3.1. Expanded Granular Sludge Bed (EGSB) Reactor.

- Also known as *attached growth anaerobic expanded-bed reactor* (AEBR)

- Packing material: generally silica sand (diameter range: 0.2-0.5 mm) and specific gravity of 2.65

- Upflow velocity in the reactor: 2 m/h. (Up to 10 m/h as maximum [18])

- Bed expansion during operation: 20%

- Suitable for medium-low strength wastewater and low temperatures (< 25 °C)

- External recirculation

- Comparable to fluidized bed reactor (FBR): similar design but BFR is operated at higher upflow liquid velocities (20 m/h) to provide 100% bed expansion. Recycle is also used.

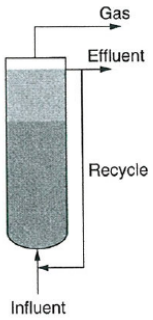


Fig. Nr. 11: Expanded Granular Sludge Bed (EGSB) Reactor

3.3.3.2. Internal Circulation (IC) Reactor.

- Requires anaerobic granular sludge
- Two internal three-phase separators
- Internal circulation of water by gas lift principle

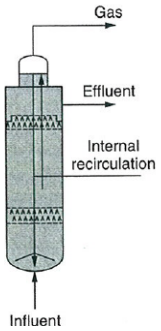


Fig. Nr. 11: Expanded Granular Sludge Bed (EGSB) Reactor

Fairly common in anaerobic treatment is the physical separation of hydrolysis and acidification in an acidifying reactor, and acetogenesis and methanogenesis in a second reactor. This phase separation is supported for some researches, who claimed that such separation would lead to better control of the overall digestion process.

Staging of anaerobic treatment systems can be considered beneficial for the treatment of various types of complex wastewaters, such as domestic sewage or wastewaters containing slowly biodegradable or inhibitory compounds. A staged reactor system will provide a higher treatment efficiency, because more difficult compounds like intermediates such as propionate, or possibly even xenobiotic compounds (when present in the wastewater), will find a more optimal environment for degradation due to the development of appropriate microbial communities in each stage. The process stability of a staged system is also substantially higher than in the present commonly practiced one-step systems.

4. FACTORS THAT AFFECT ANAEROBIC PROCESS OPERATIONS

The main factors that affect the performance of anaerobic operations are:

4.1. Temperature[12]

This effect is particularly important because of the interacting populations. For example, different species of bacteria will respond to changes in temperature in qualitatively similar but quantitatively dissimilar ways. As a result, a reactor which has been developed at one temperature is likely to have a different balance of species than a reactor developed at another temperature.

Furthermore, changes of only a few degrees can cause an imbalance between the two major populations which can lead to process failure. Consequently, the maintenance of a uniform temperature is more important than the maintenance of the temperature which gives the

maximum possible rates. Little information has been reported about the effects of temperature upon non-methanogenic bacteria.

Based on data available concerning the effects of temperature upon the utilization of VFA by the methanogenic population, it has been found that considerable reduction is reactor size can be achieved by operation at elevated temperature.

Most *mesophilic* digesters are designed to operate at a temperature between 30-35 °C. If the methane produced within the reactor is to be sufficient to supply the heat required to maintain such temperature, the amount of biodegradable COD available in the waste must exceed approximately 5000 mg/L. If it doesn't, then the design engineer must decide whether to operate at a lower temperature with a longer MCRT or to supply heat from an external source.

It is possible to operate anaerobic bioreactors in the *thermophilic* range, although different microbial species will be involved. However, experience in the field with thermophilic digestion has not been highly satisfactory and there are still considerable questions. Consequently, most anaerobic operations are designed in the mesophilic temperature range and the MCRT is adjusted to give the desired performance. Operationally if the mesophilic or thermophilic conditions are desired and the temperature becomes low, this will be compensated with longer MCRT.

In general, anaerobic digestion can occur over a wide temperature range which has generally been subdivided into three separate ranges: psychrophilic (5-25 °C), mesophilic (25-38 °C) and thermophilic (50-70 °C).

4.2. pH[12]

A pH value near neutral (7.0-7.5) is preferred and below 6.8 the methanogenic activity is inhibited.
The primary effect of pH upon the non-methanogenic population is upon the types of products formed. This parameter changes the substrates available to the hydrogenic and methanogenic bacteria, which will, in return, influence the rates at they can operate. It is not yet clear at what pH the best products are formed by the non-methanogenic bacteria, but as long as the two populations are grown together, a pH near 7,0 is optimum for the system as a whole.

4.3. Light metals ions (Na, K, Mg, Ca and Al)[6]

High salt levels cause bacterial cells to dehydrate due to osmotic pressure. Although the cations of salts in solution must always be associated with the anions, the toxicity of salts was found to be predominantly determined by the cation. The light metal ions including sodium, potassium, calcium and magnesium are required for microbial growth and, consequently, affect specific growth rate like any other nutrient. While moderate concentrations stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity.

It is believed that Aluminium inhibits growth due to competition with iron and manganese, or to its adhesion to the microbial cell membrane or wall. It has been reported for some studies that both acetogenic and methanogenic microorganisms were inhibited by addition of $Al(OH)_3$, being the specific activity of acetogenic more affected than methanogenic.

Calcium is known to be essential for the growth of certain strains of methanogens. It is also important in the formation of microbial aggregates. Excessive amounts of calcium lead to precipitation of carbonate and phosphate, which may result in: scaling of reactors and pipes, scaling of biomass and reduced specific methanogenic activity, loss of buffer capacity and essential nutrients for anaerobic degradation. Beyond this, very little is known about the toxicity of Ca^{2+}.

The optimal Magnesium concentration was reported to be 720 mg/L for some anaerobic bacteria type *Methanosarcina*. Cultures could be adapted to 300 mM Mg2+ without a change in growth rate, but growth ceases at 400 mg/L, according some authors. Magnesium ions at high concentrations have been shown to stimulate the production of single cells, according to other authors.

In case of Potassium, studies have shown that high levels of extracellular potassium (1.0 M) lead to passive influx of potassium ions that neutralize the membrane potential. In addition, potassium is one of the best extractants for metals bound to exchangeable sites in sludge. Toxic effect of potassium is rarely referenced in the literature. Low concentrations of potassium (< 400 mg/L) were observed to cause enhancement in performance in both the thermophilic and mesophilic ranges while at higher concentrations there was an inhibitory effect that was more pronounced in the thermophilic temperature range.

It seems there is more rapid adaptation of mesophilic microorganisms than thermophilic microorganism to wastewaters with high concentrations of sodium, according to researchers. In comparing VFA-degrading bacteria, sodium was more toxic to propionic acid-utilizing microorganisms than to acetic acid-utilizing ones. At low concentrations, sodium is essential for methanogens, probably because of its role in the formation of adenosine tri-phosphate or in the oxidation of NADH.

Some people reported sodium concentrations in the range 100-200 mg/L to be beneficial for the growth of mesophilic anaerobes. According to others, the optimal sodium concentration for mesophilic aceticlastic methanogens in wastewater treatment is 230 mg Na^+/L. The optimal growth conditions reported for mesophilic hydrogenotropic methanogens occurs at 350 mg Na^+/L. Other study reported sodium concentrations ranging from 3500 to 5500 mg/L to be moderately and 8000 mg/L strongly inhibitory to methanogens at mesophilic temperatures.

It Acclimation of methanogens to high concentrations of sodium over prolonged periods of time could increase the tolerance and shorten the lag phase before methane production begins. The tolerance is related to the Na^+ concentration the methanogens acclimated to and the time of exposure.

4.4. Heavy metals (Cr, Fe, Co, Cu, Zn, Cd and Ni)[6]

The heavy metals identified to be of particular concern include chromium, iron, cobalt, copper, zinc, cadmium and nickel. A distinguishing feature of heavy metals is that, unlike many other toxic substances, they are not biodegradable and can accumulate to potentially toxic concentrations. The toxic effect of heavy metals is attributed to disruption of enzyme function and structure by binding of the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups.

Analysis of ten methanogenic strains showed the following order of heavy metal composition in the cell: Fe >> Zn >> Ni > Co = Mo > Cu. Whether heavy metals would be stimulatory or inhibitory to anaerobic microorganisms is determined by the total metal concentration, chemical forms of the metals, and process-related factors such as pH and redox potential.
The relative sensitivity of acidogenesis and methanogenesis reported to heavy metals is Cu > Zn > Cr > Cd > Ni > Pb and Cd > Cu > Cr > Zn > Pb > Ni, respectively.

Industrial wastewaters or sludges generally contain many kinds of heavy metals which cause synergistic or antagonistic effects on anaerobic digestion. The level of inhibition is determined by the species and the ratio of the individual components. Although toxicity of most mixed heavy metals such as Cr-Cd, Cr-Pb, Cr-Cd-Pb, and Zn-Cu-Ni was found to be synergistic, some of the metal mixtures showed antagonistic inhibition.

The most important methods for mitigating heavy metal toxicity are precipitation, sorption and chelation by organic and inorganic ligands.

4.5. Toxicity caused by organics[13]

Many toxic and recalcitrant organic compounds are degraded under anaerobic conditions, with the compound serving as a growth substrate with fermentation and ultimate methane production. Typical examples include non-halogenated aromatic and aliphatic compounds such as phenol, toluene, alcohols, and ketones. However, most chlorinated organic compounds are not attacked easily under anaerobic conditions and do not serve as electron acceptors in anaerobic oxidation reduction reactions.

Examples of chlorinate compounds degraded under anaerobic conditions include tetrachloroethene, trichloroethene, carbon tetrachloride, trichlorobenzene, pentachlorophenol, chlrohydrocarbons and PCBs. These chlorinated compounds serve as the electron acceptor, and hydrogen produced from fermentation reactions provides the main electron donor. Hydrogen replaces chlorine in the molecule, and such reactions have generally been referred to as *anaerobic dehalogenation* or *anaerobic dechlorination*. As the number of chlorine molecules on the organic molecule decreases, the reactions tend to be slower and less complete.

4.6. Ammonia[6,13]

Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea. Ammonium ion (NH_4^+) and free ammonia (FA)(NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous solution. FA has been suggested to be the main cause of inhibition since it is freely membrane-permeable. The hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency.

Among the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition. It is generally believed that ammonia concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms.

As long as the pH is 7.2 or below, most ammonia will be in the form of ammonium ion so that total ammonia concentrations approaching 3000 mg/L can be tolerated with little effect [12].

Since the free ammonia form has been suggested to be the actual toxic agent, an increase in pH would result in increased toxicity. Since the amount of free ammonia is a function of temperature and pH, the dissociation constants for NH_3 as a function of temperature are given as follow:

Temperature (°C)	20	25	30	40
Ka_{NH3} (mol/L)x 10^{10}	5.84	5.62	5.49	5.37

As example, at a pH of 7.5 and at 30 to 35 °C, 2-4% of the ammonium present will be as free ammonia.

In stating the toxicity as total ammonium concentration [13], an author reported a toxicity concentration range of 1500 to 3000 mg/L as NH_4^+ at pH above 7.4, with 3000 mg/L being toxic at any pH. However, after long-term acclimatization, much higher NH_4^+ concentrations without toxicity have been observed. Other author found no inhibition effects for both thermophilic and mesophilic digestion of municipal sludge with NH_4^+ concentration ranging from 1900 to 2400 mg/L. Others have reported no effect of ammonia toxicity with long-term acclimatized cultures at NH_4^+ concentrations in the range of 5000-8000 mg/L.

To remove ammonia from the substrate, two physical-chemical methods are used: air stripping and chemical precipitation.

4.7. Sulphide[6, 7, 13]

Sulphide is a common constituent of many industrial wastewaters. The formation of sulphide upon reduction of sulphate and other sulphur containing compounds is one of the problems associated with anaerobic wastewater treatment. Release of sulphide is undesirable because of its smell, its toxicity and its corrosive properties. In addition, reduction of sulphate to sulphide by sulphate-reducing bacteria can be toxic to methanogenic bacteria at high enough concentrations.

Theoretically, wastewaters with a COD/sulphate ratio of 0.67 or higher contain enough COD (electron donor) to remove all sulphate by sulphate-reducing bacteria. If the ratio is lower, addition of extra COD, for example, as ethanol or synthesis gas (a mixture of H_2, CO_2 and CO) is required.[20]

Sulphate-reducing bacteria, obligate anaerobes of the domain Bacteria, are morphologically diverse, but share the common characteristic of being able to use sulphate as an electron acceptor and are divided into one of two groups depending of whether they produce fatty acids or use acetate. Based on their metabolic functionality they fall into two groups:

Group I (acetate oxidizers). They are complete oxidizers which have the ability to oxidize the organic compound to acetate and carbon dioxide. Sulphate reducers can use a diverse array of organic compounds as their electron donor and reduce sulphate to sulphide. Common genera found in anaerobic biochemical operations are: *Desulfobacter Desulfobacterium, Desulfococcus, Desulfonema, Desulfosarcina, Desulfoarculus, Desulfoacinum, Desulforhabdus, Desulfomonile as well as Desulfotomaculum sapomandens and Desulfovibrio baarsii.*

Group II (non-acetate oxidizers). They carry out incomplete oxidation of the organic compounds, like fatty acids, to acetate and carbon dioxide, while reducing sulphate to sulphide. Some species or genera are: *Desulfovibrio, Desulfomicrobium, Desulfobotulus, Desulfofustis, Desulfotomaculum, Desulfomonile, Desulfobacula, Archaeglobus, Desulfobulbus, Desulforhopalus and Thermodesulfobacterium.*

4.7.1. Microbial sulphur cycle

Sulphur occurs with three oxidation states of -2 (sulphide and reduced organic sulphur), 0 (elemental sulphur) and +6 (sulphate) being the most significant in nature. Chemical or biological agents contribute to transformation of sulphur from one state to another. A biogeochemical cycle which describes these transformations is comprised of many oxidation-reduction reactions. For instance, H_2S, a reduced form of sulphur, can be oxidized to sulphur or sulphate by a variety of microorganisms. Sulphate, in turn, can be reduced back to sulphide by sulphate reducing bacteria.

A simplified schematic of the microbial sulphur cycle demonstrating the fundamental reactions is presented in Fig. 12. The sulphur cycle consists of oxidative and reductive sides.

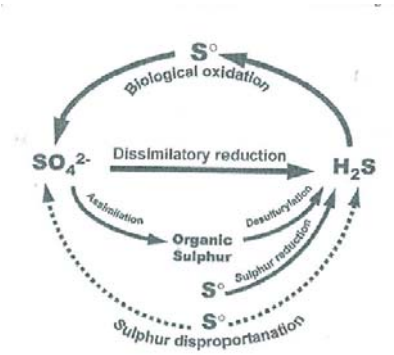


Fig Nr. 12: Schematic representation of microbial sulphur cycle

Sulphate on the reductive side functions as an electron acceptor in metabolic pathways used by a wide range of microorganisms and is converted to sulphide. On the oxidative side, reduced sulphur compounds such as sulphide serve as electron donors for phototrophic or chemolithothrophic bacteria which convert these compounds to elemental sulphur or sulphate. A situation in which the reductive or oxidative sides of this cycle are not in balance could result in accumulation of intermediates such as sulphur, iron sulphide and hydrogen sulphide.

Sulphur disproportionation, carried out by some species of sulphate reducing bacteria and other highly specialized bacteria, is an energy generating process in which elemental sulphur or thiosulphate functions both as electron donor and electron acceptor. Sulphur disproportionation results in simultaneous formation of sulphate and sulphide.

In addition to the inorganic sulphur compounds, a vast array of organic sulphur compounds (i.e. sulphur containing proteins) are synthesized by microorganisms and considered part of the microbial sulphur cycle. Other organic sulphur compounds such as di-methyl sulphide, di-methyl di-sulphide, di-methyl sulfoxide, methanediol and carbon disulphide are also involved and affect the microbial sulphur cycle.

4.7.2. Competition of SRB and other anaerobes

Two stages of inhibition exist as a result of sulphate reduction:
- Primary inhibition is due to competition for common organic and inorganic substrates from SRB, which suppresses methane production.
- Secondary inhibition results from the toxicity of sulphide to various bacteria groups

Compounds which can be completely or partially degraded by SRB include branched-chain and long chain fatty acids, ethanol and other alcohols, organic acids and aromatic compounds. Some researches ranked the affinity of SRB for reduced substrates in the order of H_2 > propionate > other organic electron donors.

SRB may compete with methanogens, acetogens or fermentative microorganisms for available acetate, H_2, propionate and butyrate in anaerobic systems (Fig. Nr.13). The outcome of the competition between SRB and other anaerobic microorganisms determines the concentration of the sulphide in the reactor system. Sulphide has been reported to be toxic to methanogens as well as to the SRB themselves. Thus the concentration of sulphide and the susceptibility of anaerobes feed back into the competition between SRB and other anaerobes.

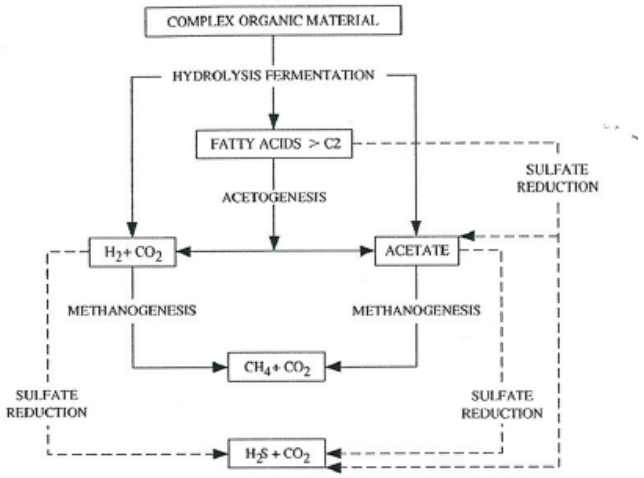


Fig. Nr. 13: Substrate competition between SRB, MPB and AB during anaerobic digestion of organic matter

4.7.2.1. Competition between SRB and hydrolytic and acidogenic bacteria

SRB do not degrade natural biopolymers such as stars, glycogen, protein or lipids and thus depend on the activity of other organisms for providing them with degradation products. Consequently, competition does not occur in the hydrolysis stage.

Although a few strains of SRB have been shown to utilize sugars and amino acids as substrate, vigorous growth of SRB on typical acidogenic substrates is not common. It is generally agreed that SRB cannot effectively compete against the fast growing fermentative microorganisms involved in monomer degradation. As an example, in experimentations in anaerobic digesters fed with glucose and lactose was observed no change in the degradation rates of SRB after they were added, indicating that SRB species did not play any substantial role in the degradation of glucose and lactose.

4.7.2.2. Competition between SRB and acetogens

From a purely thermodynamic and kinetic standpoint, SRB should out-compete other anaerobes for substrate. In practice, however, factors such as COD/SO_4^{2-} ratio, the relative population of SRB and other anaerobes, and the sensivity of SRB and other anaerobes to sulphide toxicity influence the competition. As a result, the literature on anaerobic digestion of sulphate-containing wastewaters is highly complex and often contradictory.

Propionate is a key intermediate in anaerobic digestion and a substrate for all SRB. Degradation of propionate by SRB involves an incomplete conversion to acetate. SRB show a higher affinity for propionate and faster growth rates than the propionate-utilizing syntrophic species.

Butyrate and ethanol are also important fermentation intermediates in anaerobic digestion, but SRB seem to have lower affinity for butyrate and ethanol than non-SRB which instead demonstrated effective competition.

4.7.2.3. Competition between SRB and hydrogenotrophic methanogens

From thermodynamic and substrate affinity considerations, H_2-oxidizing SRB should effectively out-compete hydrogenotropic methanogens under the conditions prevailing in anaerobic digesters. This view is supported by experimental data reporting that H_2 oxidation is almost exclusively catalyzed by SRB. Methanogenesis appeared to occur simultaneously with sulphate-reduction, but methanogens could not compete for H_2 with the SRB. The predominance of SRB in H_2 utilization has been related to the more favourable kinetic parameters for SRB.

Temperature has been reported to impact the outcome of the competition between SRB and hydrogenotrophic methane producing bacteria (MPB). SRB has been reported to dominate at mesophilic conditions (37 °C) while MPB outcompete SRB at thermophilic conditions (55 °C). No explanation about this difference has been offered.

4.7.2.4. Competition between SRB and aceticlastic methanogens

Literature data on the outcome of competition between SRB and MPB for acetate are contradictory. Various mechanisms have been proposed to explain the observed discrepancies. The most important observations found are.

- Aceticlastic MPB predominate when the COD/SO_4^{2-} was above 2.7; SRB predominate when this ratio was below 1.7. Active competition occurred between these ratios.
- Initial population of SRB plays a role in the competition between SRB and MPB.
- Successful competition of MPB is due to their superior attachment capabilities. In fixed-film reactors, better attachment of microorganisms can effectively prevent biomass washout.
- It is also believed that Aceticlastic methanogens predominate because SRB have a lower affinity for acetate than for other substrates. Under sulphate-limiting conditions, acetate was believed to be the least favoured substrate for sulphate reduction. However, the dominance of SRB in acetate degradation was attributed to the kinetic advantages of SRB over MPB.
- Higher extent of organic removal by SRB is attributed to the long HRT used in the UASB/CSTR reactor, which led to the washout of the dispersed growing MPB.

4.7.3. Sulphate/Sulphite toxicity control

Most of the alternatives to deal with SRB activity are oriented to remove sulphide, since it is sulphide that has undesirable smell, toxicity and is corrosive.

In the case of sulphate, it has been proposed the following reaction to avoid sulphate to be reduced to sulphide: the addition of barium to precipitate the barium sulphate (insoluble in water):

$$SO_4^{2-} + BaCl_2 \rightarrow BaSO_4\downarrow + 2\ Cl^-$$

In the case of sulphide, one method to prevent its toxicity is to dilute the wastewater stream, although in general this approach is considered undesirable because of the increase in the total volume of wastewater that must be treated. An alternative way to reduce sulphide concentration in an anaerobic treatment system is by incorporating a sulphide removal step in the overall process.

Sulphide removal techniques include physico-chemical techniques (stripping), chemical reactions (coagulation, oxidation, precipitation), or biological conversions (partial oxidation to elemental sulphur. A common drawback of these techniques is that they require an extra process unit, implying extra installation and operational costs.

Commercial well-established physicochemical processes are Claus, Alkanolamine, Lo-Cat and Holmes-Stretford. Operation at high pressures and temperatures, as well as the need for expensive chemicals make the physicochemical processes energy and cost intensive. In addition, the physico-chemical processes are generally developed for the treatment of gaseous streams and are feasible when large volumes of polluted stream with high sulphide content are treated.

Biological methods, by contrast, operate around the ambient temperature and pressure, can handle smaller volumes of the contaminated stream and could remove sulphide even at low concentrations. Biological alternatives for the treatment of sulphide-laden streams which rely on oxidation of sulphide to elemental sulphur or sulphate are categorized as direct and indirect.

The indirect method relies on the oxidizing power of ferric iron for conversion of sulphide to elemental sulphur, and the catalytic activity of iron-oxidizing bacteria for the regeneration of ferric iron. Chemical reaction with ferric salt is as follow:

$$3\ H_2S + 2\ FeCl_3 \rightarrow Fe_2S_3\downarrow + 6H^+ + 6Cl^-$$

where ferric sulphide is a solid, black powder but decays at ambient temperature into a yellow-green powder. This is a quite unstable artificial product not occurring in the nature.

In the direct approach, photoautotrophic or chemolitotrophic sulphide oxidizing bacteria convert the sulphide to elemental sulphur or sulphate.

Other biological alternative that it is being developed to delete or at least reduce sulphide is the introduction of limited quantities of oxygen/air in anaerobic bioreactors[8]. According to some experiments, H_2S level has been decreased in biogas to below detection thresholds, indicating that moderate oxygenation can indeed be applied successfully as a strategy for the

removal of sulphide of biogas. Supporting batch experiments showed that sulphide was oxidized mainly to species with an average oxidation state of the sulphur atoms around zero, i.e. elemental sulphur and presumably polysulphide. From the effluent data it is obvious that full re-oxidation to sulphate did not occur and neither did oxidation to thiosulphate. In addition, the reappearance of sulphide, logically due to anaerobic biological reduction of oxidized S-containing compounds (i.e. polysulfide, elemental sulphur and thiosulfate), was observed but just after complete depletion of oxygen and at a lower rate than of the sulphide of removal.

Additional alternative it is also the adaptation of the MPB to free H_2S, particularly in reactors with fixed biomass. This could increase the tolerance of MPB to sulphide. Some researches reported that acclimated aceticlastic and hydrogenotropic MPB were only slightly inhibited at more than 1000 mg/L free H_2S.

Finally, it is important to indicate that toxicity of sulphide is regarded as being pH-dependent because only the neutral undissociated hydrogen sulphide can pass the cell membrane. The hydrogen sulphide dissociates in water according to the following equations:

$$H_2S \leftrightarrow H^+ + HS^- \qquad K_{a1} = 1.00 \times 10^{-7} \text{ mol/L (25 °C)}$$
$$HS^- \leftrightarrow H^+ + S^{2-} \qquad K_{a2} = 1.00 * 10^{-19} \text{ mol/L (25 °C)}$$

In the liquid phase, the total dissolved sulphide is present as the unionized form (H_2S) and as HS^-. As the pKa value of this acid-base equilibrium is about 7, small pH variations in the pH range 6-8 will significantly affect the free (unionized) H_2S concentration. At neutral pH values, free H_2S accounts to 50% of total dissolved sulphide, whereas at pH 8 it is only around 10% (Fig. Nr. 14) [20]:

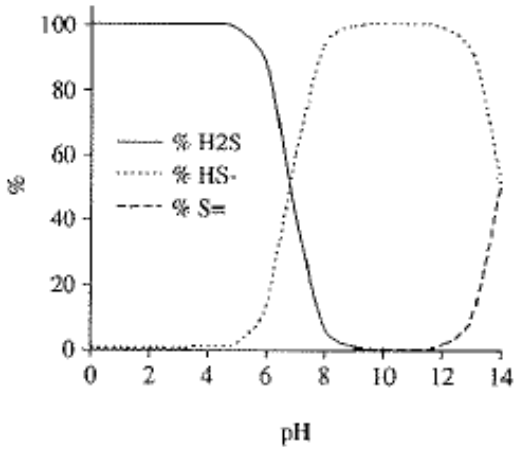


Fig. Nr. 14: Equilibrium for $H_2S/HS^-/S^{2-}$ in aqueous solutions as a function of pH

4.8. Alkalinity[13]

Because of the high CO_2 content in the gases developed in the anaerobic processes (30-50% CO_2), alkalinity concentrations in the range from 2000 to 4000 mg/L $CaCO_3$ are typically required to maintain the pH at or near neutral. The level of alkalinity needed is seldom available in the influent wastewater, but may be generated in some cases by the degradation of protein and amino-acids. The requirement to purchase chemicals for pH control can have a significant impact on the economics of anaerobic treatment.

5. GLYCOLS AND METHANOL

Methanol is also known as methyl alcohol, wood alcohol, wood naphtha or wood spirits. Its chemical formula is CH_3OH (often abbreviated MeOH). It is the simplest alcohol, and is a light, volatile, colourless, flammable, and liquid with a distinctive odour that is very similar to but slightly sweeter than ethanol (drinking alcohol). At room temperature it is a polar liquid and is used as an antifreeze, solvent, fuel, and as a denaturant for ethanol. It is also used for producing biodiesel via trans-esterification reaction.

Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria, and is ubiquitous in the environment. As a result, there is a small fraction of methanol vapour in the atmosphere. Over the course of several days, atmospheric methanol is oxidized with the help of sunlight to carbon dioxide and water.

EG (or MEG: Mono-ethylene glycol) is an organic compound widely used as automotive antifreeze and a precursor to polymers. In its pure form, it is an odourless, colourless, syrupy, sweet-tasting liquid. Ethylene glycol is toxic, and ingestion can result in death. Its IUPAC name is ethane-1,2-diol), and chemical formula is $HOCH_2CH_2OH$.

DEG (or Di-ethylene glycol) is an organic compound colourless, practically odourless, poisonous, viscous, and hygroscopic liquid with a sweetish taste. It is miscible in water, alcohol, ether, acetone and ethylene glycol. DEG is a widely used solvent. Its IUPAC name is (2-hydroxyethoxy)ethan-2-ol) and chemical formula $(HOCH_2CH_2)_2O$.

TEG (or Tri-ethylene glycol or triglycol) is a colourless odourless viscous liquid with. TEG is used by the oil and gas industry to "dehydrate" natural gas. It may also be used to dehydrate certain other gases. Its IUPAC name is 2-[2-(2-Hydroxyethoxy)ethoxy]etanol) and has a molecular formula $HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$

5.1. Glycols and methanol as production chemicals [19]

Natural gas and associated condensate are often produced from the reservoir saturated (in equilibrium) with water. Dehydration is the typical process used to remove water from natural gas and natural gas liquids (NGLs), and is required to: prevent the formation of hydrates and condensation of free water in processing and transportation facilities, meet a water content specification, and prevent corrosion.

Techniques for dehydrating natural gas, associated gas condensate and NGLs include: absorption using liquid desiccants, adsorption using solid desiccants and dehydration with $CaCl_2$. Absorption is the process often used, using typically glycols. Di-ethylene-glycol (DEG), tri-ethylene-glycol (TEG) and tetra-ethylene glycol (TREG) are used as liquid desiccants, but TEG is the most common for natural gas dehydration.

In some cases, however, dehydration may not be practical or economically feasible. In these cases, inhibition can be an effective method of preventing hydrate formation. Inhibition utilizes injection of one of the glycols or methanol into a process stream where it can combine with the condensed aqueous phase to lower the hydrate formation temperature at a given pressure. Methanol has been the most popular inhibitor, due to its cost and its effectiveness.

Ethylene-glycol (EG), di-ethylene-glycol (DEG), and tri-ethylene-glycol (TEG) have been also used for hydrate inhibition. The most popular has been EG because of its lower cost, lower viscosity, and lower solubility in liquid hydrocarbons. Glycols have higher molecular weights than alcohols. This reduces volatility and thus glycols may be recovered and recycled more readily than alcohols. The preference for methanol versus glycol may be determined by economic considerations.

Despite of methanol and glycols are recovered from the aqueous phase, regenerated and re-injected; the complete efficiency of these processes is not always reached and some appreciable amounts of these components are part of the water, product of the dehydration of the produced gas.

5.2. Treatability of Methanol, Ethylene glycol (EG) and poly ethylene glycols (PEG)[3,4,5]

It is important to indicate that methanol and ethylene glycol (EG or MEG) are part of the major fermentable substances known during anaerobic breakdown [16]. Energy-rich CoA derivatives and other energy-rich compounds are then produced (see Fig. Nr.15). On the other hand, according to some researches, it has been possible to reach anaerobic degradation of polyethylene glycols (PEG), in comparison with aerobic processes where general resistance has been found to this type of degradation. Methanogenic enrichments capable of degrading polyethylene glycols and ethylene glycol were obtained from sewage sludge. Ethanol, acetate, methane, and (in the case of polyethylene glycols) ethylene glycol were detected as products.

A number of studies document the excellent aerobic treatability of ethylene glycol in activated sludge and natural systems. Aerobic microorganisms use both EG and PEGs as sources if carbon and energy. The aerobic metabolism of EG is relatively common, and the pathways of its metabolism are known. However, the ether bond of the oligomers and polymers is comparatively resistant to microbial attack. This is specially true for the degradation of PEG with molecular weight of 20,000 (PEG-20,000; $[HO\text{-}(CH_2\text{-}CH_2\text{-}O\text{-})_{450}H]$.

Their copious use, high organic strength and aerobic biodegrability threaten to deplete oxygen from surface waters receiving uncontrolled runoff. In addition, attention has shifted to the potential toxic effects from additives, especially corrosion inhibitors. One option for treating wastewaters containing glycols -based on experiences treating aircraft de-icing fluids (ADFs) runoff- it is the use of anaerobic treatment.

The metabolic pathway for anaerobic degradation of EG was proposed in 1983 by Dwyer and Tiedje: EG undergoes an initial disproportionation reaction into ethanol and acetate. Ethanol is subsequently oxidized to acetate. Aceticlastic methanogens split acetate into methane and carbon dioxide; hydrogen-oxidizing methanogens produce methane from hydrogen liberated during ethanol oxidation:

$$4\ OH\text{-}CH_2\text{-}CH_2\text{-}OH \rightarrow 2\ CH_3CH_2OH + 2\ CH_3COOH$$
$$2\ CH_3CH_2OH + 2\ H_2O \rightarrow 2\ CH_3COOH + 4\ H2$$
$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$$

As they expected during their experimentation, the amount of methane in both consortia was one-fourth that of the final acetate concentration and, therefore, is evidence that methane was produced only as a product of ethanol oxidation.

Some author has reported that ethylene glycol was biodegradable in an activated sludge plant. Other author reported that *Pseudomonas aeruginosa* grew on ethylene glycol at a concentration of 200 mg/L and were inhibited at a concentration of 1000 mg/L[23]. This last fact coincides with other author that established that anaerobic treatment of EG at concentration greater than 10000 mg/L with bicarbonate alkalinity ranging from 4500 to 5000 mg/L as $CaCO_3$ and VSS ranging from 2000 to 2900 mg/L is not feasible due to pH inhibition. Intermediates in the brakedown of ethylene glycol were ethanol, propanol, acetaldehyde or ethylene oxide, propionic acid and acetic acid. [3]

Other author indicated that EG at concentrations from 5000-20000 mg/L, EG was readily biodegradable. The only concern is this waste stream is the high requirement for alkalinity due to volatile acids production from ethylene glycol.[23]

Reported %Carbon recovered in products for EG is about 85-89%.[5]

In the case of PEGs, few examples of anaerobic degradation are available. Some researches believe that the glycol units are released from the polymer by hydrolysis before subsequent metabolism to acetate, ethanol and methane. Because the glycol polymers are degraded by one-sixth that of the monomer degradation, it appears that the polymer must be hydrolyzed into fragments, from which monomers can then by hydrolytically removed.

Based on some experiments, same researches believe that EG, DEG and PEG-400 consortia are dominated by two morphological types of bacteria. These were isolated from DEG consortia and tentatively identified as: a *Methanobacterium sp.* and a *Desulfovibrio sp.* Likewise, tested PEG-1,000 and PEG-20,000 consortia exhibit less distinctive, more varied morphologies of bacteria, resembling *Methanosarcina sp.* [5]

It is also believed that neither the EG nor DEG consortia were able to significantly attack glycols of higher molecular weight.[5]

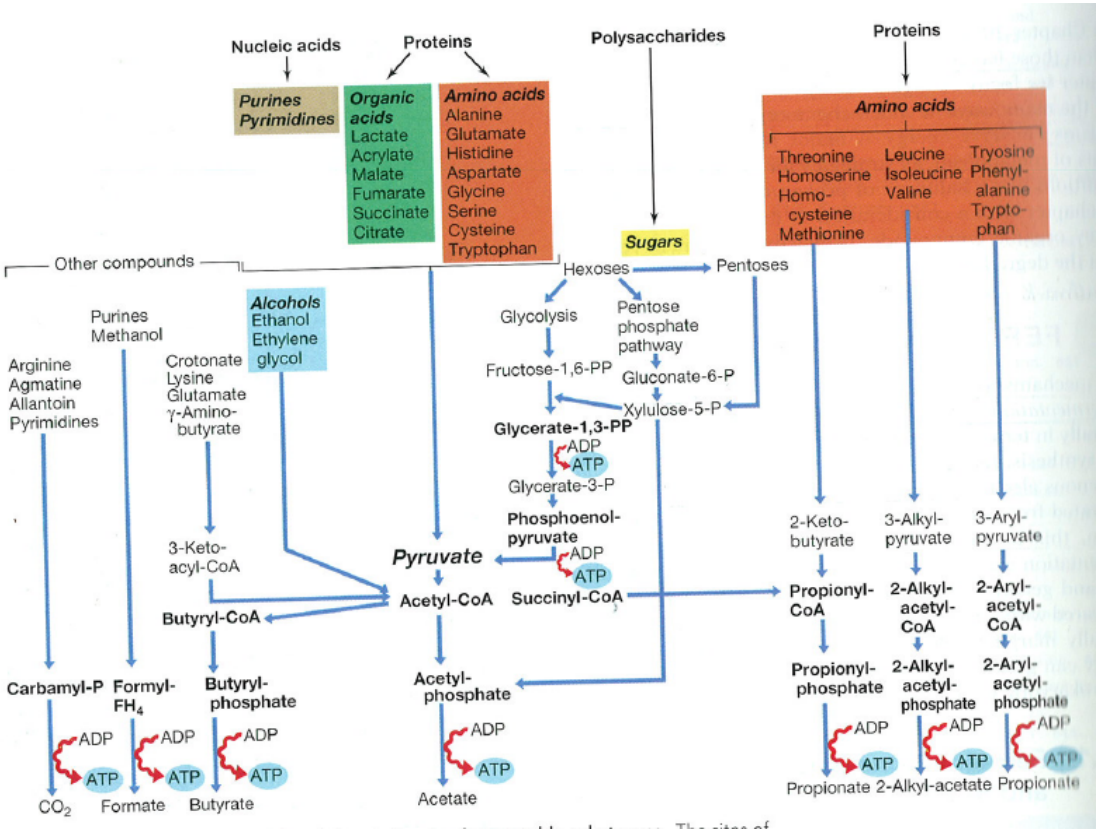
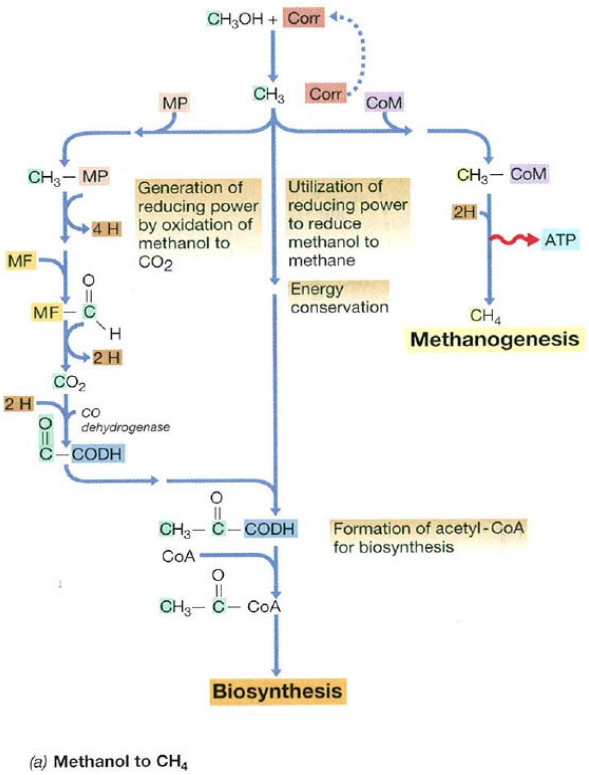


Fig. Nr. 15: Routes of the anaerobic breakdown of major fermentable substances.



(a) Methanol to CH_4

Fig. Nr. 16: Methanogenesis from methanol to CH_4. For growth on methanol, most methanol carbon is converted to CH_4, and a smaller amount is converted to either CO_2, or via formation of acetyl-CoA, is assimilated into cell material.

II. METHODOLOGY

1. THEORETICAL BASIS FOR THE EXPERIMENTAL PART

1.1. Indicators of operational performance [12]

Unfortunately, there is no single indicator which will reliably signal the imbalance between the two major populations, so that a number of indicators must be considered simultaneously

1.1.1. Volatile acids concentration

Although numbers such as 200-400 mg/L as acetic acid are often quoted as being normal in good digestion, the magnitude of the volatile acids concentration is less important than its time rate of change.

1.1.2. Bicarbonate alkalinity

Bicarbonate alkalinity indicates how much buffering capacity remains in the system. If the buffering capacity is low, relatively small increases in the concentration of volatile acids can have a severe effect upon the pH and in consequence an adverse effect upon the methanogenic population. Conversely, if the system has adequate alkalinity, it can tolerate significant fluctuations in the concentrations of volatile acids without large change in pH. It is difficult to specify a satisfactory alkalinity because the amount present will depend upon both the character of the carrier water and the concentration of the waste being treated.

Ratio of concentration of volatile acids (as mg/L of acetic acid) to the bicarbonate alkalinity (as mg/L of $CaCO_3$) is an important factor to characterize the operation. As long as this ratio is less than 0.4 the system should be able to accommodate moderate variations in the volatile acids concentrations with little fluctuation in pH. A rise in the ratio above that, however, is indicative of an imbalance within the system as well as lack of reserve buffering capacity. If the ratio rises above 0.8 the system is likely to experience a severe drop in pH from even small changes in volatile acids.

Nature of buffering system

In aqueous systems carbon dioxide is in equilibrium with carbonic acid, which dissociates to give hydrogen and bicarbonate ions. Anaerobic reactors also contain other weak acid-base systems, such as ammonia and ortophosphoric, hydrosulphuric, and volatile acids, but the carbonic acid system is the most important in determining reactor pH. This is due to several factors:

First, in most anaerobic reactors the concentrations of the orthophosphoric and hydrosulfuric acid systems are too low to provide significant buffering capacity.

Second, in the normal pH range of anaerobic reactors, the buffering actions of the volatile acids and ammonia are negligible; furthermore they are almost completely dissociated and thus act as a strong acid and a strong base respectively. As a consequence, the interaction of the carbonic acid system and the net strong base (B_{ns}), controls the pH. The term net strong base, (B_{ns}), refers to the summation of all strong acids and bases including volatile fatty acids and ammonia.

A proton balance on the system in the pH range 6.0 to 7.5 shows that the bicarbonate alkalinity will approximately equal to the concentration of net strong base, so that the pH of the system is defined by those two species.

The various components of the carbonate system are interrelated by the following equilibrium (constants at 25 °C)[22]:

$$CO_{2\,(g)} \leftrightarrow CO_{2\,(aq)} \quad ; \quad K = K_H = 10^{-1,5}$$

Where K_H is Henry's constant

$$CO_{2\,(aq)} + H_2O \leftrightarrow H_2CO_3 \quad ; K_m = 10^{-2,8}$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad ; K_{1'} = 10^{-3,5}$$

$$H_2CO_3^* \leftrightarrow H^+ + HCO_3^- \quad ; K_{a,1} = 10^{-6,3}$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad ; K_{a,2} = 10^{-10,3}$$

1.1.3. pH

It is an important parameter to good operation due to the narrow band within which methanogenic bacteria can function properly. If the pH is not maintained near neutrality, inhibition of the methanogenic population will occur and the system will enter in a failure spiral.

1.1.4. Methane production rate

The rate of methane production is a direct measure of the metabolic activity of the methanogenic bacteria and as such has great potential as a diagnostic tool of digester performance. Any rapid change in it will indicate that something has happened to the methanogenic bacteria. The feed rate to the reactor must be quite uniform to prevent normal variations in the methane production rate from masking changes caused toxicity or other problems.

1.1.5. Other indicators

Other parameters which are sometimes used to indicate the operational stability of anaerobic reactors are gas composition and total gas production rate. Both are influenced by partial pressure of carbon dioxide in the gas space, which is in turn affected by interphase gas transfer with the liquid. Changes in either of these variables represent a complex interaction among several factors, making small changes unreliable as indicators of reactor performance. An imbalance between the two major populations is likely to be manifested by a decrease in the production rate of methane and an increase in the rate of carbon dioxide production.

1.2. Quantitative evaluation of biogas production and composition[9]

In research on anaerobic digestion the production of biogas and/or methane is one of the most important parameters, not only because of its economic value but also as it is related to substrate degradation. Biogas meters on anaerobic digesters can give a good indication of chemical oxygen demand (COD) removal because almost all of the converted COD leaves the digester as methane.

There are several difficulties in measuring laboratory digester biogas flows. The consensus in the literature is that the ideal meter must be accurate, affordable, data-logging, and corrosion resistant, require little maintenance, capable of measuring a wide

flow range, produce insignificant pressure variations, and be able to operate at low gauge pressures.

1.2.1. Gas volume meters

A method for collection of biogas is the use sampling bags with extremely low permeability. This avoids the problem of absorption during long periods of contact with a barrier solution, but measurement of the gas volume still depends on accurate correction for temperature and pressure depending on the method adopted.

Other alternative is the use of manometric methods, commonly used when dealing with production of low volumes of gas. The main drawback of the manometric approach is that variation in the pressure headspace gases alters the quantity dissolved in the liquid phase, especially in the case of carbon dioxide which in turn can alter the pH and affect the experimental conditions.

The continuous flow meter is another alternative typically used in laboratories. The device works by means of an inverted tipping bucket immersed in liquid.

Finally a common method of biogas collection is by liquid displacement. Gas meters of this type are used for general laboratory-based volume measurement because they are inexpensive, easy to set up and used, robust and capable of working for long periods without maintenance, and can be connected to data acquisition systems.

The used of displacement gas meters requires that measurements taken directly from the gas column (e.g. liquid levels, pressure) are used to calculate gas volumes. As well as adjusting to standard conditions, it is also necessary to take into account the vapour content and to make a correction for any hydrostatic pressure on the gas.

There are three types of liquid displacement gasometers (Fig. Nr. 17):

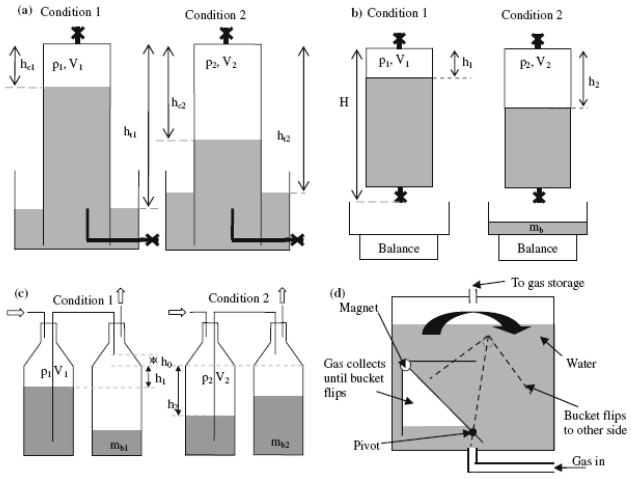


Fig. 17. Equipment design: (a) through gasometer, (b) weight gasometer, (c) bottle gasometer and (d) gas flow meter

- Height gasometer. It consists of a closed cylinder or column partly submerged in an open container of the barrier solution. Gas is introduced into the column via either the top valve (e.g. when emptying a collection bag) or the bottom valve (e.g. when connected directly to a digester), and displaces the barrier solution into the container. In some designs the level of the liquid in the container is

maintained constant by provision of an overflow ensuring that the inlet to the gasometer remains at a constant pressure relative to ambient.

- Weight gasometer. It is used for measurement of the volume in a gas sampling bag. In this type of gasometer, a column of liquid is located is located over a collection vessel. The volume of gas is introduced through a valve at the top of the column and liquid drains into the collection vessel. The liquid is then weighed and this measurement together with the heath of liquid in the column can be used to calculate the volume of gas.
- Bottle gasometer. In this type, the gas displaces the barrier solution from a sealed bottle into a second open container, and the volume is determined either by weighing the displaced liquid or by measuring the change in heights.

Barrier solutions

Consideration must also be given to the type of liquid used in the gas meter (the "barrier solution"), which can be selected either to minimise solubility of the phases or to maximise solubility of one component. In the first case, some sources simply recommend the use of acidified water, where others suggest adding salinity, and still others a combination of both acidity and salinity. In the second case, alkaline solutions have been used to absorb carbon dioxide where quantification of methane is only required. It is thought that although alkaline conditions reduce the loss of gases by diffusion through the barrier solution they cannot prevent it entirely.

According to some literature revision, some barrier solutions used for this type of tests are: tap water, acidified water (pH 2), saturated NaCl solution, acidified saturated NaCl solution (pH 2), basic solutions (like NaOH), and mineral oil.

According to some tests done, it was demonstrated that acidified water alone is not a suitable solution as a barrier solution even for short periods of storage. Tests with a standard gas (65% CH_4 and 35% CO_2) demonstrated that diffusion of these gases in both directions through the barrier solution occurs, driven by the high partial pressures.

It was also demonstrated that acidified water and mineral oil showed considerable losses in CO_2 and up to 10% loss in CH_4 over the test period.

On the other hand, saturated NaCl and acidified saturated NaCl showed similar results with the ability to retain 99% of CH_4 and 92% CO_2 in the gas meter during the test. However, use of a saturated saline solution leads to crystallisation of salt in and on experimental equipment, and from a practical point of view the use of weaker solution may be preferable.

The improved performance of saline solutions is due to the reduced solubility of gases, as the presence of dissolved solids leads to hydration ("solvation") of the solute, leaving less free solvent available for gas adsorption

Other important conclusion from this experimentation, it is a clear trend of increasing CO_2 losses with decreasing ionic strength (experimented solutions: 25%, 50%, 75% and 100% saturated NaCl). However, the performance of a 75% saturated NaCl solution was comparable to that of the saturated solution, with 96% CH_4 and 88% CO_2 remaining in the column headspace at the end of a 8-day-test period.

According to some authors, high ionic strength does not completely prevent gas from dissolving in the displaced liquid and diffusing into the surrounding atmosphere: this is the particularly case for CO_2.

Finally, a test using a 3M solution of NaOH was reported. There was no observable change in the quantity of methane in the column headspace over a 10-day period. There was however evidence of inward diffusion of air that was noted in a gas chromatograph profile after 5 days.

All other barriers solutions performed better than water tap.

Standard conditions

To adjust the gas to the standard conditions, it is also necessary to take into account the vapour content and make a correction for any hydrostatic pressure of the gas. It is also important to keep in mind that the vapour content of a saturated gas increases with increasing temperature, and is only influence by this parameter.

Researches revealed than even when gas volumes are reported as corrected to standard conditions (s.c.), more often than not the standard conditions are not given. Yet there are currently several definitions of standard conditions in widespread use, with standard temperatures between 0 and 25 °C and standard pressures between 100 and 101,326 kPa. In this case, standard conditions will be defined at 273,16 K and 100 kPa.

1.2.2. Composition of the gas

Composition varies depending upon the origin of the anaerobic digestion process, but it is mainly methane (CH_4) and carbon dioxide (CO_2). Also may have small amounts of hydrogen (H_2), sulphide, water vapour, oxygen and siloxanes. To determine its composition two procedures are typically used: (1) the gas chromatographic method and (2) the volumetric method (typically using an Orsat apparatus).

The principal advantage of gas chromatography is speed. Commercial equipment is designed specifically for isothermal or temperature-programmed gas analysis and permits the routine separation and measurement of CO_2, N_2, O_2 and CH_4 in less than 15 to 20 min.

The requirements for a recorder, pressure-regulated bottles of carrier gas, and certified standard gas mixtures for calibration raise costs to the point where infrequent analyses by this method may be uneconomical. The advantages of this system are freedom from cumulative errors found in sequential volumetric measurements, adaptability to other gas component analyses, adaptability to intermittent on-line sampling and analysis, and the use of samples of 1 mL or less. Fig. Nr. 18

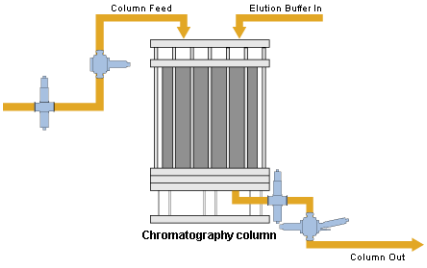


Fig. No. 18: Chromatograph analyser

On the other hand the volumetric analysis (Orsat-type gas-analysis apparatus, Fig. Nr. 19) is suitable for the determination of CO_2, H_2, CH_4 and O_2. Nitrogen is estimated indirectly by difference. Although the method is time-consuming, the equipment is relatively simple. Because no calibration is need before used, the procedure is particularly appropriate when analyses are conducted infrequently.

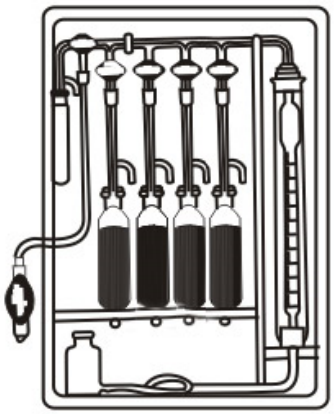


Fig. No. 19: Orsat analyzer

Physical or chemical reactions occur in several steps to remove each component[21]:

- Carbon dioxide absorption. Remove CO_2 from sample by passing it through the CO_2-absortion pipet charged with KOH solution.
- Oxygen absorption. Remove O_2 by passing sample through 0_2-absorption pipet charged with alkaline pyrogallol reagent.
- Hydrogen oxidation. Remove H_2 by passing sample through CuO assembly maintained at a temperature in the range 290 to 300 °C.
- Methane oxidation. Oxidation can be done either by catalytic oxidation or by slow-combustion process.

1.2.3. Governing equation for volume correction [9]

From hydrostatic relations and the equation of state, the equation governing height type gas meter can be derived. Gas volumes is calculated taking into the account the correction for the vapour content, the pressure head due to height of liquid in the gas meter relative to atmospheric pressure, and errors/irregularities in the column cross-sectional area.

$$V_{s.c.} = \frac{T_{s.c.} \cdot A}{T_{atm} \cdot P_{s.c.}} \left(\left(P_{atm} - P_{H_2O}(T_{atm}) - \rho_b \cdot g(h_{t2} - h_{c2}) \right) h_{c2} - \left(P_{atm} - P_{H_2O}(T_{atm}) - \rho_b \cdot g(h_{t1} - h_{c1}) \right) h_{c1} \right)$$

where:

$V_{s.c.}$ = volume at standard conditions (m^3)
$T_{s.c.}$ = temperature at standard conditions (K)
A = transversal area of gas meter (m^2)
T_{atm} = atmospheric temperature (K), 293 K
$P_{s.c.}$ = pressure at standard conditions (Pa)
P_{atm} = atmospheric pressure (Pa), 101,4 kPa

P_{H20} = saturated vapour pressure (Pa)
ρ_b = density of barrier solution (kg/m^3)
g = gravitational constant (9,8 m/s^2)
h = distance to liquid surface from a datum (m)

Based on Fig. 17 (a), the distances for the height gas meter are:
h_{c1}= height for condition 1 (from top of column to liquid level)
h_{c2}= height for condition 2 (from top of column to liquid level)
h_{t1}= distance for condition 1 (from top of column to barrier solution liquid level)
h_{t2}= distance for condition 2 (from top of column to barrier solution liquid level)

In addition, a number of assumptions are made in this derivation:

- The cross-sectional area of the column is constant
- Biogas acts as a perfect gas
- Once leaving the anaerobic digester biogas quickly cools to ambient temperature
- The biogas is saturated with vapour

The saturated vapour pressure (SVP) can be modelled by the Goff-Gratch equation:

$$p_{H_2O}(T) = 101326,6 \cdot 10^{z}$$

Where z is calculated by:

$$z = -7,90298\left(\frac{373,16}{T} - 1\right) + 5,02808 \cdot \log_{10}\left(\frac{373,16}{T}\right)$$
$$-1,3816 \cdot 10^{-7}\left(10^{\left(11,344\left(1-\frac{T}{373,16}\right)\right)} - 1\right) + 8,13289 \cdot 10^{-3}\left(10^{\left(-3,49149\left(\frac{373,16}{T}-1\right)\right)} - 1\right) + \log_{10}(1013,246)$$

Calibration of the gas volume meter

Initial tests of the height gas meter will be done using air in acid solution. Defined volumes of air will be taken from air supply valve from the lab with syringe. The volume from the syringe will be compared with that from the column. Several trials will give the margin of error of this test.

After this “calibration”, the column should be purged with biogas sample.

2. MATERIAL AND METHODS

2.1. Laboratory-scale apparatus, cultivation conditions and procedures

A schematic illustration of the experimental laboratory-scale apparatus used for sludge digestion and biogas recollection is given in the Fig. Nr. 20

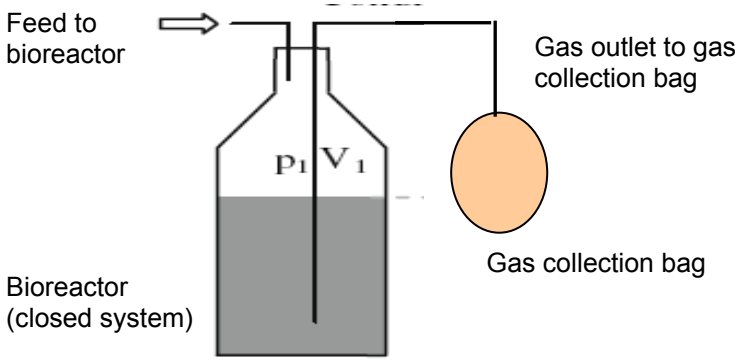


Fig. Nr. 20: Simple schematic of a closed bioreactor

The setup consisted of two components connected. An anaerobic digester (glass bottle with 2 liters of capacity) for production of biogas and a gas collection bag to gather the possible biogas generated.

The mixed sludge was prepared as follows:

- 500 mL Bio-sludge as inoculum (from IVAR Wastewater Treatment Plant, Mekjarvik)
- 200 mL Wastewater (feed stream to wastewater Treatment Plant), prepared with sugar as substrate, with a concentration of 10 g/L. The same wastewater was used as a source of nutrients
- To complete a total volume of bioreactor= 2000 mL (2 L), 1300 mL potable water were added
- Temperature in the bottle was regulated for mesophilic conditions and permanent mechanical agitation was provided for the equipment.

- Date of reception of samples (bio-sludge and feed wastewater to plant): 20/10/10, 10:10 am. Characteristics of wastewater received are given in the Appendix, Tables Nr. 02 and 03.

The activities during the whole experimentation can be divided in the following periods:
1. Biomass acclimation
2. Bioreactor fed with industrial wastewater (Sleipner)
2. Measurement of produced gas
3. Some variations in the experimental conditions/ Change of concentrations and macro and micronutrients

2.1.1. 1st Period: Biomass acclimation

The sample of sludge was incubated in the bioreactor. During 50 days the biological activity was measured after sugar and nutrients added. Initial conditions expected in the bioreactor:

Retention time: 20 days

Temperature: 30-35 °C (mesophilic conditions)

pH: near 7,0

Feed: 200 mL every 2 days (100 mL/day)

Substrate: sugar (Concentration =10 g/L, contained in the 200 mL of wastewater) because it is known substrate easily biodegrable

Source of micro/micronutrients: the same 200 mL of wastewater

Agitation provided/No recirculation provided

Additional observations:
Since recollection of gas failed using the gas recollection bag, some additional observations were done using syringes as "batch reactors":

1st *microsystem test* using syringe filled with:
5 mL (wastewater with C_{sugar}= 5 g/L) + 15 mL (bioreactor sample)

2nd *microsystem tests* using other syringes containing:
Case 1 = 5 mL (Sleipner) + 15 mL (Bioreactor sample)
Case 2 = 4 mL (Sleipner) + 15 mL (Bioreactor sample) + 1 mL wastewater with FeCl3
Case 3 = 15 mL (Bioreactor sample)+ 5 mL wastewater with sugar (10 g/L)
Case 4 = 15 mL (Bioreactor sample)

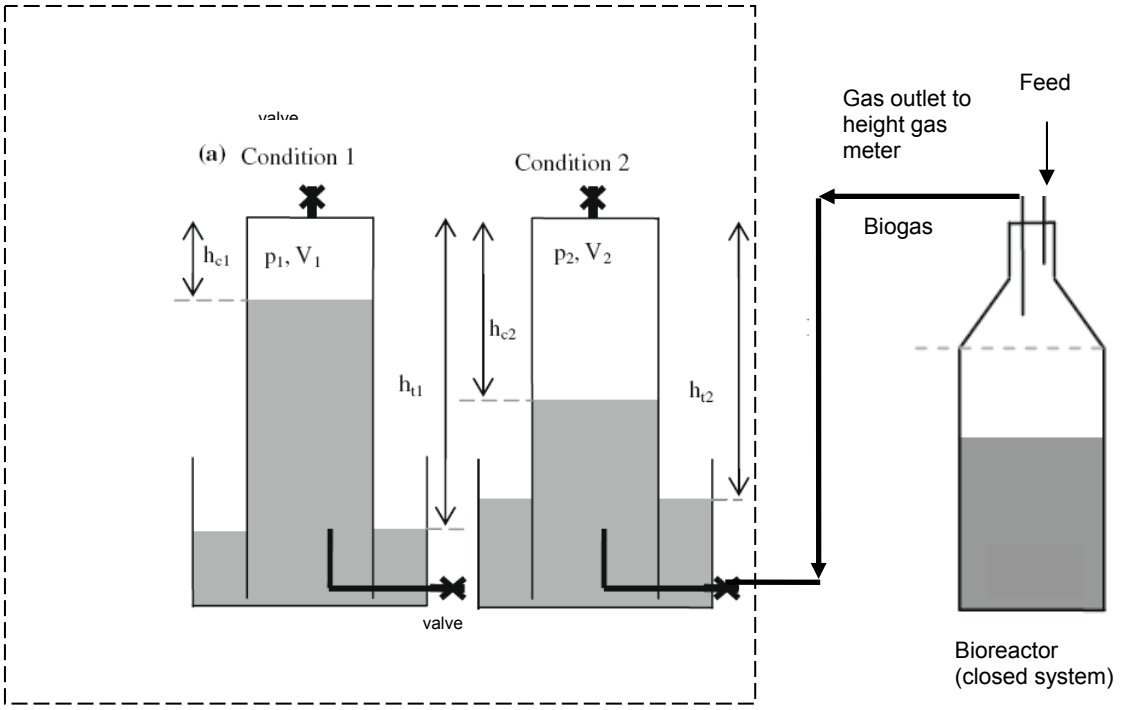
2.1.2. 2nd Period: Bioreactor fed with industrial wastewater (Sleipner)

During this period, the reactor was fed with the industrial wastewater (instead of sugar). In addition, wastewater from IVAR treatment plant was used to provide the necessary nutrients typically missing in the industrial wastewater.

2.1.3. 3rd Period: Measurement of produced gas

Gas recollection system was done first using a gas sampling bag, later a syringe and finally a gasometer (type: height meter).

Figure Nr. 21 shows this type of height meter. This gasometer consisted of a closed cylinder or column partially submerged in an open container of the barrier solution. Gas generated was displaced by the internal pressure from the bioreactor into the column. The gas was transported by a hose connected from the top of the bioreactor until the bottom of the cylinder (gasometer) to guarantee that all the gas will be inside the column. The gas coming into the gasometer displaced the barrier solution into the container.



Note for the Fig No. 21.: the ideal gas meter should have either a top valve (e.g. when emptying a collection bag or recollecting again the gas) and a bottom valve (e.g. when emptying a syringe or connected directly to a digester).

Barrier Solution

A solution of $Ca(OH)_2$ was used, to see the precipitation of $CaCO_3$ formed during the reaction of $Ca(OH)_2$ with CO_2 generated inside the bioreactor and when the solubility limit is passed.

When carbon dioxide dissolves in water, it forms carbonic acid. Lime water (water with $Ca(OH)_2$) neutralizes the carbonic acid and carbonate ion is formed. Calcium carbonate is insoluble and precipitates in the solution.

$$CO_2\,(g) + H_2O(l) \longrightarrow H_2CO_3\,(aq)$$

$$H_2CO_3\,(aq) + 2\ OH\text{-}(aq) \longrightarrow CO_3^{\,2-}(aq) + 4\ H_2O\ (l)$$

$$Ca^{\,2+}\,(aq) + CO_3^{\,2-}(aq) \longrightarrow CaCO_3(s)$$

Calcium carbonate is poorly soluble in pure water: 47 mg/L at normal atmospheric CO_2 partial pressure (P_{CO2} is around $3{,}5\text{x}10^{-4}$ atm or 35 Pa). The equilibrium of its solution is given as anywhere from $K_{sp} = 3{,}7\times10^{-9}$ to $K_{sp} = 8{,}7\times10^{-9}$ at 25 °C (depending upon the data source by the equation).

A saturated $Ca(OH)_2$ solution prepared with distilled water could be used. A concentration of lime solution about 3M was used.

2.1.1.4. 4th Period: Variation of conditions

- During this period, after seeing better stable conditions, especially keeping stable residence time, it was decided to change feed concentration and source of nutrients.

2.2. Analytical methods

- Temperature: Normal thermometer

- pH and conductivity: Multi 340i

- Alkalinity/VFA: Metrohm 632 pH-meter for pH measurement during titration and normal burette for titration. With the computer program TITRA5, values of alkalinity and short-chain fatty acids were calculated

- COD. This analysis was performed in accordance with the dichromate reflux method described in Standard Methods.

A COD balance can be used to account for the changes in COD during fermentation. Instead of oxygen accounting for the change in COD, the COD loss in the anaerobic reactor is accounted for by methane production. By stoichiometry the COD equivalent of methane can be determined. The COD of methane is the amount of oxygen needed to oxidize methane to carbon dioxide and water:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

- TSS/VSS analyses were performed by weighing after over drying, after the temperatures 105 °C and 550 °C, respectively. Filtration will be done with fiber glass filters, type C (Medium to fast, high loading), commercially known as GF/C, with 1 μm pore size.

3. RESULTS AND DISCUSSION

3. 1. Feed composition

Based on feed wastewater to water treatment plant:
The exact composition of this domestic wastewater is unknown but typically domestic water has the main macronutrients and micronutrients required for the microorganisms (Fig. Nr. 22 and 23). The experimentation was done between autumn and winter, so an increase of salinity was expected in the wastewater.
The domestic water received at IVAR has a COD about 350 mg/L and the conductivity varies between 1 and 10 mS/cm, which is high for domestic sewage indicating high concentration of dissolved ions.

Based on Sleipner composition:
As expected from industrial wastewaters, Sleipner produced water has a very low concentration of the **micronutrients** typically required for microorganisms. These are

basically calcium, potassium, magnesium, nitrogen and phosphorus. All of them are under minimum requirement.
Other important micronutrients are sodium, chlorine and sulphur. Comparing values reported for Sleipner against what some researches has defined for maximum levels for anaerobic microorganisms; sodium seems to be in excess (last reported values between 250-600 mg/L, more than max. 230 mg/L recommended to avoid inhibition of aceticlastic methanogenic bacteria, which is responsible for about 70% of methane production.

Considering **micronutrients** requirements, it has been seen that most of them are under requirement, such as manganese, molybdenum, copper, cobalt and zinc. Iodine and boron are not present. Iron might present in high proportion (more than required as micronutrient), resulting in the possibility of react and precipitate as an oxide or hydroxide. Other reported required micronutrients are selenium and nickel. Some concentration of Ni is present but requirement level is unknown. Selenium is not available in Sleipner, according to reported analysis.

3.2. Sugar as source of substrate

During a period of about 50 days of anaerobic biodegradation, the following average values are reported (Fig: Nr. 1):
Temperature: 33,9 °C (min.-max. = 31-37 °C)
pH: 6,8 (min.- max. =6,47-7,36)
Conductivity: 4,43 mS/cm (min.-max. = 3,51-4,70)
TDS= 3103 mg/L (2457-3290 mg/L)

- During the first 6 days a total decrease of pH = 0,89 or a decrease of pH = 0,15 units/day approximately was observed. This rapid decrease in pH is a possible indication of fatty acids production and stimulation of acidogenic bacteria, and in consequence, alkalinity consumption. This is in agreement with the observation of “fat stains”, and a characteristic “tarry” smell; however, this observation is a qualitative appreciation.

- After **10 days** of initiate the bioreactor, apparently gas production started (bubbles observed). Gas recollection no obtained during this phase because gas sampling bag to recollect gas had a leak (undetectable at simple view). Leakage detected 19 days later of initiation of bioreactor.

- No good stability of residence time (HRT) in the process: variations of feed flow rate to reactor and later variations in the concentration of sugar from 10 g/L to 5 g/L. Initially it was desired to have 20 days of residence times. Variations in pH (decrease) and some difficulties to get 200 mL of sample for analysis (not enough pressure in the bioreactor to extract all the volume of sample) make difficult to keep a stable residence time. However, after changing the concentration from 10 g/L to 5 g/L after 23 days of operation, the system turned more stable (as it is observed in Graphic Nr. 2). This is possibly due to an imbalance of the activity of the microorganisms predominating all those different to methanogens. After diminution of concentration, no more addition of chemicals to keep the alkalinity was required, since pH kept more stable at this concentration.

According to some authors, it is best to remove not more than 5% of the reactor contents at any one time. For this bioreactor with a operating volume of 2L means that 100 mL/day should be the maximum volume to be removed (that means a minimum residence time equal to 20 days). When there is not sufficient internal pressure to extract the volume of sample, the best way is to extract the sample in two or more parts, to let the gases (especially CO_2 which is the most soluble) to occupy the space of liquid removed from the bioreactor and then to have enough pressure to extract the rest of volume desired.

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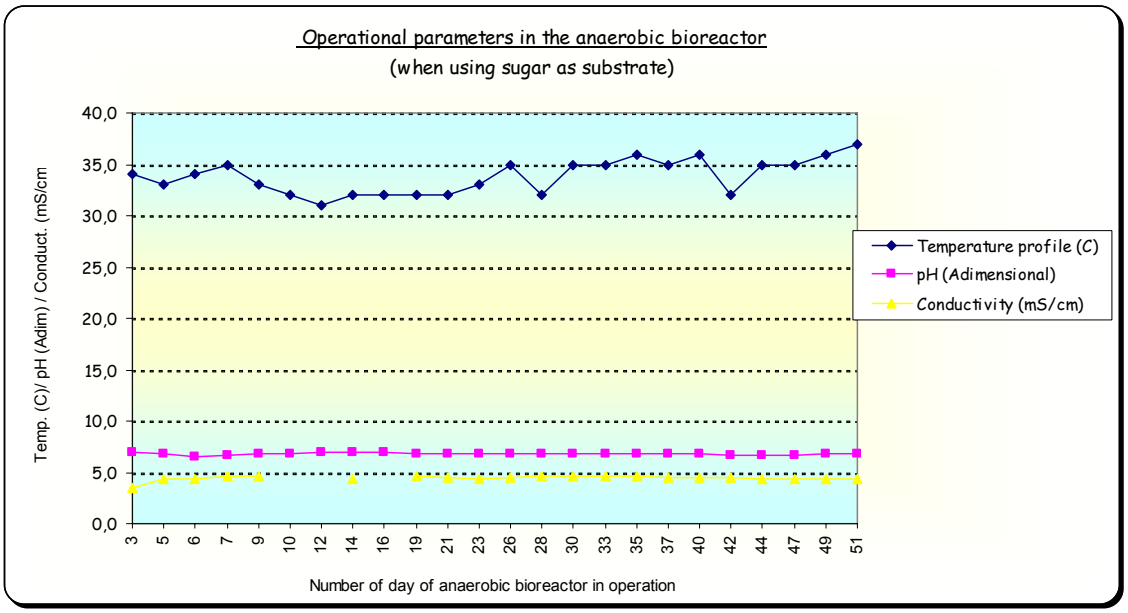
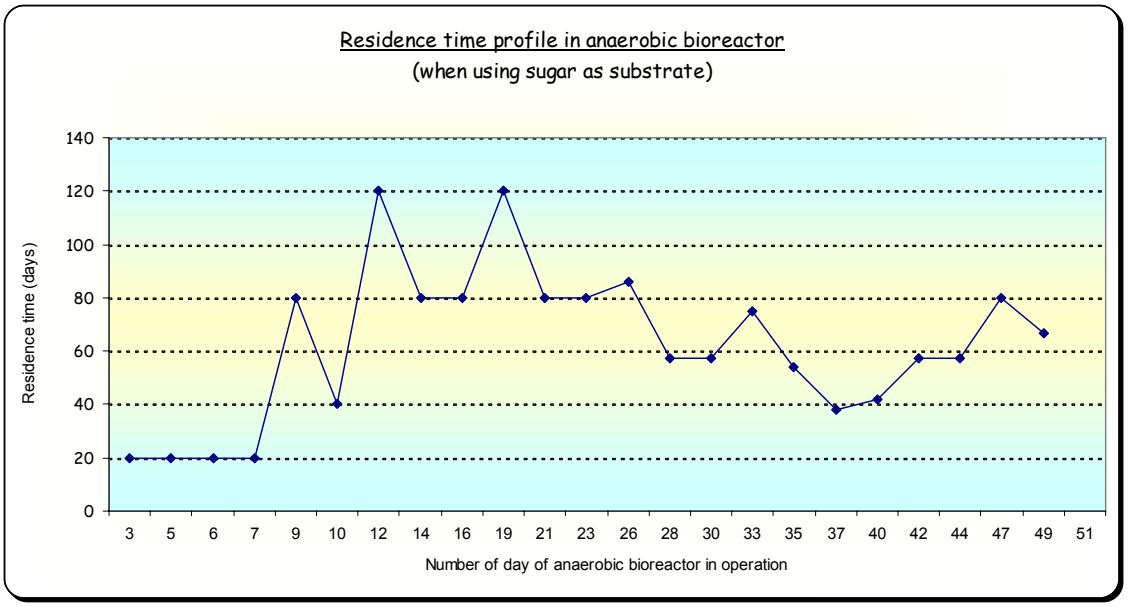
- During the time of operating the bioreactor with sugar, different ways to recollect gas were proven without success.



Graphic Nr. 1: Profile of conditions in the anaerobic bioreactor using sugar as substrate



Graphic Nr. 2: Residence time in the anaerobic bioreactor using sugar as substrate

- In the case of the 1st syringe (sugar + bioreactor sample), gas production was observed after **14 days** of being installed. The difference of this "micro system" with the bioreactor is the sugar concentration. For the bioreactor 10 g/L of sugar were used and for the syringe (micro system) a concentration of 5 g/L was implemented. In addition, this syringe was installed 26 days after the bioreactor was installed

- One important and interesting observation related with the 4 syringes (Microsystems), containing:
Case 1 = 5 mL (Sleipner) + 15 mL (Bioreactor sample)
Case 2 = 4 mL (Sleipner) + 15 mL (Bioreactor sample) + 1 mL wastewater with $FeCl_3$
Case 3 = 15 mL (Bioreactor sample) + 5 mL wastewater with sugar (10 g/L)
Case 4 = 15 mL (Bioreactor sample)

The first three samples apparently developed gas after 5 days of being installed (bubbles). After **9 days**, samples containing Sleipner substrate developed more gas production than sample containing sugar and nutrients, being the syringe with Sleipner and wastewater (nutrients) that developed more amount of gas.

General observations about using sugar as substrate

- It is believed no imbalance due to change in reactor temperature occurred during all experimentation. Even during feeding the bioreactor, the feed was heated near mesophilic temperatures to avoid disturbs in the system.

- It is also believed any kind of toxicity was not observed.

- Range of pH during all experimentation (about these 50 days) was between 6.47-6.98, meaning that H_2S was the dominant specie between H_2S/HS^- and in concentrations between 60-80% approximately of the total species $H_2S/HS^-/S^{-2}$. However, very small concentrations of sulphur seem to be contained in this effluent, so high concentration of H_2S affecting the process was not the case.

- In the case of and NH_4^+/NH_3 (free ammonia), since the system had a pH between 6.47-6.98, free ammonia concentration is supposed to be low and no toxicity was observed.

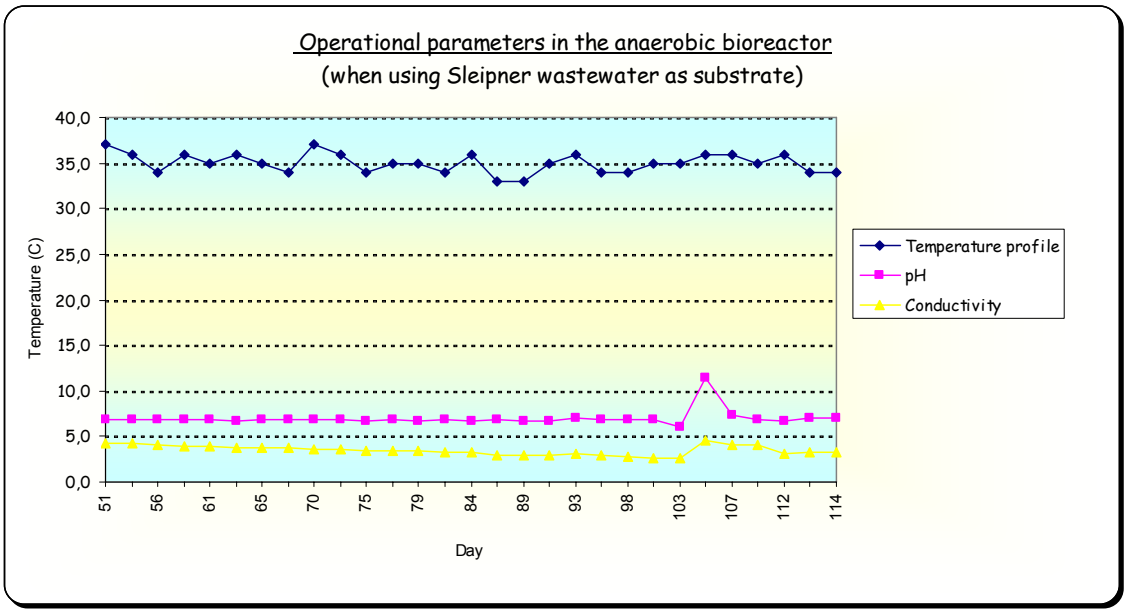
3.3. Industrial wastewater (Sleipner) as source of substrate

During a period of about 22 days of anaerobic biodegradation using Sleipner as source of substrate, the process was relatively stable. The following average values are reported (between days 51 to 112):
Temperature: 35 °C (min.-max. = 33-37 °C)
pH: 6,79 (min.- max. =6,05-6,97)
Conductivity: 3,42 mS/cm (min.-max. = 2,58-4,28)
TDS= 2136 mg/L (1613-2675 mg/L)

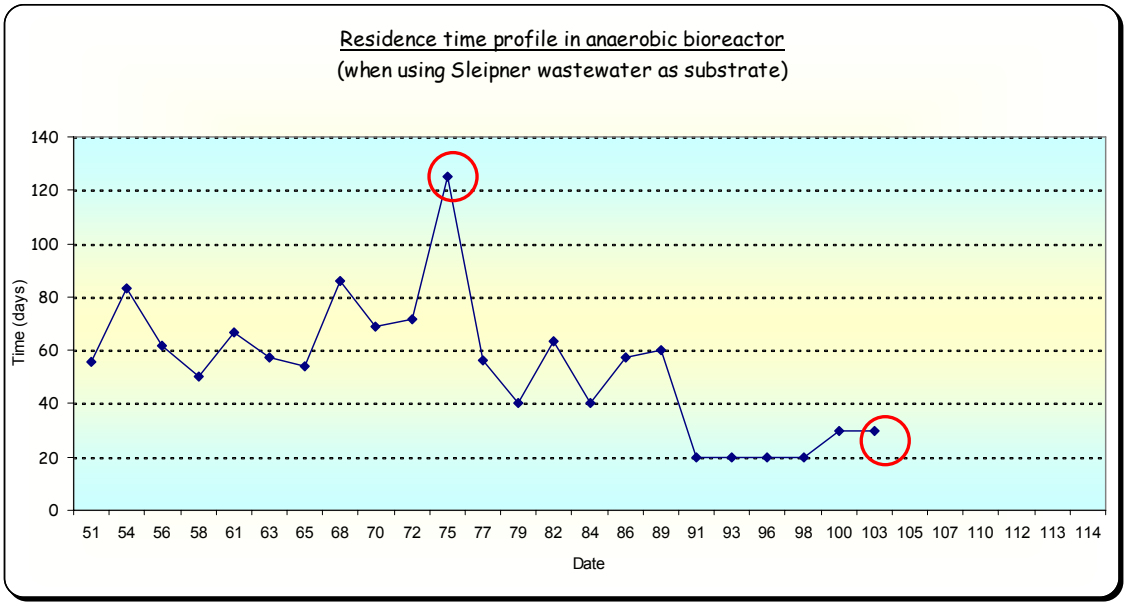
- In this case, too much effort was directed to gas recollection and measurement. The measurement of gas, initially using a syringe to recollect the gas and estimate volume was no reliable, since the syringe was jammed sometimes. In addition, when it was

possible to read some gas volume collected in the syringe, as product of the anaerobic activity in the bioreactor, the volume was at unknown pressure condition, that is, the internal pressure was unknown to determine the volume of gas measured at standard conditions. That uncertainty changes with the implementation of the gas meter, where the pressure of the gas recollected is at atmospheric pressure, due to equilibrium reached by liquid displacement.

- From Table Nr. 4 and Graphic Nr.1, it can be observed that conductivity and total dissolved solids (TDS) started to decrease when using Sleipner, indication of ions concentration decreases. Just when addition of substances (Na_2CO_3, NaOH) to increase alkalinity, increase of conductivity was measured, especially with NaOH (peak in the graphic).



Graphic Nr. 1: Profile of conditions in the anaerobic bioreactor using Sleipner as substrate



Graphic Nr. 2: Residence time in the anaerobic bioreactor using Sleipner as substrate

- Buffering system observations:
During the days 93-110 (dates 20.01.11-06.02.11), some analysis of bicarbonate alkalinity were reported (as mg $CaCO_3$/L). Calculations of short-chain fatty acids (as mg Acetic Acid/L) were automatically done (for the program TITRA5) too. Several observations might be reported:

1) Based on theoretical information (see in part I, item 3.2.3) the alkalinity concentration should be between 2000-4000 mg/L to maintain pH near neutrality. As example, for day 93, the pH was 6.97 and alkalinity was 2053 mg$CaCO_3$/L and no fatty acids were reported by the program. That probably means that the system was stable.

2) Based on theoretical observations (see in part II, item 1.1.2.) the ratio of concentration of volatile acids to bicarbonate alkalinity should less than 0.4. For day 96 the ratio was about 0.22, for day 100 ratio was 0.05, for days 103 and 107 was about 3.00 and for day 110 was 4,50 approximately (values registered in Table Nr. 04). Some explanations are offered:

* For day 96, it seems that ratio=0.22 was normal. Then a decrease of rate until 0.05 for day 100 occurred. It seems an indication of something has happened either to retard the acidogenic bacteria or to stimulate the methanogenic population. This reduction of rate coincides with the increase of substrate volume (from 20 to 40 mL), that might have caused an imbalance in the system.

* For day 100, it seems that the acidogenic bacteria responded fairly rapid to the increase of substrate by increasing its specific growth rate, thereby increasing the rate at which volatile acids, carbon dioxide, hydrogen, and other end products are produced. Then the methanogenic bacteria seemed inhibited because might not be able to remove the volatile acid intermediates as rapidly as they are produced. The pH dropped 0.77 units, indication of high presence of acid in the system.

* For day 103, the volatile acids/alkalinity is 3.0 and pH=6.05; indication of even greater imbalance between the two populations and probably further methanogenic inhibition. As a desperate measured to control the pH, NaOH was used to cause that ionic equilibrium occurs very fast. That meant also that NaOH required to remove CO_2 from the gas space to form the required bicarbonate alkalinity. That coincides with the observation that no gas was observed from that day 103 and since the NaOH was used in excess, the pH reached a peak of 11.45.

* For day 107 was observed a decrease of 4.05 units (from pH=11.45 to 7.40). This might be a clear indication that more CO_2 was generated by acidogenic bacteria (pH drops even though alkalinity remained the same). Then, after this eventuality, the pH was again between 6.6-7.0 (until final day of observation = day114).

- From the COD analysis (Table Nr. 6 in Appendixes), the average value reported for Sleipner COD is about 15200 mg COD/L. According the different values reported for TOC from Karstø Refinery (Tables Nr. 2 and 3), the average TOC is 5200 mg COD/L. This means that COD/TOC = 2.9, or inversely TOC/COD= 0.34; which is a typical value for this type of wastewater with organic compounds.

COD removal

- Efficiency of COD removal found using Sleipner as substrate and evaluated during the operation days 89 to 94 (dates: 16.01.11 to 21-01.11) was between 37 to 62% approximately (Table Nr. 6 in Appendixes), which it is considered low. Expected gas methane formation during those days, according to the COD consumed or removed, was between 0,09 to 0,15 L (90-150 mL).

Gas mesurement

- Values of gas produced using a syringe was in average 31 mL. It is believed that pressure in the syringe, after reaching equilibrium "internal pressure bioreactor-atmospheric pressure" was near atmospheric pressure, however, it is difficult to prove.

- Values of gas produced reported after installation of gas meters were between 10-200 mL, being 200 mL the maximum peak reached just in one occasion. The average volume of gas registered was about 54 mL (0,054 L). It is believed that these volumes reported with the gas meter are mainly methane, since the CO_2 was expected to react with the calcium hydroxide solution.

When using the gasometer, layers of calcium carbonate were observed on the top of barrier solution (calcium hydroxide solution) due to the reaction of CO_2, mostly from the atmosphere, but also from the gas generated in the bioreactor. As a proof of this, it was observed faster change in the colour of phenolphthalein added to the barrier solution liquid than the change in colour inside the cylinder where gas measurement was performed. Despite of being in contact both fluids, the change in colour was most evident in the beaker or vessel containing the barrier solution (in contact with the atmosphere (Reference pictures Nr .44- 51).

No correction correction/calibration factors were used to estimate the "real volumes" in the gasometer, as presented in the theoretical part, due to no determination of liquid level of barrier solution.

- After changing source of nutrients (from using wastewater from IVAR as source of nutrients to using a synthetically prepared source of nutrients, no more observations were done to the system.

- Selection of type of reactor

Sleipner is a high-strength effluent which has about 15000 mg COD/L (15 kg COD/m^3). If Sleipner volumetric flow is about 70 m3/d then 1050 kg COD could be removed per day.

The continuously stirred tank reactor (CSTR) is the most widely used since operate closest to the steady state. For design purposes it is acceptable because it is easier to model, however, the development of dynamic models has demonstrated the need for more accurate ways of examining operational problems of bioreactors. In addition, the HRT is equal to SRT (MCRT), meaning that SRT has to be big enough (maybe 20 days as minimum for this case) to provide sufficient safety factors for operation and process stability. If not enough SRT is provided, washout of the methanogenic bacteria might occurred.

Other similar examples of these *flow-through systems* are anaerobic contact processes, covered anaerobic lagoon and anaerobic sequencing batch reactor (ASBR), where sludge retention is obtained by sedimentation, but in these cases SRT > HRT. In addition, all these flow-through systems are used for relatively low volumetric loading rates.

Contact systems seem to be the best option to treat Sleipner from the operational point of view. These systems are better for wastewaters with a lower solids concentration. The biomass is retained within the reactor in a number of ways. For example, by allowing bacterial adhesion resulting in biofilms, or to develop as flocs maintained in suspension either by mechanical mixing or by upward flow of effluent through the reactor. These mechanisms enable a high retention of biomass (SRT is far greater than the HRT of the wastewater). The most significant example of reactor using stationary material is the fixed film reactors (AF), whereas the upflow anaerobic sludge bed (UASB) reactor and the expanded granular sludge bed (EGSB) reactor.

CONCLUSIONS

It is believed that Sleipner wastewater is feasible of being degraded anaerobically, since COD removal and gas formation was reported during the experimental period. Sleipner wastewater is a high-strength wastewater with about 15000 mg COD/L. Other factors also make this conversion possible is that Sleipner has low toxic compounds concentrations, such as sulphide or inhibitory organic compounds, or at least, at level tolerable for the microorganisms, but control of the buffering capacity and residence time must be improved.

A good start of a bioreactor is to consider a temperature of about 35 C (mesophilic condition). To reduce the start-up period due to slow growth of the methane producing bacteria, the seeding with anaerobic bacteria from another plant is a good option.

Despite of being able to get gas, there is still uncertainty about the amount of gas produced and the quality of the gas since the composition was unknown. It is believed that methane was recollected as main gas among other gases (such as hydrogen, water vapour, etc) when using the gas meter, since a barrier solution of calcium hydroxide was used to react with possible carbon dioxide present.

Any change in the loading of the digester must be gradual in order to ensure that the concentration of volatile acids does not exceed the normal buffering capacity of the system. Operational imbalances in this experience must be corrected in order to get steady state conditions. Once the steady state conditions are reached and parameters conditions are determined, then the design conditions will be able to be estimated reasonably.

Biogas production is a of key importance in anaerobic digestion experiments but errors in its quantification can arise unless two essential issues are properly considered: the method for collecting the biogas/methane produced without significant losses or errors, and the method for converting the observed biogas/methane production to that under standard conditions using suitable correction/calibration factors.

Using the height-based method requires appropriate sizing of the cylinder to ensure that likely errors in the height measurement are small relatively for the volumes of gas measured. Biogas not only dissolves into barrier solutions, but tends to permeate through these into the atmosphere because the partial pressures of methane and carbon dioxide in the atmosphere are much lower than in the collection column. Conversely, oxygen and nitrogen can diffuse into the collection column. The speed of diffusion is a function of the solubility of gases in the barrier solution, and therefore solutions with high ionic content are most suitable. In height gas meters, over longer contact periods may gas to diffuse through the barrier solution.

Since the volume of gas produced, there are some common errors made in the quantification of biogas from anaerobic digestion experiments. Typically errors are related with underestimation of gas diffusion, volume calculation depending on type of equipment, corrections that must be made to obtain gas volumes at standard temperature and pressure and the type of liquid used in the gas meter (the “barrier solution”).

Based on the theoretical information and the experimental activities, in the particular case of treating wastewaters containing appreciable amounts of mono-ethylene glycol (EG), such as Sleipner, the conversion of this substrate to volatile acids production consumes appreciable amounts of bicarbonate alkalinity, so a lot of attention must be put experimentally to avoid bicarbonate alkalinity to be exhausted rapidly.

From the examples of *contact systems* reactors probably the most adjusted to Sleipner might be the UASB reactor, based on theoretical aspects given previously in this document; however, more extensive and detail study of design aspects must be taken into account. Important design considerations are: wastewater characteristics in terms of composition and solids content, volumetric organic load, upflow velocity, reactor volume, physical features including the influent distribution system, and gas collection system. Factors that affect the development of the granulated solids must also be taken into account.

RECOMMENDATIONS

- Treating high-strength industrial wastewaters, anaerobic treatment has been shown to provide a very cost-effective alternative to aerobic process with saving in energy, nutrient addition and reactor volume. Sleipner effluent has been proved to be anaerobically biodegradable so it is recommendable more experimentation and more continuous follow-up in the Laboratory of the analysis and system to have a better understanding of the system.

- Despite of not knowing the composition of the gas, it is believe is feasible that biogas was produced during the experimentation so there is the possibility of treating Sleipner anaerobically, however, it is extremely important to do the chromatographic gas analysis to determine the real composition of the gas, in order to estimate how much methane is being really produced. In addition, it might recommendable to install a sort of manometer to know the internal pressure and estimate composition based on the partial pressures of components in the bioreactor too.

- It is also recommendable to do more sensibilities of cases after stable conditions are reached, to determine the boundary conditions of the anaerobic system and optimum conditions (variations of temperature, residence time, concentration load, more salinity, higher sulphur and/or nitrogen-compounds concentrations, etc)

- The design of the anaerobic system depends on more laboratory studies and the presentation of their results in graphical form. Those graphs can then be coupled with relatively simple mass balance and process design equations in order to interpolate to the desired design conditions.

- Finally it is essential to keep in mind alternatives in future when producing biogas, like promoting the growth of plant using the CO_2 from the produced from the biogas and generating O_2. In this way, it is also possible to purify the methane produced and avoiding hazardous emissions of those components besides of CO_2 that are present is traces or significant quantities in the biogas (such as siloxanes and hydrogen sulphide), depending on the source of compositions.

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APPENDIXES

Anaerobic system using sugar as substrate

Day	Date		T_{room} (°C)	T_{sample} (°C)	pH	K (mS/cm)	TDS[1] (mg/L)	TS (g/L)	TVS (g/L)	H_2CO_3 alkalinity (mg/L $CaCO_3$)	Short-chain fatty acids (mg/L Ac.Acid)[2]	Cs (mg/L)	$V_{removed}$ (mL)	Resid. Time (d)	Comments	Reference picture Nr.
1	Wednesday	20.10.2010	22,0	25,5	7,36	11,66	8162,00	32,69	17,38			10	------	------	- Analysis corresponding to biosludge received (from IVAR) - Installation bioreactor with 500 mL of bio-sludge+200 mL wastewater (with Csugar = 10 g/L and 1300 mL tap water	1-10
3	Friday	22.10.2010	22,0	34,0	6,98	3,51	2457,00	7,71	N/D			10	200	20	Not determined TVS (sample fell down)	11, 12
5	Sunday	24.10.2010	23,0	33,0	6,78	4,34	3038,00					10	200	20	No observations	
6	Monday	25.10.2010	22,0	34,0	6,47	4,29	3003,00	7,40	5,00	1600,80	0,00	10	100	20	pH is going down fast. Adjustment of pH was done using NaOH.	13-17
7	Tuesday	26.10.2010	22,0	35,0	6,72	4,70	3290,00			1160,60	0,00	10	100	20	Increase of pH was just 0,25 (too little). New adjustment was done using instead NaHCO3 (better buffer capacity). Some kinf of "fat stains" are dispersed on the top of the sample extracted.	
9	Thursday	28.10.2010	22,0	33,0	6,84	4,60	3220,00					10	50	80	Increase of pH = 0,12 in 48 hours	
10	Friday	29.10.2010	22,0	32,0	6,83		0,00					10	50	40	Presence of bubbles in the bioreactor (in the liquid) and on the bottom of the cap	18
12	Sunday	31.10.2010	22,0	31,0	6,90		0,00					10	50	120	Big bubbles (transparents) and small bubbles (green) observed	19
14	Tuesday	02.11.2010	22,0	32,0	6,97	4,29	3003,00					10	50	80	Bubbles going up. Bigger stains of "fat"	
16	Thursday	04.11.2010	21,0	32,0	6,93		0,00					10	50	80	Addition CaCO3 to increase pH (69,1 mg CaCO3)	
19	Sunday	07.11.2010	22,0	32,0	6,84	4,61	3227,00					10	50	120	Leakage was found upstream valve to the gas collector (balloon). Leakage was deleted	
21	Tuesday	09.11.2010	19,0	32,0	6,83	4,47	3129,00					10	50	80	No observations	
23	Thursday	11.11.2010	22,0	33,0	6,84	4,34	3038,00					5	50	80	New wastewater samples received from inlet wastewater plant (1 sample with FeCl3 and the other "normal inlet") Wastewater samples were prepared to "feed" the bioreactor with 2 different concentrations (5 g/L and 10 g/L) Small concentration of sugar was used this time (5 g/L)	
26	Sunday	14.11.2010	23,0	35,0	6,85	4,55	3185,00					5	70	86	First syringe prepared as a "microsystem", using 5 g/L sugar concentration and 15 mL of bioreactor sample	20-22
28	Tuesday	16.11.2010	22,0	32,0	6,88	4,60	3220,00					5	70	57	Total sugar added= 5 g/L * 0,070 L = 0,35 g sugar	23
30	Thursday	18.11.2010	21,0	35,0	6,86	4,60	3220,00					5	70	57	No observations	24
33	Sunday	21.11.2010	22,0	35,0	6,84	4,62	3234,00					7,3	80	75	43 mL of feed solution with Csugar = 5 g/L + 37 mL of feed solution with Csugar= 10 g/L was added to bioreactor (total Csugar= 7, 3 g/L, total sugar= 0,585 g). Sample of feed water with Csugar = 5 g/L run out!	
35	Tuesday	23.11.2010	22,0	36,0	6,75	4,60	3220,00					10	74	54	Since pH goes down about 0,1 units, then just feed less sugar. Assuming 0,35 g sugar consumption, then add less volume of feed water with Csugar=10 g/L. Assuming also that there is a remaining sugar of 0,585-0,35 = 0,235 g, then volume to add is 11,5 mL	25
37	Thursday	25.11.2010	22,0	35,0	6,82	4,52	3164,00					10	105	38	It is assumed requirement of 0,35 g sugar, Volume of feed solution added= 35 mL	
40	Sunday	28.11.2010	21,0	36,0	6,80	4,52	3164,00					10	95	42	After 2 weeks, increment of gas is observed in the syringe with sugar+sludge! It has been decided to install new syringes (4) for further paralell observations: Case 1= 5 mL (Sleipner)+15 mL (Bioreactor) Case 2= 4 mL (Sleipner)+15 mL (Bioreactor)+ 1mL wastewater (containing FeCl3) Case 3 = 15 mL (Bioreactor)+5mL (wastewater with sugar, 10 g/L) case 4= 15 mL (bioreactor)	26,27,29
42	Tuesday	30.11.2010	20,0	32,0	6,70	4,46	3122,00					10	70	57	Sugar added based on assumed consumption of 0,35 g/2 days	

Anaerobic system using sugar as substrate

Day	Date		T_{room} (°C)	T_{sample} (°C)	pH	K (mS/cm)	TDS[1] (mg/L)	TS (g/L)	TVS (g/L)	H_2CO_3 alkalinity (mg/L $CaCO_3$)	Short-chain fatty acids (mg/L Ac.Acid)[2]	Cs (mg/L)	$V_{removed}$ (mL)	Resid. Time (d)	Comments	Reference picture Nr.
44	Thursday	02.12.2010	20,0	35,0	6,73	4,42	3094,00					10	70	57	Sugar added based on assumed consumption of 0,35 g/2 days. Observations about new syringes installed as "micro-systems": Case 1 and Case 2= some bubbles on the top Case 3= some bubbles on the top and in the bottom. Solids try to stick together like a solid mass Case4 = nothing relevant	28, 30-31
47	Sunday	05.12.2010	21,0	35,0	6,72	4,42	3094,00					10	75	80	- Sugar added based on assumed consumption of 0,35 g/2 days. - Big syringe has about 2 mL of piston displacement! - Observations about new syringes installed as "micro-systems": Case 1= bubbles at bottom (small ones) and a bigger on the top Case 2=bubbles at bottom (small ones) and a bigger on the top (bigger than in Case 1) Case 3= more small bubbles than Cases 1 and 2. Big bubble on the top (bigger than in Case1) Case 4= no bubbles	32-35
49	Tuesday	07.12.2010	21,0	36,0	6,83	4,35	3045,00					10	60	67	Important: pH went up and conductivity down! Sugar added based on assumed consumption of 0,35 g/2 days	
51	Thursday	09.12.2010	21,0	37,0	6,78	4,28	2996,00					no sugar	72		CHANGE OF SUBSTRATE SOURCE (FROM SUGAR TO INDUSTRIAL WASTEWATERS)!	36-38

Note:

(1) TDS calculated using Conductivities values and according to Standard Method, 1998, [TDS (mg/L) = EC (µS/cm)* 0,7].

(2) Based on titration and TITRA5 program

Prosessvann 64TA206[(1)]

Analysis report		TOC	pH	Fenol	Density	Sulfide
Dato	Time	(mg/L)		(mg/L)	(at 15 C), mg/L	(ppm)
04.12.2009	08:45	5700	5,4	51,2	1,0005	<0,5
05.02.2010	10:30	4405	5,5	49,0	1,0005	<0,5
27.05.2010	13:10	5270	5,3	53,0	1,0004	<0,5
05.10.2010	14:00	5200	5,1	45,0	1,0009	<0,5

Notes:

(1) Analisis provided by Karstø Refinery

Prosessvann 64TA206 (1)

Dato		01.08.2008	17.12.2008
Analyse rap.	NOV	028836-08	058407-08
Kasium Ca oppsluttet	mg/L	93	90,9
Kalium K oppsluttet	mg/L	5,5	6,6
Magnesium Mg oppsluttet	mg/L	14	11,5
Natrium Na oppsluttet	mg/L	260	587
Svovel S oppsluttet	µg/L	6700	4000
Jern Fe oppsluttet	mg/L	42	607
Aluminium Al oppsluttet	µg/L	1000	326
Arsen As Oppsluttet	µg/L	2,9	15
Barium Ba oppsluttet	µg/L	280	634
Kadmium Cd oppsluttet	µg/L	<0.4	0,29
Kobolt Co oppsluttet	µg/L	5,4	12
Krom Cr oppsluttet	µg/L	76	234
Kobber Cu oppsluttet	µg/L	5,1	91
Mangan Mn oppsluttet	µg/L	440	5880
Nikkel Ni oppsluttet	µg/L	37	78
Bly Pb oppsluttet	µg/L	4,1	30
AntimonSb oppsluttet	µg/L	<4	2,5
Tinn Sn oppsluttet	µg/L	<2	11
Vanadium V oppsluttet	µg/L	2,2	4,1
Molybden Mo oppsluttet	µg/L	7,7	40
Sink Zn oppsluttet	µg/L	160	146
Strontium Sr oppsluttet	µg/L	2000	3340
Silisium Si oppsluttet	µg/L	1200	1685
Fosfor total	mg P/L	0,23	0,28
Nitrogen total	mg N/L	31,5	39,5
TOC	mg/L	5400	5200
Metanol	mg/L		4500
Monoethylenglycol	mg/L		2600
Diethylenglycol	mg/L		
Triethylenglycol	mg/L		
Propylenglycol	mg/L		17
Sum PAH (16)	µg/L	440	200
Naftalen	µg/L	420	176
Acenaftylen	µg/L	1,1	0,818
Acennaften	µg/L	2,2	2,52
Fluoren	µg/L	10	7,9
Fenantren	µg/L	8,1	7,6
Antracen	µg/L	0,71	0,359
Fluoanten	µg/L	0,56	0,622
Pyren	µg/L	0,51	0,517
Benzo(a)antracen	µg/L	0,08	0,09
Crysen	µg/L	0,1	0,106
Benzo(b)antracen	µg/L	0,03	0,054
Benzo(k)antracen	µg/L	0,03	0,013
Benzo(a)pyren	µg/L	0,19	0,207
Indeno(1, 2, 3, cd)pyren	µg/L	<0.1	0,012
Dibenzo(a, h)antracen	µg/L	<0.1	0,004
Benzo(g, h,i)perylen	µg/L	<0.1	0,021
Fenoler som fenol	µg/L		51000
Kvikksølv, Hg	µg/L	1,23	2,3
Bensen	µg/L	17000	29000
toulen	µg/L	9900	22000
etylbensen	µg/L	700	1100
p,m-xylen	µg/L	3800	5500
o-xylen	µg/L	1400	2000
Ftalater:			
Dimetylftalat	ng/L	<100	<50
Dietylftalat	ng/L	<100	4900
Bensylbenzoat	ng/L	<100	<50
Diisobutylftalat	ng/L	165	370
Dibutylftalat	ng/L	335	510
Dimetoksyetylftalat	ng/L	<100	<50
Diisoheksylftalat	ng/L	<100	<50
Di-2-etoksyetylftalat	ng/L	<100	<50
Dipentylftalat	ng/L	<100	<50

Dato		01.08.2008	17.12.2008
Analyse rap.	NOV	028836-08	058407-08
Di-n-heksylftalat	ng/L	<100	<50
Bensylbutylftalat	ng/L	<100	<50
Heksyl-2-etylheksylftalat	ng/L	<100	<50
Dibutoksyetylftalat	ng/L	<100	<50
Disykloheksylftalat	ng/L	<50	<50
Di-(2-etylhexyl)ftalat	ng/L	663	2500
Diisononylftalat	ng/L	<100	<50
Di-n-oktylftalat	ng/L	<100	<50
Diisodekylftalat	ng/L	<1000	<1000
Suspendert stoff, SS	mg/L	25	200
pH	pH	4,6	5,4
Sulfid	mg/L	<0.02	0,03
Fenol	ng/L		
2-metylfenol	ng/L		11600000
4-metylfenol	ng/L		6690000
4-etylfenol	ng/L		670000
2,4-dimetylfenol	ng/L		1100000
3,5-dimetylfenol	ng/L		849000
4-n-proplyfenol	ng/L		157000
2,4,6-trimetylfenol	ng/L		55100
2,3,5- trimetylfenol	ng/L		82400
4-n-butylfenol	ng/L		7730
4-tert-butylfenol	ng/L		61200
4-isoproplyl-3-metylfenol	ng/L		3860
4-n-pentylfenol	ng/L		<10
2-tert-butyl-4-metylfenol	ng/L		<10
4-tert-butyl-2-metylfenol	ng/L		<10
4-n-heksylfenol	ng/L		368
2,5-diisopropylfenol	ng/L		<10
2,6-diisopropylfenol	ng/L		<10
2-tert-butyl-4-etylfenol	ng/L		23000
6-tert-butyl-2,4-dimetylfenol	ng/L		1870
4-n-heptylfenol	ng/L		<10
2,6-dimetyl-4-(1,1-dimetylpropyl)fenol	ng/L		<10
4-(1-etyl-1metylpropyl)-2-metylfenol	ng/L		<10
2,6diisopropyl-4-metylfenol	ng/L		<10
4-n-oktylfenol	ng/L		<10
4-tert-oktylfenol	ng/L		172
2,4-di-tert-butylfenol	ng/L		<10
2,6-di-tert-butylfenol	ng/L		<10
4-n-nonylfenol	ng/L		<10
2-metyl-4-tert-oktylfenol	ng/L		<10
2,6-di-tert-butyl-4-metylfenol	ng/L		<10
4,6-di-tert-butyl-2-metylfenol	ng/L		<10
Naftalen	ng/L		541000
C1-Naftalen	ng/L		431000
C2-Naftalen	ng/L		289000
C3-Naftalen	ng/L		10700
Phenantren	ng/L		9860
Antrasen	ng/L		805
C1-Phenantren	ng/L		7430
C2-Phenantren	ng/L		640
C3-Phenantren	ng/L		536
Dibenzotiophen	ng/L		3540
C1-Dibenzotiophen	ng/L		3690
C2-Dibenzotiophen	ng/L		710
C3-Dibenzotiophen	ng/L		506

Notes:
(1) Analisis provided by Karstø Refinery

Anaerobic system using industrial wastewater as substrate

Day	Date		T_{room} (°C)	T_{sample} (°C)	pH	K (mS/cm)	TDS[1] (mg/L)	TSS (g/L)	VSS (g/L)	H_2CO_3 alkalinity (mg/L $CaCO_3$)	Short-chain fatty acids (mg/L Ac.Acid)[2]	$V_{Sleipner}$ (mL)	V_{IVAR} (mL)	V_{Water} (mL)	$V_{removed}$ (mL)	Resid. Time (d)	Gas produc. (mL)	Comments	Reference picture Nr.
51	Thursday	09.12.2010	21,0	37,0	6,78	4,28	2675,00					12	10	50	72	56		Observations about "microsystem syringes": Case 1= bubbles forming Case 2= displacement of piston (less than 1/2 mL) Case 3= bubbles forming Case 4= no changes	36-38
54	Sunday	12.12.2010	21,0	36,0	6,87	4,17	2606,25					10	10	52	72	83		To recollect gas, a big syringe was installed at the top of the tank (instead of rubber balloon used before)	39-40
56	Tuesday	14.12.2010	20,0	34,0	6,87	4,09	2556,25					10	10	45	65	62		- Wastewater from IVAR used from this day contains FeCL3 - Observations about "microsystem syringes": Case 1= more bubbles forming (at the bottom mainly). Piston displacement of 1 mL aprox. Case 2= more bubbles forming (at the bottom mainly). Piston displacement more than 1 mL Case 3= bubbles forming. No piston displacement. Just relative small amount of bubbles on top Case 4= no changes	42-43
58	Thursday	16.12.2010	22,0	36,0	6,87	4,00	2500,00					11	10	59	80	50		No observations	
61	Sunday	19.12.2010	21,0	35,0	6,84	3,92	2450,00					10	10	70	90	67	35,6	No observations	
63	Tuesday	21.12.2010	22,0	36,0	6,76	3,83	2393,75					10	10	50	70	57	1	Possibly piston of syringe jammed	
65	Thursday	23.12.2010	22,0	35,0	6,84	3,77	2356,25					10	10	54	74	54	38	No observations	
68	Sunday	26.12.2010	22,0	34,0	6,86	3,71	2318,75					10	10	50	70	86	24	No observations	
70	Tuesday	28.12.2010	22,0	37,0	6,78	3,60	2250,00					10	10	38	58	69	8	- Filtration is observed due to the presence of liquid on the top of the bioreactor. It seems that high pressure is the bioreactor is not able to be properly relieved. '- Observations about the microsystems syringes: Case 1= biggest gas volume (about 6 mL). Still bubbles coming from the bottom Case 2= appreciable gas volume (about 4,5 mL). Still bubbles coming from bottom Case 3= Small amount of gas. No piston displacement. Small bubbles in the bottom Case 4= No changes	
72	Thursday	30.12.2010	22,0	36,0	6,81	3,58	2237,50					10	10	36	56	71	29	Water vapor observed at the top of the bioreactor (inside) Piston on syringe was jammed and initial gas volumen reading was 21 mL, then after releasing piston, V=29 mL	44-45
75	Sunday	02.01.2011	22,0	34,0	6,76	3,51	2193,75					10	10	28	48	125	24	Observations about the microsystems syringes: Case 2= piston was totally displaced (no gas retained in the syringe anymore)	
77	Tuesday	04.01.2011	22,0	35,0	6,80	3,48	2175,00					20	15	46	71	56		- Increase of Sleipner volume - Installation of "gasometer"	46
79	Thursday	06.01.2011	24,0	35,0	6,71	3,40	2125,00					20	15	65	100	40		No observations	
82	Sunday	09.01.2011	22,0	34,0	6,81	3,29	2056,25					20	20	55	95	63	30	- volume of gas read in the "gasometer" (by difference of levels in the cilinder). In the gasometer are observed water vapor drops	47-48

Day	Date		T_{room} (°C)	T_{sample} (°C)	pH	K (mS/cm)	TDS[1] (mg/L)	TSS (g/L)	VSS (g/L)	H_2CO_3 alkalinity (mg/L $CaCO_3$)	Short-chain fatty acids (mg/L Ac.Acid)[2]	$V_{Sleipner}$ (mL)	V_{IVAR} (mL)	V_{Water} (mL)	$V_{removed}$ (mL)	Resid. Time (d)	Gas produc. (mL)	Comments	Reference picture Nr.
84	Tuesday	11.01.2011	22,0	36,0	6,76	3,20	2000,00					20	20	60	100	40	20		
86	Thursday	13.01.2011	21,0	33,0	6,78	2,97	1856,25					20	20	30	70	57	N/D	Gas volume not measured. It is believed some amount of $Ca(OH)_2$ went into the bioreactor	49-50
89	Sunday	16.01.2011	21,0	33,0	6,74	2,94	1837,50					20	20	60	100	60	20	No observations	51
91	Tuesday	18.01.2011	22,0	35,0	6,76	2,87	1793,75					20	20	160	200	20	200	To increase pH in bioreactor, addition of 1,0763 g Na_2CO_3	
93	Thursday	20.01.2011	20,0	36,0	6,97	3,12	1950,00	1,40	1,03	2053,20	0,00	20	20	160	200	20	N/D	No reading of gas volume due to mistake. Gasometer not connected to bioreactor	52
96	Sunday	23.01.2011	22,0	34,0	6,91	2,90	1812,50	0,95	0,67	754,80	166,90	20	20	160	200	20	10	No observations	53
98	Tuesday	25.01.2011	22,0	34,0	6,93	2,73	1706,25	0,86	0,50	748,60	0,00	40	40	120	200	20	N/D	Increase of Sleipner volume (from 20 to 40 mL). Volume of gas produced was not registered	54-55
100	Thursday	27.01.2011	22,0	35,0	6,82	2,65	1656,25	0,52	0,32	592,40	31,10	40	40	120	200	30	45	No observations	
103	Sunday	30.01.2011	22,0	35,0	6,05	2,58	1612,50			187,40	561,80	40	40	120	200	30	N/D	- No too much gas. Smell of samples is not the usual. '- Since pH was too low, it was decided to increase it. NaOH was used (in excess!, about 2 grams)	
105	Tuesday	01.02.2011	21,0	36,0	11,45	4,61	2881,25					0	0	0	0		N/D	Since increase of pH was too high, addition of Acetic acid was done to decrease pH (1,6 mL to reach a final pH=9,90). Total decrease of pH reached= 11,45-9,90=1,55. Strong smell due to presence of NaOH	
107	Thursday	03.02.2011	22,0	36,0	7,40	4,10	2562,50	0,75	0,41	501,20	1497,70	0	0	0	0		N/D	No feeding. Waiting for stabilizaton of the system	
110	Sunday	06.02.2011	22,0	35,0	6,78	4,13	2581,25	0,72		394,30	1772,50	0	0	0	0			Smell is less strong, apparently more similar to before (with presence of fatty acids, only presumibly). Bubbles of gas observed leaving the sample.	
112	Tuesday	08.02.2011	22,0	36,0	6,64	3,18	1987,50					0	0	0	0			No feeding. Waiting for stabilization of the system. Instead preparation of solution of nutrients was done to replace feeding of wastewater from IVAR (used as a source of nutrients). Addition of nutrients was done (10 mL)	
113	Wednesda	09.02.2011	22,0	34,0	6,98	3,34	2087,50											No feeding. Waiting for stabilizaton of the system	
114	Thursday	10.02.2011	22,0	34,0	7,05	3,22	2012,50											No feeding. Waiting for stabilizaton of the system	

Note:

(1) TDS calculated using Conductivities values and according to Standard Method, 1998, [TDS (mg/L) = EC (µS/cm)* 0,7].

(2) Based on titration and TITRA5 program

Composition of nutrient solution for anaerobic degradation

	Compound	Concentration (mg/L)	Element (mg/L)	Element concentration (mg/L)
Micronutrients	NH_4Cl	19500	**N**	5103
	$NaH_2PO_4 \cdot 2H_2O$	8500	**P**	1690
	$MgSO_4 \cdot 7H_2O$	7332	**Mg**	735
	KCl	1622	**K**	850
	$CaCl_2 \cdot 2H_2O$	1560	**Ca**	425
Micronutrients	$MnCl_2 \cdot 4H_2O$	120	**Mn**	33,3
	$NaMoO_4 \cdot 2H_2O$	60	**Mo**	26,3
	$CuSO_4 \cdot 5H_2O$	100	**Cu**	7,6
	$CoCl_2 \cdot 6H_2O$	150	**Co**	37,2
	$ZnSO_4 \cdot 7H_2O$	120	**Zn**	27,3
	H_3BO_3	150	**B**	26,2
	KI	180	**I**	137,6
	$FeCl_3 \cdot 6H_2O$	1500	**Fe**	309,7

Data from: "Growth requirements and growth kinetics", by Leif Ydstebø; UiS, 2008

COD Report

Sample	Date	Average COD from Diluted sample (mg/L)	factor	COD Original sample (mg/L)[1]
6	16.01.2010	37,1	10	371
7	16.01.2010	91,3	2	182,6
2	18.01.2010	45,3	10	453
3	18.01.2010	92,2	2	184,4
4	20.01.2010	35,3	10	353
5	20.01.2010	90,5	2	181
13	21.01.2010	53	5	265
8	sludge 1	183,6	10	1836
9	sludge 2	116,25	16,7	1941,4
10	sleipner 1	271	50	13550
11	sleipner 2	169,85	100	16985

Date	Initial COD (mg/L)	Addition of COD to bioreactor IVAR[2,3] (mg)	Addition of COD to bioreactor Sleipner[2,3] (mg)	Total (mg)	TOTAL COD available (mg)	COD consumed (mg)	Efficiency of COD removal (%)	CH_4 production expected[4] (L)	CH_4 production reported[5] (L)
16.01.2010	371	7	339,7	346,7	717,7	----	-----	----	----
18.01.2010	453	7	339,7	346,7	799,7	264,7	36,88	0,09	0,20
20.01.2010	353	7	339,7	346,7	699,7	446,7	55,86	0,16	No reported
21.01.2010	265	0	0	0	265	434,7	62,13	0,15	0,01

Notes:

(1) It is believed that samples more dilluted reported better results and those with low dillution factors are less exact, probably due to the presence of substances that interfer with the analysis oxidizing them and in consequence reducing COD accuracy reported (in this case, probably due to high concentration of Cl^- in the wastewater)

(2) IVAR wastewater has an average COD content of 350 mg/L (medium strength concentration) and Sleipner has 16985 mg/L approx.

(3) Values of volume added to bioreactor according volumes reported in Table Nr. 4 (Appendixes section)

(4) CH_4 volume expected to be produced estimated by: 0,35 L CH_4 produced/g COD removed, (see reference in part I, Theoretical Basis for the research, item 2.1.3.)

(5) According to experimental data reported in Table Nr. 4 (Appendixes section)

PICTURES RELATED TO EXPERIMENTATION

Picture Nr. 1: Received samples


Picture Nr. 2: Analysis of pH of bio-sludge received

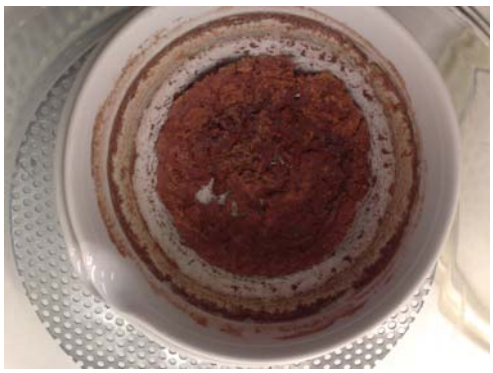

Picture Nr. 3: Bio-sludge to be evaporated for TS analysis at 105 °C


Picture Nr. 4: Bio-sludge after evaporation (for TS determination)

Picture Nr. 5: Bio-sludge after evaporation (for TS determination)

Picture Nr. 6: Bio-sludge for TVS determination (combustion at 550 °C)

Picture Nr. 7: Bio-sludge after combustion at 550 °C



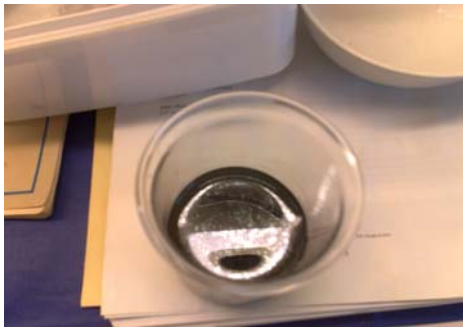
Picture Nr. 8: Bio-sludge after combustion at 550 °C

Picture Nr. 9: Installation of bioreactor

Picture Nr. 10: Gas sampling bag for gas recollection

Picture Nr. 11: Bioreactor after extraction of 200 mL of sample

Picture Nr. 12: Feeding bioreactor with IVAR wastewater and sugar (10 g/L)

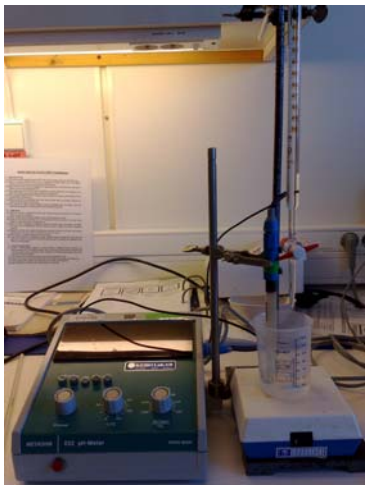


Picture Nr. 13: Presence of “fatty” stains. “Tarry” smell

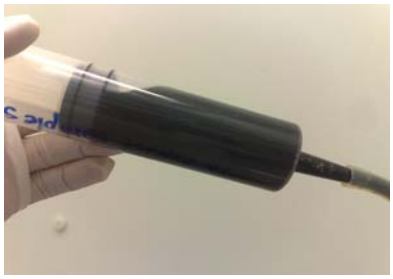
Picture Nr. 14: Presence of fatty stains

Picture Nr. 15: Water vapour on the top of bioreactor (internally)

Picture Nr. 16: Presence of gas on the top



Picture Nr. 17: Alkalinity determination



Picture Nr. 18: Presence of gas (bubbles)

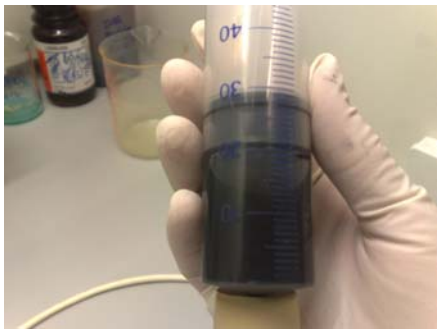
Picture Nr. 19: Presence of gas

Picture Nr. 20: “Microsystem installed” (syringe with sample of bioreactor and 5 g/L of sugar concentration). No bubbles of air were left inside.

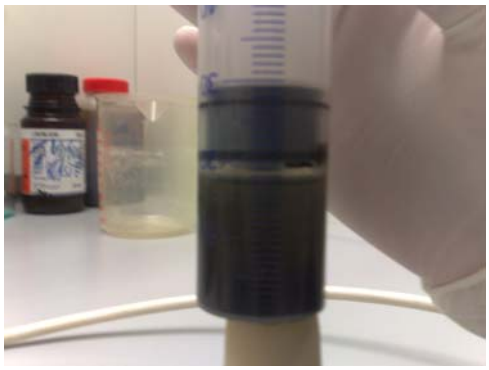
Picture Nr. 21: 2 days after microsystem was installed

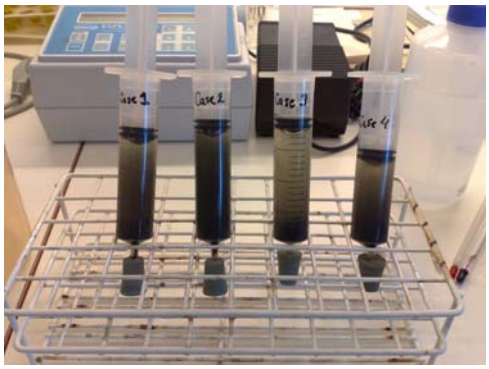
Picture Nr. 22: 5 days after microsystem was installed


Picture Nr. 23: Gas bubbles in the bioreactor



Picture Nr. 24: 2 weeks after microsystem was installed

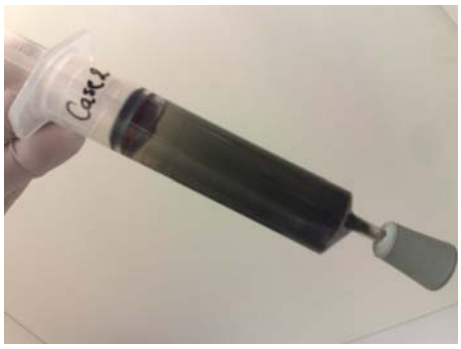

Picture Nr. 25: 2 weeks after microsystem was installed


Picture Nr. 26: Installation of 4 syringes

Picture Nr. 27: Industrial wastewater sample (Sleipner) with high content of methanol and MEG, from Karstø plant

Picture Nr. 28: 21 days after microsystem was installed

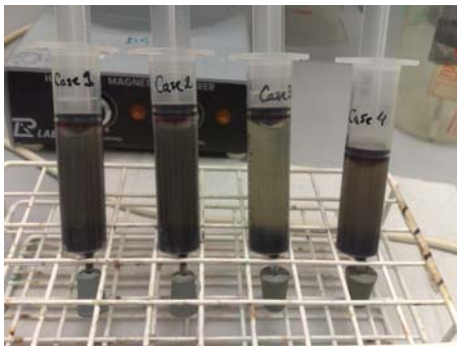
Picture Nr. 29: 1 week after installation of 4 syringes as “microsystem”. This is Case Nr. 1 (5 mL (Sleipner+15 mL of Bioreactor sample)



Picture Nr. 30: 1 week after installation of 4 syringes as “microsystem”. This is Case Nr. 2 (4mL Sleipner sample + 15 mL of Bioreactor sample+ 1mL wastewater as nutrient source)

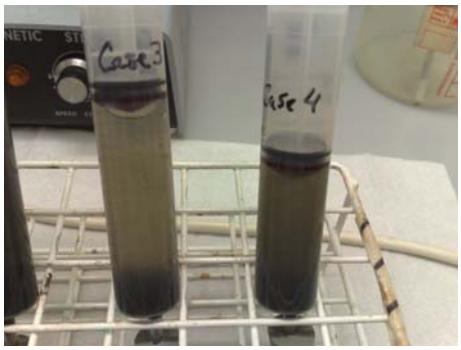
Picture Nr. 31: 1 week after installation of 4 syringes as “microsystem”. This is Case Nr. 3 (15 mL of Bioreactor sample+ 5 mL wastewater as nutrient source with sugar 10 g/L)

Picture Nr. 32: 1 week after installation of 4 syringes as “microsystem”. This is Case Nr. 4 (15 mL of Bioreactor sample)

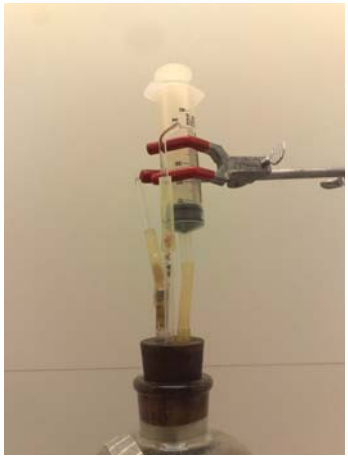


Picture Nr. 33: 11 days after the 4 syringes were installed

Picture Nr. 34: 11 days after the 4 syringes were installed (Cases 1 and 2)

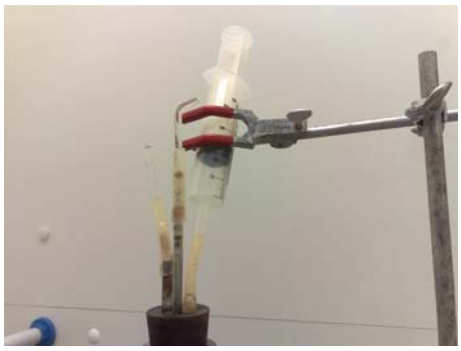


Picture Nr. 35: 11 days after the 4 syringes were installed (Cases 3 and 4)

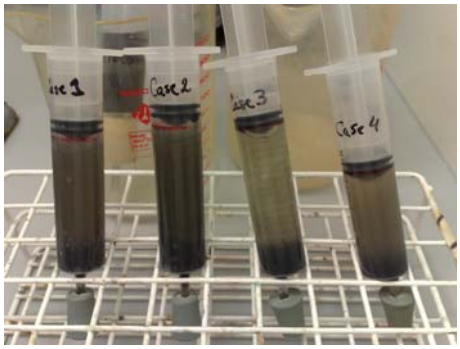


Picture Nr. 36: syringe installed to recollect gas instead of gas sampling bag

Picture Nr. 37: syringe installed to recollect gas instead of gas sampling bag



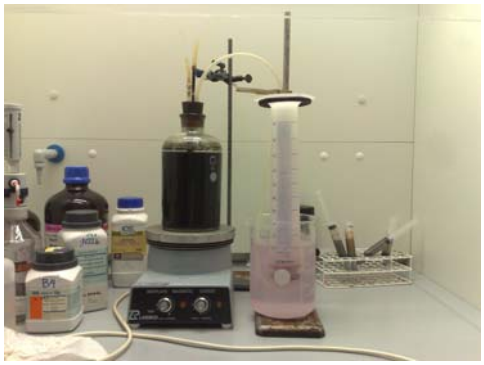
Picture Nr. 38: Gas recollected after installation of syringe (instead gas sampling bag)

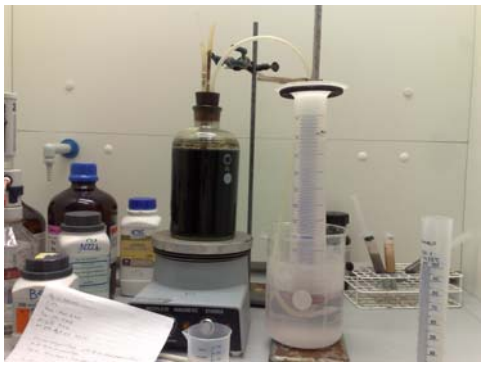


Picture Nr. 39: 16 days after the 4 syringes were installed (gas on the top)

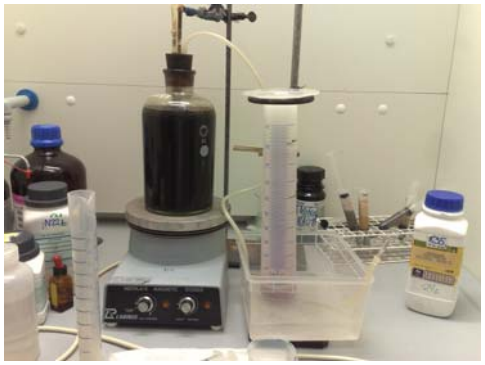
Picture Nr. 40: Gas recollected in 2 days (30.12.10)


Picture Nr. 41: Gas recollected in 2 days (30.12.10)

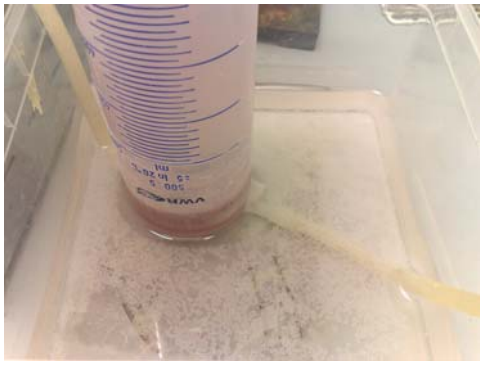

Picture Nr. 42: Installation of gasometer


Picture Nr. 43: Gasometer after 3 days in operation

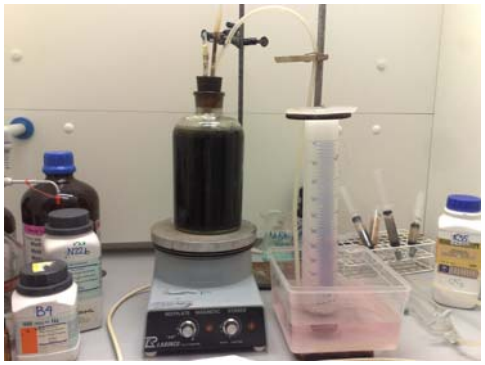
Picture Nr. 44: Precipitation of $CaCO_3$ due to reaction of CO_2 (present in the ambient and from bioreactor) with $Ca(OH)_2$



Picture Nr. 45: Gasometer 1 week after the installation

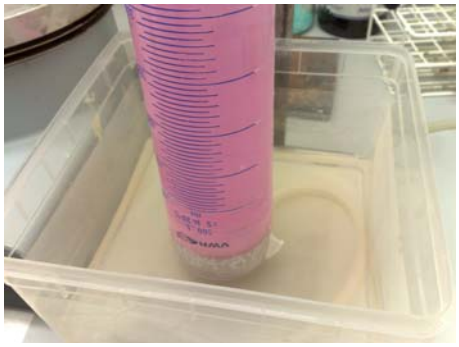


Picture Nr. 46: Change in colour of phenolphthalein and evaporation of water

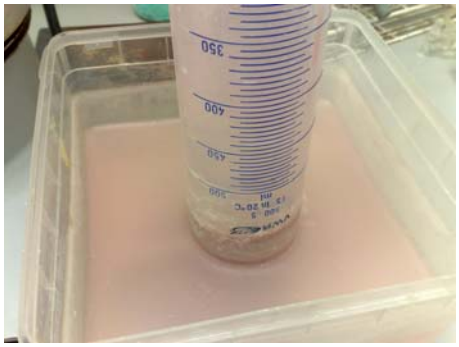


Picture Nr. 47: Gas measurement in gasometer

Picture Nr. 48: COD analysis

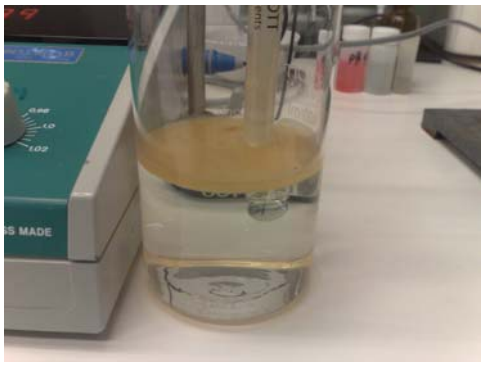


Picture Nr. 49: Comparison of colour of the same solution of $Ca(OH)_2$ between the liquid inside the gasometer and the liquid expose to the atmosphere

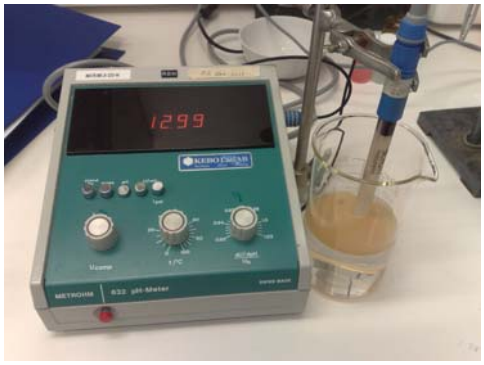


Picture Nr. 50: Gasometer liquid colour after some days and external layer of CaCO3 formed on the top of the liquid exposed to the atmosphere

Picture Nr. 51: Gas contained in the gasometer and some bubbles are also observed along the cylinder



Picture Nr. 52: Bioreactor sample after addition of NaOH in excess



Picture Nr. 53: pH of the sample after addition of NaOH in excess

Table Nr. 7: Typical composition of untreated domestic wastewater

Contaminants	Unit	Concentration[a]		
		Low strength	Medium strength	High strength
Solids, total (TS)	mg/L	390	720	1230
Dissolved, total (TDS)	mg/L	270	500	860
Fixed	mg/L	160	300	520
Volatile	mg/L	110	200	340
Suspended solids, total (TSS)	mg/L	120	210	400
Fixed	mg/L	25	50	85
Volatile	mg/L	95	160	315
Settleable solids	mL/L	5	10	20
Biochemical oxygen demand, 5–d, 20°C (BOD, 20°C)	mg/L	110	190	350
Total organic carbon (TOC)	mg/L	80	140	260
Chemical oxygen demand (COD)	mg/L	250	430	800
Nitrogen (total as N)	mg/L	20	40	70
Organic	mg/L	8	15	25
Free ammonia	mg/L	12	25	45
Nitrites	mg/L	0	0	0
Nitrates	mg/L	0	0	0
Phosphorus (total as P)	mg/L	4	7	12
Organic	mg/L	1	2	4
Inorganic	mg/L	3	5	8
Chlorides[b]	mg/L	30	50	90
Sulfate[b]	mg/L	20	30	50
Oil and grease	mg/L	50	90	100
Volatile organic compounds (VOCs)	μg/L	<100	100–400	>400
Total coliform	No./100 mL	10^6–10^8	10^7–10^9	10^7–10^{10}
Fecal coliform	No./100 mL	10^3–10^5	10^4–10^6	10^5–10^8
Cryptosporidum oocysts	No./100 mL	10^{-1}–10^0	10^{-1}–10^1	10^{-1}–10^2
Giardia lamblia cysts	No./100 mL	10^{-1}–10^1	10^{-1}–10^2	10^{-1}–10^3

[a]Low strength is based on an approximate wastewater flowrate of 750 L/capita·d (200 gal/capita·d). Medium strength is based on an approximate wastewater flowrate of 460 L/capita·d (120 gal/capita·d). High strength is based on an approximate wastewater flowrate of 240 L/capita·d (60 gal/capita·d).

From: Wastewater Engineering, Treatment and reuse. Metcalf & Eddy

Table Nr. 8: Typical mineral increase from domestic water use

Constituent	Increment range, mg/L[a,b]
Anions:	
Bicarbonate (HCO_3)	50–100
Carbonate (CO_3)	0–10
Chloride (Cl)	20–50
Sulfate (SO_4)	15–30
Cations:	
Calcium (Ca)	6–16
Magnesium (Mg)	4–10
Potassium (K)	7–15
Sodium (Na)	40–70[c]
Other constituents:	
Aluminum (Al)	0.1–0.2
Boron (B)	0.1–0.2
Fluoride (F)	0.2–0.4
Manganese (Mn)	0.2–0.4
Silica (SiO_2)	2–10
Total alkalinity (as $CaCO_3$)	60–120
Total dissolved solids (TDS)	150–380

[a] Based on 460 L/capita·d (120 gal/capita·d).
[b] Values do not include commercial and industrial additions.
[c] Excluding the addition from domestic water softeners.
Note: mg/L = g/m^3.

From: Wastewater Engineering, Treatment and reuse. Metcalf & Eddy