Assessment of the anaerobic biodegradability of bitumen fume condensate wastewater by mesophilic AnMBR

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Abstract

Certain industrial wastewaters have posed a challenge to water treatment systems because of their composition. The bitumen from the reclaimed asphalt process is heated by the BAM Infra Asfalt and produces fumes, which are usually sent to the air filters and then out through the chimney. To recover the heat lost through the fume, it is condensed. The bitumen fume condensate contains aromatic hydrocarbons of petroleum origin and this poses a threat to health and the environment. The removal of these compounds by anaerobic biodegradation was assessed with a mesophilic anaerobic membrane bioreactor (AnMBR). The bitumen condensate contained over 800 compounds, out of which some were p-cresol, o-cresol and 2-napthalenemethanol. The inhibition to the methanogenic activity and toxicity to the biomass of this wastewater on three different inocula were studied under batch-test conditions. A phenol-degrading sludge was less inhibited (IC_{50}= 870 mg $COD_{bitumen}$/L) and more resistant to the toxicity than granular sludge from a petrochemical wastewater treatment plant (IC_{50}= 187mg $COD_{bitumen}$/L) and a municipal sludge (sludge from a municipal wastewater treatment plant, IC_{50}= 127 mg $COD_{bitumen}$/L). In continuous operation, the bitumen condensate was degraded efficiently with 89% ± 12% (S.D) COD removal from the influent of the AnMBR. Maximum organic conversion rate of the bitumen condensate was 26.0 mg COD/g VSS.d. This research demonstrated the efficiency of AnMBR technology to degrade bitumen condensate. Further research must be done to improve the organic conversion rate and optimise the technology for large scale implementation.

Contents

1 Introduction

1.1 Toxic effluents from industries

A large quantity of water is used in industries for different processes, but only a small quantity of this water is present in their products or services. The rest of the water is disposed of into water bodies as wastewater or discharged into municipal sewers to be, ideally, taken to treatment plants. This wastewater contains contaminants from the industrial processes and may disrupt the natural composition of the receptor water body or the normal functioning of the treatment plants, which is harmful to the environment. Industries, considering this, try to minimise the effect of their wastes on the environment by treating their wastewater separately.

Wastewater differs from different industries. It would vary in composition, physical appearance, and toxicity, implying that different effluents will require different methods to treat the specific contaminants present in them. A thorough analysis of the wastewater is required to choose the method of treatment. In this thesis, the research has been focused on treating the organic contaminants in the effluent from an asphalt industry of BAM Infra Asfalt.

1.2 Asphalt industry - BAM Infra Asfalt

The BAM Infra Asfalt is a Dutch construction services company. One of their construction services includes the production of asphalt concrete for roads and pavements. The procedure used in the company is the reclaimed asphalt pavement (RAP) process. In this process, aggregates from old roads and pavements are reclaimed and reused with bitumen to produce new asphalt concrete. The bitumen is heated to a temperature of 105°C and mixed with dry sand and stones and the fumes from the heating of bitumen are led to a baghouse filter. The particulates present in the fume are filtered out by the filter (Figure 1). Then, the fume is let out through the chimney (Bruin, 2019). The use of RAP gives the company an advantage of reusing materials.

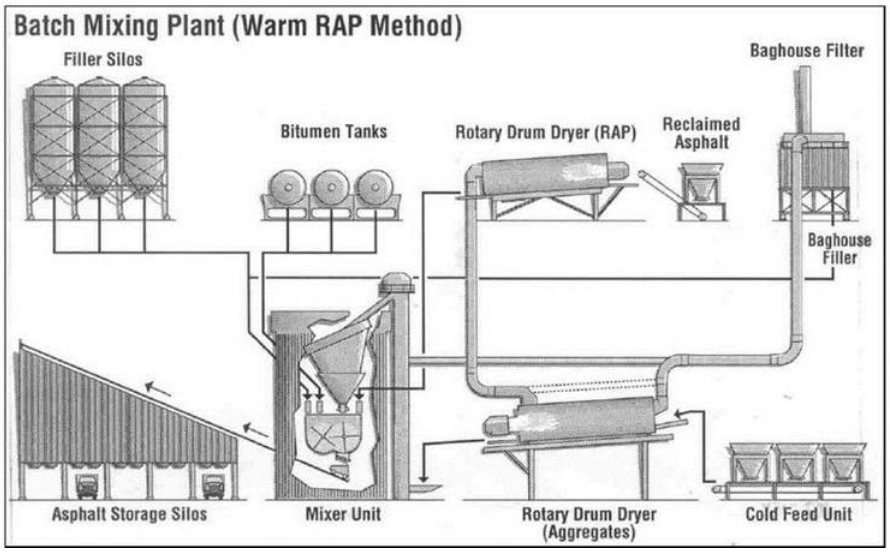

Figure 1: Process step of a batch mixing plant using the reclaimed asphalt pavement (RAP) process. The bitumen is heated and mixed with aggregates to form asphalt concrete (de Bruin, 2019).

To recover the energy lost after heating the bitumen, the fumes are condensed, and the energy recovered is added to the heat to mould the bitumen. The compounds present in the resulting condensate are contaminants and therefore should be removed from the condensate before it can be reused or discharged in water bodies.

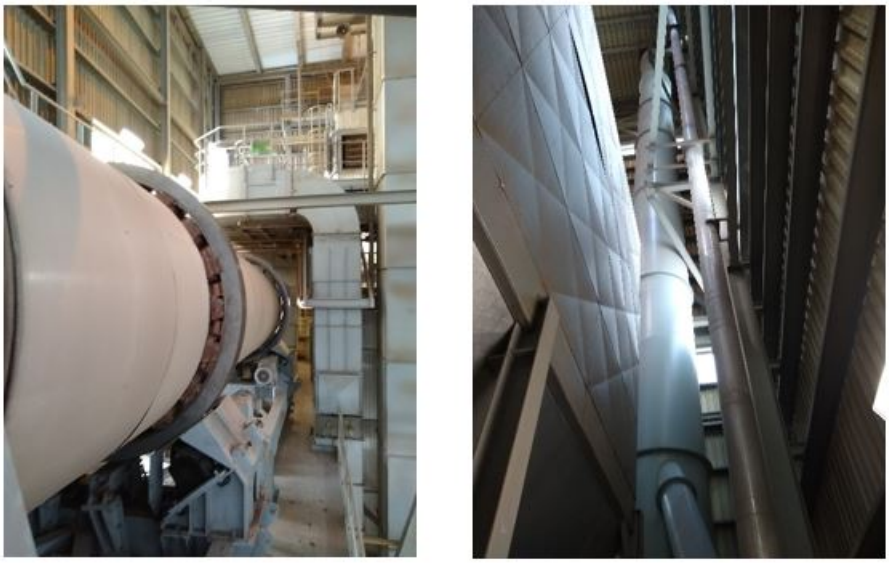

Figure 2: Air conduit for the bitumen fumes in BAM Infra plant at Bergen op Zoom, The Netherlands. The fumes are condensed and collected as a bitumen fume condensate (January 2019)

1.3 Wastewater treatment

Wastewater treatment can be categorised into three main groups, based on the methods used to remove the contaminants present in the wastewater: physical, chemical, and biological. In this research, the biological method of treatment was used. The biological methods employ microorganisms to remove contaminants and can be further categorised based on the metabolic function of the microorganisms. These include aerobic processes (in the presence of molecular oxygen) and anaerobic processes (absence of oxygen). This research studied the application of anaerobic process, also known as anaerobic digestion (AD), as a possible method for the treatment of bitumen condensate.

AD is the fermentation process in which organic material is degraded (Van Lier et al., 2008). In the AD, the final electron acceptor is carbon dioxide (CO_2), and the main product of this process is a mixture of methane (CH_4) and CO_2 which is known as biogas. AD has some advantages over the aerobic methods, for example, the energy consumption is lower as the aeration step is avoided and sludge production is minimal due to a lower growth yield of the anaerobic microorganisms. Furthermore, AD allows for recovering energy in the form of CH_4. In comparison to aerobic treatment, when degrading 1 kg of chemical oxygen demand (COD), AD can produce 13.5 MJ of energy in the form of CH_4, while aerobic methods will consume an approximate of 1 kWh^{-1} (Van Lier et al., 2008). Also, when compared to other fossil fuels, CH_4 produces less atmospheric pollutants and generates less CO_2 per unit energy when it is burned (Chynoweth et al., 2000). Another advantage is that some organic compounds can only be degraded under anaerobic (reducing) conditions. All these make the treatment of wastewater by AD an attractive option.

1.4 AD for industrial wastewater

As an energy-producing process, the anaerobic technology has become one of the preferred methods of wastewater treatment due to the simplicity of the technology and its the low space requirement in comparison to the aerobic water treatment technology (Van Lier et al., 2008). The anaerobic technology has been applied to several industrial wastewater effluents such as those coming from agro-food industry, beverage, alcohol distillery, pulp and paper, and other miscellaneous industries. The application potential of anaerobic wastewater treatment has expanded (Van Lier et al., 2008). It is being used to treat wastewaters that were not previously considered for anaerobic treatment, such as wastewaters containing toxic compounds or with complex composition. Besides, AD has expanded to wastewater from industries like petrochemical and chemical industries (van Lier et al., 2015).

Membrane technology is a highly viable option for wastewater under extreme conditions, such as high temperatures and high salinity, or wastewaters with toxic compounds. With the concept of close water cycles in industrial processes, treatment of wastewater with those characteristics is required more commonly now (Van Lier et al., 2008). The membrane retains the biomass in the reactor, overcoming the granulation deficiency problems faced by the non-membrane configurations of anaerobic reactors and producing effluents free of particulates. AnMBR could provide a solution to the treatment of industrial wastewater. The treated water could be used for other processes in the industry, such as heat integration, sanitary or landscaping purposes (Mutamim et al., 2013).

2 Literature Review

2.1 Bitumen condensate

The bitumen (or asphalt) is one of the main resources in the roads and pavement industry. It is obtained as the product of crude oil fractional distillation (Boczkaj et al., 2017). The main product of this industry, asphalt concrete, is a composite material used to make the surfaces of roads, pavements, parking lots, airports, embankment dams, etc. It consists of sand and aggregates, bound together by bitumen. To have good bonding, the sand and stones are dried in a rotary drum dryer in which bitumen is heated to 105°C to achieve a mouldable consistency. After that, sand and stones are mixed with the bitumen. Due to the heating of bitumen, fumes containing water vapour and organic compounds of petroleum origin are produced. Polycyclic aromatic hydrocarbons (PAHs) are known to be among these organic compounds. PAHs are pollutants predominantly generated during the incomplete combustion of organic material (Boczkaj et al., 2017; Binet et al., 2002; de Méo et al., 1996). Many PAHs have toxic, mutagenic or/and carcinogenic properties (Abdel-Shafy & Mansour, 2016).

In humans, occupational exposure to PAHs may occur to workers breathing exhaust fumes in industries such as asphalt industry. Routes of exposure include ingestion, inhalation, and dermal contact. The effect of PAHs on human health depends on various factors such as the extent of exposure (length of time), the concentration of the compounds during the exposure, the toxicity of the PAHs, and the route of exposure (Kim et al., 2013). These compounds cause eye irritation and skin problems on short-term exposure, and clogging in blood vessels in people with coronary heart disease. Long-term exposure to certain PAHs (for example, pyrene) has been identified as the cause of cancer in animals under experimental conditions (Kim et al., 2013). Removing PAHs by water treatment is a priority because of their effect on health.

As mentioned before in section 1.2, the bitumen fumes in BAM Asphalt industry is condensed to recover the heat. The PAHs and other organic compounds will be present in the bitumen condensate. This wastewater requires treatment to remove these contaminants before disposing or reusing the water.

2.1.1 Other treatment methods of wastewater similar to bitumen condensate

Bitumen condensate is not a common wastewater to treat. The research gap is widely present and only a few case studies have been conducted with similar wastewaters. One such case study comes from the company Enviro Concepts, Australia. In 2015, they conducted and proposed a treatment procedure for asphalt wastewater treatment. The water was contaminated by asphalt and bitumen particles due to the cleaning of the asphalt and bitumen tracks, tanks, and equipment. The process flow scheme for the water treatment was – 1. Solids settling tank, 2. Oil-water separator, 3. Dissolved air flotation (coagulation and flocculation), 4. Deep-bed media filtration, 5. Disinfection. The water could be used again as a compliant feed supply for the pressure cleaning processes. These treatment steps required the addition of chemicals (coagulation) and contaminants were removed by chemical or physical reactions (filter bed). Although these processes can remove contaminants from the water, the contaminants were only removed and not degraded, implying that they were being disposed into landfills.

Another study was conducted using advanced oxidation processes (AOPs) (Boczkaj et al., 2017). AOPs are a set of processes that can remove organic materials in wastewater by oxidation through reactions with hydroxyl radicals (OH^-). The process utilises the oxidation treatment by O_3/UV and H_2O_2/UV with suitable UV sources and appropriate photochemical reactors. Bitumen is a material obtained from vacuum distillation; the gasses emitted during this process are scrubbed using caustic soda solution. The effluent from this is a basic solution containing a condensed oil phase, sulphide ions, and organic compounds dissolved in the water phase. These contaminants was removed by oxidation process by dosing oxidants (H_2O_2 and O_3). 43% removal of COD was achieved. A higher COD removal percentage was found hard to obtain due to the presence of hydrocarbons persistent to degradation. One of the reasons AOP is not a suitable option is the high operating and maintenance costs of energy and chemical reagents required (Genesis Water Technology, 2019)

The wastewater from used industrial oils (UIOs) recycling operations has a composition similar to that of bitumen condensate (petrochemical origin). A study was done to determine the anaerobic biodegradation of the UIOs wastewater using an expanded granular sludge bed reactor (EGSB) (Garcia-Mancha et al., 2012). Anaerobic degradation was feasible with an OLR lower than 5.5 g COD/L.d. at room temperature. An increase in OLR to 10 g COD/L.d. was biodegraded at a higher temperature (mesophilic temperature). This study concluded that anaerobic treatment was a feasible option for treating UIO wastewater. After long-term operation of the bioreactor, the biomass modified its microbial community to degrade the contaminants

Bitumen condensate, having a similar composition to the wastewater from UIOs, can also be degraded by anaerobic digestion. The chemical composition of the bitumen condensate and other characteristics need to be studied. This will provide an insight to the anaerobic biodegradation of these compounds.

2.2 Possible compounds in bitumen condensate and their biodegradation

As already discussed, the bitumen condensate contains a mixture of organic and aromatic compounds derived from its production process (Roy et al., 2007). Aromatic compounds are defined as organic molecules containing one or more aromatic rings, such as benzene. These compounds are relatively stable and less easy to biodegrade than other organic compounds (Boll et al., 2002). They also pose a threat to human health as they are possible carcinogens (Clark et al., 2011). Figure 3 presents a list of compounds that have been reported in bitumen fume condensate (Boczkaj et al., 2017; Binet et al., 2002; de Méo et al., 1996) and their molecular structure.

Those compounds cause eye irritation and skin problems on contact; furthermore, aromatic compounds have been known to mutate the human DNA. Phenanthrene, acenaphthene, anthracene, benz[a]anthracene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and pyrene are known to be carcinogenic compounds (PubChem, 2019).

1-Butanol	Acenaphthene	Benzo[k]fluoranthene	Cyclohexanone	Naphthalene
1-Decanethiol	Acenaphthylene	Benzofluoranthene [b+j+k]	Cyclohexanol	Phenol
1-Heptanol	Acetaldehyde	Benzo[a]pyrene	Dibenz[a,h]anthracene	Phenanthrene
1-Hexanol	Acetophenone	Benzo[b]fluoranthene	Dibenzothiophene	Pyrene
2-Mercaptoethanol	Anthracene	Chrysene	Fluoranthene	Thioanisole
2,6-Dimethylphenol	Benzyl alcohol	o-Cresol	Fluorene	Thiophenol
4-Ethylphenol	Benz[a]anthracene	m-Cresol	Furfural	
2-Pentanone	Benz[g,h,i]perylene	Coronene	Indeno[1,2,3-c,d] pyrene	

Figure 3: Reported compounds that can be present in bitumen fume condensate (images from PubChem)

Some aromatic compounds have been reported to be biodegraded under anaerobic conditions. The initial anaerobic conversion of aromatic compounds is done by cleavage of carbon-carbon from the ring, decarboxylation, oxidation or reduction of substituent groups (Boll et al., 2002). Most of these reactions lead to lower aromatic compounds, like benzoate. Further reduction of benzoate requires high energy which can be overcome by hydrolysis (Boll et al., 2002).

Aromatic rings with no functional groups face another problem. The initial introduction of oxygen into the aromatic hydrocarbons via hydration in anaerobic process is thermodynamically unfavourable (Cao et al., 2009). They have large resonance energy (0.0454 eV per pi-electron for benzene ring), which provides great stability to the compounds. However, these compounds can be activated through biological methods (Coates et al., 2002). Anaerobic bacteria usually insert a carboxyl group from CO_2 or succinic acid to the poly-chain aromatic hydrocarbons (Seo et al., 2009). Figure 3a and 3b show the examples of the catabolic pathways of naphthalene, methylnaphthalene, and tetralin by anaerobic bacteria.

The initial activation of the hydrocarbons is an important step for AD. There are four enzymatic reactions (Foght, 2008):

- addition of fumarate to yield aromatic-substituted succinates,
- methylation of unsubstituted aromatics,
- hydroxylation of an alkyl substituent via a dehydrogenase, and
- direct carboxylation.

Foght (2008) explained the various pathways of anaerobic degradation that common aromatic compounds will take.

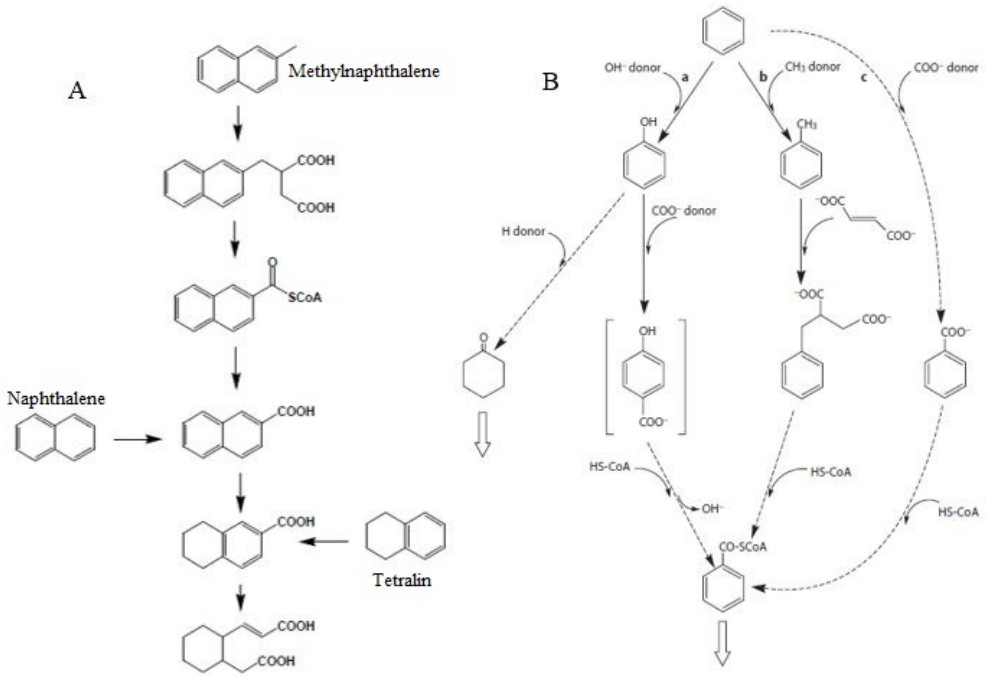

Figure 4: A: Naphthalene, methylnaphthalene, and tetralin are degraded by anaerobic bacteria by the addition of a carboxyl group from CO_2 or succinic acid (Seo et al., 2009). B: The three degradation pathways for benzene, a: hydroxylation to form phenol, cyclohexanone and benzoyl-COA, b: Alkylation to form toluene, followed by fumarate addition to form benzyl succinate and benzoyl-CoA, c: Carboxylation to form benzoate and benzoyl-CoA (Foght, 2008)

2.3 General concepts of AD

The AD is the degradation of process organic compounds by bacteria in the absence of free oxygen (Bowie et al., 2013). The process produces biogas, a mixture of mainly CH_4 and CO_2. AD can be found in common examples; it takes places in the stomachs of ruminants, municipal landfills and municipal sewers. AD takes place in four steps of series and parallel reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

During hydrolysis, enzymes break down proteins into amino acids, polysaccharides to simple sugars, and lipids to long-chain fatty acids (LCFA). This process is usually rate-limiting for the overall AD due to the low availability of the free surface area on the compounds. This implies that, for wastewaters with complex compounds, hydrolysis determines the overall process rate and thereby the reactor design.

Acidogenesis is a faster conversion step in AD. It is performed by a group of microorganisms called acidogenic bacteria. In the acidogenesis, the products from hydrolysis diffuse into the cell of the bacteria and are anaerobically oxidized. The main products of acidogenesis are alcohols, lactic acid, and volatile fatty acids (VFAs). (Henze et al., 2015).

Acetogenesis is the process, in which acetogenic bacteria transform the VFAs into acetate and hydrogen, while homoacetogenic bacteria produce acetic acid from hydrogen and CO_2. This step could limit thermodynamically the AD unless the hydrogen partial pressure is below 10^{-3} atm (Khanal, 2009). This is maintained by the presence of hydrogen-consuming organisms, such as the

hydrogenotrophic methanogens, which form a syntrophic association with the hydrogen-producing microorganisms. The balance between these two populations could be disturbed by a sudden drop in pH, or by an overload by toxic compounds affecting the methanogenic microorganisms. An imbalance in the production and consumption of hydrogen will lead to an increase in its concentration since the hydrogen-consumption will be affected. As a result of this, the VFAs concentration will increase inhibiting the methanogens even more in a process called reactor souring (Tchobanoglous et al., 2014).

The methanogenesis is the final step that converts the VFAs produced in the later stages into CH_4 and CO_2. The conversion is performed by methanogenic archaea. There are two main methanogenic pathways, the acetoclastic (Equation 1) and the hydrogenotrophic (Equation 2) pathways. Acetoclastic methanogens reduce the methyl group of the acetate to CH_4, while the carboxylic group is oxidized to CO_2.

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{1}$$

CH_4 produced by the acetoclastic methanogens accounts for 70% of the total CH_4 produced (Henze et al., 2015). On the other hand, hydrogenotrophic methanogens produce CH_4 from the reduction of CO_2 by hydrogen.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{2}$$

AD are summarized in Figure 5.

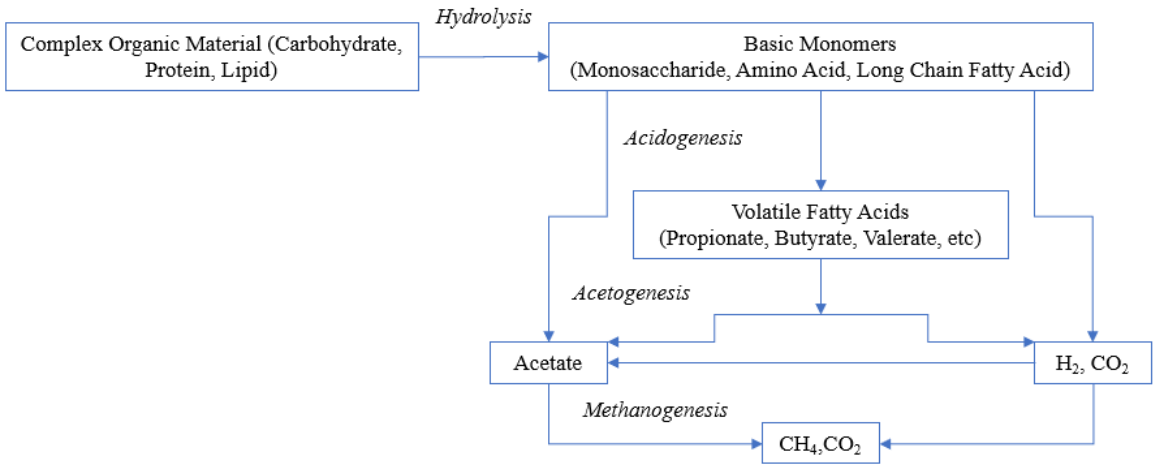

Figure 5: The AD processes. The various steps involved along with the different compounds required are depicted.

2.4 AD for industrial wastewater

With the advancement in the anaerobic wastewater treatment technology, several anaerobic reactors have been developed for the treatment of industrial wastewater. Although, one of the limitations when treating industrial wastewater is the low growth rate results in longer start-up and recovery period, along with higher sensitivity towards process changes (Khanal, 2009). Hence, the system requires close attention and continuous monitoring of the process parameters.

For the anaerobic treatment of industrial wastewater, sludge bed technology is commonly used; however, different reactor configurations can be used for the treatment of these waters (McCarty, 2001). Various configurations have been developed in the past the four decades, such as the anaerobic contact process (ACP), anaerobic filter (AF), upflow anaerobic sludge blanker (UASB) reactor, expanded granular sludge bed (EGSB) reactor, internal circulation (IC) reactor, anaerobic baffled reactor (ABR), and membrane coupled reactors. These reactor configurations usually make use of granular sludge as biomass (van Lier et al., 2015). The advantages of these reactors are their compactness, ease of operation at high loading rates and low hydraulic retention time (HRT).

2.4.1 Anaerobic membrane bioreactor (AnMBR)

The anaerobic membrane bioreactor (AnMBR) is an anaerobic system that couples anaerobic biological degradation with a membrane module for filtration of the solids from the effluent of the reactor (Wang et al., 2013). By replacing the sedimentation stage in the conventional biological process with the membrane filtration, it improves the solids retention within the digester. The membrane module can either be submerged in the reactor or connected to the reactor as a separate unit (Hillis, 2007). As a result of high biomass retention, high-quality effluents can be obtained due to low solids concentration in the permeate (Van Lier et al., 2001). AnMBR technology has the potential to maintain a slow-growing microbial population to biodegrade specific pollutants (Dereli et al., 2012). One disadvantage it faces is the limitation of its application due to higher capital costs and operational costs (Hillis, 2007). It is cost-effective when used to treat high contaminant load wastewater. Other drawbacks include membrane fouling, and high operation and capital costs (Chernicharo et al., 2015).

AnMBRs have two main configurations: submerged membrane reactor and external crossflow membrane reactor (Chang, 2014). Among these, the external cross-flow membrane reactor can be hydrodynamically controlled with more ease. The membrane modules in an external membrane bioreactor can be replaced with ease, compared to the one in a submerged membrane reactor. Due to this, it is easier to remedy the membrane fouling in an external membrane reactor. However, the energy consumed by recirculation to the membrane and back to the reactor is higher(Lin et al., 2013).

2.4.2 Membrane filtration

Membrane filtration is the physical separation of compounds in a solution using a semipermeable (permeable to the solvent) membrane. In AnMBR, the particles that are retained by filtration are sent back to the reactor as the concentrate flow, while the permeable particles are extracted along with water as the permeate (Crittenden et al., 2012). Membrane filtration can be categorised based on the driving force required for separation (pressure, temperature, or concentration), based on the pore size of the membrane (microfiltration, ultrafiltration, nanofiltration, and reverse osmosis), and based on the membrane configuration (flat sheets, hollow fibres, spiral, and flat sheets). Ultrafiltration (UF) is the generally used membrane filtration in biological treatment because UF membranes can retain protozoa, viruses, bacteria and large organic molecules (UF retains particles greater than 0.01 μm) (Judd & Judd, 2011)

Some important parameters that govern the operation of a membrane module: 1. Membrane

operation 2. Flux 3. Transmembrane Pressure (TMP) and 4. Fouling.

There are two types of membrane operations, cross-flow filtration and the dead-end filtration. If there is no retentate stream, then the operation is dead-end operation. If the filtration has a retentate stream flowing from the module outlet containing the particles and a permeate stream, the operation is called cross-flow. In cross-flow operation, only a fraction of the stream entering the module forms the retentate stream (Judd & Judd, 2011). In the dead-end filtration process, resistance due to accumulation of solids increases proportionally to the total volume of filtrate passed through the membrane. It requires periodic cleaning. In a cross-flow operation, this accumulation, also known as a cake layer, continues until the adhesive forces between the cake layer and membrane are balanced out by the scouring forces of the stream (Judd & Judd, 2011).

Flux is the flow rate of the fluid per unit area and is defined by Equation 3 (Hillis, 2007).

$$J = \frac{Q_P}{A_m} = \frac{TMP}{\mu_w \times R_m} \tag{3}$$

Where
J is Flux, [L/(m^{2}s)]
Q_P is Permeate flowrate, [L/s]
A_m is Membrane area, [m^2]
TMP is Pressure drop across the membrane, [Pa, kg/(ms^2)]
μ_w is Dynamic viscosity of water, [kg/(m.s)]
R_m is Hydraulic resistance of the membrane, [1/m]

Transmembrane pressure (TMP) is the pressure difference that enables the water to flow through the membrane. TMP is calculated as the difference in pressures from the feed-water side and the permeate side; it is given by the following equation (Judd & Judd, 2011):

$$TMP = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate} \tag{4}$$

Where
J is Flux, [L/(m^{2}h)]
P_{feed} is Pressure of the feed flow to the membrane [bar, kg/(ms^2)]
$P_{retentate}$ is Pressure of the retentate flow from the membrane [bar, kg/(ms^2)]
$P_{permeate}$ is Pressure of the permeate flow from the membrane [bar, kg/(ms^2)]

Fouling refers to the reduction in the water flow through the membrane due to the layer formed on the surface of the membrane causing plugging of the membrane pores. Fouling leads to a decline in the permeate flow and an increase in the membrane resistance to the flow (Judd & Judd, 2011). The resistance can be calculated from the flux equation by rearranging Equation 3:

$$R = \frac{TMP}{\mu \times J} \tag{5}$$

There are three types of fouling: 1. Reversible fouling which can be removed by physical cleanings, like backwashing or membrane relaxation, 2. Irreversible fouling which cannot be removed by physical cleaning but by chemical cleaning, 3. Irrecoverable fouling which cannot be removed

(Judd & Judd, 2011).

Fouling can be managed with periodical cleaning of the membrane by removing the accumulated solids. During the backwash, the filtration direction will be reversed using the permeate stream to remove the fouling compounds from the pores and surface (van Dijk, 2009). Physical cleaning methods, such as backwash and membrane relaxation, can be used. Backwashing is reversing the flow of water while membrane relaxation is ceasing the permeation while continuing to scour the membrane with air bubbles. On the other hand, chemical cleaning is done by washing the membrane with mineral or organic acids, caustic soda, or sodium hypochlorite. The membrane can be cleaned either in-situ or after removing it from the reactor (Judd & Judd, 2011). The concentration of the chemicals and the frequency of the cleaning can be adjusted based on the influent quality, membrane material, and operating conditions of the reactor.

2.5 Methods applied in this research for bitumen condensate analysis

2.5.1 GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is the most common analytical technique used for the identification and quantification of organic substances present in complex matrices. Gas chromatography and mass spectrometry can be used as two separate analytical techniques; gas chromatography separates the components of a mixture in time, mass spectrometry provides information that aids in the structural identification of each component (Sparkman et al., 2011).

2.5.2 ICPMS analysis

ICPMS combines the inductively coupled plasma (ICP) and mass spectrometry. The ICP is an ionization source that splits the sample into its constituent elements. The elements are transformed into ions. These ions are detected with mass spectrometry. It has good precision, low detection limits, multi-elemental capability and ability to do rapid isotopic analysis (Beauchemin, 2014).

2.5.3 Specific methanogenic activity, inhibition and toxicity

The specific methanogenic activity (SMA) test determines the methane-producing capability of the biomass. Biomass concentration should remain constant during the test. Reduction in the methane-producing activity by methanogens due to certain agents is attributed to inhibition. Inhibition could be caused by either physical or chemical agents, such as temperature, pH, the concentration of the medium, etc (Orozco, 2008). Toxicity is manifested as a decrease in growth rate and as the death of the cell. Depending on the concentration of the toxic compound and the history of the cell exposure to toxic compounds, the microorganisms can continue living, after adapting to the environment or die. The CH_4 producing capacity can be inhibited by either a chemical or physical agent (Orozco, 2008). Gad (2014) defined the 50% lethal concentration (LC_{50}) as the statistically calculated concentration of a material that would be expected to cause death to half of the sample of the targeted organisms.

3 Objective and research questions

The objective of this research was the assessment of the biodegradability of bitumen fume condensate under anaerobic conditions. The research was executed under the hypothesis that by the usage of an AnMBR, it is possible to develop an anaerobic microbial population capable of biodegrading the petroleum-based bitumen fume condensate. The main research question was:

What is the biodegradability of bitumen condensate in an anaerobic membrane bioreactor (AnMBR) under mesophilic conditions?

There is a wide gap in the literature regarding the bitumen condensate wastewater, its characteristics and treatment. From literature, the possible compounds in the bitumen condensate were determined. Further information about the treatment methods for this wastewater was unavailable. To answer the research question, other relevant information was required. This was obtained by answering the following secondary research questions:

- What are the main organic compounds present in the bitumen condensate?
- What is the inhibition percentage due to the bitumen condensate on the acetoclastic methanogenesis in different biomass sources?
- What is the toxicity on the anaerobic microorganisms due to the bitumen condensate in different biomass sources?
- What is the maximum organic conversion rate of bitumen condensate in a mesophilic AnMBR?

4 Materials and methods

4.1 Initial characterisation tests

4.1.1 Chemical oxygen demand and total organic carbon

The chemical oxygen demand (COD) is the concentration of oxygen required for the decomposition and total organic compounds (TOC) is the concentration of carbon found in a compounds. COD and TOC concentrations were measured with the kits from Hach Lange. For TOC, the bitumen condensate was filtered through 0.45 µm TOC filter (SpartmanTM 30, GE Healthcare, U.K) and measured with TOC cuvettes (Lange Hach Phenol Cuvette Test LCK 386) by a spectrophotometer (Lange Hach DR3900). For COD, it was measured with a COD cuvette (Lange Hach COD Cuvette Test LCK 314 and 514) by the spectrophotometer.

4.1.2 Volatile fatty acids

To analyse the volatile fatty acids (VFAs) sample taken from the bitumen condensate was filtered through 0.45 μm filter. 750 μL of this filtered sample was diluted with 750 μL pentanol (320 mg/L) at 1:2 dilution ratio in a glass vial. 10 μL of formic acid (98%) was added to the sample for acidification. The gas chromatograph (Agilenttech 7890A) used for the analysis has a capillary HP-FFAP column size of 25 m x 0.32 mm x 0.50 μm (Agilent 19091F-112) and flame ionization detector (FID). The carrier gas used was helium at 0.7 bar and 2.45 mL/min flowrate. The temperature for the detector was 240°C and the temperature for the injector was 225°C.

4.1.3 GC-MS analysis for organic compound determination

To get an insight into the organic chemical compounds present in the bitumen condensate, GC-MS analysis were done. Samples were analysed by an external laboratory (Het Waterlaboratorium, Haarlem, The Netherlands) using a Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system (Thermo Scientific, Massachusetts, USA), method and column. It was possible to choose a list of compounds to be analysed against. The list chosen had compounds reported as components of the bitumen condensate. Samples were prepared by filtering bitumen condensate through a 0.45 µm TOC filer (SpartmanTM 30, GE Healthcare, U.K), and 10 mL were collected and put in 10 mL crystal flasks (provided by Het Waterlaboratorium).

4.1.4 GC-QTOF for organic compound determination

0.45 μm filtered bitumen condensate was also sent to the Snyder Research Group at the University of Arizona. An Agilent 7200 Accurate Mass GC-QTOF (GC-quadruple time of flight) was used with a full scan analysis in electronic impact (EI) and positive chemical ionization (PCI). The column used was a DB-5 30 m x 0.25 mm x 0.25 μm capillary column with a column flow of 1.2 mL/min. The initial oven temperature is 40°C for 5 minutes. The temperature was then increased at a rate of 10°C/min to 300°C and held for 5 minutes. To identify the unknown compounds, Mass Hunter Unknown Analysis version B.08.00 was used. The mass spectral similarity search was performed using NIST MS Search 2.0 (NIST/EPA/NIH Mass Spectral Library, NIST 08, National

Institute of Standards and Technology, 2008 Gaithersburg, MD).

4.1.5 ICPMS for metallic ions determination

Bitumen condensate was analysed using ICPMS by model PlasmaQuantMS from Analytik Jena (Jena, Germany). 10 mL samples were prepared using nitric acid (HNO_3) and then filtered through a 0.45 μm polyethersulphone syringe filter. The final concentration of HNO_3 was $\approx$ 1% (v/v). Proper dilution was done to have the analytes in a concentration lower than 5 mg/L. In the analysis method, an argon flow of 9.0 L/min was used for the plasma and auxiliary flow of 1.4 L/min. The nebulizer flow was 1.10 L/min. The samples were then analysed using ICPMS. Samples were introduced into the ICP, at a temperature higher than 5700°C. The high temperature was focused on an area of 5 cm^3. The samples were introduced in the form of aerosol into the plasma. The ionized sample was extracted from plasma into the quadrupole mass spectrometer using a ReflexION (ion mirror to focus the ions).

4.2 Volatilisation test of bitumen condensate

The volatilisation of bitumen condensate was tested. Nine 250 mL Schott Duran glasses were used, with 200 mL of bitumen condensate in each. The batch test condition was replicated at a temperature of 35°C. The tests were carried out in a temperature-controlled shaker (New Brunswick Scientific, Innova 44) at 130 RPM to ensure mixing of the solution. With a sample size n = 9, the variation of COD concentration over 10 days was observed.

4.3 Batch test for determining the inhibition due to bitumen condensate

The batch test to determine the inhibition and toxicity was tested with three different biomasses:

- Municipal sludge digestate (sludge from an anaerobic reactor at Harnaschpolder),
- Phenol-degrading adapted sludge (BioXtreme sludge),
- Petroleum wastewater sludge (sludge from USAB reactor at Shell, Moerdijk).

The bottles used for the batch experiment were 250 mL Schott Duran glasses, and the working inoculum + substrate volume was 200 mL. A series of different bitumen COD concentrations were dosed to the different biomasses and changes in the SMA and cell viability were measured. 3 mL of macronutrients/g COD and 0.3 mL of micronutrients/g COD were provided for the growth of the biomass. Phosphate buffer solution was added to maintain pH levels of 6.8 - 7.4 (Hendriks et al., 2018). Inoculum to substrate (I/S) ratio used for the batch experiment was 2, based on volatile suspended solids (VSS). Each experiment was carried out in triplicates. The temperature was kept constant at 35°C. Batch tests were carried out in a temperature-controlled shaker (New Brunswick Scientific, Innova 44) at 130 RPM to ensure mixing of the solution. The COD of the solution in each bottle was measured at the beginning and end of the experiment. CH_4 production rate was measured online using the liquid displacement method by AMPTS system (Bioprocess Control, Sweden). The output was the plot of the CH_4 production (in NmL) against time.

The IC_{50} of the bitumen condensate was calculated as the bitumen condensate concentration corresponding to the 50% decrease of the maximum rate of methane production. IC_{50}, or the half maximal inhibitory concentration, trend usually follows a sigmoidal decrease with increase in the inhibitory compounds (Odriozola et al, 2019), in this case, the bitumen condensate concentration. This was evaluated using SigmaPlot using the 3-parameter logistic model. The equation used was

$$y = a \times \frac{1}{1 + (\frac{x}{x_0})^b} \tag{6}$$

Where, y = SMA value (mg COD/gVSS.d)

a = maximum SMA value (mg COD/g VSS.d)

x = bitumen condensate concentration (mg COD/L)

x_0 = IC_{50} value (mg COD/L)

b = hill slope

4.4 Flow cytometry for toxicity determination

Flow cytometry was used to obtain information about cell viability. The flow cytometer used was a BD AccuriTM C6 (BD AccuriTM cytometers, Belgium). Samples from the batch tests were collected at the beginning and the end for the batch experiment. The samples were then prepared by diluting it in a 0.22 μm-filtered phosphate buffer solution at 1:500 dilution. The phosphate solution was prepared using ultra-pure water. After the dilution, it was sonicated by a sonifer (Branson Digital Sonifer) at 100W for 3 cycles of 45 seconds. It was diluted again with the filtered phosphate buffer solution at 1:500 dilution factor. The sample was then filtered through 10 μm membrane filter into a 2 mL Eppendorf. The samples were then stained with SYBR Green and SYBR Green + Propidium Iodide. SYBR Green was used to stain all the cells present, thereby giving the total cell count (5 μL of the stain was added to 495 μL of the sample). SYBR Green + Propidium Iodide was used to stain only the dead cells (cells with broken cell membrane), thereby giving the dead cells count (5 μL of this stain was added to 495 μL of the sample). The samples were incubated at 37°C for 10 minutes. The flow cytometer was cleaned using 1% chlorine solution, soap, 70% ethanol and fresh ultra-pure water. The file was then extracted from the computer and the data were processed in FlowJoTM software.

4.5 AnMBR continuous experiment

4.5.1 Experimental set-Up

The volume of the AnMBR set-up was 6 L and working was volume 5 L. The temperature of the AnMBR set-up was set at 35oC with a water bath (Tamson TC16, Netherlands). The feed water was pumped into the reactor with a peristaltic pump (Watson-Marlow 120U, UK) and mixed with the biomass inside. From the reactor, the solution was pumped to the membrane by an internal recirculation pump (Watson-Marlow 540Du, UK). The membrane used for the reactor was a tubular PVDF (polyvinylidene difluoride) ultrafiltration (UF) membrane with a nominal pore size of 30 nm (Pentair, Netherlands). It had a diameter of 5.2 mm and length 640 mm. The surface

area was 0.0104 m^2 and the cross-sectional area was 2.12×10^{-05} m^2.

Permeate was pumped out from the membrane module (Watson-Marlow 120U, UK) and collected in a container. The concentrate from the membrane, which contains the biomass, was sent back to the reactor. The initial flow rate of the influent and the corresponding effluent flow rate was 2 L/d. The corresponding hydraulic retention time (HRT) was 2.5 days and organic loading rate was 0.2 g COD/ gVSS.d. The reactor was operated for 120 days, with a SRT 4124 days.

The biogas produced was measured by a gas meter (Ritter, MGC-10PMMA_R). Biogas was recirculated through the system to allow a better mixing of the biomass with the wastewater. The permeate pump was controlled by the volume control system of the reactor with two pressure sensors; a headspace sensor (AE Sensors ATM [0-20 mbar], Netherlands) for the gas pressure and a total pressure (headspace + hydrostatic) sensor (AE Sensors ATM [0-70 mbar], Netherlands. The TMP was governed by the inlet, outlet and permeate pressure of the membrane. Therefore, these pressures were measured by three pressure sensors (AE Sensors ATM [-800 to 600 mbar], Netherlands).

The reactor was monitored through LabView software (National Instruments, USA). This software assisted in maintaining the parameters of pressure, temperature and flow rates of the pumps. The reactor set-up is depicted in Figure 6.

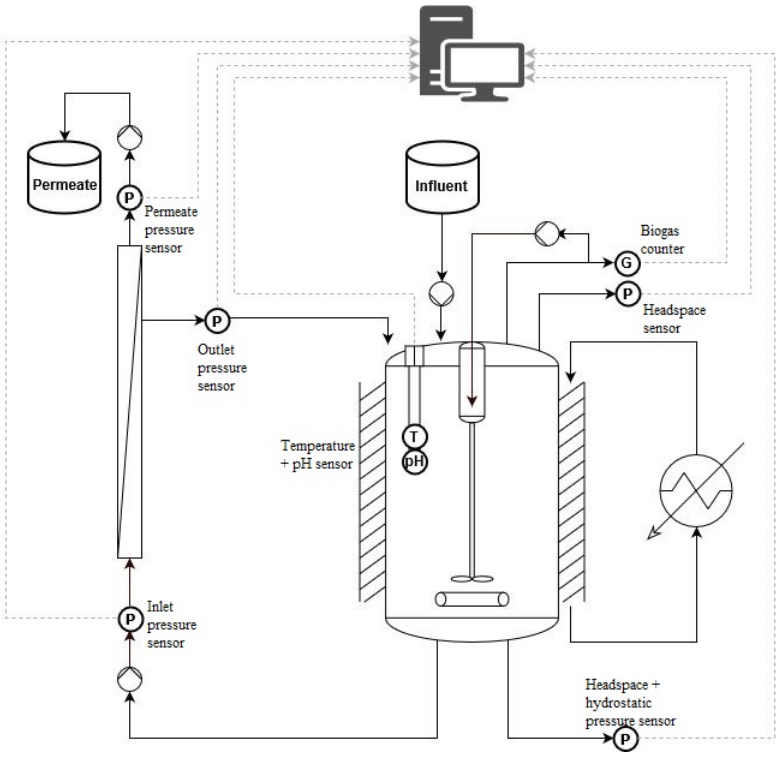

Figure 6: Scheme of the AnMBR. The set-up was connected to a computer to monitor with sensors and pumps. The pressure sensor at different points assist in determining the volume of the reactor and the TMP. The temperature and pH sensor provide information about the temperate and pH inside the reactor

The feed solution was prepared with the bitumen condensate and additional solutions required for biomass growth and maintenance. The macro-nutrients solution consisted of salts of calcium,

ammonium and magnesium (Hendriks et al., 2018). The feeding solution was summarised in table 1.

Solutions	Unit	Value
Macronutrients solution	mL/gCOD	1.53
Micronutrients solution	mL/gCOD	0.76
Solution A	mL/L	30.5
Solution B	mL/L	19.5
Yeast Extract	mgCOD/L	0.1
Acetate	gCOD/L	0.5
Phenol	gCOD/L	1.2
Bitumen condensate	gCOD/L	1.2

Table 1: Solutions and compounds required to prepare the feed solution for the reactor

The biomass (seed sludge) came from UASB reactor from a wastewater plant of Shell, used for treating petrochemical wastewater. The biomass was added at a concentration of 6 g VSS/L (initial concentration).

4.5.2 Membrane preparation and critical flux determination

The membrane was cleaned and de-coated before starting the experiment by soaking the membrane in critic acid (5 g/L) for a day and washing it off with demineralised water.

Critical flux of the membrane is the permeate flux above which fouling that appears is irreversible. The critical flux determination was done by a modified protocol (Le Clech et al., 2003). The time interval was 15 minutes and the step height of the flux was 4 L/(m^2.h). The critical flux was determined when a significant increase in the TMP (Δ P_0 = TMP_n - TMP_{n-1}) occurred.

4.5.3 Analysis methods for the biomass, biogas, effluent, and influent

The VSS concentration in the reactor was measured in triplicates according to the standard methods specified by APHA (1999).

The biogas was analysed by gas chromatography (GC, Agilenttech 7890 A), with an HP-PLOT Moleseive GC column (Agilent 19095P-MS6) of 60 m x 0.53 mm x 200 μm and a thermal conductivity detector (TCD). The carrier gas used was helium at 14.8 psi and 23 mL/min flow rate. The operation temperature was 200°C. 10 mL of the gas was drawn out into a syringe at the sampling point in gas line of reactor. This was then injected into the GC and analysed. The phenol and COD concentrations of the effluent and influent were measured with the help of the kits from Lange Hach. For phenol, the permeate was filtered through 0.45 μm and measured with phenol cuvettes (Lange Hach Phenol Cuvette Test LCK 346) by a spectrophotometer (Lange Hach DR3900). For COD, it was measured with a COD cuvette (Lange Hach COD Cuvette Test LCK 314 and 514) by the spectrophotometer.

VFAs in the permeate were analysed using the same method as in section 4.1. From this analysis, we can determine the total COD concentration of the VFAs. Knowing the total COD concentration of the effluent, the difference between the COD concentration of the effluent and the

COD concentration of the VFAs would be the COD concentration of the bitumen condensate.

$$COD_{bitumencondensate} = COD_{effluent} - COD_{acetate} - COD_{phenol} \tag{7}$$

5 Results and discussion

5.1 Initial characterisation test

5.1.1 COD concentration, TOC concentration and VFAs

Initial characteristics of the bitumen condensate were determined before starting the batch tests. These include COD, TOC concentration and VFA concentration. Due to different condensate collection methods, the COD concentration varied between 600 mg COD/L and 1000 mg COD/L and the TOC concentration varied between 200 mg/L and 300 mg/L. 1000 mg COD/L is considered as a 'medium' strength wastewater, but since bitumen condensate is petrochemical wastewater, 1000 mg COD/L of bitumen condensate is a 'high' strength wastewater (Mutamim et al., 2013). Through GC analysis of a 500 mg COD/L of bitumen condensate, it was found that there was 13.4 mg/L of acetic acid present and 8.3 mg/L of phenol.

For AD processes in general, the optimum pH range is between 6.8 to 7.2. The pH of the bitumen condensate was tested and found to be 2.81. To neutralise 50 mL of the bitumen condensate, 0.17 mmoles of sodium hydroxide solution were used. Therefore, it can be concluded that the bitumen condensate did not have any buffering capacity. The phosphate buffer solutions used in the biochemical tests helped to maintain the pH neutral and it further avoided the need to add a base solution to neutralize the condensate.

5.1.2 GC-MS analysis for organic compounds

The analysis from Het Waterlaboratorium showed a mixture of 70 compounds. The compounds with highest concentrations were naphthalene (0.7 μg/L), acenaphthylene (0.27 μg/L) and phenanthrene (0.04 μg/L). Since this analysis was target-specific, all the compounds present in the bitumen condensate were not detected. This was proved as the COD concentration of the compounds in the list chosen (0.004 mg COD/L) is less than the COD concentration of the bitumen condensate (1126 mg COD/L) provided for the analysis. Appendix B.2 shows the compounds that were identified and their concentration. The concentrations are very low, in μg/L.

5.1.3 GC-QTOF non-targeted analysis

The GC-MS analysis conducted at the University of Arizona was a non-target analysis. The data processed from GC-QTOF was analysed by the Snyder Research Group. Several phenolic compounds such as p-cresol, o-cresol and 2-naphtalenemethanol were detected. Structures of these compounds were proposed based on their accurate mass obtained from matching NIST data library. A full scan showed 933 compounds detected but only 430 matches the data base from NIST. Appendix B.1 shows the tentative compounds that correspond an 80% of a match with those founds in the library.

The compounds identified were reported in the literature studied showing that they are toxic and carcinogenic, and cause skin and eye irritation on contact (Boczkaj et al., 2017; Binet et al., 2002; de Méo et al., 1996). From literature, it is known these compounds can be anaerobically degraded as shown from figure 4. Due to the presence of various phenolic compounds in the bitumen condensate, the inhibition and toxicity of these compounds have been studied from literature. The IC_{50} for phenol for a non-adapted phenol sludge was 1.1 g COD/L and for an adapted sludge, it was 3.3 g COD/L. For cresol, the IC_{50} for a non-adapted sludge is 0.6 g COD/L and for an adapted sludge is 1.0 g COD/L (Olguin-Lora et al., 2003).

5.1.4 ICP-MS for metallic ion determination

Precipitation occurs in the feed solution for the AnMBR when the phosphate buffer solution is mixed with bitumen condensate. A non-target analysis was conducted to determine metals in bitumen condensate. It was determined that the precipitation was caused by the phosphate anions from the buffer solution. The precipitate was filtered through from the feed solution with glass-fibre filters of 0.7 μm before being used in AnMBR. The metal present in bitumen condensate precipitated as insoluble phosphate salts. The data from ICP-MS showed a high concentration of calcium (26 mg/L) and iron (190 mg/L). Other metals that were present boron (1.1 mg/L), magnesium (1.2 mg/L), sodium (0.8 mg/L) and silicon (1.2 mg/L). The concentrations of the metals are depicted in Figure 7. Bitumen condensate, being produced from the condensation of fumes, in principle, should not contain metals. The presence of the different metals could be from the corrosion in the chimney due to the acidic nature of the condensate. To avoid this, a different procedure for collection of bitumen condensate is required.

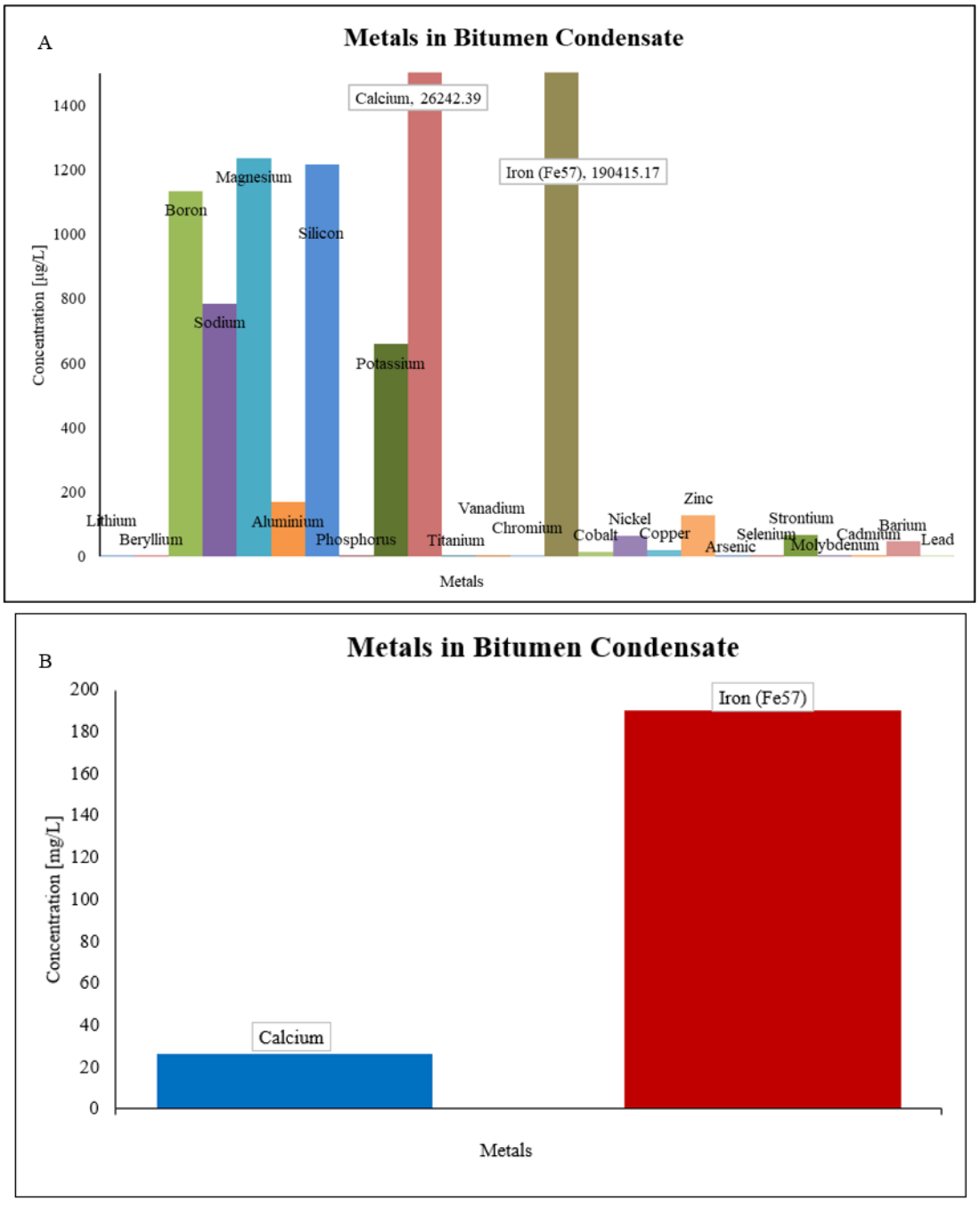

Figure 7: A: A comparison of the concentrations of the metals found in the bitumen condensate. B: Metals present in the bitumen condensate and its concentration The concentrated metals were Calcium and Iron (Fe57), this figure shows these metals with their concentrations

5.2 Volatilisation of bitumen condensate

Studies have determined that it is possible for PAHs and fatty acids can be volatilised during AD (Van Metre et al., 2012). During the AnMBR operation and biochemical tests, the compounds in

the bitumen condensate could volatilised. Day 1 is the first day of the experiment and day 11 is the last, day 7 is an intermediate day. The columns signify average of the COD concentrations of the nine bottles. No significant change in the COD concentration was observed over the 10 days as the error bars (representing 95% confidence interval) overlapped. Based on this, it can be concluded that no volatilisation of compounds took place. (Figure 8).

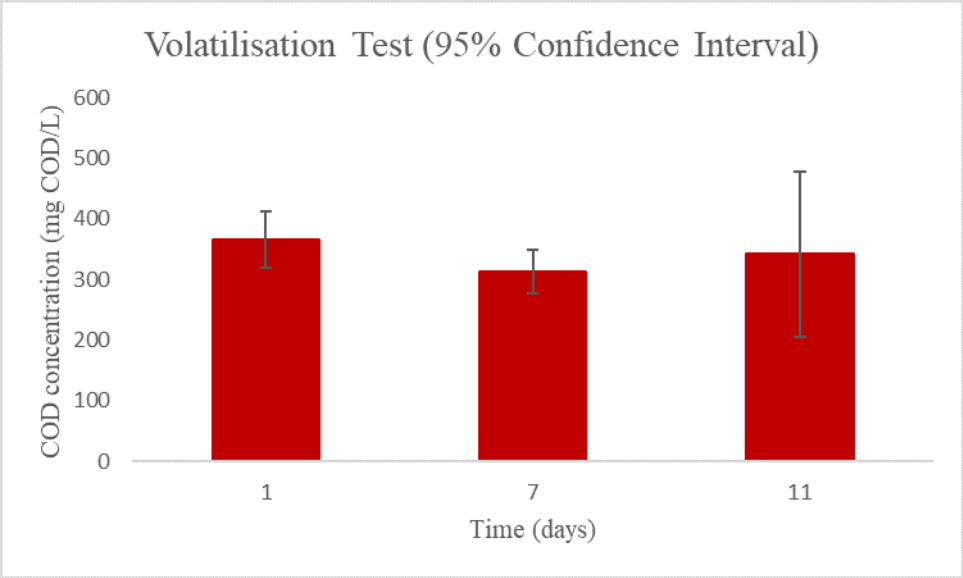

Figure 8: The variation of the average COD concentration over 10 days was studied and was seen there is not significant difference. Sample size for each condition, n = 9. The error bars represent the 95% confidence interval

5.3 Analytical results

5.4 Biochemical analysis

5.4.1 Specific methanogenic activity (SMA) and inhibition

The objective of the batch experiments was to determine if any inhibition was caused due to the presence of bitumen condensate on the CH_4 production. The CH_4 production rate and the inhibition of the condensate were studied. The batch tests were done with three different inoculums. The CH_4 production was evaluated from the three test sets.

The first sludge used was the municipal sludge digestate. Figure 9 shows the specific CH_4 production rate with the municipal sludge. The control (acetate [2 g COD/L]) produced 130 mL of CH_4 and the SMA was determined to be 0.08 g COD-CH_4/g VSS.d.

Jawed and Tare (1999) conducted an SMA test at 35°C using anaerobic biomass obtained from a digester and maintained for three months on a mixture of acetate (2–2.5 g COD/L) and propionate. The SMA value obtained was between 0.64 and 0.89 g COD-CH_4/g VSS.d (Jawed & Tare, 1999). This suggests a difference in the microbial population between the two biomasses (Hussain & Kant, 2017) and that the municipal sludge is of 'poorer methanogenic' quality than the biomass used by Jawed and Tare (1999).

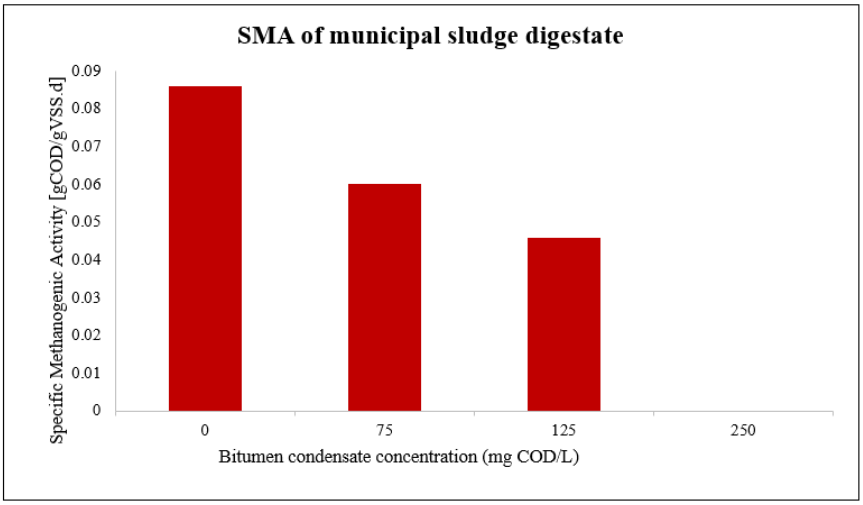

Figure 9: The average SMA values by municipal sludge digestate on varying concentrations of bitumen condensate. Each bitumen condensate concentration was tested in triplicates (n= 3)

Figure 10 shows the accumulated CH_4 produced in the different bitumen condensate concentration over time.

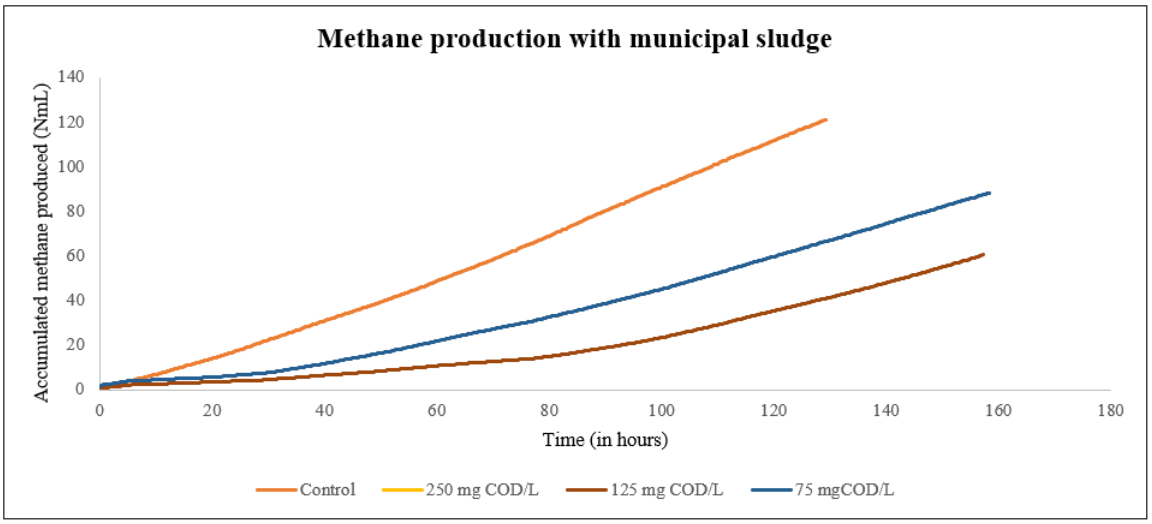

Figure 10: The accumulated CH_4 produced over time with municipal sludge with varying concentrations of bitumen condensate. The conditions were executed in triplicates and the most representative methane production lines are depicted in the figure.

For the inhibition tests, three different bitumen COD concentrations were used to assess the effect on the SMA. The SMA for 75 mg COD/L of bitumen condensate was 0.06 g COD-CH_4/g VSS.d. is lower than the SMA of the control. This trend continued with the higher bitumen condensate concentrations. The SMA for 125 mg COD/L bitumen condensate was 0.05 g COD-CH_4/ g VSS.d. 250 mg COD/L of bitumen condensate resulted in a total inhibition. Municipal

sludge is adapted to degrade sewage and solid waste containing nitrogen and phosphate compounds (Sosnowski et al., 2003). The compounds are 'simpler' COD than the COD concentration of bitumen condensate. Hence, the presence of the compounds from bitumen condensate was inhibiting the acetoclastic methanogenic activity completely at a relative low COD concentration (250 mg COD/L) (Mutamim et al., 2013).The IC_{50} of the bitumen condensate on the municipal sludge digestate was estimated by model fitting using equation 7 (figure 11) with a value of 119 mg COD/L.

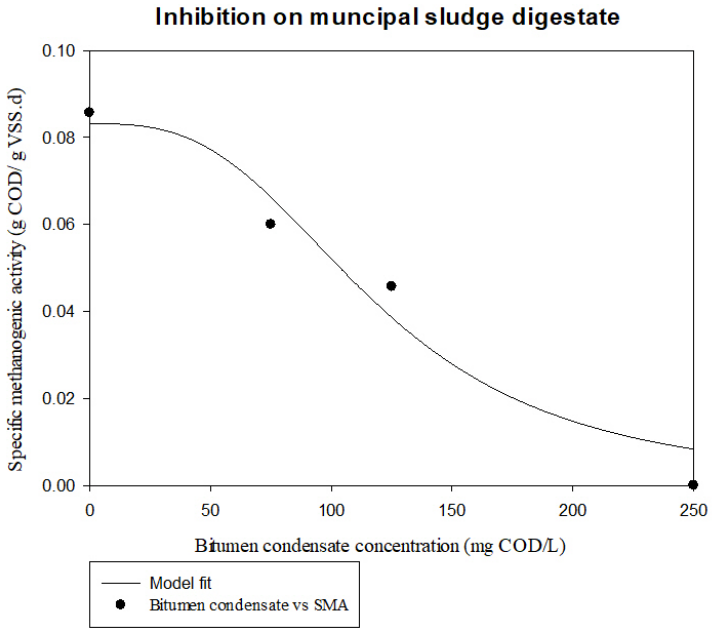

Figure 11: Inhibition in municipal sludge due to bitumen condensate is determined by plotting the average SMA values (sample size, n = 3) with the sigmoidal function

The second SMA inhibition test was run with sludge treating petrochemical wastewater (Shell, Moerdijk). Bitumen condensate used was between the range of 75 mg COD/L to 1000 mg COD/L. The SMA value of the control was 0.8 g COD-CH_4/g VSS.d. The tests with bitumen condensate concentration of 75 mg COD/L had an SMA of 0.7 g COD-CH_4/g VSS.d. The SMA value of 125 mg COD/L bitumen condensate was 0.6 g COD-CH_4/g VSS.d and for 250 mg COD/L bitumen condensate was 0.3 g COD-CH_4/g VSS.d. The SMAs for the higher bitumen condensate concentrations are lower and are depicted in Figure 12.

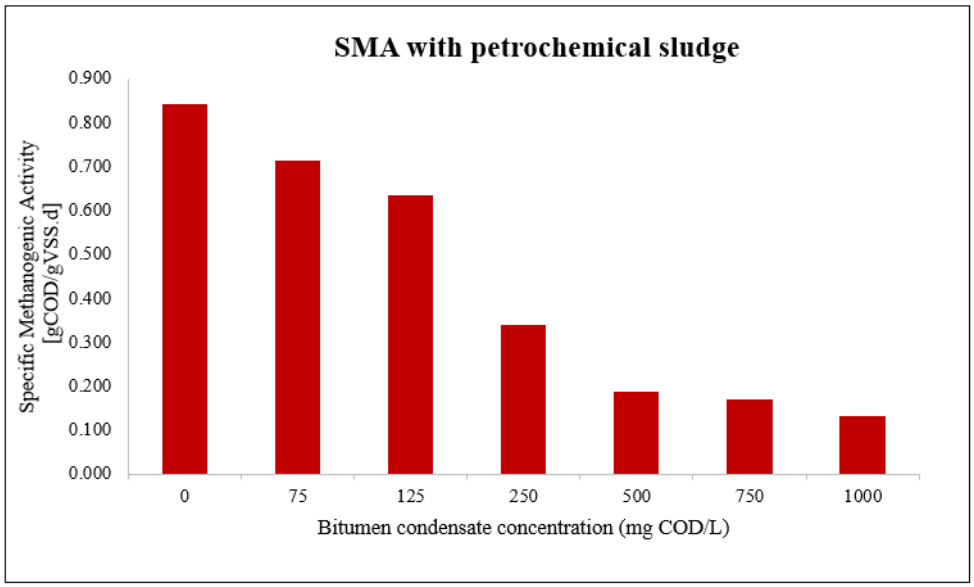

Figure 12: The average SMA values by petrochemical sludge on varying concentrations of bitumen condensate. Each bitumen condensate condition was tested in triplicates

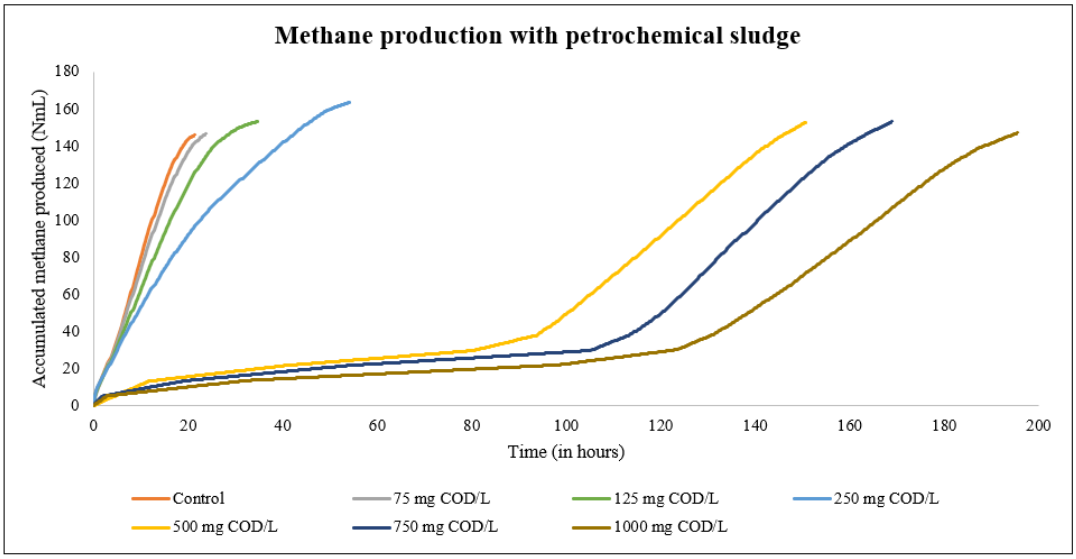

Figure 13: The accumulated CH_4 produced over time with petrochemical sludge with varying concentrations of bitumen condensate. The conditions were executed in triplicates and the most representative methane production lines are depicted in the figure.

For bitumen condensate concentrations of 500, 750 and 1000 mg COD/L, the CH_4 production had a lag phase where the rate of CH_4 production is considerably low (0.01 g COD-CH_4/g VSS.d). The higher concentrations of bitumen condensate cause a temporary inhibition of the methanogenic activity, resulting in a lag phase and reduced methane production (figure 13). The lag phase was shorter for bitumen condensate at 500 mg COD/L. The SMAs for petrochemical sludge was higher than that from the municipal sludge because the former biomass was adapted to degrading industrial wastewater while the latter was adapted to degrading municipal waste. Figure 13 shows

the accumulated CH_4 production.

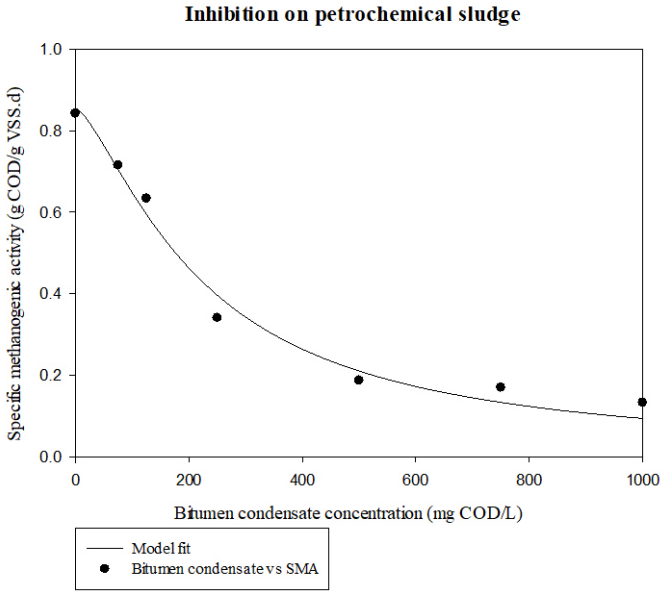

Figure 14: Inhibition in petrochemical sludge due to bitumen condensate is determined by plotting the average SMA values (sample size, n = 3) with the sigmoidal function

The IC_{50} of the bitumen condensate for the petrochemical sludge was determined to be 224 mg COD/L. The IC_{50} for the petrochemical sludge was higher than that of the municipal sludge since the latter is adapted to toxic environment provided by the bitumen condensate.

The third SMA inhibition test was done with the phenol-degrading sludge. Since bitumen condensate contains phenol and phenolic compounds (reported from the GC-MS analysis), it performed better than the petrochemical sludge. The overall acetate conversion time taken by the BioXtreme sludge is shorter than the petrochemical sludge. However, a lag phase for the higher concentrations of bitumen condensate (500, 750, and 1000 mg COD/L) was observed with this sludge as well 16. The SMA values for the different bitumen condensate COD values are depicted in Figure 15.

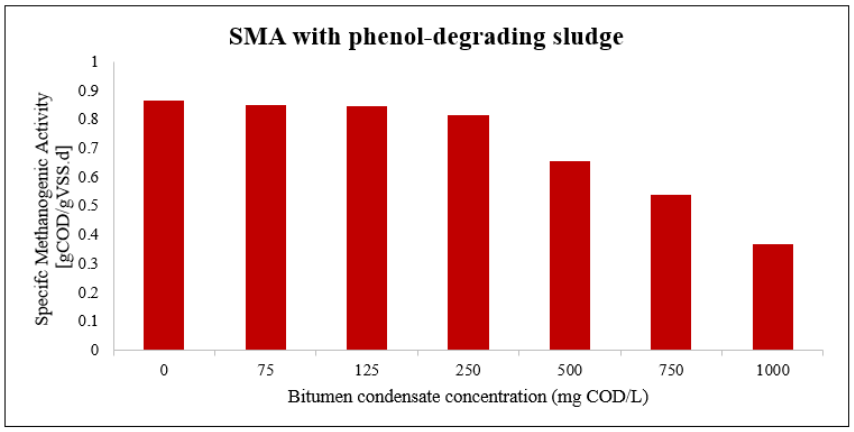

Figure 15: The average SMA values by phenol-degrading sludge on varying concentrations of bitumen condensate. Each bitumen condensate condition was tested in triplicates

The accumulated CH_4 produced in different test conditions over time is shown in Figure 16.

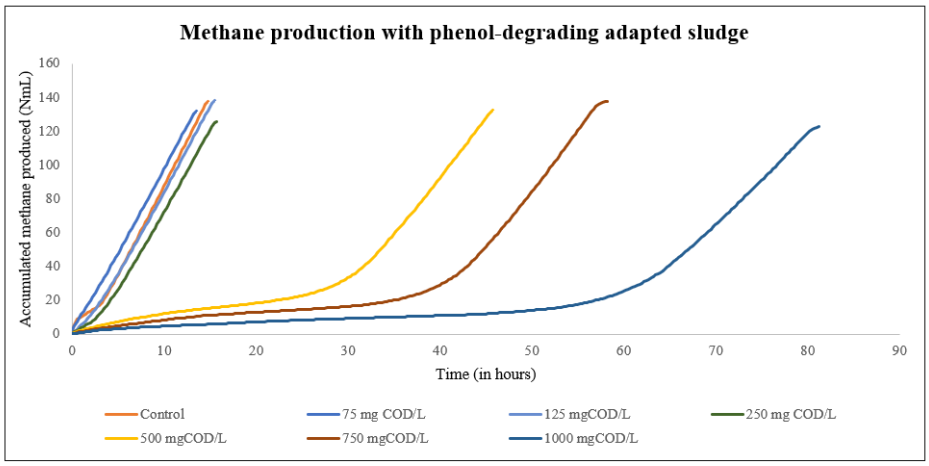

Figure 16: The accumulated CH_4 produced over time with phenol-degrading sludge with varying concentrations of bitumen condensate. The conditions were executed in triplicates and the most representative methane production lines are depicted in the figure.

The IC_{50} of the bitumen condensate for the phenol-degrading sludge was determined to be 901 mg COD/L (figure 17). Since the phenol-degrading sludge has been degrading phenol for more than one year, the compounds in the bitumen condensate are not inhibiting to the microbial community. The IC_{50} for phenol for a non-adapted phenol sludge is was 1.1 g COD/L (Olguin-Lora et al., 2003)and for an adapted, it was 3.3 g COD/L.

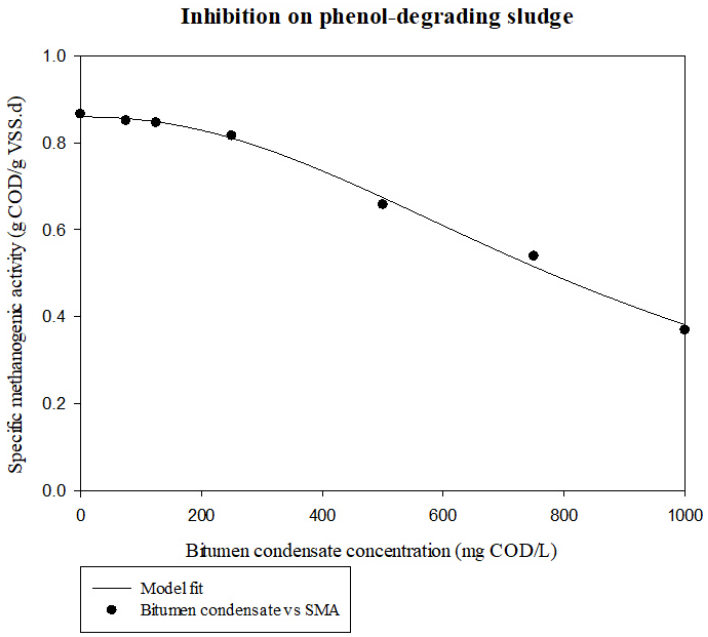

Figure 17: Inhibition in phenol-degrading sludge due to bitumen condensate is determined by plotting the average SMA values (sample size, n = 3) with the sigmoidal function

5.4.2 Toxicity determination

When the activity inhibited are vital to the cells then the substance causing inhibition is labelled as toxic. The effect of toxicity is observed in the ultimate death of the cells (Orozco, 2008). In section 5.4.1, it was showed that the addition of bitumen condensate inhibited CH_4 production. Toxicity, as membrane damage, was determined by flow cytometry. For the municipal sludge digestate, there was a decrease of 30% in the living cell percentage from the beginning of the experiments for 75 mg COD/L of bitumen condensate concentration and a 32% decrease for 125 mg COD/L (Figure 18). For 250 mg COD/L of bitumen condensate concentration,there was seen a 35.5% decrease.

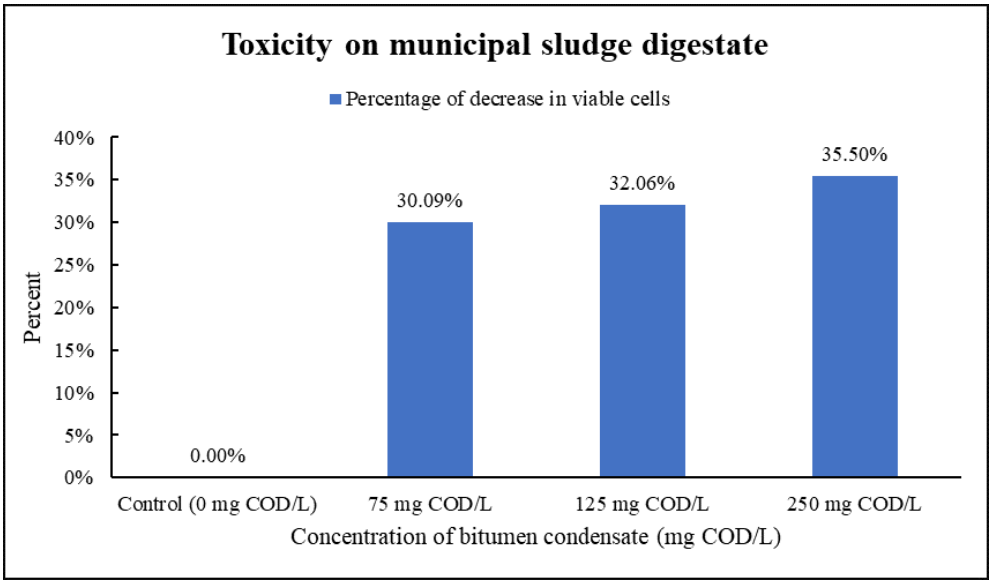

Figure 18: The toxicity of the bitumen condensate on the municipal sludge was determined as the percentage decrease in viable cells. Each bitumen condensate condition was tested in triplicates

Toxicity due to the different bitumen concentrations was lower in the petrochemical sludge and the phenol-degrading sludge than in municipal sludge (Figure 19). The decrease in cell viability between the beginning and end of the experiment for the concentration of 1000 mg COD/L for petrochemical sludge was 34%. The decrease in cell viability for the phenol-degrading sludge for the concentration of 1000 mg COD/L was 10%.

The microbial community of the petrochemical and phenol-degrading sludge were less toxicated than the microbial community of municipal sludge when exposed to bitumen condensate. This was because the petrochemical and phenol-degrading sludge have been exposed to the toxic conditions of industrial wastewater. When comparing the difference of the effect of toxicity between the petrochemical sludge and the phenol-degrading sludge, the phenol-degrading sludge was less toxicated because of its exposure to extreme toxic conditions of phenol compounds for more than a year.

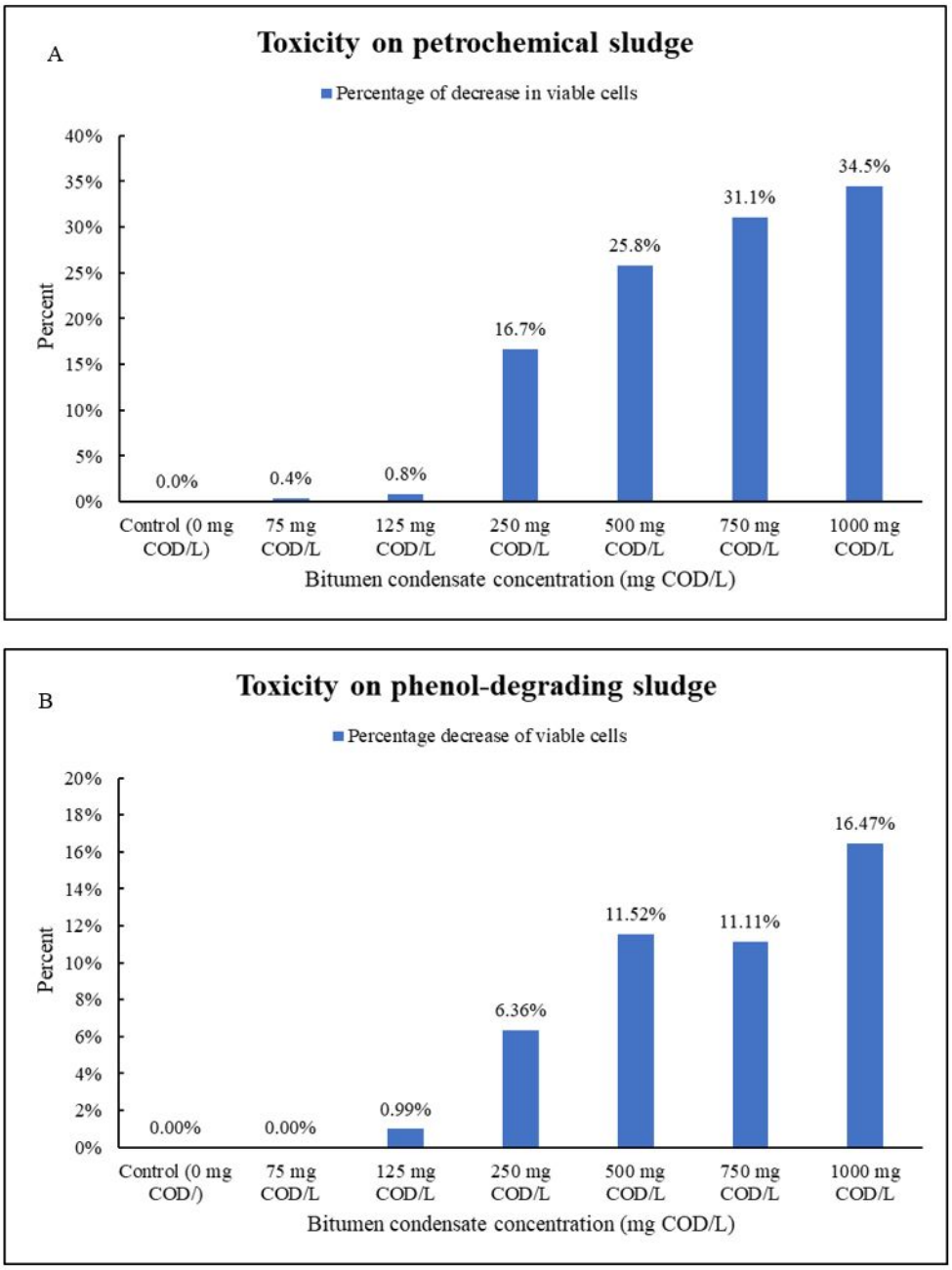

Figure 19: The toxicity of the bitumen condensate was determined as the percentage decrease in viable cells. Each bitumen condensate condition was tested in triplicates (sample size, n =3)
A: Toxicity due to bitumen condensate on petrochemical sludge, B: Toxicity due to bitumen condensate on phenol-degrading sludge. The phenol-degrading sludge was less affected by the toxicity of the bitumen condensate compared to petrochemical sludge

5.5 Continuous tests

5.5.1 Critical flux determination

The 10 min average TMP value against flux is presented in figure 20. The TMP increase became significant with a flux higher than 40 LMH. In the continuous experiment, the maximum flow rate

was 2 L/d corresponding to a flux which was substantially smaller than the critical flux (about 10L/d).

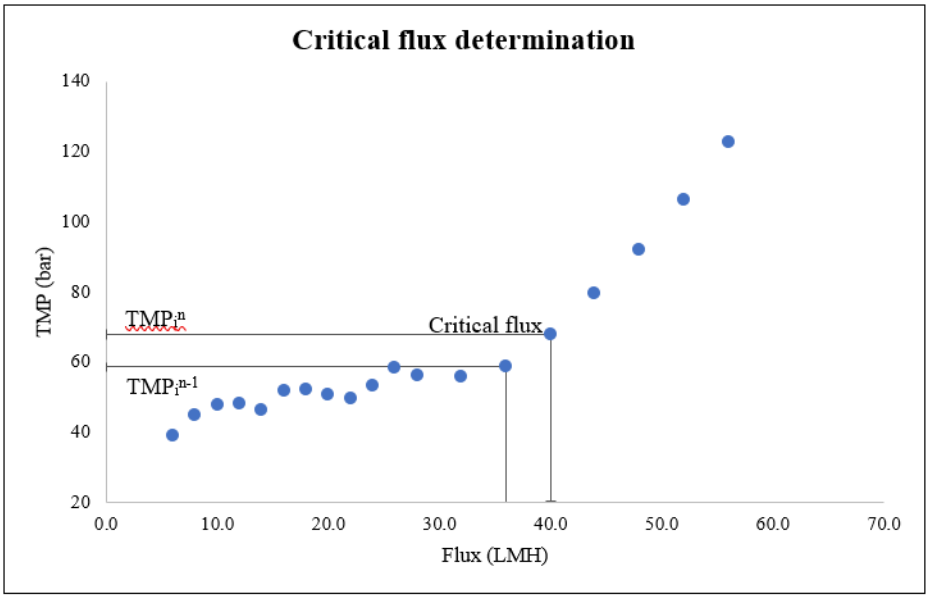

Figure 20: Critical flux determination. TMP_i^{n-1}=58.6 mbar, TMP_i^n=67.8 mbar,ΔP_0 = 9.2 mbar at flux 40 LMH. The flow rate corresponding to the critical flux was about 10 L/d.

5.5.2 COD removal from the effluent

To determine the organic conversion rate of bitumen condensate in an AnMBR, the total COD removal, phenol removal and, as a result, the bitumen condensate removal was determined (7). Acetate was added to the feedwater to ensure the methanogens will survive (0.5 g COD/L). From the SMA inhibition tests and toxicity determination, it was seen that the microbial community of the phenol-degrading sludge was better adapted to the bitumen condensate than the petrochemical sludge and the municipal sludge. This was because it had been degrading toxic compounds (phenol) for more than one year. The initial concentration of phenol of 1.2 g COD/L was added to improve the OLR.

Figure 21A shows the COD concentration in the effluent of the AnMBR and the COD removal percentage. The initial inflow COD concentration was 2.5 g COD/L. After 31 days, it was seen the removal percentage was constant at 40%. The HRT was increased to 4 days to ensure the substrate was retained in the reactor for a longer period, thereby increasing the efficiency of substrate conversion. After this,the removal percentage improved to 45%. Due to variation in bitumen condensate concentration in the feed, from the 40th day onwards, the feed had a lower COD concentration (1.9 g COD/L) than the initial concentration. The removal percentage increased to 50%. After the phenol concentration was reduced to 0.2 g/L on the 60th day, the removal percentage increased to 96%. After day 80, it reduced to 60% due to technical problems in the reactor. The maximum organic conversion rate (OCR) attainable was 38.0 mg COD/g VSS.d (73^{rd} day). The variation of the OLR and the OCR is depicted in figure 21.

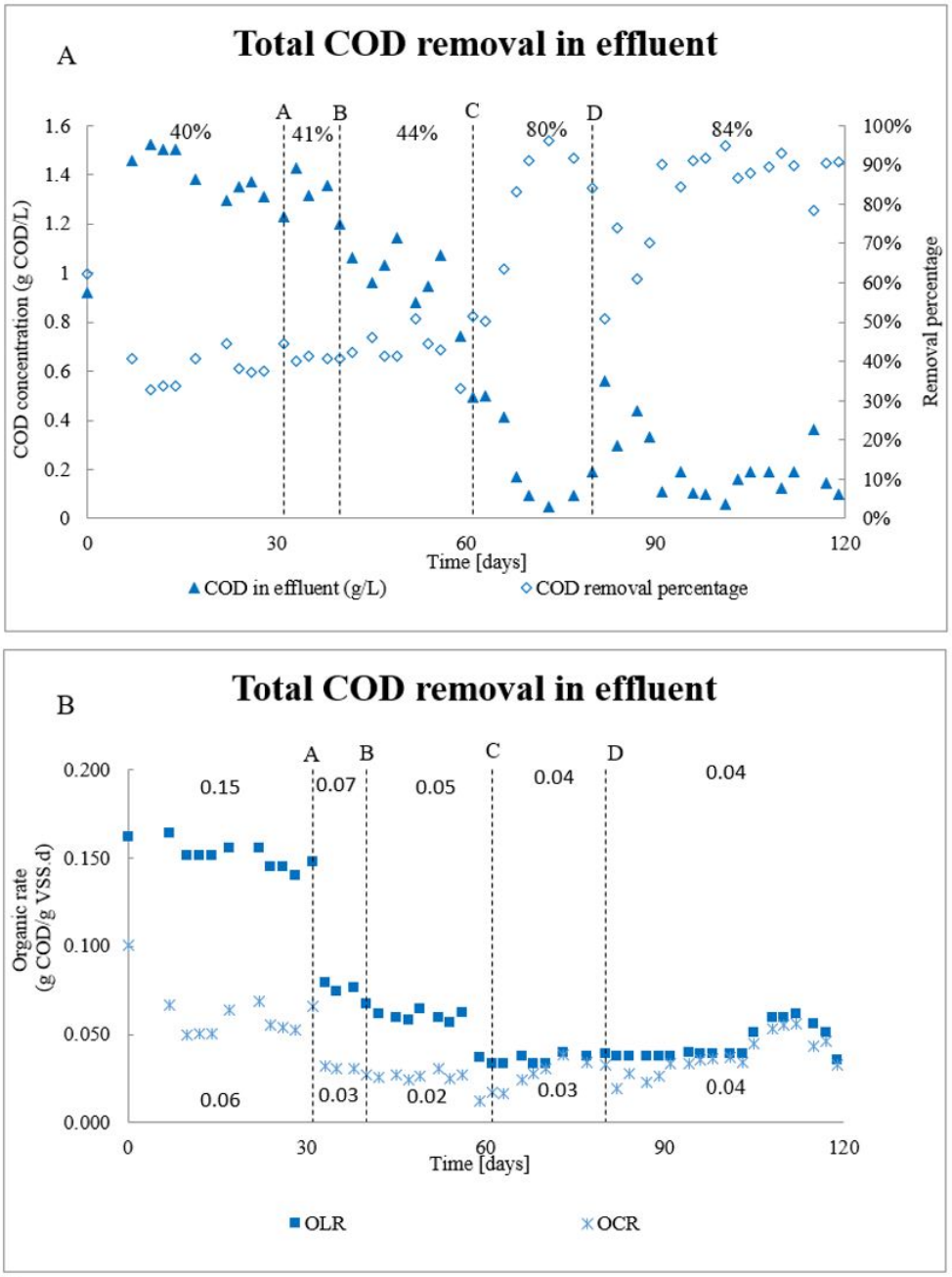

Figure 21: A-COD removal efficiency over 120 days. The efficiency increased to maximum of 96%. It reduced due to a technical problem with the reactor; this was recouped and the reactor achieved high removal efficiency. The average percentage removal during each period is depicted on top. B - The Organic Loading Rate (OLR) shows the rate of substrate entering the reactor and the Organic Conversion Rate (OCR) shows the rate of substrate converted. After 60 days, the OCR improves and all the substrate is converted. The average OLR during each period is depicted on top and the average OCR is depicted at bottom. Events A: HRT was increased to 4 days; B: bitumen condensate concentration reduced, (COD concentration 1.9 g COD/L); C:Phenol concentration was reduced, (COD concentration 1.2 g COD/L); D: technical problem in the reactor, gas leak

5.5.3 Phenol removal from the effluent

In the first 30 days, phenol removal by the AnMBR was low (below 10%, Figure 22)A. After 30 days, the HRT was increased to 4 days. The removal percentage increased to an average of 14%. After 60 days, and observing no improvement in removal percentage of phenol, it was decided to reduce the phenol concentration to 0.2 g/L. The removal percentage increased to 55% in 7 days' time. After day 70, the removal percentage increased to 96% implying that the microbial community developed to degrade phenol. However, due to technical problems in the reactor, the removal efficiency reduced to 30% but it improved and 100% removal efficiency was achieved by day 95.

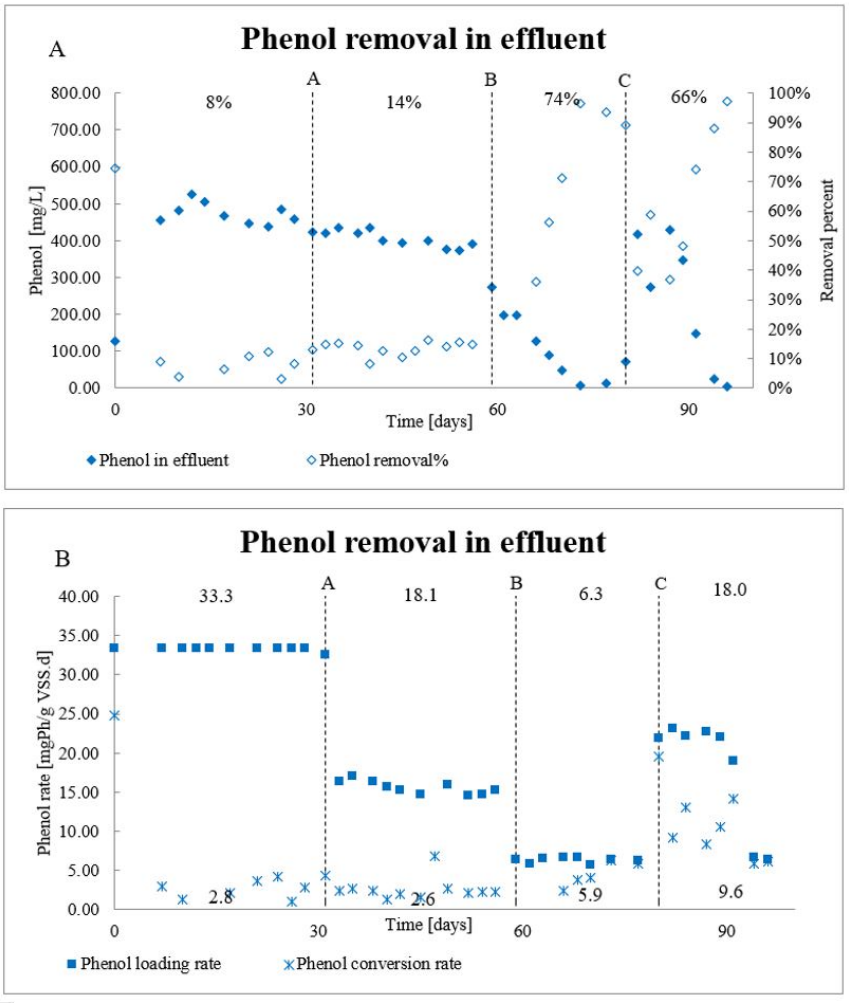

Figure 22: A-Phenol removal efficiency over 100 days. The removal improved after reducing the phenol quantity in the feed (from 0.5 g/L of phenol to 0.2 g/L of phenol)It reduced due to a technical problem with the reactor; this was recouped and the reactor achieved high removal efficiency. The average percentage removal during each period is depicted on top. B - The phenol loading rate (PhLR) shows the rate of phenol entering the reactor and the phenol loading rate (PhCR) shows the rate of phenol converted. After 90 days, the PhCR improves and all the phenol is converted. The average PhLR during each period is depicted on top and the average PhCR is depicted at bottom. Events A: HRT was increased to 4 days; B:Phenol concentration was reduced, the total concentration of influent 1.2 g COD/L; C: technical problem in the reactor, gas leak

5.5.4 VFAs removal from the effluent

From the VFA analysis, it was determined that there was no more acetate in the effluent by the end of two months (figure 23).

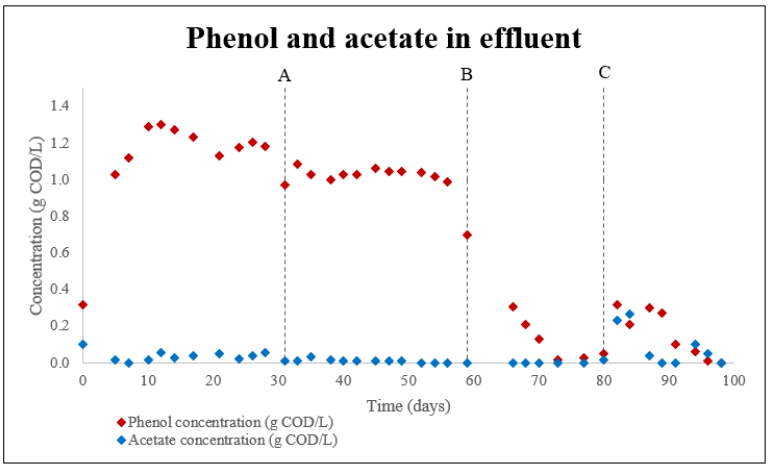

Figure 23: The concentration of phenol and acetate in the effluent. The phenol removal improved after 60 days of operation while there was no acetate concentration in the effluent, implying complete acetate degradation by the methanogens

From equation 7, the removal percentage of the bitumen condensate COD ranged between 73%-100% (average 89% ± 12% (S.D.). When the OLR was reduced after 30 days (Figure 21)B, the bitumen condensate removal percentage was reduced as well. The reactor adapted to this, and the removal of bitumen condensate improved after 10 days (Figure 24). By equation 7, it was possible to conclude that 100% removal of bitumen condensate was achieved. A maximum organic conversion rate of 26.0 mg COD/g VSS.d was achieved by the reactor.

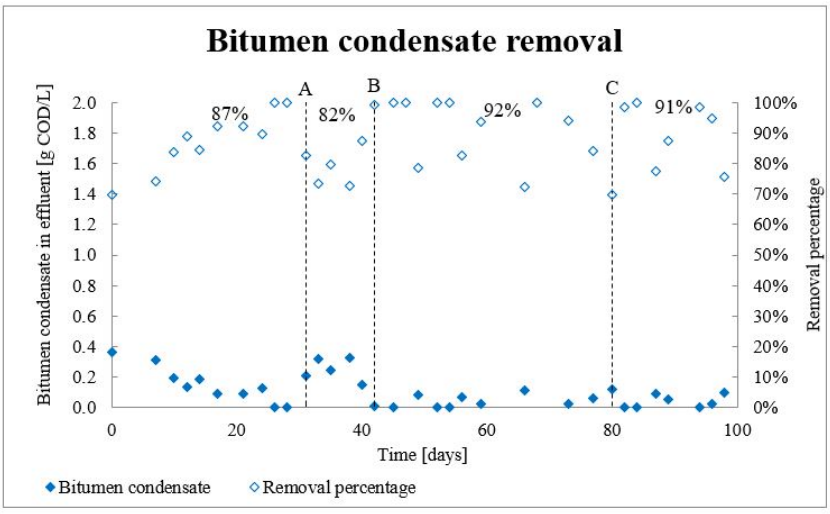

Figure 24: Condensate removal efficiency over 100 days. The high removal efficiency was achieved, efficiency varying between 70%-100% (average 89% ± 12% (S.D)). The average removal percentage is depicted on top.
Events A: HRT was increased to 4 days; B: bitumen condensate concentration reduced, the total concentration of influent 1.9 g COD/L; C: technical problem in the reactor, gas leak

6 Conclusion and recommendations

6.1 Conclusion

This research aimed to determine the biodegradability of bitumen condensate by AD using an AnMBR under mesophilic conditions.

The bitumen condensate was found to contain over 800 compounds. The main compounds were p-cresol, o-cresol, and 2-naphtalenemethanol. It was seen that while the compounds can be adsorbed on to the biomass, it does not volatilise and escape as a gas.

After testing with three different sludges, it was seen that bitumen condensate was most inhibitory for the municipal sludge (IC_{50} = 127 mg COD/L). the in the petrochemical sludge (IC_{50} = 187 mg COD/L). The phenol-degrading sludge is (IC_{50} = 870 mg COD/L).

Bitumen condensate at concentration 250 mg COD/L was toxic (cell viability reduced by 35%) of to the municipal sludge. It was not toxic to petrochemical or the phenol-degrading sludge at the same concentration of bitumen condensate. Cell viability reduced by 35% for the bitumen condensate concentration of 1000 mg COD/L for the petrochemical sludge and by 10% for the bitumen condensate concentration of 1000 mg COD/L for the phenol-degrading sludge.

After the start-up period, there was 70%-100% removal of the bitumen condensate compounds from the influent. Although due to the variation in concentration of the bitumen condensate, a stable trend in the degradation of the compounds was not achieved. The maximum organic conversion rate of the total COD was 38.0 mg COD/g VSS.d. A maximum organic conversion rate of bitumen condensate to be achieved was 26.0 mg COD/g VSS.d.

Advantages and disadvantages of using an AnMBRs for treating industrial wastewater have been discussed in section 1.3, section 1.4, and section 2.4. Compared to the mentioned two treatment methods in section 2.1.1, AD by AnMBR is the better option.The biomass, over time, developed the microbial community to degraded bitumen condensate and phenol.

6.2 Recommendations

- The bitumen condensate collection at the company is at the basic level currently, it can be improved to have a more efficient collection and better reuse of the heat. Sutter et al (2016) proposed a design for the condensate generator. This is depicted in Figure 25. Fumes generated is passed through the first glass condensation tube. It is then passed through a filter and then to the second glass condensation tube. Below each of the condensation tubes, there are glass flasks to collect the condensate. The condensation tubes are jacketed by coolant fluid flowing at -20^{o}C. The lightest vapours are passed through activated charcoal. The valve is connected to the vacuum pump which regulates the flow inside the condensation system. The typical collection time of the fumes was proposed as 5 consecutive days.

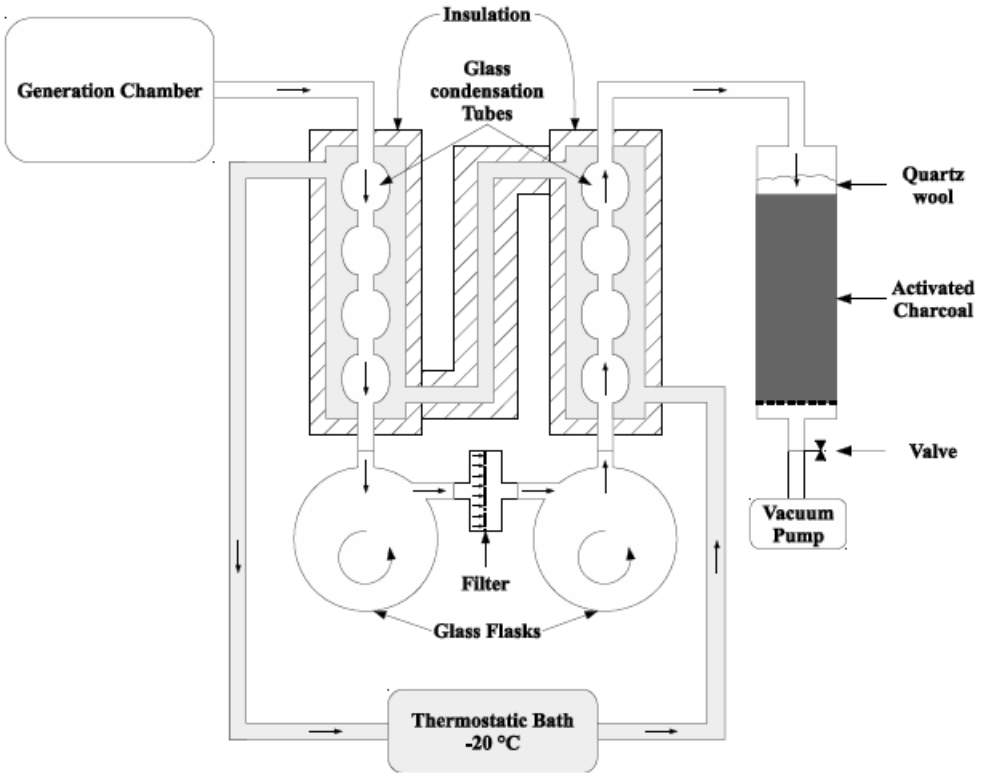

Figure 25: Design proposed by Sutter et al (2016) for the collection of generated bitumen condensate

- The bitumen condensate is acidic (2.8 pH). Large quantities of bitumen condensate should be stored in non-metal containers as it is very corrosive and can react with the metal.
- The phenol was added to improve the adaption of the petrochemical sludge to toxic compounds. The initial concentration of phenol added was 0.5 g/L. It was reduced to 0.2 g/L. The next recommended step is to stop the addition of phenol and added the bitumen condensate solely without dilution.
- The permeate from the AnMBR will contain other metal ions from the micronutrients and macronutrients. An additional treatment step is required for the removal of these inorganic compounds.

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Appendix

A Macronutrients and micronutrients composition

A.1 Macronutrients composition

Table 2: Macronutrients composition

Compounds	Concentrations g/L
NH_4Cl	170
$CaCl_2.2H_2O$	8
$MgSO_4.7H_2O$	9

A.2 Micronutrients composition

Table 3: Micronutrients composition

Compounds	Concentrations g/L
$FeCl_3.4H_2O$	2
$CoCl_2.6H_2O$	2
$MnCl_2.4H_2O$	0.5
$CuCl_2.2H_2O$	30
$ZNCL_2$	50
HBO_2	50
$(NH_4)_6Mo_7O_2.4H_2O$	90
$Na_2SeO_3.5H_2O$	100
$NiCl_2.6H_2O$	50
EDTA	1
HCl 36%	1
Resazurine	0.5
Yeast extract	2

B GC-MS analysis

B.1 Bitumen condensate compounds from non-targeted GC-QTOF analysis

Table 4: Tentative compounds present in the bitumen condensate, detected by a non-target analysis in a GC

Retention time	Compounds	Formula
7.32	Cyclopentanone	C5H8O
7.57	Urea, propyl-	C4H10N2O
7.80	2,5-Hexanedione	C6H10O2

Retention time	Compounds	Formula
7.83	Pyridine, 2,5-dimethyl-	C7H9N
8.34	2-Butyn-1-al diethyl acetal	C8H14O2
8.38	Butane, 2,2,3-trimethyl-	C7H16
8.66	2,4-Dimethylfuran	C6H8O
8.75	2H-Pyran-2-one	C5H4O2
8.86	Methacrylic anhydride	C8H10O3
9.08	Pentanoic acid, 2-methyl-, anhydride	C12H22O3
9.14	Benzonitrile	C7H5N
9.97	2-Furancarboxylic acid, tetrahydro-3-methyl-5-oxo-	C6H8O4
10.21	2-Cyclopenten-1-one, 2,3-dimethyl-	C7H10O
10.27	Methacrylic anhydride	C8H10O3
10.53	Phenol, 2-methyl-	C7H8O
10.73	Benzenemethanol, .alpha.-methyl-, (R)-	C8H10O
10.83	Phenacylidene diacetate	C12H12O5
10.93	Ethyl 4-(ethyloxy)-2-oxobut-3-enoate	C8H12O4
10.95	p-Cresol	C7H8O
11.16	Ethanone, 1-(3-thienyl)-	C6H6OS
11.26	Ethanone, 1-(3-thienyl)-	C6H6OS
11.69	Phenylethyl Alcohol	C8H10O
12.05	Phenol, 2-ethyl-	C8H10O
12.19	Benzenemethanol, 2-methyl-	C8H10O
12.29	Phenol, 3,5-dimethyl-	C8H10O
12.48	Benzenemethanol, .alpha.,4-dimethyl-	C9H12O
12.60	Phenol, 2-ethyl-	C8H10O
12.73	Phenol, 2,3-dimethyl-	C8H10O
12.95	p-Toluic acid,4-cyanophenyl ester	C15H11NO2
12.99	Phenol, 3,4-dimethyl-	C8H10O
13.21	Benzofuran	C8H6O
13.61	Benzothiazole	C7H5NS
13.64	1H-Inden-1-ol,2,3-dihydro-	C9H10O
13.74	Benzothiazole	C7H5NS
13.77	Phenol, 2-ethyl-4-methyl-	C9H12O
13.79	Benzeneacetonitrile,alpha.-methylene-	C9H7N
14.15	1-Benzocyclobutenecarbonitrile	C9H7N
14.18	Benzenepropanoic acid,3-phenylpropyl ester	C18H20O2
14.41	1H-Inden-1-one,2,3-dihydro-	C9H8O
14.80	2-Propenal, 3-(4-methylphenyl)-	C10H10O
14.96	1-Methylindan-2-one	C10H10O
15.15	Isoquinoline, 3-methyl-	C10H9N
15.29	Isoquinoline, 1-methyl-	C10H9N
15.34	1-Propanone, 3-chloro-1-phenyl-	C9H9ClO
15.42	7-Methylindan-1-one	C10H10O
15.56	1-Propanone, 3-chloro-1-phenyl-	C9H9ClO
15.79	1(2H)-Naphthalenone, 3,4-dihydro-	C10H10O

Retention time	Compounds	Formula
16.00	7-Methylindan-1-one	C10H10O
16.02	4H-1-Benzopyran-4-one	C9H6O2
16.12	Coumarin	C9H6O2
16.19	7-Methylindan-1-one	C10H10O
16.22	7-Methylindan-1-one	C10H10O
16.27	4-Methylphthalaldehyde	C9H8O2
16.45	4'-Methylbutyrophenone	C11H14O
16.60	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	C9H8O3
16.63	Ethanone,1-[4-(1-methylethenyl)phenyl]-	C11H12O
16.89	4'-Methylpropiophenone	C10H12O
16.93	4'-Methylpropiophenone	C10H12O
17.08	4'-Methylpropiophenone	C10H12O
17.27	Benzonitrile,2-ethoxy-	C9H9NO
17.87	1-Propanone,1-(2,4-dimethylphenyl)-	C11H14O
18.09	4-Ethylbenzoic acid,4-nitrophenyl ester	C15H13NO4
18.34	Benzoic acid, 3,4-dimethyl-,methyl ester	C10H12O2
18.38	2-Naphthalenemethanol	C11H10O
18.97	1-Propanone,1-(2,4-dimethylphenyl)-	C11H14O
19.39	1-Acenaphthenol	C12H10O
20.26	9H-Fluoren-9-ol	C13H10O
20.63	Benzenesulfonamide,N-butyl-	C10H15NO2S
20.70	2-Propanol, 1-chloro-,phosphate (3:1)	C9H18Cl3O4P
22.18	1H,3H-Naphtho[1,8-cd]pyran-1-one	C12H8O2
22.98	Dibenzothiophene,4,6-dimethyl-	C14H12S
24.60	Hexanoic acid,(2-hexanoylaminoethyl)-amide	C14H28N2O2

B.2 GC-MS results with concentration

Table 5: Compounds and concentrations

Compounds	Concentrations
1,2,4-Trichlorobenezene	<0.02 μg/L
1,2-Dichlorobenezene	<0.02 μg/L
1,3,5-Trichlorobenezene	<0.02 μg/L
1,3-Dichlorobenezene	<0.02 μg/L
1,4-Dichlorobenezene	<0.02 μg/L
1,2,3-Trichlorobenezene	<0.02 μg/L
Pentachlorobenezene	<0.04 μg/L
1,2,4,5-Tetrachlorobenezene	<0.04 μg/L
1,2,3,4-Tetrachlorobenezene	<0.04 μg/L
Hexachloroethane	<0.02 μg/L
Hexachlorobutadiene	<0.02 μg/L
Fluoranthene	0.02 μg/L
Antracene	<0.004 μg/L

Compounds	Concentrations
Benzo(a)antracene	<0.01 μg/L
Acenaphtylene	0.27 μg/L
Benzo(b)flouranthene	<0.008 μg/L
Benzo(ghi)perylene	<0.008 μg/L
Benzo(k)flouranthene	<0.008 μg/L
Acenaphtene	0.03 μg/L
Fluorene	0.04 μg/L
Benzo(a)pyrene	<0.006 μg/L
Pyrene	<0.006 μg/L
Indeno(1,2,3-cd)pyrene	<0.008 μg/L
Naphthalene	0.70 μg/L
Chrysene	<0.006 μg/L
Fenantrene	0.04 μg/L
2,4,4'-Trichlorobiphenyl	<0.04 μg/L
2,5,2',5'-Tetrachlorobiphenyl	<0.04 μg/L
2,4,5,2',5'-Pentachlorobiphenyl	<0.02 μg/L
2,4,5,3',4'-Pentachlorobiphenyl	<0.02 μg/L
2,4,5,2',4',5'-Hexachlorobiphenyl	<0.04 μg/L
2,3,4,2',4',5'-Hexachlorobiphenyl	<0.04 μg/L
2,3,4,5,2',4',5'-Heptachlorobiphenyl	<0.04 μg/L

C COD balance from the AnMBR

From the influent and effluent analysis, COD concentrations were measured. The COD present in biogas production was determined by multiplying the CH_4 production rate by the CH_4 COD equivalent (0.4 L CH_4/g COD). The COD fed into the reactor should be present as COD in the effluent or as COD in biogas. The missing COD is calculated as a percentage between the COD received by the reactor and the COD leaving the reactor. Table 6 shows the COD balance over the 90 days period.

Table 6: COD balance obtained from influent COD, effluent COD and biogas

Day	Influent COD g COD/d	Effluent COD g COD/d	Biogas COD g COD-CH_4/d	Missing COD %
7	4.44	2.91	1.47	1.18
10	4.14	3.04	0.44	16.08
31	5.90	2.46	0.36	52.26
61	2.62	0.49	0.04	79.88
68	2.46	0.17	0.00	93.18
70	2.54	0.09	0.00	96.40
73	3.08	0.05	0.00	98.44
77	1.96	0.09	0.00	95.22
87	0.98	0.44	0.28	26.30

It was noticed there was a technical problem with the biogas line of the bioreactor. The

biomass achieved better COD removal efficiency, which implied that most of the COD removed were converted into CH_4. However, due to a leakage that could not be fixed in the gas line connected to biogas counter, the biogas produced was not measured during 16 days corresponding to the operation day 61 to the operation day 77. After 73 days, the missing COD raised to 98%, which we assume could correspond to the not measured biogas.

D Biogas composition analysis from AnMBR

Figure 26 shows the accumulated volume of biogas produced during the AnMBR operation. After 40 days, although the removal of COD increased over time, the biogas production rate reduced. From the effluent analysis, it was seen that the influent COD was converted to biogas. The reduction in the biogas production rate was due to a technical problem with the biogas line. Therefore, it was not possible to measure the cumulated total biogas produced.

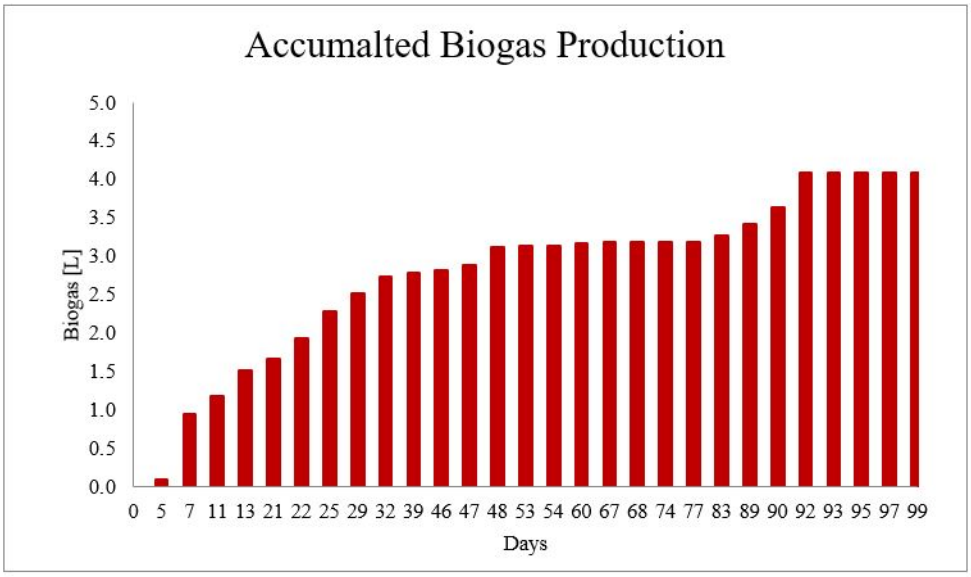

Figure 26: The accumulated biogas production over the 90 days. It demonstrates the reduction in biogas measured after 30 days due to a technical problem in the gas lines

The gas samples extracted from the gas line were a mixture of CO_2, CH_4, and nitrogen. By GC analysis, the percentage of CH_4 and CO_2 in AnMBR biogas was determined (Figure 26). For predicting the relative amount of CH_4 in the produced biogas, the COD/TOC ratio is useful. From (Van Lier et al., 2008), COD/TOC ratio of 2.7 corresponds to 65% of CH_4 in biogas from compounds like phenol and glycerine.

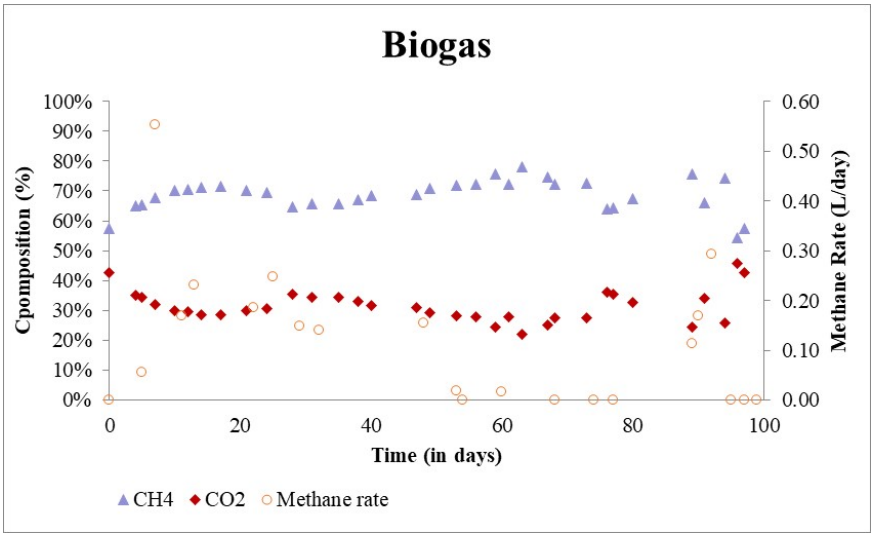

Figure 27: Composition of biogas as a percentage of CH_4 and CO_2. The percentage of CH_4 increases to 75% over time.

In the first week of operation, the CH_4 percentage was 58% (Figure 25). Phenol and phenolic compounds produce an approximate of 58% of CH_4 in biogas (Van Lier et al., 2008). The percentage of CH_4 is an average of 70% over 90 days. 60-70% of CH_4 in biogas can be produced from ethanol, benzene, and cyclohexane. These compounds were reported to be found in the bitumen condensate (Appendix B.1).