Effect of aeration by micro - bubbles on methane production in anaerobic digestion

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Abstract

Biogas produced form anaerobic digestion is a sustainable and economical way of producing energy from organic matter. Conventionally, process conditions for anaerobic digestion have strictly been without the presence of oxygen. However, in recent years, studies have suggested that introducing limited amounts of oxygen might have the potential to enhance the anaerobic digestion and energy generation processes further. A way to determine the ultimate methane generating potential for any solid substrate is through a Bio-Methane Potential (BMP) test. Moreover, this parameter is one of the most critical factors that determine the design and cost of a biogas producing plant. This study aims to look at the effect of introducing controlled amounts of oxygen on biogas quantity and quality during anaerobic digestion.

The anaerobic digestion process is studied, specifically it's biochemical processes, to understand the affect of addition of oxygen. Anaerobic sludge is aerated to mimic DAF (Dissolved Air Flotation) conditions, where tap water is pressurized (at 3 and 5 bar) and then depressurized in contact with the anaerobic sludge in a column reactor. During the course of this process, air micro-bubbles which were dissolved under high pressure are released due to contact with atmospheric conditions. To estimate and compare the methane production of the originally collected non-aerated sludge (anaerobic sludge not aerated in the column reactor, therefore, considered as 0 bar) and aerated sludge (anaerobic sludge subjected to high pressure micro-air bubbles in the column reactor at 3 and 5 bar) a BMP test is conducted. Methane production was found to be lower in the aerated sludge with the BMP value for the 0 bar sludge being 296.17 +/- 45.15 $NL_{CH4}kg^1$ and the value for 5 bar aerated sludge being 252.26 +/- 16.8 $NL_{CH4}kg^1$. Biogas composition of the aerated sludge was also examined with a Gas Chromatography (GC) machine and the percentage of methane, carbon dioxide and oxygen were measured for the 3 and 5 bar aerated sludge. For the 5 bar aerated sludge, the overall percentages are averaged at 30%, 70% and 1% respectively and for the 3 bar aerated sludge the average values are 20%, 80% and 1% respectively. Furthermore, particle size distribution (PSD) analysis was done to compare variations for particle sizes between the aerated (5 bar) and non-aerated (0 bar) sludge. Very low variation was observed between these samples with the average size of the aerated samples being marginally smaller than the non-aerated sludge, indicating poor separation efficiency for the separation method adopted.

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1 Introduction

1.1. Research Background

Anaerobic digestion is an extremely popular waste water treatment technology due to its ability to reduce pollution and generate renewable energy. During anaerobic digestion, microbial organisms help degrade and stabilize organic material under anaerobic conditions producing a renewable source of energy - biogas (mainly CO_2 and CH_4) as well as microbial biomass (Kelleher, 2002). Historical evidence suggests that the Assyria were the first to use biogas to heat bathing water in the 30th century (S. Verma, 2002) and as per documented data, the first engineered anaerobic digester was installed in 1859 in Bombay, India (F. Monnet, 2003). Regardless, it was only by the mid-nineties that fundamental biochemical processes of anaerobic digestion were beginning to be understood in depth. Buswell was the first to lead research towards identify anaerobic bacteria and the conditions required for biogas production. (F. Monnet, 2003)

Nowadays, anaerobic digestion is a very attractive technology which can handle many different types of waste water ranging from municipal, agricultural to industrial waste water (Botheju and Bakke, 2011). There are many advantages that anaerobic digestion has over alternative treatment technologies such as higher organic loading rates, less sludge production, energy production by methane and less energy conversion (Tchobanoglous et al., 1979). With the current global environment challenges of climate change and pollution rise, anaerobic digestion is a tempting choice due to its energy efficient and waste minimizing properties. Anaerobic digestion helps bring down greenhouse gas (GHG) emissions as it's required footprint is lesser than conventional technologies, bearing in mind that the methane produced is treated properly and not released directly in to the atmosphere, as it is an extremely harmful GHG gas. Another advantage of this technology is that it's digestate is rich in nutrients which can be used as fertilizer. On the down side, the main problem with anaerobic digestion is because of its complex and unstable biochemical processes. This instability could be due to accidental oxygen infiltration which might lead to adverse impact on the anaerobic digestion process, as it is extremely sensitive to operation and feed changes.(Botheju and Bakke, 2011) This is one aspect where further understanding is needed.

Conventionally, it is considered extremely crucial that there is no oxygen present for anaerobic digestion as oxygen was toxic for methanogens (Zehnder et al., 1988). However, later studies showed that methanogens would tolerate oxygen till certain concentrations

or were found to be protected by anaerobic facultative bacteria both in granular and suspended sludge (Krayzelova et al., 2015). In some studies, hydrogen sulphide oxidation with excess oxygen has also showed improved biogas quality (Tartakovsky et al., 2011). Introducing controlled air micro-bubbles to the process of anaerobic digestion may lead to benefits due to enhancement of process efficiency and eventually improve the biogas production capacity of the plant without causing harm to the methanogens (Botheju and Bakke, 2011). Therefore, a way to improve the process of anaerobic digestion could be with an additional assistance from partial aeration.

1.2. Research Scope

Based on the research background, the scope of this study is limited to collecting, aerating under high pressure (by mimicking DAF conditions at 3 and 5 bar pressure in a column reactor, part of the collected sludge) and analyzing anaerobic sludge taken form Harnaschpolder waste water treatment plant (wwtp) in the Netherlands. The anaerobic sludge is analyzed based on 3 experiments - a BMP test, which estimates and helps compares the methane production of the non-aerated (0 bar) sludge and the aerated (3 and 5 bar) sludge, a GC test which gives the biogas composition of the aerated sludge, and finally a PSD analysis which compares the particles size variations between the non-aerated and aerated sludge.

This study will only focus on examining and comparing the quantity and quality of biogas produced by the non-aerated and aerated (aerated in the lab by injecting high pressure air micro-bubbles into the sludge in a column reactor at 3 and 5 bar) anaerobic sludge based on the aforementioned analysis methods. Other aspects of the anaerobic digestion process will not be considered in this study.

2

Literature Review and Research Question

This section discusses the literature background of the study undertaken. Firstly, various processes of anaerobic digestion are discussed in depth followed by a detailed literature analysis of the effect of aeration on anaerobic digestion. Lastly, the research question is defined based in the knowledge gaps identified and the principles of Automatic Methane Potential Test System II (AMPTS II) and gas-chromotography machine are discussed.

2.1. Anaerobic Digestion

With its many significant advantages such as low energy consumption, less sludge production and renewable energy generation, anaerobic digestion is widely used as a water treatment technology (van Starkenburg, 1997). During anaerobic digestion, organic matter is anaerobically degraded in successive stages of reactions in series or parallel. These stages are hydrolysis, acidogenesis, acetogenesis and methanogenesis. In an anaerobic environment there are many different species of microorganisms co-existing resulting in several complex reactions. The main groups include - fermentative bacteria, H-producing acetogenic bacteria, H-consuming acetogenic bacteria, CO_2 reducing methanogens and aceticlastic methanogens.(van Lier et al., 2008) Figure 2.1 gives the schematic representation of the processes taking place during anaerobic digestion.

Hydrolysis is a surface phenomenon where exo-enzymes excreted by fermenative bacteria break down undissolved, complex polymeric particles in to dissolved, lesser complex molecules that the fermentative bacteria can easily take up through it's cell wall/membrane (van Lier et al., 2008). Fermentative bacteria constitutes a large part of the organisms responsible for hydrolysis and these are known to thrive with and without oxygen (Botheju and Bakke, 2011). Around 80 % of primary sludge and 45-17 % of municipal sewage is known to be made up of suspended solids of which the main bio-polymers are carbohydrates, proteins and lipids. Hydrolysis convert polysaccharide in to simple sugars, proteins to amino acids, and lipids to long chain fatty acids (LCFA). In the second stage - acidoogenesis, is a fast and common reaction which take place in the presence of non-hydrolytic and hydrolytic microorganisms. the compounds which dissolved in the fermentative bacteria, are degraded into smaller elements like volatile fatty acids (VFAs), alcohols, lactic acid, CO_2, H_2, NH_3 and NH_3 which are further excreted. (van Lier et al., 2008) The next stage is acetogenesis where all the VFA's and some short chain organic substances like ethanol are converted into, as the name suggests, acetate. This is done by only done by a group

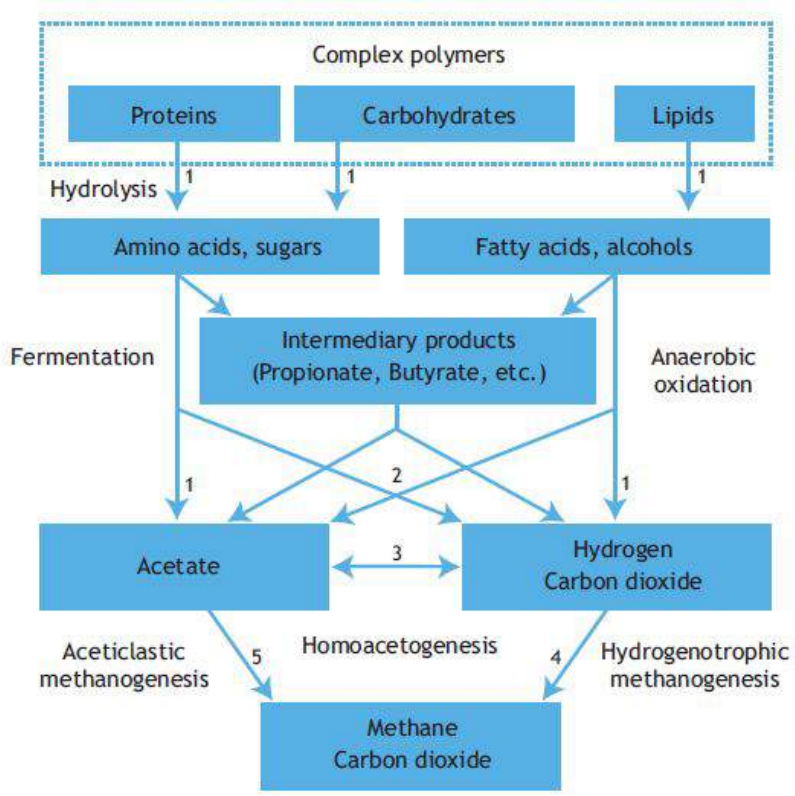

Figure 2.1: Scheme depicting the process (hydrolysis, acetogenesis, acidogenesis & methanogenesis) and bacterial groups involved in anaerobic digestion. (Gujer and Zehnder, 1983)

of anaerobic organisms called acetogens (Botheju and Bakke, 2011). This final step of the process is methanogenesis where methanogens convert acetate, hydrogen, carbonate, formate/methanol into CO_2, methane and new cell mass (van Lier et al., 2008).

Among these processes, hydrolysis and methanogenesis are generally considered as the rate limiting step (van Lier et al., 2001). The complexity and nature of the of the waste being treated eventually determines the ultimate rate limiting step. Generally, in the overall process, that process is hydrolysis for waste waters that contain suspended solids and solid substrates. Furthermore, the factors that affect the rate of hydrolysis include pH,temperature, concentration of biomass and the type of organic substance. (Visvanathan and Abeynayaka, 2012) Therefore, slower the hydrolysis, slower the production of biogas. Thus, introducing a pre-treatment step which ensures a efficient way of suspended substrate to be available for anaerobic bacteria can result in more methane generation from waste water (van Lier et al., 2001).

2.2. Micro-bubble aeration in Anaerobic Digestion

It is a common perception that the presence of oxygen in anaerobic ecosystems is toxic or inhibitory (Chu et al., 2005), (Zitomer, 1998). This is because of the presence of exclusively anaerobic groups of microorganisms - acetogens and methanogens (Whitman et al., 2006). It is also possible that soluble organic matter is converted to CO_2 aerobically which could cause instability, lesser methane production, slower process start ups or even complete reactor failures (Kato et al., 1997).

Unintentionally, it is possible that some oxygen can reach the anaerobic digester especially during mixing or feeding, however this is difficult to quantify and analyze. In literature, some studies can be found which demonstrate the effect and benefits of introducing limited amounts of oxygen to anaerobic digestion. (Botheju and Bakke, 2011). Some studies have suggested that presence of oxygen improved hydrolysis of particulate matter with carbohydrates and proteins being hydrolyzed more but no change was observed in the lipids conversion (Johansen and Bakke, 2006),(Jagadabhi et al., 2010). Moreover, under anaerobic and anoxic conditions hydrolysis rates are higher and that under optimum oxygenation amounts, more methane can be produced in anaerobic digestion. Above 10% increased methane yield was observed in studies done with minimum variations of oxidation-reduction potential (ORP) (Botheju and Bakke, 2011).

Higher COD solublisation, short chain fatty acid conversion and higher VFA accumulation were observed when micro-aeration was applied as a pre-treatment for anaerobic digestion. This was reasoned to be because the facultative organisms would have been consuming the oxygen (Lim and Wang, 2013). Hydrogen sulphide concentrations were also found to be reduced in biogas production in some studies done with oxygen present in batch tests with synthetic waste water, different sludges, substrates and starch (Botheju and Bakke, 2011),(Lim and Wang, 2013), (Johansen and Bakke, 2006). Some studies also found no impact on the methane yield and COD removal due to the addition of limited amounts of oxygen in the anaerobic digestion process (Krayzelova et al., 2014), (Díaz et al., 2011) .

2.3. Knowledge Gap

Based on the above literature review it can be seen that there is a knowledge gap in regarding the effects of limited aeration on anaerobic digestion and subsequent methane production. This study will aim to bridge the gap by evaluating the effect on methane production and biogas quality by comparing experimental data between non-aerated and pre-treated aerated sludge by using AMPTS II and gas-chromatogrpgy equipment.

2.4. Research Questions and Hypothesis

The aim of this study can be can be divided into the following research questions-

- What is the effect of adding air micro-bubbles on the methane production and biogas quality in anaerobic digestion? Will there be an increase, decrease or no change in biogas production and quality due to the addition of oxygen?
- What is the effect on production and quality of biogas by injecting different pressures of air micro-bubbles in anaerobic sludge? In terms of biogas quality, will there be more CO_2 produced if the O_2 concentration increases?
- What are the variations in Particle size distribution between the non-aerated and aerated sludge? What do these variations suggest about the separation method used and it's impact on the methane production?

Based on these questions, the hypothesis for this study is put forward as - ***By adding air micro-bubbles to anaerobic sludge, the methane production and biogas quality will be greater than in non-aerated sludge.***

2.5. Equipment Principles for biogas analysis

2.5.1. AMPTS II

Conventional measuring principles form the basis for the Automatic Methane Potential Test System AMPTS II machine, thus making them easy to compare with standard methods. The advantage that this machine has over standard methods is that the data collection is of high quality and fully automatic which reduces time and labour effort, for the long incubation period of these tests. The AMPTS set up has 3 parts - unit A, B and C. (Bioprocess Control, 2016)

Unit A is the sample incubation unit which can hold up to 15 vials of the sample containing the incubated inoculum. A slow rotating agitator mixes the vial contents and biogas is produced (from which bio-methane potential is estimated). Unit B is the CO_2 absorbing unit which is another set of 15 vials containing 250 ml alkaline solution (NaOH). This solution helps retain acid gas elements such as CO_2 and H_2S in the produced biogas and allows CH_4 to pass through. In unit C, the gas volume measuring device, the volume of CH_4 released from unit B is measured by a wet gas flow measuring device containing a multi-flow cell arrangement. This device is based on the principle of buoyancy and liquid displacement. Finally, when a defined volume of gas flows through this device, it generates a digital pulse which is recorded, analyzed and displayed on an integrated data acquisition system. (Bioprocess Control, 2016)

2.5.2. Gas Chromatography

Various gas components can be measured using the gas chromatograph (GC) machine. This analytically equipment works on the following principle - a sample solution is injected in to the instrument, it is transported to a separation tube (column) by a gas stream (helium or nitrogen). Inside, the gas is separated into it's various components and a detector measures their quantity. (Shimadzu Corporation, 2019)

3

Materials and Methodology

In this section the materials used and methodologies followed for the study are presented in detail. First, synthetic waste water as substrate and micro-aerated and non-aerated inoculums were prepared. Next, bio-methane potential (BMP) test, gas chromatography (GC) and particle size distribution analysis were carried out to estimate the methane potential and biogas composition of the aerated and non-aerated sludge. All the experiments undertaken in this study were performed at the Water Lab at TU Delft (Building 23 Stevinweg 1 2628 CN Delft, the Netherlands)

3.1. Substrate

A composition of synthetic (powdered) municipal waste-water was prepared as substrate. The recipe used was from the study done by Ozgun (Ozgun et al., 2013) with modifications. The micro-nutrients specified in the recipe were not added to the powdered mixture prepared as they were in liquid form. Table 3.1 shows the substrate composition that resembles domestic waste water. Total solids (TS) and volatile solids (VS) were determined for the 22.4 g/L of substrate prepared, based on the procedure given by APHA (APhA, 1998). Table 3.2 gives the TS and VS values for the substrate used. The detailed calculation of these values are given in Appendix A.

3.2. Inoculum

The inoculum used was anaerobic sludge collected from the anaerobic digester of Harneschpolder waste-water treatment plant (wwtp) in the Netherlands. About 10 liters of the sludge was collected from the wwtp which was then sieved using a 0.71 mm sieve and incubated in the oven at 35 °C for 5 days. The sieve dimensions were based on the guidelines given by Holliger et al. (Holliger et al., 2016) in the preparation and storage section. The TS and VS values of the non-aerated sludge and aerated (3 and 5 bar) inoculum were measured based on the APHA guidelines (APhA, 1998) and are given in Table 3.2. The detailed calculation of these values are given in Appendix A.

3.3. Experimental Setup

The experiment was conducted in 3 parts. First, 1.5 liters of the sieved and incubated sludge was mixed with 1.5 liters of pressurized tap water at 3 and 5 bar separately in a column

Macronutrient Solution			Micronutrient Solution		
Compound	Unit	Value	Compound	Unit	Value
Urea	mg/L	1200	$FeCl_3.6\ H_2O$	mg/L	1000
NH_4Cl	mg/L	2000	$CoCl_2.6H_2O$	mg/L	1000
$CH_3COONa.3\ H_20$	mg/L	7400	$MnCl_2.4H_2O$	mg/L	250
Ovalbumin	mg/L	450	$CuCl_2.2H_2O$	mg/L	15
$MgSO_4.7H_2O$	mg/L	180	$ZnCl_2$	mg/L	25
$KH_2PO_4.3H_2O$	mg/L	1400	H_3BO_3	mg/L	25
$CaCl_2$	mg/L	264.9	$(NH_4)_8Mo_7O_{24}.4H_2O$	mg/L	45
Starch	mg/L	6400	$Na_2SeO_3.H_2O$	mg/L	50
Milk powder	mg/L	1500	$NiCl_2.6H_2O$	mg/L	25
Yeast extract	mg/L	600	EDTA	mg/L	500
Sunflower oil	mg/L	1000	HCl 36%	mL/L	0.5
Micronutrients	mL/L	26.6	Resazurin sodium salt	mg/L	250
			Yeast extract	mg/L	1000

Table 3.1: Composition of the synthetic (powdered) waste-water prepared as substrate(Ozgun et al., 2013).

Parameter	TS(g/g)	VS(g/g)
0 bar	0.0236	0.0166
3 bar	0.0009	0.0005
5 bar	0.0043	0.0032
Wastewater (Powder)	0.7200	0.5100
Cellulose	0.9300	0.9500

Table 3.2: Total solids (TS) and volatile solids (VS) of inoculum, waste water and cellulose used in the experiment.

setups. The column was flushed with N_2 gas for each run and was water locked from the top to ensure that there was no infiltration of oxygen from the atmosphere. This was done to ensure that the presence of oxygen was only thought the introduction of pressurized water.

From these 2 columns runs, 1.5 liters of the supernatant for both pressures were pumped out and used as incoulum in the AMPTS machine, along with prepared blank and control bottles. A few bottles of the triplicates used for the 3 bar and 5 bar AMPTS measurement were placed in the incubator shaker as they could not be incorporated in the limited slots of the AMPTS machine. For the 15 samples placed in the AMPTS machine, methane production was recorded automatically and the methane produced by the 3 bottles in the incuba-

tor shaker were estimated by the GC method. Also, 2 separate 250ml bottles were prepared using the suparnatant inoculum of the 3 and 5 bar aerated sludge for biogas composition analysis using the GC machine (7200B GC/Q-TOF System from Agilent Technologies). Figure 3.1 depicts the schematic diagram of the overall experimental setup.

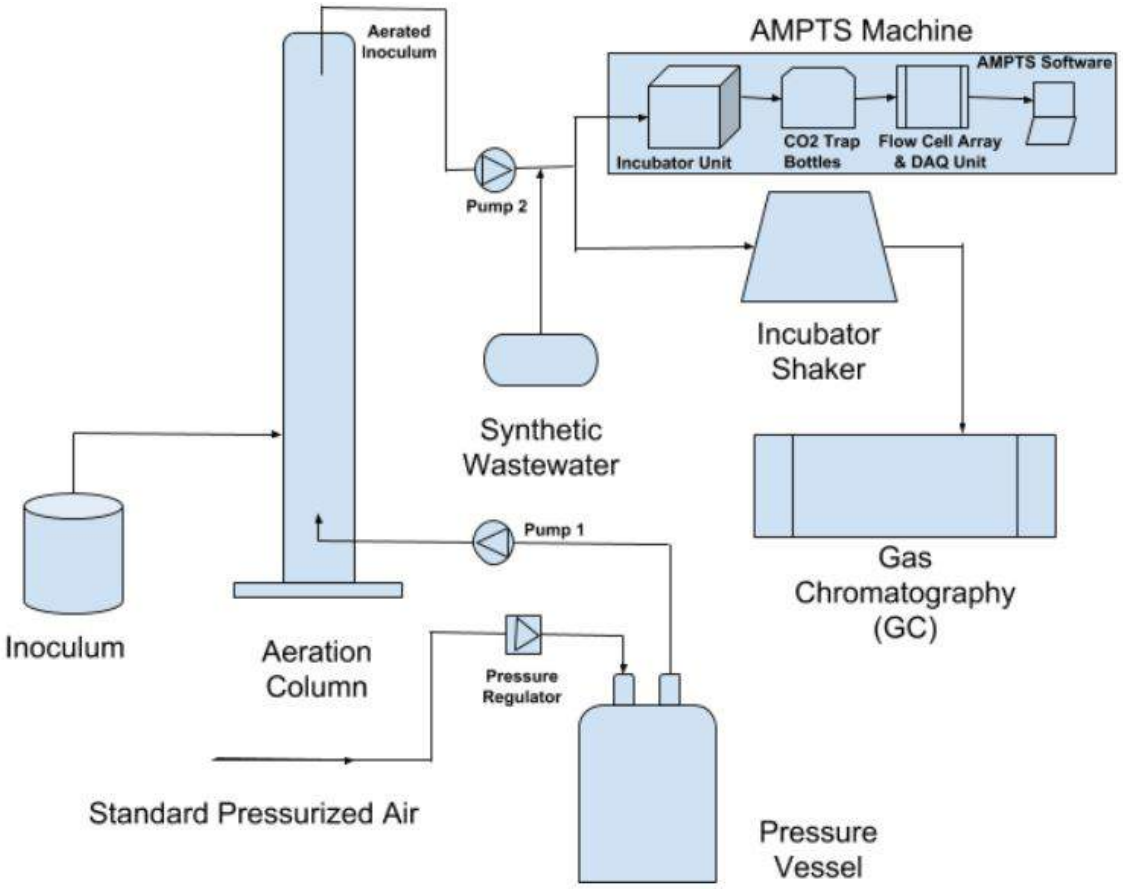

Figure 3.1: Schematic diagram of the overall experiment.

3.3.1. Micro-bubble aeration of the Sludge

Figure 3.2 shows the laboratory setup for aerating the sludge with 3 and 5 bar pressurized "white water", which is when water appears white because of the presence of pressurized micro-bubbles in it (USGC, 2016). 3 and 5 bar was chosen because literature suggests that if pressure is increased more than 5 bar, the size of the micro-bubble shows no effect (De Rijk et al., 1994). The dissolved oxygen (DO) in the pressure vessel can not be measured directly, therefore, Henry's law is used to calculate the equilibrium oxygen concentration (Henry, 1803) given by the equation;

$$C_{O2} = \frac{P_{O2}}{KH_{O2}} \tag{3.1}$$

Where, C_{O2} is the solutions oxygen concentration, P_{O2} is oxygen's partial pressure and KH_{O2} is the taken Henry's law constant. If we assume that the oxygen concentration in the air is taken as 21% and for atmospheric conditions at 20°C, the concentration of oxygen

for non-aerated sludge (at 0 bar taken as 1 atm pressure) comes out to be;

$$C_{O2} = \frac{1(atm) * 0.21 * 1600(mg/mol) * 2mol}{769.23(Latm/mol)} = 8.73mg/L \tag{3.2}$$

Similarly, for 3 bar (taken as 4 atm pressure) and 5 bar (taken as 6 atm pressure) the concentration of oxygen is given in Table 3.3.

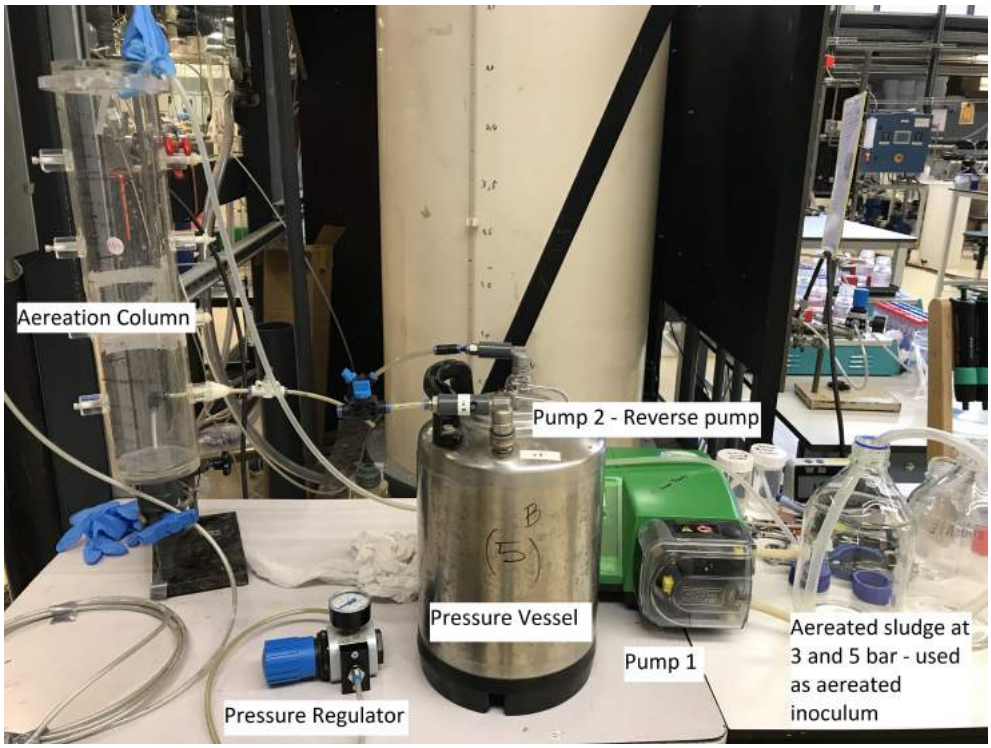

Figure 3.2: Pictorial layout of the setup made for aerating the sludge at 3 and 5 bar with pressurized white water.

Pressure (bar)	Oxygen Concentration (mg/L)
0	8.73
3	35.00
5	53.00

Table 3.3: The calculated oxygen concentrations of 0 bar, 3 bar and 5 bar sludges.

After the collected anaerobic sludge is sieved and incubated, it is aerated in a single column reactor in 2 runs - first using 3 bar pressurized water and the second using 5 bar. The pressurized water for the 2 runs was prepared in 2 different sealed pressure vessels with provision for inflow and out flow connections (Thielmann stainless steel pressure containers). The standard pressurized air inlet provided at the lab was connected to a pressure regulator (FESTO Pressure regulator LR/LRS) which was then connected via a valve (Festo HE Series Pneumatic Manual Control Valve, PBT) to the inlet of the pressure vessel containing 5 liters of tap water. The outlet closed using a valve.

Next, the pressure regulator was set to the required pressure - 3 bar for the first vessel. The valve for the standard pressurized air was opened along with the inlet valve for the pressure vessel. Thus, the standard pressurized air gets compressed to the required pressure of 3 bar by the pressure regulator ans then flows to the pressure vessel containing the tap water. This pressurized air is allowed to flow for 5 - 10 minutes and then the pressure vessel inlet valve is closed. The pressure vessels is then mixed vigorously be hand for 30 minutes to make sure the water present in it is pressurized equally and the oxygen concentration in the water had reached equilibrium. This was done on the advise of the supervisor as literature regarding the kinetics of pressuring water could not be found. The same procedure was repeated to pressurize another pressure vessel containing 5 liters to 5 bars.

After preparing the 2 pressurized water vessels, a 4 liter column reactor was used to aerate the sludge. First, the 3 bar pressurized vessel was connected to the inlet at the right bottom side of the column using a standard valve and a needle valve. 1.5 liter of the sludge was added to the column, flushed with nitrogen gas for 2 minutes and sealed from the top to prevent oxygen form entering the reactor. 1.5 liters of 3 bar pressurized water from the pressure vessel was then injected into the reactor by a pump (Watson Marlow 520s) at 110 rpm to the bottom inlet of the reactor. The valve from the outlet of the pressure vessel is opened, keeping the inlet valve closed. When the total volume in the reactor reached 3.8 liters, the pump was stopped and reversed to pump out 1.4 liters of the top part (supernatant) of the reactor. Next, the reactor was emptied, washed with tap water and the process was repeated for 5 bar of pressurized water. The 3 and 5 bar pumped supenatants were stored in glass bottles at 4°C, to be used in the AMPTS machine. Samples (10 ml) from the 5 bar aerated sludge and it's supernatant were also collected for particle size distribution analysis.

3.3.2. Bio-methane Potential(BMP) test using AMPTS and Incubator Shaker

Two experiments were run in parallel to measure the methane volume produced from the 3 different sludge used as inoculum (0, 3 and 5 bar). Automatic Methane Potential Test System (AMPTS) II manufactured by bioprocess control and the incubator shaker (Innova 44 Large-Capacity, Floor-Stackable Incubator Shaker from Eppendorf) gas-chromotographic method was used to measure the methane volume for the non-aerated (0 bar), and aerated (3 and 5 bar) sludge.

As there were 3 different incoculums for which the methane potential was to be determine in triplicate (cellulose, negative controls and samples for 0 bar sludge were taken in duplicate due to space constraints in the AMPTS machine), 18 bottles (500ml) for measuring the methane potential were prepared. Where the negative controls corresponds to the methane produced in the background only by the inoculum and cellulose represents the positive control (Holliger et al., 2016). Since the AMPTS machine only fits 15 bottles the remaining 3 bottles were measured in the incubator shaker based on the gas-chromatographic method, as their BMP was already known. Figure 3.3 shows the plan view of the arrangement of the bottles prepared. (USGC, 2016).

The substrate to inoculum ratio was taken as 1:2 for the BMP test Based on this ratio, half of the total volume (400 ml) of the bottles were taken as incoculum (200 ml). Therefore, the total VS of the total bottle was calculated by multiplying the VS of the sludge with the total volume of the inoculum taken (200 ml) for each different bottle prepared. Next, based

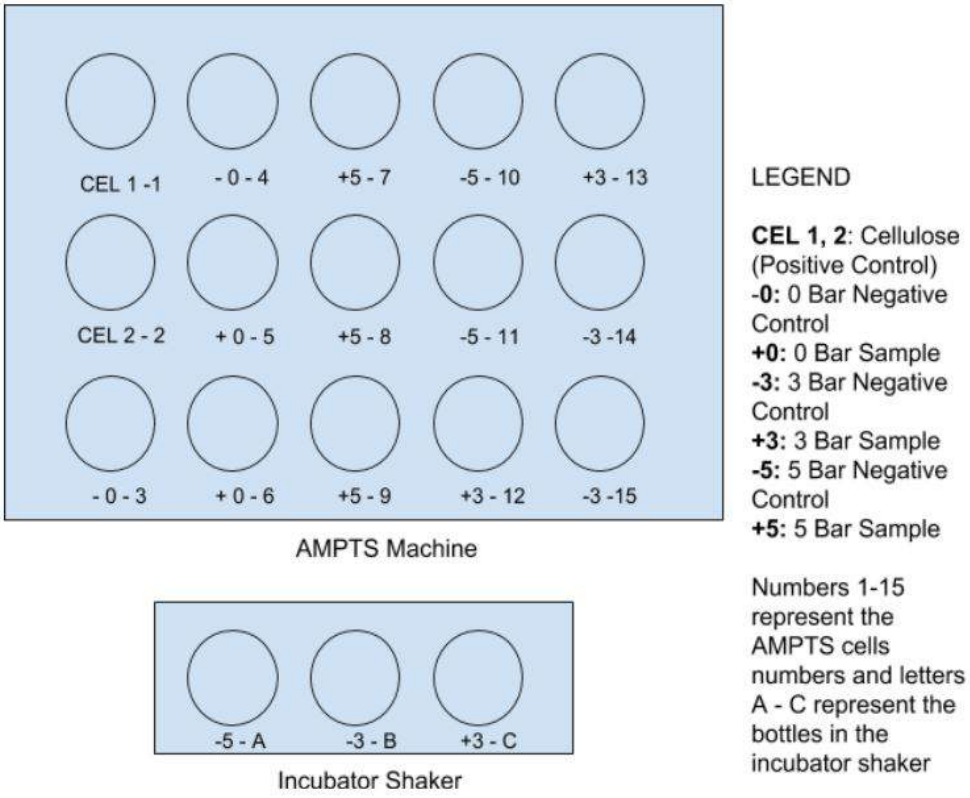

Figure 3.3: Plan view of the scheme of bottles placed in the AMPTS machine

on the inoculum to substrate ration taken as 1:2, the substrate mass (g) for each bottle was calculated. Finally the remaining volume was filled with demi-water to reach the total volume of 400 ml in each bottle. Table 3.4, 3.5, 3.6 gives the components and amounts of each of the 18 bottles prepared.

I/S Ratio (by VS)	2						
VS of Inoculum (w/w)	0.0166						
VS of Cellulose (w/w)	0.95						
VS of C (w/w)	0.5						
Total Volume (mL)	400						
Bottle No.	Sample Type	Total Volume	Inoculum Volume (mL)	Inoculum VS (g)	Substrate VS (g/g)	Substrate mass (g)	Demiwater (mL)
-0	negative control	400	200	3.32	0.00	0.00	200.00
-0	negative control	400	200	3.32	0.00	0.00	200.00
+0	Substance C	400	200	3.32	0.50	3.32	196.68
+0	Substance C	400	200	3.32	0.50	3.32	196.68
Cel 1	Positive Control	400	200	3.32	0.95	1.75	198.25
Cel 2	Positive Control	400	200	3.32	0.95	1.75	198.25

Table 3.4: Materials and their amounts used to prepare the 0 bar 500 ml bottles for the AMPTS machine.

The elements of the AMPTS machine were prepared and operated as per the user manual (Bioprocess Control, 2016) which automatically measured the methane production of 15 bottles prepared and the remaining 3 bottles measured methane potential based on the gas-chromatography method. The procedure followed for the 3 bottles was based on the method specified by Angelidaki et al. (Angelidaki et al., 2009) under data collection. Furthermore, the pH of the various bottles was recorded as it make sure each bottle prepared

I/S Ratio (by VS)	2						
VS of Inoculum (w/w)	0.00054						
VS of Cellulose (w/w)	0.95						
VS of C (w/w)	0.5						
Total Volume (mL)	400						
Bottle No.	Sample Type	Total Volume	Inoculum Volume (mL)	Inoculum VS (g)	Substrate C VS (g/g)	Substrate C mass (g)	Demiwater (mL)
-3	negative control	400	200	0.108	0.00	0.00	200.00
-3	negative control	400	200	0.108	0.00	0.00	200.00
-3	negative control	400	200	0.108	0.00	0.00	200.00
+3	Substance C	400	200	0.108	0.50	0.11	199.89
+3	Substance C	400	200	0.108	0.50	0.11	199.89
+3	Substance C	400	200	0.108	0.50	0.11	199.89

Table 3.5: Materials and their amounts used to prepare the 3 bar 500 ml bottles for the AMPTS machine.

I/S Ratio (by VS)	2						
VS of Inoculum (w/w)	0.0032						
VS of Cellulose (w/w)	0.95						
VS of C (w/w)	0.5						
Total Volume (mL)	400						
Bottle No.	Sample Type	Total Volume	Inoculum Volume (mL)	Inoculum VS (g)	Substrate C VS (g/g)	Substrate C mass (g)	Demiwater (mL)
-5	negative control	400	200	0.64	0.00	0.00	200.00
-5	negative control	400	200	0.64	0.00	0.00	200.00
-5	negative control	400	200	0.64	0.00	0.00	200.00
+5	Substance C	400	200	0.64	0.50	0.64	199.36
+5	Substance C	400	200	0.64	0.50	0.64	199.36
+5	Substance C	400	200	0.64	0.50	0.64	199.36

Table 3.6: Materials and their amounts used to prepare the 5 bar 500 ml bottles for the AMPTS machine.

had the pH > 7 and 8.5 (Holliger et al., 2016). Figure 3.4 shows the experimetnal set up for the AMPTS machine.

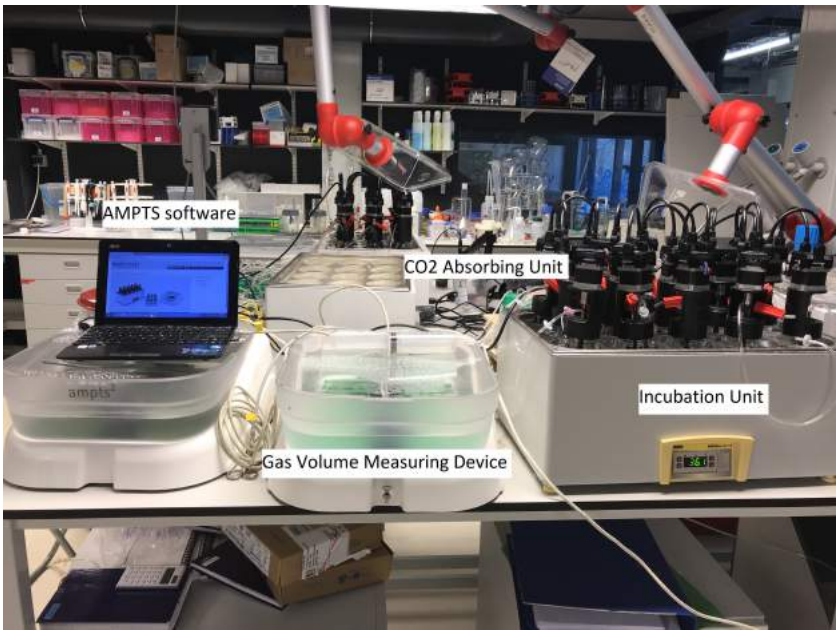

Figure 3.4: Experimental setup of the AMPTS machine

3.3.3. Bio-gas composition using Gas Chromatoraphy (GC)

Apart from the 18 (500ml) bottles for the AMPTS, 2 250 ml bottles were also prepared to measure the biogas composition of the 3 and 5 bar aerated inoculums. Table 3.7 gives the quantity of substrate and inoculum used in the bottles. Their amounts were calculated based on the same calculations done for the AMPTS machine bottles. The bottles were

Bottle No.	Sample Type	Total Volume	Inoculum Volume (mL)	Inoculum VS (g)	Substrate C VS (g/g)	Substrate C mass (g)	DW (mL)
5 Bar	Biogas Composition	150	150	0.48	0.5	0.48	99.52
3 Bar	Biogas Composition	150	82	0.04428	0.5	0.04428	167.9557

Table 3.7: Materials and their amounts used to prepare the 5 bar and 3 bar 250ml bottles for analyzing biogas composition

placed in the incubator shaker and samples for GC analysis were taken in intervals. 10 ml gas sample was extracted using a syringe from the head space of the bottles and their volume recorded. To ensure the samples were a good representation of the biogas in the bottles, the 2nd or 3rd sample extracted were used in the GC machine (by Agilent Technologies) to analyze the biogas composition. Figure 3.5 shows the samples in the incubator shaker for which the GC analysis was done.

Figure 3.5: Samples for methane production (3 - 500 ml bottles) and for biogas production (2 -250 ml bottles) in the incubator shaker.

3.4. Determining the experiment's end point

As recommended in Holliger et al. (Holliger et al., 2016), the duration of the BMP test was not set in advance. When the daily methane production was found to be <1% of accumulated methane volume for 3 consecutive days, the experiment was stopped. The experiment was started on 7th December, 2018 and terminated on 16th January 2019.

3.5. Particle Size Distribution

The 0 bar, 5 bar aerated and 5 bar supernatant samples were tested to analyze the effect of aeration on particle size distribution. The Bluewave – Particle Size Analyzer (PSA) from Microtrac was used for this analysis. The procedure followed was based on the Bulewave particle size analyzer user manual (Microtrac, 2019).

3.6. Additional Specific Methanogenic Activity (SMA) Experiment

A similar aeration run was additionally done in a larger column (30 Litres) to improve the separation efficiency and determine the SMA. Figure 3.6 and 3.7 give the respective experimental setup and column separation observed during the experiment. The set up was similar to the one done in the the main experiment mention in section 3.3.1 for this study. However it should be noted that the pressure of white water was always kept at 5 bar and the sludge used here was coagulated and flocculated sludge from the same wwtp, as compared to anaerobic sludge used in the previous experiment. Furthermore, both the sludge and white water were introduced together from the inlet with the pump kept at 25 rpm. The other crucial change to observe here is that the inlet is inverted (as compared to the initial experiment). More details of the experimental layout and other specification of this experiment are given in appendix IV.

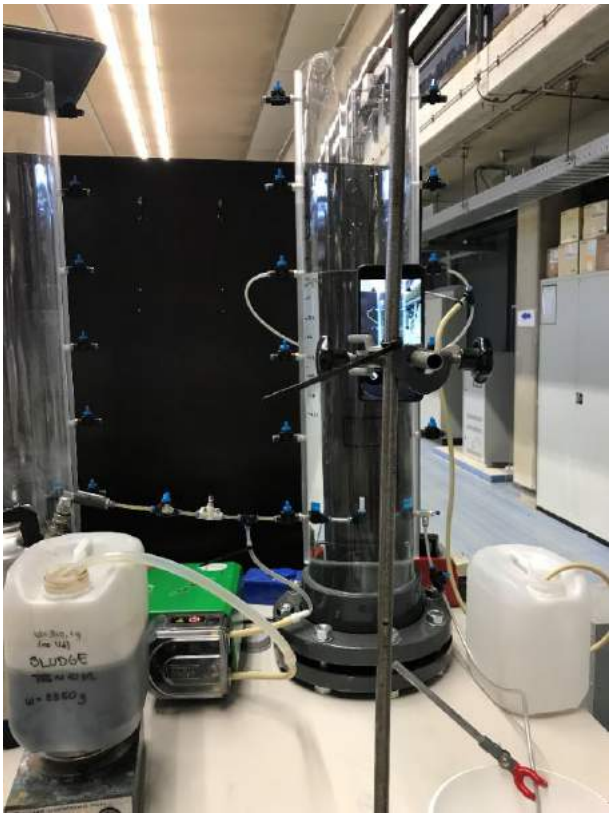

Figure 3.6: Experimental setup for the additional aeration experiment run to improve separation efficiency.

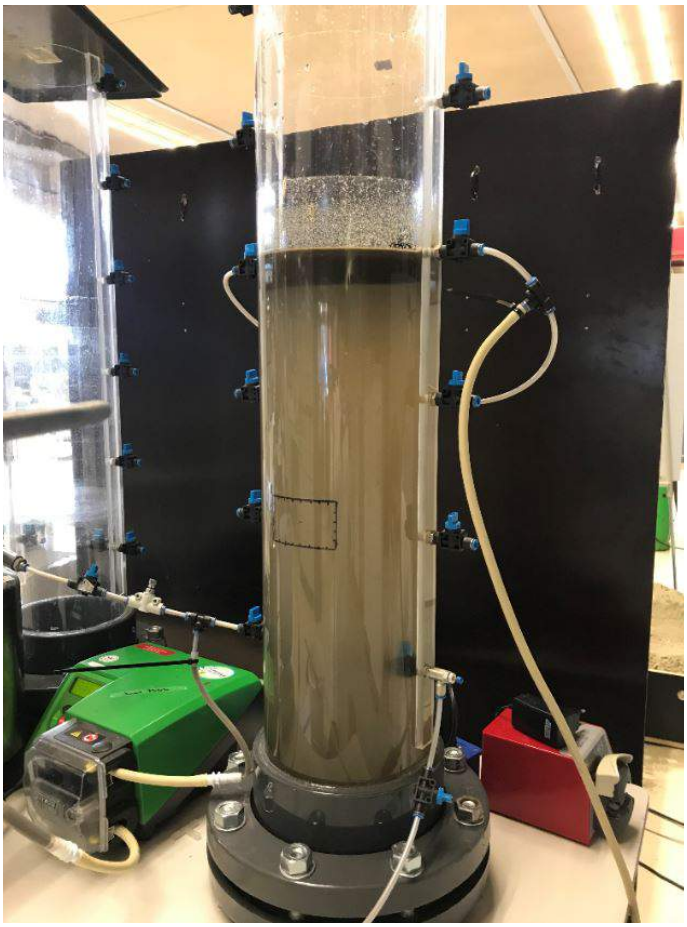

Figure 3.7: Particle separation observed in the column after the aeration.

4

Results and Discussions

4.1. Bio-methane Quantity

For the 15 bottles placed in the AMPTS machine, the cumulative methane production was automatically calculated. The methane production for the 3 bottles placed in the incubator shaker, were calculated manually using the values obtained from the GC machine and values of gas extracted. Appendix 1 gives the calculations done for the bottles in the incubator shaker. Figure 4.1, 4.2 and 4.3 show the cumulative methane production in triplicate of the 0 bar, 3 bar and 5 bar samples along with their positive and negative controls. Figure

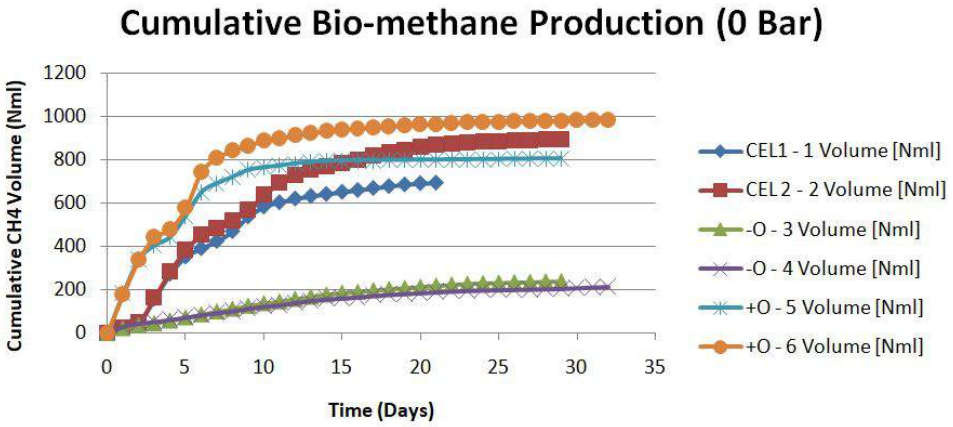

Figure 4.1: Cumulative methane production by the 0 bar substrate, blank and positive controls.

4.4 and 4.5 show a zoomed in image of the values obtained for the 5 and 3 bar cumulative substrate and negative control values.

4.1.1. Cumulative Methane Production and Maximum BMP

Comparing first 3 graph, it can be observed that the cumulative methane production by the substrate bottles is the maximum for 0 bar (891.80 Nml), lesser for 5 bar (182.90 Nml) and almost negligible for the 3 bar substrate bottles (36.80 Nml). According to Holliger et.al. (Holliger et al., 2016), BMP should be expressed as dry volume of methane gas under standard temperature (273 K) and pressure (101 kPa) per VS added. For this study, this is

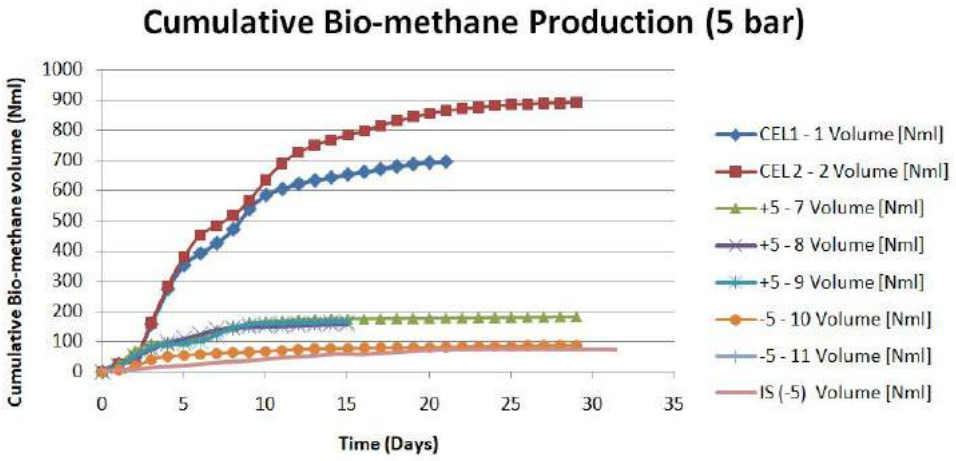

Figure 4.2: Cumulative methane production by the 5 bar substrate, blank and positive controls.

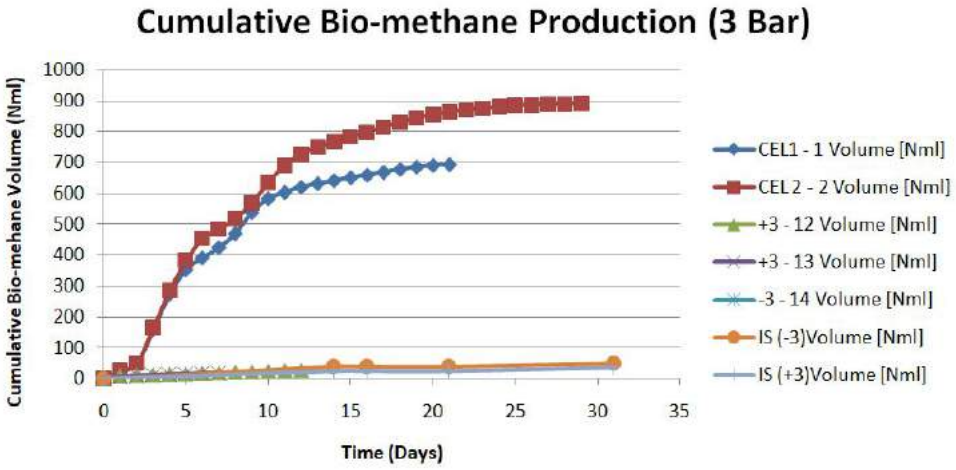

Figure 4.3: Cumulative methane production by the 3 bar substrate, blank and positive controls.

calculated by dividing the methane produced by 0, 5 and 3 bar substrates by the VS that was added. Furthermore, it mentions that the value of the substrate/positive control are determined by subtracting the methane production of the blanks from the total production. Also, the standard deviation of the blanks should be considered. This is given by the formula (Holliger et al., 2016);

$$BMP_{substrate/control} = BMP_{average,substrate/control} +/- \sqrt{(SD_{blank})^2 + (SD_{substrate/control})^2} \tag{4.1}$$

Table 4.1 gives values of the maximum cumulative methane produced and the maximum BMP values calculated for the 0, 3 and 5 bar samples based on the above equations. Appendix III give the data from the AMPTS machine and incubator shaker. These values show that the bio-methane production of the non-aerated (0 bar) sludge is more than the aerated (5 bar) the methane production per VS and the cumulative methane production for 0 bar produces almost 5 times more methane.

A reason for lower BMP value for the 5 bar aerated sludge could be because of the inefficient separation in the coulumn due to improper functioning of the bubble generating noz-

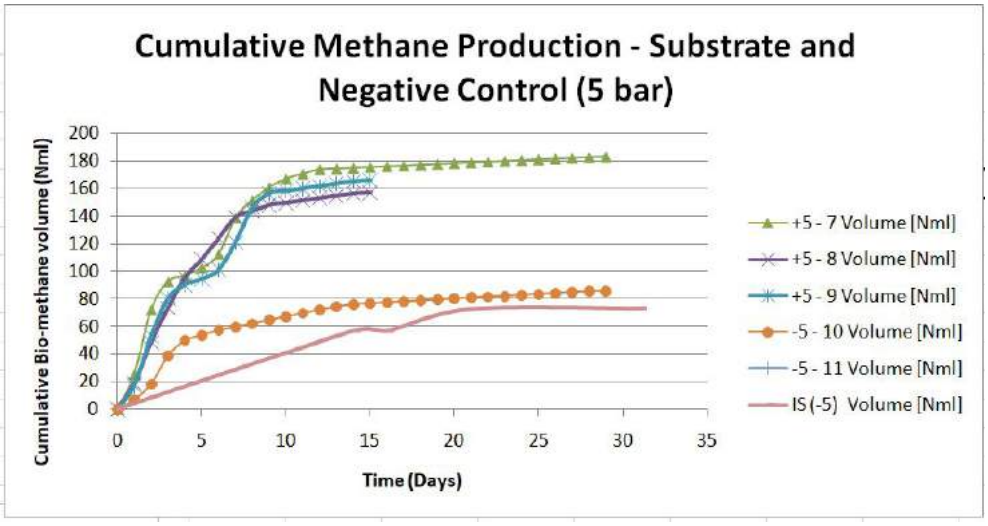

Figure 4.4: Zoomed in image of cumulative methane production by the 5 bar substrate and negative control.

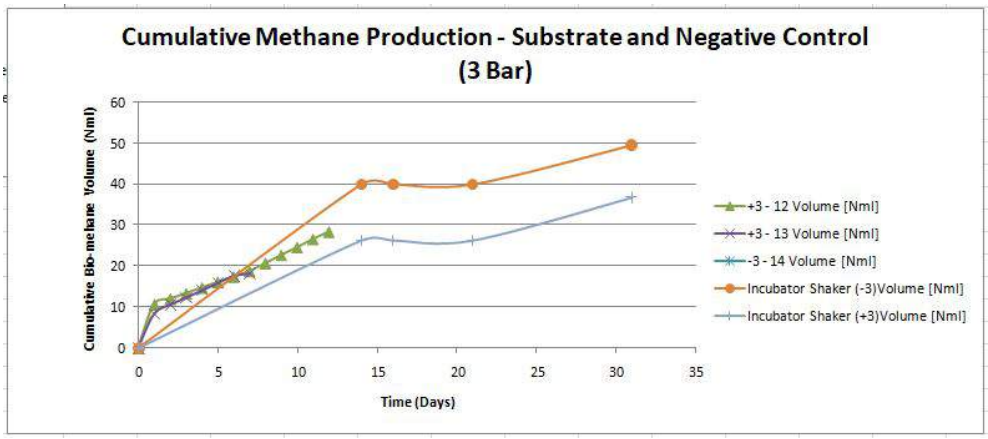

Figure 4.5: Zoomed in image of cumulative methane production by the 3 bar substrate and negative control.

Sample (bar)	Sample name	Maximum Cumulative Methane Production (Nml)	Maximum BMP (NL(CH4)/Kg (VS))	Relative Standard Deviation (RSD) (%) = (SD/Average)	VS added (g)
0	+ 0	983.30	268.84 ± 126.40	13.8	3.32
0	CEL (Positive Control)	891.80	382.69 ± 123.70	13.5	3.32
3	+ 3	40.56			0.11
5	+ 5	182.9	151.85 ± 9.15	5	0.64
5	CEL (Positive Control)	891.8	460.63 ± 121.06	13.5	3.32

Table 4.1: Maximum cumulative methane production values and maximum BMP values.

zle. This uneven separation of the anaerobic sludge by the air micro-bubbles might have led to a diluted supernatant, which was eventually used as inoculum for the BMP analysis. Because of this dilution, during the BMP test there could have been more substrate and less inoculum present, leading to an inefficient consumption of the substrate.

According to Holliger et al. (Holliger et al., 2016), the Relative Standard Deviation (RSD) for blanks/positive control should be < 5 and for a heterogeneous substrate < 10 %. Furthermore, the BMP of the positive control should be < 85% and > 100% of theoretical BMP

(for cellulose it should be < 352 $NL_{CH4}Kg_{VS}^{-1}$ and > 414 $NL_{CH4}Kg_{VS}^{-1}$. Based on the data obtained in table 4.1 it can be seen that these values are not within the limit for this study which can be attributed to the over dilution of the aerated sludge and for the cellulose, the reason could be because of using an old batch for this experiment. Preferably a fresh batch of cellulose should be used for further experiments.

The values for 3 bar aerated sludge were disregarded as there wasn't sufficient data to verify the BMP of the substrate as shown in the zoomed in image in figure 4.5. It can be observed here that the negative control is higher than the substrate curve, which renders this data unreliable. A possible reason for this could be that the cells in the AMPTS machine which were allotted to measure the methane production for the 3 bar samples and their negative controls (cells 11-14) were not functioning well which can be seen from the AMPTS data in appendix III. Thus, the data obtained from these cells is unreliable. Furthermore, the sludge source and substrate to inoculum ratio has a significant impact on the methane production (Elbeshbishy et al., 2012). Therefore, as per table 3.6 it can be observed that these values for the 3 bar aerated sludge is on the lower side.

BMP test results can further be used to get information about the substrate such as the hydrolysis rate and ultimate methane potential (Angelidaki et al., 2009).

4.1.2. Specific Methanogenic Activity (SMA)

As per the additional SMA test conducted using the AMPTS machine, the SMA values for 5 bar and 0 bar incoulums, with cellulose and acetate as substrates. Cellulose was added to indicate the overall activity of the samples, whereas acetate was added to give an idea about the methonogenic activity. After running the experiment for 5 days the following graph was observed. By measuring the slope of this graph and subtracting the slope of the blank the

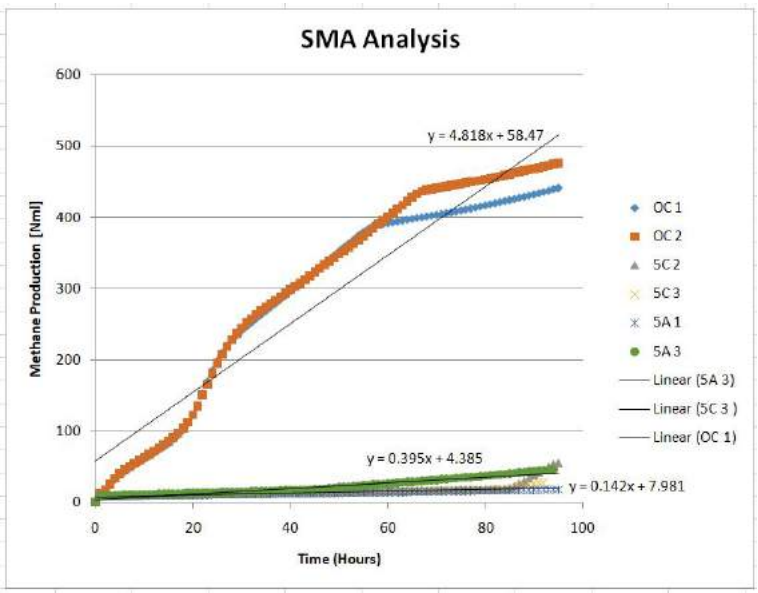

Table 4.2: Graph depicting the cumulative methane values producted and the slope of the graph for 0 bar, 5 bar cellulose and 5 bar acetate samples.

maximum rate of methane production was calculated. The equation given below is used to

calculate the maximum methane production based on COD(Nielfa et al., 2015);

$$COD_{CH4} = n_{CH4} * 64 g mol^{-1} day^{-1} \tag{4.2}$$

Here, COD_{CH4} is the amount of COD in grams and n_{CH4} the methane volume in moles. Based on this equation and the VS values obtained for 0 and 5 bar cellulose and 5 bar acetate their respective SMA's come out to be 3.05 $gCOD/gVSd$, 0.66 $gCOD/gVSd$ and 0.23 $gCOD/gVSd$ respectively. The data for these values are given in appendix IV. This shows that the SMA of the inoculum which was aerated is less indicating the rate of methane production is lesser when the sludge is aerated as compared to non-aerated sludge. We can also observe here that the rate of methane production is slightly more for 5 bar samples with cellulose and is least for the samples with 5 bar acetate. This shows that the overall rate of methane production is more as compared to for just the methanogenesis as acetate is substrate for acetoclastic methanogens and cellulose could have produced hydrogenotrophic methanogens which can consume H_2.

4.1.3. BMP rate: Kinetics

Anaerobic digestion rates have conventionally been estimated using BMP data based on methane production values. Another way to study anaerobic biodegerdation is by undertaking kinetic studies including process inhibitions (Raposo et al., 2011). Growth rate and other kinetic parameters for the microbial processes in anaerobic processes can be determined using Monod kinetic equations (Barthakur et al., 1991) and Gompertz model (Matheri et al., 2016).

4.2. Bio-gas Composition

Table 4.2 shows the schedule and total amounts of the sampling done to estimate the biogas composition and table 4.3 gives the percentages of methane and carbon dioxide recorded in the 3 and the 5 bar 250 ml bottle samples. The values obtained from the GC were

Components	Total gas extracted (ml)	Day 9	Day 14	Day 16	Day 21	Day 31	Day 34
BGC 3	40	20	0	0	0	30	40
BGC 5	120	70	90	100	0	110	120

Table 4.3: Schedule and amounts of 3 and 5 bar samples extracted for GC analysis

Pressure	Composition	Day 14	Day 31	Day 34	Day 40
5 bar	CO2	15.61%	52.54%	53.15%	28.80%
	O2	0.18%	1.71%	2.60%	0.45%
	CH4	84.21%	45.75%	44.24%	70.74%
3 bar	CO2	14.30%	16.84%	18.89%	
	O2	3.92%	2.54%	3.24%	
	CH4	81.79%	80.61%	77.87%	

Table 4.4: Percentage of methane and carbon dioxide recorded in the 5 and 3 bar samples over time.

recalculated to remove errors from peaks due to nitrogen and other minor gasses as well

as human errors such as valve adjustment. As nitrogen was only used to flush the bottles to simulate anaerobic conditions the peaks obtained due to nitrogen were disregarded.

It can be observed from the data that the 5 bar aerated sludge generated almost 3 times more volume of methane than the 3 bar sludge. Another observation that can be made that the first sample recorded for the biogas composition was on day 14 of the experiment, when most of the initial methane had already been produced. This can be inferred as on day 14, the percentage of methane is 84% and 81% respectively for the 5 bar and 3 bar sludge, and this trend continues till day 40. If samples would have been recorded before day 14, it is possible that the methane fraction would have increased and CO_2 would have decreased.

It is suggested in literature that aerated samples might produce less relative percentage of methane and more of CO_2 compared to non-aerated samples, suggesting that micro-aeration could result in lesser methane production and more CO_2 production due to oxygen favouring respiration and resulting in COD getting partially oxidized to CO_2 (Botheju and Bakke, 2011). However, to verify this additional biogas composition studies should be carried out to with aerated and non-aerated sludge and biogas samples should be taken regularly during the first few days of the experiment. The effect of the presence of hydrogen sulphide due to air micro-bubbles was not estimated due to lack of data in the GC samples taken. Some studies have found reduced hydrogen sulphide concentrations in biogas due to this. (Botheju and Bakke, 2011), (Lim and Wang, 2013), (Johansen and Bakke, 2006). Further studies are required to estimate this effect.

Figure 4.6 and 4.7 depict the gas percentages of the aerated 5 and 3 bar sludge GC analysis done.

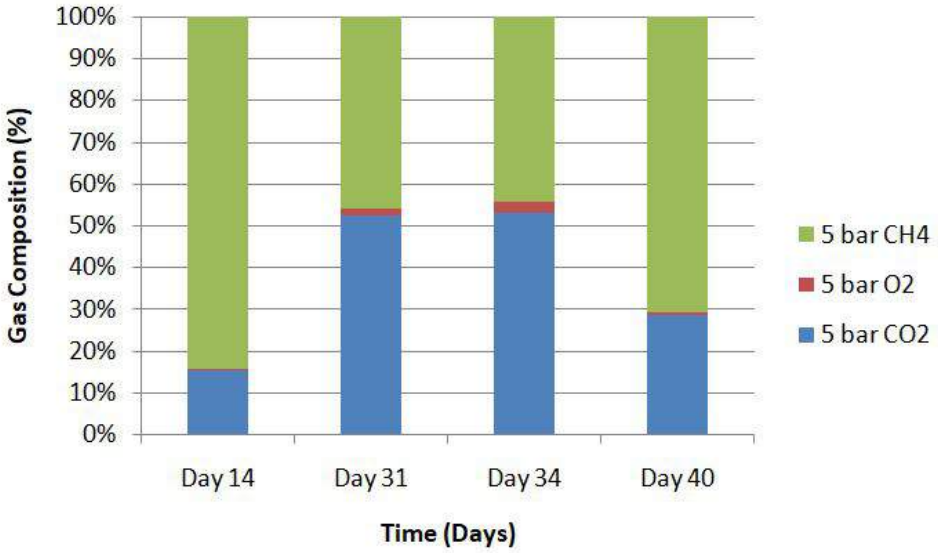

Figure 4.6: Percentage distribution of the gas composition analysis done for the 5 bar sample in the GC.

Here it can be seen that the majority of the gas in methane with the the rest being carbon dioxide and some small quantities of oxygen being observed as well. Nitrogen values were corrected for as it was only used for flushing the bottles for anaerobic conditions. The small quantity of oxygen is because of the aeration done in to the sludge. The trend seems

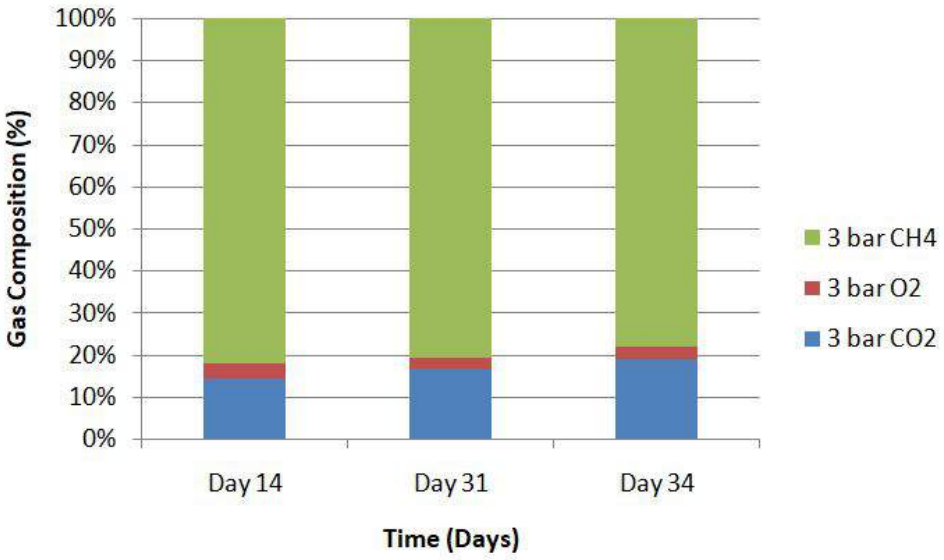

Figure 4.7: Percentage distribution of the gas composition analysis done for the 3 bar sample in the GC.

to be mostly consistent after day 14 with some differences in day 31 and 34 for the 5 bar sample. This could be due to human error or mistakes made during the GC analysis. Moreover, for a more robust analysis of the gas composition, GC analysis should be done for both the aerated (5 and 3 bar) and non-aerated sludge (0 bar). Also, regular measurements should be taken for the first few days after initialization of the experiment based on initial theoretical estimations made regarding the trend of the gas production.

4.3. Particle Size Distribution

Samples of the non-aerated (0 bar) and 5 bar aerated sample (supernatant and non-supernatant) were analyzed for particles size distribution. Table 4.8 gives the overall comparison between the various parameters of the samples. Appendix B give the reports obtained form this analysis.

It can be observed from this analysis that the 5 bar aerated suparanatant is smaller in size than the non-aerated sludge, whereas the aerated 5 bar non-suparnatant particle size is bigger. Here, from the D90 values of the aerated 5 bar supernatant and non-supernatant it can be seen that there is not much difference between the size of the particles being separated by aeration. This will result is a inefficient separation efficiency as most of the supernatant would be diluted, leading to flawed inocula for the BMP analysis. Furthermore, based on the values of mean volume between the aerated 5 bar supernatant and aerated 5 bar non-supernatant, it can be calculated that there is around 10% volume separation due to aeration. In other studies, this separation has been close to 97% (Tulleken, 2018), however it is to be noted that these studies were carried out with a higher retention time and additional flocculant input. Thus, it can be recommended that longer retention times and addition of flocculant can lead to better separation efficiency during aeration , potentially resulting in better BMP values for the aerated sludge.

Parameter	Component	0 bar	5 Bar Supernatant	5 Bar Non-Supernatant
Volume	Mean Volume (µm)	60.09	55.60	66.16
	Standard Deviation	38.14	34.03	41.14
	D10 (µm)	123.00	110.20	135.00
	D50 (µm)	45.32	42.55	47.83
	D90 (µm)	16.64	15.70	17.86
	Peak Dia (µm)	45.32	42.55	47.83
	Peak Width (µm)	76.28	68.06	82.27
Number	Mean Number (µm)	11.39	11.19	13.16
	Standard Deviation	5.82	5.60	6.50
	D10 (µm)	21.08	20.45	23.87
	D50 (µm)	8.25	8.21	9.81
	D90 (µm)	5.13	5.13	6.10
	Peak Dia (µm)	8.25	8.21	9.81
	Peak Width (µm)	11.64	11.12	13.00
Area	Mean Area (µm)	33.26	31.31	35.80
	Standard Deviation	21.18	19.95	22.05
	D10 (µm)	66.06	61.98	69.66
	D50 (µm)	24.56	23.32	26.50
	D90 (µm)	8.64	8.37	9.79
	Peak Dia (µm)	24.56	23.32	26.50
	Peak Width (µm)	42.37	39.90	44.10

Figure 4.8: Comparison obtained from the PSD machine for 0 bar, 5 bar suparnatant and 5 bar non-suparnatant samples.

4.4. Separation Efficiency and Practical Applications

An important parameter to consider for this study is the aeration mechanism. It is crucial that to estimate the bio-methane potential of the aerated sludge, the separation of the sludge by the air micro-bubbles takes place in the most efficient manner. Figure 4.9 depicts the aeration set up used for this experiment, where the outlet for the pressurized water is shown. Furthermore, how the sludge and pressurized water interact is also important. As in this study, the sludge was static in the column and aerated from a inlet, it's possible the entire surface area of the sludge did not receive uniform aeration. Further experiments need to be performed to understand the most efficient way to aerate the sludge. It is possible that due to a inefficient aeration mechanism, the aerated sludge produce lesser and diluted supernatant which could have caused lower BMP output. Some more possible configurations and their effect on particle size can found in the study done by Tulleken (Tulleken, 2018).

In practice, micro-aeration for anaerobic digestion can be implemented as a Dissolved Air Flotation (DAF) setup. DAF is used to separate solids from municipal waste water, being subjected to micro-aeration before flowing into the anaerobic digester. Furthermoe, if there is higher SMA due to micro-aeration, reactor volumes can be reduced (Wang et al., 2005). However, it should be kept in mind that there are possible limitations of micro-aeration in anaerobic digestion such as explosion risk due to mixing of oxygen with methane (Krayzelova et al., 2015). This is risk is relatively low as the concentration of oxygen is generally very low. Also, it's possible that there might be partial oxidation of the

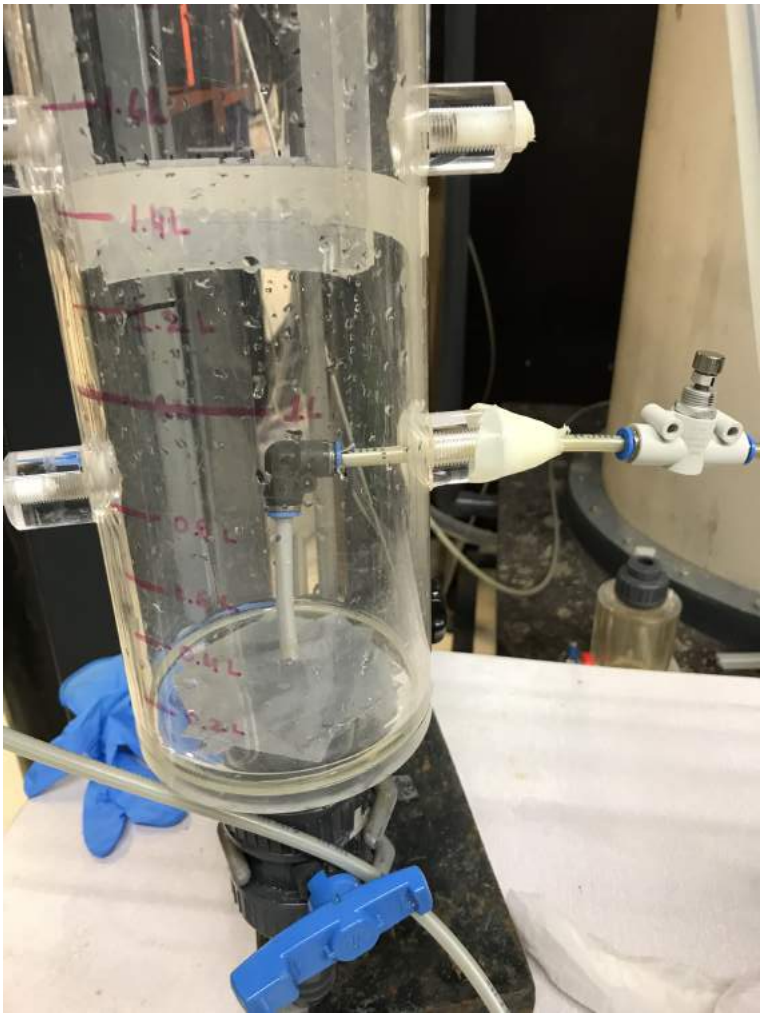

Figure 4.9: Pictorial representation of the mechanism of aeration inlet in the lab setup

substrate because of which there would be lower methane production. Hydrogen sulphide might also get oxidized into elemental sulphur and result in corrosion and clogging (Díaz et al., 2011).

5

Conclusions and Recommendation

Because of the many economical and environmental advantages of anaerobic digestion, improving it's bio-chemical processes can further accelerate it's waste water treatment capacity. A method to do so in recent times has been by introducing air micro-bubbles and investigating it's effects. This study concluded that introducing air micro-bubbles do not improve the bio-methane production in anaerobic digestion. This shows that the hypothesis of adding air micro-bubbles to anaerobic sludge to improve methane production and biogas quality could not be proved with the experimental set up and parameters considered in this study. For further research it may be a possibility to prove this hypothesis with efficient separation of particles during aeration and optimized substrate and inoculum parameters. Table 5.1 give as summary of the conclusion drawn from the 0 and 5 bar sample results obtained in the experiment done.

The results from the 3 bar substrate were discarded due to unreliable data. This can be attributed to malfunctioning of the cells in the AMPTS machine used for the 3 bar aerated sludge analysis. Moreover, the amount of substrate and inoculum added for this analysis was also on the lower side which might have led to unreliable data. For better data collection in future research, it is recommended to collect at 1.5 times more sludge from the wwtp to account for the losses during experimentation and recalculate the substrate VS to be added for better methane production. Furthermore, better AMPTS machine maintenance should be considered before starting the experiment to minimize equipment related errors.

The SMA values obtained for non-aerated (0 bar) and aerated (5 bar with cellulose and acetate substrate) sludge was calculated as 3.05 $gCOD/gVSd$, 0.66 $gCOD/gVSd$ and 0.23 $gCOD/gVSd$ respectively. We can see from these results that the rate of methane production is less for the aerated 5 bar samples, and least for the 5 bar sample with acetate as substrate. It should be noted here that the substrate for the 0 bar non-aerated sample is synthetic powdered waste water. This indicates that aeration has inhibited methanogenesis. As there is difference in the methane production between cellulose and acetate, this indicates presence of hydrogenotrophic and acetoclastic methanogens. Here the low rate shows that both these groups have been inhibited.

Parameters Compared	Conclusion
Inoculum 0 bar + substrate endogenous (no substrate) **VERSUS** Inoculums 0 bar + substrate cellulose	Maximum cumulative methane production is 200 Nml and 891 Nml respectively. This shows the difference of methane production when there is no substrate against when cellulose is taken as substrate indicating 4 times higher value when cellulose is substrate.
Inoculum 5 bar + substrate endogenous (no substrate) **VERSUS** Inoculums 5 bar + substrate cellulose	Maximum cumulative methane production is 80 Nml and 180 Nml respectively. This shows the difference of methane production when there is no substrate against when cellulose is taken as substrate indicating 2 times higher value when cellulose is substrate.
Inoculum 0 bar + substrate (wastewater) **VERSUS** Inoculums 5 bar + substrate (wastewater)	Here, the maximum methane production for the former is 891.80 Nml and a maximum BMP value is 268.84 +/- 126.40 NL_{CH4} Kg_{VS}^{-1} and for the latter is 182.90 Nml as maximum cumulative methane production and BMP value of 151.85 +/- 9.15 NL_{CH4} Kg_{VS}^{-1}. The low values for 5 bar inoculums sample could be a result of poor separation efficiency during the column aeration, leading to diluted supernatant and eventually a lower BMP value. It is recommended that to avoid such scenarios, the aeration method should be optimized by changing the inlet design, other inlet parameters or sludge – white water interaction scheme.

Figure 5.1: Summary of the conclusion drawn for the 0 bar and 5 bar results obtained in the experiment.

The biogas composition showed that more methane was produced by the aerated 5 bar sludge as compared to the aerated 3 bar sludge, and that after day 14 of the experiment the average percentage of methane produced (84% for 5 bar and 81% for 3 bar) was the most followed by carbon dioxide (16% for 5 bar and 15% for 3 bar). Small amounts of oxygen have also been observed in the aerated sludges which are a result of the aeration process. It is recommended that for an overall comparison between the aerated and non-aerated sludge, biogas composition is measured for the 0 bar sludge as well and that for a more robust gas composition trend analysis, GC measurements should be taken more regularly in the first few weeks of the experiment.

Finally, a critical parameter to consider for methane production in this experiment is the sludge interaction with the aeration mechanism. As per the particle size analysis done, it can be concluded that there is very low separation efficient of the aeration process (about 10%), which could have led to the low BMP value of the aerated 5 bar sludge. In further experiments, the pressurized water should be introduced evenly from the bottom of the reactor to make sure it's inter action with the sludge either already present in the reactor or added simultaneously, is uniform. Adding a flocculant and more retention time would also lead to better results. If there is enough availability of more sludge, multiple setups and runs are recommended.

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A

Appendix I: Cellulose and Substrate TS and VS values

Sample (no aereation)	Tray (g)	Tray + Sample (g)	Sample (g)	After 105 oven with tray (g)	Dry sample (g)	TS (g/g)	After 600 Oven with tray (g)	Dry Sample (g)	FS (g/g)	VS (g/g)
AT	22.2995	35.362	13.0625	22.633	0.3335	0.025531	22.3993	0.0998	0.007640191	0.017891
BT	23.327	36.332	13.005	23.6318	0.3048	0.023437	23.4153	0.0883	0.006789696	0.016647
CT	23.8468	36.5638	12.717	24.1258	0.279	0.021939	23.9302	0.0834	0.006558151	0.015381
									AVERAGE	0.01664
Sample (3 bar)	Tray (g)	Tray + Sample (g)	Sample (g)	After 105 oven (g)	Dry sample (g)	TS (g/g)	After 600 Oven (g)	Dry Sample (g)	FS (g/g)	VS (g/g)
1T	24.2557	35.8	11.5443	24.2636	0.0079	0.000684	24.2587	0.003	0.000259869	0.000424
2T	22.166	34.4945	12.3285	22.177	0.011	0.000892	22.1699	0.0039	0.00031634	0.000576
3T	22.8759	35.2187	12.3428	22.8886	0.0127	0.001029	22.8808	0.0049	0.000396993	0.000632
									AVERAGE	0.000544
Sample (5 bar)	Tray (g)	Tray + Sample (g)	Sample (g)	After 105 oven (g)	Dry sample (g)	TS (g/g)	After 600 Oven (g)	Dry Sample (g)	FS (g/g)	VS (g/g)
XT	21.6535	34.0149	12.3614	21.7081	0.0546	0.004417	21.6684	0.0149	0.001205365	0.003212
YT	22.868	35.1996	12.3316	22.9236	0.0556	0.004509	22.8832	0.0152	0.001232606	0.003276
ZT	23.5786	35.9761	12.3975	23.6288	0.0502	0.004049	23.589	0.0104	0.000838879	0.00321
									AVERAGE	0.003233

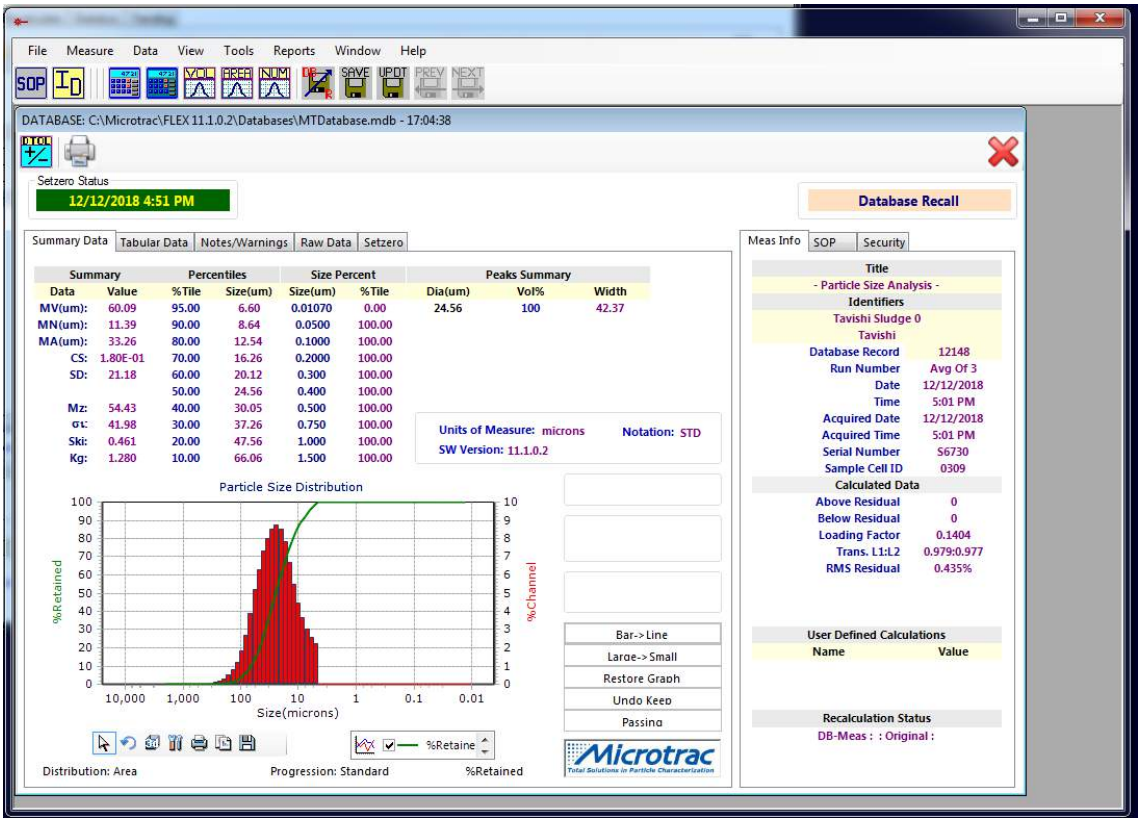
Figure A.1: Detailed calculation of TS and VS values obtained for the 0, 3 and 5 bar aerated sludge.

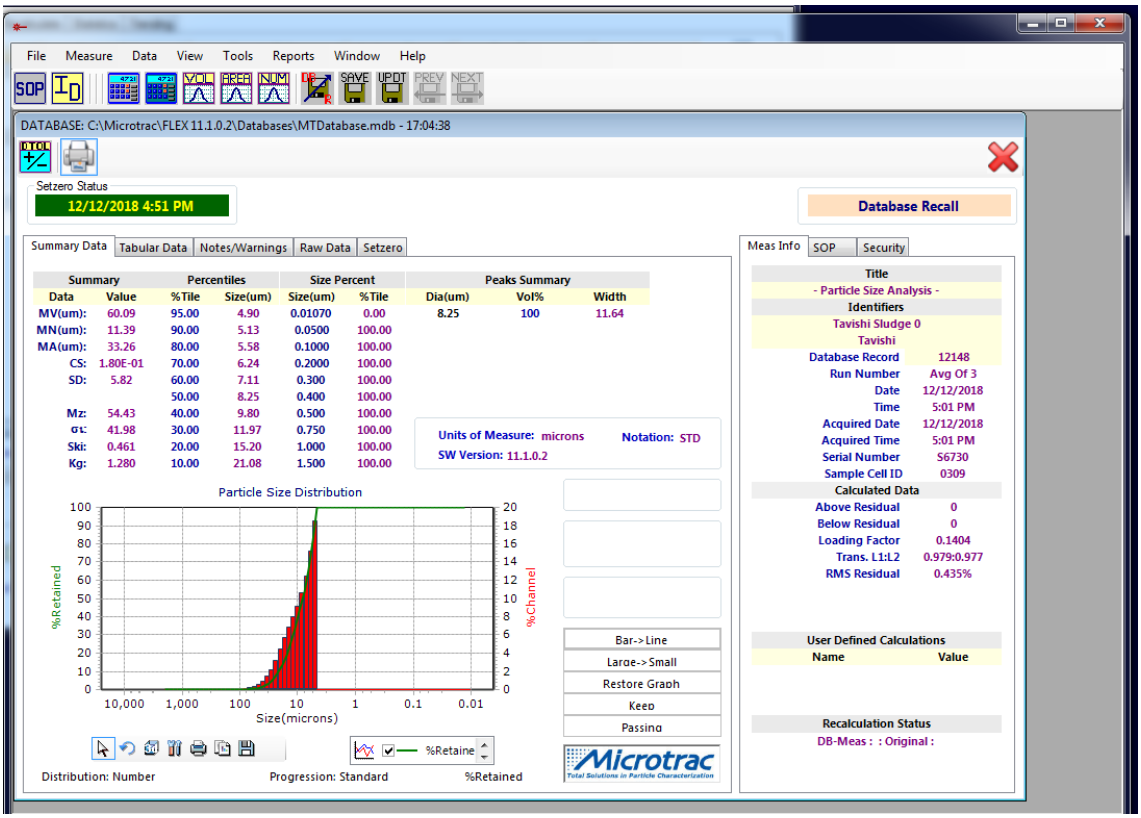
Sample (Cellulose)	Tray (g)	Tray + Sample (g)	Sample (g)	After 105 oven (g)	Dry sample (g)	TS (g/g)	After 600 Oven (g)	Dry Sample (g)	FS (g/g)	VS (g/g)
C1	23.5747	23.8715	0.2968	23.827	0.2523	0.850067	23.5751	0.0004	0.001347709	0.84872
C2	21.6548	22.6548	1	22.6086	0.9538	0.9538	21.6556	0.0008	0.0008	0.953
C3	22.8682	24.5752	1.707	24.5296	1.6614	0.973286	22.8694	0.0012	0.000702988	0.972583
									AVERAGE	0.924768
Sample (Synthetic WW)	Tray (g)	Tray + Sample (g)	Sample (g)	After 105 oven with tray (g)	Dry sample (g)	TS (g/g)	After 600 Oven with tray (g)	Dry Sample (g)	FS (g/g)	VS (g/g)
T1	18.969	19.9702	1.0012	19.6993	0.7303	0.729425	19.1922	0.2232	0.222932481	0.506492
T2	16.9163	17.919	1.0027	17.632	0.7157	0.713773	17.1146	0.1983	0.197766032	0.516007
T3	16.8547	17.8598	1.0051	17.5698	0.7151	0.711471	17.0612	0.2065	0.205452194	0.506019
									AVERAGE	0.509506

Figure A.2: Detailed calculation of TS and VS values obtained for the powdered substrate and cellulose.

A

Appendix II: Particle Size Distribution Data

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Meas Info SOP Security
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MN(um): 11.39 90.00 5.13 0.0500 100.00
MA(um): 33.26 80.00 5.58 0.1000 100.00
CS: 1.80E-01 70.00 6.24 0.2000 100.00
SD: 5.82 60.00 7.11 0.300 100.00
50.00 8.25 0.400 100.00
Mz: 54.43 40.00 9.80 0.500 100.00
σι: 41.98 30.00 11.97 0.750 100.00
Ski: 0.461 20.00 15.20 1.000 100.00
Kg: 1.280 10.00 21.08 1.500 100.00
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SW Version: 11.1.0.2
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%Channel
Size(microns)
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Large->Small
Restore Graph
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Passing
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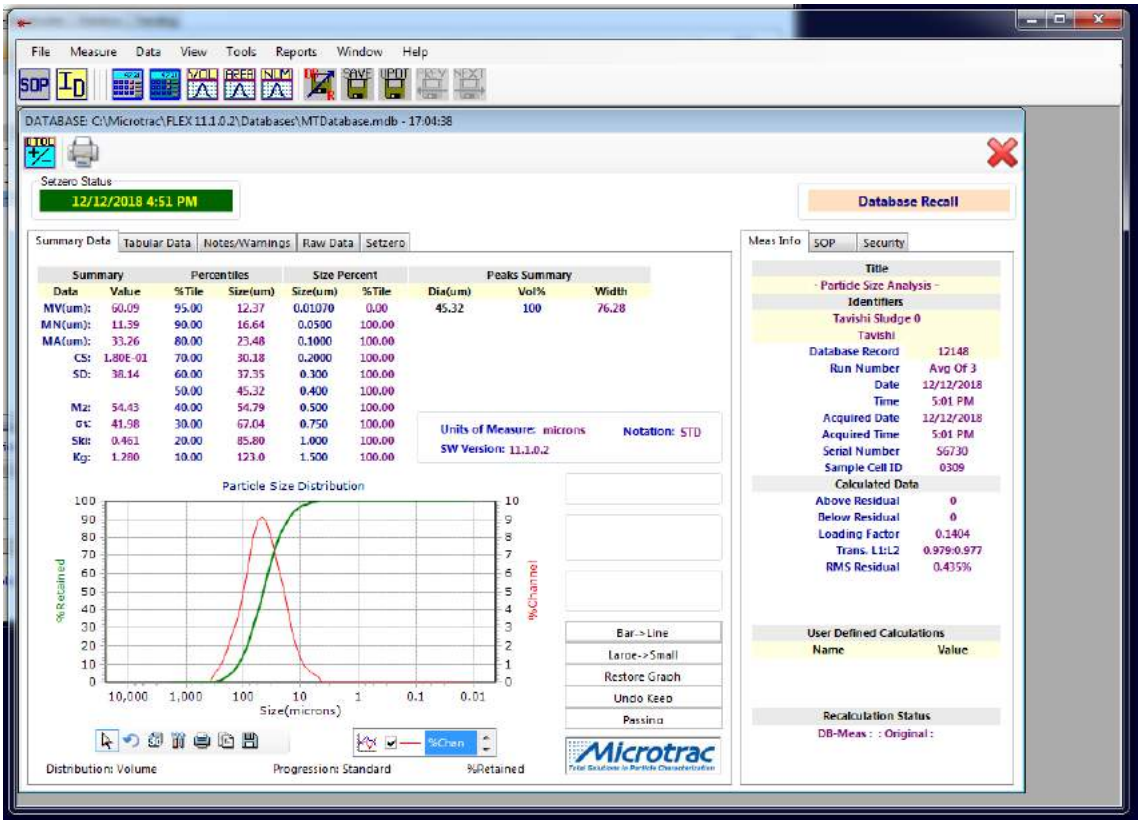
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Meas Info SOP Security
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MA(um): 33.26 80.00 23.48 0.1000 100.00
CS: 1.80E-01 70.00 30.18 0.2000 100.00
SD: 38.14 60.00 37.35 0.300 100.00
50.00 45.32 0.400 100.00
Mz: 54.43 40.00 54.79 0.500 100.00
σι: 41.98 30.00 67.04 0.750 100.00
Ski: 0.461 20.00 85.80 1.000 100.00
Kg: 1.280 10.00 123.0 1.500 100.00
Units of Measure: microns Notation: STD
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%Channel
Size(microns)
Bar->Line
Large->Small
Restore Graph
Undo Keep
Passing
Distribution: Volume Progression: Standard %Retained
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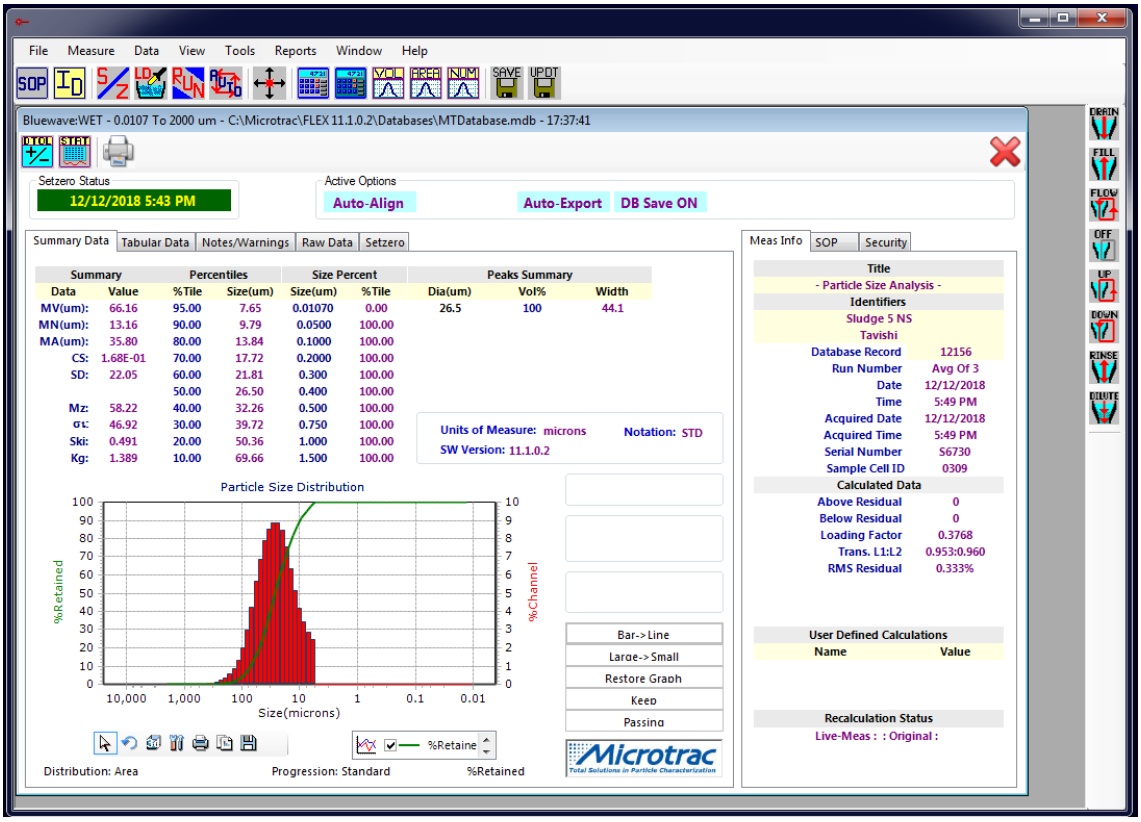
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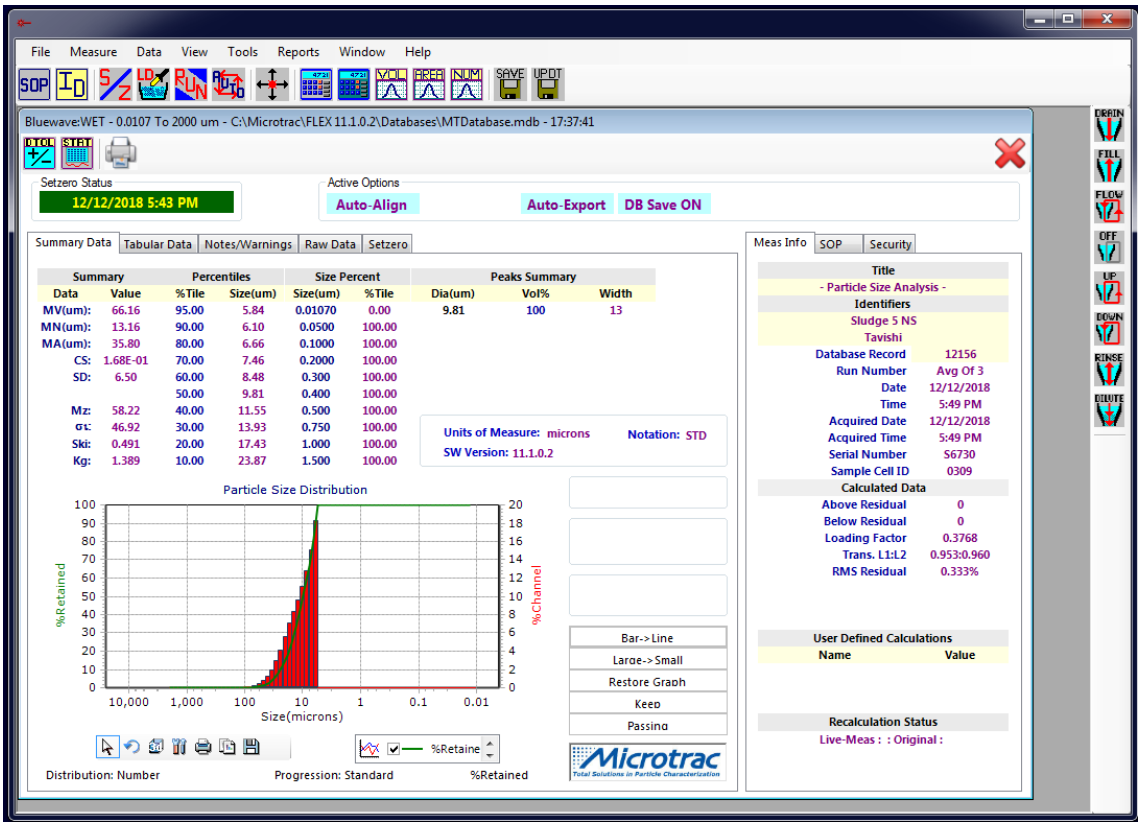
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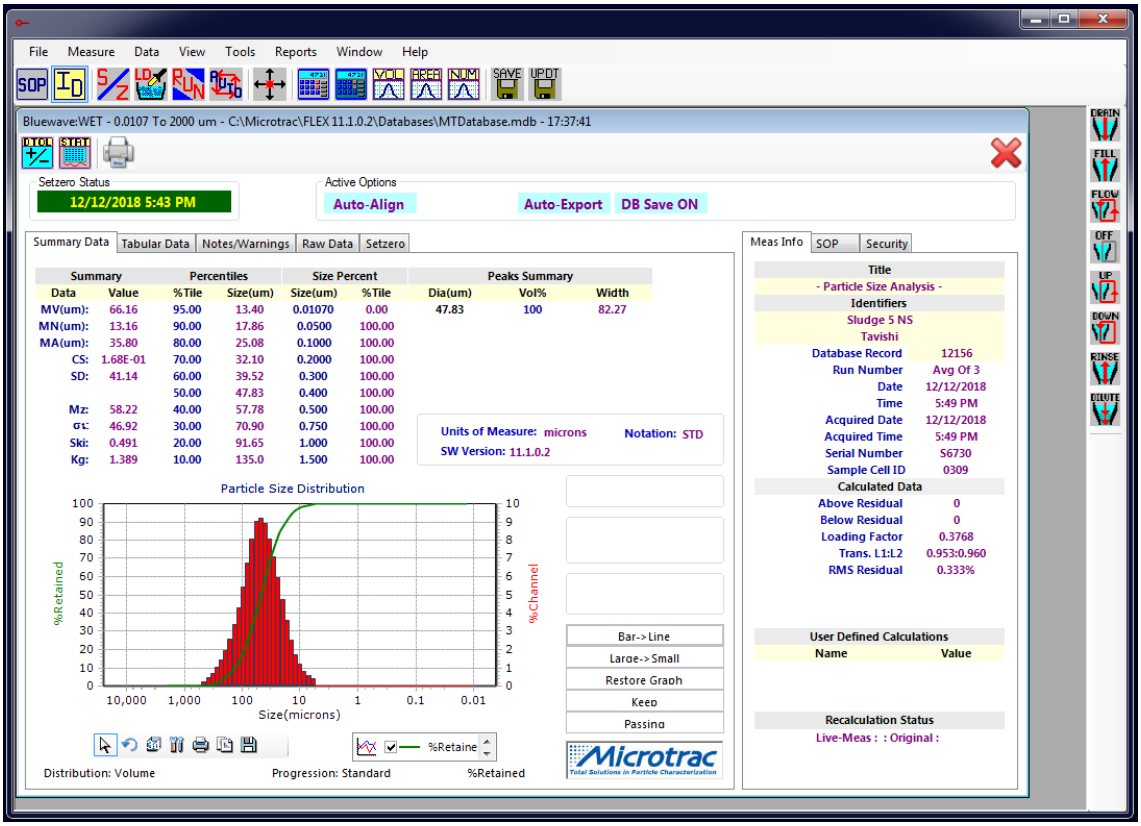
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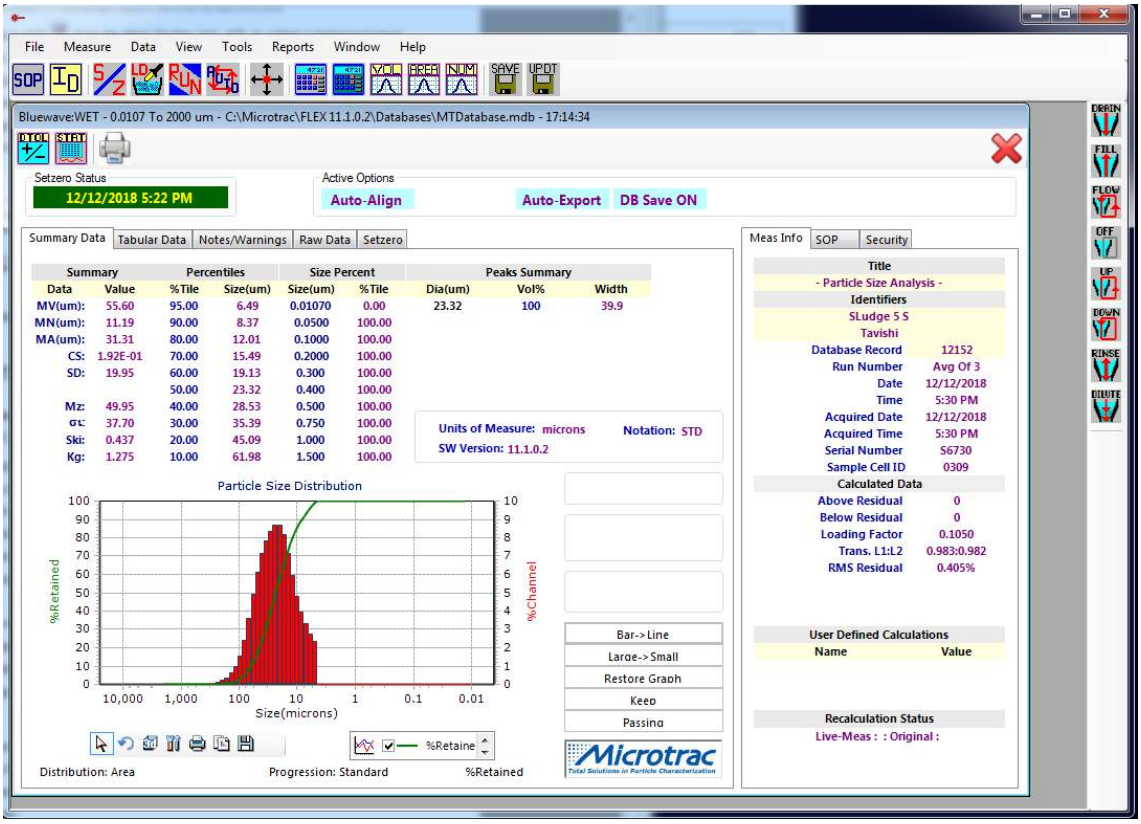
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50.00 23.32
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30.00 35.39
20.00 45.09
10.00 61.98
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0.1000 100.00
0.2000 100.00
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0.500 100.00
0.750 100.00
1.000 100.00
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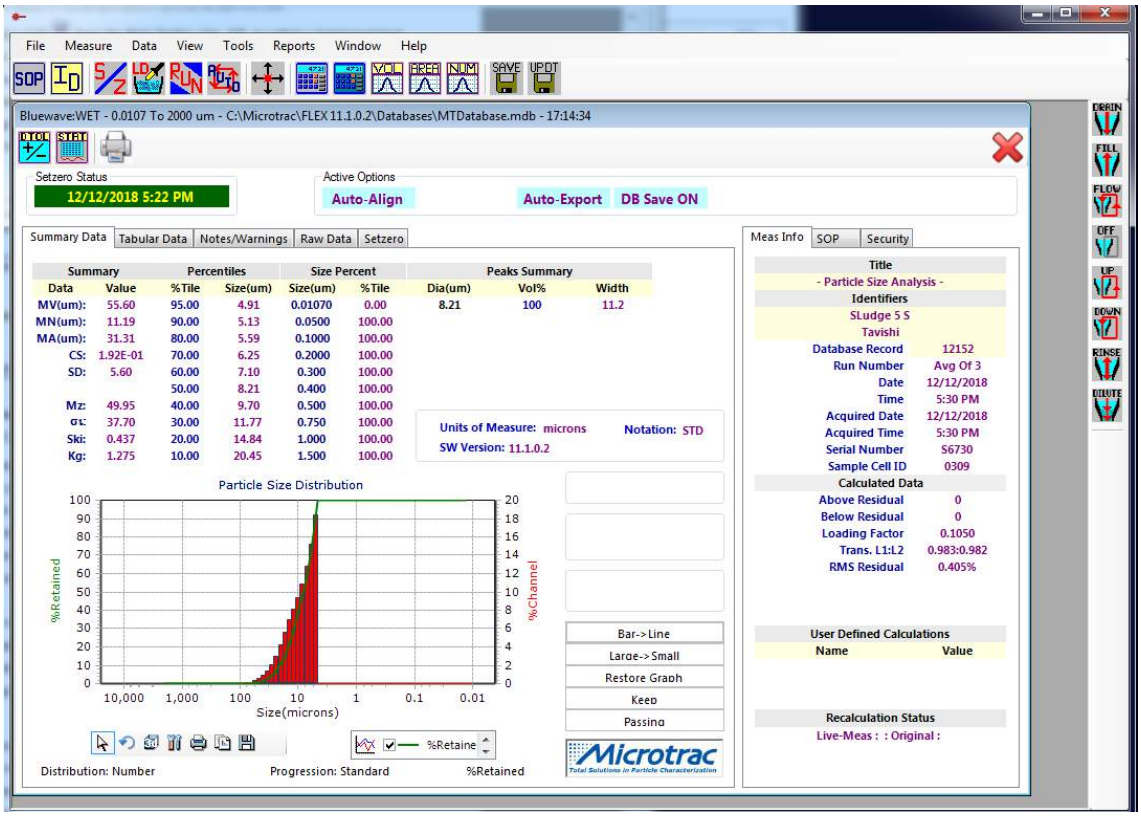
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Auto-Export
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Tabular Data
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SD: 5.60 60.00 7.10 0.300 100.00
50.00 8.21 0.400 100.00
Mz: 49.95 40.00 9.70 0.500 100.00
σι: 37.70 30.00 11.77 0.750 100.00
Ski: 0.437 20.00 14.84 1.000 100.00
Kg: 1.275 10.00 20.45 1.500 100.00
Units of Measure: microns
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Security
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Below Residual 0
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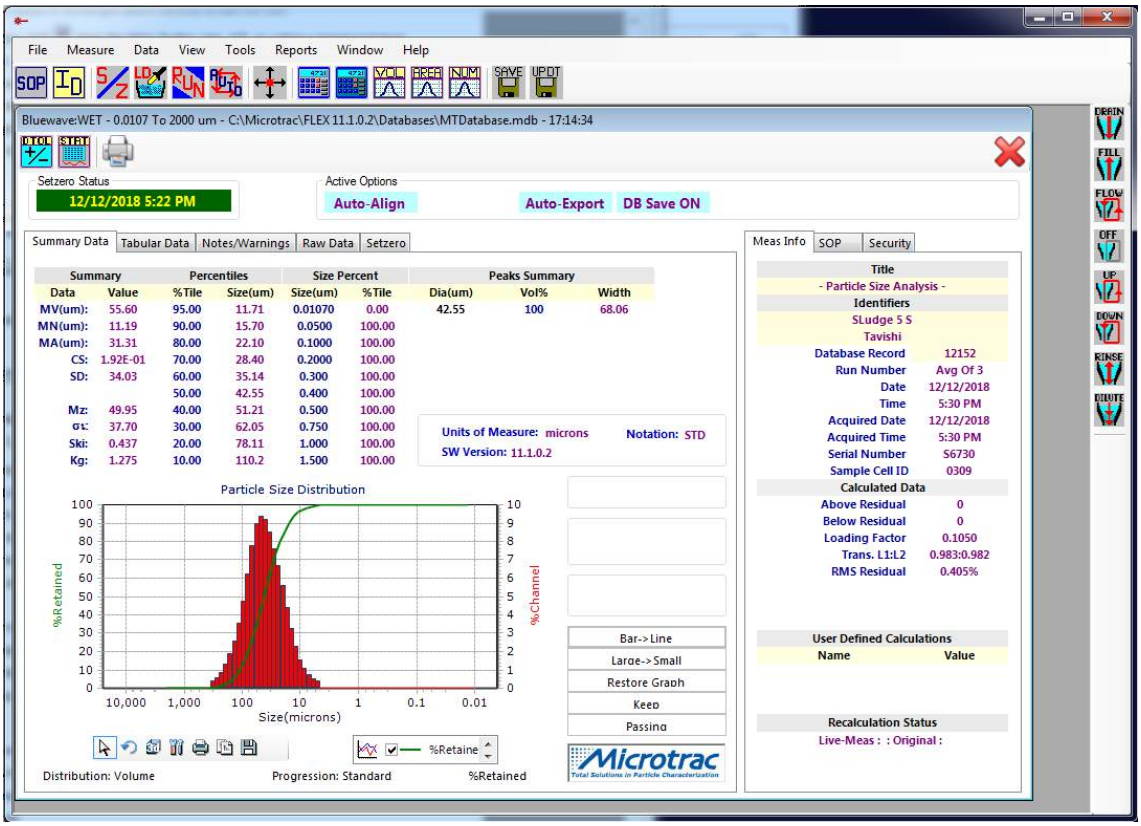
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MA(um): 31.31 80.00 22.10 0.1000 100.00
CS: 1.92E-01 70.00 28.40 0.2000 100.00
SD: 34.03 60.00 35.14 0.300 100.00
50.00 42.55 0.400 100.00
Mz: 49.95 40.00 51.21 0.500 100.00
σι: 37.70 30.00 62.05 0.750 100.00
Ski: 0.437 20.00 78.11 1.000 100.00
Kg: 1.275 10.00 110.2 1.500 100.00
Units of Measure: microns
Notation: STD
SW Version: 11.1.0.2
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%Channel
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Large->Small
Restore Graph
Keep
Passing
Distribution: Volume
Progression: Standard
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Loading Factor 0.1050
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RMS Residual 0.405%
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FILL
FLOW
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UP
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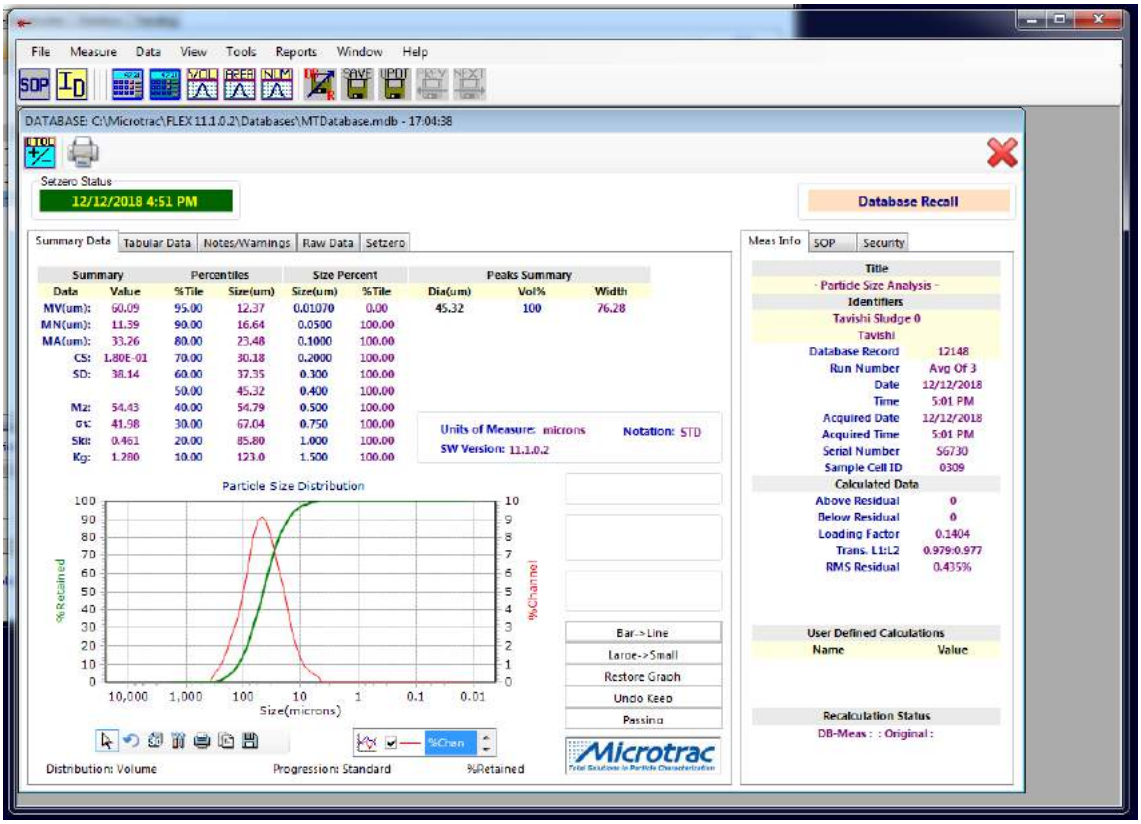
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Size Percent
Peaks Summary
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MA(um): 33.26 80.00 23.48 0.1000 100.00
CS: 1.80E-01 70.00 30.18 0.2000 100.00
SD: 38.14 60.00 37.35 0.300 100.00
50.00 45.32 0.400 100.00
Mz: 54.43 40.00 54.79 0.500 100.00
σι: 41.98 30.00 67.04 0.750 100.00
Ski: 0.461 20.00 85.80 1.000 100.00
Kg: 1.280 10.00 123.0 1.500 100.00
Units of Measure: microns
Notation: STD
SW Version: 11.1.0.2
Particle Size Distribution
%Retained
%/Channel
Size(microns)
Bar->Line
Large->Small
Restore Graph
Undo Keep
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Distribution: Volume
Progression: Standard
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User Defined Calculations
Name Value
Recalculation Status
DB-Meas : : Original :

A

Appendix III: AMPTS Data and Analysis

Day	CEL1 - 1 Volume [Nml]	CEL 2 - 2 Volume [Nml]	AVERAGE CEL [Nml]	STANDARD DEVIATION [Nml]	+O - 5 Volume [Nml]	+O - 6 Volume [Nml]	AVERAGE +O [Nml]	STANDARD DEVIATION [Nml]	-O - 3 Volume [Nml]	-O - 4 Volume [Nml]	AVERAGE -O [Nml]	STANDARD DEVIATION [Nml]	BMP CEL (AVG(CEL)-AVG(-0))	BMP CEL (AVG(+0)-AVG(-0))	Accounting for SD as per equation 4.1 (Cel)	Accounting for SD as per equation 4.1 (+O)
0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	26.3	26.20	26.25	0.07	185.20	180.30	182.75	3.46	22.90	30.60	26.75	5.44	0.00	55.05	5.45	6.45
2	48.5	49.00	48.75	0.35	341.20	338.50	339.85	1.91	36.70	45.20	40.95	6.01	4.46	102.36	6.02	6.31
3	157.8	163.80	160.80	4.24	406.40	444.00	425.20	26.59	46.00	52.60	49.30	4.67	63.71	128.07	6.31	26.99
4	273.3	284.80	279.05	8.13	443.80	478.20	461.00	24.32	58.60	62.30	60.45	2.62	124.91	138.86	8.54	24.46
5	352.8	382.30	367.55	20.86	542.20	577.10	559.65	24.68	72.50	72.60	72.55	0.07	168.57	168.57	20.86	24.68
6	391.3	452.90	422.10	43.56	650.30	743.60	696.95	65.97	86.70	83.10	84.90	2.55	192.69	209.92	43.63	66.02
7	424.7	484.70	454.70	42.43	691.60	808.90	750.25	82.94	100.40	93.30	96.85	5.02	204.49	225.98	42.72	83.10
8	469.9	517.40	493.65	33.59	720.40	841.90	781.15	85.91	112.70	101.60	107.15	7.85	220.86	235.29	34.49	86.27
9	537.9	567.40	552.65	20.86	752.70	864.60	808.65	79.13	125.50	113.20	119.35	9.40	247.31	243.57	22.88	79.68
10	583.1	635.70	609.40	37.19	764.00	888.60	826.30	88.11	138.40	122.50	130.45	11.24	273.69	248.89	38.86	88.82
11	603.5	690.30	646.90	61.38	772.60	898.80	835.70	89.24	146.80	129.10	137.95	12.52	290.83	251.72	62.64	90.11
12	620.3	726.90	673.60	75.38	782.50	912.40	847.45	91.85	158.30	139.60	148.95	13.22	299.80	255.26	76.53	92.80
13	631.9	750.00	690.95	83.51	789.60	923.90	856.75	94.96	168.60	149.00	158.80	13.86	304.09	258.06	84.65	95.97
14	641.4	767.10	704.25	88.88	794.10	932.70	863.40	98.00	178.30	156.30	167.30	15.56	306.83	260.06	90.23	98.23
15	651.1	783.40	717.25	93.55	796.00	939.70	867.85	101.61	186.40	160.90	173.65	18.03	310.63	261.40	95.27	103.20
16	659.5	797.10	728.30	97.30	796.70	944.20	870.45	104.30	191.20	165.40	178.30	18.24	314.29	262.18	98.99	105.88
17	668.6	816.50	742.05	103.87	797.30	948.50	872.90	106.91	197.80	171.50	184.65	18.60	318.51	262.92	105.53	108.52
18	677.9	829.70	753.80	107.34	798.00	952.60	875.30	109.32	204.40	176.80	190.60	19.52	321.83	263.64	109.10	111.05
19	685.3	843.90	764.60	112.15	798.60	956.80	877.70	111.86	209.20	180.50	194.85	20.29	325.57	264.37	113.97	113.69
20	690.7	855.80	773.25	116.74	799.30	961.30	880.30	114.55	214.40	184.20	199.30	21.35	327.97	265.15	118.68	116.52
21	693.3	864.50	778.90	121.06	799.90	965.50	882.70	117.10	220.10	187.90	204.00	22.77	328.51	265.87	123.18	119.23
22		869.90	863.90	121.06	800.60	968.70	884.65	118.86	224.40	191.70	208.05	23.12	378.20	266.46	123.25	121.09
23		875.40	875.40	121.06	801.20	971.80	886.50	120.63	228.50	194.70	211.60	23.90	379.31	267.02	123.39	122.98
24		880.80	880.80	121.06	801.90	974.40	888.15	121.98	231.50	196.20	213.85	24.96	381.11	267.52	123.60	124.50
25		883.70	883.70	121.06	802.50	975.60	889.05	122.40	233.20	197.80	215.50	25.03	381.83	267.79	123.62	124.93
26		885.80	885.80	121.06	803.20	976.70	889.95	122.68	235.00	199.30	217.15	25.24	382.09	268.06	123.66	125.25
27		887.90	887.90	121.06	803.80	977.80	890.80	123.04	236.80	200.80	218.80	25.46	382.34	268.31	123.70	125.64
28		890.00	890.00	121.06	804.50	979.00	891.75	123.39	238.50	202.30	220.40	25.60	382.63	268.60	123.73	126.02
29		891.80	891.80	121.06	805.00	980.10	892.55	123.81	240.10	204.10	222.10	25.46	382.69	268.84	123.70	126.40
30						981.30	981.30			207.30	207.30					
31						982.40	982.40			210.50	210.50					
32						983.30	983.30			212.90	212.90					

Figure A.1: AMPTS data and analysis for cellulose ans non-aerated sludge.

Day	CEL1 - 1 Volume [Nml]	CEL 2 - 2 Volume [Nml]	AVERAGE CEL [Nml]	STANDARD DEVIATION [Nml]		-5 - 7 Volume [Nml]	-5 - 8 Volume [Nml]	-5 - 9 Volume [Nml]	AVERAGE -5 [Nml]	STANDARD DEVIATION [Nml]		-5 - 10 Volume [Nml]	-5 - 11 Volume [Nml]	IS (-5) Volume [Nml]	AVERAGE -5 [Nml]	STANDARD DEVIATION [Nml]		BMP CEL (AVG(CEL)- AVG(-5))	BMP -O (AVG (+5) - AVG (-5))	Accounting for SD as per equation 4.1 (Cel)	Accounting for SD as per equation 4.1 (+5)
0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
1.00	26.20	26.20	26.20	0.07		25.70	18.50	17.20	20.23	4.35		6.80	6.80		6.80			11.11	21.95	0.07	4.35
2.00	48.50	49.00	48.75	0.35		72.00	48.50	53.00	57.83	12.47		18.30	18.30		18.30			17.40	61.77	0.35	12.47
3.00	167.80	163.80	165.80	4.24		92.40	73.20	80.30	82.00	9.66		29.00	29.00		29.00			63.60	67.19	4.24	9.66
4.00	273.30	294.80	279.05	8.13		96.70	96.00	90.20	93.97	3.37		49.80	49.80		49.80			131.00	69.01	8.13	3.37
5.00	352.80	382.30	367.55	20.86		102.20	108.40	94.30	101.63	7.07		53.50	53.50		53.50			179.46	76.21	20.86	7.07
6.00	391.30	452.90	422.10	43.56		112.10	123.70	101.00	112.27	11.35		57.20	57.20		57.20			208.51	86.04	43.56	11.35
7.00	424.70	484.70	454.70	42.43		138.40	138.90	121.00	132.77	10.19		69.70	69.70		69.70			225.71	114.17	42.43	10.19
8.00	463.90	517.40	490.65	33.59		151.20	143.40	146.20	146.93	3.95		62.20	62.20		62.20			246.54	132.40	33.59	3.95
9.00	537.90	567.40	552.65	20.86		160.60	147.80	156.50	154.97	6.54		64.70	64.70		64.70			278.83	141.04	20.86	6.54
10.00	583.10	638.70	610.90	37.19		165.90	149.50	158.30	158.23	8.70		67.20	67.20		67.20			308.83	142.24	37.19	8.70
11.00	603.50	618.30	610.90	9.61		170.40	151.20	160.00	160.53	9.61		69.60	69.60		69.60			323.89	142.08	9.61	9.61
12.00	620.30	726.90	673.60	75.38		173.60	152.90	161.80	162.77	10.38		72.00	72.00		72.00			343.77	141.82	75.38	10.38
13.00	631.90	799.00	715.45	83.51		174.10	154.60	163.50	164.07	9.76		74.40	74.40		74.40			352.31	140.10	83.51	9.76
14.00	644.40	792.10	718.25	98.88		174.70	156.30	165.40	165.47	9.20		76.20	76.20	56.80	76.20	13.72		364.43	154.58	98.94	18.52
15.00	657.10	788.40	722.75	93.65		174.70	156.30	166.00	165.67	9.15		76.20	76.20		76.20			367.51	154.27	93.65	9.15
16.00	658.50	797.10	727.80	97.20		175.80	156.90		166.90	9.15		77.50	77.50	56.80	77.50	14.64		377.80	153.77	96.29	17.28
17.00	668.60	815.50	742.05	103.87		176.30			176.30	9.15		78.20	78.20		78.20			379.34	153.28	103.87	9.15
18.00	677.90	829.70	753.80	107.34		176.90			176.90	9.15		78.90	78.90		78.90			385.66	153.13	107.34	9.15
19.00	685.30	843.30	764.80	112.15		177.40			177.40	9.15		79.50	79.50		79.50			391.49	152.97	112.15	9.15
20.00	690.70	855.80	773.25	116.74		178.00			178.00	9.15		80.20	80.20		80.20			396.03	152.81	116.74	9.15
21.00	693.30	864.50	778.90	121.06		178.60			178.60	9.15		80.90	80.90	72.68	76.79	5.81		401.21	159.08	121.20	10.84
22.00		869.90	869.90	121.06		179.10			179.10	9.15		81.60	81.60		81.60			450.46	152.34	121.06	9.15
23.00		875.40	875.40	121.06		179.70			179.70	9.15		82.20	82.20		82.20			453.26	152.34	121.06	9.15
24.00		880.80	880.80	121.06		180.20			180.20	9.15		82.90	82.90		82.90			455.94	152.03	121.06	9.15
25.00		883.70	883.70	121.06		180.80			180.80	9.15		83.60	83.60		83.60			457.20	151.68	121.06	9.15
26.00		885.90	885.90	121.06		181.30			181.30	9.15		84.30	84.30		84.30			458.00	151.56	121.06	9.15
27.00		887.90	887.90	121.06		181.90			181.90	9.15		84.90	84.90		84.90			458.86	151.56	121.06	9.15
28.00		890.00	890.00	121.06		182.40			182.40	9.15		85.60	85.60		85.60			458.86	151.25	121.06	9.15
29.00		892.60	892.60	121.06		182.90			182.90	9.15		85.70	85.70		85.70			459.63	151.09	121.06	9.15

Figure A.2: AMPTS data and analysis for cellulose ans 5 aerated sludge

Day	CEL1 - 1 Volume [Nml]	CEL 2 - 2 Volume [Nml]	+3 - 12 Volume [Nml]	+3 - 13 Volume [Nml]	-3 - 14 Volume [Nml]	Incubator Shaker (-3)Volume [Nml]	Incubator Shaker (+3)Volume [Nml]
0	0	0	0	0		0	0
1	26.3	26.2	10.7	8.3			
2	48.5	49	12.1	10.6			
3	157.8	163.8	13.4	12.3			
4	273.3	284.8	14.8	14.1			
5	352.8	382.3	16.2	15.9			
6	391.3	452.9	17.5	17.6			
7	424.7	484.7	18.9	18.2			
8	469.9	517.4	20.8				
9	537.9	567.4	22.8				
10	583.1	635.7	24.7				
11	603.5	690.3	26.7				
12	620.3	726.9	28.5				
13	631.9	750					
14	641.4	767.1				40	26.3
15	651.1	783.4					
16	659.5	797.1				40	26.3
17	668.6	815.5					
18	677.9	829.7					
19	685.3	843.9					
20	690.7	855.8					
21	693.3	864.5				40	26.3
22		869.9					
23		875.4					
24		880.8					
25		883.7					
26		885.8					
27		887.9					
28		890					
29		891.8					
30							
31						49.56	36.8
32							

Figure A.3: AMPTS data for cellulose ans 3 bar aerated sludge

A

Appendix IV: SMA Data

Details of the additional SMA experiment done are as follows, The duration of the experiment was 5 days and the Inoculums were taken as the DAF supernatant (0 and 5 bar). To these inoculums, Acetate and Cellulose were added as substrate and the AMPTS bottles were prepared in triplicate along with bottles for biogas analysis for the incubator shaker, Figure A.1 shows TS and VS values for the incoulums and substrates and figure A.2 gives the amounts of these parameters to be added to the AMPTS bottles in triplicate. Figure A.3 gives the layout of the AMPTS machine for the SMA experiment. Table A.4 gives the data

Parameters	TS (g/g)	VS (g/g)
0 bar	0.0236	0.0166
5 bar	0.0078	0.0052
Cellulose	0.9723	0.9780
Acetate	0.6000	0.2150

Figure A.1: TS and VS values for the additional experiment done to measure SMA.

obtained for the SMA calculation

I/S Ratio (by VS)	2		Inoculum 1:	DAF supernatant 0 bar			
VS of Inoculum 1 (w/w)	0.0166		Inoculum 2:	DAF supernatant 5 bar			
VS of Inoculum 2 (w/w)	0.0050		Inoculum 3:	HP anaerobic sludge			
VS of Inoculum 3 (w/w)	0.0258		Substrate 1:	Cellulose			
VS of Substrate 1 (w/w)	0.9700		Substrate 2:	Acetate			
VS of Substrate 2 (w/w)	0.2150		Substrate 3:	HP anaerobic sludge			
VS of Substrate 3 (w/w)	0.0258		Substrate 4:	DAF supernatant 0 bar			
VS of Substrate 4 (w/w)	0.0005		Substrate 5:	DAF supernatant 5 bar			
VS of Substrate 5 (w/w)	0.0050						
Total Volume (mL)	450						
SLUDGE ACTIVITY			1	2		3	4
Bottle No.	Sample Type	Total Volume	Inoculum Volume (mL)	Inoculum VS (g)	Substrate VS (g/g)	Substrate mass (g)	DIW (mL)
1	I: 1, S:1 (OA1)	450	300.00	4.98	2.49	2.57	147.43
2	I: 1, S:1 (OA2)	450	300.00	4.98	2.49	2.57	147.43
3	I: 1, S:1 (OA3)	450	300.00	4.98	2.49	2.57	147.43
4	I: 1, S:2 (OC 1)	450	300.00	4.98	2.49	11.58	138.42
5	I: 1, S:2 (OC 2)	450	300.00	4.98	2.49	11.58	138.42
6	I: 1, S:2 (OC 3)	450	300.00	4.98	2.49	11.58	138.42
15	I: 2, S:1 (5A 1)	450	300.00	1.50	0.75	0.77	149.23
8	I: 2, S:1 (5A 2)	450	300.00	1.50	0.75	0.77	149.23
9	I: 2, S:1 (5A 3)	450	300.00	1.50	0.75	0.77	149.23
10	I: 2, S:2 (5C 1)	450	300.00	1.50	0.75	3.49	146.51
11	I: 2, S:2 (5C 2)	450	300.00	1.50	0.75	3.49	146.51
12	I: 2, S:2 (5C 3)	450	300.00	1.50	0.75	3.49	146.51
13	I: 2, - (5 1)	450	300.00	0.15	0.00	0.00	150.00
14	I: 2, - (5 2)	450	300.00	0.15	0.00	0.00	150.00
7 (cell not working)	I: 2, - (5 3)	450	300.00	0.15	0.00	0.00	150.00
Biogas							
BG1	I: 1, S:1	250	166.67	2.77	1.38	1.43	81.91
BG2	I: 1, S:2	250	166.67	2.77	1.38	6.43	76.90
BG3	I: 2, S:1	250	166.67	0.83	0.42	0.43	82.90
BG4	I: 2, S:2	250	166.67	0.83	0.42	1.94	81.40
BG5	I: 2, -	250	166.67	2.77	0.00	0.00	83.33

Figure A.2: Amount of substrate and inoculum to be added to each AMPTS and biogas bottle prepared.

DAF 0 bar, acetate	DAF 0 bar, cellulose	DAF 5 bar acetate	DAF 5 bar, cellulose	DAF 5 bar, No Substrate
DAF 0 bar, acetate	DAF 0 bar, cellulose	DAF 5 bar acetate	DAF 5 bar, cellulose	DAF 5 bar, No Substrate
DAF 0 bar, acetate	DAF 0 bar, cellulose	DAF 5 bar acetate	DAF 5 bar, cellulose	DAF 5 bar, No Substrate
Biogas 1	Biogas 2	Biogas 3	Biogas 4	Biogas 5

Figure A.3: Layout of the bottles prepared for the AMPTS and biogas experiment.

Hour	OC 1	OC 2	5C 2	5C 3	5A 1	5A 3	Hour	OC 1	OC 2	5C 2	5C 3	5A 1	5A 3
0	0	0	0	0	0	0	46	329.7	328.3	14.3	14.2	13.7	18.6
1	10.7	12.1	9.3	9.2	9.3	9.3	47	335.3	333.4	14.4	14.3	13.8	19.1
2	16.4	17.7	9.4	9.4	9.4	9.5	48	341.2	338.5	14.5	14.4	13.9	19.7
3	23.6	25	9.5	9.5	9.5	9.7	49	347	343.4	14.6	14.5	14	20.2
4	31.8	33.5	9.6	9.6	9.6	9.9	50	352.8	348.3	14.7	14.6	14.1	20.8
5	38.8	40.1	9.7	9.7	9.7	10.1	51	358.4	353	14.8	14.7	14.2	21.3
6	43.6	45.6	9.8	9.8	9.8	10.3	52	363.9	357.8	14.9	14.9	14.3	21.9
7	48.1	50	9.9	9.9	9.8	10.5	53	369.5	363	15	15	14.4	22.4
8	52.4	54.4	10.1	10	9.9	10.7	54	374.9	368.3	15.1	15.1	14.5	23
9	56.6	58.7	10.2	10.1	10	10.9	55	380.2	373.8	15.3	15.2	14.6	23.5
10	60.7	63	10.3	10.2	10.1	11.1	56	384.4	379.3	15.4	15.3	14.7	24.1
11	64.9	67.4	10.4	10.3	10.2	11.3	57	388.6	384.7	15.5	15.4	14.8	24.6
12	69.3	71.8	10.5	10.5	10.3	11.5	58	390.1	390.3	15.6	15.5	14.9	25.2
13	73.7	76.3	10.6	10.6	10.4	11.7	59	391.4	395.7	15.7	15.6	15	25.7
14	78.2	80.8	10.7	10.7	10.5	11.9	60	392.6	401	15.8	15.7	15.1	26.3
15	82.8	85.8	10.8	10.8	10.6	12.1	61	393.8	406.5	15.9	15.8	15.1	26.8
16	88.5	91	10.9	10.9	10.7	12.3	62	395.1	411.9	16	16	15.2	27.4
17	94.8	97	11.1	11	10.8	12.5	63	396.3	417.3	16	16.1	15.3	27.9
18	102	104.2	11.2	11.1	10.9	12.7	64	397.5	422.6	16.2	16.2	15.4	28.4
19	111.6	112.5	11.3	11.2	11	12.9	65	398.7	427.9	16.4	16.3	15.5	29
20	122.8	122.7	11.4	11.3	11.1	13.1	66	399.8	432.5	16.5	16.4	15.6	29.5
21	137	135.4	11.5	11.4	11.2	13.3	67	400.9	436.8	16.6	16.5	15.7	30.1
22	153.2	150.3	11.6	11.6	11.3	13.5	68	402	438.2	16.7	16.6	15.8	30.6
23	169.7	165.4	11.7	11.7	11.4	13.7	69	403.1	439.7	16.8	16.7	15.9	31.2
24	185.2	180.3	11.8	11.8	11.5	13.9	70	404.2	441.1	16.9	16.8	16	31.7
25	198.6	194.6	11.9	11.9	11.6	14.1	71	405.3	442.5	17	16.9	16.1	32.3
26	210.2	207.5	12	12	11.7	14.3	72	406.4	444	17.1	17.1	16.2	32.8
27	219.9	218.8	12.2	12.1	11.8	14.5	73	407.6	445.4	17.2	17.2	16.3	33.4
28	228	228.7	12.3	12.2	11.9	14.7	74	409	446.6	17.4	17.3	16.4	33.9
29	234.9	237.3	12.4	12.3	12	14.9	75	410.4	447.7	17.5	17.4	16.5	34.5
30	241.2	244.9	12.5	12.4	12.1	15.2	76	411.8	448.8	17.6	17.5	16.6	35
31	247.2	251.7	12.6	12.5	12.2	15.4	77	413.2	450	17.7	17.6	16.7	35.6
32	252.7	257.8	12.7	12.7	12.3	15.6	78	414.6	451.1	17.8	17.7	16.8	36.1
33	258.2	263.4	12.8	12.8	12.4	15.8	79	416	452.2	17.9	17.8	16.9	36.6
34	263.6	268.7	12.9	12.9	12.5	16	80	417.5	453.3	18	17.9	17	37.3
35	269	274	13	13	12.6	16.2	81	419	454.4	18.1	18.1	17.1	37.9
36	274.4	279.1	13.2	13.1	12.7	16.4	82	420.5	455.9	18.2	18.2	17.2	38.5
37	279.8	283.8	13.3	13.2	12.8	16.6	83	422	457.5	18.3	18.3	17.3	39.2
38	285.4	288.6	13.4	13.3	12.9	16.8	84	423.5	459.2	18.5	18.4	17.4	39.8
39	291	293.4	13.5	13.4	13	17	85	425	460.8	19.8	19	17.5	40.5
40	296.5	298.2	13.6	13.5	13.1	17.2	86	426.5	462.5	23.1	20.4	17.6	41.1
41	302.1	303	13.7	13.6	13.2	17.4	87	428.1	464.1	26.4	21.9	17.7	41.7
42	307.7	307.9	13.8	13.8	13.3	17.6	88	429.7	465.5	30.1	23.3	17.8	42.4
43	312.9	312.7	13.9	13.9	13.4	17.8	89	431.3	467	34.1	24.7	17.9	43
44	318.1	317.9	14	14	13.5	18	90	432.9	468.4	38.1	26.1	18	43.7
45	323.9	323.1	14.1	14.1	13.6	18.2	91	434.5	469.9	42.3	27.6	18.1	44.3
							92	436.4	471.3	46.4	27.7	18.2	44.9
							93	438.2	472.8	50.5		18.3	45.6
							94	440.1	474.5	54.7		18.4	45.9
							95	441.9	476.4	55.7		18.4	

Figure A.4: Data obtained from the AMPTS for the SMA analysis.