Master Thesis

Biogas measurement techniques and the associated errors

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Foreword

This thesis is written for the fulfillment of the Master degree programme in Renewable Energy at the University of Jyväskylä, Finland. The experimental tests were carried out at the Environmental Science and Technology Laboratory. This thesis will help to understand and overcome the potential errors in biogas measurement, analysis and their relation with the anaerobic degradation process.

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ABSTRACT

There are several techniques for the measurement of laboratory scale biogas production. This thesis describes the study and evaluation of the most significant and commonly used biogas measurement techniques and analyzes the source of errors associated with the measurement of biogas production. Firstly, inaccuracy mainly due to biogas carbon dioxide (CO_2) dilution in displaceable liquids and losses to atmosphere of dissolved CO_2 was evaluated by testing solubility of CO_2 in different barrier solutions. Saturated acidified brine solution showed lower CO_2 solubility among the tested solutions. An accurate, simple, automated and easy to calibrate laboratory scale liquid displacement gas measuring device was built and tested. Headspace gas chromatography (HS-GC) analysis and errors that could arise due to solubility, concentration and temperature influence were also investigated. Experimental analysis using dry and saturated synthetic bio-gas samples were carried at different temperatures. Gas chromatography results showed significant changes in methane (CH_4) and CO_2 concentration when incubated at 5, 35, 55 & 70 °C and compared with standard prepared at room temperature (23 °C). Errors at thermphilic temperatures were much higher than at 35 or 5 °C. One of the possible and easy means of avoiding errors was found by maintaining the same temperature for both standard and samples during the entire GC experimental analysis. This was performed by cooling the assays to room temperature for a short period of measurement time. This short fluctuation of temperature and its effect on the entire anaerobic process and microbiological activity was also examined. Errors that could arise during the gas measurement and analysis were pointed out and the possible means of corrections were discussed.

CONTENTS

1 INTRODUCTION

The interest of using different renewable energy sources has been growing. More recently, one of the sources, biogas, has shown great potential and has gained importance as a CO_2-neutral fuel because of low CO_2 emissions. Biogas can be used for heat and/or electricity production or can be upgraded to vehicle fuel [1]. More efficient use of biogas as regenerative energy can be done by injecting the treated biogas into natural gas grids [2].

Anaerobic digestion provides the possibility and solution to major global concerns such as alternative energy production, proper management of human, animal, agricultural, municipal and industrial waste, controlling environmental pollution, recycling of nutrients back to soil and expanding food supplies [3].

Laboratory scale anaerobic biodegradability experiments help in determining the ultimate methane potential of substances and their rate of biodegradation. Gaseous end products and/or intermediate liquid products such as volatile fatty acids (VFA) provide important information for the evaluation of the anaerobic process and methane potential [1, 3]. Analyses of these parameters are usually carried out in laboratories by incubating the test substrate in reactors at a given set temperature. Laboratory reactors can be either batch or semi-continuous, continuously or intermittently mixed [4]. The process of anaerobic digestion is well understood and widely used. However, it suffers from severe drawbacks associated with gas volume measurement and its composition analysis. The evaluation of gas production is important because it is key indicator of any reactor performance.

It is always challenging for the researchers when the biogas quantity, composition and microbial ecology in a full scale plant is not similar to that noticed in lab-scale reactors. There are several factors associated with this type of problem. However, the differences in the results are also likely to occur due to errors in gas measurement techniques- much sensitive in case of laboratory scale experiments.

The collection and preservation of the gas generated in the digester is the first and most important operation in gas measuring technique, and numerous methods have been developed over the past 30 years. Volumetric or manometric gas measurement systems are being widely used for the quantification of gas in laboratory scale experiments [5]. Gas

chromatography (GC) is widely used and is one of the most prolific chemical analysis methods in the world today. The main reason for the popularity of GC is its capability to obtain both qualitative and quantitative information (identification of unknown components and determination of quantity of each gas components) from a complex biogas sample [6].

2 BACKGROUND

2.1 Anaerobic digestion

Anaerobic digestion is a biological process which occurs in the absence of oxygen. It helps in the breakdown of organic matter and the stabilization of these materials, by conversion to CH_4 and CO_2 gases and a nearly stable residue. The digestion of organic materials by the micro-organisms produces biogas. Biogas typically consists of 50 to 65 % (volume) CH_4, 35 to 50 % (volume) CO_2, 4 to 6 g/m³ of H_2S and 30-160 g/m^3 of water [7].

The process of anaerobic digestion involves several steps like hydrolysis, acidogenesis, acetogenesis and methogenesis reaction. These four main stages of this degradation can be distinguished [8, 9]:

a) Hydrolysis – complex organic material are broken down by enzymes to soluble products (hydrolytic fermentative bacteria)
b) Acidogenesis – generation of intermediary products such as short-chain fatty acids, (hydrogen producing and acetogenic organisms)
c) Acetogenesis – acetate production (hydrogen-producing, hydrogen-consuming acetogenic organisms)
d) Methanogenesis – CH_4 production (methane-forming bacteria)

Methane production is the final stage of anaerobic degradation. Different bacterial species are specialized in the production of methane from different compounds. The catabolic pathways of methanogens are very complicated. They can be divided into three groups: CO_2-reducing, methylotrophic and aceticlastic pathways as detailed elsewhere [10, 11].

2.2 Biogas generation during anaerobic process

The production of biogas from slurry takes with the generation of bubble in the slurry. The birth of a bubble can happen when there is excess pressure inside the bubble. According to the Laplace equation, excess pressure p = 2S/r (S = surface tension, r = bubble radius).

When the bubble is formed at depth h, from the surface of slurry, then the external pressure is,

$$P_{ex} = P_{head} + \rho gh$$

P_{head} is the pressure inside the headspace of the reactor (or atmospheric pressure). ρgh is the static pressure of liquid at bubble height h from the surface.

Formation of the bubble inside the slurry can exist only when the pressure inside the bubble balances the external pressure (P_{ex}) and the surface tension.

$$P_{in} = P_{ex} + 2S/r$$

This shows extremely high pressure is required at the time of the birth of a gas bubble (because when r is too small, 2S/r becomes significantly large). The roughness available at the micro level (such as roughness of digester wall or the specks of suspended solid particles) helps in the nucleation and provides the requirement of infinite pressure when the bubble starts from zero radius. When the partial pressure of the biogas bubbles exceeds total pressure acting on it, the bubbles will be released from the slurry into the headspace. [12, 13]

Biogas bubbles mainly include CO_2 and CH_4 which are the most oxidized and reduced state of organic carbon respectively. Ignoring the trace gases, the molecular weight of biogas depends on the relative concentration of CO_2 and CH_4. Methane being the lightest gas, when its concentration increases, the gram molecular weight of the biogas decreases [14].

2.3 Gas measurement

Monitoring of biogas production is the most common method adopted in most of the research as the level of CO_2 and CH_4 gives important information about the state of the anaerobic degradation process.

The comparison of biodegradability data from different scientific papers can be a complex task. This is not only due to the difference in environmental conditions and protocols, but also due to the variety of equipment used. The basic protocol for anaerobic gas measurement and biodegradability tests is defined in the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC,1988), Her Majesty's Stationery Office, UK (HMSO, 1988) and the International Organization for Standardization (ISO 11734, 1995). However, many researchers are improving the methods usability by developing automatically operated instruments according to the experimental requirements [1, 15].

Gas Chromatography (GC) is an optimal analytical instrument for the analysis of components such as CH_4, CO_2, H_2S and siloxanes which are present in the gas [16].

The most important factors affecting the precision of biogas volume measurement and sensitivity are errors due to varying temperatures, vapor content, solubility and pressure [17]. The gas measurement technique and process itself can result in the inhibition of anaerobic digestion. This is because the high amounts of dissolved CO_2 can affect the pH of the medium and, consequently, can alter the microbial activity [18].

2.4 Standard temperature and pressure conversion

The important parameters for gas to standard temperature and pressure (STP) conversion are the biogas temperature and pressure, temperature of anaerobic environment and ambient temperature and pressure. Most of the scientific papers in the field of anaerobic digestion simply quote gas production volumes without mentioning any correction applied to standard conditions. However, the results reported as corrected to STP more often do not provide the information of the standard conditions [17].

Correction of the measured volume of a (STP) volume of dry gas is important. Different organizations have different definitions for the standard reference conditions of temperature and pressure. The standards of the International Union of Pure and Applied

Chemistry (IUPAC) and the National Institute of Standards and Technology (NIST) are most common in use. Since there is no universally accepted set of reference conditions, the information about reported gas volumes without stating reference temperature and pressure cannot be considered accurate. The current definition of IUPAC for standard reference conditions of absolute pressure and temperature is 100 kPa (1 bar) and 0 °C (273.15 K) [19]. Similarly, for NIST the reference conditions are absolute pressure of 101.325 kPa (1 atm) and temperature of 20 °C (293.15 K) [20]. Generally, gas volumes are reported as IUPAC original standard reference conditions of 101.325 kPa (1 atm) and 0 °C (273.15 K).

2.5 Interrelation between gas measurement and anaerobic degradation

The anaerobic digestion process is carried out by the involvement of different types of microorganisms which possess a very close syntrophic relationship. The production of CH_4 is a slow and sensitive process. Favorable environmental conditions such as temperature and pH are very essential factors for the growth of micro-organisms. The steady conversion and utilization of organic acids are essential as the accumulation of these acids or decrease of pH can lead to the inhibition of the methanogenesis process.

Anaerobic digestion can be classified as a) Psychrophilic (less than 15 °C) b) Mesophilic (15 to 45 °C) and c) Thermophilic (45 to 65 °C). The mesophilic and thermophilic digestion are important because in the psychrophilic range the production of biogas is very low [9, 7]. Temperature is a very important factor as it affects the rate of reaction and also influence the effects on solubility of metals, solubility of CO_2 and consequently on the buffering and composition of the gas.

The solubility of gases increases with an increase in pressure and decreases with an increase in temperature. CH_4 can be considered a less soluble hydrocarbon in water because of its smaller size and because it has the potential of causing minimum disruption to water's hydrogen bonds. This effect is so low that its solubility in water can be considered virtually zero [21]. On the other hand, CO_2 is very polar and its solubility is high in water. The difference in the solubility of two gases in water at different temperatures is given in Figure 1.

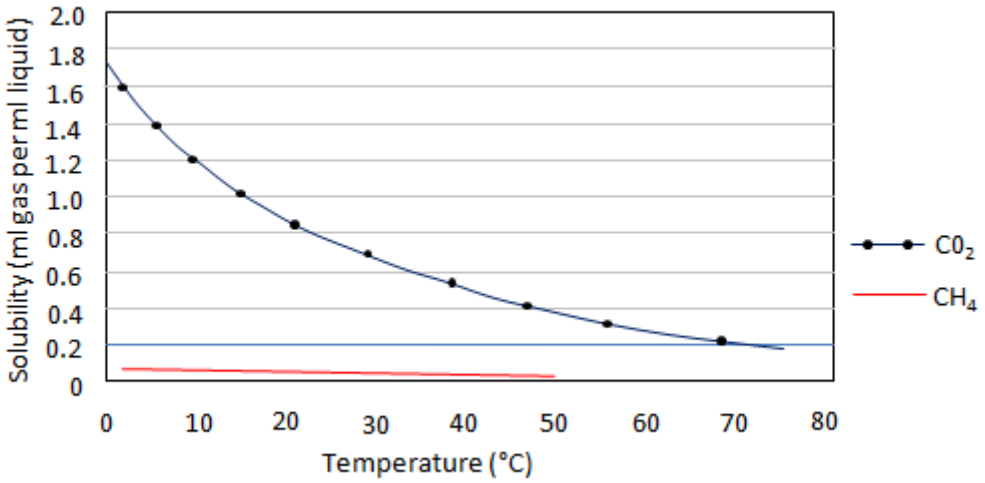

Figure 1: Solubility of CO_2 and CH_4 at 1atmosphere (101.325 kPa) and temperature [21].

The partial pressure of gas in the headspace can be held within a constant range. But CO_2 can hydrate and dissociate in the aqueous phase and vary the function of pH and other factors. The reaction scheme can be expressed as [22]:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

When pH value is < 8, the concentration of carbonate ions may be neglected and the hydration reaction can be expressed as:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

When alkalinity is less than 1000 mg/L, pH starts to change rapidly. A total alkalinity of 1.5 g $CaCO_3$/L is recommended for an adequate performance of the anaerobic systems [23, 24].

The CO_2 concentration in the reactor head space, temperature, alkalinity and volatile acids can alter the pH which can influence the anaerobic process either directly by affecting the enzymes' activity by changing their protein structure or indirectly by affecting the toxicity of the compounds. Figure 2 shows that the methanogens have optimum growth within the pH range of 6.6 to 7.4 or in a wider range of 6.0 to 8.0. The pH value outside this range is very lethal for the survival of microorganisms. However, the acid forming bacteria can still be active even for pH values as low as 4.5. Therefore, continuous acid production can occur even when the methane production gets interrupted due to low pH [25].

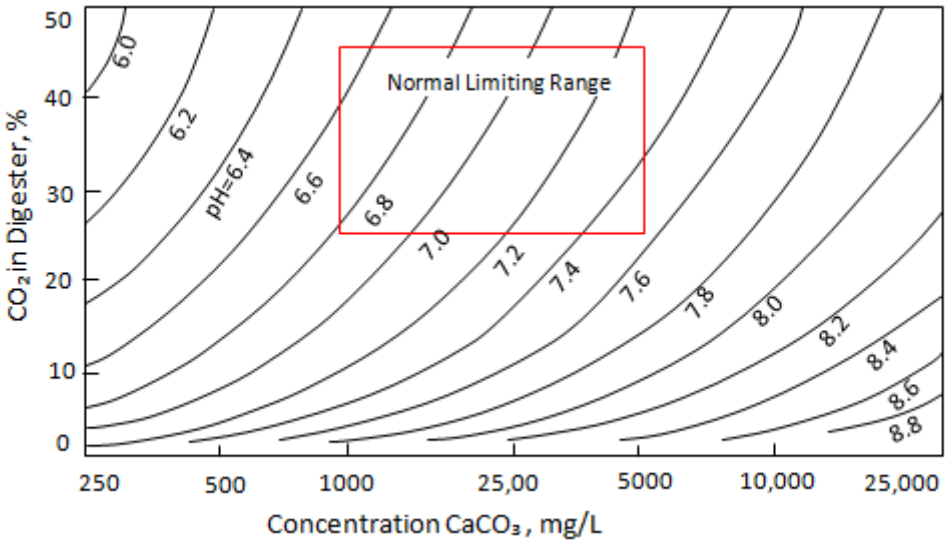

Figure 2: Relationship between pH, calcium carbonate concentration, and carbon dioxide concentration at 35 °C and 1 atm [26].

The two important factors that can influence the pH of the system are carbonic acid and volatile acids. Within the pH range of methogenic bacteria, the buffering capacity is closely dependent on CO_2/alkalinity. The concentration of the CO_2 in the gaseous phase directly influences the carbonic acid in the solution when the balance is established between the CO_2 in liquid and gaseous phase. It is also related with Henry's law which states the solubility of a gas in a liquid is directly proportional to the pressure of that gas above the surface of the solution.

The gas measurement technology is very much interlinked with the build up of gas pressure. As CO_2 is about 40 to 60 times more soluble than CH_4 in water under anaerobic conditions, the increase in digester pressure results in the increase of CO_2 concentration in the liquid, resulting in a change in pH. This can stimulate the methane production rate by changing the concentration of free ammonia.

Ammonia is always found in equilibrium with ammonium (NH_4^+) in the aqueous solution. Ammonium is not as toxic as ammonia to the microorganisms and this equilibrium is determined by several factors such as acidity, pH and temperature.

$$NH_4^+ \leftrightarrow NH_3 + H^+$$

The equilibrium of the reaction shifts to the right under the influence of high pH or high temperature resulting in a toxic environment for the anaerobic microorganisms (Figure 3).

This is why the thermophilic digestion process is more sensitive to ammonia inhibition than a mesophilic digestion process [28].

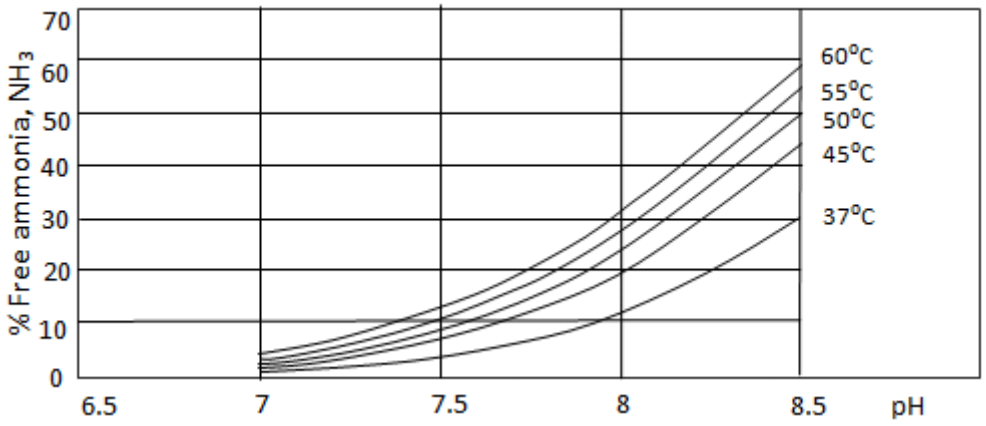

Figure 3. Effect of pH and temperature on the balance between ammonium and toxic ammonia NH_4^+/NH_3 [27].

Development of negative pressure in the biogas could drag the outside air into the anaerobic system which should not be allowed. The oxygen in the air inhibits methanogens and results in a drop in the biogas methane production rate. Negative pressure occurs when the pressure of the atmosphere (outside the anaerobic system) is greater than the inside gas pressure. Therefore, anaerobic gas collection, sampling and the feeding process should not allow the atmospheric diffusion of air with the reactor.

3 GAS MEASUREMENT TECHNIQUES

3.1 Volumetric and manometric gas measurement

Biogas measurement is done either manometrically by keeping the volume constant and measuring the pressure increase, or volumetrically by providing constant pressure conditions allowing measurement of the biogas volume [6]. The rate and volume of biogas produced from anaerobic biodegradability assays include different techniques such as lubricated syringes, volume displacement devices, pressure manometers or transducers, manometer assisted syringes, or low pressure switch meters. Measurement of gas at low headspace pressure is an important requirement to all manometric or volumetric determinations of anaerobic biodegradability [5].

Different researchers have developed different types of displacement gas measurement devices depending upon the research requirements [28-31]. The general working principle of these automatic displacement gas meters is the difference of pressure between the inlet and outlet of the meter which causes the periodic filling and emptying a defined volume of gas in the measurement chamber. The sensor operates the closing and opening of a two-way or three-way solenoid valve in order to release the collected gas and resets the whole system. The total volume of gas is the product of the number of fillings or emptyings (recorded by a counter system) times the defined volume of the chamber. The measurement of gas is independent of the flow profile.

Manometric transducers with various configurations are also widely used for the determination of produced gas volume. When the gradual increase of headspace gas pressure reaches a set value, a solenoid valve gets activated upon receiving the electric signal. Gas is allowed to be released for a few seconds of a set time period. This same process is repeated and continuous recording is done. They have a limited range of accuracy and the variation of inorganic carbon and liquid pH can inhibit the anaerobic process. The use of the pressure transducer technique for the determination of gas production has been described in most of the standards. ISO 11734 recommends taking regular intermediate pressure readings while using pressure transducers [15]. In this regard, Tagliapietra et al. [32] has compared the effects of headspace pressure on the kinetics of gas production in batch anaerobic system with automated a batch system equipped with an gas sensors and with venting valves. Theodorou et al. [33] reported that high headspace pressure can result in increased CO_2 solubility and can significantly disturb the microbial activity.

3.1.1 Liquid displacement gas measurement

The majority of laboratory volumetric gas meters are based on the liquid displacement method. These meters can be constructed with simple materials like glass/plastic jars or cylinders. Liquid displacement meters are simple, economic and they can work for a long period of time without maintenance. The preservation and collection of gases is the most important operation for any liquid displacement gasometer [34]. Gasometers are the classical gas measuring unit which works with the principle of gas storing and does not provide the flowrates directly. The collection of the gas is usually done with the use of vessels containing a suitable liquid which is displaced as the gas gets collected.

Conversion procedures of biogas from Normal conditions to Standard conditions are presented below. Fluctuation of room temperature and atmospheric pressure during the measurement of gas can contribute errors in volume calculations. Therefore, to apply corrections, the record of change of atmospheric pressure and temperature is important.

The gas pressure inside the tube collected over the liquid solution is the sum of the biogas pressure and the vapor pressure. The pressure of biogas, (P_{bio}) can be obtained by subtracting the vapor pressure of liquid (Pw) at the temperature of measurement from the pressure of collected moist gas (P).

$$P_{bio} = P - P_w$$

If the gas is collected over liquid, static pressure acts due to the difference of level (P_{level}),

$$P_{bio} = P - P_w - P_{level} \quad \text{or} \quad P_{bio} = P - P_w + P_{level}$$

The produced biogas volume in normal condition can be converted to STP using Combine Gas law:

$$V_0 = V \times \frac{T_0}{T} \times \frac{P_{bio}}{P_0}$$

Here, V is the measured gas volume, V_o is the volume of gas in standard temperature and pressure, P_o is the standard pressure, T is gas temperature at the time of measurement, and T_o is the standard temperature. Modified Arden Buck Equation (1996) can be suggested for the calculation of vapor pressure [35].

$$P_w = 6.1121 \exp\left(\left(18.678 - \frac{T_c}{234.5}\right) \times \frac{T_c}{257.14 + T_c}\right)$$

Tc is the temperature of gas in degrees Celsius. Pw is pressure in hP (1 hP = 0.1 kPa)

Gasometers are usually height or weight types. In the height gasometer, biogas can be introduced into the liquid column directly from the digester or by emptying a gas bag as shown in Figure 4 a, 4 b. Gas volume is calculated from the measurement of change in barrier solution height. Figure 5a shows weight type meter in which the gas directly from digester displaces the barrier liquid from a sealed flask into a second open container, and the gas volume is determined by weighting the displaced solution. Figure 5 b shows the weighting method of gas volume measurement by gas bag emptying.

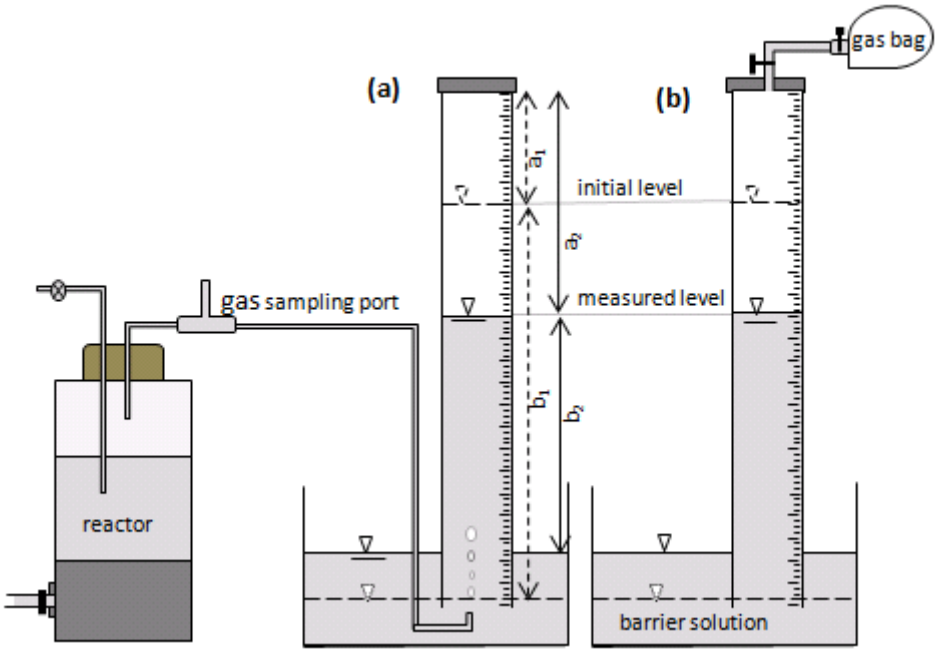

Figure 4a and 4b: Measurement of gas a) direct from a reactor using cylinder meter b) indirectly by collecting in a gas bag using height meter, modified from [17].

Equation for gas volume calculation by height measurement using height meter:

$$V_0 = \frac{T_0}{T\,P_0}\left[\left((P - P_w - \rho.g.b_1)A\,.\,a_1\right) - \left((P - P_w - \rho.g.b_2)A.ha_2\right)\right]$$

Equation for gas volume calculation by weighting displaced solution in bottle meter:

$$V_0 = \frac{T_0(m_b - m_a)}{T\,P_0\,\rho}\left[P - P_w + \rho.g\left(a_1 + a_2 + {}^{V_a}/_{A}\right)\right]$$

Equation for gas measurement by weighting displaced solution in column meter:

$$V_0 = \frac{T_0}{T\,P_0}\left\{\left[\left(P - P_w + \rho.g.\left(b_1 - \frac{m}{\rho.A}\right)\right).A.\left(a_1 + \frac{m}{\rho.A}\right)\right] - \left[(P - P_w + \rho.g.b_1).A.a_1\right]\right\}$$

Here, a and b represent heights of gas and liquid. m represents mass of liquid measured. Subscripts 1, 2 represent condition before measurement and after measurement. ρ is the density of liquid. A is cross sectional area. g is acceleration due to gravity.

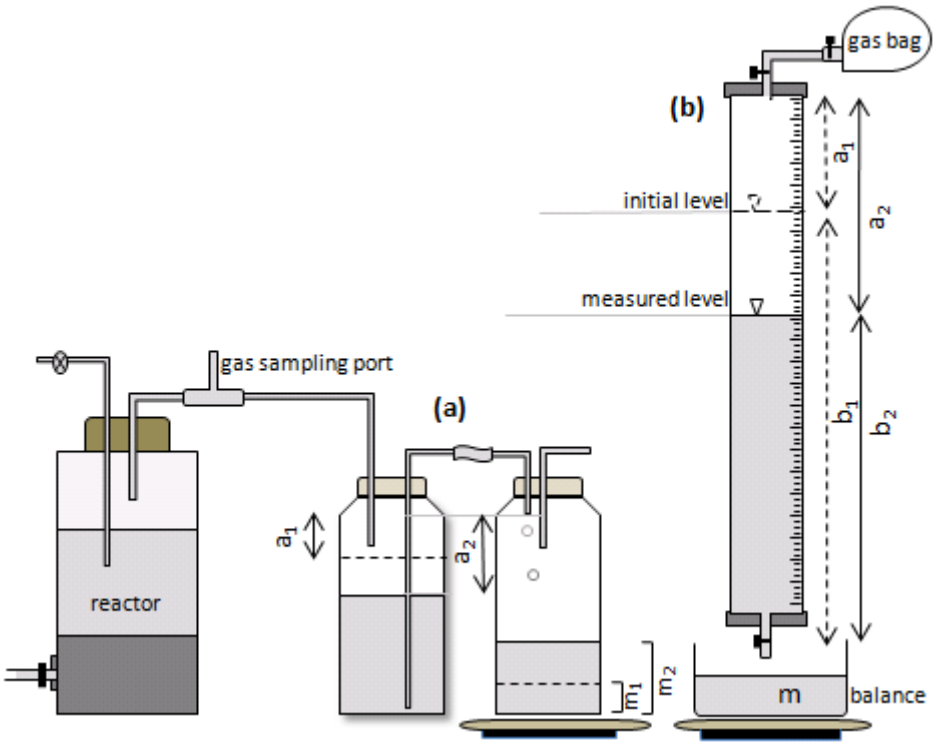

Figure 5a and 5b: Measurement of gas a) direct from reactor using bottle meter b) indirectly by collecting in gas bag and using height meter, modified from [17].

The major drawback of the liquid displacement gas collecting and measuring system is inaccuracy due to biogas solubility/diffusion through the barrier solution. Liquids such as simple tap water, oil, acidified water and carbonated water are widely used as barrier solutions. The solubility and diffusion varies with type of liquid, atmospheric pressure, temperature, density of liquid, gas composition. Therefore, the same correction factor cannot be applied for every time of gas measurement. A study reported that underestimations of CO_2 in the biogas could be as high as 30% with the use of Warburg liquid displacement gas measurement system [15]. The evaporation of barrier solutions after a long period of time can also result in inaccuracies. Gas solubility errors can be eliminated by collecting gas in gas bags and measuring the gas volume with liquid column meters (Figure 4 b, 5 b)

CO_2 is a sensitive parameter and its analysis is important for the monitoring of the anaerobic digestion process. The increase and subsequent stabilization of CO_2 content represents the progress of the process during the start-up. The CO_2 measurement during the routine operation is also important as it suggests the specific digester's operational background value.

Simple basic solutions can be used for determining the CO_2 and CH_4 concentration without chromatography analysis. This is done by allowing a known volume of biogas in contact with a saturated solution of potassium hydroxide or sodium hydroxide. The CO_2 will get dissolved rapidly in the solution and the remaining gas can be assumed to be methane [36].

$$CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O$$

Most scientific publications and standards suggest the use of either a highly acidic, saline or acidified saline solution to avoid the diffusion of CO_2 in the liquid displacement measuring gasometers [17, 37, 38, 39]. The accuracy of automatically operated displacement instruments also depends on the nature of the sealing liquid. Table 1 summarizes the barrier solutions.

Table 1 Summary of type and nature of barrier solutions proposed by different sources

S.n	Source	Suggested solution	Composition
1	Walker et al. [17]	NaCl/acid	Saturated NaCl solution, pH 2
2	ASTM D 5511 [37]	Acidified water	Water, pH not less than 2
3	ISO/DIS 14853 [38]	NaCl/ acid	200 g NaCl +1 l distilled water + 5 g citric acid
4	Apex instruments [39]	Orsat confining solution	100 g of (Na_2SO_4) + 500 ml distilled water +20 ml concentrated sulfuric acid

The questions that arise regarding the use of the various barrier/sealing solutions are:

I. Can the biogas volume determined using simple liquid displacement meters can be considered accurate?
II. What type of solution can be considered the most accurate and will avoid the loss of gas due to diffusion?

3.2 Gas chromatography

Gas chromatography (GC) is a popular instrument and has several advantages such as high resolution, high speed, high sensitivity and good quantitative results. GC is an ideal method since it is well suited for the measurement of gas which is in contact with its liquid phase [39].

Samples are inserted into the GC after running the prepared calibration standards of CO_2 and CH_4. The thermal conductivity detector (TCD) is widely used for the detection of light hydrocarbons and compounds that respond weakly to the flame ionization detector (FID). The TCD is less sensitive than the FID (10^{-5}-10^{-6}g/s, linear range: 10^3-10^4). The FID is very sensitive towards organic molecules (10^{-12} g/s, linear range: 10^6 –10^7). The FID analysis is important when measurement is required for small amounts of hydrocarbons as it can give larger signals and hence better precision than TCD [40].

3.2.1 Headspace biogas analysis with GC (HS-GC)

The measurement and analysis of gas in a closed anaerobic system to determine the reliability of activity assessment is very much related with the balance between the liquid and gas phase. In the case of chromatography analysis, the solubility of analyte gases in the liquid phase and their distribution takes place according to the thermodynamic equilibrium of the system [39]. The most important factor for headspace biogas is the consistency of temperature and pressure as it can directly influence the balance of the gas concentration. Errors can result in the GC gas measurement when the temperature of samples differs significantly from the temperature of the calibration gas [41]. A small change in temperature during GC measurement of gas can also affect the anaerobic microbiology. GC can simply help in the determination of the ultimate methane potential of substances and their rate of biodegradation.

The biogas process is commonly investigated at 35 °C and to a lesser extent at 20 °C, 55 °C and 70 °C. However, the standard methane, that is used for comparison is prepared at the laboratory room temperature (≈20 to 23 °C).

Anaerobic reaction undergoes different changes in gas pressure

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	Hydrogenotrophic methanogenesis
$CH_3COOH \rightarrow CH_4 + CO_2$	Aceticlastic methanogenesis
$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$	Acetate oxidation
$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$	Homoacetogenesis

In hydrogenotrophic reaction, 5 mole of gas is converted into 1 mole of gas resulting in the decrease of pressure. On the other hand, the conversion of acetate into CO_2 and CH_4 will results in the rise of pressure. The pressure can vary from the type of substrates used and

the products formed. In normal anaerobic $H_2 + CO_2$ culture, the pressure can vary from 0.6 to 2.5 atmp (1 atmp = 101.29 kPa) [41].

Pressure inside a closed anaerobic system will be affected by the change in temperature and vapor pressure. However, if we use a pressure-lock syringe for GC gas analysis, then the methane measurement is independent of pressure. In case of leaking during sampling, a third compound i.e. air, can diffuse into the syringe (or through the rubber septum). Temperature can easily influence the GC measurement response. Another concern is that removal of sample aliquots from the bottle should not influence the effective pressure in the bottle. Therefore, the sample portions removed should be small enough compared with the volume of the bottle to minimize this effect.

The difference in temperature between the incubated reactors and the prepared standards can be a major source of error in headspace gas analysis. It is important to maintain the equilibrium of gas concentration which is controlled by the equilibrium constant (distribution constant, partition constant, Henry's law constant) [39].

The questions that arise regarding the headspace biogas analysis are:

I. Does the biogas analyzed by GC from assays incubated at different temperature yield the same results?
II. Are the standard curves obtained from preparing the standard gas sample at room temp valid for comparison of biogas at different incubated temperatures?
III. What will be the effect of a short fluctuation of incubation temperature on GC response and on the anaerobic degradation process?

4 OBJECTIVES

Experiment was designed to aid accuracy in volumetric gas measurement with liquid displacement and to aid accuracy in HS-GC analysis of biogas. The objectives were:

I. To compare diffusion of CO_2 in different types of solutions mentioned in different publications and to find a suitable barrier/sealing solution which can be used in displacement meters.
II. To design and develop accurate, simple volumetric gas meter.
III. To evaluate the errors that could arise due to the difference in nature and temperature between the standards and samples.

IV. To analyze the effect of short time temperature fluctuation during the gas analysis from assays incubated at mesophilic and thermophilic temperature.

5 MATERIALS AND METHODS

In this thesis, three experiments are conducted. This chapter includes the following information: the materials used in each experiment; the experimental setup; the sampling and analytical methods.

5.1 Solubility test of biogas in different solutions

The solubility test of CO_2 and CH_4 in different barrier/sealing solutions was performed for a total period of 30 days in order to compare the solubility/diffusion of biogas.

5.1.1 Materials for gas solubility test

Liquid displacement method by downward displacement of gas was used to determine the solubility of biogas. Twelve different types of solutions were prepared. The quantity of each test solution prepared was 4.5 liters. Gas meters were constructed from transparent acrylic tubes of internal diameter 6.4 cm and height 1 m. The top of the gas meters were capped with an air tight rubber septum in order to take samples for GC TCD analysis.

5.1.2 Gas solubility test set up

The different solutions tested were carbonated distilled water (pH 2.5), tap water, acidified water (pH 2), acidified water (pH 1), acidified water (pH 0.5), 20% NaCl soulution (pH 1), 20% NaCl solution (pH 0.5), 40% NaCl solution (pH 0.5), 60% NaCl solution (pH 0.5), 80% NaCl solution (pH 0.5), 95% NaCl solution (pH 0.5) and Orsat solution.

A standard gas consisting of 50% CO_2 and 50% CH_4 was used as the test biogas. It was introduced from the bottom of the columns with the help of a peristaltic pump. The gas flow rate for each meter was maintained at 1.16 cm^3/sec for 1000 sec. Samples were taken with a pressure lock syringe and their CO_2 and CH_4 content was measured with GC. The change in liquid height, laboratory temperature and pressure were recorded at the time of measurement. Gas analysis was done on the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 9^{th}, 11^{th}, 14^{th}, 21^{th} and 30^{th} day of the experiment.

5.2 Gas meter design and test

A dedicated gas meter was designed and constructed which works on the principle of liquid displacement (Figure 6). Water and acidified saturated NaCl solution were used as test liquids.

5.2.1 Gas meter design

The gas meter was constructed using two coaxial chambers made of acrylic (outer) and glass (inner) cylinders. In between the walls of the cylinders, a small glass tube is placed which acts as support for the magnetic floater. The outer chamber is open in the upper area so that the liquid is at atmospheric pressure. The internal chamber is closed on the top arranged with a piping connection to the three way solenoid valve with gas inlet and exhaust. When there is no gas flow, liquid surface in both chambers is equal. As the gas production increases, it gets collected inside the internal chamber which results in the rise of the liquid level in between the walls. The change in the liquid height is proportional to the volume of gas collected inside the internal chamber and is controlled with the activation of a pre-set magnetic level sensor that regulates the operation of the solenoid valve.

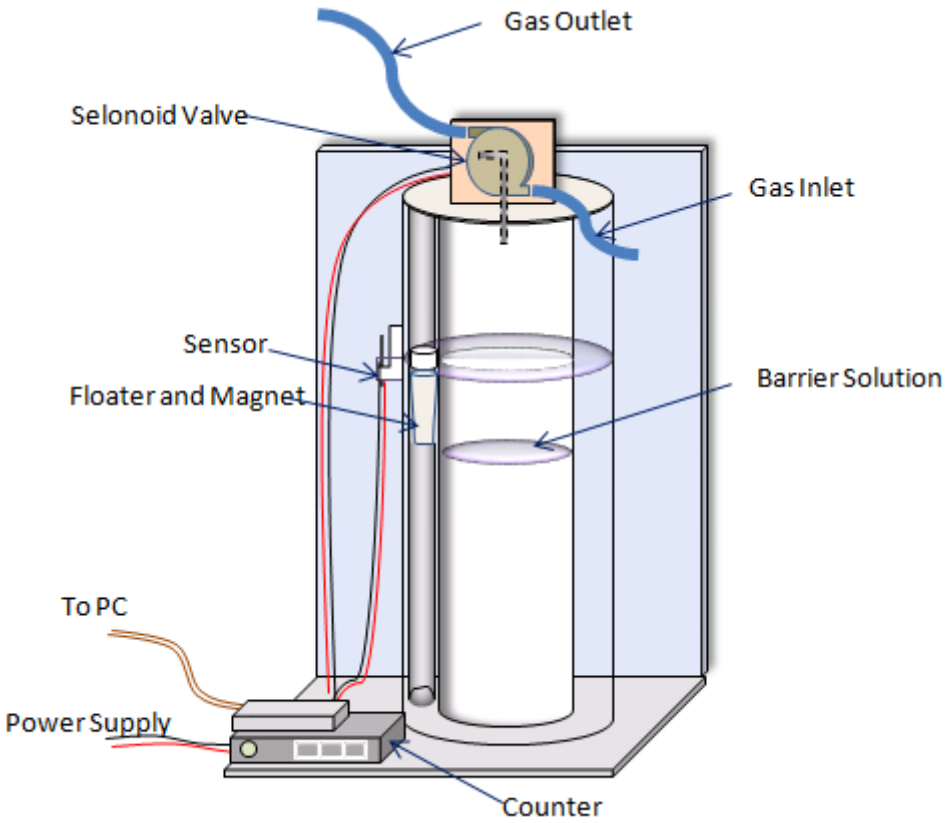

Figure 6: Layout of automatic biogas meter.

5.2.2 Gas meter test

Water and saturated acidified NaCl solutions were tested as the sealing solution in the constructed biogas meter.

The calibration idea was based on the literature [28]. Calibration was done by expelling standard saturated gas (50 % CO_2 and 50 % CH_4) from a buffer flask by injecting a known amount of solution with the help of a syringe and measuring the mass of liquid injected. Knowing the density of liquid, an accurate determination of the gas displaced volume (V) for that count of gas measured was calculated. The gas volume at standard temperature and pressure (V_o) during one cycle operation is given by.

$$V_0 = V \times \frac{T_0}{T} \times \frac{P + \Delta H - P_w}{P_0}$$

T_o, P_o are standard temperature and pressure. P is the laboratory room pressure, ΔH is the pressure difference due to difference in liquid height, P_w is vapor pressure.

Standard saturated gas was supplied continuously at a flow rate of 2.1 cm^3/min and re-calibrations was done after 10, 50, 100, 200 and 400 counts to see the performance of the barrier solutions in the gas meter.

5.3 Headspace gas chromatography analysis

This part of the experiment was designed to determine the change in response due to the difference in nature and temperature between the standards and samples. The effect of temperature fluctuation on gas measurement and anaerobic degradation was also analyzed

5.3.1 Materials for headspace gas chromatography test

Synthetic dry/humidified biogas at different incubation temperatures and biogas produced from anaerobic digestion at mesophilic and thermophilic temperatures were used for the HSGC analysis. Standard gas consisting 50% CO_2 and 50% CH_4 was used for dry biogas analysis. Deionized water was used to humidify standard gas for the gas-liquid phase headspace experiment. Insulation of the syringe was done with a silicon tube in order to minimize the effect of external temperature.

Homogeneous liquid inoculum was used for mesophilic and thermophilic anaerobic experiments. The inoculum used to seed the mesophilic and thermophilic anaerobic digestion was mesophilically digested sewage sludge brought from municipal waste water treatment plant and aged under anaerobic condition for a period of 2 weeks. Culture media contained (per liter of deionized water): 10 ml Solution A, 2 ml solution B, 1 ml solution C, 1 ml solution D, 50 ml $NaHCO_3$, 1 g yeast extract and 1ml of vitamin solution. Solution A (per liter of dionized water) contained: 100g NH_4Cl, 10g NaCl, 10 g $MgCl_2.2H_20$; Solution B contained: 200g $K_2HPO_4.3H_20$; Solution C contained: 0.5 g $C_{12}H_6NO_4Na$; Solution D contained 2380 mg of $FeCl_2.6H_20$, 500 ml Redest water, 1 ml HCl, 50 g H_3BO_3, 50 g $ZnCl_2$, 38 g $CuCl_2.2H_20$, 50 g $MnCl_2.4H_2O$, 50 mg $((NH_4)_6Mo_7O_{24})$, 50 mg $AlCl_3$, 50 mg $COCl_2.6H_2O$, 92 mg $NiCl_2.6H_2O$, 500 mg EDTA and 66 mg $Na_2SeO_3.5H_2O$. 0.5 M glucose solution was also used as stock solution.

5.3.2 Dry and humidified synthetic biogas analysis with GC TCD

This experiment was arranged in order to understand the change in chromatography response for samples incubated at different temperatures when compared to the standard prepared at room temperature.

Experimental analysis for dry and humidified synthetic gas samples were carried out at different temperatures. The composition and measurement of gases of an enclosed anaerobic system can be easily affected by different factors such as temperature, pressure and solubility. In the present study, the concentration of CO_2 and CH_4 was analyzed with GC from bottles incubated at different temperatures (4, 20, 35, 55, 70 °C). This would give the relation of CO_2 and CH_4 solubility in liquid phase and their relation with temperature.

Glass serum bottles (120 ml) were used in the study. The bottles were flushed with N_2 for 5 minutes and then with a standard gas (50 % CO_2 and 50 % CH_4) for 20 min. Assays were sealed with butyl rubber stoppers and aluminum crimps. For the preparation of saturated biogas, the bottles were filled with 20 ml distilled water prior to flushing and sealing. Gas pressure inside the bottles was maintained≈1atm. Prepared assays were incubated at 4, 20, 35, 55 and 70 °C.

Altogether 48 bottles were used Half of them (24 bottles) were for the gas phase experiment and the other half for the gas-liquid phase experiment (as shown in Table 2 below). Each group was further divided into 6 sub-groups. Therefore, each temperature set

experiment consisted of 4 bottles. Temperature was controlled by placing the bottles in a gyratory water bath. The water bath was placed very close to the GC instrument and samplings were done without removing the bottles from the bath. Standards were prepared at room temperature (23 °C).

The measurement experimental procedure was done in three steps for each set of bottles for both dry gas and saturated gas. The samples were taken directly from the headspace of the serum bottles for subsequent analysis.

Step-I – each prepared set of assays (4 numbers) was kept at room temperature 23 °C for 2 hours and gas concentration was examined using GC.

Step-II – the assays were incubated in a constant temperature water bath for 2 hours and samplings were done without removing the bottles. The effect and influence of outside environment, heat transfer from human hand to syringe, and the rapidity/delay in gas injection on GC response were also the part of research. Gas sampling was done with an insulated syringe and then by removing the insulation in order to see if there was an effect of the external temperature. The syringe was insulated with a silicon tube. Samples were injected in the CG column rapidly within 5 seconds and after delaying for 30 seconds.

Step-III – temperature of sample bottles was equalized back to the room temperature by placing the bottles in a 23 °C water bath for 2 hours and GC measurement was done again.

Table 2: Summary of dry/humidified gas experiment.

Set	Bottle	Incubation temp	Gas measurement temperature
1	1-4	0 °C	0 °C and 23 °C
2	5-8	5 °C	5 °C and 23 °C
3	9-12	20 °C	20 °C and 23 °C
4	13-16	35 °C	35 °C and 23 °C
5	17-20	55 °C	55 °C and 23 °C
6	21-24	70 °C	70 °C and 23 °C

5.3.3 Biogas from anaerobic digestion and analysis with GC- TCD/FID

The objective was to investigate the effect of fluctuation of incubation temperature during the biogas measurement and its general effect in biogas production (microbiology environment). GC gas measurement was done using both TCD and FID in order to compare the results.

The bottles were filled with 30 ml inoculum, 1 ml of 0.5 M glucose and 9 ml of batch anaerobic medium to make up the total working volume of 40 ml. Assays were made anaerobic by flushing with 30 % CO_2 and 70 % N_2 for 5 min and sealing with butyl rubber stoppers and aluminum crimps.

Altogether 16 bottles were prepared (as shown in Table 3 below). Half of them (8 bottles) were incubated at 35 °C (mesophilic condition) and the other half at 55 °C (thermophilic condition). Each group was further divided in two sub-groups with 4 bottles in each set. One set of bottles was cooled to room temperature just for a short period of time while taking the GC gas measurement. The temperature of another set of bottles was held constant during the entire experiment. At the same time, FID measurement for CH_4 was carried in order to check the differences. The anaerobic experiment was carried for a period of 40 days. Gas measurement was done on the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 12^{th}, 16^{th}, 23^{rd} and 40^{th} day of the experiment.

Table 3: Summary of mesophilic and thermophilic experiment.

Set	Bottle	Digestion temperature	Gas measurement temperature
1	1-4	Mesophilic (35 °C)	at 35 °C
2	5-8	Mesophilic (35 °C)	at 35 °C and at 23 °C
3	9-12	Thermophlic (55 °C)	at 55 °C
4	13-16	Thermophilic (55 °C)	at 55 °C and at 23 °C

5.3 Sampling and analytical methods

The ambient temperature and pressure at the time of gas measurement was noted for STP conversion in case of solution gas solubility test. Vapor pressure was calculated using the Arden Buck equation [36].

The number of replicates of the samples was four for each set of headspace gas experiment. This allows analysis of the collected data and guarantees the reproducibility of the assays.

The Gas Chromatographer used was equipped with a flame ionization detector (Perkin Elmer Clarus 500, Perkin Elmer Alumina column 30 m x 0.53 mm, carrier gas argon, oven temperature 100 °C, injection port 250 °C, detector 225 °C) and thermal conductivity detector (Perkin Elmer Calarus 500, Supelco Carboxen™ 1010 PLOT fused silica capillary column 30m x 0.53 mm, carrier gas argon, oven 200 °C, injection port 225 °C, detector 230 °C)

A special gas tight syringe (VICI Pressure-Lok® Precision Analytical Syringe) provided with removable needles was used for sampling with 0.1 ml of sample taken at a time and injected directly in the chromatographic column.

5.3.1 CG calculation

The concentration (%) of the sample gas is determined by comparing it with the number of moles in the same volume (0.1 ml) of a standard at a known methane gas concentration (X %) injected into the GC.

CO_2 and CH_4 content in the sample (%) = (Sample peak area/standard area) x X %

6 RESULTS

6.1 Effect of biogas diffusion in different barrier solutions

The effect of different barrier solutions on the solubility of CO_2 and CH_4 was studied for 30 days. The results are presented in Figure 7 and Figure 8. Carbonated distilled water as a barrier solution showed high resistance to CO_2 diffusion at the beginning of experiment. As time passed, the loss of CO_2 was much higher than any other solutions. The last day's measurement showed that 89.1 % of total CO_2 was lost during the experimental period. Acidified water with pH values 2, 1 and 0.5 also were weak to prevent CO_2 solubility. The loss of CO_2 in these three solutions was 76.6 %, 72.7 % and 71.0 %, respectively. The resistances to the diffusion of CO_2 in 20 % saturated NaCl solutions with pH value 1 and pH value 0.5 was also very weak. An increase in salt concentration showed a decrease in

gas solubility. The higher the concentration of salt, the lower was the gas loss. The loss of CO_2 in 40 % saturated NaCl with a pH value of 0.5 was 49.4% and for Orsat confining solution it was 27.3%. Similarly, the loss of CO_2 in 60 %, 80 %, and 95 % saturated solutions with pH value of 0.5 was 46.9%, 27.4% and 7.4%, respectively. Solution with 95% saturated NaCl and pH value 0.5 showed better performance in the preservation of gas than other test solutions. With time, crystallization of salt started to appear at the bottom of the gasometers containig 60%, 80% and 95% saturated NaCl solutions. As methane is slightly soluble in comparison to CO_2, the loss of methane was very small; 2.2 % in 95 % NaCl saturated solution and 6.9 % in 80 % NaCl saturated solution. Loss of methane in simple tap water and Orsat confining solution was 14.03 % and 3.76 % respectively.

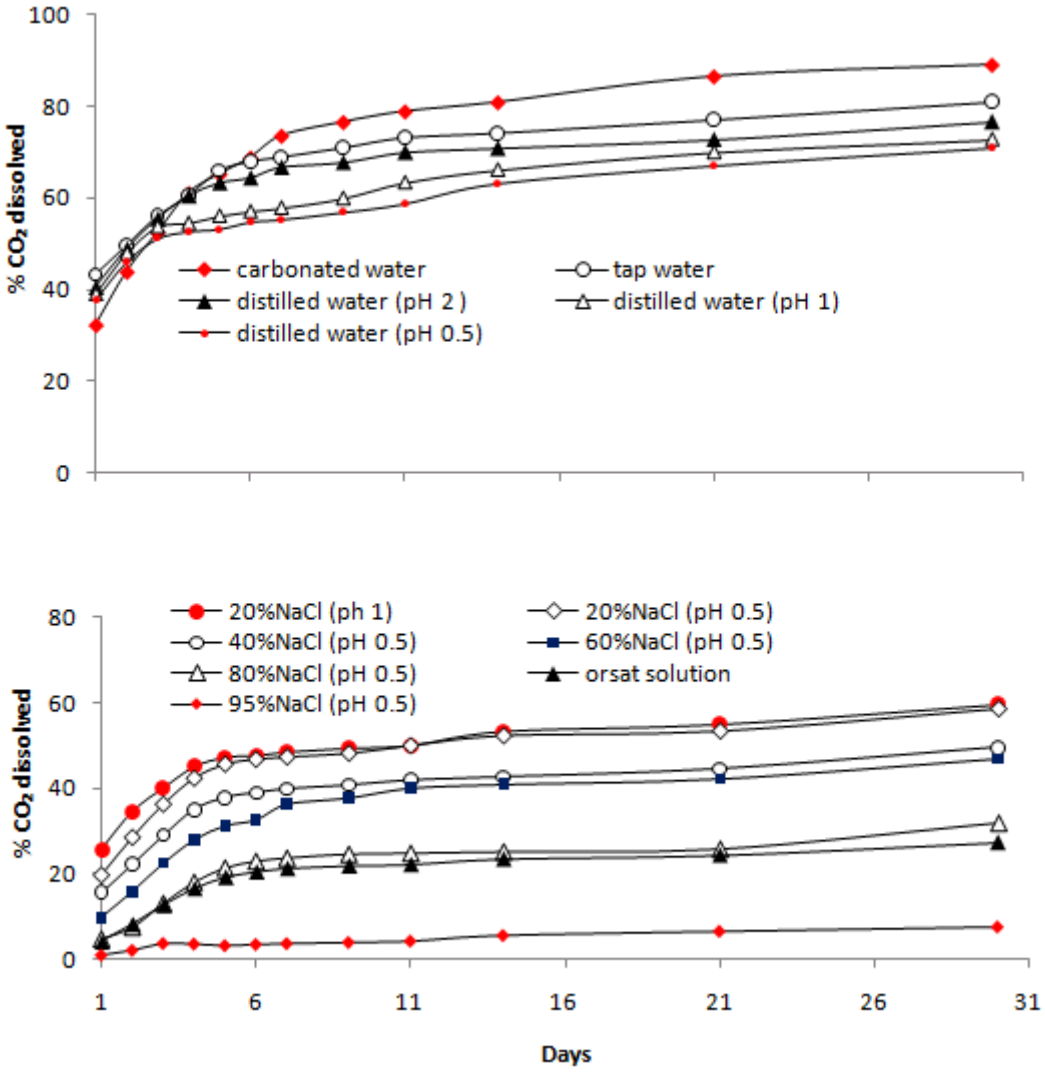

Figure7: Percentage of carbon dioxide dissolved in various barrier solutions

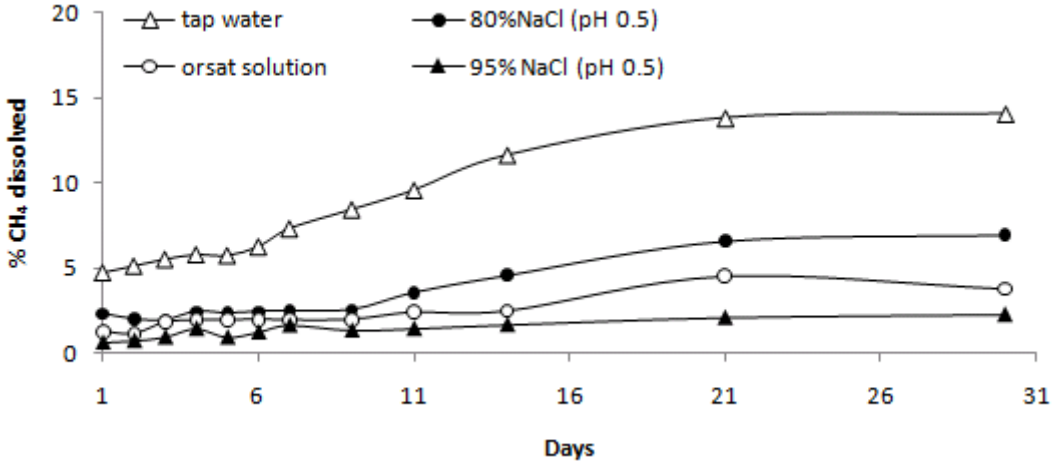

Figure 8: Percentage of methane dissolved in various barrier solutions

6.2 Gas meter performance

The gas meter test was performed with simple tap water and acidified NaCl solution as sealing liquids. Figure 9 shows the performance of two different sealing solutions, simple water and acidified saturated NaCl. When tap water was used, the biogas volume error decreased with the increase in time. The errors at the beginning of the counts were higher for tap water. The decrease in the error was due to the increase of carbon dioxide saturation in the liquid. Figure 9 shows an error of 1.5 % after 400 counts for water. NaCl solution showed a good result with .05 % after 400 counts. The volume and nature of the liquid, ratio of diameter of the cylinders, and the adjustment of sensor height volume for each count were seen to have a major influence on the error on the biogas volume measured.

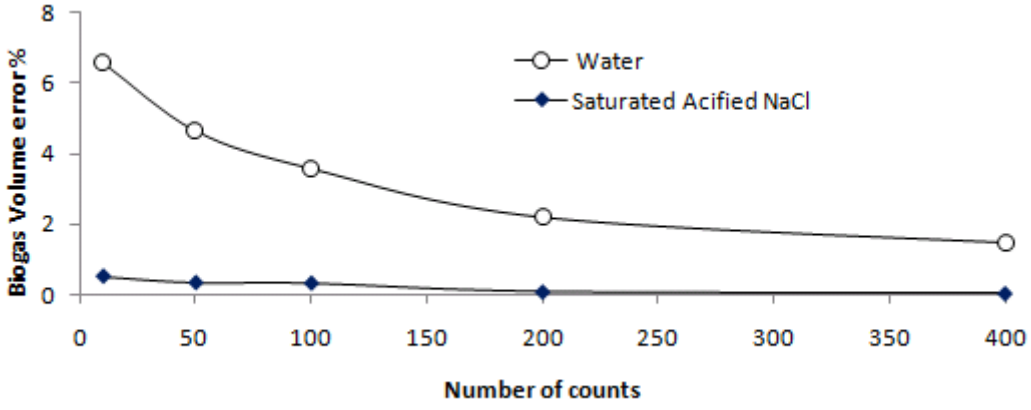

Figure 9: Percentage biogas volume error verses number of counts

6.3 Effect of dry and humidified synthetic biogas measurement with GC-TCD

A series of experiments were performed to understand the effect of gas temperature on CH_4 and CO_2 measurement using GC- TCD. Figure 10 represents the change of CH_4/CO_2 concentrations of dry biogas (gas phase alone) analysis and humidified biogas (gas liquid phase) analysis. The curved line shows the concentration of gases when the measurement was done at the incubated temperatures with the insulated syringe. The more horizontal line indicates the response of the gas concentration when the temperature of the bottles was brought back to the room temperature i.e. 23°C and gas measurement was done.

The concentration of CH_4 and CO_2 in the incubated assays at different temperatures changed from the original concentration (50% CH_4, 50% CO_2) when samplings were done at incubation temperature and compared with standards prepared at room temperature (23 °C). GC response increased with the increase of incubation temperature. However, the increment trend was not similar in all assays. The magnitude of response in the case of the dry gas was less than that of humidified gas. The CH_4 concentration for dry and humidified gas at the lower temperature of 4 °C was 46.9 % and 47.1 %, respectively. At 35 °C, the concentration of CH_4 was 52.1 % for dry gas and 53.4 % for humidified gas. The corresponding values at 55 °C were 56.9 % for dry gas and 58.8 % for humidified gas. The response was much higher at 70 °C; it was 64.2 % for dry gas and 73.2 % for saturated gas.

TCD response for CO_2 in the dry gas experiment was similar to that of CH_4. However, for the saturated phase, with the increase of temperature, there was a big increase in CO_2 concentration. At mesophilic temperature (35 °C), the humidified CO_2 concentration was 57.7 %. On the other hand at 55 °C and 70 °C, GC response increased sharply with total CO_2 concentration of 87.6 % and 124.7 %.

When the incubated bottles were brought back to the room temperature, the concentration of gases also changed back to their original levels.

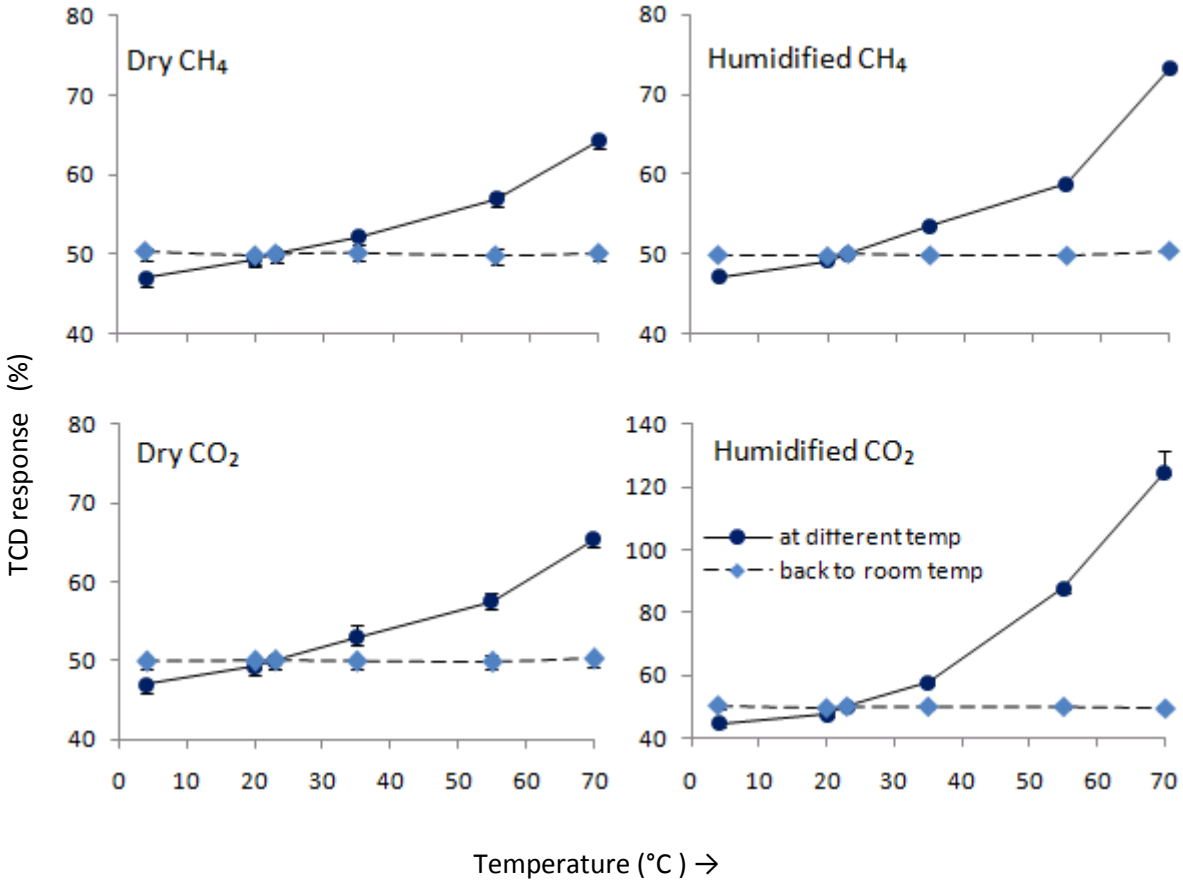

Figure 10: Change in TCD response with temperature (upper: dry/ humidified CH_4 response and lower: dry/ humidified CO_2).
The graphs represent GC TCD responses for methane and carbon dioxide at different temperatures relative to the standard prepared at room temperature. The points on the lines indicate the averages of the four replicate bottles.

6.3.1 Effect of comparison between insulated and uninsulated syringe

The effects of insulated verses uninsulated syringe samplings (influence of outside temperature) along with rapid verses delayed injection of samples were also studied. Difference in the chromatography response was observed when gas sampling was conducted with an insulated and uninsulated pressure lock syringe. GC response was also seen to have relation with the time that analytes stored inside the syringe barrel. Figures 11 and 12 show the comparison of CH_4 and CO_2 concentrations. A small increase on GC response was observed when sampled with an uninsulated syringe compared to that of an insulated one. Also the GC response was slightly lower for delayed injections (30 seconds) when compared to rapid injections.

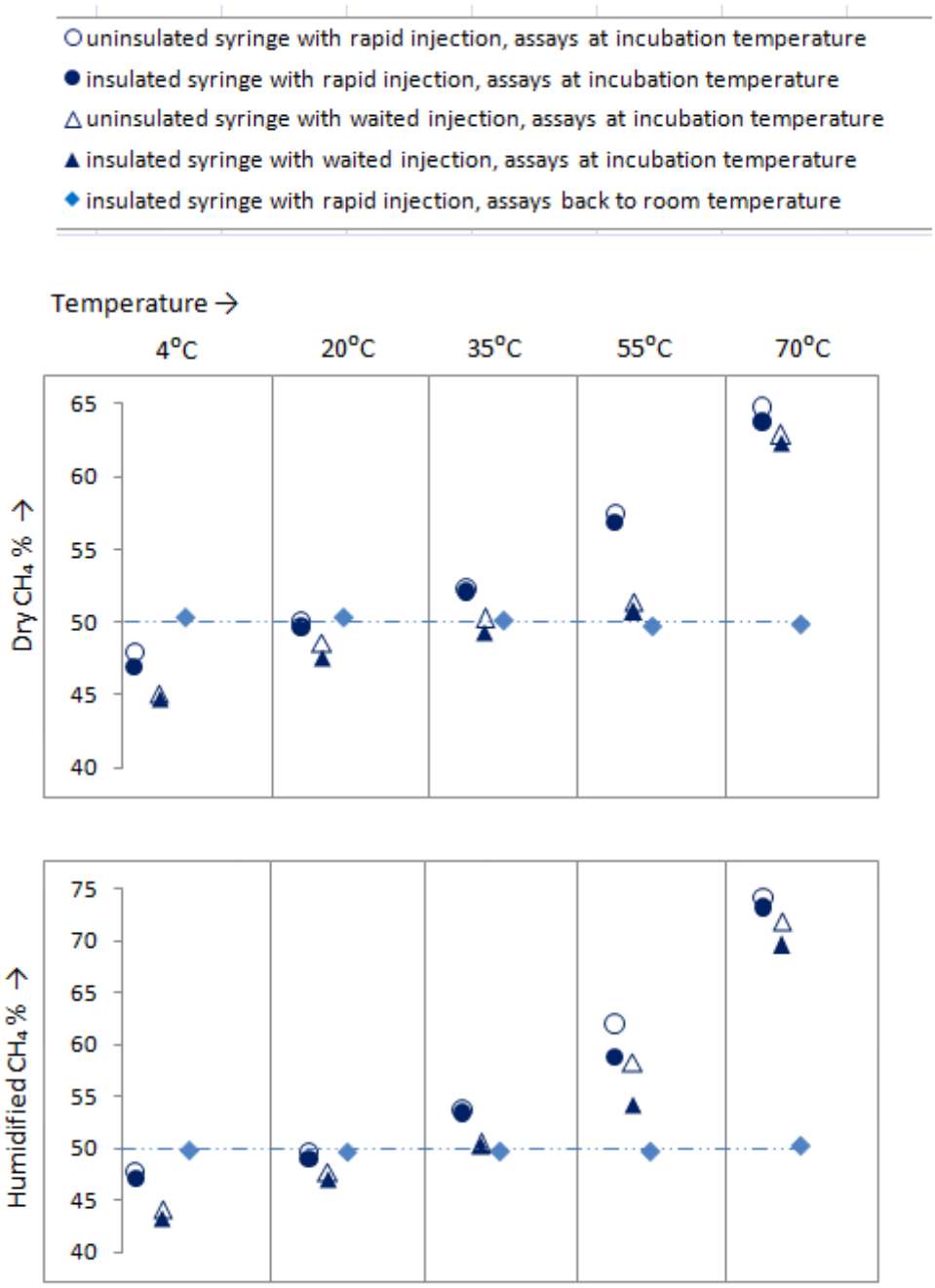

Figure 11: Comparison of TCD response between insulated and unsilutated syringes with rapid and delayed injections for dry and humidified CH_4 at different incubation temperatures.

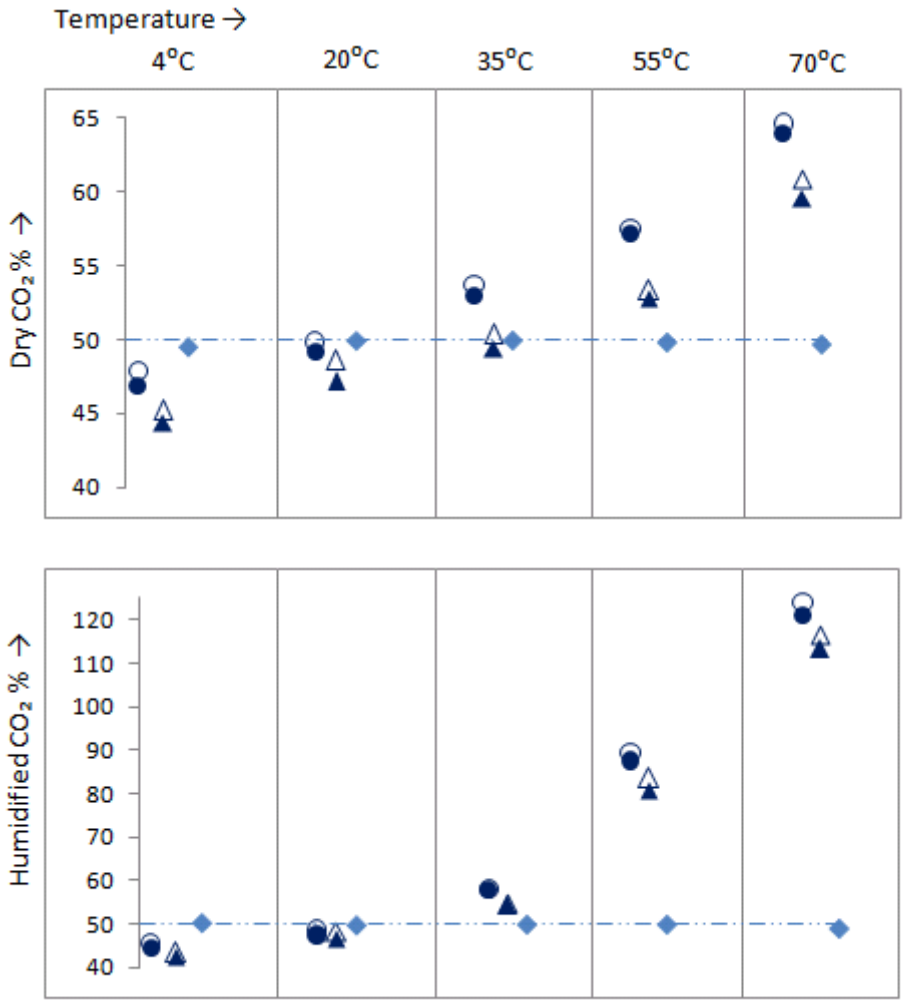

Figure 12: Comparison of TCD response between insulated and uninsulated syringes with rapid and slow injections for dry and humidified CO_2 at different incubation temperature.

6.4 Anaerobic digestion and biogas analysis with GC TCD/FID

The previous experiment (gas phase alone and gas liquid phase) showed the higher accuracy in GC gas measurement when the samples were cooled back to room temperature i.e. 23 °C. The case of headspace anaerobic gas measurement from reactors can be considered differently because the micro-organisms are very sensitive to different factors including a change in temperature. This part of the experiment incorporates mesophilic and themophilic anaerobic biogas production, GC gas measurement and their relation with a very short term cooling effect on biogas production. Figure 13 shows the difference in the concentration of CO_2 and CH_4 with TCD analysis for mesophilic and thermophilic gas measurement when gas measurement was done at an incubated temperature and after short term cooling. This effect of cooling in the anaerobic degradation process can be analyzed

by comparing the gas concentration between the first set of bottles, which were always at constant temperature, with bottles that were cooled.

The last day's CH_4 concentration before and after cooling in the case of mesophilic digestion was 64.3 % and 59.7 %, respectively. For constant temperature assays it was 60.5%. Thermophilic CH_4 concentration before and after cooling of the assays was 71.1% and 59.0 %, respectively. The corresponding value in constant temperature assays was 76.7 %. Concentration of CO_2 before and after a short time of cooling on that day was 33.3 % and 32.0 %, respectively. It was 37.72% in constant temperature assays. The last day's CO_2 concentration before cooling and after cooling in thermophilic digestion was 67.1 % and 45.6 %. This shows a difference of more than a 20 % change in CO_2 concentration in the headspace due to the change of bottle temperature. The CO_2 concentration was 63.6 % in the constant temperature assays.

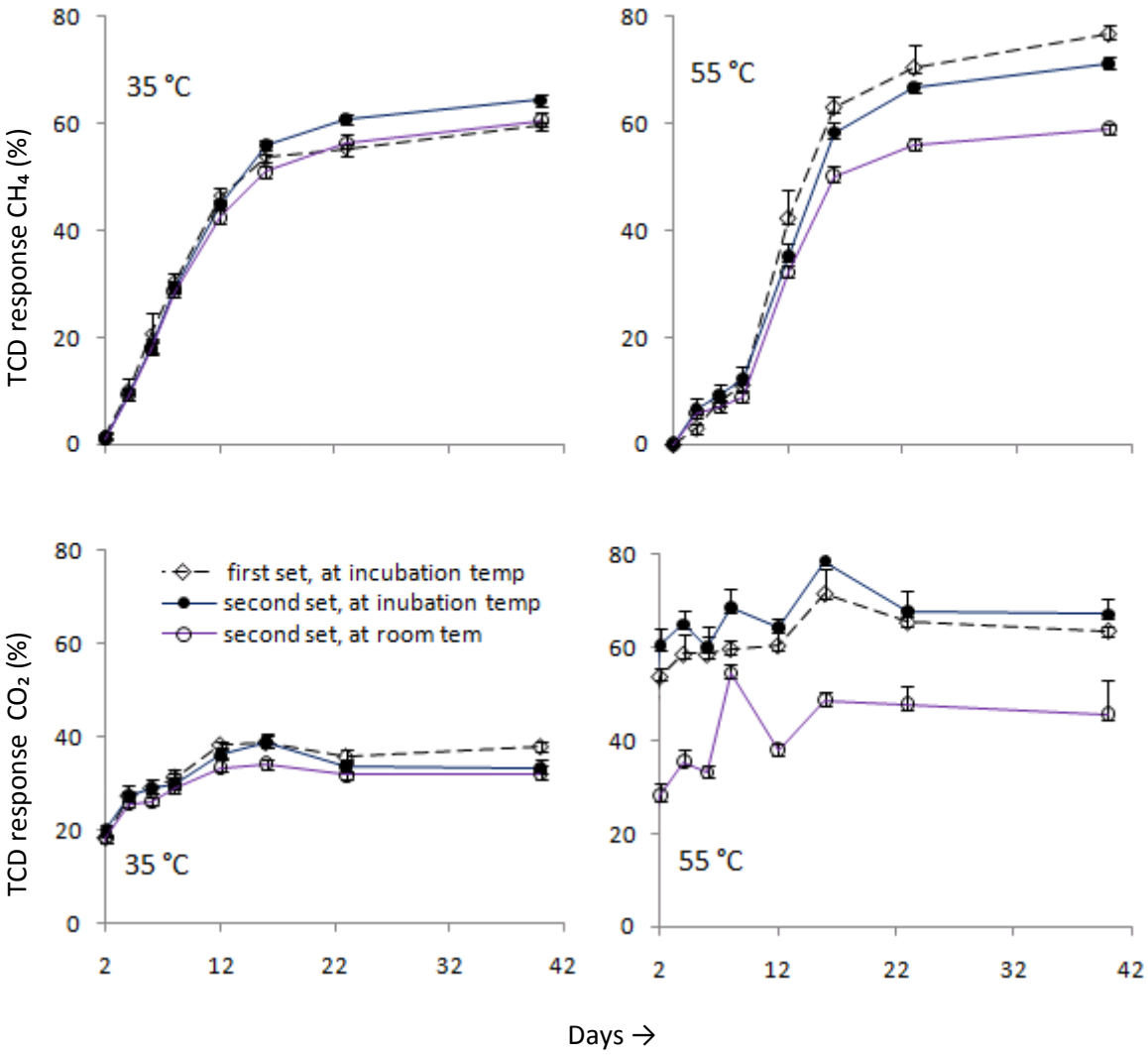

Figure 13: TCD response for CH_4 (above) and CO_2 (below) effect of short term cooling

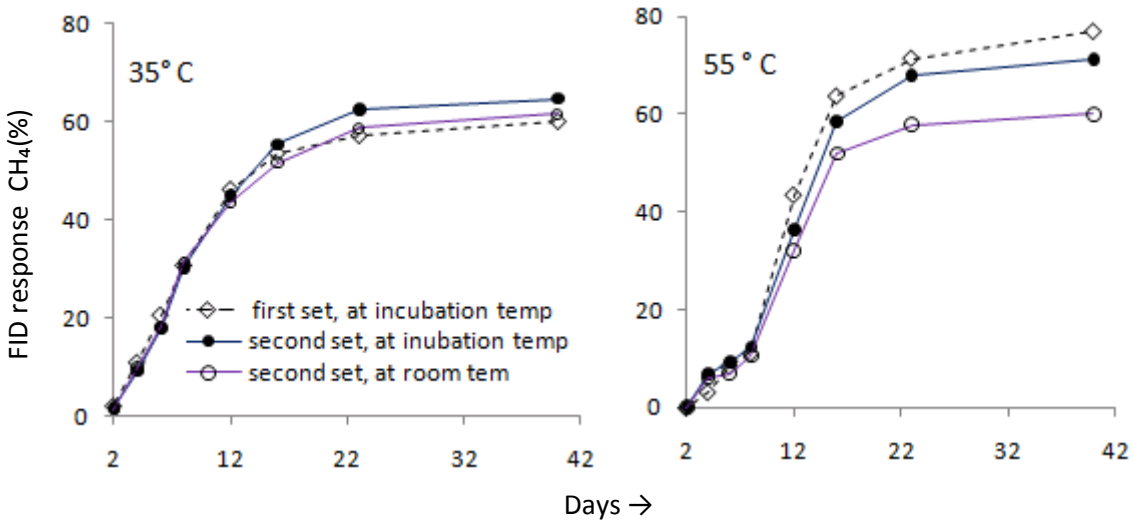

Figure 14: FID response for CH_4 and effect of short term cooling

7 DISCUSSIONS

7.1 Different barrier solutions

Our experimental analysis showed that most of the solutions that are recommended in literatures are unsuitable to be used in displacement gasometers or gas flow meters because of the high diffusion of CO_2. Although the diffusion of CH_4 in the barrier solutions is much less when compared with that of CO_2, simple liquids as barrier solution could also result in the decrease of absolute quantity of methane collected over the liquid. The rate of diffusion is the function of the gas solubility in the barrier solutions. It is suggested that a solution with high ionic strength does not completely prevent biogas (particularly CO2) from dissolving in the displaced liquid and diffusing into the surrounding atmosphere [16]. An increase of the acidity of solution alone did not prevent much in the diffusion of the biogas. However, acidified solutions with a high ionic concentration were able to preserve composition and volume of the biogas. An increase of the ionic concentration in the solution resulted in a decrease of the gas diffusion. Acidified saturated NaCl solution showed the best result in the preservation of biogas. The experimental result agrees with the trend of gas diffusion found in other studies [18, 38] and suggests the use of acidified NaCl solution as barrier solution. Many researchers have used simple liquids like paraffin oil, water, acidified water or weak brine solution as a sealing/barrier solution in displacement devices with the idea that saturation of the liquid with CO_2 will prevent

further loss of gas. From the result of biogas solubility tests in carbonated water, it can be assumed that because of the change of pressure between the outside atmosphere and the inside of the container, simple liquids just act as a bridge for the diffusion of gas which results in the continuous loss in gas volume. Solubility of biogas in barrier solutions and the further diffusion into the atmosphere can give the wrong information of the volume of measured gas. Inward diffusion of atmospheric air can also occur when barrier solutions are less resistant to gas solubility. These liquids can be used as a barrier solution when the production of gas from the digester is high and the error due to slow diffusion of gas can be neglected over the long run. For small digesters and low production of gas, the use of acidified saturated NaCl (>90%) solution is recommended. As the biogas from the reactor is always vapor saturated, the problem of crystallization may not appear. Conversion of the biogas volume at laboratory temperature and pressure to dry gas STP is important.

7.2 Gas meter

In laboratory scale experiments, the production of the biogas can be very small. Accurate measurement of the produced gas volume is not an easy task. Several devised liquid displacement gas-measuring systems such as presented in [28-31] are technically robust. However, the problem of miscounting can still occur because of the diffusion of biogas in the barrier/sealing solutions. The designed meter overcomes this problem. It is simple, durable, accurate and easy to use. Calibration of the designed automated meter is easy. The back pressure and the volume for each count to be measured can be fixed simply by adjusting the sensor height. The use of acidified saturated NaCl solution can prevent the diffusion of CO_2 and give more precise measurement of biogas.

7.3 Headspace Gas Chromatography

The results from the dry synthetic biogas experiment showed the increase of CH_4 and CO_2 concentration with increase of incubation temperature. This can be defined as an effect from the Basic Gas Law of PV=nRT, where as the temperature of the sample increases, the pressure builds inside the bottle, since the volume was constant, and when extracting the sample into the syringe with more analyte molecules than what it would get if the temperature or pressure remained the same. Therefore, the change in molar quantities at

different temperatures relative to the laboratory room temperature of 23°C can be expressed simplifying the gas law,

$PV/R = n_1T_1 = n_2T_2$ or $n_1T_1 = n_2T_2$

Here, $T_1 = 273.2 + 23^0C = 296.2K$ & $T_2 = 273.2 + x^0C$,

n_1 is the moles of gas at room temperature and n_2 at x °C. Then gas response relative to room temperature is: [41]

$$CH_4, CO_2 \text{Temperature response factor} = \frac{273.2 + x}{296.2}$$

With the increase of temperature from 23°C, there was a steady increase in GC- TCD response. The continuous increase of dry gas CH_4 and CO_2 concentration with the increment of temperature can be understood as temperature increment response relative to the standard prepared at room temperature. However, the GC response of CH_4 and CO_2 at thermophilic temperature was much high and cannot be defined by the temperature response factor. This could be due to the thermal expansion of needle and syringe barrel or because of remarkable temperature difference between the syringe barrel and the headspace gas. This GC response was even much higher in the case of the saturated gas experiment. In the case of the humidified gas experiments, the vapor undoubtedly compounded the problem. As temperature increased, it resulted in the increase of pressure inside the sample bottle even more than that of the dry gas. The rise of temperature and diffusion of gas molecules from liquid into the headspace was another reason for the high GC response.

The trend of difference in the concentration of gas before and after cooling in case of mesophilic and thermophilic digestions was similar to that of the synthetic saturated biogas experiment. The high GC response of CO_2 at mesophilic temperature was mainly because of the easy release of CO_2 from the liquid phase. CO_2 concentration was very extreme at thermophilic temperature. The humidified gas CH_4 response at mesophilic temperature was slightly higher than that of the dry gas experiment. However, there was much erroneous response at thermophilic temperature.

From the gas solubility data (Figure3) and from the Ideal Gas Law it can be calculated that at laboaratory room temperature (23 °C) and normal atmospheric pressure(≈1atm), the dissolved CH_4 in 20 ml of liquid solution would be 0.02 x 20 ml = 0.40ml of CH_4, the headspace would contain ((120-20-8)/2) = 46 ml of CH_4 (liquid and stopper volume

subtracted and divided by 2 as the test gas was a mixture of 50 % CO_2 and 50 % CH_4). This would represent 0.017 mmole of dissolved methane in liquid and 1.89 mmole in gas phase. Therefore, almost 99% of the CH_4 was in the gas phase. With the increase of incubation temperature, the headspace methane will increase by only a negligible amount. On the other hand, the solubility of CO_2 is much higher than CH_4. At 1 atm and 23 °C, dissolved CO_2 in a 20 ml of liquid solution would be 0.02 x 800 ml= 16 ml of CO_2. Temperature plays key role for the change of bicarbonate in aqueous solution which finally alters the concentration of CO_2 in headspace as it generates carbon dioxide gas upon reaction with the hydrogen ions. This can be written as [22, 42]:

$$HCO_3^- + H^+ \leftrightarrow H_2O + CO_2$$

At thermophilic temperature there was almost a complete release of CO_2 from the solutions into the headspace, shifting the above reaction towards the products. The humidified gas graphs in the gas liquid phase experiment also represent bicarbonate decomposition as an equilibrium reaction. The carbon dioxide equilibrium between the vapor and liquid phase was obtained simply by keeping the reactors temperature constant. This formation of CO_2 due to bicarbonate decomposition at higher incubation affects the measurement accuracy when it is compared with the bottles at room temperature or in the mesophilic temperature range [22]. This phenomenon suggest that anaerobic digester assays incubated at mesophilic and thermophilic temperature also follow the same trend and show more erroneous responses similar to that of the humidified gas experiment.

Higher measurement errors are likely to occur when samples are taken at higher anaerobic temperatures and standard is prepared at laboratory temperature. Also, CH_4/CO_2 biogas standards that are used in GC calibrations and baseline measurements are generally dry in nature and prepared at room temperature. The use of biogas standards prepared at the incubation temperature can only be useful in the reduction of errors of the dry gas measurement. This will not give the complete solution because biogas in the assays is always gas-liquid phase and the GC response for saturated gas with the increase of temperature is more complex.

It is important to understand the relation between the small change of temperature with gas measurement and the degradation process. In most of the cases, incubated bottles are removed from the temperature controlled environment during the measurement of gas. This change of temperature can easily affect the equilibrium between the gas and liquid

phase which can result in the change of headspace gas concentration. This can also affect the microbiology of anaerobic digestion. Analysis for mesophilc and thermophilic experiments showed the difference in production of biogas between the constant temperature set of bottles and those which were underwent temperature fluctuations. Figure 13 shows that there was an effect on the microbiology of the anaerobic degradation process due to the short temperature fluctuation.

The slightly higher GC response with the use of an uninsulated syringe is supposed to be the influence of the human hand or external temperature. GC response was also higher for those injections which were injected very rapidly. It is supposed that rapid injection is important for better accuracy. Slow injection can cause a small loss or change in the concentration of analyte molecules inside the syringe barrel.

8 CONCLUSIONS AND RECOMMENDATION

With proper displacement techniques, it is possible to determine the accurate production of biogas volume. Acidified saturated NaCl solution can be used as barrier or sealing solution in all types of liquid displacement meters as it showed the highest resistance to CO_2 solubility. The biogas measuring device presented in this paper is easy to construct, accurate and economical for monitoring of biogas production from laboratory scale digesters.

Errors in chromatography analysis of biogas are seen mainly because of temperature and composition differences of the standard calibration gas and experimental assays. Use of CH_4/CO_2 biogas standards at room temperature and cooling the reactors back to the same temperature for a short time were a good solution in the reduction of errors. However, the cooling effect in thermophilic/mesophilic experiments also proved to have its effect on the natural trend of anaerobic digestion. Therefore, cooling the digesters even for a short time during each time of gas measurement is not recommended as it showed an adverse effect on the anaerobic microbial ecology. It is advised to cool the reactors back to room temperature only on the last day of the experiment. GC response before and after cooling thus gives the difference in concentration of CH_4 and CO_2 between the incubated temperature and room temperature. This difference can be used for the calculation of correction factor for the previous GC responses. Cooling of digesters on the last day is advised when the rate of gas production, mainly methane, is the most important

indicator of operational performance. Measurement errors can also be eliminated very effectively when sampling is done at incubated temperature and humidified CH_4/CO_2 biogas standard at incubation temperature is used for GC calibration. However, the preparation of the vapor saturated standards for each experimental incubation temperature can be laborious and time consuming. Rapid injection of the samples into the column without delaying will give more precise results. It is also a laborious and time consuming process to maintain the syringe temperature equal to that of incubation temperature in case of manual GC operation. Therefore the use of an insulated syringe for gas samplings can be an easy option.

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