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Anaerobic Co- digestion of Digestate with Glycerol to enhance Biogas Production

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Abstract

The Brazilian sugarcane and ethanol industries produce lot of waste which has potential for energy production. Anaerobic digestion (AD) can be effectively utilized for producing biogas from these wastes. During the AD process, huge volumes of digestate are produced with some being employed in fertilizer application whilst large volumes are mostly stored in uncovered tanks. This result in emission of residual methane and loss of energy which can be recovered through post- digestion approaches. To analyse optimal utilization of this digestate and enhanced biogas production, co- digestion of post- digestate from a continuously stirred reactor (CSTR) performing co-digestion of sugarcane waste from Brazil (Vinasse, filter cake and straw) with addition of different glycerol concentrations were studied. The addition of glycerol characterised by its biodegradability and high organic content makes it a suitable substrate to enhance biogas production. A biomethane potential was assessed when the digestate was co-digested with 15% and 25% CODg/L of glycerol. The batch test lasted for 39 days. The results demonstrated that, co-digestion of digestate with glycerol has the potential of increasing cumulative methane and biogas yield with 25% addition producing the highest methane and biogas yield (318 Nml/gCOD and 196 Nml/gCOD) which was approximately 6 times higher compared to mono-digestion of the digestate.

Anaerobic co-digestion of digestate and glycerol was examined in two lab scale reactors (CSTR) at mesophilic conditions (35°C) and were run for 90 days. The reactor (R1) performing co-digestion increased methane and biogas production by 300% and 170% when glycerol concentrations of 15% and 25% of influent COD were added, respectively. Moreover, there was a decrease in CH₄ yield when the reactors were continuously fed with 15% and 25% CODg/L of glycerol. This was an indication that, microorganisms easily digested glycerol addition at the early stages. Glycerol addition (50% CODg/L) resulted in a decrease in CH₄ and biogas production. This result shows, CH₄ yields in the post-digester can be enhanced with glycerol addition if it does not exceed a limiting percentage of 50 of the organic loading rates of the feed.

List of Abbreviations

AD- Anaerobic digestion

ACoD- Anaerobic co-digestion

BMP- Biomethane potential

COD- Chemical oxygen demand

CSTR- Continuous stirred tank reactor

GHG- Greenhouse Gas

HRT- Hydraulic retention time

OLR- Organic loading rate

TS- Total solids

VFA- Volatile fatty acids

VS- Volatile solids

SD- Standard deviation

1.0 Introduction

Energy plays an indispensable role in human life and is an essential element to human and economic development (Surendra *et al.*, 2014). It is needed to meet basic human needs and its pivotal role in industrialization has led to an industrial revolution (Stern & Kander, 2012). The world's reliance on fossil fuels has impacted climate change due to the increase emission of Green House Gases (GHG) (Owusu & Asumadu-Sarkodie, 2016). Pazheri *et al.*, (2014) suggested that there is a big challenge to the energy sector in achieving sustainability as well as securing sustainable and clean energy to meet the increasing global demand. To tackle this challenge, the energy sector witnesses a tremendous transformation through global exploitation of renewable energy sources. The use of biofuels such as bioethanol, biogas and biodiesel to potentially harness energy from agricultural waste, crop residues, municipal solid waste, pulp and paper, sugar industries among others have been recently employed by many countries (Rao, *et al.*, 2010). According to Paoli *et al.*, (2011), reliance on agriculture crops for energy is a platform that can exacerbate food insecurity and hence a better utilization of agriculture by-products is an avenue that can curb a trade off in the food- energy nexus. The trend towards a high intensification of agriculture productions have accentuated adversities in waste management and improper management of these wastes on soil surface have caused contamination in the environment including pest generation and emission of GHG (Sousa, *et al.*, 2017). Harnessing agriculture waste and crop residues for energy production provides a salient way of reducing the migration of food products into biofuels production and GHG emissions (De Rossi *et al.*, 2017).

Sugarcane is commonly cultivated across the globe, and produced in more than 109 countries across the world with 1.91 billion tons harvested worldwide in 2013 (Dotaniya *et al.* 2016). The sugarcane industry is an important sector to the global economy especially providing income and employment to the locals in a country (Sawaengsak & Gheewala, 2017). The industry also generates large amounts of by- products that contains high amounts of carbohydrates making it an avenue of generating energy to supplement the global demand for energy. The energy potential of sugarcane makes it the most economical important energy crop and the utilization of its by-products into energy has been considered as a vital channel in the transition to sustainable use of agro- products into energy (*Sugarcane Wastes / BioEnergy Consult*,2019)

Brazil contributes enormously to sugarcane production in the world. According to Janke *et al.*, (2014), the industry contributed for USD 36 billion to the country's revenue during 2012 to 2013. Like most agriculture producing countries, increase and changes in production in the sugar cane industry is responsible and have caused a complex problem in waste generation and management. From the report of De Rossi *et al.*, (2017), the processes that are involved in the production of sugar and ethanol generate high volume of waste, such as Vinasse, straw, filtercake and baggase with high organic matter content. These by- products possess great potentials in the production of biogas due to their hemicellulose, cellulose and lignin composition (Janke *et al.* in 2014).

Another sector that has prominently contributed to world's energy matrix is biodiesel production as it offers advantages over the conventional diesel such as its renewability, biodegradability and lower emissions in GHG (Kolesárová, *et al.*, 2011). This has resulted in several countries using biodiesel. For instance in Brazil, a 4% addition of biodiesel was mandated to the country's energy mix resulting in an increase of 5% whilst in the European Union and the United States, it supplements to 5.75% and 20% of energy, respectively (Viana, *et al.*, 2012). However, Anitha *et al.*, (2016), stated that, one impediment that the biodiesel industries face is the large amounts of glycerol generated as a by- product which is produced during transesterification process. According to Viana *et al.*, (2012), glycerol production across the world was around 3 000 000 tons in 2011 and is estimated to increase to 4 600 000 tons in 2020.

One solution to utilizing these by- products is anaerobic digestion (AD). The AD process involves the breakdown of complex organic matter into simpler compounds in the absence of oxygen to produce a gas mixture of methane, carbon dioxide and other minor gases, so called biogas (Mata-Alvarez *et al.*, 2014). AD has presented as a promising way of producing biogas and digestate (i.e. residues of AD processes) from the organic waste from sugarcane processing (Janke *et al.* in 2014). The biogas can be used as fuels for heating, electricity generation and upgraded to natural gas quality (biomethane) and used as fuel for vehicles, whilst, digestate can be applied as fertilizers in agriculture (Holm-Nielsen *et al.*, 2009). In this context, AD contributes to a pathway of sustainability and plays an important role in a circular economy through its optimal utilization of waste, production of renewable energy and nutrient recycling (Ekstrand,2019)

According to Sambusiti *et al.*, (2015), the use of digestate for agriculture land as an organic fertilizer might not be feasible in the long term considering the increasing number of biogas plants in certain areas which might lead to oversupply. For instance, Menardo *et al.*, (2011) stated that due to different seasonal times in crop production and soil type, digestate are stored in tanks. The storage of these digestate especially those in uncovered tanks could result in emission of GHG such as CO₂, CH₄, N₂O into the atmosphere which affects the climate and causes atmospheric pollution. To address this situation, studies have shown that the resulting digestate from AD has the potential for further biogas production and Balsari *et al.*, (2009) proposed that, recirculation of digestate is an effective way to reduce oversupply and reduction of GHG from the storage and transportation of digestate. Since then further studies have focused on maximizing biogas potential and yield of digestate to enhance the biogas production and waste reduction efficiency of AD processes (Lindner *et al.*, 2015). Digestate treatment by different methods (pre- and post-treatment) classified as chemical, physical, biological and mechanical have been proposed by researchers as a way of improving biodegradability, breakdown of residual lignin and nutrient quality. However, according to Carlsson *et al.*, (2012), though pre and post-treatments methods have advantages of increasing methane yield and biodegradability, the process is cost intensive through the use of chemicals and other equipment and there is high chance of losing organic matter through intensive pretreatment (Lindner *et al.*, 2015; Sambusiti *et al.*, 2015).

1.1 Problem Statement, aim and research questions

As stated earlier, the use of AD to produce biogas from sugarcane by-products is an effective way to reduce waste from the sugar industries. The application of digestate as fertilizers is an avenue that AD contributes to agriculture production. AD is a complex process which involves various microorganisms that are subjected to the control of environmental conditions and the amount of biogas produced also depends on the substrates used (Hagos, *et al.*, 2017). It is reported that, the digestion of mono-substrates has been successful over the years. However, Hagos *et al.*, (2017) stated that, due to nutritional imbalances for microorganisms, the effective way to optimally generate and improve biogas and methane yield is through anaerobic co-digestion (AcoD) from different substrates. AcoD, which is a simultaneous digestion of two or more substrates either from municipal solid waste, agriculture by-products or animal manure has been identified as a pathway of minimizing the limitations and barriers associated with nutrient limitation in AD processes (Mata-Alvarez *et al.*, 2014).

Recently, AcoD of substrates from sugarcane by-products with other organic wastes such as vinasse with coffee residues , vinasse with filter cake, vinasse with chicken manure and few others have gained popularity (Pinto *et al.*, 2018; López González, *et al.*, 2017). However, solutions for a more effective handling and utilization of digestate in sugarcane processing industry have not yet been established. Hence, utilizing the digestate through co-digestion methods with highly degradable substrates such as glycerol can be a way to enhance its degradation. In addition, poor quality of glycerol from biodiesel due to high impurities and high cost of glycerol purification have led to a lower market demand and its large amounts produced have created a market surplus (Beschkov, *et al.*, 2017). The need to utilize and transform glycerol to a valuable product is important as it will enhance the economic and environmental suitability to biodiesel production (Siles *et al.*, 2010). This research aims to assess the biogas potential of glycerol and digestate (from anaerobic digestion of vinasse, straw and filtercake) in an AcoD as a solution for enhancing energy recovery from wastes produced in sugarcane processing and biodiesel industries. The main reason behind this research is to find an alternative approach to reduce cost intensity such as high energy consumption, chemical cost and the possibilities of inhibition from by- products related digestate handling and also create a market for the oversupply and storage of digestate and glycerol through biogas production,

The study addresses the following research questions:

1. What are the biomethane potential of digestate, glycerol and a combination of the two?
2. What is the biogas and methane yield of digestate and its co-digestion with glycerol in a continuous experiment?
3. Is glycerol a suitable substrate for increasing biogas production of digestate from anaerobic digestion of vinasse, straw and filtercake?

2.0 Background

This section provides an overview of background information related to anaerobic digestion. It emphasizes on ACoD of digestate, glycerol and other substrates that are related to the subject. Biomethane potential is also discussed in this section.

2.1 Anaerobic Digestion

AD has been identified as an avenue for sustainable waste treatment due to its potential energy recovery (Mata-Alvarez *et al.*, 2014). In recent years countries like Sweden, Germany, Austria and others have deployed its use as an alternative energy due to its renewability, economic development and environmental benefits by means of offsetting CO₂ emission (Rao *et al.*, 2010). It is the degradation of organic matter to biogas in the absence of oxygen. There are four biochemical processes involved in anaerobic digestion: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The process also depends on the interaction of numerous microorganisms that occurs in the four different stages (Surendra, *et al.*, 2014). Two major gases produced from AD process are CH₄ and CO₂ with CH₄ (50–75%) and CO₂ (25–50%), and digestate. Figure 1 depicts the four stages involved in AD.

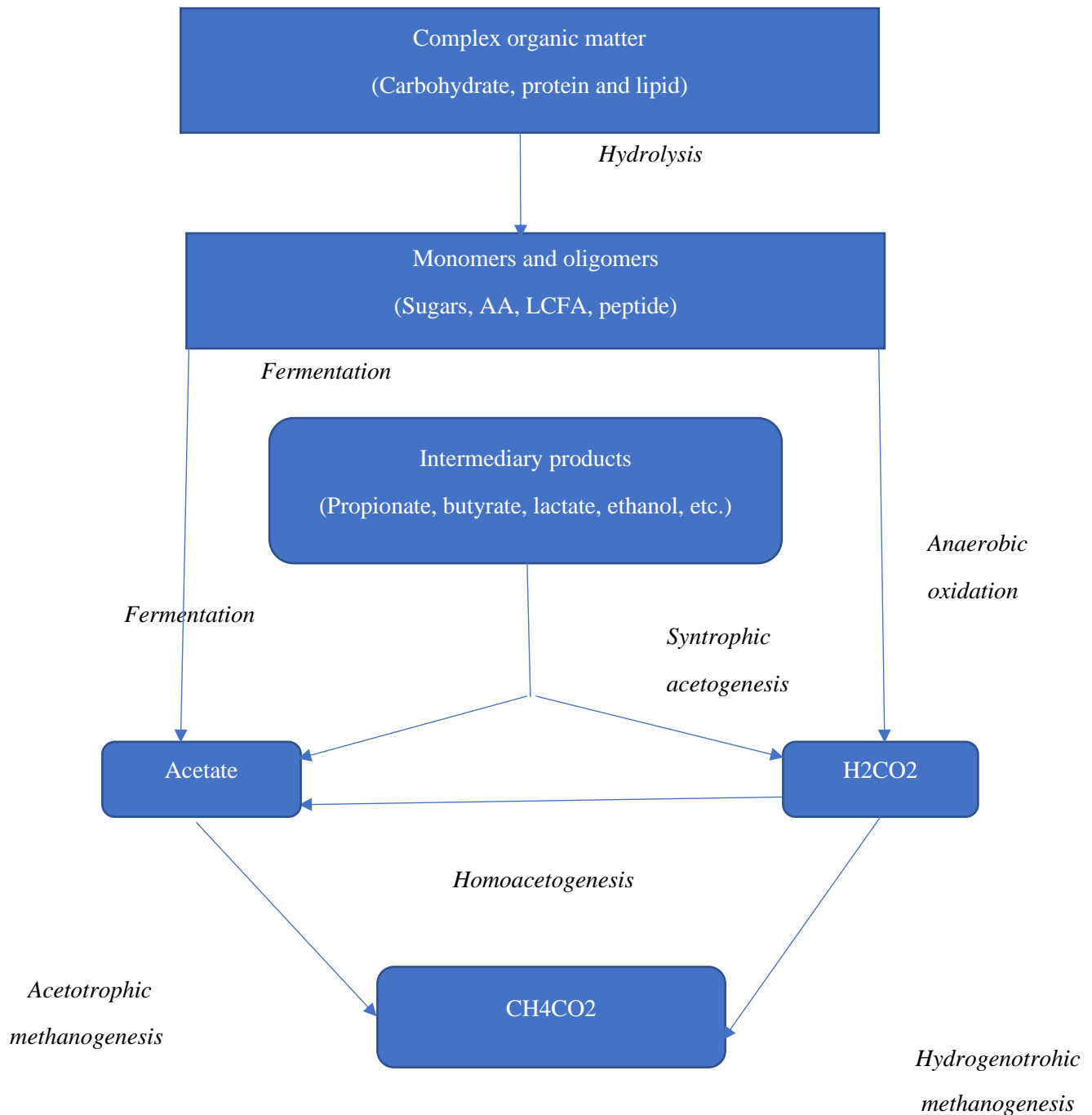


Fig. 1. Process involved in AD. Modified from (Surendra *et al*, 2014)

The hydrolysis stage involves the decomposition of complex organic compounds such as protein, fat, and carbohydrate. These compounds are fermented and converted into H₂, CO₂, VFAs such as acetic acids by the acidogenic bacteria. VFAs are metabolized by bacteria and converted into acetate in the acetogenesis stages. Finally, methanogenic bacteria convert the acetate, CO₂ or H₂ into CH₄ (Surendra, *et al*, 2014).

2.2 Factors influencing Anaerobic Digestion Process

AD is a complex process due to the interaction of various microorganisms and environmental factors. The characteristics of these organisms vary within the digester. Maintaining a balance in microbial population and monitoring various operating conditions is important to achieve a successful process and optimal methane yield. Factors such as temperature, pH, alkalinity, total solids (TS) and volatile solids (VS), the type of substrates, organic loading rate (OLR) and hydraulic retention time (HRT) should be meticulously monitored since it can affect the efficiency of biogas yield and microbial process which can cause AD instability (Fahriansyah and Andrianto, 2017).

2.2.1 Temperature

Temperature is a crucial factor to consider in AD process. It can affect the physiochemical properties of substrates and methanogens are also sensitive to changes in temperature, hence sudden changes can affect the process (Kim *et al.*, 2017). AD process operates at two temperatures, mesophilic 30–40°C and thermophilic 50–60°C. Methane yield is the main disparity between mesophilic and thermophilic. A small change in temperature from 30- 32°C can affect the bacteria which in turn affects biogas yield. Yang *et al.*, (2018), suggested that different types of microorganisms favor mesophilic and hence makes it more stable compared to thermophilic. Stable production of methane is known to produce at 32-35 °C (Sorathia *et al.*, 2012). Kim *et al.*, (2017), study on the effect of temperature on AD process showed that at an organic loading rate of 6.7 g COD·L⁻¹·d⁻¹(HRT 30 d) and 5.0 g COD·L⁻¹·d⁻¹(HRT 40 d) for mesophilic and thermophilic conditions respectively the highest methane yield was evident under mesophilic conditions whilst it also showed low concentration of propionate and acetate. Other studies suggest thermophilic digestion yields higher CH₄ compared to mesophilic because methanogens are known to prefer higher temperatures, however Yang *et al.*, (2018), stated increase in VFAs and ammonia are the impediments related to high temperatures. Wilson *et al.*, (2008), studies also revealed that reactors operated at highest temperature of 53°C were prone to VFA accumulation and gaseous hydrogen.

2.2.2 pH

pH concentration is an important factor that influence AD process. It affects methane yield and solubilization of organic matter (Feng *et al.*, 2015). pH is mostly low in the early stages of AD process due to the formation of organic acids. The hydrolysis and acidogenic stages operates at pH values between 5.5-6.5 whilst microorganisms in the methanogenic stage prefer pH value of 6.5-7.8, (Siddique & Wahid, 2018). It is considered important as it determines a favorable environment for microbes in a digester (Nguyen, 2014). Various microorganisms are favorable to different pH values and Feng *et al* (2015) stated that to attain an optimum methane yield, the most favorable pH values should be in the range of 6.8 and 7.2. Keeping pH neutral in AD is very paramount as low pH can cause the accumulation of VFAs and inhibition methanogenic growth (Nguyen, 2014). The addition of alkali such as lime is a way to help overcome low pH.

2.2.3 VFAs

Volatile fatty acid (VFAs) are important intermediary compounds which can cause an imbalance in AD process. High concentrations of VFAs causes a decrease in pH and primarily causing system failures. Constant monitoring and examining of VFAs is imperative to enable good performance in a digester (Wang, *et al*, 1999). Easily degradable substrates have the tendency of accumulating VFAs which affect the development of methanogens or microbial stress (Nordlander, *et al*, 2017). Though monitoring of all VFAs are important, key attention to butyric acids have shown to be a proper way of ensuring system stabilization since butyric acid are inhibitory to methanogenic bacteria (Nguyen, 2014).

2.2.4 TS and VS

The water or moisture content of a substrate is a common and important parameter that impact AD process. The water content enhances growth of bacteria and movement of nutrients. It also minimizes the mass transfer of substrate. In view of this, Tsunatu, *et al.*, (2014) suggested TS of a substrate requires monitoring in AD process as continuous increase in TS causes a drop-in water content which affect microbial activity. TS is the dissolved organic matter of a substrate expressed as the percentage of the total weights in grams per kg (Orhorhoro *et al.*, 2017). TS is determined by weighing the substrate and drying it in an oven at a temperature of 105 until its water content evaporates to zero. Studies by Orhorhoro *et al.*, (2017): Tsunatu, *et al.*, (2014) reported that an increase in TS causes a decrease in biogas production.

Volatile Solids is the organic fraction of TS, its determination is important as it aid in investigating the amount of organic matter of a substrate. VS content of a substrate is a way to determine the biogas potential of a substrate, Tsunatu, *et al.*, (2014) and shows how much organic matter is degraded. To determine the VS, the residue from TS is burned in a muffle furnace at 550 °C for at least two hours. The crucibles are then cooled in a desiccator till it reaches room temperature and weighed. Orhororo *et al.*, (2017) studies on effect of TS and VS on biogas yield concluded that, increasing percentage of VS correlates to an increase biogas yield and vice versa. In their research it was observed that, 91.1% (2.88kg) volatile solids generated the highest amount of biogas.

2.2.5 Loading

The loading rate mostly referred as Organic loading rate (OLR) is defined *as the amount of dry solids that is fed to a reactor per unit volume of a digestion process*. The OLR should be carefully fixed and adapted to active microorganisms as low OLR results in inefficiency whilst higher OLR thus beyond certain range can cause VFA accumulation (Siddique & Wahid, 2018). Again, the fact that there can be inconsistencies in the supply of feedstock or substrates requires that there should be proper management to prevent any unplanned circumstances. A study by Bi *et al.*, (2019) on the effects of OLR of chicken manure showed at 1.6 and 2.5 kg loading rate high OLR of 2.5kg lead to an increase in VFA accumulation suppressing methanogenic activities especially in thermophilic reactors. The study further suggested both thermophilic and mesophilic reactors were efficient at low(1.6kg) OLR. (Ferguson, *et al.*, 2016) posits high concentration of glycerol causes inhibition and system instability. The accumulation of VFAs was result of high (more than 1% addition) organic rate of glycerol as demonstrated by Fountoulakis *et al.*, (2010) whose experiment was on co- digestion of sewage sludge and glycerol.

2.3 Biomethane Potential (BMP)

The microorganism inherent in a feedstock are sensitive to environmental conditions. This requires that certain characteristics of these feedstock such as biodegradability needs to be known as it affects efficient operation of AD (Filer, *et al.*, 2019). An experimental test that has proven to be successful in determining the biodegradability of a substrate is the biomethane potential test (Viana *et al.*, 2011). It aids in determining the methane potential of a substrate and has proven to be a useful tool in providing significant predictions for a large-scale anaerobic digester compared to theoretical calculations of methane potential (Esposito *et al.*, 2012). Theoretical calculations mostly provide higher results. Various literatures have different approaches to BMP as there is no defined standard protocols for BMP. However, BMP tests require a blank, substrate and control Filer, *et al.*, (2019) and according to Holliger *et al.*, (2016) one compulsory element for the validation of BMP tests is conducting it in at least triplicates. They are mostly performed in bottles and filled with inoculum and substrate in a ratio of 2:1 (inoculum: substrate). They are then subjected to a temperature of 35 C or 55 C (Filer, *et al.*, 2019). The methane potential of a substrate is determined by subtracting the methane of the inoculum mostly known as blank from the methane production of the substrate and are expressed in terms of COD or VS of the substrate (Holliger *et al.*, 2016).

2.4 Anaerobic Digestion in the Brazilian sugar and ethanol industry

Brazil is currently the world's leading producer in sugarcane as favorable climatic conditions and soil types has resulted in an increase production of the crop (FAO, 2012). In 2014, the estimated sugar production was 40.97 million tons (Botellho *et al.*, 2014). The sugar industries producing sugar and ethanol are mainly responsible for generating by-products such as vinasse, filtercake, straw, and bagasse (De Rossi, 2017). These wastes are known to have high commercial importance. However, filtercake and vinasse have been applied as fertilizer for sugarcane production, whilst straw are indiscriminately managed and left to decay (Janke *et al.*, 2015). The application of vinasse as fertilizer poses numerous environmental impacts on surface and ground water (Moraes *et al.*, 2017). Filtercake which is obtained during the crushing stages of sugarcane juice is rich in organic matter and a source of carbon and nitrogen making it a good potential for biogas production. Sugarcane straw is a fibrous residue consisting of 40% cellulose, 35% hemicellulose and 15% lignin and is generated from the crushing of sugarcane. Vinasse is a by-product of ethanol distillation and it is estimated that a

liter of ethanol production produces 8-18 liters of vinasse. It has a high amount of organic matter making it beneficial for fertilizer application and biogas production (Botellho *et al.*, 2014). Various studies have recently been done on utilizing sugarcane by-products for biogas production. In recent years the sugarcane industry has developed new methods and processes to create an energy balance in utilizing these waste, Janke *et al.*, (2014) studied the biomethane potential of the Brazilian sugarcane industry in Sao Paulo and concluded that the biogas yield for filter cake, vinasse, bagasse and straw were 486, 647,528, and 395 NmL/gVS respectively. The study further suggested that full utilization of filter cake and vinasse to biomethane could supplement 10% and 17% of the total natural gas consumption in Sao Paulo. Again, a quarter and half of bagasse and straw conversion into biomethane could add 54% of energy. Furthermore, digestate from the anaerobic reaction of these waste can also be applied as fertilizer in sugarcane production. This is considered to be sustainable compared to that of direct application of filtercake and vinasse as fertilizer (Moraes *et al.*, 2017).

2.5. Digestate

The potential of the Brazilian sugar industry's waste does not contribute only to energy but also to agriculture production through the application of digestate as fertilizers (Janke *et al.*, 2014). Digestate which is a by- product of AD is mainly composed of 90% water, organic matter and undegraded digestate (Sambusiti *et al.*, 2015). It is mostly applied as fertilizers due to the availability of nutrients like nitrogen, phosphate and potassium (Sogn *et al.*, 2018). Before applied as fertilizers, digestate is mostly stored in uncovered tanks for 180 days. This result in the emission of GHG gases such as CO₂, N₂O and CH₄ which affect the environment. Sambusiti *et al.*, (2015) suggested that, due to different seasons in farming and the increasing number of biogas plants in certain areas, digestate in the long term will be stored for a longer period which will exacerbate its effect on the environment. Such acts have prompted some European countries to store digestate in covered tanks resulting in an advantage of capturing methane (Menardo *et al.*, 2011). Some authors stated that resulting digestate have the potential of methane yield (Balsari *et al.*, 2009; Kaparaju and Rintala, 2003). The former is known to first identify the methane yield in digestate through recirculation whilst the latter through digestate methanation suggested a 15% increase in methane yield. Though methane yield has been investigated by the researchers, the amount of methane that could be recouped from digestate also depends on the organic matter content (Sambusiti *et al.*, 2015). Digestate is known to have higher ammonia but decreased carbon and organic matter content and low

biodegradability, the carbon to nitrogen ratio (C/N) is smaller with high pH (Möller & Müller, 2012). To improve this barrier and enhance biogas production most research have focused on pre and post treatment (Sambusiti *et al.*, 2015 ; Lindner *et al.*, 2015; Menardo, *et al.*, 2011). A study by Solé-Bundó *et al.*, (2017) compared digestates of different origin and stated that, the organic matter of digestate is lower (<40%), these values differ due to the type of substrate and operating conditions of reactors. The study also found low C/N ratio (around 3) and hence co-digestion with carbon rich substrates will increase the carbon content and methane yield. It will be irrelevant to investigate methane yield from digestate which has less methane potential or exhausted (Menardo, *et al.*). These authors in a batch study on the methane yield of different digestate from anaerobic digestion plants suggested a heterogeneous methane yield between 2.88 and 37.63 NL/kgVS. The study concluded that methane yield was highly influenced by OLR, hydraulic retention time and stock quality. A 70 NmL CH₄/g VS and 90 NmL CH₄/g VS, methane yield was discovered on post- treatments of agriculture digestate (Sambusiti *et al.*, 2015). However, the use of chemical like alkaline treatment causes inhibition in AD process and its cost is an impediment to full- scale biogas plants (Lindner *et al.*, 2015). The physical and chemical compositions (TS, VS, pH and ammonia) of digestate from different plants investigated by (Menardo, *et al.*,2011) is shown in table 1.

Table 1. Chemical and physical parameters of digestate samples from four CSTR biogas plants

Sample (Digestate)	Initial pH	Final pH	Initial TS(%)	Final TS	Initial VS	Final VS	Initial Ammonia	Final Ammonia
A	7.9	8.0	9.6	8.2	77.0	67.5	2.0	2.6
B	7.8	7.9	5.4	4.9	74.1	69.2	3.0	3.5
C	8.0	8.2	3.7	3.5	67.4	64.0	8.1	9.1
D	8.1	8.2	1.7	1.6	62.1	62.1	8.7	8.9

Sample A and C, slaughter waste, tomato skins and glycerol as substrates. Sample B, straw and cut maize. Sample D, swine effluents (Menardo, *et al.*, 2011).

2.6 Anaerobic Co-Digestion (AcoD)

Producing energy from AD is predicted to be a sustainable and renewable energy for the future, however, there has been some hindrances in the use of mono substrates for digestion. Low organic loads, high concentrations of heavy metals, high and lack of nitrogen characterized in certain substrates can result in methanogenic inhibition and also system failures, (Mata-Alvarez *et al.*, 2014). Anaerobic Co-digestion, which is a mixture of two or more substrates have proven to resolve the difficulties associated with AD of mono substrates. Studies on AcoD have shown significant improvement in biodegradability, synergetic effects, nutrient balance, system

stabilization and above all enhance biogas production both in large scale plants and laboratory works (Hagos *et al.*, 2017). An imperative factor to consider in AD is the (C/N) ratio, as an imbalance in the ratio can cause system failure, through AcoD an optimal C:N ratio can be achieved (Nordlander *et al.*, 2017). Co-digestion has improved the biogas production of lignocellulosic waste (Mata-Alvarez *et al.*, 2014). Though AcoD is a promising technology and brings several advantages, special considerations should be focused on the type of substrate and the proximity in obtaining the different substrates to reduce shipping costs (Mata-Alvarez *et al.*, 2014).

2.6.1 Anaerobic Co-Digestion of Glycerol

The growing concerns of GHG emissions have brought about alternative energy sources such as biogas and biodiesel. Biodiesel which is produced through transesterification of vegetable oil produces a by-product in the form of glycerol (Viana *et al.*, 2011). Approximately, 10kg of glycerol is generated from every 100kg of biodiesel produced (Santibáñez, *et al.*, 2011). This implies a huge chunk of glycerol is generated as waste from the biodiesel industry especially relating to the increase amount of the industry. Countries like Brazil have increased its volume of biodiesel, whilst the European Union has about 5.75% increase in biodiesel production, (Viana *et al.*, 2012). The growing market has brought about an environmental concern in managing large amounts of impure glycerol produced as it is considered to generate surplus in future (Santibáñez, *et al.*, 2011). Glycerol is highly biodegradable and contains considerable amount of carbon, making it a good co-substrate for anaerobic digestion, (Silva, *et al.*, 2018). Though there are new technologies in utilizing glycerol, anaerobic digestion is economically feasible and offers significant advantages (Viana *et al.*, 2011). The refining processes involved in purifying crude glycerol for use in food, cosmetic and pharmaceutical industries is considered to be expensive, Kolesárová *et al.*, (2011), the resulting digestate from AD of glycerol is very stable making it a good source of fertilizer. Due to its high carbon content, studies have shown that, co-digestion of glycerol with recalcitrant substrates have enhanced biodegradability resulting in improvement in biogas production (Santibáñez, *et al.*, 2011). The use of both pure and crude glycerol in anaerobic digestion have been studied as both primary and co- substrate ((Siles *et al.*, 2010; Nghiem *et al.*, 2014). Study on the use of different types of glycerol in AD suggested that, the use of any type of glycerol is a good co- substrate since the CH₄ yield were comparable among the substrates. Nghiem *et al.*, (2014) conducted a study on co-digestion of sewage sludge with both crude and pure glycerol and suggested that, despite

the level of impurities, crude and pure glycerol have a similar COD content of approximately 1 kg/L. In using glycerol addition of (0.25% and 0.5%, v/v) their study concluded that, the addition of 0.5%(v/v) of glycerol resulted in a higher production than the addition of 0.25%. Again, a higher methane yield was produced from pure glycerol during the first ten days of the test but the specific methane production at the end of the experiment (30 days) were identical approximately 0.67 m³/L glycerol between both pure and crude glycerol. Siles *et al.*, (2010) using organic loading rate of 0.27–0.36 g COD/g VSS d in anaerobic co-digestion, concluded that both crude and pure glycerol is 100% biodegradable. Various works on AD of glycerol have emerged that small amount of glycerol enhances biogas production, implying that glycerol can be applied advantageously (Viana *et al.*,2011). However, there are no clear specifications of the amount to be applied (Beschkov, *et al.*, 2017). Different proportions have been studied by various authors and have efficiently increased the amount of methane yield, however, it was suggested by (Santibáñez, *et al.*, 2011) that large loading of glycerol can hinder AD process such as high acetic concentrations. They further suggested loading rate of glycerol should be increased gradually. Co-digestion of glycerol with municipal solid waste, sewage sludge, slaughterhouse wastewater and maize silage have been evaluated by several authors, Fountoulakis *et al.*, (2010) in using a temperature of 35°C reported, a 1% (v/v) addition of glycerol with sewage sludge boosted biogas yield. The control reactor with only sewage sludge produced 1106 ± 36 ml CH₄/d, whilst the addition of glycerol enhanced methane yield to 2353 ± 94 ml CH₄/d. They concluded further increase of glycerol above 1% results in imbalance of the AD process. Sulaiman *et al.*, (2009) studied co-digestion of palm oil mill effluent with refined glycerin. 1% (v/v) addition of glycerol showed both COD removal efficiency and methane yield at 90% and 505 m³ day⁻¹, respectively. An increase percentage of 5.25% showed high COD removal efficiency but less methane yield of 307 m³ day⁻¹. In the experiment most of methane production from glycerol appeared within the first 7-8 days due to the readily biodegradability of glycerol. Work on co-digestion of crude glycerin with cassava waste water was done by Larsen, *et al.*, (2013), their results indicated, the addition of 2% and 3 % of glycerol showed high COD removal between the range of 91.54 to 98.69% for OLR of 3.05; 9.32 and 14.83 g L⁻¹ d⁻¹. There were also average removal efficiencies of TS and VS averaging 81.19 to 55.58% for TS and 90.21 to 61.45% for VS. The addition of 2% glycerol showed high product of biogas compared to 3% addition. Though the use of glycerol has been a good substrate for AD, its high biodegradability can cause accumulation of VFAs and drop in pH due to inhibition (Viana *et al.*,2011). Inhibition can affect AD process by causing decrease in

methane yield or failure in the process. Alternatives to overcome these barriers include addition of alkaline or the removal of acid intermediates. However the most efficient way is to use small amount of glycerol and gradually increase the OLR (Beschkov, *et al.*, 2017). An essential nutrient in AD process is Nitrogen, however, glycerol lacks this nutrient and hence co-digestion with nitrogen rich substrates is important. The addition of Nitrogen also helps to stabilize pH especially from highly degradable substrates (Bouallagui *et al.*, 2005). Different concentrations of glycerol used in Larsen *et al.*, (2013) study with OLR, TS, VS, COD and pH are presented in table 2.

Table 2. Concentrations of Glycerol added to cassava wastewater with OLR, TS and VS removal, pH and biogas production.

OLR (g COD L-1 d-1)	Concentrati on Of Glycerol (% v/v)	Removal TS (%)	Removal VS (%)	COD Removal (%)	pH	Biogas Production L L-1 d-1
3.05	0	81.19 ±2.96	90.21 ±2.30	96.41±0.42	7.71±0.3	1.168±0.17
9.32	2	75.47 ±6.43	81.18 ±9.50	98.69±0.69	7.48±0.7	1.979±0.42
14.83	3	68.79 ±9.79	72.24±10.06	98.59±0.12	7.82±0.30	1.305±0.24
13.59	2	55.58±12.9	61.45±13.27	91.54±23.88	4.85±0.10	0.861±0.37

(C. Larsen *et al.*, 2013) : *anaerobic co-digestion of crude glycerin and starch industry effluent*

The initial COD and pH of the crude glycerol used in their study was 1,900g O₂ L⁻¹ and 8.77 respectively.

3.0. Material and Methods

3.1. Experimental Setup

3.1.1 Feedstock

Effluents from an anaerobic stirred tank reactor (CSTR) digesting (vinasse, straw and filtercake) was used as substrate. Vinasse, Straw and Filter cake were obtained from sugarcane industry and ethanol distillery from Brazil and were then transported to Sweden. A synthetic glycerol was used (98% pure, $C_3H_8O_3$). During the experiment, crude glycerol could not be obtained from an industry and avoiding the effects of impurities from an industrial glycerol led to the choice of using a synthetic one. It was observed from literature that, despite crude glycerol having higher levels of impurity (such as salts, fatty acids and soap) both pure and crude glycerol showed similar range of biogas potentials (Nghiem *et al.*, 2014), implying marginal effects of impurities of AD of glycerol.

3.1.2 Inoculum

An inoculum was taken from an anaerobic digester treating effluents from primary and secondary treatment stages at Nykvarn wastewater treatment plant in Linköping, Sweden. The digester from which the inoculum was collected operated at 38°C with an OLR of ca 2kg VS/m³d, HRT of 20 days and reactor volume of 6000m³.

3.1.3. CH₄ Potential of Digestate and Glycerol

The BMP test was conducted to measure the methane yield of digestate, glycerol and inoculum (Fig.1). The test was analyzed according to methods reported by (Ekstrand *et al.*, 2013). The BMP test was done under controlled conditions in a 330 ml bottle sealed with rubber stopper. The experiment was constructed in triplicate for each treatment. Each bottle was filled with 20 ml of inoculum which contained 3,7gTS/L, 69gVS/L and 66.3g/LCOD and substrate in a ratio of 2:1(inoculum: substrate) on the basis of COD concentration. Water was added up to a 100ml total volume. The batch assays were marked based on the proportion of glycerol and digestate in the substrate as G (100 % of gCOD glycerol), D (100% of gCOD digestate), 15G-D (15% of gCOD glycerol and 85% of gCOD digestate), and 25G-D (25% of gCOD glycerol and 75% of gCOD digestate). Three control bottles were set up only with inoculum (marked as I). The head space in each bottle was 230mL. Before filling with inoculum and substrates, each bottle was flushed with Nitrogen (N₂) to minimize oxygen exposure. The bottles were then placed in

a water bath with temperature maintained at 37°C. To ensure homogenous solution, the bottles were stirred manually once a every day.

Figure 2 shows the batch test set up. Whilst table 3 shows the OLR.

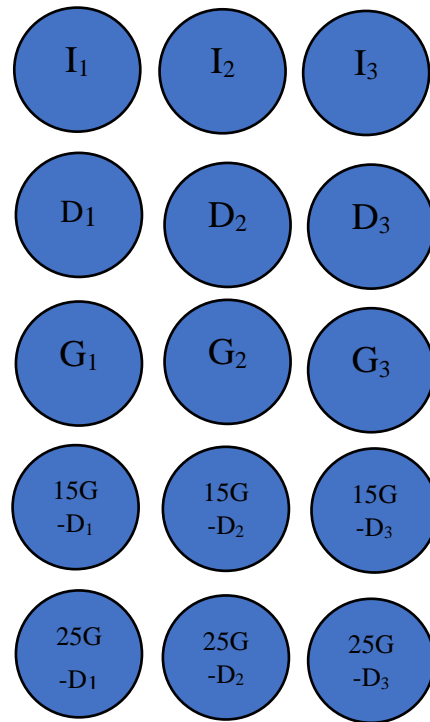


Figure 2. BMP setup, I₁, I₂, I₃ inoculum: D₁, D₂, D₃ digestate: G₁, G₂, G₃, 100% glycerol: 15G-D₁, 15G-D₂, 15G-D₃: 15% glycerol+digestate, 25G-D₁, 25G-D₂, 25G-D₃ 25% glycerol+digestate

Table 3. The volume of organic load used in the batch test, including percentages of glycerol added.

Treatment	Inoculum (CODg/L)	Glycerol (CODg/L)	Digestate (CODg/L)
Glycerol	20	0.62	-
15% G-D	20	0.09	12.6
25% G-D	20	0.15	11.1
Digestate	20	-	14.8
Inoculum	20	-	-

3.3 Analytical measurements

3.3.1 TS and VS

The TS and VS for the inoculum and all treatments were measured according to the standard methods (APHA, 2012). Each test was done in duplicate. This was done by marking six crucibles with pencils and weighed. The crucibles were then filled with the samples and weighed again. To obtain the TS each sample was placed in a 105°C oven for 20hrs. The crucibles were then taken out of the oven and put in a desiccator to cool down after which they were weighed and taken to the muffled oven and heated at a temperature of 550°C for two hours. They were then brought back to the desiccator to cool down and weighed after. The TS and VS were then calculated by the equations below

$$TS (\%) = \frac{\text{dry weight at } 105^{\circ}\text{C} \times 100}{\text{Wet weight}} \quad \text{Equation 1}$$

$$VS (\% \text{ of } TS) = \frac{(\text{Dry Weight at } 105^{\circ}\text{C} - (\text{weight after combustion at } 550^{\circ}\text{C})) \times 100}{\text{Dry weight at } 105^{\circ}\text{C}} \quad \text{Equation 2}$$

3.3.2 COD

Chemical Oxygen Demand (COD) analyses were performed before and after the batch test. All analyses were done in duplicate to each treatment. This was done by using 1000-10000 mg/L O₂ COD kit- LCK014 from (Hach- Lange, Germany). The analyses were done according to the manufacturer's instructions by first inverting the kit a few times to allow for sediment suspension. Samples were pipetted into the cuvette. It was then closed and inverted again. The cuvette was then heated in a thermostat for 2 hours after which it was taken out and inverted twice and allowed to cool at room temperature. The COD was then evaluated in a spectrophotometer. The values of the COD recorded were then multiplied by their dilution factor.

3.3.3 pH analysis

The pH of the samples in the bottles, thus the substrates and the inoculum solution were determined according (NS-EN 12176, 1998) by using pH meter (WTW inoLab pH 7310, Germany). For a verified result, each measurement was done three times. This was done before and after the BMP test.

3.4 Biogas and Methane Measurement

The pressure for each bottle was measured once a day within the first three days and was subsequently measured once a week. The pressure was measured by a pressure meter (Testo Inc., USA). To measure the overpressure, a needle attached to the pressure meter was inverted into the lid of each batch bottle and the pressure displayed on the meter was recorded in mbar. The pressure gauge was then calibrated after each measurement against the atmospheric pressure after each measurement. Determination of total biogas was done by converting the pressure to atmospheric pressure. The correction of this volume was done by multiplying the headspace volume with the atmospheric pressure to achieve the total gas volume in the headspace. The gas volumes were normalized to 1 atm pressure and 273 K and presented as (NmL) using the ideal gas law.

$$V_{STP} = \frac{P_{gas}}{P_{stp}} \times \frac{T_{stp}}{T_{gas}} \quad \text{Equation 3}$$

To determine the methane content, gas samples were collected from each batch bottle in syringes and transferred to a bottle with volume of 31ml. The methane analysis of the headspace was done by transferring gas from the 31ml bottle to Hewlett Packard HP HP5880A GC Gas chromatograph (GC-FID). Three standards with methane contents of 0.07 %, 0.63 % and 1.71% were injected into the GC-FID and calibration was done by linear regression.

3.4.1 Methane Yield

The net volume of CH₄ yield was achieved by subtracting the methane volume of the inoculum from the CH₄ volume of each treatment. This was done to know the volume of CH₄ produced from substrate added to each bottle.

$$\text{Net CH}_4 \text{ (mL)} = \text{CH}_4 \text{ of treatment} - \text{CH}_4 \text{ of Inoculum.} \quad \text{Equation 4}$$

The total methane yield of each treatment was determined by dividing the net accumulated CH₄ yield of each treatment by COD of substrate added to each bottle.

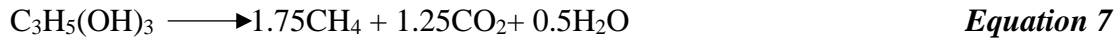
$$\text{Accumulated CH}_4 \text{ yield} = \frac{\text{Net Accumulated CH}_4 \text{ (mL)}}{(\text{gCOD of substrate/L}) \times \text{Active Volume in the bottle}} \quad \text{Equation 5}$$

The modified Gompertz model was used to determine the methane potential, maximum methane production rate and lag phase.

$$M = P \times \exp \left(-\exp \frac{(R_m \times e \times (L - t))}{p} + 1 \right) \quad \text{Equation 6}$$

Where M is the total CH_4 yield ($\text{NmLCH}_4/\text{gCOD}$), P is the methane potential ($\text{NmLCH}_4/\text{gCOD}$), R_m is the maximum methane production rate (NmL/d), L is the lag phase (d) and t is the time at which the corresponding CH_4 yield is recorded. Parameters, M , R_m , and L were determined by fitting the Gompertz equation to the accumulated CH_4 yield curves by non-linear using SPSS software (IBM, USA).

The theoretical methane yield for glycerol converted to methane was calculated from the Buswell equation and the ideal gas law according to (Viana *et al.*, 2011) and is given in appendix 2



$$\text{Gas Law: } PV = nRT \quad \text{Equation 8}$$

Where P is the normal atmospheric pressure (101300 pa), V is the volume of gas (L), n is the number of moles, R is the gas constant $8.314\text{J}(\text{mol K})$, and T is the temperature in K (310 K corresponds to 37°C).

3.6 Reactors Operation

Two lab scale continuous stirred- tank (CSTR) reactors (R1 and R2) with 5 L volume were used in this experiment. At the start up, the reactors were inoculated with sludge from Nykvarn wastewater treatment plant in Sweden. The study employed mesophilic digestion at 37°C and continuously stirred at 100 rpm. The experiment was carried out for 94 consecutive days. The digestate used as feed stock was collected every day from two anaerobic reactors performing co-digestion of vinasse, straw and filtercake. The reactors were continuously fed manually with 250ml of digestate and after 30 days of HRT, R1 was fed with different mixtures of digestate and glycerol and the control reactor R2 was fed with only digestate. The organic loading rate (table 4) of the feed stock changed over time due to changes in OLR of the digestate source. The reactor (R1) that served as co digestion was supplemented with increasing concentrations of glycerol at 15%, 25%, 50% of the OLR of the reactor. 15% addition was added on the 30th day, whilst 25% and 50% on day 60 and 85, respectively. For comparable results to know the extra methane and biogas produced from glycerol, the total volume of gas from the control reactor R2 was subtracted from R1 as shown in section 4.4

Table 4 OLR of digestate and glycerol in reactors

Glycerol Concentration (%)	Day	Digestate (gCOD/Ld)	Glycerol (gCOD/Ld)
15	30- 45	0.32	0.20
15	46-59	0.35	0.30
25	60-70	0.87	0.44
25	71-84	0.92	0.55
50	85-95	1.84	0.95

3.6.1 Reactor Monitoring

The reactors and digestion performance were continuously monitored through measurements in TS, VS, pH, COD and VFAs according to the methods described in previous sections. During the first half of the experiment pH was measured daily and was done once a week when constant values were reached. TS, VS and VFAs were performed once a week, whilst COD analysis were done once in every two weeks. Gas production and biogas compositions (CH₄, CO₂, O₂, H₂) from R1 were recorded daily from a gas meter attached to the reactor whilst for R2 biogas compositions (CH₄, CO₂, O₂, H₂) were measured with BIOGAS 5000 (Scantec Nordic) once a week. All gas measurements were corrected to standard temperature and pressure (273.15 K and 101.325 kPa). Whilst methane yield was calculated by multiplying the biogas production by the methane concentrations and dividing by the COD of the substrate presented in (Nml/gCOD).

The collected data were analyzed in Excel and graphs presented with Origin 2017 program.

4.0 RESULTS AND DISCUSSION

4.1 Batch Experiment

4.1.1 Composition of Digestate and Glycerol

Tables 5 shows the physical and chemical compositions of digestate and glycerol used in the batch test. The digestate used in this experiment had TS contents of 65% with low organic matter (COD of 66.29g/L) compared to the COD of the glycerol (1074 g COD/L) used and as stated in literature, digestate have low carbon and organic matter with considerable amounts of nitrogen (Möller & Müller, 2012), hence the exceeding amounts of glycerol at percentages of (5% and 25% gCOD/L) of the effluents were used to improve organic content to enhance biogas production. The COD values of the glycerol was in line with the results from previous works (Nghiem *et al.*, 2014: Larsen *et al.*, 2013) reported high values in COD for glycerol with the former obtaining 1900g COD/L whilst the latter reported 1050 g COD/L. The pH of

6.7 of the digestate was within the optimum pH range in an anaerobic digester, 6.5-8.0 (Cioabla, *et al.*, 2012) whilst the pH of the glycerol was 6.8.

Table 5. Composition of Digestate and glycerol

Parameter	Digestate	Glycerol
pH	6.7	6.8
COD(g/L)	66	1074
TS (% of dry weight)	1.8	92
VS (% of TS)	65	98

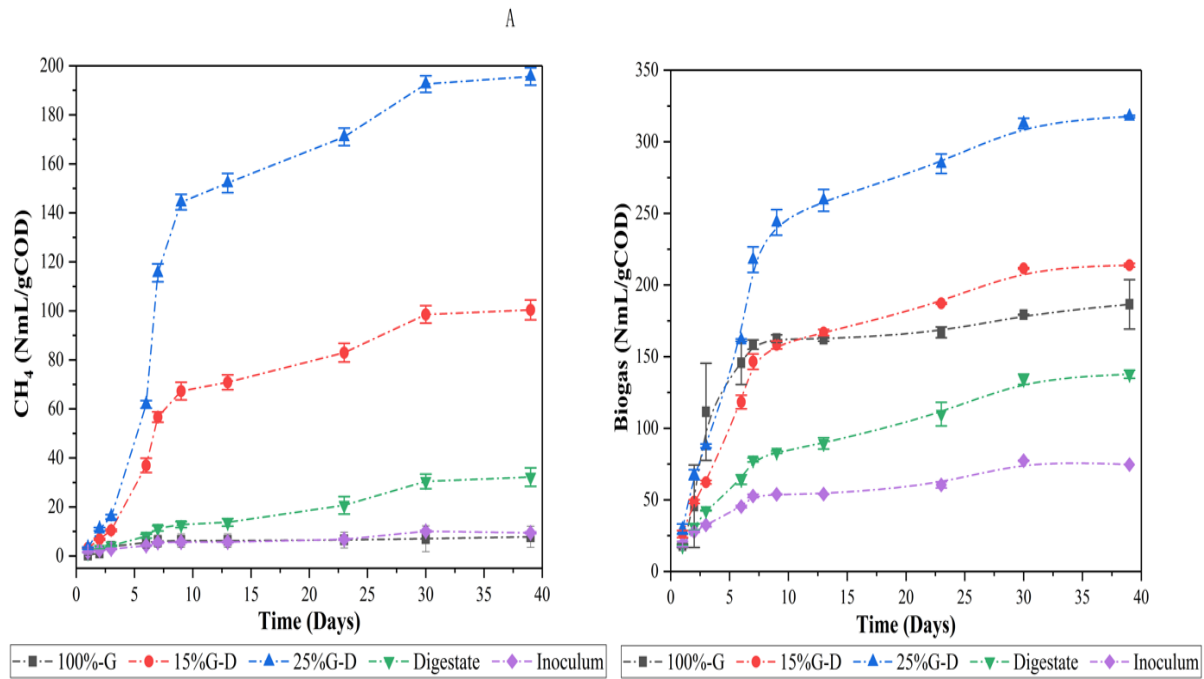
4.2 Biogas and Methane yield

The cumulative methane yield per unit mass of COD (NmL/gCOD) and biogas production from co- digestion of different concentrations of glycerol and digestate, are presented in (Figure 3 and Table 6). The goodness- of- the fit for the modified Gompertz models R^2 ranged from 0.92-0.98 showing a reasonable accuracy of fitted models. The estimated lag phase (L) values were between 0.8 and 1.7 for the highly degradable treatments (100% G, 15% G-D and 25% (G-D)). The longer lag phases were evident in the inoculum and digestate 5.3 and 1.9d. compared to treatments with glycerol 0.8 – 1.7d. Both digestate and inoculum had low maximum CH₄ production rates of 0.5 and 0.1 NmL/d, respectively, due to their less biodegradability, while the highest maximum CH₄ production rate of 12.5ml/gCOD was observed for 25G-D. In assays with 100% Glycerol as the substrate, methane production initiated at the early stages with lag phase of 0.8d, but stopped after the 8th day of the experiment. Santibáñez, *et al.*, (2011) suggested high concentrations and quick digestion of glycerol will cause VFA accumulation due to organic overloading, leading to acidification and decrease in methane yield. In all treatments, most of the methane production were observed within the first 8 days of the batch test. Treatments with glycerol had the shortest time in reaching half of its methane potential of 3.7, 6.9 and 7.2d for 100%G-D, 15%G-D and 25%G-D respectively, compared to treatment with only digestate (14.5d). This shows the quick adaptation of microorganisms to glycerol addition which improved methanogenesis rate (Sulaiman *et al.*, 2009).

Table 6. Overview of biogas production, methane concentrations, methane yield, lag phase

Substrate	Predicted CH ₄ Yield NmL/gCOD	Standard error	Cumulative CH ₄ Yield NmL/gCOD	Cumulative Biogas NmL/gCOD	Methane concentration (% of biogas)
100% G	11.6±4.1	0.5	8	187	8
15% G-D	45.4±17.7	0.4	100	214	33
25% G-D	99.2±41.2	0.6	196	318	49
Digestate	15.1±5.0	0.4	32	138	11

Substratd	Lag Phase (d)	R ² (%)	Rm(NmL/d)	time _{half} (d)
100% G	0.8	95	2.3	3.7
15% GD	1.7	97	4.5	7.2
25% GD	1.6	98	12.5	6.9
Digestate	-1.9	97	0.5	14.5
Inoculum	-5.3	92	0.1	8.0

**Figure 3.** shows Cumulative CH₄ and biogas yield (NmL/gCOD) of 100% G, 15% G-D, 25%, digestate and inoculum at standard conditions (273. K, 1 atm) mean of triplicates with SD over time (days)

In general, the highest methane and biogas yield (196 NmL/gCOD and 318 NmL/gCOD), respectively was obtained from the addition of a mixture of 25% glycerol and 75% digestate on gCOD basis corresponding to methane concentration of (59%). Whilst 15% G-D gave rise to yields of (100 NmL/gCOD, and 214 NmL/gCOD) with CH₄ concentration of 52%. It can be

elucidated from the batch test results that, the addition of both 15% and 25% of glycerol enhanced methane and biogas yield of digestate. This result agrees with literature by several authors, (Nghiem *et al.*, 2014; Fountoulakis *et al.*, 2010; Amon *et al.*, 2006; Astals *et al.*, 2010). Astals *et al.*, (2011) reported an increase in methane production in a batch test when pig manure was co-digested with glycerol. Treatments with pig manure and glycerol with boosted methane yield by (215 mL CH₄/g COD) which was about 125% more than when pig manure was mono-digested (96 mL CH₄/g COD). Amon *et al.*, 2006 also reported methane increase from 569 to 679 CH₄ g/VS) and biogas. The batch test from this study also saw a considerable amount of CH₄ and biogas production from the digestate (32 Nml/gCOD and 138 mL/gCOD). The methane production from glycerol was obtained by subtracting the volume from produced by digestate from treatments with glycerol addition (15 and 25%). Hence the resulting methane and biogas from glycerol with 15% addition was (68 Nml/gCOD and 76 Nml/gCOD) whilst 25% had volumes of (164 Nml/gCOD and 180 Nml/gCOD).

4.2.1 COD and VS Removal

The initial pH, COD, TS/VS and VS reduction are presented in table a 7. Treatments with 15% and 25% showed a stable pH from the initial and final stages of the test, implying the supplementation of glycerol is good for co-digestion as it did not cause any inhibition that could have resulted in VFA accumulation leading to a drop in pH. However, in this study, treatment with only glycerol (100%) with an OLR of 0.6 gCOD/L resulted in formation of acids after the experiment (pH=5.32) which indicate the amount of glycerol used resulted in an organic overload and inhibition of methanogenesis, which has also been observed in other AD systems with high loads of glycerol (Nghiem *et al.*, 2014 : Nuchdang & Phalakornkule, 2012). In an anaerobic co-digestion of glycerol and pig manure, by Nuchdang & Phalakornkule, (2012) at higher OLR of 5.4 g COD/L of glycerol the propionic acid to acetic acid ratio was found to be higher than the threshold for metabolic imbalance. Viana *et al.*, (2011), suggested the COD range of glycerol (925 and 1600g/L) is a hindrance to using only glycerol as substrate in AD. They further suggest, dilution of glycerol with water will be a solution to this problem. The highest pH was observed in the digestate in both initial and final stages of the test (8.11 and 8.14). These results are comparable with studies on digestate by Menardo, *et al.*, (2011) and Möller and Müller, (2012). The authors presented initial and final pH of digestate (8.1 and 8.2) and stated that, digestate have high pH levels. From table 7 it was observed that, all treatments showed satisfactory amounts of COD removal ranging between

81.7% and 88.7%. However, treatment supplemented with 15% and 25% addition of glycerol showed the highest efficiencies in COD removal (85.2% -88.7%) and VS reduction of (81.7%-88.7%). This result was not surprising as these treatments had the highest efficiency in consuming organic matter and producing the highest amount of methane. This observation was in line with Sulaiman *et al.*, (2009) and Larsen *et al.*, (2013) whose studies presented a COD removal of 90% with glycerol addition. They related the high COD removal to the high biodegradability of glycerol which is easily utilized by microorganisms.

Table 7. Initial and final pH values, TS, Vs, COD removal (%) and Vs reduction (%)

Initial Values	Substrate	pH	TS	VS	COD
	Glycerol	8.1±0.2	3.7±0.2	72±2.9	96.5±0.4
	15% G-D	7.9±0.1	1.9±0.4	69±2.3	60.9±4.2
	25% G-D	8.0±0.1	2.7±2.1	69±2.6	63.6±2.8
	Digestate	8.0±0.2	1.8±0.6	66±2.6	57.1±2.1
	Inoculum	8.1±0.02	3.7±4.7	69±2.4	66.3±2.5
Final Values		pH	TS	VS	COD
	Glycerol	5.3±0.3	nd	nd	17.7±4.0
	15% G-D	7.7±0.04	0.7±0.1	60±0.5	9.0±0.2
	25% G-D	7.7±0.1	0.5±0.2	59±3.3	7.2±4.5
	Digestate	7.9±0.1	0.7±0.02	61±0.9	9.2±0.5
	Inoculum	8.1±0.01	1.6±0.1	61±0.2	nd
				COD removal (%)	VS reduction (%)
	Glycerol			81.7	nd
	15% G-D			85.2	68.0±2.5
	25% G-D			88.7	84.1±2.1
	Digestate			83.4	64.1±2.8
	Inoculum			nd	61.8±3.1

nd= not determined

4.3 Reactors performance

The reactor performance in terms of biogas and methane production at normalized conditions are shown in fig 4. There were considerable amounts of CH₄ production yield of 150NmL/gCOD at the initial stages. The reason was due to the high organic matter of the inoculum source and was evident in the initial COD of both R1 and R2 (13.3and 18.4g/L, respectively). After the acclimation period of 30 days, the co- digestion with glycerol was divided into three stages. In the first stage, addition of glycerol as 15% of influent COD (0.2 – 0.3 gCOD/L.d; Table 4) to R1 resulted in the highest increase in methane and biogas production (340 and 760 Nml/gCOD Table 4) almost 300% higher than the control between operational days of 30 and 40. In the second stage, increase in glycerol to 25% of influent COD corresponding to an OLR of (0.44 – 0.55 gCOD/L; Table 4) with methane and biogas production of 220 and 500 Nml/gCOD approximately 170% higher than the control reactor between operational days of 60- 70. The percentage increase in biogas production was higher

than a previous work by Nghiem *et al.*, (2014) on co- digestion of sewage sludge with 0.63% and 3% (v/v) addition of glycerol. In their study, the highest methane yield (80%) higher than the control reactor was observed in the high concentration of glycerol (3%) whilst the lowest (50%) was observed in low concentration (0.63%).

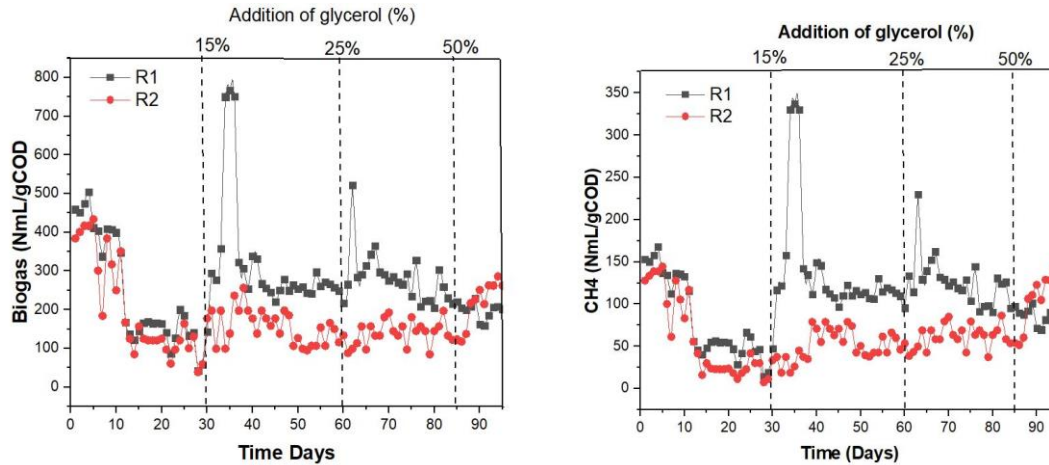
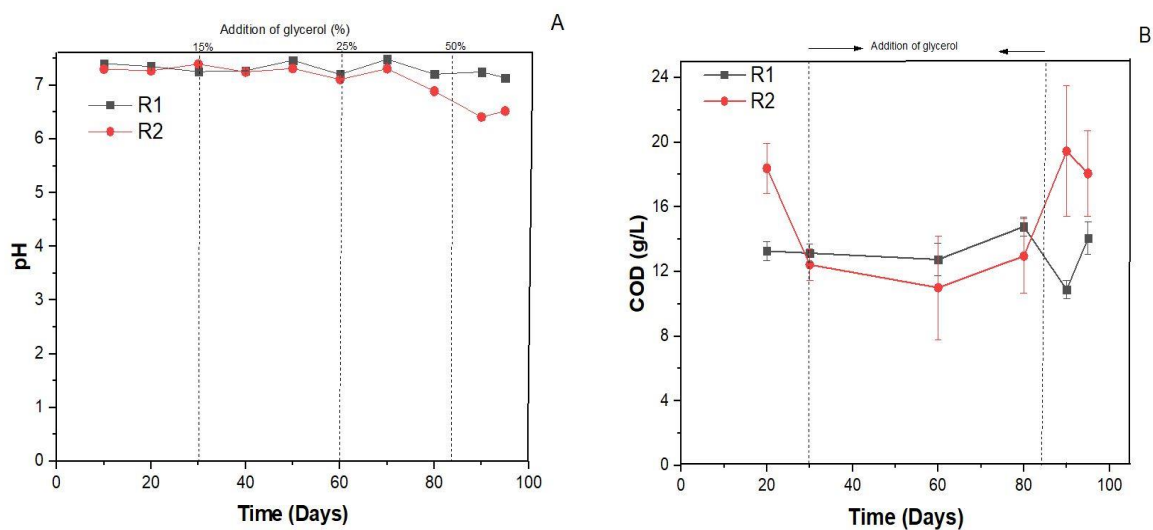


Fig. 4. Methane and biogas yield for R1 and R2 (NmL/gCOD) with glycerol addition

In the last stage, a further increase in glycerol concentration of 50% (0.95 gCOD/L; Table 4) exhibited a decline in methane production 75 NmL/gCOD of approximately 50% decrease. This was a consequence of the further increase in glycerol resulting to high OLR and the availability of increase amount in biodegradable organic matter. Studies on co -digestion of glycerol with other substrates posits that, further increase in glycerol concentration results in decrease in biogas production (Astals *et al.*, 2010: Fountoulakis *et al.*, 2010: Nghiem *et al.*, 2014 : Sulaiman *et al.*, 2009). A decrease in biogas production in an anaerobic co- digestion of palm oil mill effluent with glycerol was due to an organic overload as posited by Sulaiman *et al.*, (2009). In their study an increase in glycerol addition from 5.0 to 5.25% (v/v) caused a further reduction of biogas by 47%. They explained this to the limited amount and less survival of protozoa and methanogenic archaea after been exposed to high OLR. In this study, an increase in OLR from 0.2 to 0.95 gCOD/Ld for digestate and glycerol ,respectively, during co- digestion led to a decrease in biogas production. The decrease in biogas production in proportion to the increase in OLR was also suggested by Astals *et al.*, (2011) as glycerol being high biodegradable, high amount of organic matter at high OLR results in accumulation of VFAs and reactor acidification resulting to low methane production. However, both reactors in this study showed no VFA accumulation. This reason could be explained in two folds, firstly the 15% and 25% of influent COD addition did not cause an organic overload hence resulting

in no VFAs accumulation thereby leading to a stable process. Another reason could be that further addition of glycerol 50% at OLR of 0.95 gCOD/L which caused a decrease in biogas production did not stimulate additional fermentation of solids. *The optimization of sludge fermentation for VFAs production* was studied by Sultan and Andrew, (2019). In their study, glycerol co-fermentation with primary solids showed that, glycerol increased VFAs production because glycerol addition stimulated further fermentation of primary solids. The pH was stable except for the latter parts of the experiment which saw a drop in pH from 7.04 to 6.4 (Fig.5A) during the addition of 50% concentration of glycerol. Measurements of VFAs could not be performed in the later stage of operation due to practical limitations at the laboratory. Therefore, it can be assumed, the sustained high loading of glycerol led to VFA accumulation and a substantial drop in pH (6.4) which resulted in a decrease in methane production. Again, fermentative utilization of glycerol results to products such as 1,3- Propanediol and 1,2 Propanediol (Clomburg & Gonzalez, 2013). These products were not monitored during the experiment, hence accumulation of these products might have led to an unstable process.



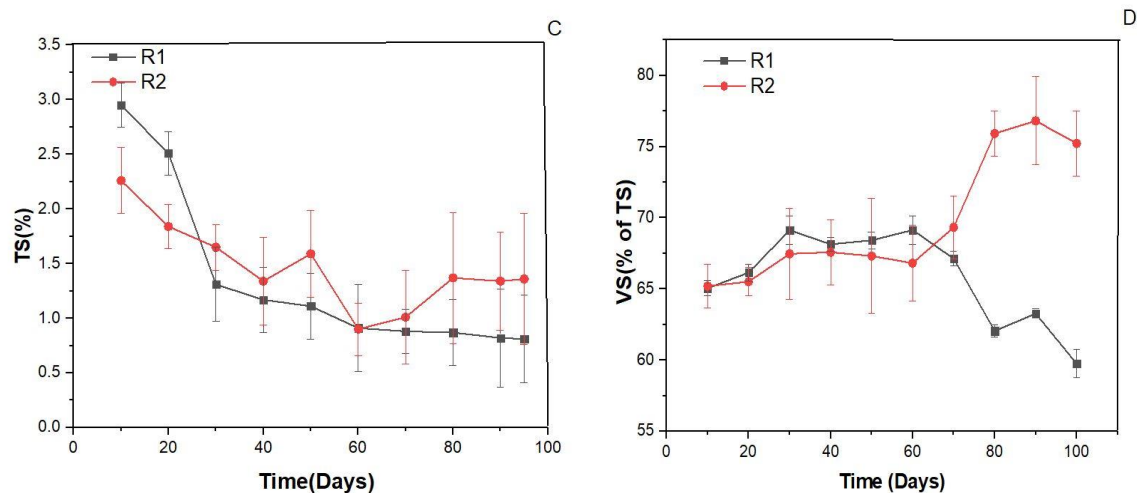


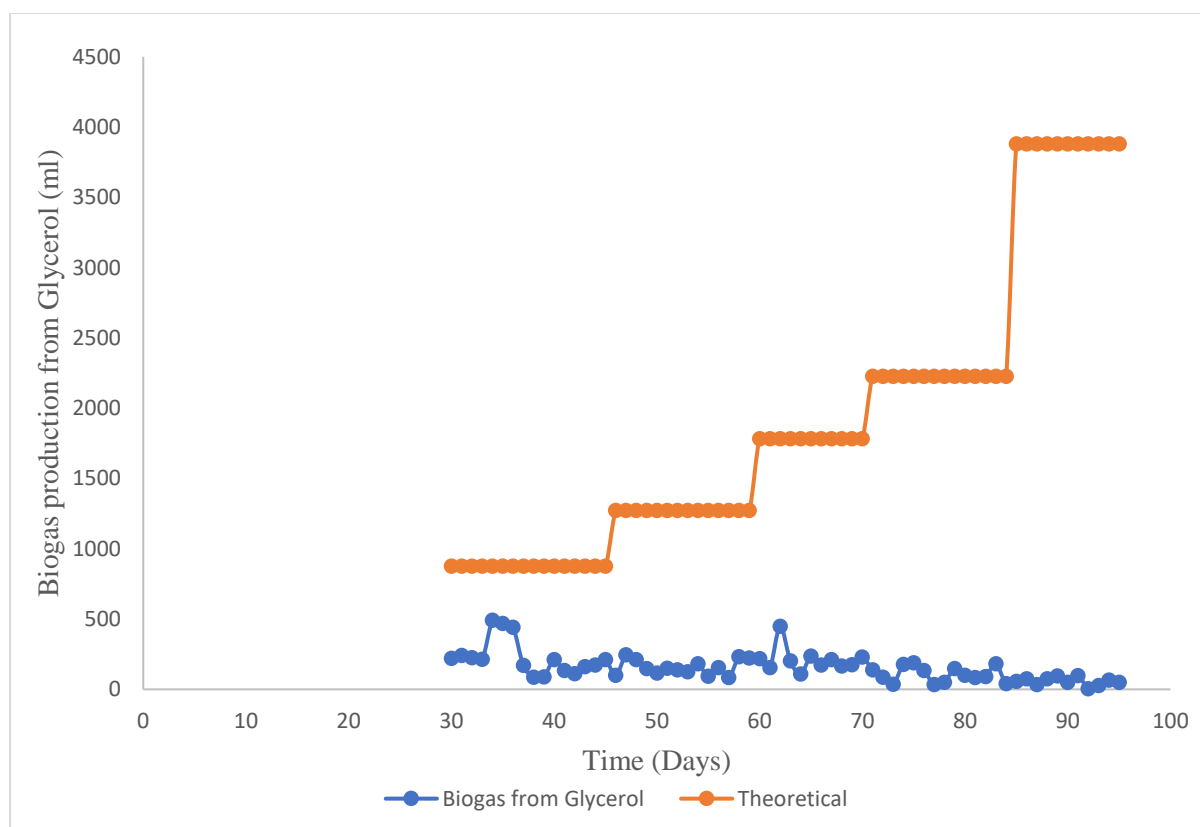
Fig. 5. Parameters monitored during the continuous experiment for R1 and R2. (B) pH value; (C) COD; (D) total solids and (E) volatile solids with SD

There appears to be a decrease methane production when the reactors were continuously fed at both 15% and 25% glycerol, indicating the microbial consortium easily adjusted and digested glycerol addition at the early stages and converted them into biogas. (Astals, *et al.*, 2011). Again, the reason could also relate to the changes increase in OLR during feeding. Clomburg & Gonzalez, (2013) suggest glycerol requires numerous microorganisms to utilize the available reduced carbons required to enhance yield in anaerobic fermentation, however, the number of microorganisms required during fermentation of glycerol is limited in the absence of external electron acceptors. Therefore, it could be explained that, further feeding (increase OLR) with glycerol did not have enough microorganisms for its utilization. This could result in undigested glycerol causing reduction in methane production. Though these factors could be the primary reasons to the decrease in methane production. It should also be noted that, in the course of the experiment there was a problem with R1 which led to fluctuations in gas production and low concentrations of CH_4 . For instance, some operational days recorded methane concentration as low as 9 and 13 percent. The addition of glycerol concentrations augmented VS and COD of R1 compared to R2. This may be explained due to the high organic matter and COD content of glycerol which resulted in high efficiency of biogas production. This increase was evident between operational days of 30 and 65 (fig 5 B and D) with corresponding methane increase occurring in day 30 to 60 (fig 4 A).

Table 8. Comparison of experimental and theoretical methane in R1

Parameters	15% glycerol	25% glycerol	% Theoretical
Experimental Methane production (Nml/gCOD)	340	220	67
Theoretical methane production (ml/gCOD)	508	663	33
Operational days	30-45	60-70	

In using the OLR of glycerol fed to the reactor, the theoretical methane production in R1 based on the Buswell formula and the ideal gas law was estimated at an average of 508 and 663 (ml/gCOD) of CH₄ between operational days of 30-45 and 60-70 respectively. According to literature, the amount of methane yielded theoretically should be higher than the actual methane yield, because not all substrate in the digester will be degraded and also used for cell growth, (Esposito *et al.*, 2012). The fluctuations in methane and gas productions during operational days could have accounted to the low (33%) of the theoretical methane production.

**Fig.5.** Biogas production and theoretical biogas potential from glycerol.

The daily biogas production from glycerol was obtained by subtracting R2 (control reactor) from R1. The results were then plotted with the theoretical biogas potential of glycerol. The highest amount of biogas production from glycerol at 15% influent COD (0.2 COD/L.d) was obtained between operational days of 30-45. However a continuous increase in OLR (0.3-0.95 COD/L.d) during the experiment resulted in a decrease in biogas production. This is an indication that, R2 showed the highest efficiency in biogas production at low OLR (0.2 COD/L.d). The theoretical values were higher than the experimental values and according to Schnürer and Jarvis (2018) the theoretical and experimental values are not consistent because not all materials fed to the reactors are digested.

4.4 Implementation of Co- digestion of glycerol

The idea of using glycerol in this study was because it is an easily biodegradable carbon source. As pointed out earlier, 100 kg of biodiesel production generates approximately 10 kg of glycerol, this has necessitated advancement to utilize this waste. A preliminary analysis by Viana *et al.*, (2011) on the use of glycerol in AD for a biogas plant showed that, 1.2GW of electricity and 4.4GW of heat could be produced from a reactor treating 25m³ of glycerol. From this study, a stable anaerobic digestion of glycerol can be achieved by increasing the OLR approximately 3 times of influent COD of glycerol by co- digestion with digestate. The glycerol added generated biogas amounts of 9519 Nml/gCOD) This is an indication that, by having a minimum input costs (without further refinery by the biodiesel industry) waste product from biodiesel (glycerol) could be reduced through AD and hence contribute to a sustainable environment. The amount of biogas generated from co-digestion of digestate with glycerol can also contribute to the energy sector, through fuel production and electrical energy.

5.0 Conclusion and Recommendations

Anaerobic co-digestion of digestate (effluents from a CSTR reactor performing co- digestion of sugarcane waste vinasse, filter cake and straw) with glycerol was investigated in this study. Firstly, a BMP test was conducted to determine the methane and biogas potential of digestate and glycerol as co- substrate. Results from the experiment indicated that, at ratio of 2:1 of inoculum to substrates, the addition of glycerol with digestate corresponding to 25% of influent COD yielded highest biogas and CH₄ yield of 318 Nml/gCOD and 196 Nml/gCOD respectively compared to mono- digestion of digestate (138Nml/gCOD and 32 mL/gCOD)

The results from co- digestion of digestate with different concentrations of glycerol in a continuous reactor showed that, glycerol addition of 15% and 25% increased biogas and CH₄ production from 340 to 760 Nml/gCOD and to 130 and 295 Nml/gCOD corresponding to a percentage increase of 300% and 170% respectively, whilst exceeding amounts of 50% glycerol resulted in decrease in methane yield. The control reactor R2 digesting only digestate produced a total methane and biogas volume of (5909 and 16355 Nml/gCOD), indicating the digestate used produced considerable amount of gas volumes which had an impact in the co-digestion process. R1 performing co-digestion of digestate and glycerol produced total methane and biogas volume of (10621 and 25874 Nml/gCOD). Hence the contributing amount of methane and biogas production from glycerol was (4712 and 9519 Nml/gCOD) respectively. Given the observed yields, the impact of the co-digestion process on the digestibility of the digestate itself is unclear.

The results from both the batch and continuous experiment demonstrated that co-digestion of digestate and glycerol was feasible as methane and biogas production was enhanced by glycerol addition. Thus, the result presented a promising alternative to both the sugarcane and glycerol industries to curb the challenges in waste management of their by- products. However, for a stable digestion process and to enhance methane yield, glycerol addition should not exceed 25% of influent COD with a corresponding OLR of 0.22-0.23gCOD/L.

Recommendations for future studies for this study could be:

1. A pilot scale study could be done to know how different concentrations of glycerol in (v/v) will enhance biogas production

2. Performing full microbial analysis with co-digestion of glycerol and digestate is recommended.
3. The use of crude glycerol from an industry for co-digestion is also recommended.

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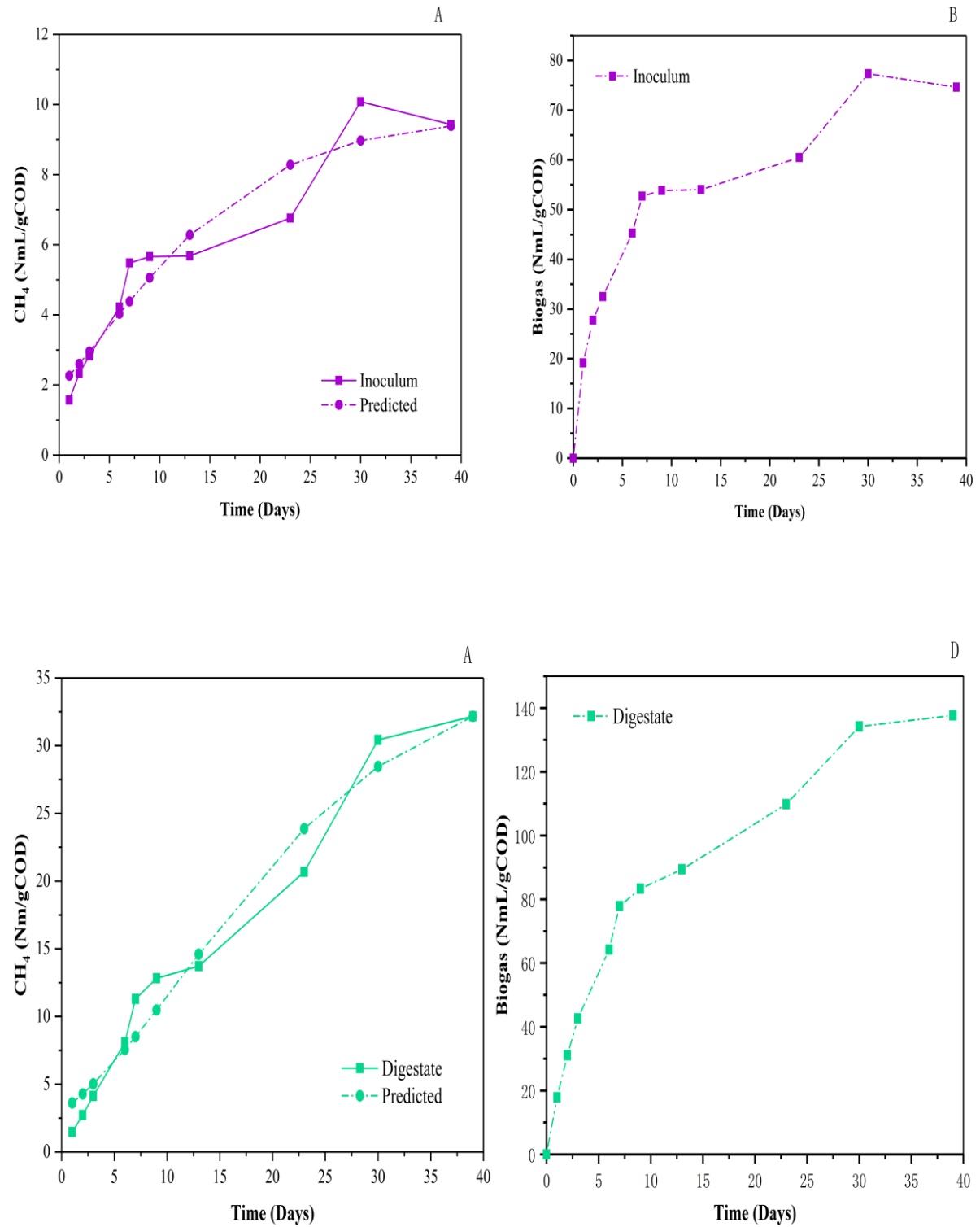
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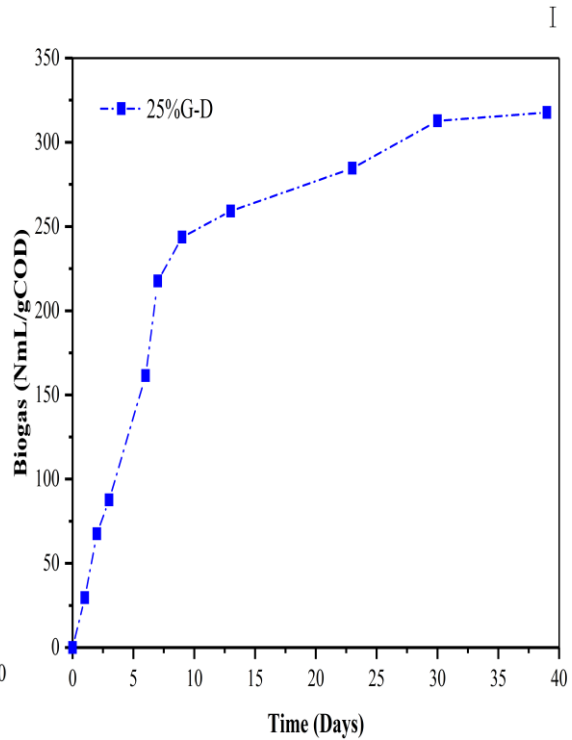
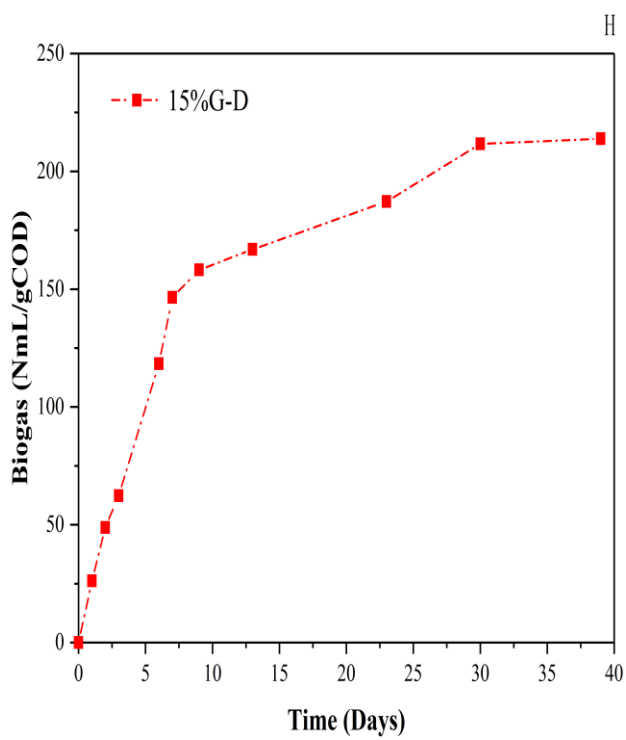
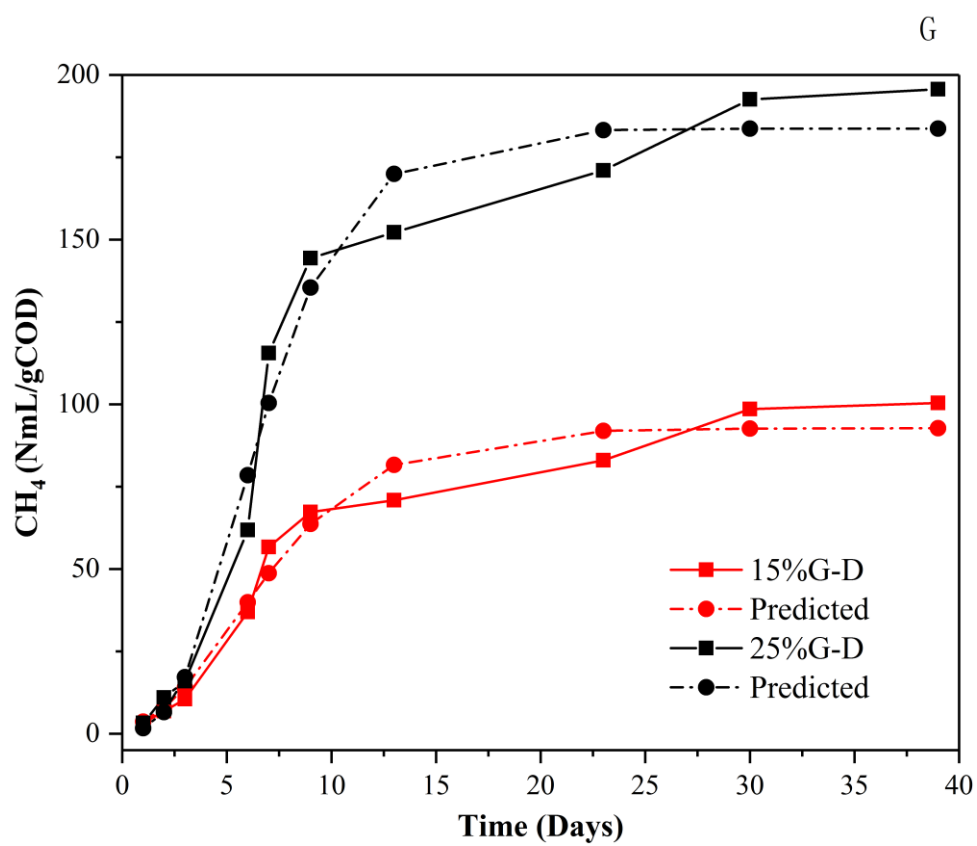
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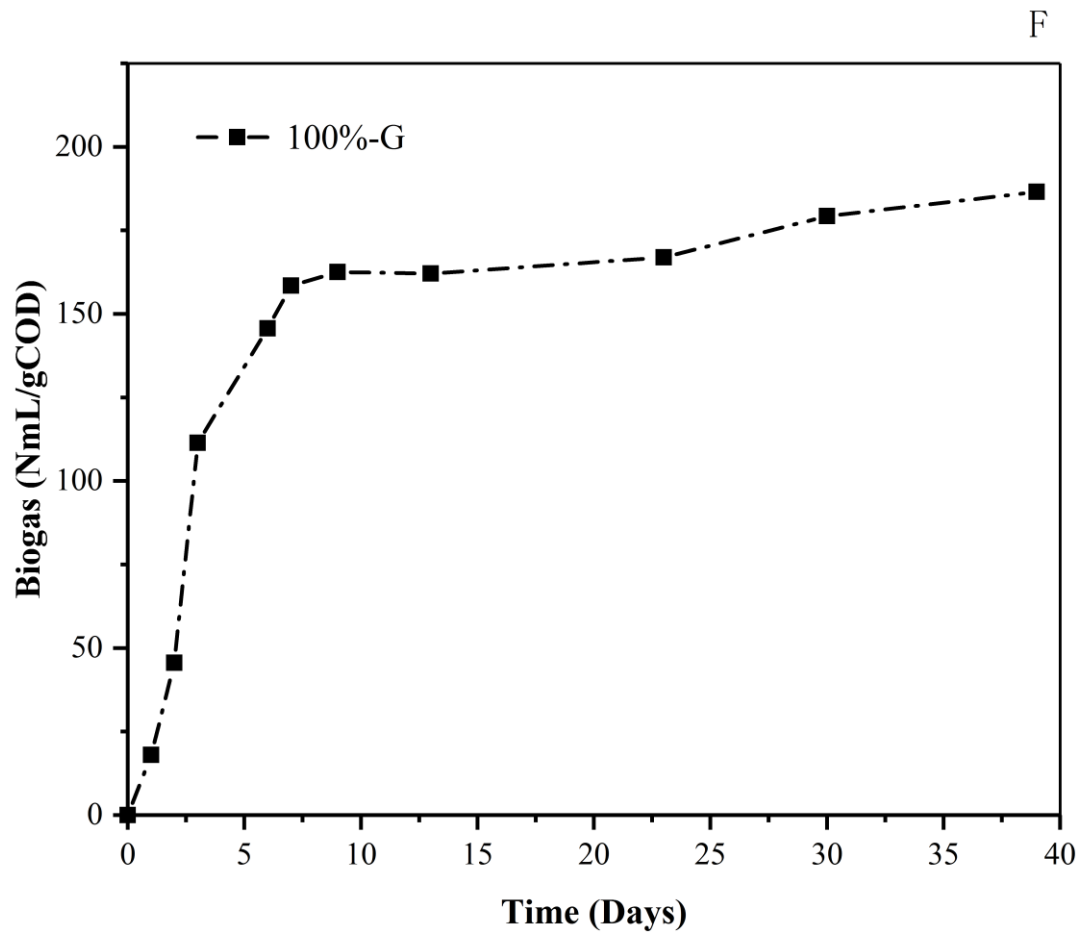
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7.0 Appendix

7.1 Appendix 1. Results from batch test for various treatment showing biogas production and data after being fitted in Gompertz model.







7.2 Appendix 2

Theoretical methane calculation using the Busswel equation and the ideal gas law

Amount of glycerol added to reactor between operational days of 30-45 is 0.2gCOD/L/d. the reactor volume is 5L, therefore 0.2*5= 1 g glycerol was added to the digester every day. Molecular weight of glycerol is 92g, therefore this corresponds to 1/92= 0.011mols of glycerol addition per day.

From Buswells formula, $C_3H_5(OH)_3 \rightarrow 1.75CH_4 + 1.25CO_2 + 0.5H_2O$ according to (Viana *et al.*,2011) 1 mol of glycerol produces 1.75 mols of CH_4 , hence 1.75*0.011= 0.02mol of CH_4 .

From $pV=nRT$

$$V = \frac{nRT}{p}$$

$$= \frac{0.02 \cdot 8.314 \cdot 310}{101300}$$

V= 508mL of CH_4 from glycerol

Image 1 BMP setup



Image 2 reactor setup

