Feasibility of Integrated Biohydrogen and Biogas Production from Sweet sorghum

Ms Divya Joslin Mathias
B.Tech, M.E (Chemical)

School of Engineering and Built Environment

Griffith University

Submitted in fulfilment of the requirements of the degree of
Doctor of Philosophy

October 2023

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)__[redacted]__

Name of Student: DIVYA JOSLIN MATHIAS

ACKNOWLEDGEMENT

I would like to thank my principal supervisor, Prof Prasad Kaparaju, for his valuable guidance, constant encouragement, support and understanding throughout my candidature. He constantly found time to check on the progress of my research amidst his very busy schedule. He always stood by me without judgements through the ups and downs of my PhD journey. His passion for research and his students always inspired me.

I also thank my associate supervisor, Assoc Prof Tony Green for his encouraging words and constant support. His words always gave me the confidence to carry on. I also extend my thank to Assoc Prof Thiago Edwiges for reviewing my publications and providing me with valuable inputs that helped me improve my objectives further.

I also wish to thank Mr Rajinder Singh. He provided me with raw materials for my project and provided valuable inputs to improve my analytical, experimental, and statistical skills.

I thank Erika for helping me get acquainted with laboratory methods and always immediately offering a helping hand during the lab sessions when requested. I also extend my thanks to Shweta, Tirthankar and Amal for helping me out with analysis of my samples and setting up the BMP experiments.

I also thank the Griffith University for providing financial support for this research through the Griffith University Postgraduate Research Scholarship and Griffith University International Postgraduate Research Scholarship. I thank the Griffith Graduate Research School for helping me throughout the scholarship extension and the thesis submission processes.

Above all, I thank God Almighty for giving me the wisdom and resources to complete this journey.

ABSTRACT

Sweet sorghum (*Sorghum bicolor* (L.) Moench), an annual C4 plant, is considered as a suitable biomass for biofuel production due to its high biomass yields, rapid growth rate, and low water and nutrient needs. In an integrated biorefinery concept, sweet sorghum has the potential to produce high-value products such as animal feed, renewable fuels, chemicals, and fertilisers, unlike any other annual crop. This integrated biorefinery approach will allow us to utilise the whole crop biomass more efficiently to generate renewable biofuels, high-value by-products such as organic acids, thereby enhancing the economic viability of the project through crop biorefinery concept. The aim of this PhD study is to evaluate the feasibility of utilising sweet sorghum, grown on fallow land of sugarcane farming, as a feedstock to produce biohydrogen (H_2), organic acids, and methane (CH_4) in an integrated biorefinery concept. To optimise the individual bioprocesses, chemical composition analysis and lab-scale experiments were conducted, followed by a feasibility of farm-scale biogas plant and evaluate the biogas utilisation options.

Nine sweet sorghum cultivars were grown on sandy loam soil during September 2020 in North Queensland, Australia. At first chemical composition, biochemical methane potentials, and kinetic models were employed to determine the H_2 and CH_4 potentials. In subsequent batch anaerobic digestion experiments, the effects of harvest time, inoculum to substrate ratio (ISR), and inoculum source on methane yields from sweet sorghum cultivars were investigated. The chemical composition results showed that the total solids (TS) content in the nine sweet sorghum cultivars ranged between 12.05% (SE-23) and 21.40% w/w (Mega Sweet). A similar trend was also noticed with volatile solids (VS). The high VS/TS of 0.89–0.95 and low carbon to nitrogen (C/N) ratio of 40-80 indicates that the tested cultivars were rich in organic matter and thus can be potential feedstocks for biofuels production. The carbohydrate content of the tested sweet sorghum cultivars was 45–60% (w/w) and the lignin content was 16–25% (w/w), indicating that sweet sorghum is an ideal feedstock for biohydrogen, organic acids, and methane production. Given that plant height is determined by crop maturity and

internode length, there is a strong relationship between plant height and crop biomass yield.

The effect of harvest time (50, 66, and 80 days after sowing, DAS) on chemical composition and the methane yields of SE-19, SE-45 and Mega Sweet showed that higher biomass and methane yields were obtained when the crop was harvested at 80 DAS. After 80 DAS, highest biomass yields of 174 t/ha FM was obtained for SE-45 followed by 132 t/ha and 71 t/ha for SE-19 and Mega Sweet, respectively. Methane yields of 168.99, 184.39 and 243.47 NL $CH_4/kgVS_{added}$ were obtained for SE-19, SE-45 and Mega Sweet after 80 DAS, respectively. The results demonstrated that longer the crop growth periods, the higher the sweet sorghum biomass production and, consequently, higher the methane yields.

The effect of inoculum to substrate ratio (ISR) of 2, 4 and 6 on methane yields of nine sweet sorghum cultivars (SE-1, SE-5, SE-23, SE-35, SE-42, SE-45, SE-81, SE-86 and Mega Sweet) showed that methane yields increased with increasing ISR and was also dependent on the chemical composition of sweet sorghum cultivar. At ISR 2, methane yields ranged from a low 175.93 NL $CH_4/kgVS_{added}$ (SE-1) to the highest value of 291.27 NL $CH_4/kgVS_{added}$ (SE-35). Increase in ISR from 2 to 4 resulted in an increase in methane by 67.6-76.4%. Further increase in ISR to 6 resulted in an increase in methane yields by 46.8% to 78.1%. Overall, SE-35 was the best cultivar followed by SE-5, SE-23, SE-42, and SE-45 (342-349 NL $CH_4/kgVS_{added}$). Among the tested cultivars, SE-1 had the lowest methane yields of 175.93-306.86 NL $CH_4/kgVS_{added}$). On the other hand, SE-35 had the highest methane yields of 227, 291, and 375.91 NL $CH_4/kgVS_{added}$ and were obtained at ISRs of 2, 4, and 6 for SE-35, respectively. In our study, we had used a fixed seed rate per ha. SE-35 had biomass yields of 82.3 t/ha but the low methane yields of 29.83 tons per fresh matter was due to its lower volatile solids content. Thus, the SE-81 cultivar was selected as a promising sweet sorghum cultivar due its relatively high methane yields (3,059.18 m^3/ha) and gross energy yield. The estimated gross energy per ha of the SE-81 cultivar was 29.55 MWh/ha or equivalent to 37.98 passenger cars per hectare per annum.

Effect of rumen fluid, sewage sludge digestate, and cow manure digestate as inoculum source on methane yields of SE-1, SE-19, SE-20, SE-35 and SE-122 was investigated. Results showed that methane yields were profoundly influence by type of inoculum use. Among the tested inocula, cow manure digestate was found to be the best inoculum with a maximum methane yield of 241.81-281 $NLCH_4/kgVS_{added}$. Although rumen fluid was found to be a good source of cellulolytic bacteria, cow manure digestate had good proportion of both hydrolytic bacteria and methanogenic organisms and thus provided an ideal consortium for a stable anaerobic digestion process.

The effect of organic loading rate (OLR) on biohydrogen production rate and yields of three sweet sorghum cultivars (Mega Sweet, SE-35 and SE-45 was investigated in batch dark fermentative H_2 reactor studies at 55 °C. Results showed that biohydrogen yields were dependent on OLR and chemical composition especially total soluble sugar content of sweet sorghum cultivars. Highest biohydrogen production of 40.68 NL H_2/gVS_{added} was obtained for Mega Sweet at load rate 16 gVS/L. The high biohydrogen production noticed with Mega Sweet was attributed due to the high concentration of readily available soluble sugars in Mega Sweet than in SE-35 and SE-45.

Finally, the effect of OLR (0.5-3.5 gVS/L/d) on process performance and methane yields during the mono-digestion and co-digestion of sweet sorghum with cow manure in CSTR reactor at 37 °C and HRT of 25 d was investigated. Three sweet sorghum cultivars: Mega Sweet, SE-45 and SE-35 were used as substrates. The ratio between sweet sorghum and cow manure during co-digestion was fixed at 85:15 on VS%, respectively. Maximum methane yields of 229.70 NL $CH_4/kgVS_{fed}$ was obtained when sweet sorghum was co-digested with cow manure. The low methane yields and biodegradability for the mono-digestion of sweet sorghum could be attributed to high carbon to nitrogen ratio. This study also revealed that addition of cow manure as a substrate is a viable strategy for improving the process performance and methane production from sweet sorghum. Based on the results obtained from the experiments, a feasibility on use of sweet sorghum cultivated on sugarcane fallow for biogas production in a farm-scale biogas plant was evaluated. For this feasibility study, sweet sorghum cultivar SE-81 was used. The amount of SE-81 biomass harvested from 25 ha of fallow land was calculated to be 1,938 tFM/yr. To assess the feasibility of the proposed study, three different scenarios on the market opportunities for biogas uses were evaluated:

- Scenario 1: CHP—Biogas is used for electricity and heat generation in a combined heat and power (CHP) plant.
- Scenario 2: CHP+ BioRNG—Similar to Scenario 1 but the biogas is upgraded to renewable natural gas (BioRNG) for grid injection.
- Scenario 3: CHP + BioCNG—A portion of biogas is used for CHP to generate electricity and heat to meet the parasitic demand of the biogas plant and the remaining biogas is upgraded and compressed (BioCNG) for vehicle fuel or distribution via virtual pipeline (road transport).

Results showed that farm-scale biogas plant (1,300 m^3) when fed at 28.7 t/d of sweet sorghum and chicken manure can produce biogas production of 840.15 m^3/d across all scenarios. In Scenario 1, CHP system (84 kW generator) produced 2,520 GJ/a of electricity and 2,917 GJ/a of heat. After the meeting the parasitic energy demands, 523 MWh/a of electricity can be fed to grid. For Scenario 2, 784 GJ/a of electricity and 965 GJ/a of heat was generated. The exportable BioRNG was calculated to be 4,652 GJ/a. Whilst, Scenario 3 generated 927 GJ/a of electricity and 1,141 GJ/a of heat. The exportable BioCNG in Scenario 3 was estimated to be 4,283 GJ/a. The study thus show that Scenario 1 is the most feasible option for growing sweet sorghum on sugarcane fallow and using the produced biogas for heat and generation in a combined heat and power plant.

Keywords: Anaerobic Digestion; Biohydrogen; Biogas; Sweet sorghum; Biorefinery;

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AD	Anaerobic digestion
BMP	Biochemical methane Potential
CH4	Methane
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demands
CSTR	Continuous Stirred tank reactor
DAP	Days after planting
DAS	Days after sowing
DBY	Dry biomass yield
FIA	Flow injection Analysis
FM	Fresh matter
GC	Gas Chromatography
HRT	Hydraulic Retention Time
ICP	Inductive Coupled Plasma
ISR	Inoculum to substrate ratio
k	Hydrolysis Constant
OLR	Organic loading rate
SMY	Specific methane yield
TKN/P	Total Kjeldahl Nitrogen/Phosphorous
TVFA	Total Volatile Fatty Acids
VFA	Volatile Fatty acids

CHAPTER 1
INTRODUCTION

One of the current strategies to produce alternative energy id the use of biomass to produce biofuels and is considered as a viable option for to replace fossil fuels especially in farm scale applications [1]. Biofuels technologies includes the technologies to produce bioethanol, biodiesel, biogas and biohydrogen and use them for sustainable transport and/or energy generation use [2]. Based on the type of biomass feedstocks used, biofuels technologies are classified as first-, second- and third-generation biofuels. First generation (1G) biofuels are produced from the edible plant parts of the crops such as maize kernels, sugarcane juice, sugar beets etc. Second generation (2G) biofuels are produced from lignocellulosic or structural plant parts of crops and/or plants such as wheat straw, sugarcane bagasse, sugar beet tops. Third generation (3G) biofuels involve production of biofuels from cultivated microalgae.

First generation biofuels are shown to have the potential for large-scale production in terms of energy production and availability of the biomass resources. However, the key challenge in 1G biofuel technologies is its unsustainability practices due to competition over food for fuel and land usage for energy crop cultivation [3]. However, viable solutions to overcome these challenges include crop rotation strategies, biorefinery concepts and use of non-arable land for growth of energy crops [4]. Among the energy crops, maize, sugar beet and sweet sorghum has gained a considerable interest for various forms of biofuel production.

Sweet sorghum is a drought tolerant C4 annual grain crop with high biomass yields per hectare [5]. The crop can grow on marginal lands with low fertiliser and water requirements. Due to its rich sugar content, it was first introduced as a food crop for sugar production and subsequently recommended as a viable feedstock for biofuel production [6]. Sweet sorghum has been predominantly investigated for cellulosic ethanol and biogas production [7]. Although sweet sorghum is mainly cultivated for ethanol production through stalk juice extraction, anaerobic digestion (AD) of sweet sorghum is gaining equal consideration as an alternative to natural gas due its simple installation and lower costs of production [8].

Anaerobic digestion is a sustainable environmental technology that utilises organic wastes from agriculture, municipality and industries to produce biogas and nutrient-rich digestate. The produced biogas can be used as renewable energy source for generation of heat and electricity through cogeneration in a combined heat and power plant (CHP) and/or biogas is cleaned and upgraded to produce biomethane for injection in the natural gas grid (BioRNG) or compressed to produce compressed biomethane (BioCNG) for vehicle use. In addition, nutrient-rich digestate can be used as a biofertilizer and recycle the nutrients (nitrogen and phosphorus) for cultivation of sweet sorghum [9]. Use of sweet sorghum as feedstock for hydrogen, H_2 (20-40%), methane, CH_4 (50-60%) and carbon dioxide, CO_2 (20-40%) production through AD technology was successfully demonstrated [10]. Similarly, bioethanol produced from sweet sorghum can be blended with gasoline for transport use and/or as a solvent for industrial applications.

AD of sweet sorghum is highly sensitive to the process conditions such as temperature, pH, inoculum to substrate ratio (ISR), hydraulic retention time (HRT) and dry matter content [17]. Therefore, optimisation of AD process is required to improve process performance and methane yields. In this study, nine different sweet sorghum cultivars were grown during the fallow period of sugarcane cultivation in Northern Queensland. At first, the effect of harvest time on crop phenology, biomass yields and productivity along with chemical composition and methane yields was investigated. Later, the effect of ISR and inoculum source on methane yields was investigated in batch experiments. The effect of organic loading rates on biohydrogen and volatile fatty acids (VFAs) production through dark fermentative H_2 process was investigated in batch fed reactor at 55 °C. The effect of organic loading rate on process performance and methane yields during mono-digestion and co-digestion of sweet sorghum with cow manure was investigated in continuous stirred tank reactor (CSTR) at 37 °C. Finally, a farm-scale biogas plant was designed to evaluate the feasibility of using sweet sorghum grown on fallow land of 25 ha for biogas production. To assess the feasibility of the proposed study, three different scenarios were designed with an aim to evaluate the possible market opportunities for biogas uses.

- Scenario 1: CHP—Biogas is used for electricity and heat generation in a combined heat and power (CHP) plant.

- Scenario 2: CHP + BioCNG—A portion of biogas is used for CHP to generate electricity and heat to meet the parasitic demand of the biogas plant and the remaining biogas is upgraded and compressed (BioCNG) for vehicle fuel or distribution via virtual pipeline (road transport).
- Scenario 3: CHP+ BioRNG—Similar to Scenario 2 but the biogas is upgraded to renewable natural gas (BioRNG) for grid injection.

1.1 Aim and Objectives

The aim of this study is to assess the feasibility of biohydrogen, organic acids and methane production from sweet sorghum grown on fallow land of sugarcane cultivation. The main objectives of the study are as follows.

1. To study the effect of harvest time on chemical composition and methane yields of sweet sorghum cultivars.
2. To study the effect of inoculum to substrate ratio on the methane yields of sweet sorghum.
3. To study the effect of inoculum source on methane production rates and yields from sweet sorghum cultivars.
4. To study the effect of inoculum to substrate ratio on volatile fatty acid production rates and yields from sweet sorghum cultivars.
5. To study the effect of organic loading rate on process performance and biohydrogen yields during dark H_2 fermentation of sweet sorghum cultivars.
6. To compare the process performance and methane yields during anaerobic mono-digestion and co-digestion of sweet sorghum with cow manure.
7. To assess the feasibility of farm-scale biogas plant using sweet sorghum grown for biogas production and uses.

CHAPTER 2
LITERATURE REVIEW

Fossil fuels have a global demand for energy and chemical production across the world. However, considering the time span required for producibility of fossil fuels in comparison to the consumption poses a major threat to the energy sources available to the world. Some of the major challenges associated with use of these fuels is the amount of greenhouse gases (GHG) released during their consumption [18]. Over the years, researchers have approached this issue with several alternative pathways to produce energy resources through renewable sources. One of the predominant ways incorporated to overcome the energy demand is by production of biofuels. Biofuels are produced from plant based materials or biodegradable wastes using various microbial fermentation processes [19]. They are highly sustainable, easy to produce and most importantly an efficient replacement for petroleum-based products. Moreover, in several countries that are predominant producers of energy crops, they can help reduce the expenses for import of petroleum products. Biofuels can be classified into several categories based on the raw materials used: First, second and third generation fuels. First generation fuels rely directly on energy crops that are also used as food source [20]. The second-generation fuels are generally manufactured from food or agricultural wastes obtained as residue from agro and food industries. Considering the amount of sustainable feedstocks available in Australia, the potential to produce renewable energy is huge and untapped.

Globally, the potential for energy generation using major available feedstocks is 10,100 – 14000 TWh and includes heat, electricity or biomethane generation [21]. Most commonly used energy crops around the world are corn, sugarcane and wheat. According to the European Council for Bioenergy, if 7% of agricultural land was to be dedicated for energy crops for biofuel production, there would be a potential to produce about 3350 -5000 TWh of energy through AD [22]. European Union (EU) has also set standards for gross consumptions of bioenergy for each sector as follows; 21% for heating, 34% for electricity and 10% for transport fuel by the year 2020 [23]. The biogas and biomethane potential in Australia was about 35 million tons of oil equivalent in 2018 and there is still scope of improvement [24].

Australia possesses a large landmass spread across various states with arid and semi-arid climatic conditions [25]. Furthermore, power generation of Australia is about 3314 GWh of bioenergy of which about 6% is from renewable energy generation. [27] The applications of bioenergy resources are not limited to electricity production but can also be applied to transport fuels, direct heat, biochemicals bioplastics, biofertilizers, detergents, oils, etc. [28].

Currently, commercial production of biofuels using first generation techniques still have a limited contribution towards fuel economy due to the cost involved in pre-processing, energy recovery and food to fuel competition. However, reducing the costs and energy input by optimizing low-cost pre-treatment processes, complete exploitation of biomass and other waste residues produced during the processes can result in commercialization of energy crops [29]. Moreover, biogas from energy crops like Sweet sorghum can help thrive local and regional economies through agriculture and energy [30]. Selection of an appropriate energy crops is highly depending on the biomass yields, bioenergy potential and low production costs at a particular region [30]. In a study conducted in Greece, seven different energy crops were used to evaluate the biogas production considering the climatic and economic aspects. These results illustrated that use of drought resistant crops that have high biomass yields were more favourable for dry climatic conditions [31].

2.1 Anaerobic Digestion

Anaerobic digestion (AD) refers to degradation of organic substrates by several microorganisms in the absence of oxygen to form biogas (methane, carbon dioxide and trace gases such as H_2S, NH3, etc)[32]. The process can be categorized into four steps: Hydrolysis, acidogenesis, acetogenesis and methanogenesis [34].

2.1.1 Hydrolysis

The hydrolysis of complex organic matter (carbohydrates, proteins and lipids) is carried out by extracellular enzymes that are excreted by different bacteria. These hydrolysed molecules include soluble sugars, amino acids, long chain fatty acids (LCFA) and alcohols. . For instance, the lipids, proteins and carbohydrates react with enzymes such lipases, cellulases, proteases released by the microorganisms and get reduced to long chain fatty acids, reducing sugars and amino acids, respectively.. The standard pH for AD process using lignocellulosic crops is 6.5 -7.5. During the hydrolysis step if the accumulation of VFAs is large, the pH decreases, thereby inhibiting the AD process. Similarly, higher accumulation of ammonia leads to increase of pH above 7.9. Carbon to nitrogen (C/N) ratio is another major factor in AD process, which is generally maintained at 25 – 30 [35],[36]. Higher amount of nitrogen leads to higher accumulation of ammonia (NH_3) which would directly impact on lowering methane production.[37]. Therefore, hydrolysis is considered as the rate limiting step of the whole anaerobic digestion [38].

2.1.2 Acidogenesis

During the acidogenesis step, the products of hydrolysis are converted into short chain VFAs (acetic acid, propionic acid, butyric acid, valeric acid and caproic acid), alcohols, NH_3, water, carbon dioxide (CO_2), etc [39]. Sometimes, excessive amount of substrate can lead to overaccumulation of VFAs thereby reducing the pH of the system [36]. When the pH of the system gets extremely low, the methanogenesis process cannot proceed as the growth of methanogens is inhibited at low pH. Therefore, it is essential to provide optimum ratio of inoculum to substrate (ISR) to avoid overaccumulation of intermediate compounds e.g., VFAs and NH_3 leading to fluctuation in pH.

2.1.3 Acetogenesis

During acetogenesis the short chain fatty acids such as acetic acid, propionic acid and butryric acid converted into alcohols and acetate. During this phase to pathways of acetate production occur. The first phase the homoacetogenic bacteria reduce the hydrogen and water into acetate. However, these bacteria cease to convert acetate if the hydrogen partial pressure is higher. During this phase the hydrogenotrophic bacteria start to overtake the acetate production and gradually decreasing the hydrogen partial pressure..[40].

2.1.4 Methanogenesis

Methanogenesis is the process by which acetic acid, carbon dioxide, and hydrogen are converted into methane and carbon dioxide [41]. In general, methane concentrations in lignocellulosic substrates range from 55-60%, while CO_2 concentrations range from 25-30%. Because microorganisms are sensitive to extremely high or low pH ranges, the pH must be 6.5 -7 during this process [42]. High pH range is resulted as an accumulation of ammonia and nitrate compounds in the system. A brief overview of the AD is demonstrated in Figure 1.

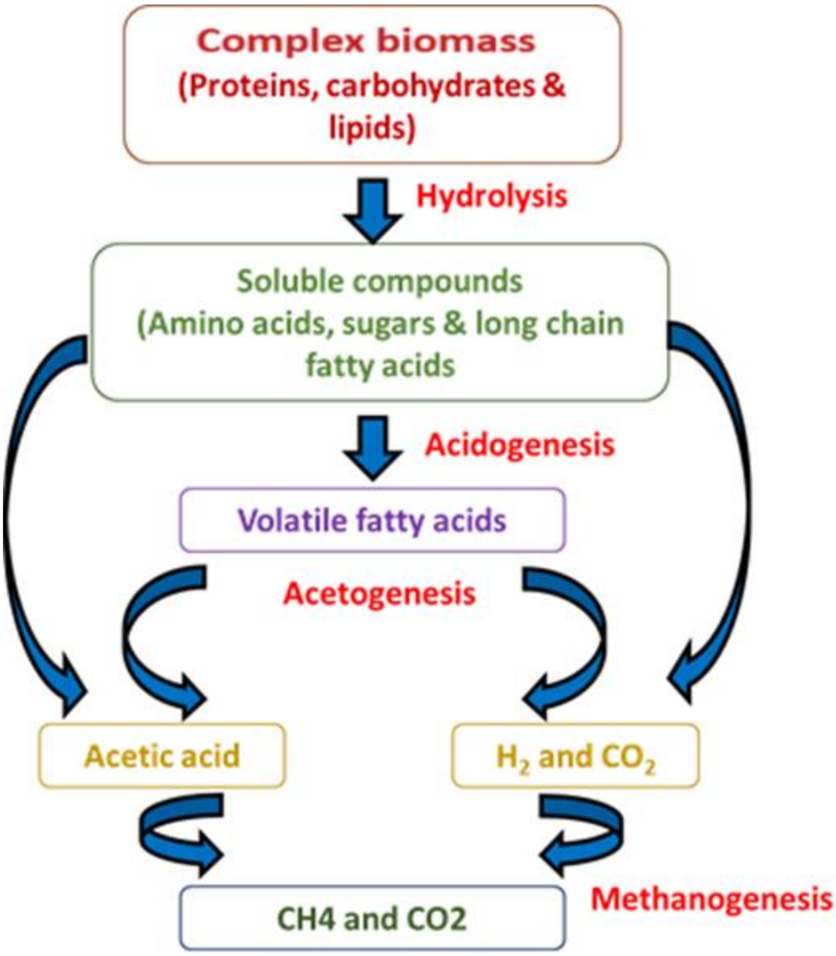

Figure 1. Overview of Anaerobic digestion process

2.2 Factors effecting Anaerobic Digestion.

As a source of renewable energy, the AD process has several advantages. The energy consumed during this process is compensated by methane recovery from the AD process and can be used as fuel or electricity. To improve crop recovery, anaerobic sludge can be used as a biofertilizer[43]. The operational and construction costs are significantly lower than those of any other conventional treatment. For instance , The initial infrastructure costs of biogas plant is much cheaper than installation costs of other renewable energy source such as solar panels, dams for hydroelectricity, etc. Because it is a biological process, the retention time is typically long, and the process is highly sensitive to changes in process parameters, resulting in low methane yields. The following are some of the major factors that interfere with the AD process.

It is necessary for all microorganisms to coordinate in order to prevent excessive accumulation of intermediate products for a stable AD process[44]. A stable anaerobic digestion under mesophilic conditions requires a pH range of 6.5 to 7.5 [45]. A study conducted by Cioabla and Ionel [46] on wheat bran for production of biogas demonstrated that highest biogas yields of 68%/32% of CH_4/CO_2 mixtures were obtained at pH ranges 6.9-7.1. Lower methane yields may be the result of any process that takes place outside of this range. Similarly, excessive levels of VFAs, ammonia, and trace elements like Mg, Fe, Pb etc. have a significant impact on the methanogenesis process[47].

2.2.1 Effect of chemical composition of the substrate on methane production

Understanding the mechanisms of anaerobic digestion and determining the process parameters required for maximum efficiency involves knowing of the chemical composition of the substrates. Prior to conducting biogas experiments on lignocellulosic substrates, the following factors must be considered: biomass yields, carbohydrates and lignin content, C/N ratio, total nitrogen and phosphorus content, total solids (TS), and volatile solids (VS). Crop biomass yields refer to the amount of plant biomass produced per hectare of agricultural land. Chemical composition of crop substrates refers to presence of carbohydrates and lignin for a particular substrate. For developing an optimal biogas process, it is essential to choose a substrate that has higher cellulosic fraction and lower lignin [48]. However, determination of lignin composition can also help us to elucidate and develop an optimal pre-treatment process [49]. Most cases, the higher carbohydrate composition leads to higher biomethane production. Dry matter content of the substrates, also termed as TS, directly influences the hydrolysis process [50]. During the AD process, the VS degrade into short chain fatty acids, reducing sugars, and amino acids. Higher substrate VS leads to greater accumulation of VFAs, which may result in a decrease in system pH. Similarly, excessive nitrogen concentration results in increased ammonia production, which increases the pH to an alkaline state. Nonetheless, if the same process must be modified for VFAs production and H_2 production, it is necessary to maintain a higher substrate VS concentration for maximum VFAs production [51]. As a result, VS concentration is an important parameter in determining the best ISR ratio for batch studies and OLR for reactor studies.

2.2.2 Effect of ISR on methane yields

Inoculum to substrate (ISR) ratio plays a crucial role in the biochemical methane potential (BMP) testing of any substrate for batch scale applications [52]. Lower ISR ratios result in higher accumulation of VFAs. VFA accumulation tends to lower the pH of the batch digestion system [53]. Reduction in pH inhibits the methanogenic microorganisms to produce methane. In addition, low pH results in a longer lag phase for AD process. A study conducted on AD of swine wastewater, demonstrated that higher inoculum concentration promoted higher methane concentration in biogas production [54]. However, at extremely high inoculum concentrations, the methane production rapidly increases for first few days. All the substrates are consumed by the microorganisms resulting in gradual decrease in methane production.

Several studies on studying the effect of Inoculum to substrate ratios have stated that ISR 2- ISR 4 have the capability to produce maximum methane concentration. A particular study on co-digestion of slaughterhouse wastes with sewage sludge demonstrated that ISR 4 was the optimum ratio since it produced maximum biogas yields. Also, there was no significant change in pH of the system which indicated a stable anaerobic digestion process when compared with other ISR [55]. Another factor for choosing an optimum ISR is by estimating the TS % required for each substrate. Increasing the TS of substrate can lead to increased acidification and ammonia inhibition [56]. Therefore, it is always important to select an ISR that maintains an optimum TS% and controlled VFA production.

2.2.3 Effect of inoculum source on methane yields

Anaerobic digestion of any substrate also depends on the microbial consortium present in the inoculum source provided. The source of inoculum provided to the substrates irrespective of the crop variety has a huge impact on the anaerobic digestion. For most studies, anaerobic sludge obtained from domestic wastewater treatment plants have been used [57]. The major reason for this is, it is abundant, renewable, and already has a rich microbial consortium of fermenting organisms that can be directly used for anaerobic fermentation processes. In addition, it contains an optimum mixture of microorganisms that can perform acidogenesis, acetogenesis, hydrolysis and methanogenesis. Anaerobic sludge used as inoculum is obtained from wastewater treatment plants and have a diverse consortium of microbes that are responsible for degradation of organic substrates into methane carbon dioxide and other trace gases. These microbes are already acclimatized to mesophilic conditions and can be used in biogas plants. Ina addition they save the extra added expenses needed to cultivate pure microbial strains in laboratories for the AD experiments.

However, over the recent years, some researchers have also explored the feasibility of cattle manure and synthetic microorganisms for enhancing biochemical methane yields for energy crops [58]. Studies on cattle manure for lignocellulosic crop have demonstrated that it can reduce the hydrolysis time during an AD process [59]. This phenomenon is due to the presence of lignocellulytic enzymes in the rumen fluid that can easily digest the complex lignocellulosic matrix. In addition, The hydrolysed substrate is further digested by E.coli and certain heterotrophic fungi that act on the VFA and convert them into methane and carbon dioxide [60]. Although Cattle manure is also produced in large extent and is renewable, yet its potential is still untapped. Cattle manure has demonstrated several environmental impacts as it tends to release methane into the atmosphere. Therefore, utilization of cattle manure as an inoculum source for production of various biofuels and value added chemicals can help decrease these emissions while utilizing it to produce biogas via a closed anaerobic digestion process [61]in addition, the cattle manure can be stored for longer durations without depletion of microbial communities [62]. There is also tremendous scope for research on cattle manure for producing enzymes and biofuels such as hydrogen [63].

Rumen fluid is yet another rich source of inoculum obtained from ruminants that feed on highly fibrous and cellulolytic materials. The major function of rumen fluid is to ferment plant polymers to short chain fatty acids, carbon dioxide and hydrogen [64].The further decomposition of the cellulosic compounds occurs in the gut of the ruminants. However, majority of the methane is produced in the rumen and is released through belching. There have been several studies on anaerobic digestion of cattle manure using rumen fluid as inoculum and these studies have said to have improves the methane yields by 2-3 times when rumen fluid was used. A comparative study conducted by Budiyono and Widiasa [65] stated that rumen fluid improved the biogas production efficiency two- three times more than the manure substrate without the rumen fluid. In another study conducted by Xing and Han [66], an artificial rumen fluid system was established by modifying a continuously stirred tank reactor (CSTR) that operated 33 -100 days and fed each day with an organic loading rate

(OLR) 2 gVS/l/d. This method demonstrated secretion of fairly high levels of extracellular multienzymes, as determined by enzyme content and gel filtration chromatography analysis. When this rumen fluid was tested with mixture of corn straw and food wastes, a high biochemical methane potential and biogas production was obtained. Hence, the type of inoculum source used for anaerobic digestion plays a substantial role in the biogas yield [67].

There has been very limited research stating the effects of inoculum source used for lignocellulosic energy crops. Most of the anaerobic digestion processes are conducted using anaerobic sludge and cattle manure primarily due to their rich microbial consortium and their abundant reservoirs [68]. A relative study of cattle manure vs rumen fluid, established that rumen fluid increased the biogas yield of cattle manure to 25-50% than just the cattle manure [65]. Therefore, a comprehensive study on the potential of rumen fluid in anaerobic digestion can help in developing an artificial rumen reactor system to improve biogas production on a large scale [69]. Aside from the microbial community and the origin of inoculum, the chemical composition of inoculum also plays a crucial role in anaerobic digestion. For instance, during the anaerobic digestion process excessive amount of protein rich substates tend to produce ammonia leading to increase in ph to basic. During this the methanogenic archaea shift their focus towards using the ammonia instead of converting carbon into methane and carbon dioxide resulting in lower methane yields.. The process inhibition by ammonia can occur due to change in pH and acclimatization of the microbes [70]. Therefore, choice an appropriate inoculum also requires adjusting the process conditions such as C/N ratios, pH adjustment and substrate loading.

2.2.4 Effect of harvest time on methane yields from energy crops

Harvest time and crop varieties are additional physiological factors that play a major role in biochemical methane yield of lignocellulosic crops. In a study conducted by Boob and Elsaesser [71], the time of harvest of hay meadows played a major influence in its final biogas yield. The crops that were harvested at a later growth stage showed less degradation in the biomass quality. Likewise, a study on Sweet sorghum stated that harvesting it at growing stages also resulted in diminished cellulose content [72]. To achieve maximum biogas yield, most crops are harvested in the late flowering stage due to their rich carbohydrate composition and dry Fiber content. The feedstock harvested at earlier stage of maturity is more suitable for cattle feed or ensilage process [73]. Early-stage harvest would lead to loss of biodiversity of the crop over a long term. Several studies on harvest time have been performed on miscanthus crop across the globe. Miscanthus is a perennial crop that can be grown with low inputs and can grow even in unfavourable conditions. A study conducted by Da Borso and Di Marzo [74] proved that late harvest of miscanthus provided a higher methane yield proving that dry matter of the crop played a major role in biogas production. Contrarily, Sweet sorghum tends to have a faster cellulose degradation rate at various growth stages and the highest cellulose content is observed at flowering stage [75].

2.2.5 Carbon nitrogen ratio

To obtain a high rate of organic material decomposition and hence maximise the potential of bioprocesses, the C/N ratio should be continuously adjusted and monitored. The substrates' nutritional makeup and the C/N ratio are connected. Co-digestion of animal manure which has a low C/N ratio, and carbon-rich substrates, such as crop silage, is an illustration of a balanced C/N ratio [76]. Unfavourable effects on the rate of methane production and the activity of microbial culture are associated with above- or below-optimal C/N ratios. The range between 20 and 30 is the AD's ideal C/N ratio. In addition to the C/N ratio, phosphorus and sulphur are also crucial for the AD-related bacteria to grow and function at their full potential.

2.3 Applications of anaerobic digestion

Although anaerobic digestion process was primarily focused on production of biogas and biofertilizers, it can now be optimized to produce several intermediate products such as biohydrogen, volatile fatty acids and bioethanol. However in this study he primary focus is on production of biogas and biofertilisers for developing a biogas refinery to produce electricity, fuel and fertlizers.2.3.1 Biohydrogen

At present, the prominent methods to produce hydrogen involves fossil fuels energy, pyrolysis, or water electrolysis. However, these methods consume a lot of energy and prove detrimental to the environment by releasing pollutants. Nonetheless, biohydrogen production is an interesting strategy as it is renewable, it's ease of production and low environment toxicity [77]. Due to its clean, renewable nature and high energy content (122 kJ/g), hydrogen is a potential replacement for conventional fossil fuels [78]. It is regarded as a secondary form of energy and can be obtained from natural and biological resources. H_2 fuel has a 2.75-times higher energy yield than hydrocarbon fuels [79]. Water is the main combustion by-product when hydrogen is utilised, and it may be recycled to produce additional hydrogen. Hydrogen is a clean fuel that does not emit CO_2 or other harmful pollutants. It has become more well-known as a green energy policy issue because to its use in fuel cell electricity generation, transportation fuel, and combustion. In addition, H_2 gas is used for steel processing, desulfurization, and re-formulation of gasoline in refineries, and the hydrogenation of fats and oils to produce a variety of chemicals in the petrochemical industry. It can be produced in two different methods: photo fermentation and dark fermentation.

Dark fermentation method for biohydrogen is the most promising method in comparison to the photo fermentation due to its simple infrastructure, less maintenance and energy input [80]. Hydrogen production occurs in the first two steps during anaerobic digestion process i.e., hydrolysis and acidogenesis. During acidogenesis the reduced monomers such as soluble sugars, amino acids and fatty acids are converted into volatile fatty acids during which hydrogen is liberated [81]. Acetate and butyrate are major by-products during biohydrogen production. These chemicals have industrial applications as food additives, pharmaceuticals, and solvents. Microorganisms such as *clostridium, Enterobacter* and *bacillus species* are the main producers of hydrogen during anaerobic digestion. These species can either be used as pure cultures or mixed to form cocultures to improve hydrogen production [82]. Generally it is challenging to isolate pure culture, therefore most studies are focused towards seeding the anaerobic digestors with mixed flora obtained as anaerobic sludge or dairy wastes [83]. Although the microbial communities in the inoculum cannot be controlled, it is possible to adjust the process parameters based on the type of product that needs to be produced. Theoretical calculation for biohydrogen production states that for every mole of glucose, 4 moles of hydrogen are liberated [84]. However, the experimental hydrogen yields are much lower owing to the presence of inhibitory substances such as lactic acid, propionic acids etc., [85].

The production of hydrogen using dark fermentation process is limited to pH 5.5 in mixed cultured inoculum. However hydrogen production largely depends on the pH, temperature and the type of substrates [86]. The presence of toxic compounds such as furfurals can impede the process [87]. In addition, dark fermentation is also largely impacted by low substrate conversion, low yields, and low production rate. Unlike anaerobic digestion, dark fermentation for higher yield of hydrogen production also may require an additional pre-treatment strategy [88]. Currently, the lower production rate and hydrogen yield during microbial fermentation are two major issues for commercial production of hydrogen [89]. To mitigate these issues, researchers are trying to develop integrated production strategies through several biomass feedstocks for optimizing hydrogen yields.

Improving hydrogen yields can be achieved in several ways: choosing appropriate microbial strains, developing a sophisticated bioreactor design and improving the process conditions [90]. *Clostridium thermocellulm* is currently the most researched microbial species for biohydrogen production due to its tolerance at high temperature and capability to produce several enzymes that can easily degrade complex lignocellulosic materials [91].

Although hydrogen production using dark fermentation is sustainable and environmentally friendly, it is essential to develop an optimized reactor process for large scale hydrogen production with significant reduction in production costs. The major cost factor for biohydrogen production lies in the availability and pre-processing of the raw materials. While energy crops have an excellent potential for hydrogen production, it is also necessary to look out for the food to fuel competition for these crops [3]. One way to enhance biohydrogen production from energy crops is by developing an integrated biorefinery system incorporating hydrogen production alongside other valuable products such as biogas, bioethanol, and other value-added chemicals [92]. Through this approach the cost involved with pre-processing and production of these fuels are balanced by the supply and demand for these products across the globe. One of the researchers also stated that application of dark fermentation technologies for biohydrogen production also helps reduce the infrastructure costs due to reduction of size of bioreactors involved [84].

2.3.2 Volatile fatty acids

Volatile fatty acids (VFA) are intermediate products formed during anaerobic digestion in which long chain fatty acids, carbohydrates and amino acids are transformed to volatile fatty acids by acidogenic and acetogenic bacteria. Currently the larger percent of VFAs utilized for production of value-added chemicals are supplied by petrochemical derivatives [93]. Biobased production of VFAs through fermentation and digestion processes are not popular due to their extremely low productivity and limited knowledge on effective substrates

[93]. However, due to the current demand for alternative pathways to reduce dependence on fossil fuel derivatives, fermentation and other microbial extraction processes are gaining importance.

Although biobased production of VFAs is still in the rudimentary phase of production, large scale production of VFAs has several advantages over petrochemical resources. Some of the advantages include low environmental effects, low production costs, abundance of raw materials and enables good waste management practices. But to achieve the commercialization of VFA production using biorefinery approach it is essential to develop a robust experimental setup and an optimized process conditions for improving productivity. Over the past few years, researchers have developed and performed several experiments for optimization of VFA production using various organic substrates such as wastewater, agricultural residues and energy crops.

The concept of VFA production through anaerobic digestion is simulated from digestion of lignocellulytic substance in ruminants. Methanogens present in their rumen fluids tend to convert VFAs into methane causing belching and bloating in ruminants [94]. Similar to this system, to enhance VFA production, it is necessary to either reduce or completely eliminate the methanogenic microorganisms present in the inoculum source [95] Its been demonstrated that after the components produced during the acetogenesis step during AD that are hydrogen and carbon dioxide are converted to methane and carbon dioxide by the methanogens. However, since we are looking to improve hydrogen yields its necessary to cease the methanogenesis step by the elimination of methanogens in the inoculum. Methanogens cannot survive higher temperature and acidic conditions..[96]. Therefore, to reduce the methanogenic process, the inoculum must be heat treated prior to VFA production. Heat treatment or extreme acidic conditions serves to destroy the methanogenic bacteria. In addition, VFA production can also be enhanced by overwhelming the system with substrates, maintaining the pH at a constant 5.5 – 6 or reducing the hydraulic retention time [95].

Volatile fatty acids can be used to produce wide range commodities such as pharmaceuticals, food additives, bioplastics, cosmetics, etc. VFA production can be achieved by several different methods depending on the type of lignocellulosic substrates and the inoculum source provided. Interrupting the methanogenesis process by manipulating process parameters such as pH., ISR ratio and temperature adjustment is the key to produce higher amount of volatile fatty acids [97]. Temperature plays a dominant role in VFA production as it interferes with enzymatic activity of microorganism. Studies have demonstrated that higher temperature ranges up to 65 ºC resulted in higher yields of VFAs with acetic acid as the major fermentation product. However, further increase of temperature led to gradual decrease of VFA yields. This is possible since the microorganism cannot resist extreme high temperatures. Therefore, determination of an optimum temperature range for VFA production is necessary especially when mixed source of microflora is used. The major volatile acids produced during anaerobic digestion are acetic acid, propionic acid, butyric acid.

Table 1 illustrates some examples of waste biomass used for hydrogen and volatile fatty acid production at various operating conditions, most lignocellulosic biomass such as duckweed, sweet sorghum stalks, sweet sorghum leaves have acetic acid as the major byproduct during dark fermentation usually in the range of 1- 3 g/L. The maximum hydrogen of 2.6 mol/mol glucose was observed for sweet sorghum bagasse followed by 2.3mol/mol glucose. The corresponding acetic acid yield was 2 g/L. Most of the batch studies were conducted at mesophilic temperature ranging between 35 – 37 º C. however it is also important to note that the higher yields in sweet sorghum bagasse were obtained due to pretreatment with an alkali at elevated temperature.

Table 1. Coproduction of hydrogen and volatile fatty acid for various organic substrates

Biomass used	Operational conditions	H_2 Yields	VFA Production	Reference
Depackaging wastes	2.5 L bioreactor at 35°C, stirring rate 200 rpm, pH regulated at 7	Cum H_2 volume – 5342 L	1577 g/L Acetic acid – 14.65%, Propionic acid-70.70% & Butyric acid – 14.65%	Noblecourt and Christophe [98]
Cane molasses & Groundnut deoiled cake	10,000 L Batch reactor operated at 37°C with automatic pH Control	Cumulative H_2 (mL L−1) 3370 ± 20 Ml/L	Acetic acid – 982 ± 24 mg/L, Butyric acid -1612 ± 20 mg/L	Balachandar and Varanasi [99]
Cashew Apple Bagasse	Pretreatment with alkaline H_2O_2 Batch studies with 125 mL serum bottles at 38°C (24h)	12.57 (mLH_2/L h)	Acetic acid – 220 ± 3 mg/L, Butyric acid – 119 ± 3 mg/L	Silva and Mendes [100]
Cheese whey permeate	Semi CSTR with sugar (25 g/L), temperature at 30°C, pH at 5.5	365.67 mmol H_2/L Day	Acetic acid – 2.5 g/L, Propionic acid – 2.2 g/L.	Romão and Silva [101]
Duckweed	Batch anaerobic studies in 125 mL bottles at 35°C with 72h incubation time, pH 7.0	Max H_2 production – 162.4 mL /gdw	Acetate -1.04 ± 0.05 g/L, Butyrate- 1.52 ± 0.09 g/L	Mu and Liu [102]
Ground wheat residue	Batch assays at 37°C	63 ml H_2 /g starch	Total VFA- 35-40g/l	Ozmihci [103]
Sugarcane vinasse	Batch fermentation assays at 37 °C	4.75 mmol H_2/ gCOD	44,01 mg g−1COD	Magrini and de Almeida [104]
Sweet sorghum Stalks	Batch fermentation at 55°C, 12-48 h,	5.1 mmol/g-substrate	acetic acid (1.27 g/L) and butyric acid (1.05 g/L)	Islam and Zhang [105]

Table 1. Coproduction of hydrogen and volatile fatty acid for various organic substrates (contd)

Biomass used	Operational conditions	H_2 Yields	VFA Production	Reference
Sucrose	Batch studies	2.3 mol H_2/mol sucrose Added	Acetic acid – 294 ± 15mM, Propionic acid -387 ± 17 mM , Butyric acid – 28 ± 3 Mm	Tunçay and Erguder [106]
Jatropha pruning	CSTR operated at 37ºC	14.5 ± 0.8 (mL H_2/L-h)	Acetic acid – 4.3 ± 0.5 g/L; Butyric acid – 3.6 ± 0.2 g/L	Serrato-Nerio and Díaz-Hinojosa [87]
Macrocystis pyrifera biomass residues	Batch assays at 37ºC	11.38 mL/g (volatile solids, VS),	0.055 g/g (VS)	Zhao and Fan [107]
Sweet sorghum Bagasse	Pretreatment with NaOH at S: L - 1:15, 70°C and 4 h followed by batch scale fermentation at pH 6.8	2.6 mol/mol C6 sugar	Acetic acid – 2.3 g/L	Panagiotopoulos and Bakker [108]
Sweet sorghum leaves	Acid pretreatment 5.95% HCl and heat application followed by bench scale bioreactor 37.5°C, 250 rpm and 84 h	213.14 ml H_2/g FS.	-	Rorke and Gueguim Kana [109]
Food waste (FW)	acidogenic bioreactor (total/working volume 10/8 m^3; L/D ratio 2)	54288 L (155.10 LH_2/kg COD)	25.77 g L^{-1} of VFA composed of acetic (H_{Ac}: 15.2 ± 1.98 g L^{-1}), propionic (H_{Pr}: 4.89 ± 1.26 g L^{-1}) and butyric (H_{Bu} 5.67 ± 0.96 g L^{-1})	Sarkar and Katakojwala [110]

2.3.3 Codigestion

Until recently, the knowledge on anaerobic digestion was only focused towards the optimization of process parameters for the monodigestion of substrates. Mono digestion of substrates present some major challenges during AD such as high carbon concentrations leading to overaccumulation of VFAs, slower decomposition and longer time for biogas production [111]. However, due to further developments in the research co-digestion studies have substrates with contrasting characteristics have been recognized while designing a full-scale biogas plant. Unlike pretreatment of substrates, co-digestion of a carbon rich substrate with nitrogen-rich substrates has said to improve methane productivity in the long run [112, 113]. It's also beneficial, especially in farm scale sectors where there is a lot of livestock manure produced in addition to agricultural wastes from the harvesting of crops [114]. Codigestion of substrates improves the nutrient availability for microbes during AD process [115]. Many articles have proved that the carbon-nitrogen balance is a crucial factor in determining the methane yields for a continuously operating reactor [116, 117]. When lignocellulosic substrates are used as the primary substrate, the most suitable carbon nitrogen ratio falls in the range of 20-40 [118, 119]. However, choosing a suitable substrate for codigestion also depends on its availability. Most studies on codigestion across the world have used animal manure as the codigestion substrate as they are abundantly available round the year. Besides these wastes are responsible for greenhouse gas emissions if otherwise left out in the open. While utilization of animal manure alone also can produce biogas, it can inhibit methanogenic archaea by releasing excess ammonia leading to system failure after few runs [120, 121]. While there are several methods to manage livestock manure in farms, biogas recovery through anaerobic digestion can have several benefits to farmers ranging from energy recovery, production of mineral rich digestate or production of compost for crops [122, 123] Some examples of codigestion of agricultural residues/energy crops with animal manure are presented in Table 2. It is noticed that carbon nitrogen ratio for most codigestion experiments lie within the range of 20-30 after addition of nitrogen rich substrates. While most lignocellulosic crops/biomass have methane yields of 200-400 $NL/kgVS_{added}$ after addition of codigestion substrates the methane yields have increased by 20-30% of the yields.it is also noticed that the carbon rich substrates comprised of 60-75% of the feed mixture as compared to nitrogen rich substrates. Excessive amount of protein rich substrates may lead to accumulation of ammonia in the reactor leading to slow depreciation of methane yields.

.

Table 2. Codigestion of agricultural residues with animal manure

Substrates	Operating conditions	C/N	Methane yields	Reference
Cattle manure, chicken manure with maize silage	CSTR; 1:1:1 on VS basis	-	6580 m^3/day	Yangin-Gomec and Ozturk [124]
Rabbit manure (12.3% w/w), sorghum crops(1.3% w/w) and water (86.4% w/w)	CSTR	15.1	134 ml CH_4/gVS	Adrover and Cotabarren [125]
Corn stover and Chicken manure	CSTR; 4 gVS/L/d	20	223 ± 7 mL/gVSadded	Li and Zhang [126]
Sorghum biomass (95%) and Liquid cow manure (5%)	CSTR; 1.49-1.86 kgVS/m^3·d;	-	295.3 mL/gVS_{added}	Dareioti and Tsigkou [127]
sorghum stem and cow manure	Batch; ISR 1	25	478 mL/gVS_{added}	Zhang and Zhang [128]
sugarcane bagasse (30) and fruit/vegetable waste (70)	Batch		2.7 L	Vats and Khan [129]
Sugarcane bagasse (35%) and poultry waste (65%)	Batch; ISR 1	23.52	9693 mL	Vats and Khan [129]
Sugarcane bagasse (75%) and mill mud (25%)	Batch	24.7	22.2 mL/gVS	Talha and Ding [130]
Sugarcane bagasse (80.684%) and cow manure (4.295%)	CSTR; 3.45 kgVS/m^3 ·day		69 ml/gVS	Darwin and Fazil [131]

2.3.4 Biogas Use

Biogas collected from crop digestion can be burnt in a gas boiler for heating or in a combined heat and power (CHP) turbine to generate heat and electricity[132]. Approximately two-thirds of the energy contained in biogas is converted into heat in CHP units; therefore, year-round heat consumption must be guaranteed [133]. This is not always possible, as some rural communities may not require heating in the summer. Utilisation and sale of thermal energy generated by a biogas cogeneration facility are fundamental to its business model [134].

Upgrading biogas to natural gas quality (pure methane, termed biomethane) enables more efficient use of biogas's energy content. Biomethane can be utilised as a transport fuel or injected into the natural gas infrastructure for off-site use.

2.3.5 Digestate Management

The digestate resulting from AD of agricultural waste may be rich in ammonium and other nutrients that are important for plant growth and therefore could be used as organic fertiliser [135, 136]. Further, substrate type and composition, nutrient concentration and form (mainly N, P and K) in the digestate and nutrient requirements of the crop determine whether the digestate/liquid can be used as fertiliser. Furthermore, co-digestion with other suitable feedstocks can improve the digestate nutrient composition by diluting toxic compounds and at the same time improve the economics of the biogas plants by operating at higher organic load, better buffering condition favouring microbial synergy and growth, and improving biogas production [137, 138]. For instance, anaerobic co-digestion with molasses was shown to improve nutrient balance and methane yields of cattle slurry, chicken manure, and activated sludge [139-141]. Similarly, green grass and yard waste were shown to ideal feedstocks for fertiliser and soil amendment due to their high N, P and K content [142, 143]. Furthermore, digestates produced by AD of clean feedstocks such as yard and agricultural waste do not pose any health risk associated with pathogenic bacteria that are noticed in untreated digested residue of animal waste. For instance, digestate from mesophilic anaerobic digestion of Sudan grass contained lower concentrations of N, P and K nutrients and heavy metals than those prescribed by the European Union [142]. Mostafazadeh-Fard *et al.* [143] demonstrated the feasibility of liquid organic fertiliser production through hydrolysis and acidification without production of biogas in a leachbed reactor. In the above study, fresh grass clippings containing a mixture of creeping bentgrass (*Agrostis stolonifera* “Penncross” and other cultivars), common bermudagrass (*Cyndon dactylon*), hybrid bermudagrass (*Cyndon dactylon* and *C. transvaalensis*), rough bluegrass (*Poatrivialis*) and annual bluegrass (*Poa annua*), were used to recover N–P–K and other micronutrients. Results showed that use of higher organic load produced much higher concentrations of TN–TP–K nutrients in the leachate. The TN, TP, and K concentrations in the leachate were as high as 10,800, 2315 and 7400 mg/L, respectively, and N–P–K ratios of 1.08–0.23–0.74. Finally, application of plant-based liquid fertiliser produced from AD of grass waste for the cultivation of chilli pepper in greenhouses showed to be effective nitrogen source with comparable or better yields than the inorganic nitrogen source (i.e., chemical fertiliser at an equivalent nitrogen application rate) for the chilli pepper crop in soils with low, moderate and high salinity levels [144], [145].

The most common method for managing digestate is to usedas fertiliser without any prior processing. Its physical and chemical properties significantly support its agricultural application. Low dry mass, a relatively high organic matter content, a slightly acidic pH, and an abundance of nitrogen, phosphorus, and potassium are just some of the parameters that support the use of the product of anaerobic digestion as a fertiliser. Before using the digestate, however, the presence of heavy metals and pathogens must be confirmed. Exceeding permissible standards could render the digestate unusable for fertilisation. Managing biodegradable waste and manure in this manner can, among other things, increase energy production from RES, reduce the amount of waste that decomposes uncontrollably, lower the carbon footprint, and introduce environmentally beneficial fertilisers to the soil [146].

Nutrient recovery from digestate is necessary to produce an end product with a high nutrient concentration that is nearly as effective as an organic fertiliser using a cost-effective separation method.

The physiochemical properties of the substrates utilised in anaerobic digestion have a substantial impact on the digestate properties. In addition, process parameters such as moisture content, hydraulic retention time, and pH play a role in altering the characteristics of the digestate.

Total solids

During the anaerobic process, the solids are consumed by the microbe and converted into volatile fatty acids and biogas, resulting in a reduction of the solids content by up to 60% of the initial material for lignocellulosic plant materials [147]. Nevertheless, the change in TS is dependent on the substrates employed. For example, energy crops or woody material have greater dry matter due to the presence of lignin and other non-degradable substrates. In contrast, substrates such as food wastes, distillery wastes, and slaughterhouse wastes, which typically contain a greater amount of fats, lipids are degraded up to 70% and result in a lower dry matter content in the digestate [148]. .

In recent years, the number of biogas plants and/or the amount of raw digestate produced per day has increased globally, leading to overproduction of raw digestate for local use. Moreover, local and regional transportation of raw digestate of more than 5–10 km has shown to exceed the costs of its fertiliser value [149] and consumes large amounts of fossil fuel [150]. Therefore, solid-liquid separation of raw digestate has been recommended to reduce the cost of digestate transportation for use [151].

Solid-liquid separation of digestate is generally carried out by using either sieves, double circle bow sieve, sieve belt press, sieve drum press, press screw/auger separator, sieve centrifuge or decanting centrifuge (see e.g. [152]). the most common solid–liquid separation technology used in full-scale biogas plants are screw press, screening drum press (vibrating screen) and centrifuge [153]. Use of precipitating agents such as aluminium sulphate (Al_2SO_4), ferric chloride ($FeCl_3$), ferric sulphate ($Fe_2(SO_4)_3$) and lime ($Ca(OH)_2$), flocculants or organic polymers (acrylamide) and functionalised chitosan have been shown to improve and/or facilitate solid-liquid separation [154-156].

The solid fraction of digestate is generally used as nutrient source directly for crop cultivation and soil conditioner [150] or after composting, as organic fertiliser [157]. In many biogas plants, the solid fraction of digestate is dried either using belt dryer, drum dryer, feed-and-turn dryer, fluidised bed dryer or solar drying system and even palletised to be sold as bio-fertilisers [155] or solid fuel [149]. Owing to the high organic fraction as well as high nutrient contents, new technologies have been proposed for solid digestate valorisation [158], such as production of biofuel in domestic furnaces [159], production of biochar [160, 161], post treatments (thermal, alkaline and enzymatic) for methane recovery [162, 163] or bioethanol production .

2.3.6 Methods for digestate separation

Some well-known digestate separation processes include belt press, screw press, and centrifugation, which separates liquid digestate from solid digestate [164]. The separation technique used is determined by the consistency of the digestate and the amount of dry matter required in each fraction. For example, if the liquid fraction is required, the dry matter content of the fraction must be kept low to avoid clogging in downstream processing or to make it easy to transport.

Screw Press

These separators are frequently used with substrates containing high fibre content, such as plant-based wastes or digestate residues derived from wood. The digestate is pressed against the cylindrical screen by the screw, and the liquid oozes out into the sieve, where it is collected separately. The solid exits the end of the separator into a hopper and is collected at the bottom at the conclusion of the operation. Screw press separators are incapable of separating solids with minute particles.

Belt Press

A belt press is made of a closed loop of belts wound horizontally by cylinders. Gravity separates the excess water from the digestate applied at the beginning of the belt, which is then transported between belts by rotating cylinders. A solid, rich filter cake is created during this process, which completely squeezes the water out of the digestate. At the belt press’s far exit, the dewater filter cake finally leaves. Throughout the entire process, the liquid fraction is collected at the bottom of the press.

Decanter Centrifuge

In order to separate slurries with colloids or smaller particles, decanter centrifuges are typically used. The decanted fraction's solids content is high in phosphorous, which is kept intact during the AD.. The digestate is fed into the centre of the centrifuge, where centrifugal force is used to separate the solids from the liquid. The outlet is used to release the solid particles that have built up at the walls after being pushed by the screws. In the meantime, liquid exits the centrifuge through the opposite 'end's outlet.

Several studies show that , decanting centrifuges are more effective than screen-based separators at removing total phosphorous and total solids According to Møller and Lund [165] the ability of decanter centrifuges to separate even finer solids led to a higher efficiency of nitrogen separation. The liquid fraction has inorganic dissolved ammoniacal nitrogen, while the solid fraction typically contains trace amounts of organic nitrogen (NH_3-N).

2.3.7 Biomethane

The final step of the anaerobic digestion process is the production of biomethane. Methanogenic archaea convert acetate, hydrogen, and carbon dioxide into methane and carbon dioxide during this process. The methanogenic archaea can be categorised as either hydrogenotrophic or acetoclastic. The hydrogenotrophic groups convert hydrogen and carbon dioxide into methane and water while the acetoclastic groups directly convert acetate into methane and carbon dioxide [95]. For the majority of lignocellulosic substrates, a mixture of 55 to 40 percent methane and carbon dioxide is generated. Similar to natural gas, biomethane can produce heat and electricity for industrial or residential use. It can also be used to power natural gas-powered vehicles [166]. Using agricultural feedstocks to produce biomethane is one of the most cost-effective methods. This system reduces transportation challenges and allows farmers to implement on-site waste management strategies. Additionally, the end products of anaerobic digestion can be used directly as fertilisers to promote crop growth. [167]. Biomethane is also considered a carbon-neutral fuel because the carbon dioxide emitted during the AD process is offset by the carbon dioxide absorbed by the energy crops during their growth period [168]. A recent study suggested that the implementation of biogas production through AD is an economically and environmentally viable technology. Therefore, commercial production of methane along with other intermediate fuels such as hydrogen, ethanol, etc. is a crucial step in the development of green technologies. [169].

Biomethane produced through anaerobic digestion is a low carbon gas, as the carbon dioxide consumed by plant substrates during photosynthesis almost offsets the carbon dioxide released during biogas production [170]. Biomethane has multiple applications, including transportation fuels, grid injection for electricity generation, industrial heating source, and residential use [22, 171]. Additionally, the residue obtained after anaerobic digestion is rich in minerals such as phosphates and nitrates and can therefore be utilised as fertilisers [172]. Numerous studies on diverse lignocellulosic substrates have been conducted to produce multiple bioproducts via an integrated biorefinery strategy. This strategy can aid in the development of a circular bioeconomy that makes more sustainable and economical use of organic substrates.

2.4 Energy crops as substrates for biogas production

There have been numerous studies on energy crops cultivated in marginal lands, in crop rotation techniques or fallow land which have proven to be beneficial in retaining soil nutrients and provide extra market income to the farmers.

Studies on lignocellulosic substrates have proven that they are effective source for biofuel production due to their high organic component that can be converted into several forms of liquid fuels (bioethanol, etc.), gaseous fuels (biogas, biohydrogen, etc.) and solid (biofertilizers). Most of the lignocellulosic substrates consist of 40-50% carbohydrates, 15-20% Lignin and 20-30% hemicellulose that vary quantitatively and qualitatively for various substrates.

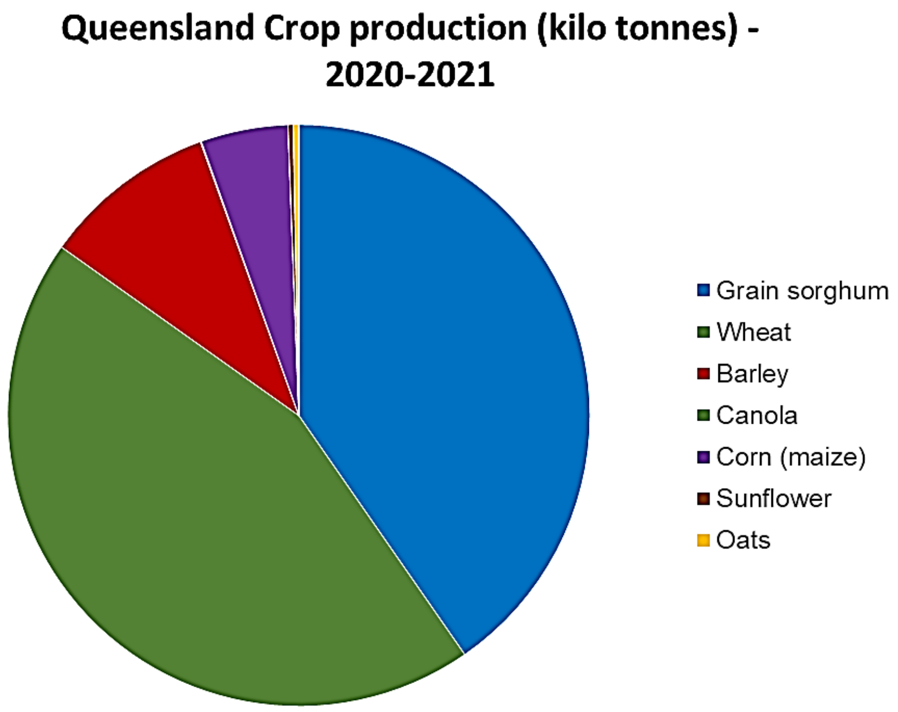

Figure 2. Queensland Crop Production forecasts – 2020-2021

Energy crops such as wheat, sorghum, maize have been proved to be effective substrates in biogas and bioethanol production in major countries like USA, Brazil, Europe, and Australia. However, the major challenge associated with the use of energy crops is that they pose a competition over food crops [173]. On the contrary, cultivation of energy crops help conserve the biodiversity and optimize land management [174]. There have been a wide range of studies across the globe proving that these substrates have a potential for large scale energy production and transportation fuel substitution. Meanwhile the food to fuel conflict in using energy crops can also be avoided by considering various cropping scenarios such as crop rotation with other food crops, utilization of marginal lands or using fallow land for energy crop productions. Crop rotation involves cultivation of two or more crops in the same agricultural land in a span of few years. For instance, a study was conducted by Murphy and Power [175], involving three crops wheat, barley and oats were cultivated in 48,000 hectares of land over a three-year period dedicated 16,000 ha per crop. This study concluded that total biomethane involving all three crops was 118,188,017 m^3/a when compared to cultivation of wheat alone at 90,482,57 m^3/a [175]. An article stated that due to the declining number of livestock's Poland had 3.6 x 10^6ha of unused meadows and pastures with good potential for energy production [176]. Likewise, fallow land is another strategy that can be incorporated in bioenergy production from specific energy crops. Hammar [177] showed that willow grown on fallow land in Sweden could not only be used to generate energy but also simultaneously remove carbon from the atmosphere through enhanced soil carbon sequestration. Yet another article also stated that biogas produced from grass grown in fallow land helped reduce the emission of greenhouse gases by 79% as compared to diesel fuel [178]. Therefore using fallow land to produce energy crops biomass production can be achieved without competing with food or fodder crop demand [179]. Methane yields of some lignocellulosic substrates obtained from Biochemical methane potential experiments are presented in Table 3.

Table 3. Methane yields of various energy crops/crop biomass

Crops	**Methane yields m^3/kgVS**	**References**
Straw of oat	0.32±0.02	[180]
Straw of rapeseed	0.24±0.02	[180]
Grass silage	0.296±0.02	[187]
Sugar beet tops	0.353±0.003	[181]
Oat straw	0.203±0.025	[182]
Maize	0.205±0.450	[21]
Barley	0.353±0.658	[183]
Hemp	0.355±0.409	[183]
Potatoes	0.276±0.400	[183]
Sorghum	0.295±0.372	[183]

VS: Volatile solids;

2.5 Sweet sorghum as energy crop for biofuel production

Sweet sorghum crop is gaining considerable interest as a lignocellulosic crop for biofuel production due to its rich carbohydrate composition and ability to grow in temperate climatic regions [184]. The plant roughly grows to a height of 5m and is a rich source of fermentable sugars when juice is extracted.

The cultivation of sweet sorghum is expected to be economically advantageous due to its propagation method involving seeds with a high multiplication ratio, in contrast to sugarcane which relies on stem cuttings with a lower multiplication ratio. This crop demonstrates a high degree of adaptability to unfavourable environmental conditions and necessitates minimal resource inputs. Water scarcity is a significant constraint on crop productivity in numerous regions across the globe. According to Dutra *et al.* [185], Sweet sorghum necessitates only one-third of the water needed for sugarcane and half of the water needed for maize. Sweet sorghum is considered to be a sustainable and renewable feedstock option for the production of bioethanol. It is well-known for its ability to efficiently utilise water and nitrogen resources [186]. The potential impact of drought on the survival and sugar production of sweet sorghum is negligible. According to Almodares *et al.* [187], in regions characterised by hot and arid climates, the cultivation of Sweet sorghum can result in a production of 80 tonnes of stalks, 5 tonnes of grains, and 15 tonnes of green leaves per hectare. The crop's ability to adapt to arid farming conditions rendered it a viable option for the production of biofuels. According to Vinutha *et al.* [188], the cultivation of Sweet sorghum crop can be effectively managed with minimal fertiliser application, distinguishing it from other feedstocks such as sugarcane, maize and sugar beetroot. Improper utilisation of nitrogen-based fertiliser not only contributes to the release of greenhouse gases but also escalates the expenditure associated with inputs. The application of nitrogen has a significant impact on various yield parameters, including dry matter yield, stem juice yield, brix, fermentable sugar yield, theoretical juice ethanol yield, theoretical lignocellulosic ethanol yield, and total theoretical ethanol yield, with the exception of brix [189]. The measurement of plant height is a significant characteristic of plant structure that exhibits a strong association with biomass productivity. Undoubtedly, Sweet sorghum cultivars exhibit a remarkable height exceeding three metres, enabling them to generate a substantial amount of biomass ranging from 58.3 to 80.5 tonnes of fresh stems per hectare specifically in semi-arid regions [190]. The hybrid's impressive biomass yield per hectare at all latitudes (up to 95 tonnes of fresh biomass per ha per cycle) is a result of their high photosynthetic efficiency (3–4%). Several cultivars of sorghum, including sweet, grain, and high energy varieties, were subjected to testing in order to assess their anaerobic biodegradability. According to Pazderu *et al*. [191], the fibre content of the stalk, leaves, and partially the panicle is a determining factor for the production and quality of biogas (methane yield). Continuous ripening results in an increase in barely fermented lignin content and a decrease in fibre degradability.

The average area of cultivation of sweet sorghum is 470,000 ha and 160,000 ha in Queensland and NSW annually. About 60% of total Australian grain sorghum is cultivated in Queensland due to its warm climatic conditions. As shown in Figure 2, currently sorghum is the highest produced crop in Queensland with yields reaching up to 2.6 t/ha [192]. There have been numerous studies on Sweet sorghum demonstrating that it is a feasible alternative to sugarcane and maize for production of biofuels [193]. In addition, it is also used for ensilage as feed for cattle manure and VFA production. A particular study attempted to produce bacterial cellulase using Sweet sorghum substrate [194]. In another attempt an effective strategy to produce hydrogen through an integrated two step fermentation of Sweet sorghum stalks using alkaline pre-treatment and enzymatic hydrolysis [195]. Another study was performed on whole Sweet sorghum plant to produce biobutanol where 100000 tonnes/year dry Sweet sorghum stalks could produce a maximum biobutanol of up to 10,000 – 14,000 t/year [196]. These studies have established that sweet sorghum is a viable substrate for several forms of biofuel production.

Often research on Sweet sorghum around the globe is focused towards optimizing the fermentation processes from juice extracted from the stalk [197]. Sweet sorghum was first introduced in the United States in the year 1852 for its rich composition of carbohydrate [198]. Although it has been primarily grown as sugar substitute to sugarcane, it has also been discovered as a potent feedstock for various biofuel products. Ethanol produced from sorghum consumes only the starch leaving protein rich by-products that can be used as cattle feed [199]. Similarly, sorghum used in biogas production leaves a mineral rich filter cake that can be use as biofertilizers [128] Therefore, the energy-food cycle is restored with limited waste liberated. In addition, Sweet sorghum is highly suitable for dry and horrid climatic conditions and can be grown 1-2 times a year [24].

A study conducted on carbohydrate rich crops stated that as the plant matures the carbohydrates are translocated from the stalks to the head to make starch for seeds [200]. This suggests the increase in the biogas yields when the Sweet sorghum varieties reach the flowering stage [201]. Also, sugar content in the Sweet sorghum tends to be the highest when there is low rainfall and extreme sunny weathers [202]. Sweet sorghum has been investigated as a drought tolerant crop, with rich genetic diversity that can sustain in low water, nutrients and has a short growth period. Therefore, due to these characteristics, instead of competing with arable land for food crops, it can also be cultivated in marginal lands converted into agricultural land [202]. Despite the excellent potential for sweet sorghum crops in biohydrogen and volatile fatty acid production, there is no sufficient data to determine the worldwide biorefinery potential of sweet sorghum as a whole crop.

2.5.1 Hydrogen production from sweet sorghum

Sorghum biomass contains a high amount of sugars that can be easily fermented, making it a great choice for producing hydrogen through fermentation. While numerous studies have investigated the impact of environmental factors on hydrogen production from different synthetic substrates and wastes, there is still a lack of information on the statistical optimization of environmental factors for biohydrogen production from sweet sorghum using mixed cultures. The potential production of hydrogen from sweet sorghum was calculated assuming efficiencies that seem presently within reach for the thermophilic fermentation This meant that for the fermentative conversions, efficiencies of 80% of the theoretical maximum were used in the calculations.

2.5.1.1 One-stage hydrogen production

In several experimental trials, Sweet sorghum plants were subjected to a process of leaf and stem removal, followed by the extraction of available sugars using water at a temperature of 30°C, as described in[203].The portion of the sample that consisted of easily accessible sugars contributed to 7.5% of the total production of H_2, while the leaves and stems contributed to 53.2% and 39.3% respectively. The cumulative hydrogen (H_2) evolution observed from the three fractions was equivalent to that of the homogenised whole plants. In their study, Ntaikou *et al*. [203] employed a mesophilic pure culture of R. albus to generate hydrogen gas from wet Sweet sorghum biomass. The researchers documented a hydrogen productivity of 14.5 L H_2/kg wet sorghum biomass, which is equivalent to approximately 58 L H_2 /kg dry biomass. Similarly, Ivanova and Rákhely [204] reported an H_2 yield of 1.75 mol H_2/mol Hexose when Sweet sorghum whole plant was used.

In a study conducted by Antonopoulou *et al.* [205] on batch fermentative H_2 production, it was observed that the maximum rate of H_2 production per kilogram of wet sorghum biomass was 2,550 mLH_2 per day, with a retention time of 6 hours. In a separate investigation conducted by Nagaiah *et al*. [206], the researchers employed the Sweet sorghum variety SSV74 to investigate the impact of pH, substrate to inoculum ratio, and incubation temperature on the generation of H_2. The highest amount of H_2 produced was 328 mL per 3.25 g of glucose equivalents under the optimal conditions pH 6.0, substrate to inoculum ratio of 0.75 and incubation temperature of 35°C. The impact of substrate loading rate on H2 yield accounted for 53% in the optimised fermentation parameters, resulting in a significant increase of 190% in overall H_2 yields. According to Nagaiah *et al*. [206], the utilisation of Sweet sorghum stalk juice has been proposed as a promising prospective resource for the production of hydrogen through fermentation. Some additional literature for one-stage and two-stage hydrogen and methane production are presented in Table 4.

2.5.1.2 Two-stage hydrogen and methane production from sweet sorghum

Two-phase systems provide the potential for optimising the biological process due to the fact that the conversion of an organic substrate into methane involves a four-step process, with each step being carried out by multiple bacterial species [207]. These bacterial species exhibit distinct preferences for specific environmental variables, including variations in pH and temperature, which are crucial for their optimal growth and metabolic activities. In the context of a single-phase biogas process, all four processes occur within a singular fermenter, wherein the temperature and pH conditions are carefully adjusted to accommodate the requirements of the methanogenic archaea. The organisms at the top of the food chain are considered to be the most sensitive, whereas the hydrolytic bacteria exhibit greater flexibility and adaptability to the environmental conditions of the methanogenic bacteria [208].

Table 4. Literature on one-stage/two stage fermentation of energy crops

Substrate	Inoculum	Hydrogen yield	Methane yield	References
Sweet sorghum biomass	Indigenous microflora	10.4 L kg/VS	107L/kgVS	[205]
Wheat straw hydrolysate	Anaerobic granular sludge	89.1 L kg/VS	-	[209]
Sugarcane bagasse	-	0.14 Nm^3 H_2 /kg TOC	2.6 Nm^3 CH_4/kg TOC	[210]
Rye Straw	-	134.71 dm^3/kgVS	189.94 dm^3/kgVS	[211]
Grass silage	Cow manure	6.46L/kgVS	495 L/kgVS	[212]
Lipid-extracted microalgae biomass	Anaerobic sludge	46.0L/kgVS	393.6L/kgVS	[213]
Corn stover	*Clostridium butyricum* AS1.209	68.0L/kgVS	-	[214]
Beer lees	Mixed microflora	53.0L/kgVS	-	[215]
Garden Waste (GW)	Co-culture *C. Saccharolyticus/ C. bescii*	98.3L/kgVS	322 ± 10 L /kg (COD_t)	[216]
Sorghum biomass	Ruminococcus albus	59 l H_2/kg FM	-	[203]
Sweet sorghum extract	Indigenous microflora	10.51 L H_2/kg FM	-	[217]
Sweet sorghum biomass	Indigenous microflora	10.4 L/kgVS	35.3 L CH_4/$kg_{sorghum}$	[205, 218]
Wheat straw hydrolysate	Activated sludge	4.4 ml H_2/g TS	234 ml CH_4/g TS	[219]
Sorghum	Activated sludge	21.1 ml H_2/g TS	438 ml CH_4/g TS	[219]

A recent study conducted by Giordano *et al.* [220], evaluated a simple bench-scale batch procedure for measuring the biochemical hydrogen and methane potential of organic substances as a two-step simulated process. The hydrogen yields achieved in this investigation, using the byproducts of the potato-processing sector, ranged from 153 to 186 L H_2/kgVS. These values exhibit a minor decrease compared to the findings reported by Xie *et al.* [221], which ranged from 200 to 271 L H_2/kgVS. However, the above authors utilised fresh potatoes as a substitute for food trash, whereas this study employed actual food waste, perhaps resulting in a decreased quantity of readily biodegradable organic material due to industrial processing.

In conclusion, the optimisation of key parameters such as VLR (Volatile Fatty Acid Loading Rate), substrate-to-biomass ratio, and F/M (Food to Microorganism) ratio is crucial in order to achieve maximum biohydrogen generation. C. tyrobutyricum, the predominant bacteria responsible for hydrogen generation in the hydrogen bioreactor, is believed to be utilising lactate and acetate as substrates for the creation of biohydrogen [222]. Meanwhile adjustment of pH for two stage reactors is crucial as hydrogenotrophic microbes require acidic conditions (pH 5.5) while methanogenic archaea require a pH of 6.8-7.5 [223]. The two-stage process is more stable than the single-stage process because it selects and enriches different bacteria in each anaerobic reactor, controls the acidification phase in the first digester, and prevents the inhibition of the methanogenic population in the second digester [224].

2.5.2 Methane production from sweet sorghum

The methane yields of several sorghum substrates used as energy crops were reported in the literature [225-228]. For instance, Mahmood and Ullah [229] tested 14 sorghum cultivars and reported methane yields of 250-354 NL CH_4/kgVS_{added}. However, the methane yields per ha in the above study from (3,924 to 7,120 Nm^3 per ha) were higher than in the present study, which were ranging from 1,664 to 3,059 (Table 7). The discrepancy is attributed to the fact that cultivars with high biomass yields per ha and methane yields had resulted in the overall high methane yield per hectare, and vice versa. Similarly, Sambusiti and Ficara [230] reported methane yields ranging from 270 NL CH_4/kg to 335 NL

$CH_4/kgVS_{added}$ for five untreated Sweet sorghum hybrids and observed that alkaline pretreatment did not improve the methane yields (288 – 336 NL /kgVSadded). The above result suggests that the effect of sodium hydroxide pretreatment on methane yields is dependent on the composition and type of substrate employed. Finally, methane yields reported in this study were also within the values of 232-316 NL $CH_4/kgVS_{added}$ Mahmood and Honermeier [231], 216-316 NL $CH_4/kgVS_{added}$ Barbanti and Di Girolamo [232] and 338 NL $CH_4/kgVS_{added}$ [233] reported for sorghum cultivated at higher latitudes and temperate climates. The results thus suggests that methane yields from sorghum are not much influenced by the extent of its cultivation but by the degradability of biomass and its chemical composition.

2.6 Techno economic feasibility study for biogas plant

An economic operation of energy crop digestion can only be achieved if high crop and biogas yields can be achieved at reasonable low investment-, raw material-, and production costs, according to practical experience[183]. Additional benefits, such as gate fees for co-substrates, on-farm local crop production, and subsidized feed-in power tariffs, significantly enhance the overall economics and must be carefully considered when evaluating the economic process. One major question that arises while setting up a biorefinery is if the feedstock is immediately available around the premises or need to be transported from certain distance. In most cases, setting up a biorefinery closer to the farms growing the energy crops is beneficial [234]. choosing a feedstock that has high dry matter content >50% reduces the chances of degradation of organic matter prior to anaerobic digestion. However, in case of wet feedstocks, options such as drying operations, silage preservation can be considered to avoid carbohydrate degradation. A mechanical pretreatment is necessary to reduce the particle size in order to prevent clogging or provide sufficient surface area for microorganism to degrade the substrates.

The entire anaerobic digestion system requires integrated tanks, mixers, covers, and heating systems. A complete mix digester usually only has one large tank. Within this tank, all phases of digestion take place (mixing, moisture addition, hydrolysis, methane production and extraction, and so on). Depending on their size and required throughput capacity, multiple digester tanks can be used in parallel to increase overall production rate. Typically, continuous mixing is accomplished by injecting biogas back into the organic material, either hydraulically with high-velocity mixing jets or mechanically with propellers or turning wheels. To prevent corrosion, digester tanks can be made of reinforced concrete (precast or cast in place), stainless or carbon steel, and their interiors can be coated with glass or painted epoxy. The circular tank walls are protected by a tank cover. These fixed or floating covers expand and contract in response to changes in interior gas pressures.

The AD is influenced by process parameters such as temperature, pH, trace elements, NH_3 accumulation, and volatile fatty acids. In general, a mesophilic digester for AD is adequate for the climate in Queensland [235]. During the winter, additional energy input may be necessary to maintain a constant temperature for the AD process. This can be reduced by closing down the digester temporarily for maintenance inspections. Organic loading rate and Hydraulic retention time are the two major deciding factors for improving the methane yields. Generally, in case of lignocellulosic substrates high biogas yields are achieved when a high OLR and low HRT is maintained [236]. However, selecting an optimum OLR and HRT completely depends on the physiochemical composition of the substrates. However, an HRT below 10 days is not recommended in order to prevent the washout of the useful microbes in the reactor. Likewise, extremely high OLRs >3kg/VS/day may lead to overaccumulation of volatile fatty acids or NH_3 that can inhibit the AD process leading to lower methane yields. In order to determine the optimum HRT and OLR for the large-scale digester it is important to conduct several lab scale studies with analytical evidence. pH adjustment is another parameter that needs to be monitored on a daily basis in order to maintain the stability of the AD process. pH is directly influenced by the presence of VFA's or ammonia in the reactor.

In addition to the basic process requirements for developing a farm-scale biorefinery, a number of additional factors must be considered to assure the biorefinery's viability. Prior to further biogas upgradation, a desulfurization unit is required to prevent contamination of the upgradation unit by trace quantities of H_2S, NH_3, etc. that are released from the biogas produced during AD. Before composting or further pretreatment for fertilizer application, the digestate produced by anaerobic digestion must undergo a hygienization process due to the presence of pathogens that are detrimental to humans. The hygienization procedure involves heating the digestate to temperatures between 70 and 120 ºC to prevent the growth of pathogens that are harmful to the environment [237]. Prior to land application as fertilizer, the digestate must be collected in final storage containers in all scenarios. To prevent the emission of greenhouse gases such as methane and ammonia, storage containers must be fitted with gas-tight covers. The additional biogas production collected from digestate storage tanks typically reimburses cover investments within a brief period. Additionally, covering the storage containers prevents the plant from emitting odors. Periodic residual fermentation potential experiments must be conducted on the to-be-stored digestates in order to ensure complete degradation. Lastly, odor- and ammonia-emitting emissions from the land application of digestates as fertilizer must be prevented through the use of appropriate application technologies. In most instances, digestates can be applied directly to adjacent agricultural land after production.

Biorefinery concept has been an attractive pathway for many industrialists looking to develop a sustainable way for biofuel production. The sequential production of several substances from a single raw substrate will help in reducing overall operational and capital costs of the biorefinery. In addition, the expenses used to develop and run the process is balanced by the biofuels, biofertilisers and energy output [238]. Some studies on sequential production of hydrogen, VFA and methane using a single substrate are shown in Table. [239, 240].

Based on the scale of operation, quantity of agricultural wastes/feedstocks the biogas digesters can be classified into centralised or farm scale plants. Large-scale digesters (i.e., hundreds to thousands of cubic metres) have historically been more common in developed nations due to higher infrastructural and capital investment requirements. The biogas produced though the AD is used for combined heat and power (CHP) purposes, injected as RNG into the natural gas grid or upgraded for use as transportation fuel. A centralised or combined system codigests agricultural leftovers, food waste, the organic part of MSW, and animal manure from multiple farms. A portion of the digestate is returned to the farms under this model to be used as fertiliser, while the remainder is sold to other farms. Currently, few countries like Denmark, Finland or Germany are the pioneers in centralised biogas plants having a capacity of above 8000m^3 [241]. Farm-scale AD plants are often constructed in big dairy or swine farms or small croplands and have digestion capacities ranging from 200 to 1,200 m^3. They combine agricultural waste and other readily available organic matter, such as energy crops grown on the same farm, with animal manure from one to three farms [242].

The economic advantages associated with the establishment and operation of a biogas plant.

- The minimisation of foreign imports of fossil fuel supplies
- The reduction in demand for fertilisers, chemical herbicides, and pesticides can be achieved by implementing compost production from anaerobic digestate.
- The treatment method effectively reduces the demand for fossil energy by directly utilising energy produced, such as biogas, electricity, and heat.
- The generation of revenue by means of selling compost and energy (such as biogas, electricity, and heat) to the public or the public grid.
- The utilisation of digestate as a biofertlizer has been shown to have long-term benefits on soil structure and fertility, leading to improved soil and agricultural output.
- The reduction of landfill area and the subsequent decrease in land costs[243]

In order to maximise profitability and contribute to glasshouse gas (GHG) mitigation, it is advisable for biogas plants to consider utilising additional substrates that possess a negative price, such as food waste, animal manure. By incorporating these substrates into their operations, biogas plants can not only enhance their attractiveness as viable options, but also capitalise on the potential for higher profits through energy recovery [244]. One important aspect to consider is the utilisation of animal manure, namely its valorisation through the energetic conversion into biogas. This can be achieved through the implementation of co-digestion plants that combine manure with energy crops. Such an approach has the potential to enhance the profitability of the operators managing these plants. Manure possesses the potential to be utilised in biogas plants without incurring any cost, and its utilisation is often linked with subsidies [245]. A study conducted by Song and Zhang [246] also suggested that The utilisation of crop straw for lignocellulosic biomass hydrolysis encounters challenges, which restrict its practical application. Consequently, it is imperative to conduct relevant investigations to enhance fermentation technology. These studies should encompass the determination of the ideal carbon to nitrogen ratio for co-digestion, examination of the impact of crop straw pretreatment, and exploration of the role of microbes in the fermentation process. The viability of generating heat and electricity through biogas combined heat and power (CHP) systems appears to be unfavourable once the subsidy schemes are no longer in effect. Due to the increasing presence of intermittent renewable energy sources such as solar and wind, the rates of electricity in the day-ahead market have significantly decreased, rendering the operation of biogas economically unfeasible. Conversely, the substantial integration of intermittent renewable energy sources within energy networks creates an opportunity for biogas combined heat and power (CHP) systems to operate exclusively during periods characterised by elevated balancing prices.

Few instances on farm scale biogas plant utilizing energy crops and animal manure are as follows.

Mussoline and Esposito [247] conducted a technoeconomic study on co-digestion of untreated rice straw with piggery wastewater manure using two pilot-scale experiments by varying temperature and feed volume. Using these values, assuming a 100-ha rice farm, two hypothetical farm-scale scenarios were created (taking into account both untreated and pretreated rice straw). In either case, 100,000 m^3 CH_4 can be produced annually, producing 328 MWh. Likewise, Akbulut [248] conducted a techno-economic analysis of power generation from the anaerobic digestion (AD) process in a specific biogas plant situated in Çiçekdağı, Kırşehir. This study primarily investigated the implementation of an integrated manure management strategy on a farm, utilising anaerobic digestion. The hydraulic retention time (HRT) of the anaerobic digestion (AD) plant, which had a volume of 2713 m^3, was observed to be 33 days. During this period, the plant exhibited consistent and stable operations, resulting in a total biogas yield per hour of around 2851.6 m^3. In the given circumstances, the daily production of electricity energy and heat energy amounts to 277.99 kWh and 320.76 kWh, respectively. Animal manure, energy crops, and agricultural residue feedstock in the AD process in farm practices provide environmentally safe digestate disposal.

On the other hand, the use of organic fertilizer allows for integrated waste management with energy generation. The following studies offer significant insights that can be applied by policymakers or other proponents of sustainable technologies. The adoption of sustainable technologies is frequently an extremely fragile and lengthy process.

2.8 Pilot scale study on sweet sorghum

From the diverse studies conducted on Sweet sorghum , it can be inferred that Sweet sorghum has a great potential for production of multiple products through a biorefinery approach [249]. As stated by Kaparaju and Serrano [250], production of several biofuels through a single substrate is an effective approach leading to large scale production of sustainable fuels. They developed an integrated process that incorporated simultaneous production of ethanol from the pre-treated liquor and biogas production from the solid pre-treated residue. It is a challenge to any biofuel sector to produce an economically viable and sustainable biorefinery.

Since Sweet sorghum has demonstrated an excellent potential for both bioethanol and biogas production there is a positive scope for developing a multi-stage reactor system to simultaneously produce hydrogen, VFAs, Bioethanol and Biogas. A study conducted by Barcelos and Maeda [251] demonstrated that Sweet sorghum whole crops has the capacity to produce 13,600 L ethanol.ha^{-1} with 79% of conversion efficiency. Another study demonstrated that Sweet sorghum for bioethanol production could solve fuel issues by increasing the octane number of gasoline and reduce the overall greenhouse gas impacts to the environment especially in hot and dry countries [184]. Subsequent anaerobic digestion of ethanol stillage can be an attractive strategy for complete utilization of sweet sorghum substrate as it utilizes all the remaining components that could not be degraded during fermentation. In addition, it also enriches the nutrient content of the digestate during biogas production which can be used as a biofertilizer to enrich crop characteristics [252].

In an attempt to produce biofuels from Sweet sorghum, Yu and Zhang [253] developed a method for ethanol fermentation from Sweet sorghum juice followed by pre-treatment of bagasse in order to produce butanol. This study demonstrated that a biorefinery approach to produce multiple bioproducts improves the demand for sweet sorghum biorefinery. Therefore, an integrated modelling of a pilot scale plant is required provide a deeper insight from farm to biorefinery continuum of sweet sorghum substrate. In addition setup of biorefineries in rural sectors has been said to increase the economic and rural development especially to farmers [254].

Despite the several obstacles involved in cultivation of Sweet sorghum , the current economic, rural and scientific conditions highly favour the use of Sweet sorghum over conventional crops such as sugarcane, maize in Australia [255]. An economic feasibility study on Sweet sorghum whole crop in biobutanol production suggested that complete process of sugar extraction, pre-treatment of Sweet sorghum bagasse and production of biobutanol resulted in a higher butanol yield than untreated stalk and bagasse. P. Nghiem and M. Nguyen [256], attempted to simulate a biorefinery for sweet sorghum whole crop to simultaneously produce fuel ethanol and co-produce industrial value-added products such axaxanthin and D-ribose from the fermented bagasse. This study also demonstrated that combination of sweet sorghum stalk juice with the glucose extracted from residual solids of enzymatic hydrolysis, resulted in higher ethanol concentration. This study directs us towards the susceptibility of recycling of co-products to improve biofuel yields.

In due course, selection of appropriate crop for bioenergy production should be based on its high biomass yield, high biogas potential and low production costs [257]. It can be proposed that sequential combination of several biochemical processes in a single biorefinery approach can help exploit maximum potential of organic substrates and help in development of a sustainable solution for renewable energy. Thus, this study aims to develop an integrated process for integrated production biohydrogen, VFA, and biogas from the whole sweet sorghum crop. The overview of the project is shown in Figure 3.

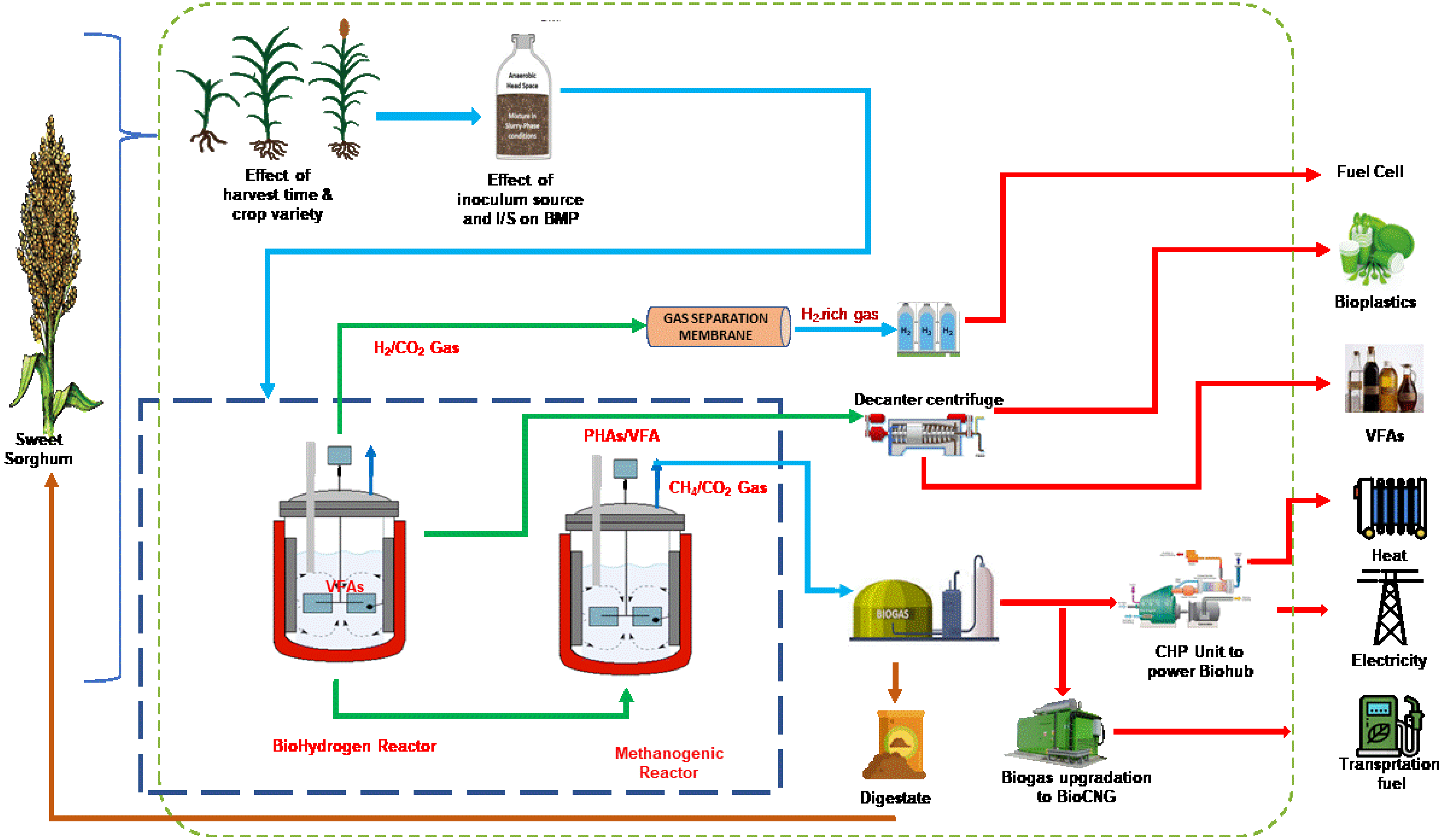

Figure 3. Complete illustration of Biorefinery application for sweet sorghum energy crop.

CHAPTER 3
METHODOLOGY

3.1. Substrates and Inocula

Nine commercial sweet sorghum cultivars were screened for their suitability in biohydrogen, and methane production. The samples used in these screening experiments were obtained from Singh Farming, Cairns, North Queensland in 2020. These cultivars were used in the study on the effect of Inoculum to substrate ratio on Biochemical methane potential and chemical composition. Meanwhile , three cultivars among the nine cultivars were used in the batch scale hydrogen production and codigestion experiment study using CSTR. Similarly, five sweet sorghum cultivars (SE-1, SE-19, SE-20, SE-35 and SE-122) were obtained from a farm in South Australia. These cultivars were used in the study on the effect of inoculum source on biochemical methane potential and VFA production. All samples were shredded to approximately 1 cm particle size by using garden shredder (Ozito 2400W). Samples were further oven dried at 45°C for 3-4 days and stored hermetically in plastic containers until further use.

Table 5. Sweet sorghum cultivars used in this study.

S.No.	Sweet sorghum cultivar
1	SE-1
2	SE-5
3	SE-19
4	SE-20
5	SE-23
6	SE-35
7	SE-42
8	SE-45
9	SE-81
10	SE-86
11	SE-122
12	Mega Sweet

Cow manure was collected from a dairy research station at the College of Veterinary Sciences, Gatton Campus, University of Queensland, Queensland.

Three different Inocula were collected and used in this study. Rumen fluid was collected from a fistulated cow at the College of Veterinary Sciences, Gatton Campus, University of Queensland, Queensland. Anaerobically digested material from a full-scale biogas plant treating sewage sludge and source separated organic fraction of municipal waste (Luggage Point, Queensland Urban Utilities, Brisbane) was collected and stored at 4 ºC until further use. Prior to the start of the experiments, inoculum was degassed by incubating at 37 °C for 5-7 days until all the residual methane was released [258]. For the experiments, inoculum was subjected to heat-treatment by boiling it for 30 minutes to inactivate methanogens and to enrich spore-forming H_2-producers [259].

3.2 Effect of harvest time on chemical composition and methane yields of sweet sorghum cultivars

Three sweet sorghum cultivars (SE-19, SE-45 and Mega Sweet) were selected for this study. These varieties were harvested at three different crop growth stages, generally correspond to the vegetative (50 days after sowing DAS), flowering (66 DAS) and pre-maturity stage (80 DAS). Harvested biomass was transported quickly to laboratory and stored as described in Section 3.1. Batch experiments were performed in 500 ml glass bottles with a working volume of 400 mL. To each assay, 250 mL of anaerobically digested material from a full-scale biogas plant treating sewage sludge (Luggage Point, Queensland Urban Utilities, Brisbane)was used as inoculum. Substrates were added to achieve an inoculum to substrate ratios (ISR) of 0.3, 0.6 and 2 calculated as $VS_{inoculum}$ to $VS_{substrate}$ on weight basis (w/w). Distilled water was added to achieve the designed working volume. Headspace in the assays was purged with pure nitrogen (N_2 99.99%) for 3 minutes to create anaerobic conditions. Assays were prepared in triplicate. Prepared assays were sealed immediately with butyl rubber stoppers and caps and incubated statically at 37 °C. Assays with inoculum alone were used as controls and the methane produced from the control assays was subtracted from the sample assays. The gauge pressure (in mbar) in the headspace was measured by using a pressure transducer and absolute pressure was calculated. The methane concentration in the assay was analysed by using gas chromatograph (GC). Description on the chemical analyses and GC analyses is presented in Section 4.1 Chemical composition of the samples before and after the experiment are presented in **Error! Reference source not found.**.

3.3 Effect of inoculum to substrate ratio on methane yields of sweet sorghum.

Nine sweet sorghum cultivars (SE-1, SE-5, SE-23, SE-35, SE-42, SE-45, SE-81, SE-86 and Mega Sweet) were selected to study the effect of inoculum to substrate ratio at three different ISR'S (2,4 and 6).. The designed ISR ratios were based on the literature data [260]. To each assay, 90 mL of inoculum and a known amount of substrate were simultaneously at an inoculum to substrate ratios (2,4 and 6) on volatile solids basis. Distilled water was used to achieve the desired working volume of 100 mL. Thereafter, the assays were prepared and incubated as mentioned in Section 3.2.

3.4. Effect of inoculum source on methane production rates and yields from sweet sorghum cultivars.

The effect of inocula sources on methane production rates and yields from sweet sorghum was performed by using rumen fluid, anaerobically digested sewage sludge and cow manure as inoculum source. Five sweet sorghum cultivars SE-1, SE-35, SE-20, SE-35 and SE-122 obtained from a farm in South Australia were used in this study. Batch experiments were conducted in 161 mL glass serum bottles at 37 °C. To each assay, a known amount substrate and inoculum (90 mL) were added to achieve the designed ISR ratio of 2 on VS basis. Distilled water was used to achieve the desired working volume of 100 mL. Thereafter, the assays were prepared and incubated as mentioned in Section 3.2. and incubated statically at 55°C. Assays with inoculum alone were used as controls.

3.5. Effect of inoculum to substrate ratio on volatile fatty acid production rates and yields from sweet sorghum cultivars.

The effect of ISR of 0.3, 0.6, and 2 on the VFA production rates and yields was conducted in batch experiments using 161 mL glass serum bottles. Sweet sorghum cultivars SE-1. SE-19, SE-20, SE-35, and SE-122 were used as substrates. Anaerobically digested material from a full-scale biogas plant treating sewage sludge and source separated organic fraction of municipal waste (Luggage Point, Queensland Urban Utilities, Brisbane) was used as inoculum (refer to Section 3.1). Prior to experiments, inoculum was subjected to heat treatment by pre-heating at 90ºC for 15 minutes to inhibit the hydrogen and acetate consuming methanogenic archaea. [259]. To each assay, 90 g of heat-treated inoculum and the required amounts of substrates were added to achieve $VS_{substrate}$ to $VS_{inoculum}$ ratio of 0.3, 0.6, and 2, respectively. Distilled water was added to attain a liquid volume of 100 ml and pH was adjusted to 5.5 with 2 N NaOH or 5M HCl. The prepared bottles were then sealed as mentioned above in Section 3.2 and incubated statically at 55°C. Assays with inoculum alone were used as controls.

3.6 Effect of organic loading rate on process performance and biohydrogen yields during dark H_2 fermentation of sweet sorghum cultivars.

Three sweet sorghum cultivars (Mega Sweet, SE-35 and SE-45) were dried at 45°C for three days, milled and stored in vacuum bags until further use. Anaerobically digested material from a full-scale biogas plant treating sewage sludge and source separated organic fraction of municipal waste (Luggage Point, Queensland Urban Utilities, Brisbane) was used as inoculum (refer to Section 3.1). Prior to experiments, inoculum was subjected to heat treatment by pre-heating at 90°C for 15 minutes to inhibit the hydrogen consuming methanogenic archaea [261].

Three laboratory-scale batch bottles were used as the reactors for the dark fermentative H_2 production studies. The H_2 reactor temperature was maintained at 55 °C by using a thermostatically controlled water bath which provides an accuracy of ±0.1°C. The working volume for H_2 reactor was 2 L. The reactor contents were mixed mechanically at a range of 50–90 rpm. The gas volumes produced were recorded with gas meters. The H_2 reactors were operated at 4 d HRT, pH of 5–5.5 and organic loading rate (OLR) of 16 kgVS/m^3/d. The pH of the reactor was monitored every day manually adjusted to 5.5 using 1M sodium hydroxide The fresh matter was calculated on the basis of organic loading rate and adjusted with distilled water.

3.7 To compare the process performance and methane yields during anaerobic mono-digestion and co-digestion of sweet sorghum with cow manure.

The effect of OLR on process performance and methane yields during the mono-digestion and co-digestion of sweet sorghum with cow manure was conducted using two laboratory-scale continuous stirred tank reactors (CSTR). Reactor 1 (R1) was fed with equal mixture of sweet sorghum cultivars (SE-35(33%, SE-45 (33%) and Mega Sweet (33%)) alone and the second reactor (R2) was fed with sweet sorghum mixture (85%) and cow manure (15%) on VS basis of the designed OLR.The reactor temperatures were maintained at 37±0.1 °C. The working volume for both reactors were 10 L and the reactor contents were mixed mechanically at a range of 50–90 rpm. The timer-controlled stirrer was able to mix the reactor contents for 10 min at every 2 h interval. The produced biogas was monitored by using gas meters (Rittermilligas counter, Ritter GmBH, Germany). Both the reactors were operated at HRT of 14 d, pH of 7.5 and organic loading rate (OLR) of 0.5 - 3 kgVS/m^3/d. Samples from the reactors were collected and analysed on weekly basis.

3.8. To assess the feasibility of farm-scale biogas plant using sweet sorghum grown for biogas production and uses.

3.8.1 Biogas plant design calculations and assumptions

A farm-scale biogas plant was designed to carry out a technical feasibility study of using sweet sorghum grown on fallow land of 25 ha in Tablelands, Queensland. The plant is located at a sugarcane farm in Tablelands, Queensland. Based on the best methane yields obtained in the BMP study, sweet sorghum SE-81 was selected as the feedstocks. Chicken manure was used as co-substrate to obtain an optimal C/N ratio of 30:1. The biogas plant will be operated all year round with some days allocated for repair and maintenance. The plant will operate 24/7 and is expected to be equipped with sufficient instrumentation and telemetry to allow for remote access and control of the plant. An estimated operational period of 8,300 h/year was considered.

The plant will be operated as follows:

- Sweet sorghum will be harvested during the crop growing season and ensiled with lactic acid for round the year supply of feedstock.
- Chicken manure will be delivered to biogas plant 5 days/week.
- Biogas generation 24/7, year-round with 95% plant availability
- BioCNG/RNG/electricity production 24/7, year-round
- Power demand (where applicable) 24/7, year-round
- Liquid digestate discharge 24/7, year-round
- Solid digestate offtake 4–5 days/week, year-round.

Microsoft® Excel was used in conducting feasibility study. The first step in the analysis was to add the chemical analysis, methane yields obtained from batch scale and CSTR experiments. Biogas plant was designed based on the amount of sweet sorghum produced from 25 ha of fallow sugarcane land. SE-81 was the cultivar selected and it can produce 1,9838 t/y of biomass. Using the C/N ratio of SE-81, the amount of chicken manure required to achieve an optimal C/N ratio of 30:1 was calculated.

Feed was prepared by mixing the substrates and water to achieve a total solids content of 8%. The feed was prepared once in a week and the prepared feed was fed semi-continuously once in a day Monday through Sunday. An equal amount of digestate was removed and pumped to post-storage tank. The digestate was stored for 1 month before it is used on the sugarcane fields as biofertilizer. Screw press was used to separate the digestate into solid and liquid fractions.

To assess the feasibility of the proposed study, three different scenarios were designed with an aim to evaluate the possible market opportunities for biogas uses.

- Scenario 1: CHP—Biogas is used for electricity and heat generation in a combined heat and power (CHP) plant.
- Scenario 2: CHP + BioCNG—A portion of biogas is used for CHP to generate electricity and heat to meet the parasitic demand of the biogas plant and the

remaining biogas is upgraded and compressed (BioCNG) for vehicle fuel or distribution via virtual pipeline (road transport).

- Scenario 3: CHP+ BioRNG—Similar to Scenario 2 but the biogas is upgraded to renewable natural gas (BioRNG) for grid injection.

For the purpose of energy calculations, the following conversion values were used. 1 m^3 of biomethane has 9.96 kW of energy production potential. Methane conversion efficiency in a CHP was 85% efficiency with 42.4% electrical and 42.6% for heat efficiencies, respectively. Biogas loses of 1% was assumed during the biogas storage, 2% during the biogas upgrading and compression processes. Decanter centrifuge was used for the digestate separation into solid and liquid fractions. A fuel consumption rate of 12.66 km/m^3 of biomethane in passenger car was assumed. Average travel distance of 10,000 km per year was assumed for passenger cars. Transport distance for trucking chicken manure was 20 km and rigid truck (17t capacity) was used in the study. Biogas was stored in a separate double membrane biogas storage tank for up to 6hrs. Energy required for biogas upgrading was 1.08 MJ/m^3 raw biogas whilst the electricity requirement for compression of upgraded biomethane was 1.08 MJ/m^3 biomethane. The upgraded biomethane was compressed to 250 bars and bottled in 50 kg gas bottles.

Density of methane (0.7157 kg/m^3) and CO_2 (1.98 kg/m^3) were used to calculate the mass of methane and CO_2.

3.9 Kinetic modelling

Kinetics of methane production and hydrolysis constant values were evaluated by using three different kinetic modelling tools such as first-order. Ultimately the model with lower % difference and lower root mean square error (RMSE%) is assumed to be the best fit.

3.9.1 First-order kinetic modelling

First-order model is the simplest model used to predict the hydrolysis constant with the assumption that hydrolysis is the rate limiting step [262]. This model helps predict the time delay and methane yields during the process (Equation 1). The time delay corresponds to the lag phase during which the bacteria try to acclimatize in the environment [263].

$$B(t) = B0 * (1 - \exp(-kt)) \quad (1)$$

Where B_0 = maximum specific methane production potential of the substrate (NmL CH_4/gVS$_{added}$), k_{hyd} *is* the hydrolysis rate constant (d^{-1}), B(t) = cumulative methane yield at digestion time *t* (d) (NmL CH_4/gVS$_{added}$).

3.9.2 Modified-Gompertz modelling

Gompertz model was first used to identify the relation between specific growth rate and bacterial population density [264]. However, it has been modified further to Modified-Gompertz, with the assumption that methane production rate in batch digestion is proportional to growth rate of methanogenic bacteria.

$$B(t) = B0 * \exp\left\{- \exp\left[\frac{Rmax*e}{B0} * (\lambda - t) + 1\right]\right\} \quad (2)$$

Where, B(t) = cumulative methane yield at digestion time *t* (d) (NmL CH_4/gVS$_{added}$), B_0 is the maximum specific methane production potential of the substrate (NmL CH_4/gVS$_{added}$), Rmax = maximum methane production rate (NmL CH_4/gVS$_{.added}$), λ = lag phase (day), t = time (day), e = exp (1) = 2.7183.

3.9.3 Chen and Hashimoto model

Chen and Hashimoto assumed that organic matter degradation is directly proportional microbial growth rate. It also helps to predict the ultimate biochemical methane potential, if all the substrate is degraded [263].

$$G(t) = G_0 * \left(1 - \frac{K_{CH}}{\mu_m * HRT + K_{CH} - 1}\right) \quad (3)$$

Where, *G(t)* = cumulative methane yield at digestion time *t* (d) (mL CH_4 /gVS$_{added}$), G_0 is the maximum specific methane production potential of the substrate (mL CH_4 /gVS$_{added}$), μm = maximum specific growth rate of microorganisms (1/day), K_{CH} = Chen and Hashimoto kinetic constant (dimension less), HRT = digestion time or hydraulic retention time (days).

3.10 Analytical methods and calculations

Total solids (TS) and volatile solids (VS) were analysed according to the Standard Methods [265]. Soluble sugars from starchand inulin were extracted by using a mild acid hydrolysis method and quantified with the anthrone method [266]. Structural carbohydrates (cellulose and hemicellulose) as well as uronic acids, i.e., galacturonic and glucuronic acids coming from cellulose, hemicelluloses and pectins were quantified in triplicates using a strong acid hydrolysis according to the protocol described elsewhere [267] .

Cellulose and hemicelluloses contents were estimated as shown below :

Cellulose (%TS) = Glucose (%TS)/1.11

Hemicelluloses (%TS) = [Xylose(%TS) + Arabinose (%TS)]/1.13

where, 1.11 is the conversion factor for glucose-based polymers (hexose sugars) to monomers and 1.13 is the conversion factor for xylose-based polymers (pentose sugars) to monomers.

Proteins were determined by multiplying Total Kjeldhal Nitrogen (TKN) with 6.25 [268]. TKN was analysed by the standard method using a Buchi digestion unit K438 and a Buchi 370-K distillator/titrator [162]. VFA composition in the liquid phase, i.e. acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and iso-valeric (C5 and iC5) and caproic (C6) acids, were determined by using a gas chromatograph fitted with a flame ionization detector (FID) as per the protocol described elsewhere [269]. Finally, Total phenols in liquid fractions where determined by using a microtube test (Spectroquant, Merck) followed by a 4-aminoantipyrine colorimetric measurement after a two-hundred dilution [270].

Biogas volume in the H_2/CH_4 reactor experiments was monitored by gas meters (Ritter Milligascounter, Ritter, Germany). Biogas composition (CH_4, CO_2, H_2)

were analysed by using a gas chromatograph (Shimadzu 2014) equipped with thermal conductivity detector (TCD).

The biogas production potential (defined as H_2 or CH_4 potential) of the substrates in one-stage process were given as the amount of H_2 or CH_4 produced in ml/gVS added. In some H_2 assays, H_2 content in the biogas phase was shown to peak at various times. In such cases the higher peak value was used in calculating H_2 potential.

$$BMP_t - \frac{ml\ CH4_{sample\ assay} - ml\ CH4_{inoculum\ assay}}{VSadded} \qquad (4)$$

where (ml CH_4 sample assay – ml CH_4 inoculum assay) is the net methane volume (ml) obtained from the one substrate only, adjusted to the standard temperature (0 °C) and pressure (1 atm) condition (STP); VS_{added} is the mass of substrate VS in the sample bottle (g).

The following conversion factors were used to convert the g of organic compound to their equivalent gCOD:1.07 for carbohydrates, 2.91 for lipid, 1.5 for protein, 1.066 for acetic acid; 1.512 for propionic acid, 1.816 for butyric and iso-butyric acids, 2.036 for valeric and iso-valeric acids [271].

3.11 Statistical Analyses

The results of the chemical analysis and methane and biohydrogen yields were presented as the mean values with standard error of the mean of the three replicates used in the study (n=3). Methane yields were subjected for one-way analyses of variance (ANOVA), or t-test followed by Tukey's post hoc test using IBM SPSS Statistics® software. The significant test was fixed at a significance level of 0.05 ($p < 0.05$).

CHAPTER 4
RESULTS AND DISCUSSION

Statement Of Contribution to Co-Authored Manuscript

This chapter includes in section 4.2 a co-authored paper titled: “Sweet sorghum as a Potential Fallow Crop in Sugarcane Farming for Biomethane Production in Queensland, Australia”, which was published in Energies Journal. My contribution to the paper involved: Conceptualization, methodology, software, data validation, investigation, and writing of original draft.

Paper citation: Mathias, D.J.; Edwiges, T.; Ketsub, N.; Singh, R.; Kaparaju, P. Sweet sorghum as a Potential Fallow Crop in Sugarcane Farming for Biomethane Production in Queensland, Australia. Energies 2023, 16, 6497. https://doi.org/10.3390/en16186497

[redacted]

Divya Joslin Mathias Date: 10 October 2023

Corresponding author of paper:

[redacted]

Professor Prasad Kaparaju Date 10 October 2023

4.1 Effect of harvest time on chemical composition and methane yields of sweet sorghum cultivars

4.1.1 Effect of harvest time on chemical composition of sweet sorghum cultivars

Three sweet sorghum cultivars SE-19, SE-45 and Mega Sweet were harvested at 50, 66 and 80 days after sowing (DAS). The harvesting times at 66 and 80 DAS were related to the periods between the mid-stage of inflorescence (earing) and the mid-stage of milk ripeness. The chemical composition of the tested sweet sorghum cultivars harvested at 50, 66 and 80 DAS is presented in Table 6. Overall, TS content and VS/TS ratio of the sweet sorghum cultivars increased with increase in crop maturity. However, TS content in SE-45 decreased from 23.2% after 66 DAS to 22.55% after 80 DAS, apparently due to the loss of soluble sugars after flowering stage. This was also indicated by the higher nutrients content in the crop from the later harvest (Table 6). The opposite was true with SE-19 and Mega Sweet cultivars. These findings are consistent with those of Chattha and Hassan [272], Ram and Suresh [273], and Ashiq and Sartaj [274], who also reported a significant increase in DM yield with increasing maturity.

At 80 DAS, TS and VS content in SE-19 and Mega Sweet were 26.25% and 26.2%. The corresponding values for SE-45 was 22.55. TKN content varied from 26.37 gN/kgTS in SE-45 to 5.24 gN/kgTS in SE-19 (50 DAS). After 66 DAS, TKN content was reduced to 10.13 gN/kgTS in SE-19 and 9.36 gN/kgTS in SE-45 (Table 6). Carbon content had low variation among the tested cultivars with the values ranging from 43 to 45% TS after DAS 66. The C/N ratio of the tested cultivars varied from 21 (SE-19) to 25.6 (Mega Sweet) after 50 DAS. The corresponding C/N ratios after 80 DAS were 34.6 in Mega Sweet and 60 in SE-19 indicating that nitrogen assimilation is dependent on the crop growth stage and varied from flowering to dough stage in the tested cultivars. It is also observed that the both carbon and nitrogen content decreased throughout the harvest periods. The Carbon content of all three cultivars decreased from 35.90-76.07 (DAS 50) to 42.50-43.16 (DAS 80).

Table 6. Effect of harvest time on chemical composition of sweet sorghum cultivars harvested at 50, 66 and 80 DAS.

Sample	Days after sowing **(DAS)**	Total solids **(% w/w)**	Volatile solids **(% w/w)**	Total Kjeldahl Phosphorus **(g/kg TS)**	Total Kjeldahl Nitrogen **(g/kg TS)**	Carbon **%TS**	Nitrogen **%TS**	**C/N**
SE-19	50	13.65	12.67	5.86	26.37	35.90	1.68	21.15
SE-45	50	12.41	11.25	0.97	5.24	76.07	3.30	23.22
Mega Sweet	50	14.24	13	6.18	34.97	61.66	2.39	25.58
SE-19	66	24.78	23.32	1.41	9.36	44.47	0.85	52.4
Mega Sweet	66	22.64	21.03	1.46	9.67	45.54	0.71	66.1
SE-45	66	23.2	22.15	1.21	10.13	42.84	0.65	64.87
SE-19	80	26.25	25.02	2.21	7.58	43.16	0.72	60.02
SE-45	80	22.55	21.53	2.62	9.40	42.66	0.98	43.54
Mega Sweet	80	26.2	24.76	2.14	7.79	42.52	1.22	34.44

4.1.2 Effect of harvest time on methane yields of sweet sorghum cultivars

The effect of harvest time on methane production from SE-19, SE-45 and Mega Sweet cultivars was determined in batch experiments at 37 °C and the results are presented in Figure 4 and Table 7. Results showed that harvest time and cultivar type had a profound influence on methane yields. Methane production started immediately with all tested cultivars and at all harvest times. However, higher methane production rates and yields were noticed with cultivars harvested at 66 and 80 DAS than at 50 DAS.

After 50 DAS, Mega Sweet had the highest methane yields (108.64 NL $CH_4/kgVS_{added}$) than SE-19 and SE-45. However, there was no significant difference in the methane yields among the three tested cultivars after 66 DAS. After 80 DAS, highest methane yields of 243.47 NL $CH_4/kgVS_{added}$ was obtained for Mega Sweet. The corresponding methane yields obtained for SE-19 and SE-45 after 80 DAS were 168.99 and 184.39 NL $CH_4/kgVS_{added}$, respectively.
The results for sorghum cultivars in this study were comparable to those of Amon and Amon [275] , who reported BMP values for winter wheat at the milk stage, dough stage, and at maturity of 0.254, 0.228, and 0.245 LCH_4/gVS_{added}, respectively. The vast majority of research indicate a gradual decrease in the growth of biomass as it reaches maturity. The duration required for plant biomass to reach a plateau and cease significant development varies depending on the specific cultivar and environmental conditions, typically occurring within a range of 90 to 100 days after sowing (DAS). This phenomenon typically takes place prior to the complete maturation of the plant, as a result of the distinct impact of the continuous buildup of assimilates in the stem and reproductive structures, along with the shedding of dead leaves. In contrast, research on a number of other crop species, including reed canary grass [276], switchgrass [277], napiergrass [278], cereals [275], and maize [279], also discovered a declining effect of progressive maturity and harvest date on SMY of these crops. The lignin content of many grass species rose throughout increasing maturation, as shown by Dien and Jung [280]; it may therefore be inferred that the same holds true for sweet sorghum cultivars used in this study..

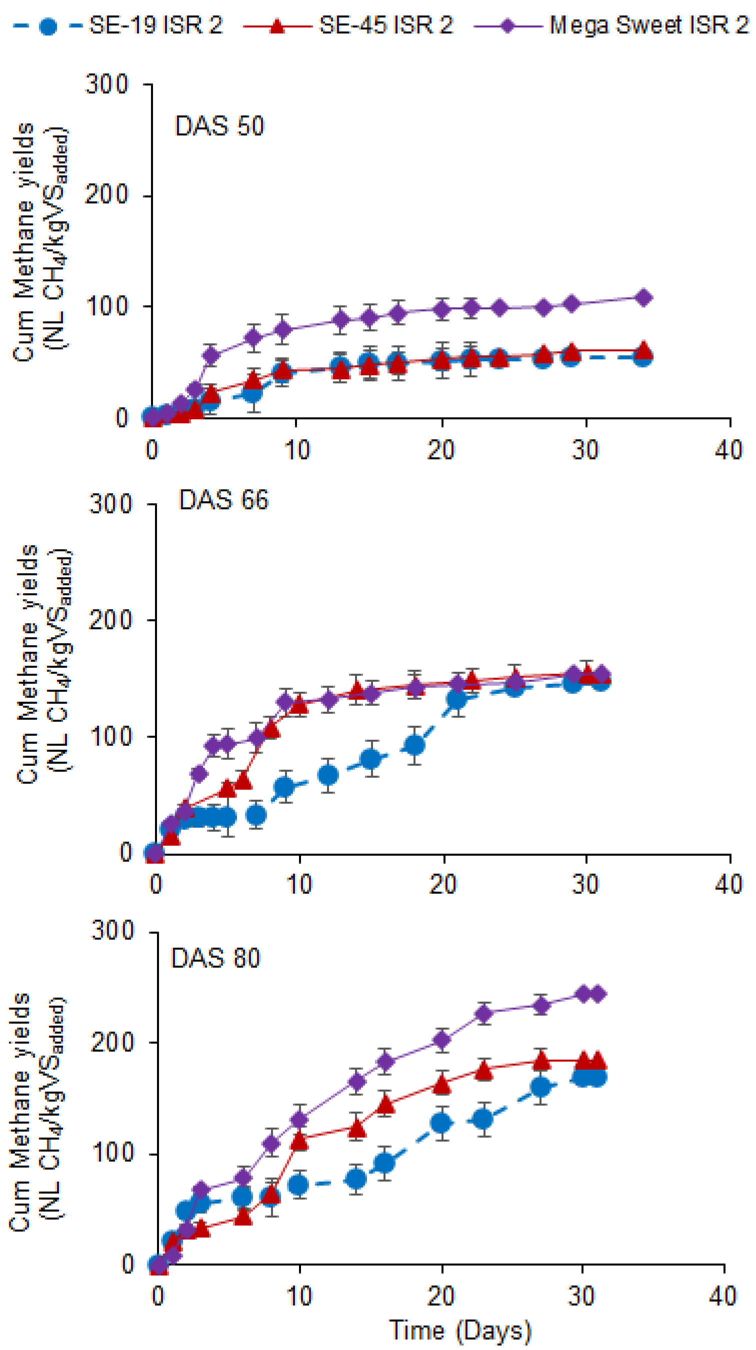

Figure 4. Effect of harvest time (50, 66 and 80 DAS) on methane yields from SE-19, SE-45 and Mega Sweet incubated in batch assays at 37 °C.

Table 7. Biomass yields, and methane yields obtained for the tested sweet sorghum cultivars harvested after 50, 66 and 80 DAS.

DAS	Sweet sorghum Cultivar	Biomass yields (t/ha)	Methane yields				
			($Nm^3/kgVS_{added}$)	($Nm^3/kgTS$)	(Nm^3/tFM)	($Nm^3/ha/a$)	% Increase
50	SE-19	81	0.055	0.01	10.95	889.22	-
	SE-45	79	0.061	0.01	6.89	541.55	-
	Mega Sweet	61	0.109	0.02	14.13	865.16	-
66	SE-19	111	0.148	0.03	31.92	3549.05	37.16
	SE-45	102	0.155	0.04	34.39	3501.25	39.35
	Mega Sweet	60	0.155	0.04	32.68	1954.46	70.32
80	SE-19	132	0.169	0.04	42.26	5561.54	32.54
	SE-45	174	0.184	0.04	39.71	6893.37	33.15
	Mega Sweet	71	0.243	0.06	60.29	4268.59	44.86

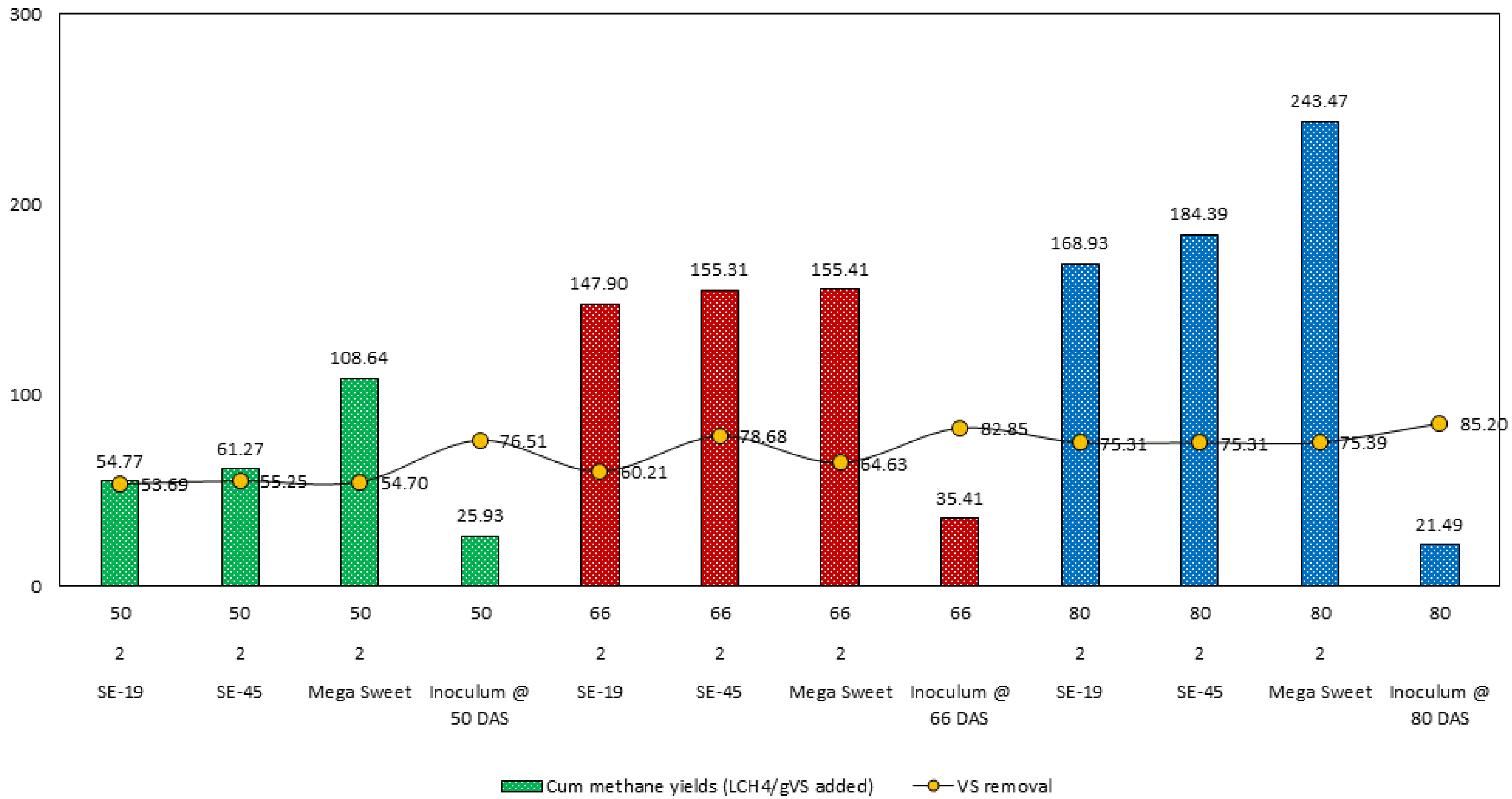

Figure 5. VS removal (%) and mean methane yields obtained for sweet sorghum cultivars SE-19, SE-45 and Mega Sweet harvested at 50, 66 and 80 DAS.

4.1.3 Assessment of digestate quality of the three cultivars harvested at DAS 50,66 and 80

The effect of harvest time on digestate of assays from SE-19, SE-45 and Mega Sweet cultivars was determined and the results are presented in Table 8 and Table 9. The change in pH values observed in digestates primarily result from the decomposition of volatile fatty acids (VFA) and the generation of ammonia (NH_3) during the anaerobic digestion process. It is noticed that at slightly higher pH values , there is higher ammonia accumulation in the assays after 30-day incubation period. Additionally, there is decrease in ammonia concentration from DAS 50 (977.68 – 1060.50 mg/L) to DAS 80 (871.63 – 880.72 mg/L). This can be attributed to higher carbon nitrogen of the cultivars at later harvest stages. However, ammonia accumulation in each of the assays are negligible to inhibit the methanogenesis process as it is < 3 g/L.

Table 8. Digestate parameters of BMP assays of three studied cultivars at 50,66 and 80 days after sowing (DAS).

Sample	pH	DAS	NO_2-N	NOx-N	NH_4-N	PO_4-P
			mg/L			
SE-19	7.45	50	1.33	64.32	977.68	54.74
SE-45	7.43	50	0.74	65.11	1060.50	55.65
Mega Sweet	7.42	50	1.09	64.86	1050.40	54.54
SE-19	7.48	66	0.00	79.79	926.17	103.02
SE-45	7.46	66	1.01	78.78	894.86	63.63
Mega Sweet	7.39	66	0.00	79.79	903.95	89.89
SE-19	7.41	80	0.00	77.77	874.66	79.79
SE-45	7.38	80	1.01	76.76	880.72	65.65
Mega Sweet	7.35	80	0.00	76.76	871.63	108.07

As shown in Table 9, the amount of volatile fatty acids (VFAs) exhibited a slight increase with increase in DAS of the cultivar added in the assay and unregulated pH. However, it is noteworthy that these concentrations remained significantly lower compared to those observed under alternative pH circumstances. The results of this study provide confirmation that acidogenesis is effectively suppressed at the pH range 6.8- 7.5. Acetic acid was the major VFA component in all assays ranging up to 69- 89% of the total VFA composition. Additionally, the acetic acid composition also decreased as the DAS increased.

These results were in line with a study conducted by Kreuger and Prade [281],The hemp substrate harvested in July and August exhibited a consistent trend of lower pH levels and higher concentrations of volatile fatty acids (VFAs) compared to those collected in September and October. However, it is noteworthy that the pH values of all the analysed samples remained above 7.

Table 9. Digestate quality of 3 sweet sorghum cultivars at DAS 50,66 and 80

Sample	DAS	Ethanol	Propanol	Butanol	1-Hexanol	Total VFA	Acetic acid	Propionic acid	iso-Butyric acid	Butyric acid	iso-Valeric acid	Valeric acid	4-Methyl valeric acid	Hexanoic acid
SE-19	50	0.9	0	0	0	45.7	40.7	3	0	0	0	2	0	0
SE-45	50	0	0	0	0	39	30.8	4.2	0	0	0	0	0	4
Mega Sweet	50	0	0	0	0	35.8	29.5	4	0	0	0	2.3	0	0
SE-19	66	0	0	0	0	76.75	64.29	10.26	0	0	0	2.2	0	0
SE-45	66	0	0	0	0	43	27	0	0	6.1	0	1.8	2.6	5.5
Mega Sweet	66	0	0	0	0	46.8	29.62	11.92	0	3.01	0	2.25	0	0
SE-19	80	0	0	0	0	8	6.2	0	0	0	0	1.8	0	0
SE-45	80	0	0	0	0	38.5	25.2	0	0	5.3	0	2.1	0	5.9
Mega Sweet	80	0	0	0	0	80.1	50.2	0	0	18.4	1	2.5	0	8

4.1.4 Energy production potential for studied sweet sorghum cultivars at various harvest times.

To evaluate the best sweet sorghum cultivar as feedstock for methane production in a farm-scale biogas plant, biomass yields and methane yields per hectare were calculated by using the biomass yields per hectare. In addition, methane yields based on VS, TS and FM were also calculated. The results are presented in Table 7and Figure 4. As expected, biomass yields per hectare increased with increase in crop duration. After 80 DAS, highest biomass yields of 174 t/ha FM was obtained for SE-45 followed by 132 t/ha and 71 t/ha for SE-19 and Mega Sweet, respectively. Overall, highest methane yields per tonne of VS and fresh biomass were obtained with Mega Sweet (80 DAS) than with SE-19 and SE-45. With only 71 t/ha of biomass yields, Mega Sweet had the lowest methane yields on per hectare basis. Interestingly, SE-45 with biomass yields of 174 t/ha and methane yields of 0.184 Nm^3/t VS_{added}, had the lowest methane yields per t of FM and highest methane yields of per hectare (Table 9). The highest methane yields of 6,893.37 Nm^3 CH_4/ha noticed for SE-45 is equivalent to a gross energy potential of 66.59 MWh/ha or 85.57 passenger cars transport fuel consumption (Table 10). Due to the apparent low biomass yields per hectare, the methane yields per hectare for Mega Sweet remained relatively low (4268.59 m^3 CH_4/ha).

The level of methane yields obtained in this experiment, i.e. 6,893.37 Nm^3 CH_4/ha at DAS 80 (Table 10), is higher than that obtained in studies conducted at intermediate latitudes under temperate climate by Mahmood and Honermeier [231] (4,100 Nm^3 /ha); Barbanti et al. (2014) Barbanti and Di Girolamo [232] (6,000 Nm^3 /ha) and [282] (3,653 Nm^3 /ha). This was the result of higher Dry biomass yields (DBY) despite equivalent Specific Methane yields (SMY).

Table 10. Methane yields per hectare, gross energy potential per hectare, heat, and electricity generation potential along with vehicle fuel and the kilometres driven per hectare of sweet sorghum cultivars harvested at 50, 66 and 80 DAS.

DAS	Cultivar	Gross energy potential (MWh/ha/a)	CHP (85%)		BioCNG (kWh/ha)	Passenger cars (km/ha)
			Electricity (kWh_{el}/ha	Heat (kWh_{th}/ha		
50	SE-19	8.59	3,642.08	3,659.26	871.43	11.04
	SE-45	5.23	2,218.11	2,228.57	530.72	6.72
	Mega Sweet	8.36	3,543.56	3,560.27	847.86	10.74
66	SE-19	34.28	14,536.34	14,604.91	3,478.07	44.06
	SE-45	33.82	14,340.57	14,408.21	3,431.23	43.46
	Mega Sweet	18.88	8,005.15	8,042.91	1,915.37	24.26
80	SE-19	53.72	22,779.19	22,886.64	5,450.31	69.04
	SE-45	66.59	28,234.15	28,367.33	6,755.51	85.57
	Mega Sweet	41.23	17,483.48	17,565.95	4,183.22	52.99

Note: $1m^3$/ton biomethane produces 9.66KWh energy; CHP is calculated as 85% of Gross Energy Potential (Heat – 42%; Electricity – 42%); BioCNG – 98% Biomethane produced.

4.1.4 Kinetics of methane production during the anaerobic digestion studied substrates at different harvest times.

Table 11 presents the kinetic parameters for first-order and modified Gompertz models for the cumulative methane production for the three tested sweet sorghum cultivars SE-19, SE-45 and Mega Sweet harvested at 50, 66 and 80 DAS. These parameters are used to establish a fit between the experimental and the predicted methane yields. Hydrolysis process is considered as the rate-limiting step in the first-order kinetic model, whereas the bacterial growth and inhibition process is considered as the rate-limiting step of the modified Gompertz model [283].

The hydrolysis coefficient, k_{hyd}, in the first-order kinetic model described the rate of hydrolysis of the substrate. A large k_{hyd} value implies that the hydrolysis of the substrate is at a faster rate. Overall, Mega Sweet had the highest k_{hyd} value of 0.21 d^{-1} (50 DAS), 0.20 d^{-1} (66 DAS) and 0.06 d^{-1} (80 DAS) indicating that the rate of hydrolysis for Mega Sweet was the fastest among the three tested cultivars and at all three harvest times (Table 11). This would be possible due to the presence of higher amount of soluble organic compounds in Mega Sweet than in SE-19 and SE-45. This was also evident from the highest ultimate methane yields (B_o) noticed for Mega Sweet than for SE-19 and SE-45. It can be noticed that the k_{hyd} value decreased with an increase in the harvest time (Table 11). On the other hand, the B_o value increased with an increase in the harvest time. Both these results indicate that the rate of hydrolysis decreased with maturity.

Modified Gompertz model uses three parameters for the prediction of methane production from the assays. Maximum methane potential of the assay (B_0), specific rate constant (R_m) and the lag phase (λ). Results showed that lag phase decreased with crop maturity. SE-19 had the highest lag phase of 1.56 d at 50 DAS. The corresponding values for SE-45 and Mega Sweet were 0.7 (Table 11). The higher value of R_m will result in a faster rate of methane production. R_m showed an increasing trend with harvest time. Cultivars harvest at 66 DAS had the highest R_m than at 50 and 80 DAS. Among the cultivars, Mega Sweet had the highest R_m at all harvest times. However, highest R_m values noticed at 66 DAS did not result in highest G_0 values indicating accumulation of organic acids and methane production was inhibited. Thus, it can be concluded that harvest time had a profound influence on both methane production and its rate of production.

Table 11. Kinetic parameters for first-order and modified Gompertz models for the three sweet sorghum cultivars harvested at 50, 66 and 80 DAS and incubated in batch experiments at 37 °C.

		First order							Modified Gompertz							
Substrate	DAS	Exp BMP	B_0	t-delay	khyd	R^2	RMSE %	% Difference	Rmax	λ	G_0	R^2	RMSE %	% Difference	T_{90}	T_{eff}
SE-19	50	52.34	56.13	1.76	0.14	0.9872	6.89	0.75	4.96	1.54	53.87	0.9907	5.86	1.25	16.55	15.01
SE-19	66	147.90	303.70	0.00	0.02	0.9649	13.28	12.88	5.79	0.00	190.05	0.9755	11.11	11.31	21.42	21.42
SE-19	80	159.29	254.32	0.00	0.03	0.9218	16.50	3.99	5.72	0.00	456.13	0.9502	13.17	3.03	25.97	25.97
SE-45	50	55.03	58.98	1.39	0.14	0.9836	7.32	1.61	5.19	0.72	56.50	0.9746	9.11	1.29	22.17	21.45
SE-45	66	155.27	161.08	0.33	0.13	0.9738	8.88	1.34	14.03	0.45	154.07	0.9828	7.20	0.96	13.66	13.21
SE-45	80	184.15	237.73	0.00	0.05	0.9770	9.70	1.27	10.16	0.50	193.77	0.9853	7.75	1.14	20.44	19.94
Mega Sweet	50	98.76	101.51	0.93	0.19	0.9862	6.15	0.92	10.61	0.00	99.43	0.9732	8.56	0.22	10.31	10.31
Mega Sweet	66	145.61	150.25	0.10	0.20	0.9847	5.81	1.46	19.85	0.00	145.24	0.9701	8.12	0.36	16.02	16.02
Mega Sweet	80	234.31	291.41	0.01	0.06	0.9941	4.80	0.08	12.94	0.00	248.81	0.9872	7.05	0.03	14.74	14.74

Note: DAS- Days after sowing; SD: Standard Deviation; Expt BMP: Experimental Biochemical methane yields (NmL/gVS_{added}); R_{max} = Maximum methane production rate (NmL/gVS.d); (G_0): Maximum cumulative methane yield (NmL/gVS_{added}); λ = lag phase (d); (B_0): Maximum cum methane yield (NmL/gVS_{added}); k_{hyd} (d^{-1}): Hydrolysis constant; T-delay: Time delay (d).

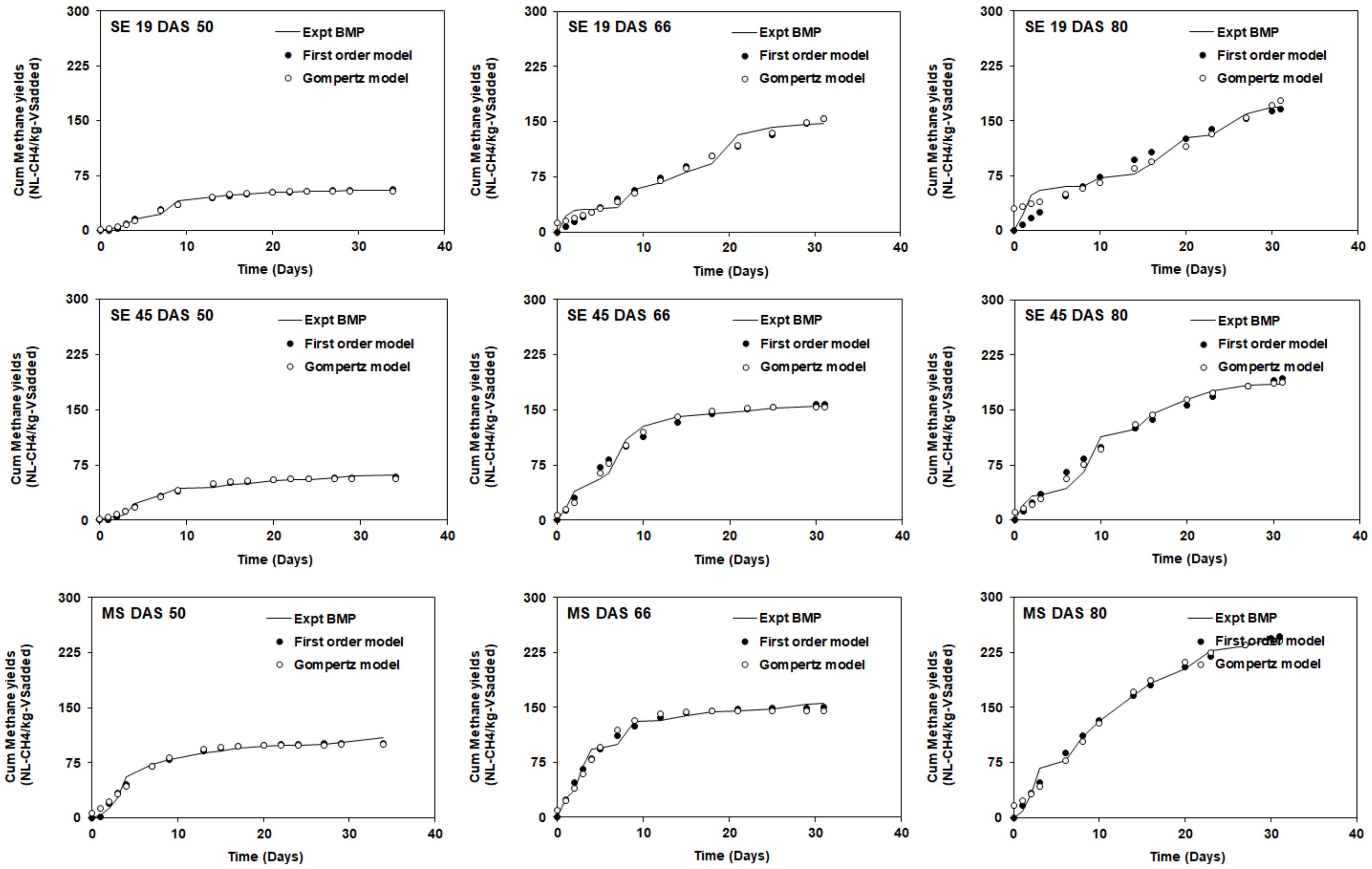

Figure 6. Kinetic parameters during the anaerobic digestion of three sweet sorghum cultivars incubated in batch digestion at DAS 50,66 and 80

It can be observed that the difference between experimental and model data decreased as DAS increased from 50 to 80. This is obviously due to the higher carbon content in the biomass present at DAS 80 and thus required a shorter adaptation time for the methanogenic bacteria to overcome the inhibition due to metabolites. This is evident from the decreasing lag value for the assays as DAS increased gradually (Table 11).

The data shows that all cultivars exhibited the best Rmax value ranging from 10.61-19.84 at DAS 80. The longest lag phase was seen when cultivar SE-19 was utilized at all DAS, with a duration ranging from 0.72 to 1.54 days The observed occurrence can be attributed to the higher lower carbon nitrogen ratio and lower volatile solids compared to the other two cultivars resulting in lower methane yields.. The correlation coefficients (R^2 - 0.997-0.999) for the various inoculum sources indicate that the first order model exhibit strong correlations and are better suited for modelling methane production and estimating the lag phase. Compared to the first order kinetic model, the modified Gompertz model produced a smaller difference between the measured and predicted gas yield and a larger R^2 value. Among the two utilized models, the modified Gompertz model was found to be more compatible with experimental findings.

Technical digestion time (T90), defined as the time required to produce 90% of the maximum methane production was calculated to analyse the performance of the process. T90, as an indicator of the methane production rate, is generally used to estimate the hydraulic retention time of a continuous reactor.

The T_{90} for all cultivars decreased with each DAS i.e., 16.55-25.97 days for DAS 50, 13.66 – 22.17 days for DAS 66 and finally 10.31-16.02 days for DAS 80. The result mentioned showed that the DAS 80 cultivar's duration was shorter than the cultivars harvested at DAS 50 and 66.

4.2 Effect of ISR ratio on methane yields from sweet sorghum

4.2.1 Chemical composition of the studied sweet sorghum cultivars

The effect of ISRs of 2, 4, and 6 on methane yields from nine sweet sorghum cultivars was determined in batch experiments at 37 °C. Table 12 presents the chemical composition of the studied sweet sorghum cultivars. Results showed that TS content in the nine sweet sorghum cultivars ranged between 12.05% (SE-23) and 21.40% w/w (Mega Sweet). A similar trend was also noticed with VS. The high VS/TS of 0.89-0.95 indicates that all the tested cultivars were rich in organic matter and are considered as ideal substrates for biogas production. This result agrees with past studies were substrates having high VS/TS where rich in organic matter [284]. The carbon content in sweet sorghum cultivars was more or less similar. SE-1 had the highest carbon content of 48.8 %TS whilst SE-42 and SE-23 had the lowest carbon content of 47.0 %TS. On the other hand, nitrogen content in the tested cultivars was in the range of 0.6 to 1.10 %TS. Thus, carbon to nitrogen (C/N) ratio ranged from a low 43.33 (SE-45) to high value of 81.36 (SE-1). However, the optimum C/N ratio for anaerobic digestion (AD) is 20-30:1 and is considered to provide sufficient nitrogen that can promote the growth of microbes and boost the degradation of organic carbon [285]. Nonetheless high VS/TS and C/N ratios indicates that the substrates are rich in carbon that maybe difficult to degrade during the AD process due to the presence of lignin and hydrolysis might be the rate limiting step for these substrates. The concentrations of TKN and TKP in the substrates are presented inTable 12. TKN values ranged from 4.79gN/kgTS in SE-1 to 7.11gN/kgTS in Mega Sweet. Conversely, SE-23 had the highest TKP (2.50 gN/kgTS). The chemical composition of sweet sorghum determined in this study were in line with literature data. The carbon nitrogen ratio of sweet sorghum in this study were similar to literature studies and ranged from 11-36.9 Gunaseelan [286]; 36-48 Sambusiti and Ficara [287] The cellulose composition in % also ranged between 6.2 – 34.5% FM. Sorghum and other forms of terrestrial biomass feedstocks generally exhibit total solids contents within the range of 20% to 40% dry matter[288]. The anaerobic fermentation of these substrate, with minimal water addition, allows for reduced energy requirements for heating the feed, increased organic loadings, and smaller digester volumes and/or longer hydraulic retention times (HRTs) compared to fermentation systems with more diluted feeds (less than 20% dry matter).

The soluble sugar, carbohydrate and lignin content in the studied cultivars is presented in Table 13. Total soluble sugar content varied from cultivar to cultivar and was shown to be dependent on the plant height and duration. Plant height is highly correlated with crop biomass yield as plant height is dependent on the crop maturity and internode length [289, 290]. The longer the sweet sorghum plant remain in vegetative stage, the greater the number of leaves and nodes it will produce. Thus, late-flowering cultivars are generally taller than early flowering cultivars. On the other hand, in the hybrids, plant height is controlled by dwarf genes and attain same number of leaves and leaf area by shortening of internode length [291]. The high total soluble sugar content in the SE-86 cultivars than in SE-5 or SE-23 cultivars is attributed to the fact that sugar accumulation generally takes place in stem and thus tall plants tend to accumulate more total soluble sugars[292] . In the present study SE-86 is 3.26 m tall compared to SE-5 (2.8 m) or SE-23 (1.96 m). The number of leaves for taller plants with heights >2.5 also increased to 13- 14. However, higher leaves do not necessarily guarantee higher total soluble sugars further indicating that soluble sugars are predominantly located in the stalk of sorghum cultivars rather than leaves. These results agree with previous studies where sucrose concentration in Sweet sorghum was shown to increase rapidly after flowering [293] and was shown to correlate with carbohydrate metabolism enzymes and the onset and extent of sugar accumulation in both sugarcane (Saccharum spp.) and sweet and grain (non-sweet) sorghums [294-296]. However, a decline in sucrose-degrading activity was shown to be a prerequisite for sugar accumulation [297]. However, there is no apparent difference in activities of specific enzymes and higher stem sugar concentration in sweet vs grain Sweet sorghum cultivars [298]

Table 12. Chemical composition of sweet sorghum cultivars

Substrate	TS (% w/w)	VS (% w/w)	Moisture (%)	VS/TS	C (%TS)	N (%TS)	C/N	TKN (gN/kgTS)	TKP (gP/kgTS)
Inoculum	2.98	2.07	97.0	0.70	34.5	6.2	5.5	55.9	26.8
SE-1	20.31	19.32	79.6	0.95	48.8	0.6	81.3	4.7	1.5
SE-5	15.24	14.29	84.7	0.94	48.7	0.7	69.5	5.9	1.5
SE-23	12.05	10.71	87.9	0.89	47.0	1.0	47.0	8.5	2.5
SE-35	14.25	13.11	85.7	0.92	47.8	1.1	43.5	6.0	1.8
SE-42	19.67	18.54	80.3	0.94	47.0	0.9	52.2	5.2	2.3
SE-45	18.26	17.24	81.7	0.94	47.7	1.1	43.3	6.9	1.5
SE-81	19.36	18.25	80.6	0.94	48.4	0.8	60.5	6.0	2.2
SE-86	19.61	18.54	80.3	0.95	47.3	0.8	59.1	5.7	1.7
Mega Sweet	21.40	19.83	78.6	0.93	47.5	1.	47.5	7.1	2.3

The cellulose content in the tested sweet sorghum cultivars ranged between 25.80 and 37.50 %TS. SE-42 had the highest cellulose content (37 %TS) whilst the lowest cellulose content was noticed in SE-81 (25.81 %TS). A similar trend was also noticed with hemicellulose and lignin. However, no significant difference in the lignin composition across the tested cultivars was noticed suggesting that the cellulose content did not increase proportionately with increase in lignin content. Both these results suggests that biomass yields were directly related to the total insoluble sugars especially with carbohydrate and lignin composition in a cultivar (Table 13). In Sweet sorghum, carbohydrates are generally accumulated and stored in the stems. This limits the amount of carbohydrates available for storage in the grain [299]. Opposite is true with grain sorghum, where most of the carbohydrates are diverted and stored in the grain [291].

Table 13. Plant height, biomass yields (65 days after sowing, DAS) along with total soluble and insoluble sugar content in the nine sweet sorghum cultivars.

Substrate	Plant Height (m)	Number of leaves	Fresh Biomass (t/ha)	Total soluble Sugars (%TS)	Cellulose (%TS)	Xylan (%TS)	Arabinan (%TS)	Galactan (%TS)	Lignin (%TS)	Total soluble Sugars (t/ha)	Total insoluble Sugars (t/ha)	Total Sugars (t/ha)
SE-1	1.86	11	67	12.3	33.2	18.2	1.6	2.4	19.2	8.24	37.12	45.36
SE-5	2.8	14	57.4	1.4	31.8	18.4	1.5	2.4	24.5	0.80	31.05	31.86
SE-23	1.96	12	78.4	1.4	35.5	19.3	1.1	1.7	22.3	1.10	45.16	46.26
SE-35	2.56	12	82.3	3.1	30.3	17	0	2.1	24.4	2.55	40.66	43.21
SE-42	3.93	14	67	2.1	37.5	21.1	1.4	2.3	25.1	1.41	41.74	43.15
SE-45	3.58	14	58.4	11.9	28.1	16.4	1.2	2.1	23.2	6.95	27.92	34.86
SE-81	3.12	14	77.5	9.2	25.8	14.4	1	1.8	23.2	7.13	33.33	40.46
SE-86	3.26	13	70.8	13.5	27.5	15.6	1.1	2	22.8	9.56	32.71	42.27
Mega Sweet	2.88	13	46.9	9.6	26.9	15.3	1.3	1.9	24.1	4.50	21.29	25.80

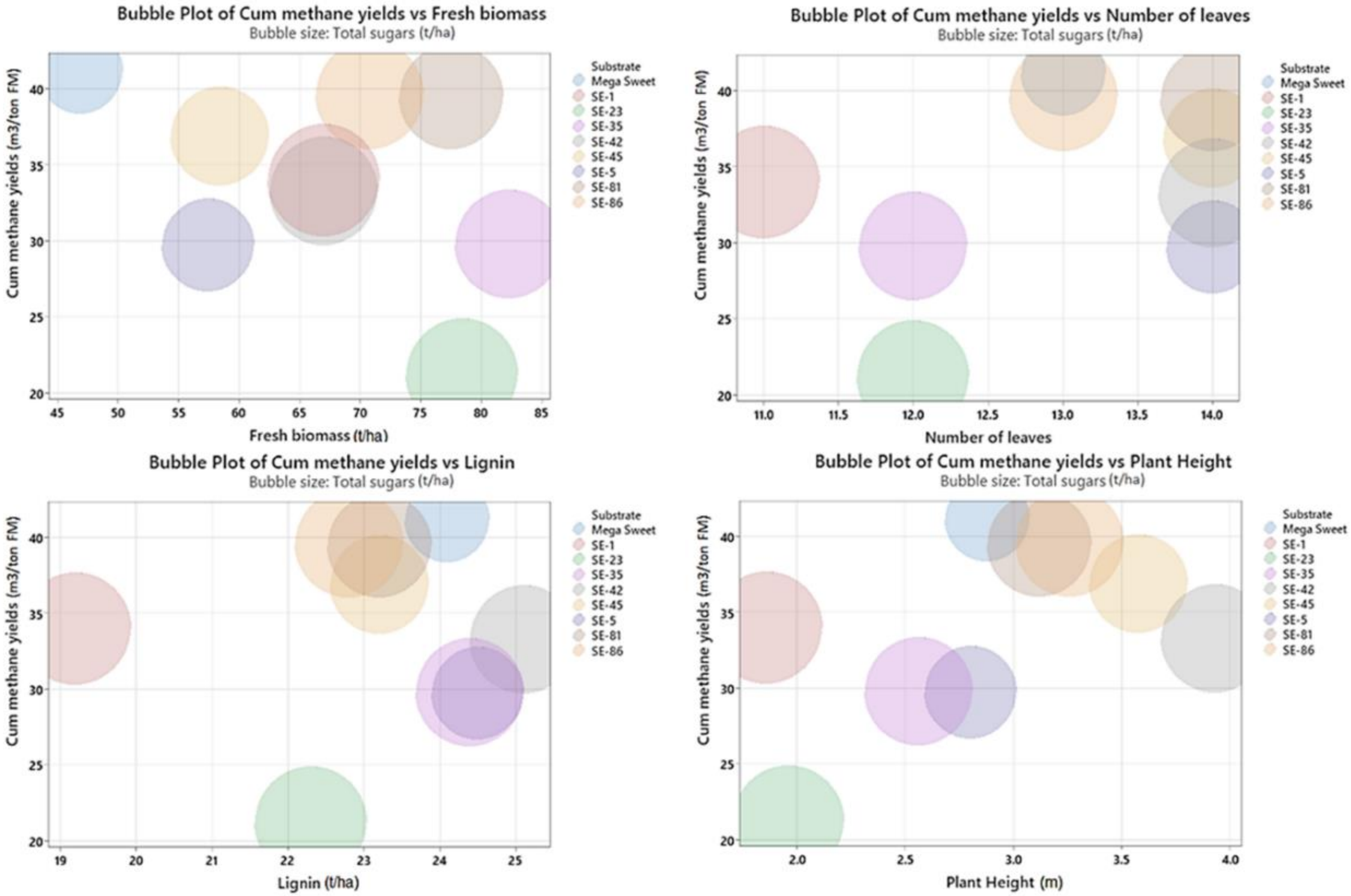

Figure 7. Bubble plots representing relationship of chemical composition and cumulative methane yields from the tested nine sweet sorghum cultivars.

4.2.2 Effect of ISR on biochemical methane potential of studied substrates

Results on the effect of ISR ratios of 2, 4, and 6 on the methane yields of nine sweet sorghum cultivars are presented in Table 12 and Figures 8 to 10. Results showed that ISR had a profound influence on methane production, with methane yields increasing with increase in ISR ratio. This is due to the fact that use of higher amount of inoculum will lead to an improved biodegradability due to high microorganism per assay and have sufficient amount of inoculum to overcome the inhibition due to metabolites and/or can adapt to inhibition [300]. Methane production started immediately in all assays with a slight lag phase (0.5 to 1.2 days) at ISR 2. No lag phase was noticed at higher ISRs of 4 and 6. However, higher methane production rates and yields were noticed at ISR 6 than ISR 4 and 2 (Figure 8 and Table 14).

Methane production rates and yields were also influenced by sweet sorghum cultivars. Highest methane yields were obtained for SE-35 at all tested ISR ratios indicating that higher the organic matter, and better VS/TS ratio, the higher the methane yields (Table 13). Further, high methane yields obtained for SE-35 at all tested ISR ratios could also be due to lowest C/N ratio of 43.5 among the studied sweet sorghum cultivars (Table 12). The optimal C/N ratio for AD process is around 20-30:1 [285]. On the other hand, the low methane yields noticed for other tested cultivars suggests that the high C/N ratio might have led to build-up of organic acids and lack of sufficient inoculum to adapt, especially at low ISR of 2 [301]. This limitation can be overcome by addition of more inoculum or codigestion with nitrogen rich substrates such as slaughter waste or food wastes that can adjust the C/N ratio optimal for AD process.

The BMP of each cultivars found in this study when the ISR ratio was greater than 2 is significantly similar than the range discovered in the literature (280-366 LCH_4 /$kgVS_{added}$) [279]. Likewise similar studies on inoculum to substrate ratios conducted by Alzate and Muñoz [302] and Zhou and Zhang [303] have both reported a comparable pattern in methane yields in relation to substrate loading. They found that the highest methane yields were achieved at inoculum to substrate ratios of 2 and 1.67, respectively. Therefore, it can be inferred that higher methane yields are achievable when inoculum to substrate ratios lie within

2-6 for lignocellulosic substrates. In addition, influence of cultivar on methane yields in our study were in the range of 175 - 227 NL/gVS$_{added}$. These results are consistent with those of several previous studies where sweet sorghum hydrids were used for methane production. Herrmann et al.[304] reported that sorghum hybrid (Sorghum bicolor x Sudanese) VS-based methane production was 317.1 Nm^3/Mg. The production of methane in Mahmood and Honermeier's [231] study was comparable to that observed in the current investigation. Nevertheless, these authors reported a higher VS-based production of methane for Rona 1–387 Nm^3/Mg than the results of the current study–349 Nm^3/MgVS (337 Nm^3 /Mg TS). In the Czech Republic, Pazderu et al.[191] noted that methane production was substantially affected by sorghum hybrids and varied between 207 and 246 Nm^3 /Mg TS. In a separate study conducted in Germany, sorghum hybrids produced biogas at substantially different rates [201]. The most significant determinants of methane production from sorghum are hybrid selection and the choosing of an appropriate harvesting date [305]. According to the findings of Herrmann and Idler [306], crops exhibiting lower amounts of acid detergent fiber (ADF) and acid detergent lignin (ADL) were associated with higher methane output. Thus, it can be inferred that sorghum cultivar in our study has a reduced methane output as the lignin content is higher. Moreover, the methane yield derived from the biochemical methane potential (BMP) test frequently exceeds the yield observed in continuous studies. According to the findings of Richards et al. (1991), the BMP test demonstrated that sorghum has the potential to achieve a methane yield of 320 mL-CH_4/g-VS when subjected to a temperature of 55°C. In their study, Sambusiti and Ficara [287] documented a methane yield of 269 mL-CH_4/g-VS through a biochemical methane potential (BMP) test conducted at a temperature of 35°C. Notably, this methane yield was achieved without any form of pretreatment, using a Sorghum Sudanese hybrid as the substrate.

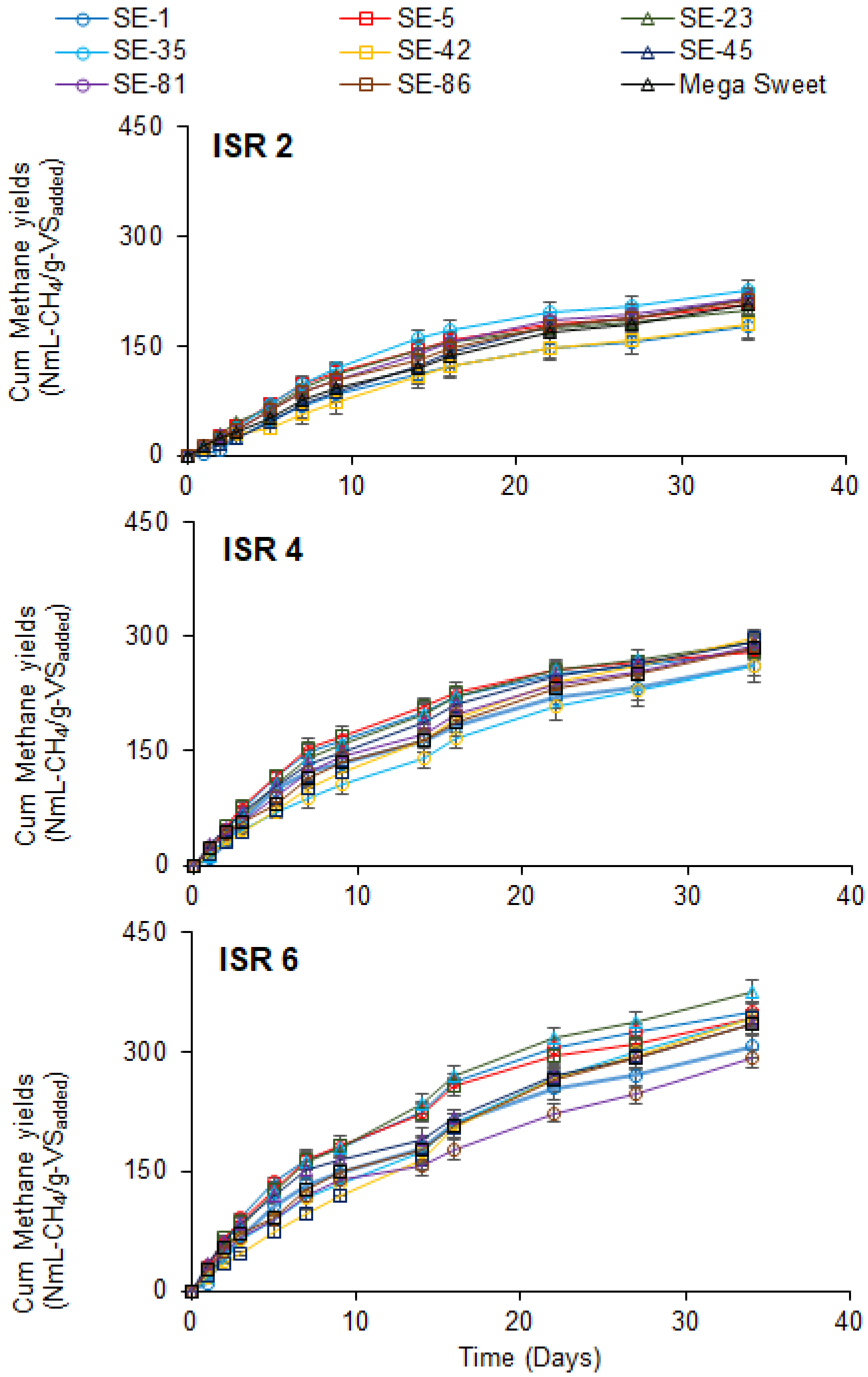

Figure 8. Effect of ISR (2,4 and 6) on methane yields during the anaerobic digestion of nine sweet sorghum cultivars in batch experiment at 37°C.

Figure 8 and Table 14 presents the methane yields obtained at the tested three ISR ratios for the nine sweet sorghum cultivars. At ISR 2, methane yields ranged from a low 175.93 NL CH_4/kgVS_{added} (SE-1) to the highest value of 291.27 NL CH_4/kgVS_{added} (SE-35). Increase in ISR from 2 to 4 resulted in an increase in methane by 67.6-76.4%. Further increase in ISR to 6 resulted in an increase in methane yields by 46.8% to 78.1%. Overall, SE-35 was the best cultivar followed by SE-5, SE-23, SE-42, and SE-45 (342-349 NL CH_4/kgVS_{added}). Among the tested cultivars, SE-1 had the lowest methane yields of 175.93-306.86 NL CH_4/kgVS_{added}). Despite SE-1 having a cellulose content >30 %TS and lower lignin content of 19.20 %TS, lower methane yields were obtained for this cultivar. This suggests that the AD efficiency cannot be calculated solely based on the carbohydrate composition as it is also influenced by other physiochemical factors of the cultivar as well as process conditions provided for AD.

4.2.3 Kinetics of methane production rates and yields with the nine cultivars at ISR ratios 2,4 and 6

The kinetics of the methane production was studied through first-order model and the modified Gompertz equations. The models were used to analyse the methane production potential (B_o), the maximum methane production rate (R_{max}) and lag phase (d) parameters. These parameters were obtained after adjusting the experimental data to the first-order model and to the modified Gompertz equation in order to identify the best fit and to analyse the influence of the ISR on the kinetic parameters. A summary of the kinetic parameters obtained from both models is presented in Table 14. The experimental data of methane production was compared with the equivalent parameters B_o and G_o obtained from first-order and modified Gompertz equations, respectively. It can be observed that the difference between experimental and model data increased as ISR increased from 2 to 6, in particularly with first-order model. This is obviously due to the low amount of inoculum present at ISR 2 and thus required a longer adaptation time for the methanogenic bacteria to overcome the inhibition due to metabolites. This is evident from the higher lag value for the assays incubated at ISR 2 (Table 12). The results also showed that first-order model best fitted the experimental data mainly because no lag phase was observed. The first-order model does not consider the presence of a lag phase and describes the AD process once the exponential phase of methane production has started. However, when the process needs longer adaptation periods modified Gompertz equations fits better and is evident from the higher determination coefficients (R^2).

It can be observed that the highest R_{max} value was noticed for SE-35 followed by SE-23 and SE-5 (Table 14). The value of time delay λ determines the minimum time required for biogas production [307]. Despite higher biogas production rate, the time constant for SE-35, according to Gompertz model, was higher than the substrate producing lower biogas yields such as Mega Sweet. This could be possible due to the readily available sugars in Mega Sweet that are easily degraded to produce biogas. In addition, the time delay (λ) constant decreased with increase in ISR indicating that the growth rate of microorganisms was faster at higher inoculum loading rates than lower loading rates. Similarly, hydrolysis constant in first-order modelling decreased with increase in ISR.

Irrespective of the ISR, the T_{90} for all cultivars had similar ranges (24 – 29 days). This suggests that in spite of higher inoculum addition, the substrate has reached its maximum degradability by T_{90}. Additionally, T_{eff} increased with increase in ISR for all cultivars. Maximum T_{eff} was noticed for SE-86 (29.46 days) and minimum T_{eff} of 24.38 for SE-5. Higher R^2 values and shorter lag phases were observed for Modified Gompertz model . Therefore, it can be inferred that Modified Gompertz model best fitted the current experimental results.

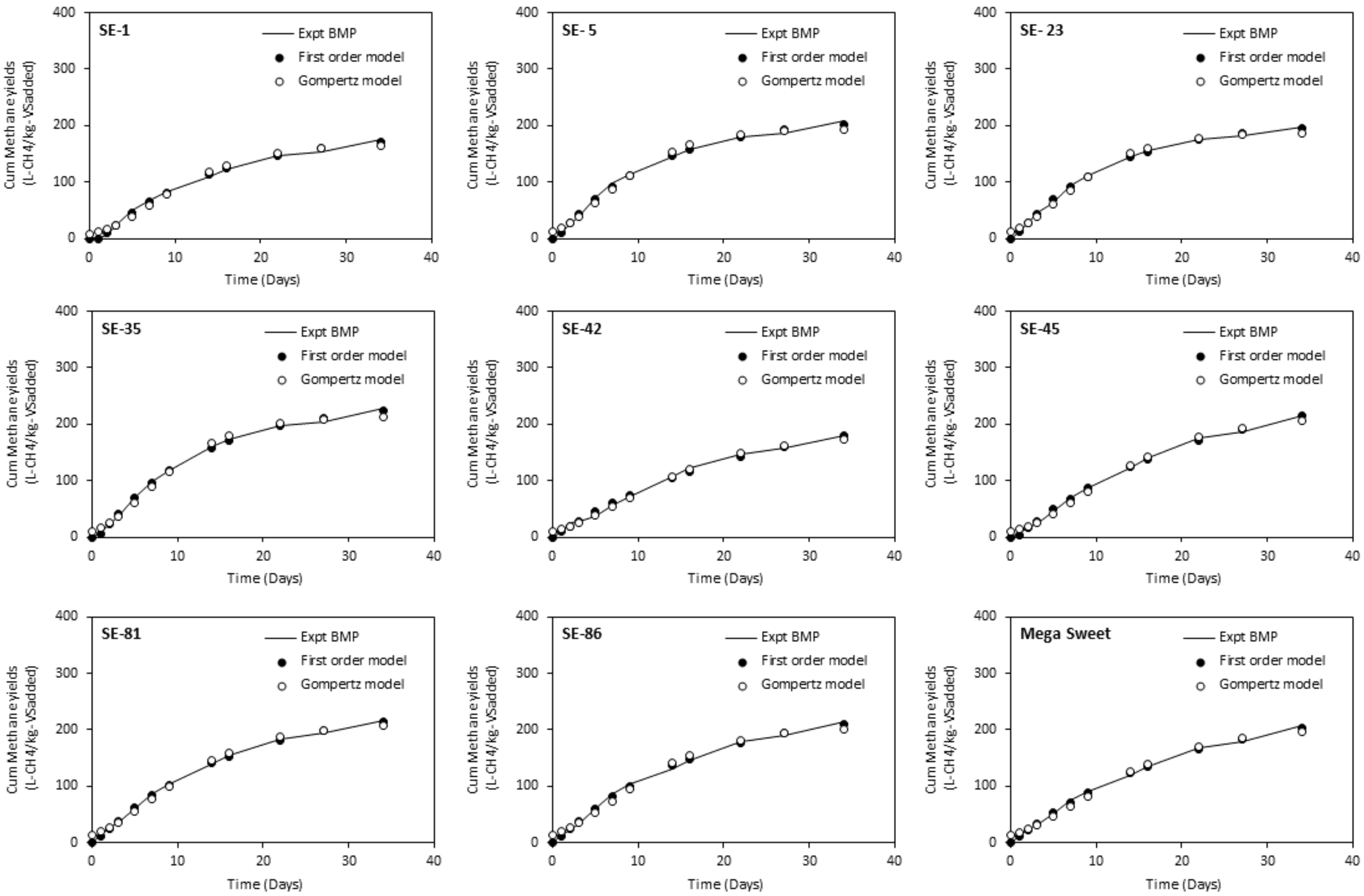

Figure 9. Kinetic parameters during the anaerobic digestion nine sweet sorghum cultivars incubated in batch digestion at ISR 2.

Table 14. Experimental and modelling methane yields obtained at different ISR for the tested sweet sorghum cultivars.

Cultivar	ISR	Expt BMP	SD	First-order						Modified Gompertz						
				B_o	T-delay	k_{hyd}	R^2	RMSE %	% Difference	R_{max}	λ	G_o	R^2	% Difference	T_{90}	T_{eff}
SE-1	2	175.93	15.1	188.61	1.22	0.07	1	4.09	2.80	9.61	0.93	166.62	0.98	6.96	28.30	27.37
	4	262.35	11.2	274.76	0.02	0.07	0.99	6.88	3.86	14.73	0	242.81	0.97	8.73	27.82	27.82
	6	306.86	14.2	333.14	0	0.06	0.99	6.73	3.41	15.73	0	290.7	0.97	7.24	28.14	28.14
SE-5	2	208.05	9.81	214.27	0.46	0.09	1	2.90	2.74	12.69	0.1	194.88	0.99	7.21	26.99	26.89
	4	283.27	2.24	285.69	0.02	0.1	1	3.86	3.06	19.62	0	261.25	0.98	8.35	24.43	24.43
	6	349.76	11.9	366.52	0	0.08	0.99	5.41	2.19	20.67	0	331.95	0.97	6.09	24.38	24.38
SE-23	2	198.37	9.5	206.9	0.32	0.09	1	2.44	1.35	12.29	0	188.79	0.99	5.60	24.32	24.32
	4	280.37	17.7	283.77	0.09	0.1	1	2.93	1.81	20.53	0	262.95	0.98	6.66	21.47	21.47
	6	342.45	11.3	350.59	0	0.08	0.99	5.14	3.66	20.81	0	317.93	0.97	8.07	26.71	26.71
SE-35	2	227.48	12.8	238.4	0.75	0.08	1	2.69	1.95	13.93	0.58	214.51	0.99	6.61	27.05	26.46
	4	291.27	16.2	301.22	0.39	0.09	1	3.71	2.09	17.82	0	275.19	0.98	6.36	24.32	24.32
	6	375.91	14.8	414.29	0	0.07	1	4.09	1.81	19.81	0	360.04	0.98	5.99	27.33	27.33
SE-42	2	179.66	20.8	236.24	0.07	0.04	1	4.92	0.36	8.16	0.42	181.05	0.99	3.67	28.49	28.07
	4	260.29	7.3	336.06	0	0.04	1	3.90	0.90	11.06	0	266.96	0.99	3.39	28.30	28.30
	6	342.47	10.7	433.63	0	0.04	1	5.20	1.08	14.31	0	350.94	0.99	2.90	28.56	28.56
SE-45	2	214.3	11	273.84	0.71	0.05	1	3.32	0.11	10.03	1	214.02	0.99	4.19	28.54	27.53
	4	297.6	3.57	395.87	0.07	0.04	1	2.47	0.14	12.87	0.2	303.26	0.99	3.63	28.33	28.13
	6	341.86	9.52	446.3	0	0.03	1	13.68	13.98	13.96	0.6	360.64	0.99	17.45	28.82	28.62

Note: SD: Standard Deviation; Expt BMP: Experimental Biochemical methane yields (mL/gVS); R_{max} = Maximum methane production rate (mL/gVS. d); G_0: Maximum cumulative methane yield (mL/gVS); λ = lag phase (d); B_0: Maximum cum methane yield (mL/gVS); k_{hyd} (d^{-1}): Hydrolysis constant; T-delay: Time delay (d).

Table 15. Experimental and modelling methane yields obtained at different ISR for the tested sweet sorghum cultivars (Contd).

Cultivar	ISR	Expt BMP	SD	First-order						Modified Gompertz						
				B_o	T-delay	k_{hyd}	R^2	RMSE %	% Difference	R_{max}	λ	G_o	R^2	% Difference	T_{90}	T_{eff}
SE-81	2	216.31	11.3	245.53	0.24	0.06	1	2.06	0.61	11.08	0.08	210.83	0.99	4.71	27.05	26.97
	4	291.87	1.39	306.49	0	0.08	1	4.46	3.04	16.57	0	274.81	0.97	7.05	26.66	26.66
	6	334.31	3.78	344.61	0	0.07	0.98	9.33	6.08	17.8	0	310.86	0.96	8.87	28.44	28.44
SE-86	2	213.75	12.2	242.33	0.2	0.06	1	3.46	1.72	10.57	0	206.51	0.99	5.84	28.03	28.03
	4	286.7	22.8	313.72	0	0.06	1	4.35	2.87	14.77	0	272.75	0.98	6.81	27.81	27.81
	6	290.94	9.43	316.05	0	0.06	0.98	9.42	6.29	13.9	0	273.54	0.96	8.12	29.46	29.46
Mega Sweet	2	207.47	14.8	259.42	0	0.05	1	3.12	1.83	9.18	0	206.28	0.99	5.01	28.86	28.86
	4	284.55	11.8	325.6	0	0.06	1	4.85	2.44	13.49	0	277.86	0.98	5.43	28.02	28.02
	6	333.93	13.2	389.88	0	0.05	0.99	7.58	2.98	15.15	0	330.29	0.98	4.21	28.37	28.37

Note: SD: Standard Deviation; Expt BMP: Experimental Biochemical methane yields (mL/gVS); R_{max} = Maximum methane production rate (mL/gVS. d); G_0: Maximum cumulative methane yield (mL/gVS); λ = lag phase (d); B_0: Maximum cum methane yield (mL/gVS); k_{hyd} (d^{-1}): Hydrolysis constant; T-delay: Time delay (d).

4.2.4 Correlation of chemical composition parameters and methane yields using principal component analysis

Table 16 presents the principal component analysis (PCA) results to analyse the relationship between chemical composition, biomass yields, total/volatile solids and, C/N ratio on methane yields. Results showed that methane yields and the other parameters were significantly correlated, as indicated by correlation coefficients over 0.6. To determine a meaningful association between several operational factors and total methane production, a Pearson correlation matrix was used. According to Table 13, Positive loading was visible in the first factor space for cumulative methane yields, lignin, and biomass yields (>0). A similar positive loading was also noticed with feed VS and the C/N ratio in the second factor space, F2. The biplot was drawn for these two components because, according to the screen plot, F1 and F2 account for 70% of the variation in the analysis.

Table 16.Prinicpal component analysis showing the relation between different parameters at three loadings.

	F1	F2	F3
FM VS (%)	-0.008	0.850	-0.305
C/N	-0.536	0.586	0.347
Cellulose	-0.868	-0.475	-0.106
Hemicellulose	-0.889	-0.357	-0.268
Lignin	0.410	-0.486	-0.662
Biomass yields (t/ha)	0.012	-0.513	0.751
Methane yields (NL $CH_{4/}gVS_{added}$)	0.939	-0.218	0.123

Figure 10 shows that Mega sweet, SE-86, SE-81, and SE-45 all have similar characteristics and can be grouped together. Table 17 also provides a detailed explanation of how the factors influence the grouping of these observations. According to this graph, lignin%, biomass yields, and cum methane yields have a negative impact on these cultivars while having a positive impact on the plant SE-35. This is explained by Table 12, which shows that the highest methane and biomass yields of SE-35 resulted in higher cum methane yields. Nonetheless, the cultivars SE-45, SE-81, SE-86, and Mega Sweet produce less methane and contain more volatile solids. As a result, it can be deduced that these cultivars were grouped together based on volatile solids content. This statement can be corroborated from Table 12, where the volatile solids range from >20% cultivars fall beyond 20%.

Table 17. Correlation factors between variables and observations

Variables	FM VS (%)	C/N	Cellulose	Hemicellulose	Lignin	Biomass yields (t/ha)	Cum methane yields (NL CH_4/kgVS$_{added}$)
FM VS (%)	1	0.319	-0.341	-0.224	-0.156	-0.494	-0.287
C/N	0.319	1	0.144	0.194	-0.589	-0.072	-0.498
Cellulose	-0.341	0.144	1	0.967	-0.048	0.173	-0.726
Hemicellulose	-0.224	0.194	0.967	1	-0.009	-0.038	-0.775
Lignin	-0.156	-0.589	-0.048	-0.009	1	-0.149	0.433
Biomass yields (t/ha)	-0.494	-0.072	0.173	-0.038	-0.149	1	0.167
Cum methane yields (NL CH_4/kgVS$_{added}$)	-0.287	-0.498	-0.726	-0.775	0.433	0.167	1

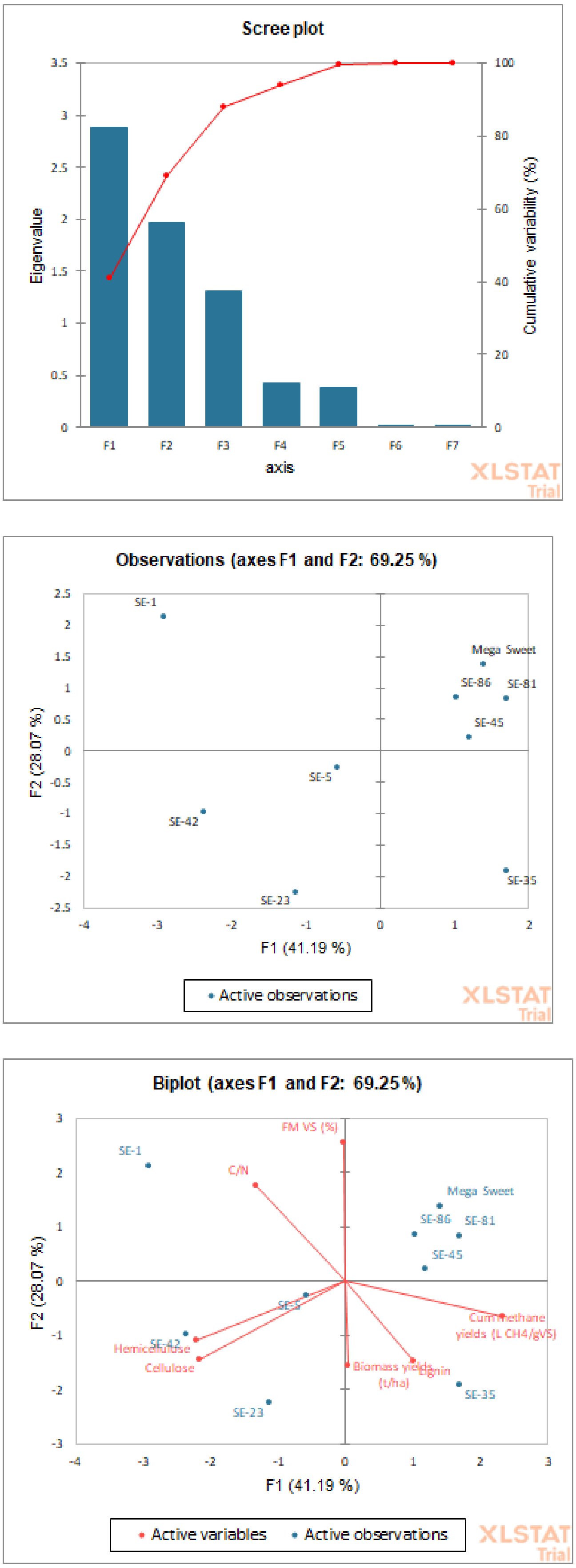

Figure 10. Scree plot, biplot for active variables and observation for the studied cultivars.

4.3 Effect of inoculum source on methane production rates and yields from sweet sorghum cultivars

4.3.1 Chemical composition of inoculum on substrates

The effect of different inocula sources such as rumen fluid along with anaerobically digested cow manure, and sewage sludge on methane production rates and yields of five sweet sorghum cultivars (SE-1, SE-19, SE-20, SE-35 and SE-122) obtained from South Australia was studied. The chemical composition of three inocula is presented in Table 18. It is noticed that the C/N ratio of both cow manure digestate and rumen fluid fall in the range of 10-16 while sewage sludge digestate had a C/N ratio of 6. For a stable anaerobic digestion process, a C/N ratio ranging between 20-40 was reported as an optimal ratio. On the other hand, sweet sorghum cultivars were rich in carbon source and have significantly higher C/N ratio of 24.7-30.3. One of the reasons for lower methane production from these substrates can be attributed to lack of nitrogen supplements during the AD process. As a result, changing inoculum source to a nitrogen rich medium such as manure or slaughterhouse or food wastes are beneficial to improve the methane yields of sweet sorghum. In addition, microbiology composition of the inoculum is a primary factor that influences the biochemical methane potential of the substrates.

Table 18. Chemical composition of five sweet sorghum cultivars and three inocula used for this study

Substrate	TS (% w/w)	VS (% w/w)	VS/TS	Moisture (% w/w)	C (%TS)	N (%TS)	C/N	TKN (g N/kgTS)	TKP (g P/kgTS)
SE-1	26.56	25.83	0.97	73.44	44.5	1.8	24.72	16.93	1.74
SE-19	43.9	41.89	0.95	56.1	45.5	1.6	28.44	15.36	1.95
SE-20	41.74	39.8	0.95	58.26	45.5	1.5	30.33	16.31	1.28
SE-35	26.8	25.29	0.94	73.2	42.7	1.6	26.69	16.42	2.10)
SE-122	31.15	28.81	0.92	68.85	43.5	1.9	22.89	18.79	2.52
Anaerobic sludge	2.98	2.07	0.69	97.02	32.36	5.19	6.24	50.59	28.35
Cow Manure digestate	4.35	3.29	0.76	95.65	38.9	2.9	13.41	25.59	13.24
Rumen fluid	2.91	1.99	0.68	97.09	29.55	2.14	13.81	17.76	10.54

4.3.2 Effect of different inoculum source on methane yields of sweet sorghum cultivars

The effect of inoculum source on methane production rates and yields of five sweet sorghum cultivars is presented in Figure 11 and Table 19 respectively. Results showed that inoculum had a profound influence on the methane production rates and methane yields. With sewage sludge and cow manure digestate, methane production started immediately and showed a higher methane production rate than with rumen fluid for the initial 20 days of incubation. On the other hand, methane production in assays with rumen fluid showed a lag phase of 1-2 and the production rates were low for the initial 20 days. Thereafter, methane production rates in assays with cow manure digestate were higher than with rumen fluid and sewage sludge. Overall, higher methane yields were obtained with cow manure digestate followed by rumen fluid than with sewage sludge. The methane yields clearly indicate that hydrolysis of lignocellulosic feedstocks was the rate limiting step and was influenced by different inoculum sources [308]. Among the tested inoculum sources, rumen fluid contains large quantities of cellulolytic bacteria that can efficiently degrade complex lignocellulosic structures into simple sugars, as determined by microbiological analysis (Data not sown). Matsuki et al. [309] reported that methane production increased by 1.5-3 times when the substrate was pre-treated with rumen fluid prior to anaerobic digestion. Despite the addition of rumen fluid, which has lignin-degrading microorganisms and fibrolytic microorganisms capable of anaerobic lignin degradation the degradation of lignocellulosic components into volatile fatty acids (VFAs) remained poor, as demonstrated by measurements of methane yields and chemical composition of the digestate. These results were also in agreement with a similar study conducted by Takizawa and Baba [310]. However, the maximum methane production was noticed when cow manure digestate was used as inoculum. It is also noticed that VFA accumulation was the lowest for cow manure. While rumen fluid is rich in cellulolytic microorganisms, digestate from cow manure is rich in both hydrolytic and methanogenic microorganisms. A similar study conducted also stated that highest removal of carbohydrates were removed from cow manure digestate which resulted in higher methane production [311]. Furthermore, cow manure digestate contains an additional source of trace elements and nitrogen, which further improves the C/N ratio in assays.

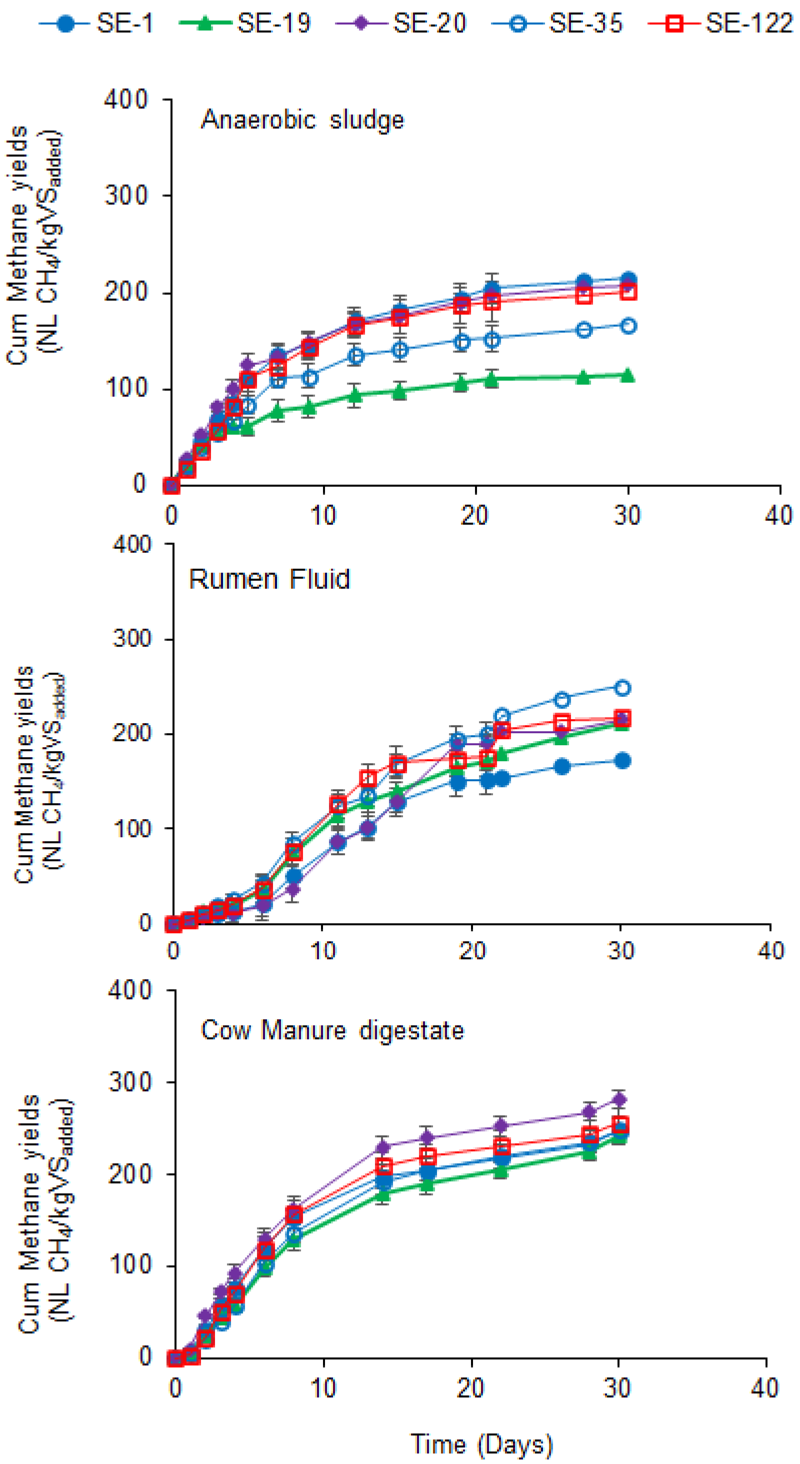

Figure 11. Methane yields of five sweet sorghum cultivars with anaerobic sludge , rumen fluid and cow manure digestate

Mean cumulative methane yields with the studied inocula sources rumen fluid and anaerobic sewage sludge are presented in Table 16. Results showed that methane yields were profoundly influenced by the tested inocula sources. Methane yields in assays incubated with rumen fluid was 172.45-249.75 NL $CH_4/kgVS_{added}$. The corresponding values with anaerobic sewage sludge and cow manure digestate were 114.19-215.35 NL $CH_4/kgVS_{added}$ and 241.82 – 281.43 NL $CH_4/kgVS_{added}$, respectively.

Table 19. Cumulative methane yields of sweet sorghum for different inoculum source.

Sweet sorghum cultivars	Rumen Fluid	Anaerobic sewage sludge	Cow manure digestate
	Cum CH_4 yields (NL CH_4/kg-VS_{added})	Cum CH_4 yields (NL CH_4/kg-VS_{added})	Cum CH_4 yields (NL CH_4/kg-VS_{added})
SE-1	172.45±7.45	215.35±6.49	246.99±9.74
SE-19	211.24±9.33	114.19±7.53	241.82±2.08
SE-20	214.89±2.87	207.9±9.05	281.43±2.32
SE-35	249.73±7.45	167.35±13	248.01±3.75
SE-122	216.67±10.1	202.09±13	256.08±2.33
Inoculum source	28.82±3.51	90.7±53.5	41.1±1.13

With rumen fluid, relatively high methane yields were noticed with all sweet sorghum cultivars except for SE-1 (Figure 11). The increase in methane yields over SE-1 was 49.22-84.99%. The improved methane yields noticed in assays with rumen fluid was probable due to the difference in the chemical composition of the sweet sorghum. Rumen fluid is premeditated to breakdown cellulose and lignin so that cattle can easily digest the complex lignocellulosic crops [312]. Rumen fluid is generally rich in hydrolytic bacteria that can easily degrade the complex carbohydrates and proteins from crops [69]. Some studies suggest that rumen fluid consists of certain bacterial species that can liberate cellulolytic enzymes that cannot be normally degraded by anaerobic sludge and thereby improve methane yields [313],[309]. Therefore, use of rumen fluid as inoculum to improve the hydrolysis and hydrogen/VFA production from lignocellulose substrate through two-stage AD process consisting of first-stage dark H_2 fermentation and second-stage methane fermentation can improve the hydrolysis and methane yields from the methanogenic reactor and the overall energy balance.

In the present study, rumen fluid produced 4-fold higher VFAs than with anaerobic sludge demonstrating that rumen fluid is much more efficient in hydrolysis than anaerobic sludge [314]. Further, ammonium concentration increased sharply in assays incubated with rumen fluid especially at the initial stages of the incubation. Although rumen fluid was found to be more potent inoculum source for anaerobic digestion, it is necessary to account for the ammonia production. Excessive ammonia production in the AD indicates that the microorganisms have degraded the proteins to produce amino acids and ammonia [315]. However, as the pH increases, the microorganism cease methane production and focus on degrading the ammonia to stabilize the pH thereby resulting in lower methane yields. Although it is not feasible to produce rumen fluid on a large scale, there is a scope for extracting the rumen fluid bacteria and enriching them via an artificial rumen fluid system. In addition, the potential of rumen fluid for biohydrogen production needs to be investigated. Unlike rumen fluid, cow manure is essentially another rich source of anaerobic mixed culture for AD of lignocellulosic crops [316]. In addition to high nitrogen content, cow manure digestate already contains cellulolytic microbes from the rumen of the cattle and thus it has perfect balance of the microbial consortium required for a stable AD process. Finally, it is also noticed in Figure 11, that the lag phase for hydrolysis in all assays incubated with cow manure digestate was lower than rumen fluid. Therefore, use of cow manure digestate as an inoculum source for anaerobic digestion of sweet sorghum is an ideal option for rural biogas plants. As can be seen in our study that cow manure digestate produced the highest methane yields for all cultivars. In fact, the methane yields of all cultivars were increased by 14-43% respectively as compared to anaerobic sludge. The methane yields were also consistent with similar study conducted by Riau and Burgos [317], where rye grass and black oats were used to produce 276-287 NL CH_4/kgVS when cow manure digestate was used.

The VFA results for five sweet sorghum cultivars (SE-1, SE-19, SE-20, SE-35 and SE – 122) for various inoculum sources are presented in Table 20. It is noticed that total VFA ranged from 21.51 – 68.94 mg/L for anaerobic sludge , 5.16 – 17.02 mg/L for cow manure digestate followed by 1.93- 4.49 mg/L for rumen fluid, the accumulation of total VFA was observed for anaerobic sludge as an inoculum source at end of the BMP assay. The VFA results for five sweet sorghum cultivars (SE-1, SE-19, SE-20, SE-35 and SE – 122) for various inoculum sources are presented in Table 20. It is noticed that total VFA ranged from 21.51 – 68.94 mg/L for anaerobic sludge ,5.16 – 17.02 mg/L for cow manure digestate followed by 1.93- 4.49 mg/L for 112 rumen fluid. While the maximum accumulation of total VFA was observed for anaerobic sludge as an inoculum source at end of the BMP assay. Total VFA measured at the end of the BMP test cannot be used to represent the maximum accumulation of total VFA during a BMP. Essentially there is no correlation between maximum accumulation and the VFA concentration at the end.

Table 20. Effect of inoculum source on digestate of five sweet sorghum cultivars at ISR 2

Sample	**Inoculum source**	**ISR**	**Ethanol**	**Propanol**	**Butanol**	**1-Hexanol**	**Total VFA**	**Acetic acid**	**Propionic acid**	**iso-Butyric acid**	**Butyric acid**	**iso-Valeric acid**	**Valeric acid**	**4-Methyl valeric acid**	**Hexanoic acid**
SE-1	AS	2	0	0	0	0	30.69	18.63	7.56	0	2.7	0	1.8	0	0
SE-122	AS	2	0	0	0	0	21.51	13.05	2.61	0	0	0.81	2.16	0	2.88
SE-19	AS	2	0	0	0	0	23.13	16.83	0	0	3.06	0.72	2.52	0	0
SE-20	AS	2	0	0	0	0	68.94	42.57	3.96	0	4.5	0	1.98	0	15.93
SE-35	AS	2	0	0	0	0	31.32	15.12	1.35	0	3.15	0	2.07	4.5	5.13
SE-1	CMD	2	0	0	0	0	10.18	2.12	0.24	0.52	0.85	0.14	0.33	4.1	1.88
SE-122	CMD	2	0	0	0	0	12.6	2.22	0.21	0.16	0.52	0	0.27	8.49	0.73
SE-19	CMD	2	0	0	0	0	5.16	1.49	0	0.13	0.58	0	0.28	1.75	0.93
SE-20	CMD	2	0	0	0	0	17.02	1.77	0.22	0.34	0.7	0.14	0.25	12.64	0.96
SE-35	CMD	2	0	0	0	0	9.46	2.15	0.22	0.29	0.54	0.29	0.25	4.76	0.96
SE-1	RF	2	0	0	0	0	2.55	1.48	0.34	0	0.24	0.25	0.24	0	0
SE-122	RF	2	0	0	0	0	4.49	1.82	0.35	1.18	0.28	0.67	0	0.18	0
SE-19	RF	2	0	0	0	0	2.43	1.52	0.23	0	0.14	0.26	0.19	0	0.09
SE-20	RF	2	0	0	0	0	2.37	1.31	0.41	0.41	0	0.25	0	0	0
SE-35	RF	2	0	0	0	0	1.93	1.03	0.34	0	0.27	0.28	0	0	0

Note: AS: Anaerobic sludge; CMD : Cow manure digestate; RF: Rumen fluid;

In their study, Wang and Zhang [318] examined the impact of varying concentrations of volatile fatty acids (VFAs) on the growth of methanogenic microorganisms and the subsequent production of methane. The researchers observed that the presence of propionic acid at concentrations below 300 mg/L did not affect the activity of methanogenic and acidogenic bacteria. However, a notable suppression of these bacterial activities was observed when the initial propionic acid concentration reached 900 mg/L. Degradation of fibrous or cellulosic materials is likely to produce a higher molar proportion of acetate and a lower proportion of propionate. This is corroborated by the extremely low propionic acid yields() in Table 20 for all inoculum sources. However, feed with low fibre content would be expected to result in a reduction in the acetate: propionate ratio during rumen fermentation [319]. Methane is produced mainly when feedstocks are fermented to acetate and butyrate, with propionate yielding gas only due to buffering of acid [320].

4.3.3 Kinetics of methane production rates and yields with various inoculum sources

A summary of the kinetic parameters obtained for both first order and modified Gompertz models is shown in Table 21**.** The experimental methane yields data was compared with the equivalent parameters B_o and G_o obtained from first order and modified Gompertz equations, respectively. For rumen fluid, modified Gompertz model fitted well with the experimental data. On the other hand, first order model fitted well for the experimental data obtained with anaerobic sludge. The first order model best fitted the experimental data with anaerobic sludge mainly because no lag phase was observed. The first order model does not consider the presence of a lag phase and describes the AD process once the exponential phase of methane production has started. However, when the process needs longer adaptation periods modified Gompertz equations fits better and is evident from the higher determination coefficients (R^2) for assays incubated with rumen fluid.

The data shows that the SE-20 exhibited the best Rmax value when used with anaerobic sludge. Conversely, the SE-122 demonstrated the highest R_{max} value when combined with rumen fluid and cow manure digestate. The longest lag phase was seen when rumen fluid was utilized as the source of inoculum, with a duration ranging from 2.4 to 5.7 days. The observed occurrence can be attributed to the buildup of volatile fatty acids (VFAs) resulting from the metabolic activity of cellulolytic bacteria during the hydrolysis stage of anaerobic digestion [321]. As a result, the process of methanogenesis in rumen fluid exhibited an extended period. The correlation coefficients (R^2 - 0.997-0.999) for the various inoculum sources indicate that the first order model exhibit strong correlations and are better suited for modelling methane production and estimating the lag phase.

The T_{90} values for all cultivars were showing similar values for each inoculum source used i.e., 16.95 -19.89 days for anaerobic sludge, 16.41 – 24.27 days for rumen fluid and finally 20.64 – 24.25 days for cow manure digestate. The result mentioned showed that the cow manure digestate's AD duration was shorter than that of the control group. Additionally, a shorter lag phase, higher T90, and higher methane production rate were found in the cow manure digestate through combined analysis of the maximum cumulative methane production potential [241.82 - 281.43 NL CH_4/kgVS$_{added}$], indicating that using cow manure digestate could hasten the AD process and produce more methane at a faster rate.

Table 21. Experimental and modelling methane yields obtained for the tested sweet sorghum cultivars incubated with rumen fluid, cow manure digestate and anaerobic sludge at ISR 2.

Inoculum source		First-order							Modified Gompertz							
	Substrate	Exp BMP	B_0	t-delay	khyd	R^2	RMSE %	% Difference	Rm	λ	G_0	R^2	RMSE %	% Difference	T_{90}	T_{eff}
AS	SE-1	215.35	215.62	0.16	0.13	0.9971	2.64	1.00	19.90	0.00	202.98	0.9788	7.16	1.83	18.91	18.91
AS	SE-19	114.19	110.26	0.00	0.18	0.9759	6.49	2.12	13.77	0.00	105.54	0.9248	11.45	4.40	17.15	17.15
AS	SE-20	207.90	203.41	0.00	0.16	0.9902	4.52	0.65	22.69	0.00	192.36	0.9619	8.93	2.80	18.25	18.25
AS	SE-35	167.35	165.05	0.09	0.14	0.9949	3.44	2.41	15.95	0.00	155.66	0.9794	6.90	1.35	19.29	19.29
AS	SE-122	202.09	201.09	0.53	0.15	0.9954	3.45	0.24	19.39	0.10	192.06	0.9847	6.33	0.57	17.06	16.95
RF	SE-1	173.26	182.29	5.09	0.11	0.9890	6.58	0.24	11.85	3.88	174.24	0.9957	4.10	2.20	22.58	18.70
RF	SE-19	221.00	249.07	2.63	0.07	0.9895	6.00	2.82	11.84	2.42	215.03	0.9878	6.47	6.26	26.84	24.42
RF	SE-20	223.51	250.67	5.70	0.09	0.9785	9.75	4.10	14.68	5.53	226.10	0.9921	5.91	0.36	21.94	16.41
RF	SE-35	265.54	318.41	2.48	0.06	0.9917	5.33	2.96	13.35	2.50	264.87	0.9918	5.28	6.64	26.57	24.07
RF	SE-122	236.60	249.83	2.91	0.08	0.9780	8.88	9.01	14.89	3.01	220.43	0.9793	8.63	12.65	25.51	22.50
CMD	SE-1	246.99	242.06	0.86	0.13	0.9962	4.23	4.49	21.78	0.71	226.96	0.9880	7.51	8.58	23.81	23.09
CMD	SE-19	241.82	247.29	0.85	0.09	0.9964	4.39	4.32	16.78	0.68	223.75	0.9873	8.28	8.45	25.93	25.25
CMD	SE-20	281.43	283.08	0.59	0.12	0.9983	2.79	2.77	21.99	0.23	265.72	0.9897	6.79	6.08	22.36	22.12
CMD	SE-35	248.01	251.40	1.36	0.11	0.9978	3.50	2.94	19.28	1.12	232.85	0.9919	6.75	6.71	23.36	22.24
CMD	SE-122	256.08	252.99	1.36	0.14	0.9981	3.16	3.26	23.52	1.14	239.53	0.9926	6.19	6.80	21.78	20.64

Note: AS: Anaerobic sludge; CMD : Cow manure digestate; RF: Rumen fluid; SD: Standard Deviation; Expt BMP: Experimental Biochemical methane yields (mL/gVS); Rmax = Maximum methane production rate (mL/gVS. d); (G_0): Maximum cumulative methane yield (mL/gVS); λ = lag phase (d); (B_0): Maximum cum methane yield (mL/gVS); k_{hyd} (d^{-1}): Hydrolysis constant; T-delay: Time delay (d);

4.4 Effect of ISR on volatile fatty acid production rates and yields from sweet sorghum cultivars.

The aim of this study is to identify sweet sorghum cultivars that can produce excessive amounts of volatile fatty acids (VFA) for bioplastic production. Production of VFAs for bioplastics can be achieved by optimising the hydrolysis and acidogenesis step of conventional two-stage anaerobic digestion process. This will promote VFA production and accumulation in the hydrolysis reactor and inhibit the methanogenesis. Inhibition of methanogenesis can be performed by adjusting the pH, adding a chemical inhibitor, such as 2-bromoethanesulfonate (BES), by the presence of O_2, as well as by increasing the substrate loading. In this study, the effect of 0.3, 0.6 and 2 ISR ratios on VFA production from five sweet sorghum cultivars was investigated. Anaerobic digestion was performed at an initial pH of 7.5 and pH was not adjusted thereafter during the 30-d incubation. The chemical composition of sweet sorghum cultivars used in this study is presented in **Error! Reference source not found.**. Chemical analyses showed that TS content varied from a low 26% w/w to 43.9 %w/w. Among the tested cultivars, SE-18 and SE-20 had the highest TS content. In addition, SE-20 also possesses a high C/N ratio and relatively high TKN content compared to other cultivars (Table 17) .

Total VFA and the concentration of individual VFA components are presented in Table 20 and Figure 12 . Highest total VFA value of 5.4 g/L was reported for SE-20 followed by SE-122 (1.6 g/L) at ISR 0.3. Total VFA at ISR 0.6 and 2 were well below the 50 mg/L. The composition of VFAs and their concentrations also varied at the tested ISRs and was dependent on the composition of sweet sorghum cultivars. Among the VFAs, acetate (22-73% of total VFA) was the dominant VFA followed by propionate and butyrate in all assays incubated at ISR of 0.3. However, higher amount of propionate (68% of total VFA of 1.6 g/L) was noticed in SE-122. Among the studied substrates, SE-20 is the only cultivar that produced all VFAs. The major composition of VFAs in SE-20 at ISR of 0.3 was acetate (40%) followed by caproic acid (32%), propionate (10%) and butyrate (9%). On the other hand, SE-122 had propionate (68%) followed by acetate (22%) as the dominant VFAs.

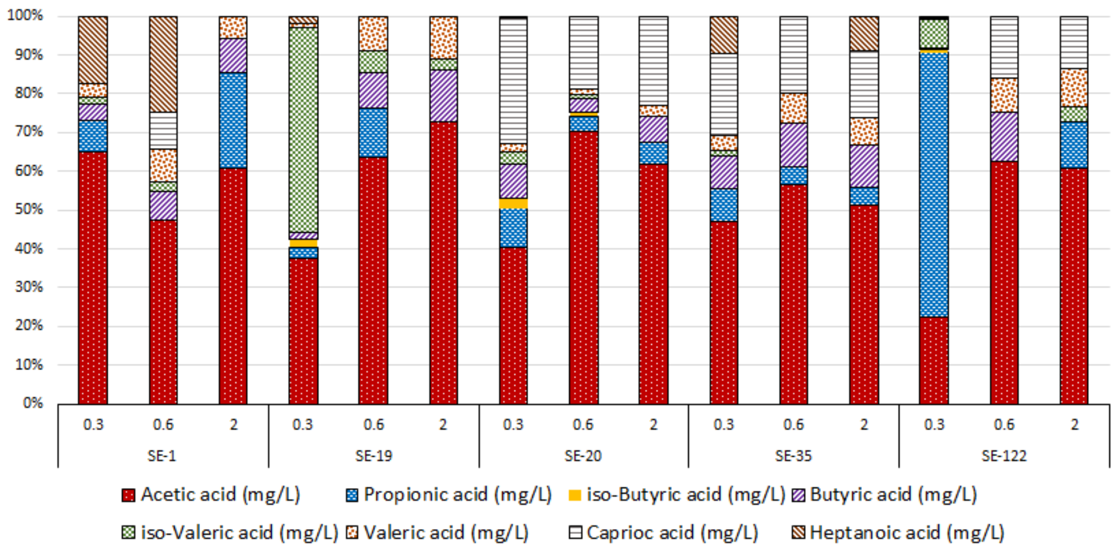

Figure 12. Percentage volatile fatty acids during the anaerobic digestion of sweet sorghum cultivars at ISR 0.3, 0.6 and 2 incubated in batch assays at 37 °C.

Table 22. Effect of ISR on VFA production (mg/L) for sweet sorghum cultivars

Cultivars	ISR	Total VFA	Acetic acid	Propionic acid	iso-Butyric acid	Butyric acid	iso-Valeric acid	Valeric acid	Caproic acid
	0.3	70.92	45.99	5.94	0	2.88	1.17	2.52	0
SE-1	0.6	37.62	17.82	0	0	2.79	0.99	3.15	3.51
	2	30.69	18.63	7.56	0	2.7	0	1.8	0
	0.3	237.15	85.68	6.21	4.68	4.23	119.79	2.7	0
SE-19	0.6	26.82	17.1	3.33	0	2.52	1.44	2.43	0
	2	23.13	16.83	0	0	3.06	0.72	2.52	0
	0.3	5,419.26	2,180.16	549.54	139.59	484.2	173.43	116.19	1,746.45
SE-20	0.6	216.27	148.32	8.1	2.43	6.93	2.16	3.15	39.69
	2	68.94	42.57	3.96	0	4.5	0	1.98	15.93
	0.3	45.63	21.51	3.87	0	3.78	0.72	1.71	9.63
SE-35	0.6	29.07	16.47	1.35	0	3.24	0	2.25	5.76
	2	33.93	15.12	1.35	0	3.15	0	2.07	5.13
	0.3	1,578.33	349.74	1,075.05	13.41	7.2	115.92	6.39	4.68
SE-122	0.6	22.05	13.77	0	0	2.79	0	1.98	3.51
	2	21.51	13.05	2.61	0	0	0.81	2.16	2.88

Although the assays demonstrated the presence of VFAs, there was no significant change in the pH of the system. Flow injection analysis of the assays indicated the presence of ammonium and nitrates, which possibly might have influenced the pH. Higher accumulation of ammonium, nitrogen leads to inhibition of methanogenesis process [322]. Previous studies have shown that low ISR ratios can enhance VFA and hydrogen production [228]. The maximum total volatile fatty acid production was observed for SE-20 at ISR 0.3 (5419.26 mg/L) with acetic acid comprising of 60% of total VFAs. however, the TVFA concentration at the end of the batch test decreased from 5419 mg/L to 68.94 mg/L with increase in ISR. Likewise, SE-122 also showed similar VFA concentrations ranging from 1578.33mg/l at ISR 0.3 to 21.51 mg/L at ISR 2. This indicates that extremely low Inoculum to substrate ratios below 0.5 can be used to optimize dark fermentation process to produce hydrogen and volatile fatty acids. Among the studied cultivars, SE-20 also produced high caproic acid concentration of 1746.45 mg/L at ISR 0.3.

Using the VFA to COD conversion factors, the total VFA in mgCOD and the calculated VFA yield (mgCOD/gVS) are presented in Table 15. Results showed that highest VFA yields of 495.95 mgCOD/gVS was obtained with SE-20 followed by SE-122 (130.6 mgCOD/gVS). Both these results indicate that SE-20 is an ideal feedstock for hydrogen/VFA production. The high VFA yields in SE-20 could be attributed to the biomass composition and high solubilization of the organic materials to VFA production at ISR 0.3.

Table 23. Total VFA (mgCOD) and individual VFA components (mgCOD) along with the VFA yields (VFAmgCOD/gVS) produced during the anaerobic digestion of sweet sorghum cultivars at ISR 0.3, 0.6 and 2 in batch assays at 37 °C.

Cultivars	ISR	Total VFA	Acetic acid	Propionic acid	iso-Butyric acid	Butyric acid	iso-Valeric acid	Valeric acid	Caproic acid	VFA yield (VFAmgCOD/gVS)
SE-1	0.3	70.75	49.03	8.98	0.00	5.23	2.38	5.13	0.00	4.01
	0.6	40.24	19.00	0.00	0.00	5.07	2.02	6.41	7.75	4.57
	2	39.86	19.86	11.43	0.00	4.90	0.00	3.66	0.00	13.57
SE-19	0.3	375.92	91.33	9.39	8.50	7.68	243.89	5.50	0.00	21.34
	0.6	35.72	18.23	5.03	0.00	4.58	2.93	4.95	0.00	4.05
	2	30.09	17.94	0.00	0.00	5.56	1.47	5.13	0.00	10.25
SE-20	0.3	8,738.32	2,324.05	830.90	253.50	879.31	353.10	236.56	3854.42	495.95
	0.6	291.25	158.11	12.25	4.41	12.58	4.40	6.41	87.60	33.06
	2	98.73	45.38	5.99	0.00	8.17	0.00	4.03	35.16	33.62
SE-35	0.3	61.85	22.93	5.85	0.00	6.86	1.47	3.48	21.25	3.51
	0.6	42.78	17.56	2.04	0.00	5.88	0.00	4.58	12.71	4.86
	2	43.92	16.12	2.04	0.00	5.72	0.00	4.21	11.32	14.95
SE-122	0.3	2,301.02	372.82	1625.48	24.35	13.08	236.01	13.01	10.33	130.60
	0.6	31.52	14.68	0.00	0.00	5.07	0.00	4.03	7.75	3.58
	2	30.26	13.91	3.95	0.00	0.00	1.65	4.40	6.36	10.30

4.5 To study the effect of organic loading rate on process performance and biohydrogen yields during dark H_2 fermentation of sweet sorghum cultivars.

In this study, the effect of one-stage reactor configuration consisting of batch dark fermentative H_2 reactor was used to assess the biohydrogen yields from sweet sorghum cultivars. Sweet sorghum cultivars Mega Sweet, SE-45 and SE-35 were used as substrates. Batch dark fermentative biohydrogen experiments were operated for 20 days. Results are presented in Table 25 and Figure 15. Results showed that biohydrogen production rate increased systematically with increase in OLR for all tested sweet sorghum cultivars. The high biohydrogen production noticed with Mega Sweet indicates the presence of high concentration of readily available soluble sugars in Mega Sweet than in SE-35 and SE-45. This also suggests that pretreatment step is necessary to improve biohydrogen yields from SE-45 and SE-35.

Table 24. Composition of 3 cultivars used in biohydrogen production

Sample	TS % (w/w)	VS % (w/w)	Moisture	VS/TS	C (%TS)	N)%TS)	C/N	TKN (g/kgTS)	TKP (g/kgTS)
SE-35	14.25	13.11	85.75	0.92	47.78	1.06	45.25	0.86	0.27
SE-45	18.26	17.24	81.74	0.94	47.67	1.12	42.53	1.26	0.28
Mega Sweet	21.4	19.83	78.6	0.93	47.49	1.01	47.04	1.52	0.50
Anaerobic sludge	2.71	2.00	97.29	0.74	38.43	5.91	6.51	54.67	24.03

Table 25. Effect of organic loading rate (OLR) on process performance and methane yields during the anaerobic mono-digestion of sweet sorghum at 55 °C in CSTR.

Cultivar	OLR (gVS/L/d)	Mean Biogas production (mL/d)	Biogas production (mL/mL feed)	H_2 (%)	Daily H_2 Production (NmL/L/d)	H_2 yields (NL H_2/kg-VS_{ed})	pH	NH_4-N (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	TVFA (mg/L)
SE-35	14	33.87	0.18	43.55	21.35	3.05	5.34	454.50	3,431.15	189.52	664.39	4,445.17
	16	184.61	0.59	44.47	139.43	24.40	5.39	125.93	1,643.86	112.72	1,358.83	3,278.50
SE-45	14	308.09	1.34	52.17	230.35	16.49	5.23	694.88	1,253.60	45.77	388.72	1,688.09
	16	106.15	0.30	40.50	57.50	3.59	5.11	372.69	1,991.50	166.97	1,049.67	3,208.14
Mega Sweet	14	688.08	0.75	25.23	18.39	1.32	5.26	38.89	2,173.39	162.21	1,376.90	3,795.56
	16	3,360.00	3.50	38.07	650.90	40.68	5.21	2.33	2,180.93	140.74	930.62	3,449.48

The TVFA production rate reached up to 5g/L suggesting the potential application for all cultivars in commercial volatile fatty acid production. Simultaneously it is observed that ammonia production was higher for Mega Sweet (694.88mg/L) which was 2 times higher than SE-35. The volatile fatty acid rich slurry shows acetic acid as the dominant acid followed by butyric acid and can be integrated to produce bioplastics using an additional fermentation reactor. The volatile fatty acid composition obtained in this study were in line with similar studies in literature. Zhu and Stadnyk [323] conducted a study where potato waste was used as a substrate for the production of biohydrogen. The resultant process yielded acetic acid and butyric acid at concentrations of 1866 and 1372 mg/L, respectively. It must be emphasized that no pretreatment was performed prior to the start-up pf the reactor. Based on the above results, it can be inferred that the optimal HRT for hydrogenic reactor is 2 days. The decreased hydrogen yield for all cultivars indicates the influence of pH and over accumulation of VFA's in the system. The optimum pH for hydrogen production lies between 4.9-5.5.

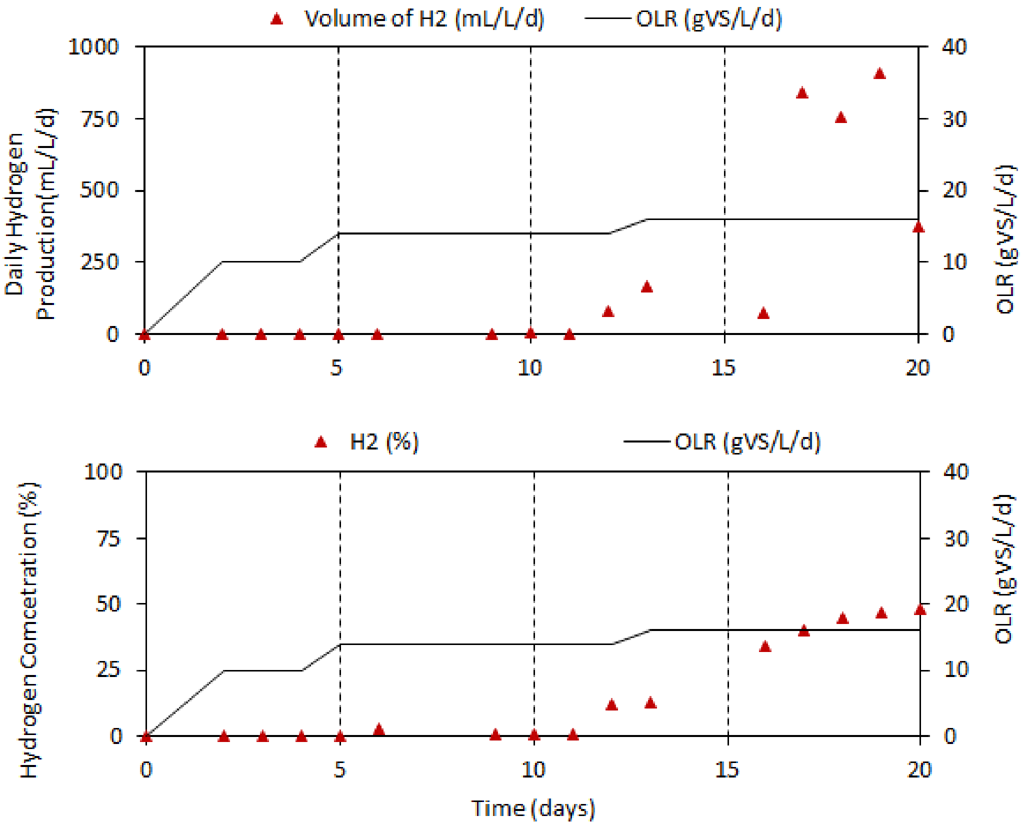

Figure 13. Biohydrogen production rates and yields during the anaerobic dark fermentation of sweet sorghum cultivar Mega Sweet

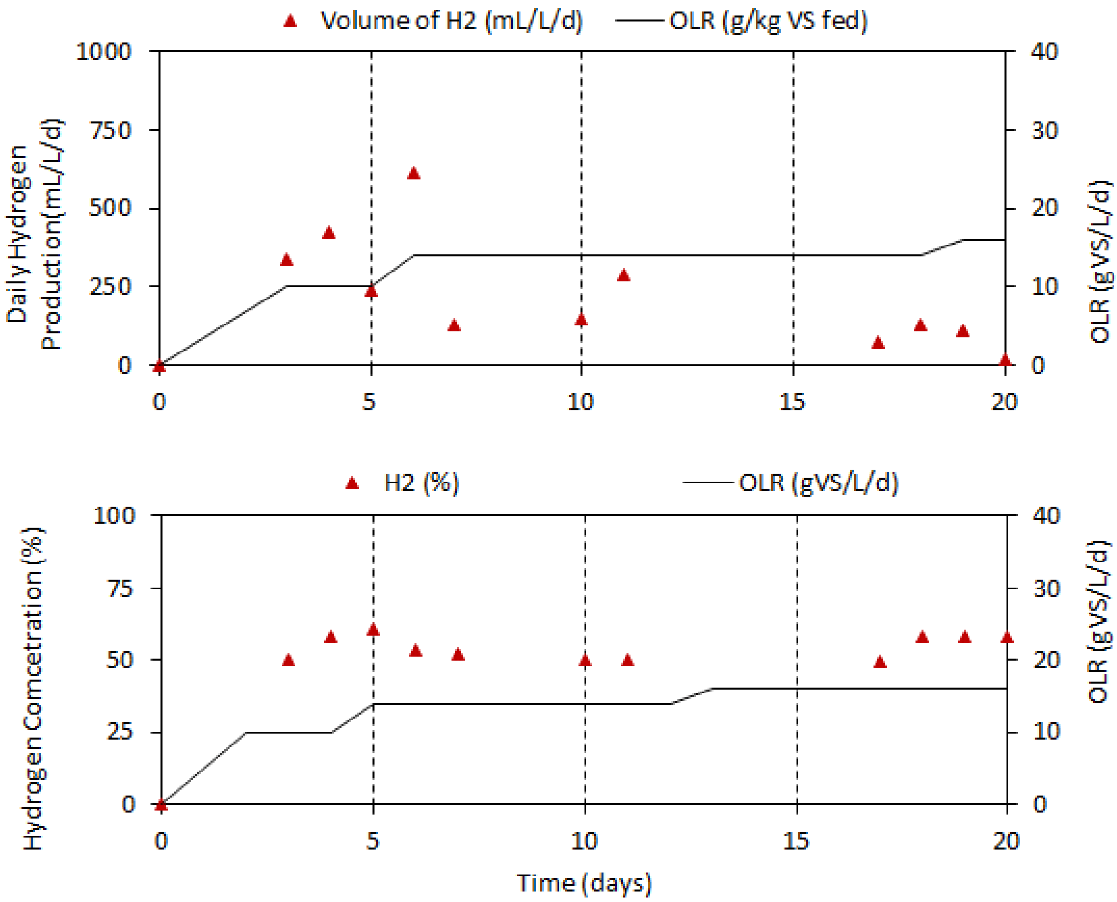

Figure 14. Biohydrogen production rates and yields during the anaerobic dark fermentation of sweet sorghum cultivar SE-45

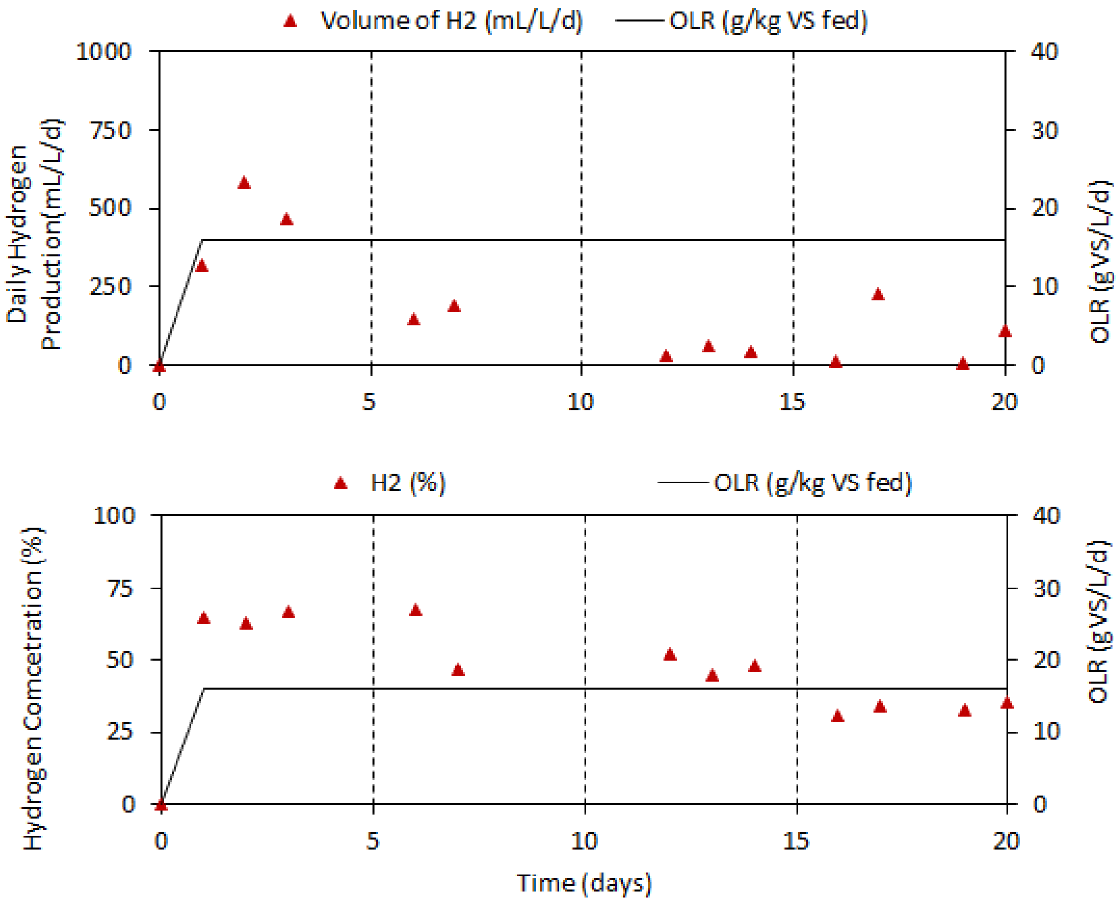

Figure 15. Biohydrogen production rates and yields during the anaerobic dark fermentation of sweet sorghum cultivar SE-35

According to Xie and Cheng [221], it is theoretically possible to convert 1 mol of glucose into 4 mol of hydrogen and 2 mol of methane in a dark fermentation process. The energy derived from hydrogen in this process might potentially contribute to 37.6% of the total energy recovery. However, achieving a molar ratio of 4 mol-H_2/mol glucose is not feasible in practical applications due to the creation of many metabolites, including butyrate and propionate, as well as the generation of cell biomass [324]. Furthermore, it is worth noting that the organic wastes in question may also contain protein and lipids, in addition to carbohydrates. However, it is important to acknowledge that these particular components are not considered ideal substrates for hydrogen synthesis. Conversely, they do serve as favourable substrates for methane production. The primary metabolite seen in our study for the purpose of hydrogen generation was butyrate followed by acetic acid [325].

The H_2 yields (24.40-40. 68 NL H_2/kgVS$_{added}$) obtained in this study matched yields from other solid substrates obtained in literature. For instance, waste from the production of bean curd yielded 14–21 mL H_2/gVS, rice yielded 31–61 mL H_2/gVS [326], wheat yielded 10-43 mL H_2/gVS, cabbage yielded 26–62 mL H_2/g°VS, and rice yielded 19–96 mL H_2/gVS [327], paper and pulp mill (42.1 mL H_2/gVS) [328]. However, it would seem that under these conditions, there is little chance of producing energy from grass silage by dark fermentation. In their studies, Zhang et al. (2007) and Zhang and Fan [329] investigated the process of hydrogen production from corn stalks using dark fermentation. They achieved a maximum hydrogen yield of 149 ml/gVS utilising pure cultures. Likewise, several others also reported improved hydrogen yields after using acid pretreatments and mixed cultures. For instance, H_2 yields of 1.73 mol H_2/mol, 2.61 mol/mol and 107.9 ml/g-TVS were reported by Pattra and Sangyoka [330], Lu and Lai [331] and Pan and Zhang [332], respectively.

Table 26. H_2, energy yields of the three sweet sorghum cultivars.

Cultivar	Biomass yields	Hydrogen yields	Biohydrogen	Annual energy production
	t/ha	(Nm^3 CH4/t-FM)	m^3/ha	kWh/ha
SE-35	82.30	0.40	32.94	98.82
	82.30	3.20	263.28	789.83
SE-45	72.00	2.84	204.63	613.89
	72.00	0.62	44.60	133.81
Mega Sweet	49.00	0.26	12.83	38.48
	49.00	8.07	395.28	1,185.83

Conversion factors of 3 kWh per 1 Nm^3 have been used for H_2 [333]

Previous studies have revealed that it is still viable to produce H_2 from energy crops like sweet sorghum without first pre-treating the substrate. According to other studies [323, 334, 335], the potential for energy production per hectare in the form of H_2 from sweet sorghum through dark fermentation under these conditions remains low when compared to CH_4 production. The annual energy production in the form of hydrogen from one hectare of sweet sorghum would only be roughly 12.83-395.28 m^3/ha, which is equivalent to about 1.12 MWh/ha, based on the current results from batch testing (Table 26). The yields of hydrogen from these energy crops could be increased considerably more under ideal experimental settings. The H_2 yield from sweet sorghum was average and did not have a yield as high in energy as the CH_4 yields in the Section 4.2.

To boost total energy efficiency, effective pre-treatment technologies or two-stage systems (combined H_2 and CH_4 synthesis) may be required if sweet sorghum or other lignocellulosic material is to be utilized for H_2 production.

4.6 To compare the process performance and methane yields during anaerobic mono-digestion and co-digestion of sweet sorghum with cow manure.

4.6.1 Effect of chemical composition of cultivars on methane yields

The effect of chemical composition on methane yields from the three sweet sorghum cultivars was determined in CSTR experiments at 37 °C. Table 27 presents the chemical composition of the studied sweet sorghum cultivars. Results showed that TS content in the three sweet sorghum cultivars ranged between 14.25% (SE-45) and 21.40% w/w (Mega Sweet). A similar trend was also noticed with VS. The high VS/TS of 0.89-0.95 indicates that all the tested cultivars were rich in organic matter and are considered as ideal substrates for biogas production [292]. This result agrees with past studies were substrates having high VS/TS where rich in organic matter. The carbon content in sweet sorghum cultivars was more or less similar (Table 1). SE-35 had the highest carbon content of 47.78 %TS whilst SE-45 and Mega Sweet had the lowest carbon content of 47.49 %TS. On the other hand, nitrogen content in the tested cultivars was in the range of 1.01 to 1.12 %TS. Thus, carbon to nitrogen (C/N) ratio ranged from a low 42.53 (SE-45) to high value of 47.04 (Mega Sweet). Nonetheless high VS/TS and C/N ratios indicates that the substrates are rich in carbon that maybe difficult to degrade during the AD process due to the presence of lignin and hydrolysis might be the rate limiting step for these substrates. The concentrations of TKN and TKP in the substrates are presented in Table 27. TKN values ranged from 0.86 gN/kgTS in SE-35 to 1.52 gN/kgTS in Mega Sweet.

Table 27. Chemical composition of three sweet sorghum cultivars and cow manure used in this study

Sample	TS % (w/w)	VS % (w/w)	Moisture (% w/w)	VS/TS	C (%TS)	N (%TS)	C/N	TKN (g/kgTS)	TKP (g/kgTS)
SE-35	14.25	13.11	85.75	0.92	47.78	1.06	45.25	0.86	0.27
SE-45	18.26	17.24	81.74	0.94	47.67	1.12	42.53	1.26	0.28
Mega Sweet	21.40	19.83	78.60	0.93	47.49	1.01	47.04	1.52	0.50
Cow Manure	14.64	11.64	85.36	0.79	43.45	2.81	15.49	25.16	12.23

Anaerobic sludge	2.71	2.00	97.29	0.74	38.43	5.91	6.50	54.67	24.03

The effect of organic loading on process performance and methane yields during mono-digestion of sweet sorghum (Reactor, R_1) and co-digestion of sweet sorghum with cow manure (Reactor, R_2) is presented in Table 28 and Table 29 and Figure 16 and Figure 17. Three different sweet sorghum cultivars were used during the reactor studies. The three cultivars and their feeding times are as follows: Mega Sweet (Day 0 to 13), SE-35 (Day 16 to 27) and SE-45 (Day 30 to 116). After the initial start-up, both reactors were operated at an OLR of 0.5 gVS/L/d (Day 1 to 13) and fed with Mega Sweet cultivar. Methane production responded to 0.5 OLR. Biogas production in R_1 was 2,450 mL/d with methane content of 60.43%. The calculated methane yields obtained at 0.5 OLR during mono-digestion in R_1 were 297.10 NL CH_4/kg-VS_{fed}. A similar response was also noticed during co-digestion of sweet sorghum with cow manure in R_2 but resulting in lower methane yields of 279.10 NL CH_4/kgVS_{fed}.

The increase in methane yields with increase in OLR from 0.5 to 1.0 was obviously due to optimised digestion performance through improved C/N ratio and use of existing buffering capacity of the inoculum.

Increase in OLR from 0.5 to 1.0 (Days 16-27) and from 1.0 to 1.5 (Days 30-44) and to 2.0 (Days 45-83) resulted in a decrease in methane yields in R_1 (Table 23). During this period methane yields decreased from 276.79 to 142.76 NL CH_4/kgVS_{fed}. At the same time, methane yields in R_2 also followed the same trend but the decrease in methane yields was less dramatic in R_2 than in R_1. Methane yields in R_2 decreased from 297.10 (0.5 OLR) to 137.07 (2.0 OLR) NL CH_4/kgVS_{fed}. The possible reason for lower methane yields in R_2 than in R_1 was obviously due to the relative increase in the amount of cow manure during this period.

A further increase in OLR from 2 to 2.5 gVS/L/d (Days 85-97) and then to 3 gVS/L/d (Days 100-116) resulted in a sharp increase in methane production rate and yields in R_2. On the other hand, methane production rates and yields decreased sharply in R_1 when fed at OLR of 3 gVS/L/d. This decrease in methane yields in R_1 was attributed due to lack of nitrogen and trace elements in the reactor that are essential for microbial metabolism and/or insufficient buffering capacity after completion of 4 HRTs. Sweet sorghum cultivars used in this study had a high C/N ratio of 47.4 (Mega Sweet), 43.5 (SE-35) and 43.4 (SE-45). On the other hand, cow manure had a C/N ratio of 15.49 (Table 27). Thus, the C/N ratio in R_1 and R_2 were below the optimal C/N ratio of 20-40 that is required for a stable anaerobic digestion process [336]. These results are in agreement with previous study where the increased specific methane yields during co-digestion was also attributed due to the synergistic effects of a more balanced nutritional composition and C/N ratio of the two feedstocks Lehtomäki and Huttunen [182].

Table 28. Effect of organic loading rate (OLR) on process performance and methane yields during the anaerobic mono-digestion of sweet sorghum at 37 °C in CSTR.

OLR (gVS/L/d)	Operation Days	Mean Biogas production (mL/d)	Biogas production (mL/mL feed)	Methane content (%)	Daily Methane Production (NmL/L/d)	Methane yields (NL CH_4/kg-VS_{fed})	pH	TVFA (mg/L)	NH_4-N (mg/L)	TKN (mg/L)	VS Removal (%)
0.5	Day 1 to 13	2,450.22	11.45	60.43	148.55	297.1	7.46	27.02	362.09	603.48	18
1	Day 16 to 27	2,814.48	12.7	58.92	166.46	166.46	7.46	43.49	957.48	1595.8	45.78
1.5	Day 30 to 44	4,240.03	22.81	61.14	259.02	172.68	7.46	53.2	681.08	1135.133	62.98
2	Day 45 to 83	4,769.46	27.21	59.89	285.51	142.76	7.44	1,264.73	462.58	770.9667	74.03
2.5	Day 86 to 97	8,995.23	41.61	58.02	519.5	207.8	7.43	60.45	740.14	1233.567	80.87
3	Day 100 to 116	4,966.29	32.75	59.29	293.66	97.89	7.48	41.81	502.48	837.4667	83.99

Table 29. Effect of organic loading rate (OLR) on process performance and methane yields during the anaerobic co-digestion of sweet sorghum with cow manure at 37 °C in CSTR.

OLR (gVS/L/d)	Operation Days	Mean Biogas production (mL/d)	Biogas production (mL/mL feed)	Methane content (%)	Daily Methane Production (NmL/L/d)	Methane yields (NL CH_4/kg-V_{fed})	pH	TVFA (mg/L)	NH_4-N (mg/L)	TKN (mg/L)	VS Removal (%)
0.5	Day 1 to 13	2,441.75	11.35	57.15	137.89	276.79	7.39	35.21	374.21	623.68	20
1	Day 16 to 27	3,469.10	16.33	56.7	196.36	212.88	7.49	76.41	1,111.00	1,851.667	50.34
1.5	Day 30 to 44	4,983.64	25.87	58.17	292.37	184.68	7.38	55.47	772.75	1,287.917	56.1
2	Day 45 to 83	4,989.14	28.96	57.96	289.71	137.07	7.35	29.68	821.13	1,368.55	74.21
2.5	Day 86 to 97	8,470.34	37.85	55.98	470.75	222.39	7.4	43.15	841.45	1,402.417	79.85
3	Day 100 to 116	8,146.32	45.22	59.37	486.5	229.76	7.38	601.38	150.49	250.816	82.49

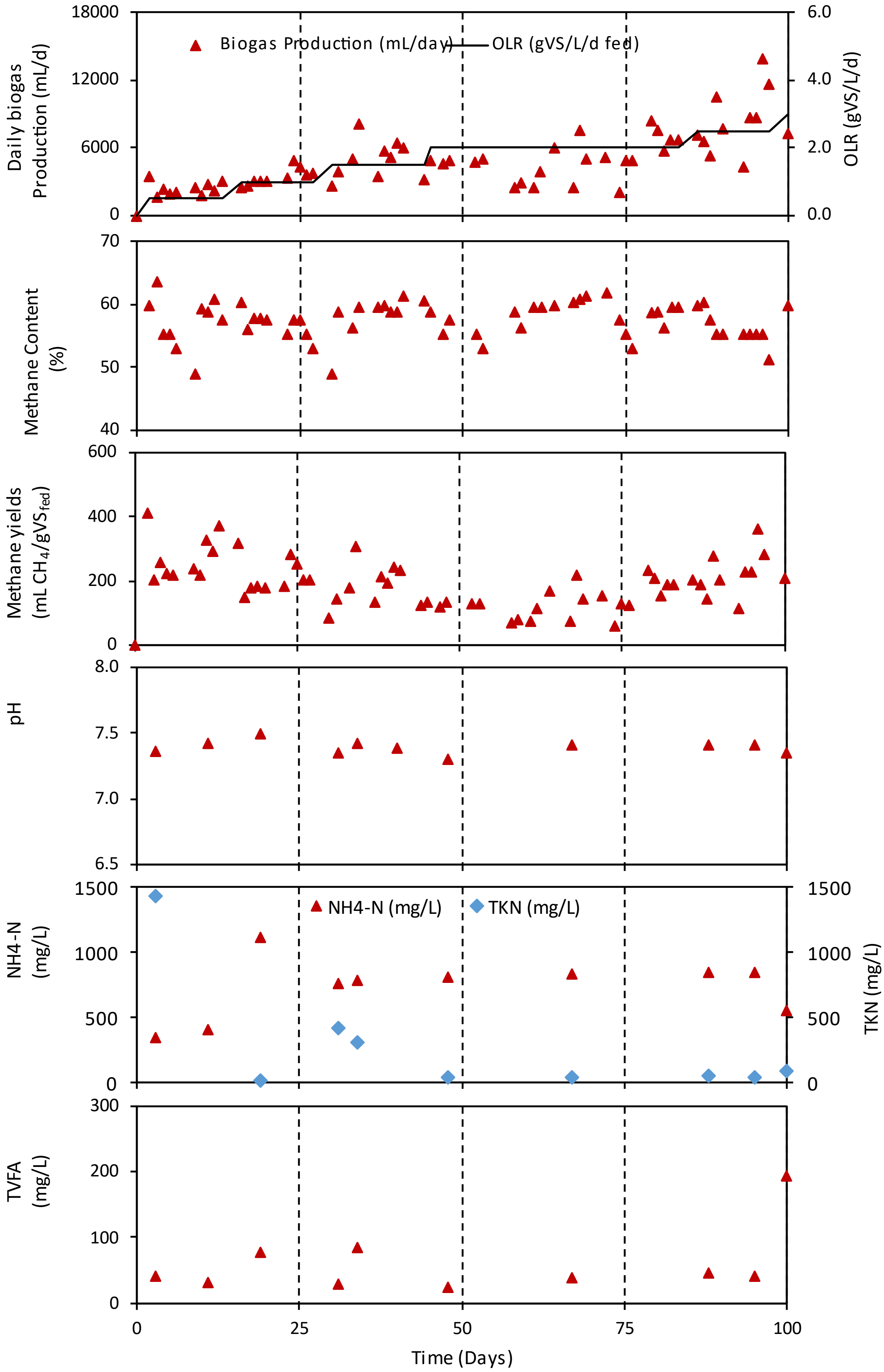

Figure 16. Process performance and methane yields during the anaerobic mono-digestion of sweet sorghum cultivar SE-45 in CSTR at 37 °C.

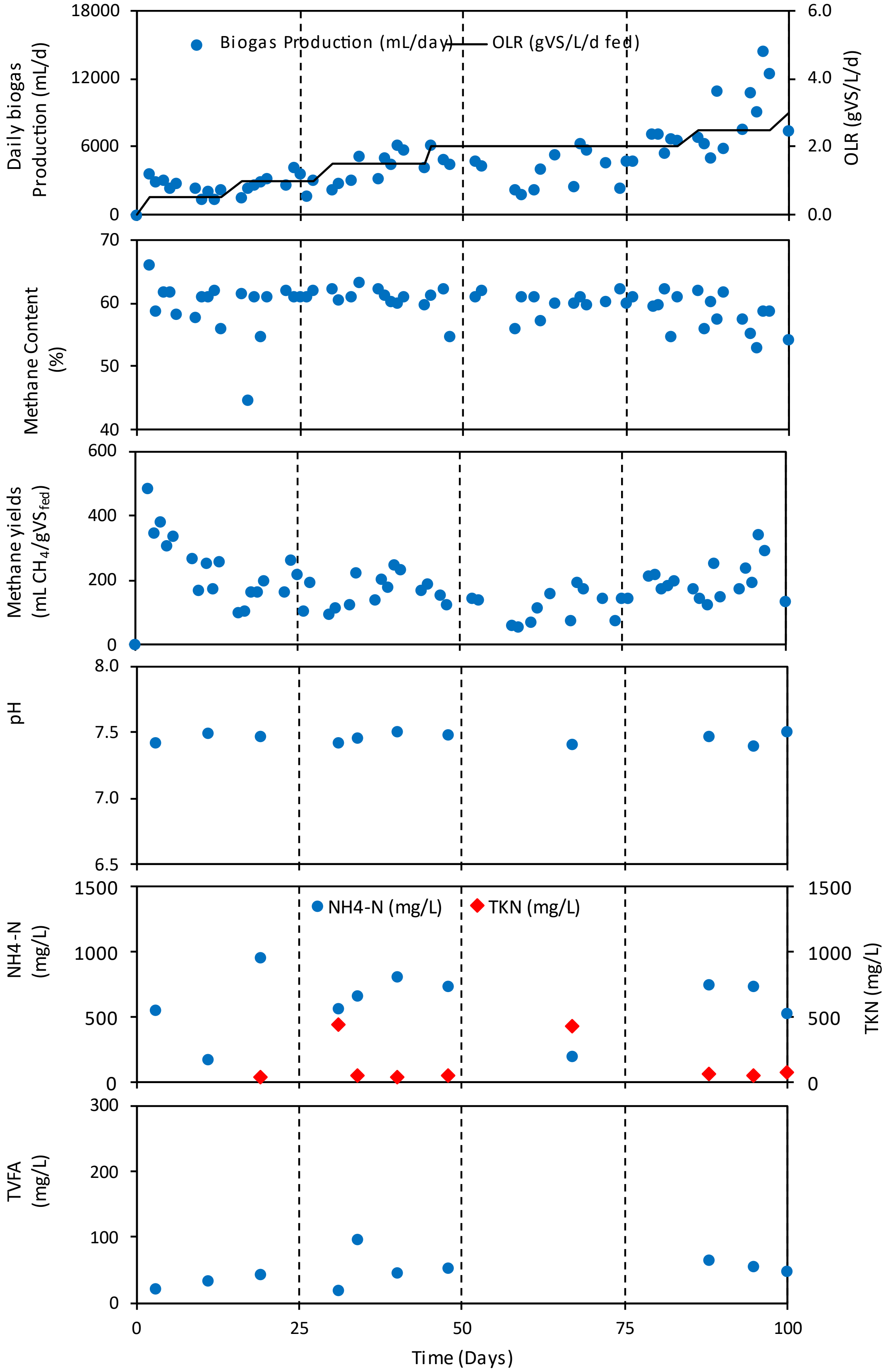

Figure 17. Process performance and methane yields during the anaerobic co-digestion of sweet sorghum cultivar SE-45 with cow manure in CSTR at 37 °C.

Overall, 2.5 gVS/L/d was found to be the optimal OLR during the mono-digestion of sweet sorghum. However, reactors can be operated at an OLR of 3 gVS/L/d if co-digestion with cow manure was practised. Maximum methane yields of 229.70 NL CH_4/kgVS$_{fed}$ could be obtained when sweet sorghum was co-digested with cow manure at 85:15 ratio on VS basis and operated at HRT of 25 d under mesophilic conditions. This was evident from a relatively stable process and higher methane yields in R_2 than in R_1 throughout the experimental period. The pH in both reactors R_1 and R_2 remained stable and there was no accumulation of ammonia or VFAs in the reactors (Table 28 and Table 29). The result also suggests that use of cow manure as co-substrate at 15% of total feed VS with sweet sorghum was sufficient to improve the process stability and methane yields through introduction of cellulolytic microorganisms and buffering capacity. The sharp decrease in methane yields in R_1 at an OLR of 3 gVS/L/d suggests that of 25 d HRT was not sufficient for efficient degradation at this OLR owing to the increased carbon source but insufficient nitrogen through sweet sorghum alone [182]. Nevertheless, the methane production seen in continuous experiments was much lower compared to the methane yield achieved in batch experiments. In a study conducted by Sambusiti and Ficara [269], a mesophilic continuously agitated reactor was used to determine the methane yield of a Sorghum Sudanese hybrid. The obtained methane yield was 237 NL-CH_4/kg-VS$_{fed}$. The organic loading rate (OLR) during the experiment was maintained below 1.8 kg-VS/m^3/d. In a study conducted by Jerger and Chynoweth [288], it was found that a mesophilic continuous stirred-tank reactor (CSTR) attained a methane yield of 260 NL-CH4/kg-VS$_{fed}$ while utilizing 'Rio' sweet sorghum (Sorghum bicolor (L.) Moench.) as the substrate. This result was obtained under two different organic loading rates (OLR) of 1.6 and 3.2 gVS/L/d. However, the methane yields in the monodigestion reactor obtained in this study were in line with the literature

This study suggests that anaerobic co-digestion of sweet sorghum with cow manure should be carried out at an OLR of 2.5-3.2 gVS/L/d with 15% of total feed VS should be coming from cow manure. The increase in process stability and methane yields of 7% at OLR of 2.5 can improve the overall economics of farm-scale biogas plant. The increased revenue could cover the costs for cow manure purchase and the associated transport costs. Further, use of cow manure can improve the digestate nutrient quality and also the sale of digestate as a biofertilizer. However, pretreatment of sweet sorghum and/or use of other co-substrates such as chicken manure or slaughterhouse waste within 20 km of the farm-scale biogas plant could further improve the methane yields and economics of farm-scale biogas production.

4.7 To assess the feasibility of farm-scale biogas plant using sweet sorghum grown for biogas production and uses.

The feasibility of sweet sorghum cultivated on sugarcane fallow and use of produced biomass for biogas plant in a farm-scale biogas plant was evaluated. For this feasibility study, sweet sorghum cultivar SE-81 was used based on the methane yields obtained in Section 4.1. It was assumed that sweet sorghum was grown on a rotational basis over 25 ha of fallow land of the total 226 ha of sugarcane land. The amount of SE-81 biomass harvested from 25 ha of fallow land was calculated to be 1,938 tFM/yr. As the carbon content in SE-81 was 48.4%, 621 tFM/yr of chicken manure was used as co-substrate to achieve an optimal carbon to nitrogen (C/N) ratio of 30:1. Further, 7,900 m^3/yr of water was required to achieve a total solids (TS) content of 8% in the feed. The characteristics and methane yield of sweet sorghum SE-81 and chicken manure used in the study are presented in Table 30.

Table 30. Characteristics of sweet sorghum and chicken manure used in the study.

Parameters	Sweet sorghum	Chicken manure
Biomass (tFM/a)	1938	621
TS (%w/w)	19.4	74.31
VS (%TS)	94.3	83
Methane yields ($Nm^3/kgVS_{added}$)	0.216	0.271
Methane content (%)	55	62

To assess the feasibility of the proposed study, three different scenarios were designed with an aim to evaluate the possible mass and energy balances along with the market opportunities for biogas uses (Table 31).

- Scenario 1: CHP—Biogas is used for electricity and heat generation in a combined heat and power (CHP) plant.

- Scenario 2: CHP + BioCNG—A portion of biogas is used for CHP to generate electricity and heat to meet the parasitic demand of the biogas plant and the remaining biogas is upgraded and compressed (BioCNG) for vehicle fuel or distribution via virtual pipeline (road transport).
- Scenario 3: CHP+ BioRNG—Similar to Scenario 2 but the biogas is upgraded to renewable natural gas (BioRNG) for grid injection.

Under both Scenarios 2 and 3, the carbon dioxide (CO_2) will be recovered and liquified to be sold as food grade BioCO_2.

Table 31.Biogas use options for the three studied scenarios.

Project parameters	Scenario 1	Scenario 2	Scenario 3
Biogas use	Electricity generation, grid supply CHP	30% electricity generation, 70% biogas upgrading CHP + BioCNG	30% electricity generation, 70% biogas upgrading CHP + BioRNG
Biogas plant outputs	Electricity Digestate	Electricity BioCNG BioCO_2 Digestate	Electricity BioRNG BioCO_2 Digestate
Grid electricity required for biogas plant	No	No	No

4.7.1. Farm-scale biogas plant design and operation

The farm-scale biogas plant concept and process flowchart for CHP generation (Scenario 1) and for CHP + BioCNG with additional BioCO_2 production (Scenario 2) are presented in

Table 32, Figure 18 and Figure 19 , respectively. The process flowchart for Scenario 3 is not presented as it is similar to Scenario 2, with only minor changes in biomethane use equipment. Sweet sorghum will be harvested during December (Section 4.1) and the produced biomass will be ensilaged and stored as silage in concrete bunker silos. Two concrete bunker silos (10 m × 4.5 m × 2.5 m) were considered for the ensilation process. Lactic acid bacteria at the rate of 4–5% on fresh weight basis will be used for ensilation process. Possible storage losses were not considered in this study. On the other hand, chicken manure (1.7 t/d) will be procured from poultry farms in Mareeba, Queensland.

The feeding technology consists of maceration coupled with an augur feeding system and feed buffer tank. At first, sweet sorghum (5.31 tFM/d) will be macerated, mixed, homogenised and fed into the buffer tank. In the buffer tank, chicken manure (1.7 t/d) is added along with process water (265 kL/d) and liquid fraction of the digestate, to adjust the solids content to <8%. The feed buffer tank (200 m^3) was designed to prepare feed for seven working days (Monday through Sunday). Feed rates were designed so that the feedstocks will be consumed evenly throughout the year. The prepared feed (28.65 t/d) is then fed to the biogas reactors (Figure 18 and Figure 19).

One semi-continuously stirred tank reactor (16.8 m diameter x 5.9 m height) system (1,300 m^3) with a working volume of 1,005 m^3 was designed to enable the implementation of the proposed co-digestion system in a farm-scale biogas plant. CSTR technology is widely applied in the European biogas plants for a range of residues including energy crops and livestock manure AD [337]. The reactors have mechanical agitators to mix the reactor contents. The rectors will be operated at an organic loading rate (OLR) of 2 kgVS/m^3/d, hydraulic retention time (HRT) of 35 d and process temperature of 37 °C. The well-insulated upright galvanised steel CSTR reactor has sufficient headspace for biogas to evolve and flow to a post-storage tank.

The biogas and digestate produced from the farm-scale biogas plant is stored in a biogas storage tank (250 m^3) and the post-storage tank (840 m^3), respectively. Every day, 28.7 tFM/d of digestate is produced (Figure 18 and Figure 19). Digestate is separated into solid and liquid fractions by using a decanter centrifuge (1.6 m^3/h). The solid fraction is sold to compost facility or used on the farm as soil conditioner. On the other hand, liquid fraction is sent to pasteurisation (70 °C for 1 h) to kill the zoonotic pathogens. The pasteurised liquid fraction is either returned as process water to dilute the incoming feedstock or sold as liquid fertilisers.

Table 32. Plant sizing parameters for the studied farm scale biogas plant

Plant sizing	Scenario 1	Scenario 2	Scenario 3
Sweet Sorghum (tFM/d)	5.31	5.31	5.31

Chicken manure (tFM/d)	1.7	1.7	1.7
Water (kL/d)	21.64	21.64	21.64
Feed (t/d)	272	272	272
Feed tank (m^3)	200	200	200
Feed prepared for (Days)	7	7	7
Feed solids (TS% w/w)	8	8	8
Total reactor volume (m^3)	1,300	1,300	1,300
Reactor working volume (m^3)	1,104	1,104	1,104
Number of reactors	1	1	1
Biogas storage tank (m^3)	250	250	250
Biogas storage time (h)	6	6	6
Digestate storage tank (m^3)	840	840	840
Digestate storage time (Month)	6	6	6
Pasteurisation tank (m^3)	2.3	2.3	2.3
Decanter centrifuge (kWh/d)	102	102	102
CHP plant size (kW)	84	26	26
CHP electrical efficiency (%)	39.4	38.2	38.2
CHP thermal efficiency (%)	49.3	49.1	49.1
Biogas upgrading unit ((m^3/h raw biogas)	NA	35	35
Operation hours (h)	8,300	8,300	8,300
Diesel for transport of feedstock (L/a)	1,960	1,960	1,960
Natural gas for parasitic heating (m^3/a)	707	67,242	61,694

The biogas train consists of a biogas blower, desulphurisation unit and a flare. In Scenario 1, 840 m^3/d of desulphurised biogas is fed to the CHP plant (84 kW) by a biogas blower (20,160 m^3/h) to produce heat and electricity (

Table 32). One stationary internal combustion engine MAN 110 kW (MAN E0836, 6 cylinder and 1500 rpm (50 Hz)) with a combined electrical and thermal conversion efficiency of 88.7% was used (Engine version LE 302 for special gas). After meeting the parasitic electricity needs, the surplus electricity is fed to the grid. Heat from the CHP plant (Coolant heat 77 kW and exhaust heat, 55kW, based on 120 °C) is used internally for heating the reactors.

In Scenario 2 &3, approximately 268 m^3/d of biogas (32% of total production) is fed to the CHP plant (26 kW) to produce heat and electricity to meet the parasitic demands of the biogas plant. One stationary internal combustion engine MAN 80 kW (MAN E0834, 4 cylinder and 1500 rpm (50 Hz)) with a combined electrical and thermal conversion efficiency of 87.2% was used (Engine version LE 322 for special gas). After meeting the parasitic electricity needs (134 GJ/a), the surplus electricity is fed to the grid (784 GJ/a). Heat from the CHP plant (Coolant heat 61 kW and exhaust heat, 35 kW, based on 120 °C) is about 2,938 GJ/a and is used internally for heating the reactors. However, a deficit of 2,326 GJ/a of thermal energy makes the plant to import 65,535 m^3/a of natural gas.

The remaining biogas (571 m^3/d) is fed to a membrane biogas upgrading unit (30 m^3/h raw biogas) coupled with CO_2 recovery and liquefaction plant (10 m^3/h) to produce BioCNG and bioCO_2, respectively. The PurePac Mini biogas upgrading system (30–60 Nm^3/h raw biogas) and CO_2 recovery system, manufactured by Bright Biomethane (the Netherlands) was considered. A methane yield of 99.5% is guaranteed in this highly efficient membrane upgrading technology. A 2% biomethane slip was considered during biogas upgrading. This reflects the worst-case scenario within the range (1–2%) usually observed in upgrading technologies (Muñoz *et al.*, 2015). In case of emergency, biogas can be flared on site by using the biogas flare (2 x 350 m^3/d).

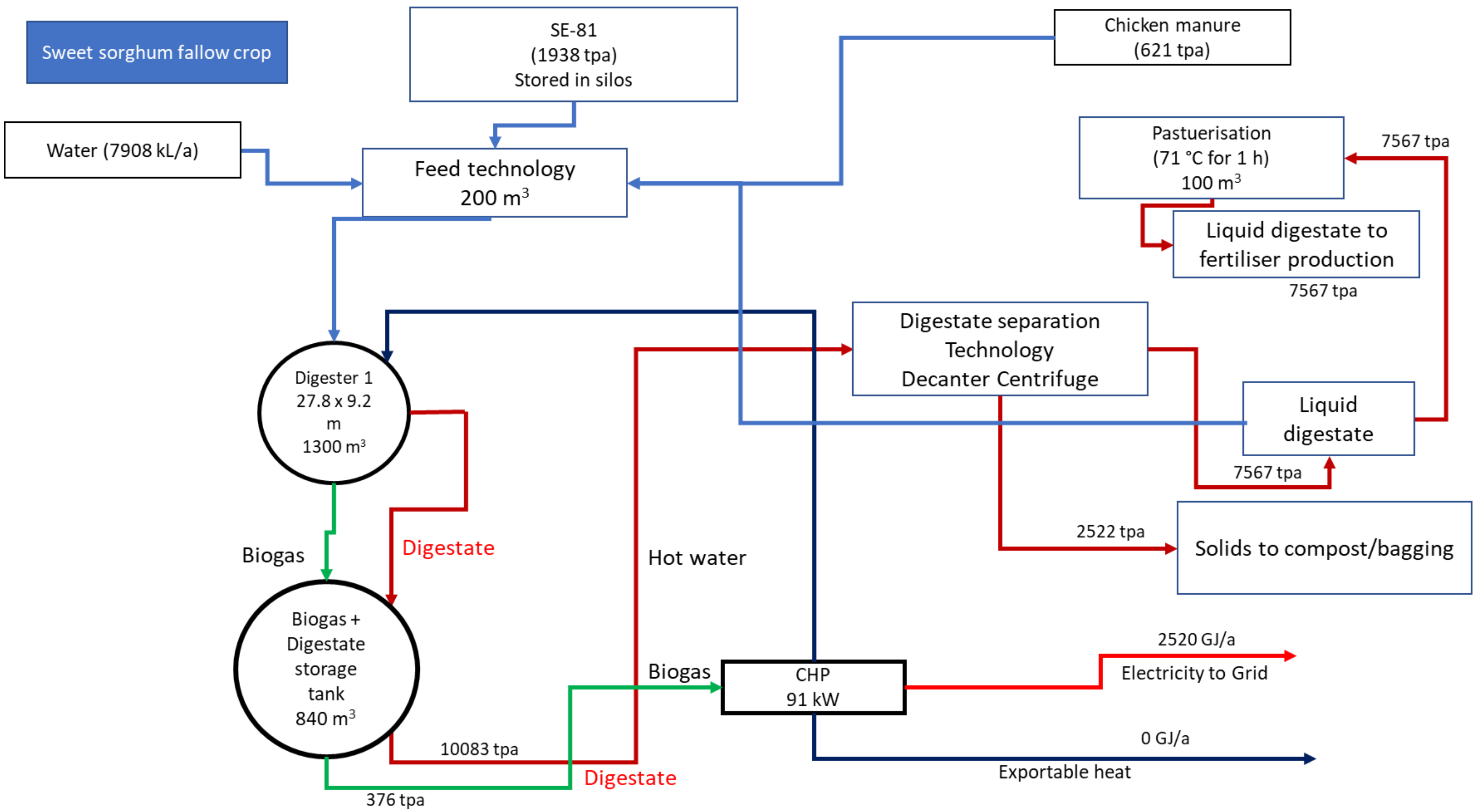

Figure 18. Process flow diagram for Scenario 1 (CHP Pathway from biomethane)

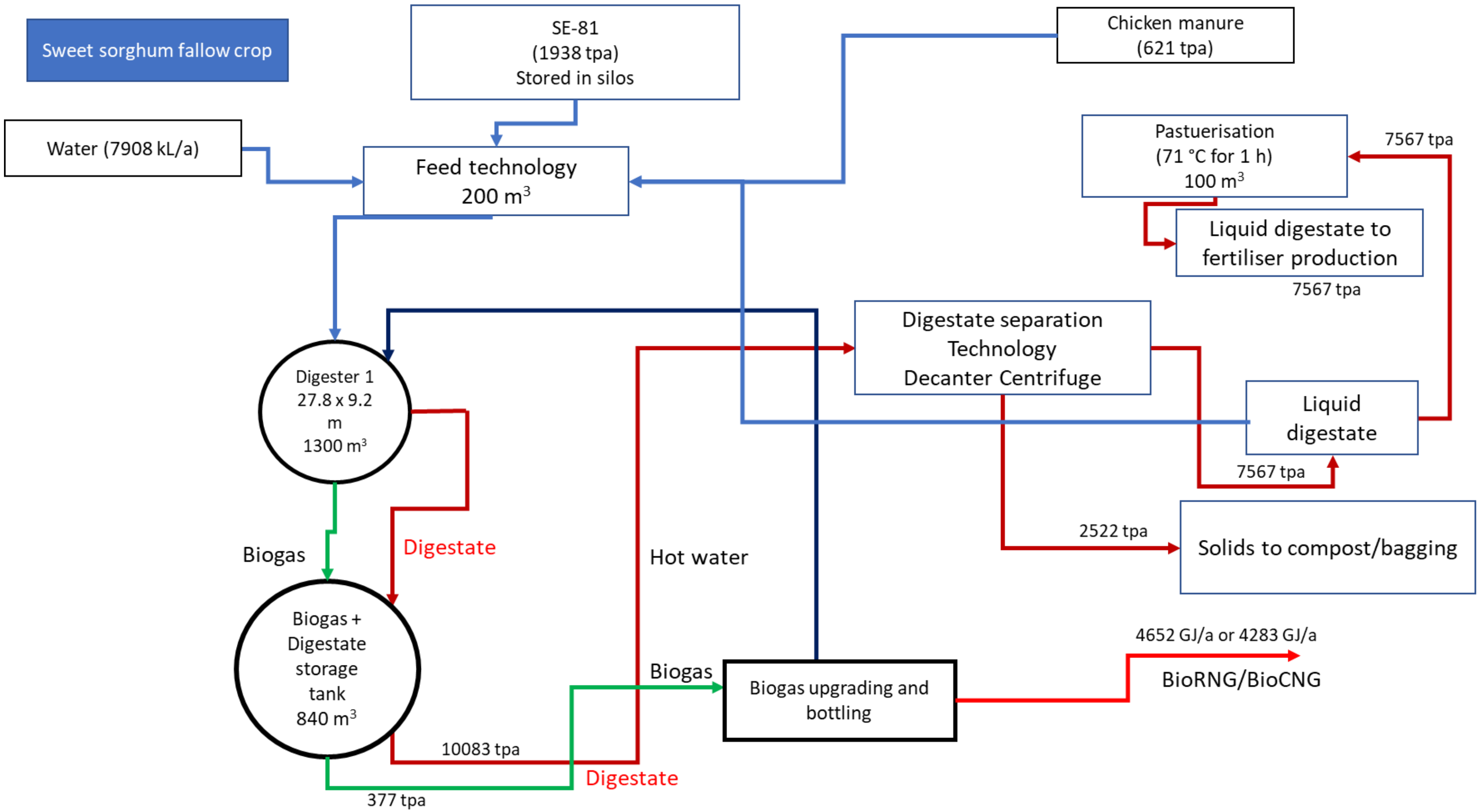

Figure 19. Process flow diagram for Scenario 2 and Scenario 3 (BioCNG and BioRNG)

4.7.3 Mass balance

Table 33 presents the mass balance for the studied three scenarios. For all three scenarios, anaerobic co-digestion of 1,938 t/a of sweet sorghum with 621 t/a of chicken manure and 7,908 kL/a of water will produce 306,653 Nm^3/a of biogas (376 t/a) and 10,083 t/a of digestate. Separation of the digestate into solid and liquid fractions by using a decanter centrifuge resulted in 2,521 t/a of solid fraction and 7,562 t/a of liquid fraction. The solid fraction (11.2 %TS) accounted for 25% of the total digestate (Table 37). The liquid fraction had a solids content of 2.4%. This liquid fraction will be used as process water to dilute the feedstocks to achieve 8% solids or sold as liquid fertiliser.

Table 33. Mass Balance of anaerobic co-digestion of sweet sorghum (SE-81) with chicken manure.

Mass Balance	Scenario 1	Scenario 2	Scenario 3
Sweet sorghum (tFM/a)	1,938	1,938	1,938
Chicken manure (tFM/a)	621	621	621
Water (kL/a)	7,908	7908	7,908
Digester loading rate (kgVS/m^3 d)	2	2	2
Total digester capacity (m^3)	1,104	1,104	1,104
Hydraulic retention time (d)	35	35	35
Digester operating temp (°C)	37	37	37
Methane produced (N m^3/a)	180,378	180,865	180,865
= Methane produced (t/a)	129.10	129.44	129.44
Carbon dioxide produced (N m^3/a)	126,274	126,572	126,572
= Carbon dioxide produced (t/a)	250.02	250.61	250.61
Biogas produced (N m^3/a)	306,653	307,437	307,437
= Biogas produced (t/a)	376	377	377
Digestate produced (t/a)	10,083	10,083	10,083

4.7.4 Energy balance for the studied scenarios

Table 34 shows with the energy balance for the studied scenarios. Loading the reactor at 28.7 t/d of feed rate yielded a daily biogas production of 840.15 m^3/d across all scenarios. In Scenario 1, the use of biogas for electricity and heat generation in a CHP system (84 kW generator) produced 2,520 GJ/a of electricity and 2,917 GJ/a of heat. Considering the energy performance of Scenario 1, the biogas plant has an installed capacity of 0.07 MW. For Scenario 2, a smaller CHP plant (26 kW) can be installed in addition to biogas upgrading unit to take care of the parasitic energy demands. Heat and electricity generation in Scenario 2 were 784 GJ/a of electricity and 965 GJ/a of heat. Similarly, CHP of 31 kW was used in Scenario 3 to generate 927 GJ/a of electricity and 1,141 GJ/a of heat.

Table 34. Energy balance during the anaerobic co-digestion of sweet sorghum (SE-81) with chicken manure for the three studied scenarios

Energy balance	Scenario 1	Scenario 2	Scenario 3
Waste transport fuel direct (GJ/a)	70	70	70
Waste transport fuel indirect (GJ/a)	8	8	8
CHP electricity produced (GJ/a)	2,520	784	927
= CHP electricity produced (kWh/a)	700,122	217,818	257,577
Generator Capacity (kW)	84	26	31
CHP Heat produced (GJ/a)	2,917	965	1,141
Biomethane produced (m^3/a)	--	129,669	119,557
CHP supplied parasitic electricity (GJ/a)	503	784	927
CHP supplied parasitic heat (GJ/a)	2,938	2,938	2,938
Imported decanter electricity (GJ/a)	134	134	134
Imported natural gas for heat energy (GJ/a)	25	2,321	2,114

From Scenario 1, it can be estimated that aside from the electricity demand for the farm-scale plant about 1,883 GJ/a of electricity can be fed to the electricity grid. This is equivalent to 523 MWh/a of exportable electricity. However, all the electricity produced in Scenario 2 and 3 will be utilized for farm-scale biogas plant operations. On the other hand, 4,652 GJ/a of biomethane (BioRNG) can be exported to natural gas grid in Scenario 2. Correspondingly, 4,283 GJ/a of compressed biomethane (BioCNG) in Scenario 3 can be sold as vehicle fuel for transport vehicle.

Table 35. Exportable energy from the studied scenarios

On-site boiler / CHP	Scenario 1	Scenario 2	Scenario 3
Energy in methane produced (GJ/a)	6,461	6,461	6,461
Generated electricity (GJ/a)	2,520	784	927
Generated heat (GJ/a)	2,917	965	1,141
Exportable electricity (GJ/a)	1,883	0	0
= Exportable electricity (MWh/a)	523	0	0
= Exportable electricity (MW)	0.06	0	0
Exportable heat (GJ/a)	0	0	0
= Exportable heat (MWh/a)	0	0	0
Energy in upgraded CH_4 ((GJ/a))	0	4,652	4,283

4.7.5 Parasitic electrical demand

The parasitic energy demands for Scenarios 1, 2 and 3 are presented in Table 36. For all scenarios the electricity demand for digester, pasteuriser, biogas holder and feed tank were calculated and presented. Results show that the parasitic energy demands were 3,575, 3,856 and 3,999 GJ/a, for Scenarios 1, 2 and 3, respectively. In all three scenarios, parasitic electricity demand was met by the CHP electricity. A small amount of electricity (134 GJ/a) was imported for operation of the decanter centrifuge for digestate separation. On the other hand, to meet the parasitic heat demands, natural gas had to be imported at 25, 2,321 and 2,114 GJ/a for Scenarios 1, 2, and 3, respectively.

Table 36.Parasitic energy demand for all studied scenarios

Embodied Energy	Scenario 1	Scenario 2	Scenario 3
Digester (GJ/a)	111.7	111.7	111.7
Pasteuriser (GJ/a)	1.9	1.9	1.9
Biogas holder (GJ/a)	1.6	1.6	1.6
Feed Tank (GJ/a)	0.3	0.3	0.3
CHP (GJ/a)	7.2	6.4	6.5
Biogas upgrading (GJ/a)	0	195	248.6
Digestate separation (GJ/a)	1.4	1.4	1.4
Digestate storage (GJ/a)	11.8	11.8	11.8

4.7.6 Digestate management and use

Annually 10,083 t of digestate was produced. The solids content of the digestate was 4.6%. A decanter centrifuge was used to separate the digestate into solid and liquid fractions in an attempt to improve the nutrient content. Table 37 presents the nutrient composition and fertiliser values of the various digestate fractions. N, P_2O_5, and K_2O fertilisers were calculated based on the N, P, K content of the feedstocks. Digestate as such was made up of 1:0.9:0.3 of N, P_2O_5, and K_2O. The corresponding values for solid fraction of digestate was 1:0.9:0.3 N: P_2O_5: K_2O, respectively. Drying and/or sale of the solid as soil conditioner would generate some additional revenue. The solid fraction can be sold for $10/t based on its nutritional content. The liquid fraction, on the other hand, had N: P_2O_5: K_2O concentration of 1:0.2:0.9 and can either be recycled as process water or sold at $80/t to replace inorganic fertilizers. Alternatively, digestate can also be used as biofertilisers for the cultivation of sweet sorghum and/or sugarcane and thereby reduce the costs associated with the purchase of inorganic fertilisers. Concentrate the nutrients in the whole digestate or in liquid fraction may require higher investments but can generate additional revenue. The feasibility and economic viable of increasing the concentration of nutrients in digestate or liquid fraction was not carryout in this study.

Table 37. Mass balance of nutrients in the digestate before and after solid-liquid separation using a decanter centrifuge.

Digestate Composition	Parameters	Solid fraction (kg)	Liquid fraction (kg)
Scenario 1-3	TS (%)	11.1	2.4
	N	4,517	10,540
	P_2O_5	4,491	2,418
	K_2O	1,181	7,900

Table 38 presents the annual greenhouse (GHG) emissions that could be avoided on adopting farm-scale biogas plant for generating renewable biogas and using it for heat and electricity generation (Scenario 1), BioCNG (Scenario 2) and BioRNG (Scenario 3). The avoided GHG emissions were compared to coal-based grid electricity and natural gas based thermal energy. Diesel was the fuel used for the transport of waste. Total GHG emissions from fossil fuel use in the transport of 621 t/a of chicken manure has been estimated to be 6.23 tCO_2-e/a. Emissions from the transport of sweet sorghum was not included as the feedstock is available on site. GHG emissions from import of natural gas to meet the thermal demand of biogas plant was 1.43, 136.31 and 125.06 tCO_2-e/a for Scenario 1, 2 and 3, respectively. Similarly, import of electricity for decanter centrifuge of 34.14 tCO_2-e/a was accounted for Scenario 2 and 3 only. Renewable energy in the form of heat and electricity generation in Scenario 1 could replace 512.77 tCO_2-e/a of emissions generated from equivalent heat produced from natural gas and electricity from coal, respectively. Similarly, use of biogas for BioCNG production and use in Scenario 3 could avoid 281.37 tCO_2-e/a emissions from natural gas. The corresponding value for Scenario 2 on the use of biogas for BioRNG production and use could avoid 304.77 tCO_2-e/a. Finally, the annual emissions saved on the use of anaerobic digestate in the studied scenarios is about 31,000 tCO_2-e/a. Overall, the net GHG emissions avoided in Scenario 1 were 536.10 tCO_2-e/a, which is 342.88-389.16 tCO_2-e/a more than the values obtained for Scenarios 2 and 3. The study thus show that Scenario 1 is the most feasible option for growing sweet sorghum on sugarcane fallow and using the produced biogas for heat and generation in a combined heat and power plant.

It should be noted that the total investment required for the project was not examined in this study and is beyond the scope of the study. Moreover, the economic viability of the project was not evaluated as the viability of the project is dependent on the revenues generated through the sale of electricity, BioRNG and/or BioCNG in the respective scenarios. The feedstock cost of $40/t of chicken manure could incur an operating cost of $24,840 per annum. Other operating costs would include fossil fuel electricity cost at $160/MWh to meet parasitic electrical and heating demand of the biogas plant, ensilation costs of $0.16/kg and biomethane grid connection cost of $0.8/GJ.

Table 38. Annual greenhouse gas (GHG) emissions (tCO_2-e/a) avoided for implementation of the farm-scale biogas plant under all studied scenarios.

Emission from fossil fuel	Scenario 1	Scenario 2	Scenario 3
Transport of chicken manure	6.23	6.23	6.23
Import of electricity for decanter centrifuge	--	34.14	34.15
Imported natural gas for heat demand of AD plant	1.43	136.31	125.07
Electricity Generation	-512.77	--	--
BioRNG	--	-304.77	--
BioCNG	--	--	-281.37
Biofertilisers (Nitrogen fertiliser)	-31.00	-31.00	-31.00
Avoided GHG emissions	536.10	193.22	146.94

The revenues for farm-scale biogas plant would originate from the sale of electricity to grid injection through feed-in tariffs at $85/MWh, BioRNG to grid at $8.5/GJ, $16/GJ for BioCNG, green certificates at $3/GJ and $200/t for uncompressed food-grade $BioCO_2$. Sale of solid digestate at $10/t as soil conditioner and Australian Carbon Credit Units (ACCUs) at $30/t CO_2-e would generate additional revenue but are not guaranteed. Inclusion of ACCUs and green certificates can improve the overall economics of the project. However, ACCUs are not currently available in Australia for such projects. But it is anticipated that ACCUs could be introduced in the future for selected bioenergy projects and is regulated by the Clean Energy Regulator (CER). As the ACCUs represent the project's emissions reductions, the project proponents can receive funding by entering their projects into a CER-run competitive auction.

5. CONCLUSION

This study demonstrates that the anaerobic digestion process can effectively convert sweet sorghum cultivars into hydrogen and/or methane. The present study aimed to investigate the impact of harvest time, inoculum to substrate ratio (ISR), and inoculum source on methane production from sweet sorghum cultivars in following batch anaerobic digestion studies. The chemical analysis revealed that the total solids (TS) content of the nine sweet sorghum cultivars varied from 12.05% (SE-23) to 21.40% w/w (Mega Sweet). A similar trend was also observed in relation to volatile solids (VS). The observed high VS/TS values ranging from 0.89 to 0.95, along with a low carbon to nitrogen (C/N) ratio ranging from 40 to 80, suggest that the examined cultivars include a substantial amount of organic matter.

The sweet sorghum cultivars that were evaluated also revealed a carbohydrate content ranging from 45% to 60% (w/w), whereas the lignin level ranged from 16% to 25% (w/w). These findings suggest that sweet sorghum possesses favorable characteristics as a feedstock for the generation of biohydrogen, organic acids, and methane.

The effect of different harvest times (50, 66, and 80 days after sowing, DAS) on the chemical composition and methane yields of SE-19, SE-45, and Mega Sweet varieties was investigated. The results indicated that harvesting the crop at 80 DAS resulted in increased biomass and methane yields. Following 80 days after sowing (DAS), the cultivar SE-45 showed the maximum biomass production of 174 t/ha fresh matter (FM), whereas SE-19 and Mega Sweet achieved biomass yields of 132 t/ha and 71 t/ha, respectively. The methane yields for SE-19, SE-45, and Mega Sweet were measured as 168.99, 184.39, and 243.47 NL CH_4/kgVSadded, respectively, after 80 days after sowing (DAS).

The effect of inoculum to substrate ratios (ISR) of 2, 4, and 6 on the methane yields of nine different sweet sorghum cultivars (SE-1, SE-5, SE-23, SE-35, SE-42, SE-45, SE-81, SE-86, and Mega Sweet) was studied. The results indicated that methane yields showed an increase as the ISR increased, while also being influenced by the specific chemical composition of each sweet sorghum cultivar. At an inoculum to substrate ratio (ISR) 2, the methane production exhibited a wide range of values, with the lowest recorded yield being 175.93 NL

$CH_4/kgVS_{added}$, and the maximum value seen at 291.27 NL $CH_4/kgVS_{added}$. The increase in the Inoculum to substrate ratio (ISR) from 2 to 4 led to a corresponding increase in methane levels ranging from 67.6% to 76.4%. The methane yields experienced a significant rise of 46.8% to 78.1% as a direct consequence of further raising the initial solid-to-liquid ratio (ISR) to 6. Within these cultivars, it was observed that SE-1 had the lowest methane yields, ranging from 175.93 to 306.86 NL $CH_4/kgVS_{added}$. In contrast, it was noticed that SE-35 exhibited the most notable methane yields, measuring 227, 291, and 375.91 NL $CH_4/kgVS_{added}$, which were achieved at ISRs of 2, 4, and 6 for SE-35, respectively. The SE-35 cultivar had the highest biomass yields, reaching 82.3 t/ha. However, its per hectare methane yield was very low at 29.83 m^3/tFM, which can be attributed to its low plant population. Therefore, the SE-81 cultivar was chosen as a potentially advantageous sweet sorghum cultivar based on its comparatively high methane yields of 3,059.18 m^3/ha and overall energy yield. The SE-81 cultivar was found to have an estimated gross energy yield of 29.55 MWh/ha, which is equivalent to the energy consumption of approximately 37.98 passenger automobiles per hectare per year.

Rumen fluid, sewage sludge digestate, and cow dung digestate as inoculum sources on the methane yields of SE-1, SE-19, SE-20, SE-35, and SE-122 revealed a significant impact on methane yields of each cultivar. The results of the experiment indicated that cow dung digestate had the highest efficiency as an inoculum, yielding methane in the range of 241.81-281 NL $CH_4/kgVS_{added}$.

The influence of organic loading rate (OLR) was studied within the range of 0.5-3.5 gVS/L/d to determine the efficiency of the anaerobic digestion process and the resulting methane yields. In the context of mono-digestion of sweet sorghum, it was determined that an optimal organic loading rate (OLR) of 2.5 gVS/L/d was observed. In the context of co-digestion, it is recommended to operate the reactor at an Organic Loading Rate (OLR) of 3 gVS/L/d. The co-digestion of sweet sorghum with cow dung resulted in a maximum methane output of 229.70 NL $CH_4/kgVS_{added}$. The results of this study also indicate that the use of cow manure as a substrate represents a feasible approach for enhancing both the operational efficiency and methane generation from sweet sorghum.

The evaluation of the feasibility of utilizing sweet sorghum cultivated on sugarcane fallow for biogas production in a farm-scale biogas plant was conducted based on the data obtained from the experiments. The quantity of SE-81 biomass obtained from a 25-hectare area of uncultivated land was determined to be 1,938 metric tons of fresh matter per year. The results of this study show that a biogas plant with a capacity of 1300 m^3, when supplied with a daily feedstock of 28.7 t of sweet sorghum and chicken manure, can produce a daily biogas production of 840.15 m^3 under various conditions. In the first scenario, the combined heat and power (CHP) system, which consists of an 84 kW generator, generated an annual electricity output of 2,520 GJ and a heat output of 2,917 GJ. Following the conclusion of the conference, it has been determined that a total of 523 MWh per annum of power can be supplied to the grid in order to satisfy the parasitic energy requirements. In Scenario 2, the generation of electricity amounted to 784 GJ/a, while the production of heat reached 965 GJ/a. The calculated annual energy output of the exportable BioRNG was determined to be 4,652 GJ. In Scenario 3, the power generated amounted to 927 GJ/a, while the heat generated reached 1,141 GJ/a. The projected amount of exportable BioCNG in Scenario 3 was 4,283 GJ/a. The outcomes of this study indicate that Scenario 1 is the most viable choice for cultivating sweet sorghum on sugarcane fallow land and utilizing the resultant biogas for heat and electricity generation in a combined heat and power facility.

BIBLIOGRAPHY

1. Gielen, D., F. Boshell, D. Saygin, M.D. Bazilian, N. Wagner, and R. Gorini, *The role of renewable energy in the global energy transformation.* Energy Strategy Reviews, 2019. **24**: p. 38-50.
2. Kucharska, K., P. Rybarczyk, I. Holowacz, R. Lukajtis, M. Glinka, and M. Kaminski, *Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes.* Molecules, 2018. **23**(11).
3. Valentine, J., J. Clifton-Brown, A. Hastings, P. Robson, G. Allison, and P. Smith, *Food vs. fuel: The use of land for lignocellulosic 'next generation' energy crops that minimize competition with primary food production.* GCB Bioenergy, 2011. **4**: p. 1-19.
4. Mitchell, R.B., M.R. Schmer, W.F. Anderson, V. Jin, K.S. Balkcom, J. Kiniry, A. Coffin, and P. White, *Dedicated Energy Crops and Crop Residues for Bioenergy Feedstocks in the Central and Eastern USA.* BioEnergy Research, 2016. **9**(2): p. 384-398.
5. Boboescu, I.-Z., J. Damay, J.K.W. Chang, J.-B. Beigbeder, X. Duret, S. Beauchemin, O. Lalonde, and J.-M. Lavoie, *Ethanol production from residual lignocellulosic fibers generated through the steam treatment of whole sorghum biomass.* Bioresource Technology, 2019. **292**: p. 121975.
6. Carvalho, W.S., J.A. Santana Junior, and C.H. Ataíde, *Catalytic pyrolysis of sweet sorghum bagasse in the presence of two acid catalysts.* Journal of the Energy Institute, 2020. **93**(1): p. 185-197.
7. Propheter, J., S. Staggenborg, X. Wu, and D. Wang, *Performance of Annual and Perennial Biofuel Crops: Yield during the First Two Years.* Agronomy Journal - AGRON J, 2010. **102**.
8. Salimbeni, A., *SWEET SORGHUM BIOGAS PLANT IN TEMPERATE REGIONS (BELGIUM) -DEMONSTRATION PLANT FOR BIOGAS AND HIGH VALUE BIOFERTILIZER PRODUCTION*. 2013.
9. Koszel, M. and E. Lorencowicz, *Agricultural Use of Biogas Digestate as a Replacement Fertilizers.* Agriculture and Agricultural Science Procedia, 2015. **7**: p. 119-124.
10. Khan, I., *Waste to biogas through anaerobic digestion: Hydrogen production potential in the developing world - A case of Bangladesh.* International Journal of Hydrogen Energy, 2020. **45**(32): p. 15951-15962.
11. Janusz, G., A. Pawlik, J. Sulej, U. Swiderska-Burek, A. Jarosz-Wilkolazka, and A. Paszczynski, *Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution.* FEMS Microbiol Rev, 2017. **41**(6): p. 941-962.
12. Singh, A.K., M. Bilal, H.M.N. Iqbal, A.S. Meyer, and A. Raj, *Bioremediation of lignin derivatives and phenolics in wastewater with lignin modifying enzymes: Status, opportunities and challenges.* Science of The Total Environment, 2021. **777**: p. 145988.
13. Mafa, M.S., S. Malgas, A. Bhattacharya, K. Rashamuse, and B.I. Pletschke, *The Effects of Alkaline Pretreatment on Agricultural Biomasses (Corn Cob and Sweet Sorghum Bagasse) and Their Hydrolysis by a Termite-Derived Enzyme Cocktail.* Agronomy, 2020. **10**(8).
14. Syamsu, K., L. Haditjaroko, and E.A. Syadiah, *Bio-ethanol production from sweet sorghum bagasse by engineered simultaneous saccharification and fermentation technology using Trichoderma reesei and Saccharomyces cerevisiae.* IOP Conference Series: Earth and Environmental Science, 2020. **472**: p. 012025.

15. Pramasari, D.A., L. Haditjaroko, T.C. Sunarti, E. Hermiati, and K. Syamsu, *The effectiveness of physical and alkali hydrothermal pretreatment in improving enzyme susceptibility of sweet sorghum bagasse.* Jurnal Bahan Alam Terbarukan, 2017. **6**(2): p. 117-131.
16. Mirfakhar, M., M.A. Asadollahi, H. Amiri, and K. Karimi, *Co-fermentation of hemicellulosic hydrolysates and starch from sweet sorghum by Clostridium acetobutylicum: A synergistic effect for butanol production.* Industrial Crops and Products, 2020. **151**: p. 112459.
17. Antonopoulou, G., H.N. Gavala, I.V. Skiadas, and G. Lyberatos, *Effect of substrate concentration on fermentative hydrogen production from sweet sorghum extract.* International Journal of Hydrogen Energy, 2011. **36**(8): p. 4843-4851.
18. Rafiee, A. and K.R. Khalilpour, *Chapter 11 - Renewable Hybridization of Oil and Gas Supply Chains*, in *Polygeneration with Polystorage for Chemical and Energy Hubs*, K.R. Khalilpour, Editor. 2019, Academic Press. p. 331-372.
19. Roberts, L.G. and T.J. Patterson, *Biofuels*, in *Encyclopedia of Toxicology (Third Edition),* P. Wexler, Editor. 2014, Academic Press: Oxford. p. 469-475.
20. Dahman, Y., K. Syed, S. Begum, P. Roy, and B. Mohtasebi, *14 - Biofuels: Their characteristics and analysis*, in *Biomass, Biopolymer-Based Materials, and Bioenergy*, D. Verma, et al., Editors. 2019, Woodhead Publishing. p. 277-325.
21. Carlu, E., T. Truong, and M. Kundevski, *Biogas opportunities for Australia March 2019.* 2019: France. p. 53.
22. Scarlat, N., J.-F. Dallemand, and F. Fahl, *Biogas: Developments and perspectives in Europe.* Renewable Energy, 2018. **129**: p. 457-472.
23. Klessmann, C., A. Held, M. Rathmann, and M. Ragwitz, *Status and perspectives of renewable energy policy and deployment in the European Union—What is needed to reach the 2020 targets?* Energy policy, 2011. **39**(12): p. 7637-7657.
24. Ahmad Dar, R., E. Ahmad Dar, A. Kaur, and U. Gupta Phutela, *Sweet sorghum-a promising alternative feedstock for biofuel production.* Renewable and Sustainable Energy Reviews, 2018. **82**: p. 4070-4090.
25. Grove, A.T., *The geography of semi-arid lands.* Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 1977. **278**(962): p. 457-475.
26. Kundevski, M., *Biogas: The energy wonder that's under our noses.* Ecogeneration, 2020(116): p. 26-29.
27. Clean Energy, C., *Clean energy Australia report 2020.* 2020.
28. Stucley, C., S. Schuck, R. Sims, J. Bland, B. Marino, M. Borowitzka, A. Abadi, J. Bartle, R. Giles, and Q. Thomas, *Bioenergy in Australia: status and opportunities.* 2012.
29. Pires, J.R., V.G. Souza, and A.L. Fernando, *Valorization of energy crops as a source for nanocellulose production–current knowledge and future prospects.* Industrial Crops and Products, 2019. **140**: p. 111642.
30. Leontopoulos, S. and G. Arabatzis, *The contribution of energy crops to biomass production.* Low Carbon Energy Technologies in Sustainable Energy Systems, 2021: p. 47-113.
31. Markou, G., M. Brulé, A. Balafoutis, M. Kornaros, D. Georgakakis, and G. Papadakis, *Biogas production from energy crops in northern Greece: economics of electricity generation associated with heat recovery in a greenhouse.* Clean Technologies and Environmental Policy, 2017. **19**(4): p. 1147-1167.
32. Laiq Ur Rehman, M., A. Iqbal, C.-C. Chang, W. Li, and M. Ju, *Anaerobic digestion.* Water Environment Research, 2019. **91**(10): p. 1253-1271.

33. Gopal, P.M., N.M. Sivaram, and D. Barik, *Chapter 7 - Paper Industry Wastes and Energy Generation From Wastes*, in *Energy from Toxic Organic Waste for Heat and Power Generation*, D. Barik, Editor. 2019, Woodhead Publishing. p. 83-97.
34. Xu, F. and Y. Li, *Biomass Digestion*, in *Encyclopedia of Sustainable Technologies*, M.A. Abraham, Editor. 2017, Elsevier: Oxford. p. 197-204.
35. Khanal, S.K., T.G. Tirta Nindhia, and S. Nitayavardhana, *Chapter 11 - Biogas From Wastes: Processes and Applications*, in *Sustainable Resource Recovery and Zero Waste Approaches*, M.J. Taherzadeh, et al., Editors. 2019, Elsevier. p. 165-174.
36. Lu, D., X. Liu, O.G. Apul, L. Zhang, D.K. Ryan, and X. Zhang, *Optimization of biomethane production from anaerobic Co-digestion of microalgae and septic tank sludge.* Biomass and Bioenergy, 2019. **127**: p. 105266.
37. Rajagopal, R., D.I. Massé, and G. Singh, *A critical review on inhibition of anaerobic digestion process by excess ammonia.* Bioresource technology, 2013. **143**: p. 632-641.
38. Pramanik, S.K., F.B. Suja, S.M. Zain, and B.K. Pramanik, *The anaerobic digestion process of biogas production from food waste: Prospects and constraints.* Bioresource Technology Reports, 2019. **8**: p. 100310.
39. Dahiya, S., O. Sarkar, Y. Swamy, and S.V. Mohan, *Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen.* Bioresource technology, 2015. **182**: p. 103-113.
40. Drake, H.L., *Acetogenesis*. 2012: Springer Science & Business Media.
41. Fenchel, T., G.M. King, and T.H. Blackburn, *Chapter 1 - Bacterial Metabolism*, in *Bacterial Biogeochemistry (Third Edition)*, T. Fenchel, G.M. King, and T.H. Blackburn, Editors. 2012, Academic Press: Boston. p. 1-34.
42. Wu, C., Q. Huang, M. Yu, Y. Ren, Q. Wang, and K. Sakai, *Effects of digestate recirculation on a two-stage anaerobic digestion system, particularly focusing on metabolite correlation analysis.* Bioresource Technology, 2018. **251**: p. 40-48.
43. Chojnacka, K., K. Moustakas, and A. Witek-Krowiak, *Bio-based fertilizers: A practical approach towards circular economy.* Bioresource Technology, 2020. **295**: p. 122223.
44. Santos, C.A. and A. Reis, *Microalgal symbiosis in biotechnology.* Applied Microbiology and Biotechnology, 2014. **98**(13): p. 5839-5846.
45. Mao, C., Y. Feng, X. Wang, and G. Ren, *Review on research achievements of biogas from anaerobic digestion.* Renewable and Sustainable Energy Reviews, 2015. **45**: p. 540-555.
46. Cioabla, A.E., I. Ionel, G.-A. Dumitrel, and F. Popescu, *Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues.* Biotechnology for Biofuels, 2012. **5**(1): p. 39.
47. Wintsche, B., K. Glaser, H. Sträuber, F. Centler, J. Liebetrau, H. Harms, and S. Kleinsteuber, *Trace Elements Induce Predominance among Methanogenic Activity in Anaerobic Digestion.* Frontiers in microbiology, 2016. **7**: p. 2034-2034.
48. Tsapekos, P., P.G. Kougias, and I. Angelidaki, *Mechanical pretreatment for increased biogas production from lignocellulosic biomass; predicting the methane yield from structural plant components.* Waste Management, 2018. **78**: p. 903-910.
49. Patinvoh, R.J., O.A. Osadolor, K. Chandolias, I. Sarvari Horvath, and M.J. Taherzadeh, *Innovative pretreatment strategies for biogas production.* Bioresour Technol, 2017. **224**: p. 13-24.
50. Hübner, M., H. Oechsner, S. Koch, A. Seggl, H. Hrenn, B. Schmiedchen, P. Wilde, and T. Miedaner, *Impact of genotype, harvest time and chemical*

composition on the methane yield of winter rye for biogas production. Biomass and Bioenergy, 2011. **35**(10): p. 4316-4323.
51. Bouzas, A., C. Gabaldón, P. Marzal, J.M. Penya-roja, and A. Seco, *Fermentation of Municipal Primary Sludge: Effect of Srt and Solids Concentration on Volatile Fatty Acid Production.* Environmental Technology, 2002. **23**(8): p. 863-875.
52. Cremonez, P.A., S.C. Sampaio, J.G. Teleken, T.W. Meier, J. Dieter, and J. Teleken, *Influence of inoculum to substrate ratio on the anaerobic digestion of a cassava starch polymer.* Industrial Crops and Products, 2019. **141**: p. 111709.
53. Zhang, W., L. Li, W. Xing, B. Chen, L. Zhang, A. Li, R. Li, and T. Yang, *Dynamic behaviors of batch anaerobic systems of food waste for methane production under different organic loads, substrate to inoculum ratios and initial pH.* Journal of Bioscience and Bioengineering, 2019. **128**(6): p. 733-743.
54. Cordoba, V., M. Fernandez, and E. Santalla, *The effect of substrate/inoculum ratio on the kinetics of methane production in swine wastewater anaerobic digestion.* Environ Sci Pollut Res Int, 2018. **25**(22): p. 21308-21317.
55. Latifi, P., M. Karrabi, and S. Danesh, *Anaerobic co-digestion of poultry slaughterhouse wastes with sewage sludge in batch-mode bioreactors (effect of inoculum-substrate ratio and total solids).* Renewable and Sustainable Energy Reviews, 2019. **107**: p. 288-296.
56. Pastor-Poquet, V., S. Papirio, E. Trably, J. Rintala, R. Escudié, and G. Esposito, *High-solids anaerobic digestion requires a trade-off between total solids, inoculum-to-substrate ratio and ammonia inhibition.* International Journal of Environmental Science and Technology, 2019. **16**(11): p. 7011-7024.
57. Hanum, F., L.C. Yuan, H. Kamahara, H.A. Aziz, Y. Atsuta, T. Yamada, and H. Daimon, *Treatment of Sewage Sludge Using Anaerobic Digestion in Malaysia: Current State and Challenges.* Frontiers in Energy Research, 2019. **7**(19).
58. Das, S., S.T. Jeong, S. Das, and P.J. Kim, *Composted Cattle Manure Increases Microbial Activity and Soil Fertility More Than Composted Swine Manure in a Submerged Rice Paddy.* Frontiers in Microbiology, 2017. **8**(1702).
59. Li, Y., J. Zhao, J. Krooneman, and G.J.W. Euverink, *Strategies to boost anaerobic digestion performance of cow manure: Laboratory achievements and their full-scale application potential.* Science of The Total Environment, 2021. **755**: p. 142940.
60. Nimame, P. and R. Ekemube, *INVESTIGATION INTO THE MICROBIAL ACTIVITIES IN BIOGAS PRODUCTION USING COW DUNG.* 2020. **8**: p. 11-19.
61. Im, S., S.O. Petersen, D. Lee, and D.-H. Kim, *Effects of storage temperature on CH4 emissions from cattle manure and subsequent biogas production potential.* Waste Management, 2020. **101**: p. 35-43.
62. Pandey, P., C. Chiu, M. Miao, Y. Wang, M. Settles, N.S. Del Rio, A. Castillo, A. Souza, R. Pereira, and R. Jeannotte, *16S rRNA analysis of diversity of manure microbial community in dairy farm environment.* PloS one, 2018. **13**(1): p. e0190126-e0190126.
63. Gupta, K.K., K.R. Aneja, and D. Rana, *Current status of cow dung as a bioresource for sustainable development.* Bioresources and Bioprocessing, 2016. **3**(1): p. 28.
64. Yue, Z.-B., W.-W. Li, and H.-Q. Yu, *Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass.* Bioresource technology, 2012. **128**.
65. Budiyono, B., I.N. Widiasa, S. Johari, and S. Sunarso, *Increasing Biogas Production Rate from Cattle Manure Using Rumen Fluid as Inoculums.* International Journal of Science and Engineering, 2014. **6**(1).

66. Xing, B.-S., Y. Han, X.C. Wang, J. Ma, S. Cao, Q. Li, J. Wen, and H. Yuan, *Cow manure as additive to a DMBR for stable and high-rate digestion of food waste: Performance and microbial community.* Water Research, 2020. **168**: p. 115099.
67. Rajput, A.A. and Z. Sheikh, *Effect of inoculum type and organic loading on biogas production of sunflower meal and wheat straw.* Sustainable Environment Research, 2019. **29**(1): p. 4.
68. Ali Shah, F., Q. Mahmood, M. Maroof Shah, A. Pervez, and S. Ahmad Asad, *Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis.* The Scientific World Journal, 2014. **2014**: p. 183752.
69. Ozbayram, E.G., O. Ince, B. Ince, H. Harms, and S. Kleinsteuber, *Comparison of rumen and manure microbiomes and implications for the inoculation of anaerobic digesters.* Microorganisms, 2018. **6**(1): p. 15.
70. Jiang, Y., E. McAdam, Y. Zhang, S. Heaven, C. Banks, and P. Longhurst, *Ammonia inhibition and toxicity in anaerobic digestion: A critical review.* Journal of Water Process Engineering, 2019. **32**: p. 100899.
71. Boob, M., M. Elsaesser, U. Thumm, J. Hartung, and I. Lewandowski, *Harvest time determines quality and usability of biomass from lowland hay meadows.* Agriculture (Switzerland), 2019. **9**(9).
72. Waliszewska, H., M. Zborowska, B. Waliszewska, S. Borysiak, A. Antczak, and W. Czekała, *Transformation of Miscanthus and Sorghum cellulose during methane fermentation.* Cellulose, 2018. **25**(2): p. 1207-1216.
73. Baute, K., L. Van Eerd, D. Robinson, P. Sikkema, M. Mushtaq, and B. Gilroyed, *Comparing the Biomass Yield and Biogas Potential of Phragmites australis with Miscanthus x giganteus and Panicum virgatum Grown in Canada.* Energies, 2018. **11**(9).
74. Da Borso, F., C. Di Marzo, F. Zuliani, F. Danuso, and M. Baldini, *Harvest time and ensilage suitability of giant reed and miscanthus for bio-methane production and characterization of digestate for agronomic use.* Agronomy Research, 2018. **16**(1): p. 22-40.
75. Chamarthy, R., S.K. Chakravarthy, V. Komala, U. Chavan, and J. Patil, *Sweet Sorghum as Feedstock for Biofuel Production: A Review.* Sugar Tech, 2011. **13**.
76. Neshat, S.A., M. Mohammadi, G.D. Najafpour, and P. Lahijani, *Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production.* Renewable and Sustainable Energy Reviews, 2017. **79**: p. 308-322.
77. Wang, H., J. Xu, L. Sheng, X. Liu, Y. Lu, and W. Li, *A review on bio-hydrogen production technology.* International Journal of Energy Research, 2018. **42**(11): p. 3442-3453.
78. Goria, K., R. Kothari, H.M. Singh, A. Singh, and V. Tyagi, *Biohydrogen: potential applications, approaches, and hurdles to overcome*, in *Handbook of Biofuels*. 2022, Elsevier. p. 399-418.
79. Kapdan, I.K. and F. Kargi, *Bio-hydrogen production from waste materials.* Enzyme and microbial technology, 2006. **38**(5): p. 569-582.
80. Sarangi, P.K. and S. Nanda, *Biohydrogen Production Through Dark Fermentation.* Chemical Engineering & Technology, 2020. **43**(4): p. 601-612.
81. Pavlostathis, S.G., *6.31 - Kinetics and Modeling of Anaerobic Treatment and Biotransformation Processes*, in *Comprehensive Biotechnology (Second Edition)*, M. Moo-Young, Editor. 2011, Academic Press: Burlington. p. 385-397.
82. Monlau, F., A. Barakat, E. Trably, C. Dumas, J.P. Steyer, and H. Carrère, *Lignocellulosic materials into biohydrogen and biomethane: Impact of structural features and pretreatment.* Critical Reviews in Environmental Science and Technology, 2013. **43**(3): p. 260-322.

83. Pachapur, V.L., P. Kutty, P. Pachapur, S.K. Brar, Y. Le Bihan, R. Galvez-Cloutier, and G. Buelna, *Seed pretreatment for increased hydrogen production using mixed-culture systems with advantages over pure-culture systems.* Energies, 2019. **12**(3): p. 530.
84. Levin, D.B., L. Pitt, and M. Love, *Biohydrogen production: prospects and limitations to practical application.* International journal of hydrogen energy, 2004. **29**(2): p. 173-185.
85. Bhatia, S.K., S.S. Jagtap, A.A. Bedekar, R.K. Bhatia, K. Rajendran, A. Pugazhendhi, C.V. Rao, A. Atabani, G. Kumar, and Y.-H. Yang, *Renewable biohydrogen production from lignocellulosic biomass using fermentation and integration of systems with other energy generation technologies.* Science of The Total Environment, 2020: p. 144429.
86. Sivagurunathan, P., G. Kumar, A. Mudhoo, E.R. Rene, G.D. Saratale, T. Kobayashi, K. Xu, S.-H. Kim, and D.-H. Kim, *Fermentative hydrogen production using lignocellulose biomass: An overview of pre-treatment methods, inhibitor effects and detoxification experiences.* Renewable and Sustainable Energy Reviews, 2017. **77**: p. 28-42.
87. Serrato-Nerio, H.E., A. Díaz-Hinojosa, and F.J. Cervantes, *Vacuum-assisted production of hydrogen and volatile fatty acids from lignocellulosic biomass derived from energy-crops pruning.* International Journal of Hydrogen Energy, 2020. **45**(53): p. 28499-28504.
88. Buitrón, G., J. Carrillo-Reyes, M. Morales, C. Faraloni, and G. Torzillo, *9 - Biohydrogen production from microalgae*, in *Microalgae-Based Biofuels and Bioproducts*, C. Gonzalez-Fernandez and R. Muñoz, Editors. 2017, Woodhead Publishing. p. 209-234.
89. Lee, H.-S., W.F. Vermaas, and B.E. Rittmann, *Biological hydrogen production: prospects and challenges.* Trends in biotechnology, 2010. **28**(5): p. 262-271.
90. Show, K.-Y., Y. Yan, and D.-J. Lee, *Chapter 16 - Bioreactor and Bioprocess Design for Biohydrogen Production*, in *Biohydrogen (Second Edition)*, A. Pandey, et al., Editors. 2019, Elsevier. p. 391-411.
91. Wang, J. and Y. Yin, *Progress in microbiology for fermentative hydrogen production from organic wastes.* Critical Reviews in Environmental Science and Technology, 2019. **49**(10): p. 825-865.
92. Arevalo-Gallegos, A., Z. Ahmad, M. Asgher, R. Parra-Saldivar, and H.M.N. Iqbal, *Lignocellulose: A sustainable material to produce value-added products with a zero waste approach—A review.* International Journal of Biological Macromolecules, 2017. **99**: p. 308-318.
93. Ramos-Suarez, M., Y. Zhang, and V. Outram, *Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste.* Reviews in Environmental Science and Bio/Technology, 2021. **20**(2): p. 439-478.
94. Moate, P., T. Clarke , L. Davis , and R. Laby *Rumen gases and bloat in grazing dairy cows.* The Journal of Agricultural Science, 1997. **129**: p. 459-469.
95. Wainaina, S., Lukitawesa, M. Kumar Awasthi, and M.J. Taherzadeh, *Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review.* Bioengineered, 2019. **10**(1): p. 437-458.
96. Yuan, Y., X. Hu, H. Chen, Y. Zhou, Y. Zhou, and D. Wang, *Advances in enhanced volatile fatty acid production from anaerobic fermentation of waste activated sludge.* Science of The Total Environment, 2019. **694**: p. 133741.
97. Zhou, M., B. Yan, J.W.C. Wong, and Y. Zhang, *Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways.* Bioresource Technology, 2018. **248**: p. 68-78.

98. Noblecourt, A., G. Christophe, C. Larroche, and P. Fontanille, *Hydrogen production by dark fermentation from pre-fermented depackaging food wastes.* Bioresource Technology, 2018. **247**: p. 864-870.
99. Balachandar, G., J.L. Varanasi, V. Singh, H. Singh, and D. Das, *Biological hydrogen production via dark fermentation: A holistic approach from lab-scale to pilot-scale.* International Journal of Hydrogen Energy, 2020. **45**(8): p. 5202-5215.
100. Silva, J.S., J.S. Mendes, J.A.C. Correia, M.V.P. Rocha, and L. Micoli, *Cashew apple bagasse as new feedstock for the hydrogen production using dark fermentation process.* Journal of Biotechnology, 2018. **286**: p. 71-78.
101. Romão, B.B., F.T.M. Silva, H.C. de Barcelos Costa, T.S. do Carmo, S.L. Cardoso, J. de Souza Ferreira, F.R.X. Batista, and V.L. Cardoso, *Alternative techniques to improve hydrogen production by dark fermentation.* 3 Biotech, 2019. **9**(1): p. 18.
102. Mu, D., H. Liu, W. Lin, P. Shukla, and J. Luo, *Simultaneous biohydrogen production from dark fermentation of duckweed and waste utilization for microalgal lipid production.* Bioresource Technology, 2020. **302**: p. 122879.
103. Ozmihci, S., *Performance of batch solid state fermentation for hydrogen production using ground wheat residue.* International Journal of Hydrogen Energy, 2017. **42**(37): p. 23494-23499.
104. Magrini, F.E., G.M. de Almeida, D. da Maia Soares, L. Fuentes, C. Ecthebehere, L.L. Beal, M.M. da Silveira, and S. Paesi, *Effect of different heat treatments of inoculum on the production of hydrogen and volatile fatty acids by dark fermentation of sugarcane vinasse.* Biomass Conversion and Biorefinery, 2020.
105. Islam, M.S., C. Zhang, K.-Y. Sui, C. Guo, and C.-Z. Liu, *Coproduction of hydrogen and volatile fatty acid via thermophilic fermentation of sweet sorghum stalk from co-culture of Clostridium thermocellum and Clostridium thermosaccharolyticum.* International Journal of Hydrogen Energy, 2017. **42**(2): p. 830-837.
106. Tunçay, E.G., T.H. Erguder, İ. Eroğlu, and U. Gündüz, *Dark fermentative hydrogen production from sucrose and molasses.* International Journal of Energy Research, 2017. **41**(13): p. 1891-1902.
107. Zhao, X.-x., X.-l. Fan, Z.-x. Xue, Z.-m. Yang, X.-z. Yuan, Y.-l. Qiu, and R.-b. Guo, *Simultaneous production of hydrogen and volatile fatty acids from anaerobic digestion of Macrocystis pyrifera biomass residues.* Journal of Central South University, 2017. **24**(6): p. 1281-1287.
108. Panagiotopoulos, I.A., R.R. Bakker, T. de Vrije, E.G. Koukios, and P.A.M. Claassen, *Pretreatment of sweet sorghum bagasse for hydrogen production by Caldicellulosiruptor saccharolyticus.* International Journal of Hydrogen Energy, 2010. **35**(15): p. 7738-7747.
109. Rorke, D. and E.B. Gueguim Kana, *Biohydrogen process development on waste sorghum (Sorghum bicolor) leaves: Optimization of saccharification, hydrogen production and preliminary scale up.* International Journal of Hydrogen Energy, 2016. **41**(30): p. 12941-12952.
110. Sarkar, O., R. Katakojwala, and S. Venkata Mohan, *Low carbon hydrogen production from a waste-based biorefinery system and environmental sustainability assessment.* Green Chemistry, 2021. **23**(1): p. 561-574.
111. Velásquez Piñas, J.A., O.J. Venturini, E.E. Silva Lora, and O.D. Calle Roalcaba, *Technical assessment of mono-digestion and co-digestion systems for the production of biogas from anaerobic digestion in Brazil.* Renewable Energy, 2018. **117**: p. 447-458.

112. Herrmann, C., N. Kalita, D. Wall, A. Xia, and J.D. Murphy, *Optimised biogas production from microalgae through co-digestion with carbon-rich co-substrates.* Bioresource Technology, 2016. **214**: p. 328-337.
113. Zahan, Z., S. Georgiou, T.H. Muster, and M.Z. Othman, *Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: A case study on the effect of carbon/nitrogen ratio, substrates mixing ratio and organic loading.* Bioresource Technology, 2018. **270**: p. 245-254.
114. Koul, B., M. Yakoob, and M.P. Shah, *Agricultural waste management strategies for environmental sustainability.* Environmental Research, 2022. **206**: p. 112285.
115. Karki, R., W. Chuenchart, K.C. Surendra, S. Shrestha, L. Raskin, S. Sung, A. Hashimoto, and S. Kumar Khanal, *Anaerobic co-digestion: Current status and perspectives.* Bioresource Technology, 2021. **330**: p. 125001.
116. Ghaleb, A.A., S.R. Kutty, G.H. Salih, A.H. Jagaba, A. Noor, V. Kumar, N.M. Almahbashi, A.A. Saeed, and B.N. Saleh Al-dhawi *Sugarcane Bagasse as a Co-Substrate with Oil-Refinery Biological Sludge for Biogas Production Using Batch Mesophilic Anaerobic Co-Digestion Technology: Effect of Carbon/Nitrogen Ratio*. Water, 2021. **13**, DOI: 10.3390/w13050590.
117. Xue, S., Y. Wang, X. Lyu, N. Zhao, J. Song, X. Wang, and G. Yang, *Interactive effects of carbohydrate, lipid, protein composition and carbon/nitrogen ratio on biogas production of different food wastes.* Bioresource Technology, 2020. **312**: p. 123566.
118. Kamperidou, V. and P. Terzopoulou *Anaerobic Digestion of Lignocellulosic Waste Materials*. Sustainability, 2021. **13**, DOI: 10.3390/su132212810.
119. Tira, H.S., Y.A. Padang, and I.M.P. Salman, *EFFECT OF CARBON TO NITROGEN RATIO ON MIXTURE OF COW AND CHICKEN MANURE ON THERMOPHILIC FERMENTATION IN PRODUCING BIOGAS.*
120. Aboudi, K., X. Gómez-Quiroga, C.J. Álvarez-Gallego, and L.I. Romero-García *Insights into Anaerobic Co-Digestion of Lignocellulosic Biomass (Sugar Beet By-Products) and Animal Manure in Long-Term Semi-Continuous Assays*. Applied Sciences, 2020. **10**, DOI: 10.3390/app10155126.
121. Czatzkowska, M., M. Harnisz, E. Korzeniewska, and I. Koniuszewska, *Inhibitors of the methane fermentation process with particular emphasis on the microbiological aspect: A review.* Energy Science & Engineering, 2020. **8**(5): p. 1880-1897.
122. Malhotra, M., K. Aboudi, L. Pisharody, A. Singh, J.R. Banu, S.K. Bhatia, S. Varjani, S. Kumar, C. González-Fernández, S. Kumar, R. Singh, and V.K. Tyagi, *Biorefinery of anaerobic digestate in a circular bioeconomy: Opportunities, challenges and perspectives.* Renewable and Sustainable Energy Reviews, 2022. **166**: p. 112642.
123. Farghali, M., A.I. Osman, K. Umetsu, and D.W. Rooney, *Integration of biogas systems into a carbon zero and hydrogen economy: a review.* Environmental Chemistry Letters, 2022. **20**(5): p. 2853-2927.
124. Yangin-Gomec, C. and I. Ozturk, *Effect of maize silage addition on biomethane recovery from mesophilic co-digestion of chicken and cattle manure to suppress ammonia inhibition.* Energy Conversion and Management, 2013. **71**: p. 92-100.
125. Adrover, M.E., I. Cotabarren, E. Madies, M. Rayes, S.B. Rodriguez Reartes, and M. Pedernera, *Anaerobic co-digestion of rabbit manure and sorghum crops in a bench-scale biodigester.* Bioresources and Bioprocessing, 2020. **7**(1): p. 42.
126. Li, Y., R. Zhang, Y. He, C. Zhang, X. Liu, C. Chen, and G. Liu, *Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR).* Bioresource Technology, 2014. **156**: p. 342-347.

127. Dareioti, M.A., K. Tsigkou, A.I. Vavouraki, and M. Kornaros *Hydrogen and Methane Production from Anaerobic Co-Digestion of Sorghum and Cow Manure: Effect of pH and Hydraulic Retention Time*. Fermentation, 2022. **8**, DOI: 10.3390/fermentation8070304.
128. Zhang, Z., G. Zhang, W. Li, C. Li, and G. Xu, *Enhanced biogas production from sorghum stem by co-digestion with cow manure.* International Journal of Hydrogen Energy, 2016. **41**(21): p. 9153-9158.
129. Vats, N., A.A. Khan, and K. Ahmad, *Effect of substrate ratio on biogas yield for anaerobic co-digestion of fruit vegetable waste & sugarcane bagasse.* Environmental Technology & Innovation, 2019. **13**: p. 331-339.
130. Talha, Z., W. Ding, E. Mehryar, M. Hassan, and J. Bi, *Alkaline pretreatment of sugarcane bagasse and filter mud codigested to improve biomethane production.* BioMed Research International, 2016. **2016**.
131. Darwin, D. and A. Fazil, *Kinetics on anaerobic co-digestion of bagasse and digested cow manure with short hydraulic retention time.* Research in Agricultural Engineering, 2017. **63**(3): p. 121-127.
132. Khan, I.U., M.H.D. Othman, H. Hashim, T. Matsuura, A. Ismail, M. Rezaei-DashtArzhandi, and I.W. Azelee, *Biogas as a renewable energy fuel–A review of biogas upgrading, utilisation and storage.* Energy conversion and management, 2017. **150**: p. 277-294.
133. Eksi, G. and F. Karaosmanoglu, *Combined bioheat and biopower: A technology review and an assessment for Turkey.* Renewable and Sustainable Energy Reviews, 2017. **73**: p. 1313-1332.
134. Mertins, A. and T. Wawer, *How to use biogas?: A systematic review of biogas utilization pathways and business models.* Bioresources and Bioprocessing, 2022. **9**(1): p. 59.
135. Adekunle, K.F. and J.A. Okolie, *A review of biochemical process of anaerobic digestion.* Advances in Bioscience and Biotechnology, 2015. **6**(03): p. 205.
136. Samani, Z. and P. Bandini. *Valorization of agricultural residuals through biophysical transformation into an organic soil enhancer*. in *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY*. 2016. AMER CHEMICAL SOC 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
137. El-Mashad, H.M. and R. Zhang, *Biogas production from co-digestion of dairy manure and food waste.* Bioresource technology, 2010. **101**(11): p. 4021-4028.
138. Khalid, A., M. Arshad, M. Anjum, T. Mahmood, and L. Dawson, *The anaerobic digestion of solid organic waste.* Waste management, 2011. **31**(8): p. 1737-1744.
139. Misi, S. and C. Forster, *Batch co-digestion of multi-component agro-wastes.* Bioresource technology, 2001. **80**(1): p. 19-28.
140. Sarker, S. and H.B. Møller, *Regulating feeding and increasing methane yield from co-digestion of C5 molasses and cattle manure.* Energy conversion and management, 2014. **84**: p. 7-12.
141. De Vrieze, J., K. Plovie, W. Verstraete, and N. Boon, *Co-digestion of molasses or kitchen waste with high-rate activated sludge results in a diverse microbial community with stable methane production.* Journal of environmental management, 2015. **152**: p. 75-82.
142. Voća, N., T. Krička, T. Ćosić, V. Rupić, Ž. Jukić, and S. Kalambura, *Digested residue as a fertilizer after the mesophilic process of anaerobic digestion.* Plant Soil Environ, 2005. **51**(6): p. 262-266.
143. Mostafazadeh-Fard, S., Z. Samani, and P. Bandini, *Production of liquid organic fertilizer through anaerobic digestion of grass clippings.* Waste and Biomass Valorization, 2019. **10**(4): p. 771-781.

144. Huez-Lopez, M.A., *Comparative responses to inorganic and organic fertilization of chile pepper plants grown under saline conditions*, in *Thesis (Ph. D.)--Plant and Environmental Sciences, New Mexico State University, 2007.* 2007, Thesis (Ph. D.)--New Mexico State University, 2007.
145. Huez-López, M.A., A.L. Ulery, Z. Samani, G. Picchioni, and R.P. Flynn, *Response of chile pepper (Capsicum annuum L.) to salt stress and organic and inorganic nitrogen sources: I.Growth and yield.* Tropical and Subtropical Agroecosystems, 2011. **14**(1): p. 137-147.
146. Jurgutis, L., A. Šlepetienė, J. Šlepetys, and J. Cesevičienė, *Towards a full circular economy in biogas plants: Sustainable management of digestate for growing biomass feedstocks and use as biofertilizer.* Energies, 2021. **14**(14): p. 4272.
147. Kainthola, J., A.S. Kalamdhad, and V.V. Goud, *A review on enhanced biogas production from anaerobic digestion of lignocellulosic biomass by different enhancement techniques.* Process Biochemistry, 2019. **84**: p. 81-90.
148. Crolla, A.M., *Anaerobic Digestion of Dairy Manure with Food and Industry Wastes–Enhanced Biogas Production and Digestate Quality*. 2017, Université d'Ottawa/University of Ottawa.
149. Kratzeisen, M., N. Starcevic, M. Martinov, C. Maurer, and J. Müller, *Applicability of biogas digestate as solid fuel.* Fuel, 2010. **89**(9): p. 2544-2548.
150. Rehl, T. and J. Müller, *Life cycle assessment of biogas digestate processing technologies.* Resources, Conservation and Recycling, 2011. **56**(1): p. 92-104.
151. Delzeit, R. and U. Kellner, *The impact of plant size and location on profitability of biogas plants in Germany under consideration of processing digestates.* Biomass and Bioenergy, 2013. **52**: p. 43-53.
152. Burton, C.H. and C. Turner, *Manure management: Treatment strategies for sustainable agriculture*. 2003: Editions Quae.
153. Al Seadi, T., B. Drosg, W. Fuchs, D. Rutz, and R. Janssen, *The Biogas Handbook, The Biogas Handbook.* Elsevier. doi, 2013. **10**(9780857097415.2): p. 267.
154. David, G., C. Negrell, L. Vachoud, E. Ruiz, M. Delalonde, and C. Wisniewski, *An environmental application of functionalized chitosan: enhancement of the separation of the solid and liquid fractions of digestate from anaerobic digestion.* Pure and Applied Chemistry, 2016. **88**(12): p. 1155-1166.
155. Drosg, B., W. Fuchs, T. Al Seadi, M. Madsen, and B. Linke, *Nutrient recovery by biogas digestate processing*. Vol. 2015. 2015: IEA Bioenergy Dublin.
156. Meixner, K., W. Fuchs, T. Valkova, K. Svardal, C. Loderer, M. Neureiter, G. Bochmann, and B. Drosg, *Effect of precipitating agents on centrifugation and ultrafiltration performance of thin stillage digestate.* Separation and Purification Technology, 2015. **145**: p. 154-160.
157. Ganesh, R., M. Torrijos, P. Sousbie, J.P. Steyer, A. Lugardon, and J.P. Delgenes, *Anaerobic co-digestion of solid waste: effect of increasing organic loading rates and characterization of the solubilised organic matter.* Bioresource technology, 2013. **130**: p. 559-569.
158. Monlau, F., C. Sambusiti, E. Ficara, A. Aboulkas, A. Barakat, and H. Carrère, *New opportunities for agricultural digestate valorization: current situation and perspectives.* Energy & Environmental Science, 2015. **8**(9): p. 2600-2621.
159. Pedrazzi, S., G. Allesina, T. Belló, C.A. Rinaldini, and P. Tartarini, *Digestate as bio-fuel in domestic furnaces.* Fuel Processing Technology, 2015. **130**: p. 172-178.
160. Monlau, F., C. Sambusiti, N. Antoniou, A. Zabaniotou, A. Solhy, and A. Barakat, *Pyrochars from bioenergy residue as novel bio-adsorbents for lignocellulosic hydrolysate detoxification.* Bioresource technology, 2015. **187**: p. 379-386.

161. Stefaniuk, M. and P. Oleszczuk, *Characterization of biochars produced from residues from biogas production.* Journal of Analytical and Applied Pyrolysis, 2015. **115**: p. 157-165.
162. Sambusiti, C., F. Monlau, E. Ficara, A. Musatti, M. Rollini, A. Barakat, and F. Malpei, *Comparison of various post-treatments for recovering methane from agricultural digestate.* Fuel Processing Technology, 2015. **137**: p. 359-365.
163. Kaparaju, P., S. Luostarinen, E. Kalmari, J. Kalmari, and J. Rintala, *Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation.* Water Science and Technology, 2002. **45**(10): p. 275-280.
164. Al Seadi, T., B. Drosg, W. Fuchs, D. Rutz, and R. Janssen, *Biogas digestate quality and utilization*, in *The biogas handbook*. 2013, Elsevier. p. 267-301.
165. Møller, H.B., I. Lund, and S.G. Sommer, *Solid–liquid separation of livestock slurry: efficiency and cost.* Bioresource Technology, 2000. **74**(3): p. 223-229.
166. Urban, W., *16 - Biomethane injection into natural gas networks*, in *The Biogas Handbook*, A. Wellinger, J. Murphy, and D. Baxter, Editors. 2013, Woodhead Publishing. p. 378-403.
167. Adams, P., *Chapter 9 - GHG Emissions From Biomethane Gas-to-Grid Injection via Anaerobic Digestion*, in *Greenhouse Gas Balances of Bioenergy Systems*, P. Thornley and P. Adams, Editors. 2018, Academic Press. p. 141-158.
168. Miyamoto, M., Y. Nakashimada, and S. Uemiya, *12 - Membrane reactors for biogas production and processing*, in *Membrane Reactors for Energy Applications and Basic Chemical Production*, A. Basile, et al., Editors. 2015, Woodhead Publishing. p. 345-365.
169. Buitrón, G., R. Cardeña, and J.S. Arcila, *Chapter 5.9 - Bioelectrosynthesis of Methane Integrated With Anaerobic Digestion*, in *Microbial Electrochemical Technology*, S.V. Mohan, S. Varjani, and A. Pandey, Editors. 2019, Elsevier. p. 899-919.
170. Bacenetti, J. and M. Fiala, *CARBON FOOTPRINT OF ELECTRICITY FROM ANAEROBIC DIGESTION PLANTS IN ITALY.* Environmental Engineering & Management Journal (EEMJ), 2015. **14**(7).
171. Patterson, T., S. Esteves, R. Dinsdale, and A. Guwy, *Life cycle assessment of biogas infrastructure options on a regional scale.* Bioresource Technology, 2011. **102**(15): p. 7313-7323.
172. Battista, F., N. Frison, and D. Bolzonella, *Energy and Nutrients' Recovery in Anaerobic Digestion of Agricultural Biomass: An Italian Perspective for Future Applications.* Energies, 2019. **12**(17).
173. Koçar, G. and N. Civaş, *An overview of biofuels from energy crops: Current status and future prospects.* Renewable and Sustainable Energy Reviews, 2013. **28**: p. 900-916.
174. Tilvikiene, V., Z. Kadziuliene, I. Liaudanskiene, E. Zvicevicius, Z. Cerniauskiene, A. Cipliene, A.J. Raila, and J. Baltrusaitis, *The quality and energy potential of introduced energy crops in northern part of temperate climate zone.* Renewable Energy, 2020. **151**: p. 887-895.
175. Murphy, J.D. and N. Power, *Technical and economic analysis of biogas production in Ireland utilising three different crop rotations.* Applied Energy, 2009. **86**(1): p. 25-36.
176. Statystyczny, P.G.U., *Rocznik statystyczny województw.* (No Title), 2006.
177. Hammar, T., P.A. Hansson, and C. Sundberg, *Climate impact assessment of willow energy from a landscape perspective: a Swedish case study.* GCB Bioenergy, 2017. **9**(5): p. 973-985.

178. Nilsson, J., C. Sundberg, P. Tidåker, and P.-A. Hansson, *Regional variation in climate impact of grass-based biogas production: A Swedish case study.* Journal of Cleaner Production, 2020. **275**: p. 122778.
179. Carlsson, G., L.-M. Mårtensson, T. Prade, S.-E. Svensson, and E.S. Jensen, *Perennial species mixtures for multifunctional production of biomass on marginal land.* GCB Bioenergy, 2017. **9**(1): p. 191-201.
180. Lehtomäki, A., T. Viinikainen, and J. Rintala, *Screening boreal energy crops and crop residues for methane biofuel production.* Biomass and bioenergy, 2008. **32**(6): p. 541-550.
181. Lehtomäki, A., T.A. Viinikainen, and J.A. Rintala, *Screening boreal energy crops and crop residues for methane biofuel production.* Biomass and Bioenergy, 2008. **32**(6): p. 541-550.
182. Lehtomäki, A., S. Huttunen, and J.A. Rintala, *Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: Effect of crop to manure ratio.* Resources, Conservation and Recycling, 2007. **51**(3): p. 591-609.
183. Braun, R., P. Weiland, and A. Wellinger. *Biogas from energy crop digestion*. in *IEA bioenergy task*. 2008.
184. Dar, R., E. Dar, A. Kaur, and U. Phutela, *Sweet sorghum-a promising alternative feedstock for biofuel production.* Renewable and Sustainable Energy Reviews, 2017. **82**.
185. Dutra, E.D., A.G.B. Neto, R.B. de Souza, M.A. de Morais Junior, J.N. Tabosa, and R.S.C. Menezes, *Ethanol production from the stem juice of different sweet sorghum cultivars in the state of Pernambuco, Northeast of Brazil.* Sugar Tech, 2013. **15**: p. 316-321.
186. Gardner, J., J. Maranville, and E. Paparozzi, *Nitrogen use efficiency among diverse sorghum cultivars.* Crop Science, 1994. **34**(3): p. 728-733.
187. Almodares, A. and M. Hatamipour. *Planting sweet sorghum under hot and dry climatic condition for bioethanol production*. in *World Renewable Energy Congress*. 2011.
188. Vinutha, K., L. Rayaprolu, K. Yadagiri, A. Umakanth, J. Patil, and P. Srinivasa Rao, *Sweet sorghum research and development in India: status and prospects.* Sugar Tech, 2014. **16**: p. 133-143.
189. Maw, M.J., J.H. Houx III, and F.B. Fritschi, *Sweet sorghum ethanol yield component response to nitrogen fertilization.* Industrial Crops and Products, 2016. **84**: p. 43-49.
190. Wang, F. and C.-Z. Liu, *Development of an economic refining strategy of sweet sorghum in the Inner Mongolia region of China.* Energy & Fuels, 2009. **23**(8): p. 4137-4142.
191. Pazderu, K., J. Hodoval, J. Urban, J. Pulkrabek, V. Pacuta, and J. Adamcik, *The influence of sweet sorghum crop stand arrangement on biomass and biogas production.* Plant, Soil and Environment, 2014. **60**(9): p. 433-438.
192. Australian Government Department of Agriculture, W.a.t.E., *Australia Crop Report: February Edition.* 2021.
193. Tan, T., J. Yu, and F. Shang, *2.58 - Biorefinery Engineering*, in *Comprehensive Biotechnology (Second Edition)*, M. Moo-Young, Editor. 2011, Academic Press: Burlington. p. 815-828.
194. Wang, Q., P.C. Nnanna, F. Shen, M. Huang, D. Tian, J. Hu, Y. Zeng, G. Yang, and S. Deng, *Full utilization of sweet sorghum for bacterial cellulose production: A concept of material crop.* Industrial Crops and Products, 2021. **162**.
195. Islam, M.S., Z. Zhang, S.B. Qu, C.L. Liu, C. Guo, and C.Z. Liu, *Coproduction of hydrogen and volatile fatty acids via integrated two-step fermentation of sweet*

sorghum stalks by alkaline and enzymatic treatment. Biomass and Bioenergy, 2021. **145**.

196. Dehghanzad, M., M. Shafiei, and K. Karimi, *Whole sweet sorghum plant as a promising feedstock for biobutanol production via biorefinery approaches: Techno-economic analysis.* Renewable Energy, 2020. **158**: p. 332-342.
197. Ratnavathi, C.V., V.V. Komala, and U. Lavanya, *Chapter 4 - Sorghum Uses—Ethanol*, in *Sorghum Biochemistry*, C.V. Ratnavathi, J.V. Patil, and U.D. Chavan, Editors. 2016, Academic Press: San Diego. p. 181-252.
198. Lima, I., R. Bigner, and M. Wright, *Conversion of Sweet Sorghum Bagasse into Value-Added Biochar.* Sugar Tech, 2017. **19**(5): p. 553-561.
199. Houx, J., A. Craig, and F. Fritschi, *Evaluation of Sweet Sorghum Bagasse as an Alternative Livestock Feed.* Crop Science, 2013. **53**: p. 1784.
200. Yahia, E.M., A. Carrillo-López, and L.A. Bello-Perez, *Chapter 9 - Carbohydrates*, in *Postharvest Physiology and Biochemistry of Fruits and Vegetables*, E.M. Yahia, Editor. 2019, Woodhead Publishing. p. 175-205.
201. Mahmood, A., H. Ullah, M. Ijaz, M. Javaid, A. Shahzad, and B. Honermeier, *Evaluation of sorghum hybrids for biomass and biogas production.* Australian Journal of Crop Science, 2013. **7**: p. 1456-1462.
202. Mathur, S., A.V. Umakanth, V.A. Tonapi, R. Sharma, and M.K. Sharma, *Sweet sorghum as biofuel feedstock: recent advances and available resources.* Biotechnology for Biofuels, 2017. **10**(1): p. 146.
203. Ntaikou, I., H.N. Gavala, M. Kornaros, and G. Lyberatos, *Hydrogen production from sugars and sweet sorghum biomass using Ruminococcus albus.* International Journal of Hydrogen Energy, 2008. **33**(4): p. 1153-1163.
204. Ivanova, G., G. Rákhely, and K.L. Kovács, *Thermophilic biohydrogen production from energy plants by Caldicellulosiruptor saccharolyticus and comparison with related studies.* International Journal of Hydrogen Energy, 2009. **34**(9): p. 3659-3670.
205. Antonopoulou, G., H.N. Gavala, I.V. Skiadas, K. Angelopoulos, and G. Lyberatos, *Biofuels generation from sweet sorghum: fermentative hydrogen production and anaerobic digestion of the remaining biomass.* Bioresource technology, 2008. **99**(1): p. 110-119.
206. Nagaiah, D., R. Prakasham, A. Umakanth, A. Uma, and P. Srinivasa Rao, *Sweet sorghum juice as an alternate substrate for fermentative hydrogen production: Evaluation of influencing parameters using DOE statistical approach.* Sugar Tech, 2013. **15**: p. 338-344.
207. Gerardi, M.H., *The microbiology of anaerobic digesters*. 2003: John Wiley & Sons.
208. Angelidaki, I., L. Ellegaard, and B.K. Ahring, *A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition.* Biotechnology and bioengineering, 1993. **42**(2): p. 159-166.
209. Kongjan, P., S. O-Thong, M. Kotay, B. Min, and I. Angelidaki, *Biohydrogen production from wheat straw hydrolysate by dark fermentation using extreme thermophilic mixed culture.* Biotechnology and bioengineering, 2010. **105**(5): p. 899-908.
210. Adarme, O.F.H., B.E.L. Baêta, D.R.S. Lima, L.V.A. Gurgel, and S.F. de Aquino, *Methane and hydrogen production from anaerobic digestion of soluble fraction obtained by sugarcane bagasse ozonation.* Industrial Crops and Products, 2017. **109**: p. 288-299.
211. Domański, J., O. Marchut-Mikołajczyk, W. Cieciura-Włoch, P. Patelski, U. Dziekońska-Kubczak, B. Januszewicz, B. Zhang, and P. Dziugan, *Production of*

Methane, Hydrogen and Ethanol from Secale cereale L. Straw Pretreated with Sulfuric Acid. Molecules, 2020. **25**(4): p. 1013.
212. Pakarinen, O., H. Tähti, and J. Rintala, *One-stage H2 and CH4 and two-stage H2+ CH4 production from grass silage and from solid and liquid fractions of NaOH pre-treated grass silage.* Biomass and Bioenergy, 2009. **33**(10): p. 1419-1427.
213. Yang, Z., R. Guo, X. Xu, X. Fan, and S. Luo, *Hydrogen and methane production from lipid-extracted microalgal biomass residues.* international journal of hydrogen energy, 2011. **36**(5): p. 3465-3470.
214. Li, D. and H. Chen, *Biological hydrogen production from steam-exploded straw by simultaneous saccharification and fermentation.* International Journal of Hydrogen Energy, 2007. **32**(12): p. 1742-1748.
215. Cui, M., Z. Yuan, X. Zhi, and J. Shen, *Optimization of biohydrogen production from beer lees using anaerobic mixed bacteria.* international journal of hydrogen energy, 2009. **34**(19): p. 7971-7978.
216. Abreu, A.A., F. Tavares, M.M. Alves, and M.A. Pereira, *Boosting dark fermentation with co-cultures of extreme thermophiles for biohythane production from garden waste.* Bioresource Technology, 2016. **219**: p. 132-138.
217. Antonopoulou, G., H.N. Gavala, I.V. Skiadas, and G. Lyberatos, *Influence of pH on fermentative hydrogen production from sweet sorghum extract.* International Journal of Hydrogen Energy, 2010. **35**(5): p. 1921-1928.
218. Antonopoulou, G., H.N. Gavala, I.V. Skiadas, and G. Lyberatos, *ADM1-based modeling of methane production from acidified sweet sorghum extract in a two stage process.* Bioresource Technology, 2012. **106**: p. 10-19.
219. Eskicioglu, C., F. Monlau, A. Barakat, I. Ferrer, P. Kaparaju, E. Trably, and H. Carrère, *Assessment of hydrothermal pretreatment of various lignocellulosic biomass with CO2 catalyst for enhanced methane and hydrogen production.* Water Research, 2017. **120**: p. 32-42.
220. Giordano, A., C. Cantù, and A. Spagni, *Monitoring the biochemical hydrogen and methane potential of the two-stage dark-fermentative process.* Bioresource Technology, 2011. **102**(6): p. 4474-4479.
221. Xie, B., J. Cheng, J. Zhou, W. Song, J. Liu, and K. Cen, *Production of hydrogen and methane from potatoes by two-phase anaerobic fermentation.* Bioresource technology, 2008. **99**(13): p. 5942-5946.
222. Cheng, H.-H., L.-M. Whang, C.-W. Wu, and M.-C. Chung, *A two-stage bioprocess for hydrogen and methane production from rice straw bioethanol residues.* Bioresource Technology, 2012. **113**: p. 23-29.
223. Din, M.F.M., S. Krishnan, Y. Li, and Y. Qin, *Biohythane (methane and hydrogen) regeneration from agricultural biomass residue: possible or utopia?* Journal of Sustainability Science and Management, 2020. **15**(2): p. 1-5.
224. Demirel, B. and O. Yenigün, *Two-phase anaerobic digestion processes: a review*. 2002, Wiley Online Library.
225. Monteiro, J., S. Di Berardino, J. Barbosa, J. Duarte, and B. Havrland, *SWEET SORGHUM ENERGETIC VALORIZATION.*
226. Cao, W., C. Sun, X. Li, J. Qiu, and R. Liu, *Methane production enhancement from products of alkaline hydrogen peroxide pretreated sweet sorghum bagasse.* RSC advances, 2017. **7**(10): p. 5701-5707.
227. Szlachta, J., H. Prask, M. Fugol, and A. Luberański, *Effect of mechanical pre-treatment of the agricultural substrates on yield of biogas and kinetics of anaerobic digestion.* Sustainability, 2018. **10**(10): p. 3669.

228. Matsakas, L., U. Rova, and P. Christakopoulos, *Evaluation of dried sweet sorghum stalks as raw material for methane production.* Biomed Res Int, 2014. **2014**: p. 731731.
229. Mahmood, A., H. Ullah, M. Ijaz, M.M. Javaid, A.N. Shahzad, and B. Honermeier, *Evaluation of sorghum hybrids for biomass and biogas production.* Australian Journal of Crop Science, 2013. **7**(10): p. 1456-1462.
230. Sambusiti, C., E. Ficara, F. Malpei, J.P. Steyer, and H. Carrère, *Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum.* Energy, 2013. **55**: p. 449-456.
231. Mahmood, A. and B. Honermeier, *Chemical composition and methane yield of sorghum cultivars with contrasting row spacing.* Field Crops Research, 2012. **128**: p. 27-33.
232. Barbanti, L., G. Di Girolamo, M. Grigatti, L. Bertin, and C. Ciavatta, *Anaerobic digestion of annual and multi-annual biomass crops.* Industrial Crops and Products, 2014. **56**: p. 137-144.
233. de la Lama-Calvente, D., M.J. Fernández-Rodríguez, J. Gandullo, I. Desena, C. de la Osa, A.B. Feria, A. Jiménez-Rodríguez, and R. Borja, *Valorization of different landrace and commercial sorghum (Sorghum bicolor (L.) Moench) straw varieties by anaerobic digestion.* GCB Bioenergy, 2023. **15**(3): p. 332-345.
234. Gabrielle, B., L. Bamière, N. Caldes, S. De Cara, G. Decocq, F. Ferchaud, C. Loyce, E. Pelzer, Y. Perez, and J. Wohlfahrt, *Paving the way for sustainable bioenergy in Europe: technological options and research avenues for large-scale biomass feedstock supply.* Renewable and Sustainable Energy Reviews, 2014. **33**: p. 11-25.
235. Schmidt, T., P. Harris, S. Lee, and B.K. McCabe, *Investigating the impact of seasonal temperature variation on biogas production from covered anaerobic lagoons treating slaughterhouse wastewater using lab scale studies.* Journal of Environmental Chemical Engineering, 2019. **7**(3): p. 103077.
236. Janke, L., A. Leite, M. Nikolausz, T. Schmidt, J. Liebetrau, M. Nelles, and W. Stinner, *Biogas production from sugarcane waste: assessment on kinetic challenges for process designing.* International journal of molecular sciences, 2015. **16**(9): p. 20685-20703.
237. Fischer, T., A. Krieg, K.J. Chae, S.K. Yim, K.H. Choi, W.K. Park, and K.C. Eom, *Farm-scale biogas plants.* Journal of the Korean Organic Waste Recycling Council, 2002. **9**(4): p. 136-144.
238. Yin, Y., S. Chen, X. Li, B. Jiang, J.R. Zhao, and G. Nong, *Comparative analysis of different CHP systems using biogas for the cassava starch plants.* Energy, 2021. **232**: p. 121028.
239. Tomasini, M., M. de Oliveira Faber, and V.S. Ferreira-Leitão, *Sequential production of hydrogen and methane using hemicellulose hydrolysate from diluted acid pretreatment of sugarcane straw.* International Journal of Hydrogen Energy, 2023. **48**(27): p. 9971-9987.
240. Rawoof, S.A.A., P.S. Kumar, D.-V.N. Vo, and S. Subramanian, *Sequential production of hydrogen and methane by anaerobic digestion of organic wastes: a review.* Environmental Chemistry Letters, 2021. **19**: p. 1043-1063.
241. Nielsen, H.B. and I. Angelidaki, *Codigestion of manure and industrial organic waste at centralized biogas plants: process imbalances and limitations.* Water Science and Technology, 2008. **58**(7): p. 1521-1528.
242. Wilkinson, K.G., *A comparison of the drivers influencing adoption of on-farm anaerobic digestion in Germany and Australia.* Biomass and bioenergy, 2011. **35**(5): p. 1613-1622.

243. Taleghani, G. and A. Shabani Kia, *Technical–economical analysis of the Saveh biogas power plant.* Renewable Energy, 2005. **30**(3): p. 441-446.
244. Bedoić, R., F. Jurić, B. Ćosić, T. Pukšec, L. Čuček, and N. Duić, *Beyond energy crops and subsidised electricity – A study on sustainable biogas production and utilisation in advanced energy markets.* Energy, 2020. **201**: p. 117651.
245. Balussou, D., A. Kleyböcker, R. McKenna, D. Möst, and W. Fichtner, *An Economic Analysis of Three Operational Co-digestion Biogas Plants in Germany.* Waste and Biomass Valorization, 2012. **3**(1): p. 23-41.
246. Song, Z., C. Zhang, G. Yang, Y. Feng, G. Ren, and X. Han, *Comparison of biogas development from households and medium and large-scale biogas plants in rural China.* Renewable and Sustainable Energy Reviews, 2014. **33**: p. 204-213.
247. Mussoline, W., G. Esposito, P. Lens, G. Garuti, and A. Giordano, *Design considerations for a farm-scale biogas plant based on pilot-scale anaerobic digesters loaded with rice straw and piggery wastewater.* Biomass and Bioenergy, 2012. **46**: p. 469-478.
248. Akbulut, A., *Techno-economic analysis of electricity and heat generation from farm-scale biogas plant: Çiçekdağı case study.* Energy, 2012. **44**(1): p. 381-390.
249. Whitfield, M.B., M.S. Chinn, and M.W. Veal, *Processing of materials derived from sweet sorghum for biobased products.* Industrial Crops and Products, 2012. **37**(1): p. 362-375.
250. Kaparaju, P., M. Serrano, A.B. Thomsen, P. Kongjan, and I. Angelidaki, *Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept.* Bioresour Technol, 2009. **100**(9): p. 2562-8.
251. Barcelos, C.A., R.N. Maeda, L.M.M. Santa Anna, and N. Pereira, *Sweet sorghum as a whole-crop feedstock for ethanol production.* Biomass and Bioenergy, 2016. **94**: p. 46-56.
252. Nkoa, R., *Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review.* Agronomy for Sustainable Development, 2014. **34**(2): p. 473-492.
253. Yu, J., T. Zhang, J. Zhong, X. Zhang, and T. Tan, *Biorefinery of sweet sorghum stem.* Biotechnology Advances, 2012. **30**(4): p. 811-816.
254. Caffrey, K.R., M.W. Veal, and M.S. Chinn, *The farm to biorefinery continuum: A techno-economic and LCA analysis of ethanol production from sweet sorghum juice.* Agricultural Systems, 2014. **130**: p. 55-66.
255. Tang, S., Z. Wang, C. Chen, P. Xie, and Q. Xie, *The prospect of sweet sorghum as the source for high biomass crop.* Journal of Agricultural Science and Botany, 2018. **02**.
256. P. Nghiem, N., C. M. Nguyen, C. M. Drapcho, and T. H. Walker, *Sweet Sorghum Biorefinery for Production of Fuel Ethanol and Value-Added Co-products.* Biological Engineering Transactions, 2013. **6**(3): p. 143-155.
257. Petersson, A., M.H. Thomsen, H. Hauggaard-Nielsen, and A.-B. Thomsen, *Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean.* Biomass and Bioenergy, 2007. **31**(11): p. 812-819.
258. Paulose, P. and P. Kaparaju, *Anaerobic mono-digestion of sugarcane trash and bagasse with and without pretreatment.* Industrial Crops and Products, 2021. **170**: p. 113712.
259. Mthethwa, N.P., *Enhancement of biohydrogen production from the aquatic weed Pistia stratiotes through a dark fermentation process*. 2019.
260. Raposo, F., C.J. Banks, I. Siegert, S. Heaven, and R. Borja, *Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests.* Process Biochemistry, 2006. **41**(6): p. 1444-1450.

261. Rafieenia, R., *Enhancement of hydrogen and methane production through anaerobic digestion using pre-treatments.* 2019.
262. Yu, L., P.C. Wensel, J. Ma, and S. Chen, *Mathematical modeling in anaerobic digestion (AD).* J Bioremed Biodeg S, 2013. **4**(2).
263. Kafle, G.K. and L. Chen, *Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models.* Waste Management, 2016. **48**: p. 492-502.
264. Tjørve, K.M.C. and E. Tjørve, *The use of Gompertz models in growth analyses, and new Gompertz-model approach: An addition to the Unified-Richards family.* PloS one, 2017. **12**(6): p. e0178691-e0178691.
265. Apha, *Standard methods for the examination of water and wastewater*. 1985: Apha.
266. Brummer, Y. and S.W. Cui, *Understanding carbohydrate analysis.* Food carbohydrates: chemistry, physical properties and applications, 2005: p. 1-38.
267. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Determination of structural carbohydrates and lignin in biomass, in: Laboratory Analytical Procedure (LAP).* National Renewable Energy Laboratory, 2008.
268. Triolo, J.M., L. Pedersen, H. Qu, and S.G. Sommer, *Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production.* Bioresource technology, 2012. **125**: p. 226-232.
269. Sambusiti, C., E. Ficara, F. Malpei, J.-P. Steyer, and H. Carrère, *Benefit of sodium hydroxide pretreatment of ensiled sorghum forage on the anaerobic reactor stability and methane production.* Bioresource technology, 2013. **144**: p. 149-155.
270. Lacoste, R., S. Venable, and J. Stone, *Modified 4-aminoantipyrine colorimetric method for phenols. Application to acrylic monomer.* Analytical Chemistry, 1959. **31**(7): p. 1246-1249.
271. Kaparaju, P.L.-N. and J. Rintala, *Thermophilic anaerobic digestion of industrial orange waste.* Environmental technology, 2006. **27**(6): p. 623-633.
272. Chattha, M.U., M.U. Hassan, I. Khan, M.B. Chattha, A. Mahmood, M.U. Chattha, M. Nawaz, M.N. Subhani, M. Kharal, and S. Khan, *Biofortification of wheat cultivars to combat zinc deficiency.* Frontiers in plant science, 2017. **8**: p. 281.
273. Ram, S., G. Suresh, and K. Singh, *Effect of planting geometry and cutting management on productivity, quality and economics of mixed pasture under rainfed conditions.* Indian Journal of Agronomy, 2007. **52**(3): p. 251-256.
274. Ashiq, H., K. Sartaj, and M. Dost, *Forage yield and nutritive value of oat cultivar fatua at various intervals of harvesting.* Pakistan Journal of Agricultural Research, 2002. **17**(2): p. 148-152.
275. Amon, T., B. Amon, V. Kryvoruchko, A. Machmüller, K. Hopfner-Sixt, V. Bodiroza, R. Hrbek, J. Friedel, E. Pötsch, and H. Wagentristl, *Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations.* Bioresource technology, 2007. **98**(17): p. 3204-3212.
276. Kandel, T.P., S. Sutaryo, H.B. Møller, U. Jørgensen, and P.E. Lærke, *Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency.* Bioresource technology, 2013. **130**: p. 659-666.
277. Massé, D., Y. Gilbert, P. Savoie, G. Bélanger, G. Parent, and D. Babineau, *Methane yield from switchgrass and reed canarygrass grown in Eastern Canada.* Bioresource Technology, 2011. **102**(22): p. 10286-10292.
278. Shiralipour, A. and P.H. Smith, *Conversion of biomass into methane gas.* Biomass, 1984. **6**(1-2): p. 85-92.

279. Amon, T., B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, and L. Gruber, *Biogas production from maize and dairy cattle manure—influence of biomass composition on the methane yield.* Agriculture, Ecosystems & Environment, 2007. **118**(1-4): p. 173-182.
280. Dien, B.S., H.-J.G. Jung, K.P. Vogel, M.D. Casler, J.F. Lamb, L. Iten, R.B. Mitchell, and G. Sarath, *Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass.* Biomass and bioenergy, 2006. **30**(10): p. 880-891.
281. Kreuger, E., T. Prade, F. Escobar, S.E. Svensson, J.E. Englund, and L. Björnsson, *Anaerobic digestion of industrial hemp–Effect of harvest time on methane energy yield per hectare.* Biomass and Bioenergy, 2011. **35**(2): p. 893-900.
282. Hassan, M.U., M.U. Chattha, A. Mahmood, and S.T. Sahi, *Performance of sorghum cultivars for biomass quality and biomethane yield grown in semi-arid area of Pakistan.* Environ Sci Pollut Res Int, 2018. **25**(13): p. 12800-12807.
283. Monlau, F., C. Sambusiti, A. Barakat, X.M. Guo, E. Latrille, E. Trably, J.-P. Steyer, and H.l.n. Carrere, *Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials.* Environmental science & technology, 2012. **46**(21): p. 12217-12225.
284. Anyango, B.N., S.M. Wandera, and J.M. Raude, *Abattoir Wastewater Treatment in Anaerobic Co-Digestion with Sugar Press Mud in Batch Reactor for Improved Biogas Yield.* Water, 2022. **14**(16): p. 2571.
285. Macias-Corral, M.A., J.A. Cueto-Wong, J. Morán-Martínez, and L. Reynoso-Cuevas, *Effect of different initial C/N ratio of cow manure and straw on microbial quality of compost.* International Journal of Recycling of Organic Waste in Agriculture, 2019. **8**(1): p. 357-365.
286. Gunaseelan, V.N., *Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition.* Bioresource Technology, 2007. **98**(6): p. 1270-1277.
287. Sambusiti, C., E. Ficara, F. Malpei, J. Steyer, and H. Carrère, *Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum.* Energy, 2013. **55**: p. 449-456.
288. Jerger, D.E., D.P. Chynoweth, and H.R. Isaacson, *Anaerobic digestion of sorghum biomass.* Biomass, 1987. **14**(2): p. 99-113.
289. Burks, P.S., C.M. Kaiser, E.M. Hawkins, and P.J. Brown, *Genomewide association for sugar yield in sweet sorghum.* Crop Science, 2015. **55**(5): p. 2138-2148.
290. Felderhoff, T., S. Murray, P. Klein, A. Sharma, M. Hamblin, S. Kresovich, W. Vermerris, and W. Rooney, *QTLs for energy-related traits in a sweet× grain sorghum [Sorghum bicolor (L.) Moench] mapping population.* Crop Science, 2012. **52**(5): p. 2040-2049.
291. Shukla, S., T.J. Felderhoff, A. Saballos, and W. Vermerris, *The relationship between plant height and sugar accumulation in the stems of sweet sorghum (Sorghum bicolor (L.) Moench).* Field Crops Research, 2017. **203**: p. 181-191.
292. Mathias, D.J., T. Edwiges, N. Ketsub, R. Singh, and P. Kaparaju, *Sweet Sorghum as a Potential Fallow Crop in Sugarcane Farming for Biomethane Production in Queensland, Australia.* Energies, 2023. **16**(18): p. 6497.
293. Zegada-Lizarazu, W. and A. Monti, *Are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices.* Biomass and Bioenergy, 2012. **40**: p. 1-12.
294. Lingle, S.E., *Sugar metabolism during growth and development in sugarcane internodes.* Crop Science, 1999. **39**(2): p. 480-486.

295. Qazi, H.A., S. Paranjpe, and S. Bhargava, *Stem sugar accumulation in sweet sorghum–activity and expression of sucrose metabolizing enzymes and sucrose transporters.* Journal of Plant Physiology, 2012. **169**(6): p. 605-613.
296. Liu, Y., B.-Q. Dun, X.-N. Zhao, M.-Q. Yue, M. Lu, and G.-Y. Li, *Correlation analysis between the key enzymes activies and sugar content in sweet sorghum ('Sorghum bicolor'L. Moench) stems at physiological maturity state.* Australian Journal of Crop Science, 2013. **7**(1): p. 84-92.
297. Hoffmann-Thoma, G., K. Hinkel, P. Nicolay, and J. Willenbrink, *Sucrose accumulation in sweet sorghum stem internodes in relation to growth.* Physiologia Plantarum, 1996. **97**(2): p. 277-284.
298. Zhu, Y.J., E. Komor, and P.H. Moore, *Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase.* Plant Physiology, 1997. **115**(2): p. 609-616.
299. McBee, G.G. and F. Miller, *Carbohydrates in Sorghum Culms as Influenced by Cultivars, Spacing, and Maturity over a Diurnal Period 1.* Crop Science, 1982. **22**(2): p. 381-385.
300. Elbeshbishy, E., G. Nakhla, and H. Hafez, *Biochemical methane potential (BMP) of food waste and primary sludge: Influence of inoculum pre-incubation and inoculum source.* Bioresource Technology, 2012. **110**: p. 18-25.
301. Paul, S. and A. Dutta, *Challenges and opportunities of lignocellulosic biomass for anaerobic digestion.* Resources, Conservation and Recycling, 2018. **130**: p. 164-174.
302. Alzate, M., R. Muñoz, F. Rogalla, F. Fdz-Polanco, and S. Pérez-Elvira, *Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment.* Bioresource technology, 2012. **123**: p. 488-494.
303. Zhou, Y., Z. Zhang, T. Nakamoto, Y. Li, Y. Yang, M. Utsumi, and N. Sugiura, *Influence of substrate-to-inoculum ratio on the batch anaerobic digestion of bean curd refuse-okara under mesophilic conditions.* Biomass and Bioenergy, 2011. **35**(7): p. 3251-3256.
304. Herrmann, C., M. Heiermann, and C. Idler, *Effects of ensiling, silage additives and storage period on methane formation of biogas crops.* Bioresour Technol, 2011. **102**(8): p. 5153-61.
305. Wannasek, L., M. Ortner, B. Amon, and T. Amon, *Sorghum, a sustainable feedstock for biogas production? Impact of climate, variety and harvesting time on maturity and biomass yield.* Biomass and Bioenergy, 2017. **106**: p. 137-145.
306. Herrmann, C., C. Idler, and M. Heiermann, *Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics.* Bioresource Technology, 2016. **206**: p. 23-35.
307. Budiyono, I.S. and S. Sumardiono, *Kinetic model of biogas yield production from vinasse at various initial pH: comparison between modified Gompertz model and first order kinetic model.* Research Journal of Applied Sciences, Engineering and Technology, 2014. **7**(13): p. 2798-2805.
308. Phuttaro, C., C. Sawatdeenarunat, K. Surendra, P. Boonsawang, S. Chaiprapat, and S.K. Khanal, *Anaerobic digestion of hydrothermally-pretreated lignocellulosic biomass: Influence of pretreatment temperatures, inhibitors and soluble organics on methane yield.* Bioresource technology, 2019. **284**: p. 128-138.
309. Baba, Y., Y. Matsuki, Y. Mori, Y. Suyama, C. Tada, Y. Fukuda, M. Saito, and Y. Nakai, *Pretreatment of lignocellulosic biomass by cattle rumen fluid for methane production: bacterial flora and enzyme activity analysis.* Journal of bioscience and bioengineering, 2017. **123**(4): p. 489-496.

310. Takizawa, S., Y. Baba, C. Tada, Y. Fukuda, and Y. Nakai, *Pretreatment with rumen fluid improves methane production in the anaerobic digestion of paper sludge.* Waste Management, 2018. **78**: p. 379-384.
311. Li, Y., J. Zhao, S. Achinas, Z. Zhang, J. Krooneman, and G.J.W. Euverink, *The biomethanation of cow manure in a continuous anaerobic digester can be boosted via a bioaugmentation culture containing Bathyarchaeota.* Science of The Total Environment, 2020. **745**: p. 141042.
312. Fasake, V. and K. Dashora, *Characterization of raw and anaerobic digested cattle dung fibers: a sustainable source of non-wood material.* Biomass Conversion and Biorefinery, 2022.
313. Pang, J., Z.-Y. Liu, M. Hao, Y.-F. Zhang, and Q.-S. Qi, *An isolated cellulolytic Escherichia coli from bovine rumen produces ethanol and hydrogen from corn straw.* Biotechnology for biofuels, 2017. **10**(1): p. 1-10.
314. Nguyen, L.N., A.Q. Nguyen, M.A.H. Johir, W. Guo, H.H. Ngo, A.V. Chaves, and L.D. Nghiem, *Application of rumen and anaerobic sludge microbes for bio harvesting from lignocellulosic biomass.* Chemosphere, 2019. **228**: p. 702-708.
315. Chen, Y., J.J. Cheng, and K.S. Creamer, *Inhibition of anaerobic digestion process: a review.* Bioresource technology, 2008. **99**(10): p. 4044-4064.
316. Sarode, S.D., D. Kumar, D. Mathias, D. McNeill, and P. Kaparaju *Anaerobic Digestion of Spoiled Maize, Lucerne and Barley Silage Mixture with and without Cow Manure: Methane Yields and Kinetic Studies*. Energies, 2023. **16**, DOI: 10.3390/en16176179.
317. Riau, V., L. Burgos, F. Camps, F. Domingo, M. Torrellas, A. Antón, and A. Bonmatí, *Closing nutrient loops in a maize rotation. Catch crops to reduce nutrient leaching and increase biogas production by anaerobic co-digestion with dairy manure.* Waste Management, 2021. **126**: p. 719-727.
318. Wang, Y., Y. Zhang, J. Wang, and L. Meng, *Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria.* Biomass and bioenergy, 2009. **33**(5): p. 848-853.
319. Moss, A.R., J.-P. Jouany, and J. Newbold. *Methane production by ruminants: its contribution to global warming*. in *Annales de zootechnie*. 2000. EDP Sciences.
320. Getachew, G., P. Robinson, E. DePeters, and S. Taylor, *Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds.* Animal feed science and technology, 2004. **111**(1-4): p. 57-71.
321. Hu, Z.-H. and H.-Q. Yu, *Anaerobic digestion of cattail by rumen cultures.* Waste management, 2006. **26**(11): p. 1222-1228.
322. Haider, M.R., Zeshan, S. Yousaf, R.N. Malik, and C. Visvanathan, *Effect of mixing ratio of food waste and rice husk co-digestion and substrate to inoculum ratio on biogas production.* Bioresource Technology, 2015. **190**: p. 451-457.
323. Zhu, H., A. Stadnyk, M. Beland, and P. Seto, *Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process.* Bioresour Technol, 2008. **99**(11): p. 5078-84.
324. Ueno, Y., H. Fukui, and M. Goto, *Operation of a two-stage fermentation process producing hydrogen and methane from organic waste.* Environmental Science & Technology, 2007. **41**(4): p. 1413-1419.
325. Luo, G., L. Xie, Q. Zhou, and I. Angelidaki, *Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process.* Bioresource Technology, 2011. **102**(18): p. 8700-8706.
326. Noike, T. and O. Mizuno, *Hydrogen fermentation of organic municipal wastes.* Water Science and Technology, 2000. **42**(12): p. 155-162.

327. Okamoto, M., T. Miyahara, O. Mizuno, and T. Noike, *Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes.* Water Science and Technology, 2000. **41**(3): p. 25-32.
328. El-Qelish, M., P. Chatterjee, M. Kokko, F. El-Gohary, M. Abo-Aly, and J. Rintala, *Conversion of boreal lake sedimented pulp mill fibre into biogas: a two-stage hydrogen and methane production.* Biomass Conversion and Biorefinery, 2022.
329. Zhang, M.-L., Y.-T. Fan, Y. Xing, C.-M. Pan, G.-S. Zhang, and J.-J. Lay, *Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures.* Biomass and Bioenergy, 2007. **31**(4): p. 250-254.
330. Pattra, S., S. Sangyoka, M. Boonmee, and A. Reungsang, *Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by Clostridium butyricum.* International Journal of Hydrogen Energy, 2008. **33**(19): p. 5256-5265.
331. Lu, Y., Q. Lai, C. Zhang, H. Zhao, K. Ma, X. Zhao, H. Chen, D. Liu, and X.-H. Xing, *Characteristics of hydrogen and methane production from cornstalks by an augmented two- or three-stage anaerobic fermentation process.* Bioresource Technology, 2009. **100**(12): p. 2889-2895.
332. Pan, C., S. Zhang, Y. Fan, and H. Hou, *Bioconversion of corncob to hydrogen using anaerobic mixed microflora.* International Journal of Hydrogen Energy, 2010. **35**(7): p. 2663-2669.
333. Pakarinen, O., *Methane and hydrogen production from crop biomass through anaerobic digestion.* Jyväskylä studies in biological and environmental science, 2011(229).
334. Zhao, C., O. Sompong, D. Karakashev, I. Angelidaki, W. Lu, and H. Wang, *High yield simultaneous hydrogen and ethanol production under extreme-thermophilic (70° C) mixed culture environment.* international journal of hydrogen energy, 2009. **34**(14): p. 5657-5665.
335. DiStefano, T.D. and A. Palomar, *Effect of anaerobic reactor process configuration on useful energy production.* Water research, 2010. **44**(8): p. 2583-2591.
336. Hills, D.J. and D.W. Roberts, *Anaerobic digestion of dairy manure and field crop residues.* Agricultural Wastes, 1981. **3**(3): p. 179-189.
337. Janke, L., S. Weinrich, A.F. Leite, H. Sträuber, C.M. Radetski, M. Nikolausz, M. Nelles, and W. Stinner, *Year-round biogas production in sugarcane biorefineries: Process stability, optimization and performance of a two-stage reactor system.* Energy Conversion and Management, 2018. **168**: p. 188-199.