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The Role of Pyrolysis Product Biochar in Advancing Anaerobic Digestion Efficiency

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Summary:

Anaerobic digestion (AD) is a renowned technology for the conversion of waste into bio-energy. It can harness energy from the waste and mitigate the impacts related to environmental problems. Despite the high ability of AD, the problems related to low methane yield, VFA accumulation, and process instability often occurs. The addition of biochar to enhance the production of methane in AD was investigated in this thesis.

Biochar is a carbon rich material obtained from the pyrolysis of different organic waste feedstocks. This thesis aims to investigate the potential of biochar derived from different feedstock to enhance the production of methane in the anaerobic digestion process. A biochemical methane potential (BMP) test was carried out with the use of automatic methane potential test system (AMPTS II) under mesophilic condition, to determine the methane potential of the samples that contains biochar.

In this study, the effect of three types of biochar obtained from garden waste, digested sewage sludge and waste on the anaerobic digestion was investigated. All the biochar samples showed higher methane yield as compared to the control sample. The results obtained from the study showed that methane production could be considerably enhanced by 24% with the use of biochar. The maximum cumulative methane yield of 401 Nml $g^{-1}VS^{-1}$ was obtained from the waste timber (WT) biochar sample pyrolyzed at 600^{o}C with high electrical conductivity and specific surface area. The further laboratory analysis revealed that the VFA concentration in the biochar samples was relatively low as compared to the control sample indicating the higher degradation of VFA yielding more methane.

Overall, the biochars had the positive effect on the production of methane improving the efficiency of AD. Biochar potential to mitigate the challenges faced during AD emphasizes its potential role in the sustainable waste management and renewable energy solutions.

Preface

This thesis has been performed on the topic "The Role of Pyrolysis Product Biochar in Advancing Anaerobic Digestion Efficiency" in the autumn of 2023 to fulfill the master's exchange program requirement of the Energy and Environmental Technology at the University of South-Eastern Norway. The exchange program is funded by NORPART Re-Tech project.

I would like to express my deepest appreciation to my main supervisors, Thea Indrebø, Sunil Prasad Lohani, Anish Ghimire, and Nabin Aryal for providing continuous supervision, guidance and encouragement during laboratory work and thesis writing, which resulted in the completion of this thesis work.

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Special appreciation to the external partner Lindum AS and Scanship AS for providing some technical information and knowledge related to the experiment.

I am also thankful to my family members for their continuous love and support throughout this academic journey.

Porsgrunn, 20.01.2024

Ashish Dutta Bhatta

Contents

Nomenclature

AD	Anaerobic Digestion
AMPTS	Automatic Methane Potential Test System
ANOVA	Analysis of Variance
BET	Brunauer-Emmett-Teller method
BJH	Barret-Joyner-Halenda method
BMP	Biochemical Methane Potential
C/N	Carbon to Nitrogen Ratio
COD	Chemical Oxygen Demand
DIET	Direct Interspecies Electron Transfer
DSS	Digested Sewage Sludge
EC	Electrical Conductivity
FA	Free Ammonia
GAC	Granular Activated Carbon
GW	Garden Waste
H/C	Hydrogen to Carbon ratio
IIET	Indirect Interspecies Electron Transfer
NmL	Normalized volume of methane
O/C	Oxygen to Carbon ratio
OLR	Organic Loading Rate
PV	Pore Volume
SCOD	Soluble Chemical Oxygen Demand
SSA	Specific Surface Area
TAN	Total Ammonia Nitrogen
TCOD	Total Chemical Oxygen Demand
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids
WT	Waste Timber

1 Introduction

Our dependence on non-renewable energy sources has brought about the release of carbon dioxide (CO_2), adding to worries like ozone harming substance like greenhouse gas (GHG) emission issues and natural contamination (Qazi et al., 2019). Environmentally friendly renewable sources, for example, biogas, solar energy, wind energy, hydropower, and geothermal power can possibly address worries about energy security, non-renewable energy reliance, and natural degradation (Obileke et al., 2021).

There is a worldwide interest in anaerobic digestion (AD), a cycle that changes waste into bio-energy. Hydrolysis, acidogenic fermentation, hydrogen-delivering acetogenesis, and methanogenesis are the four phases of the AD (Y. Li et al., 2019). Biogas is normally employed in areas like cooking, lighting, electricity generation and heating purposes. To widen its application, different upgrading techniques should be utilized to eliminate contaminations like carbon dioxide (CO_2), hydrogen sulfide (H_2S), and other impurities. The end of these mixtures is a substantial stage in creating biomethane with a methane content more significant than 90% (Kapoor et al., 2019).

Prevailing studies has shown that the use of conductive materials like carbon nanotubes, biochar, carbon cloth, granular activated carbon (GAC), and magnetite in AD can promote the production of methane (Gahlot et al., 2020). Biochar serves different capabilities in the AD process. It upholds rate-restricting hydrolysis, keeps a consistent pH, and encourages microbial growth. These approaches help to overcome the potential inhibition and the enhancement of the AD processes. Biochar use in AD advances biogas production, changes the metabolic systems of intermediate products like ammonia and acids, and improves the microbial community (Qiu et al., 2019).

1.1 Problem Statement

Anaerobic digestion is a renowned technology for converting waste into bio-energy. Despite this, the challenges due to low methane yield, VFA accumulation and process instability still persist which hinders the process efficiency of anaerobic digestion process. This study aims to address these challenges in anaerobic digestion by exploring the use of biochar as a potential solution to enhance the production of methane. Specifically, how biochars obtained from

different feedstock can influence the anaerobic digestion efficiency, particularly focusing on the methane yield and consumption of VFA.

1.2 Research Questions

The list of potential research questions for this study are:

i. How does the use of biochar impact the methane yield during the anaerobic digestion process?
ii. How the methane production is correlated with the characteristics of biochar?

1.3 Aim and Objectives

The main objective of this study is to investigate the potential of biochar derived from different feedstock to enhance the production of methane in the anaerobic digestion process. The specific objectives of the study are:

i. Investigate the influence of different biochar addition obtained from garden waste, digested sewage sludge and waste timber on methane production during the anaerobic digestion process.
ii. To correlate the methane production from biochar with its specific characteristics like carbon content, specific surface area and electrical conductivity.

1.4 Structure of the Report

There are seven principal chapter in this study. The first chapter gives an outline of the introduction, problem statement, research questions and aims and objectives to have introductory information and a legitimate information of the topic. The second chapter incorporates the literature review connected with anaerobic digestion processes. The third chapter is about the material and methods utilized in this study. Reactor arrangement, analytical procedures were incorporated in this chapter. The fourth chapter incorporates the results obtained during the study. The fifth chapter is the discussion, which discusses the results obtained with appropriate arguments. The sixth chapter covers overall conclusion and findings during the study. The seventh chapter presents the recommendation/suggestions for the future study/research.

2 Literature Review

This chapter provides a concise introduction to anaerobic digestion (AD) and its operational parameters, followed by a comprehensive exploration of methane optimization techniques. The strategies for enhancing methane production by the use of biochar is thoroughly investigated in this section.

2.1 Anaerobic Digestion

According to a natural viewpoint, anaerobic digestion promotion decreases the natural substance of waste, making it less contaminating when discarded and possibly usable as fuel for vehicles (Náthia-Neves et al., 2018). Under anaerobic circumstances, the microbial breakdown of organic matter results in the creation of biogas, which contains methane, a high-energy component (Schnürer, 2016). The principal parts of biogas are methane (CH_4) and carbon dioxide (CO_2), joined by minor amounts of hydrogen (H_2), nitrogen (N_2), hydrogen sulfide (H_2S), oxygen (O_2), water (H_2O), and soaked hydrocarbons like ethane and propane (Bharathiraja et al., 2018). To empower safe application and moderate limits, for example, diminished energy thickness, machine part erosion, it is vital to redesign biogas by tending to impurities like carbon dioxide, hydrogen sulfide, and siloxanes (Mulu et al., 2021). Biomethane, which is created by refining the biogas through the removal of carbon dioxide and other impurities, has turned into a feasible way to relieve the difficulties related to petroleum derivative-based energy utilization (Werkneh, 2022). The utilization of biomethane as an upgraded form of biogas has emerged as a favourable option for food-based crop biofuels in the replacement of fossil fuels within the transportation sector. This option is liked because of its lessened ecological effect, limited circuitous results, and lower ozone-harming substance outflows (Scarlat et al., 2018).

2.2 Stages of Biogas Production Using AD

The anaerobic digestion (AD) process involves four major phases that contribute to the development of biogas from various organic resources inside an anaerobic digester. These stages, precisely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, are represented in Figure 2.1. Inside an oxygen free environment, the promotion cycle includes the breakdown

of organic substance into methane, carbon dioxide, inorganic supplements, and fertilizer (Sawyerr et al., 2019; Weiland, 2010).

2.2.1 Hydrolysis

During the underlying stage, known as the hydrolysis step, acidogenic microbes separate or depolymerize huge natural polymers like starches, cellulose, proteins, and fats. This breakdown is worked with by hydrolytic exo-proteins (e.g., cellulase, amylase, protease, and lipase) that are discharged by fermentative microorganisms. During this stage, carbohydrate, proteins, and lipids go through a change into dissolvable particles, which incorporate monosaccharides, amino acids, and unsaturated fats (Aryal et al., 2018; Bajpai, 2017).

2.2.2 Acidogenesis

During acidogenesis step, acid-forming microorganisms transform the organic materials into higher organic acids, including propionic acid, butyric acid, and acetic acid, as well as hydrogen and carbon dioxide. The higher organic acids are consequently changed into acetic acid and hydrogen by acetogenic microbes (Aryal et al., 2018; Bajpai, 2017).

2.2.3 Acetogenesis

During the acetogenesis interaction, acidogenesis microorganisms convert VFAs, for example, propionic acid and butyric acid into acetic acid derivation, while likewise producing hydrogen and carbon dioxide. The transformation of these moderate VFAs to acetate is urgent for the resulting activity of methanogenic microbes, although acetate derivation created during acidogenesis is as of now accessible for this reason (Aryal et al., 2018; Bajpai, 2017).

2.2.4 Methanogenesis

During the last step of methanogenesis, methane is produced through the action of methanogenic microbes. These microscopic organisms can process different mixtures like formic acid, acetic acid, methanol, carbon monoxide, carbon dioxide, and hydrogen, eventually changing over them into methane. Methanogenic microorganisms assume an essential part in the anaerobic assimilation process, as they have a slow development rate and are significantly delicate to ecological variances. Furthermore, they can adapt to a restricted scope of somewhat basic substances (Aryal et al., 2018; Bajpai, 2017).

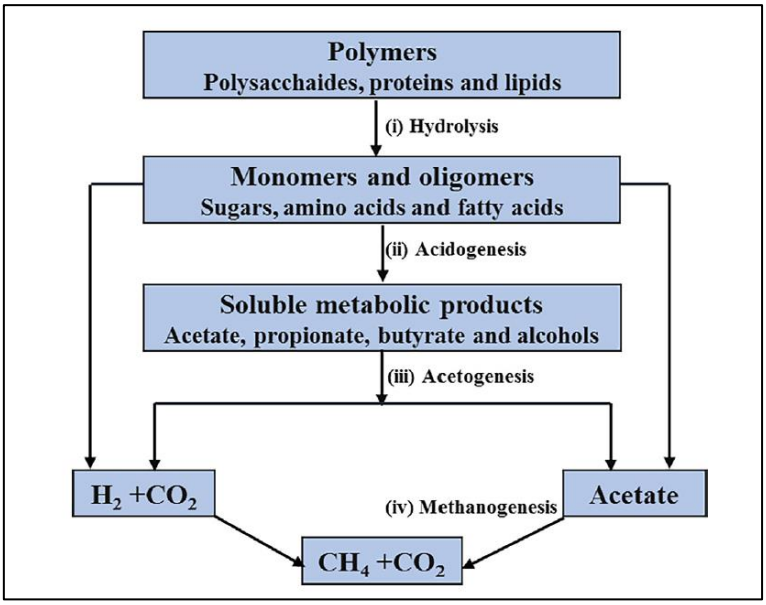


Figure 2.1: Stages of anaerobic digestion for biogas production (Aryal et al., 2018).

2.3 Factors Affecting Biogas Production in AD

Biogas production, which is a complex biological cycle, is impacted by various ecological components. The shared dependence among microscopic organisms assumes a critical role in the biogas cycle. At the point when the activity becomes unsteady, different intermediates like volatile fatty acids and alcohols accumulate at different rates, contingent upon the sort of substrate and the particular problem causing the instability (Angelidaki et al., 2009).

2.3.1 Temperature

Temperature assumes a vital part in the anaerobic digestion (AD) process. It impacts different parts of AD, including enzyme activity, methane yield, and the nature of the digestate or effluent. Anaerobic microscopic organisms can flourish under various temperature ranges, including psychrophilic (10-30°C), mesophilic (30-40°C), and thermophilic (50-60°C) conditions.

The exhibition of AD improves with increasing temperature, especially in thermophilic activity. This is because of higher metabolic rates, explicit metabolic rates, microbe extinction, and biogas production. Thermophilic digestion is less restrained by ammonia accumulation contrasted with mesophilic processing. Biogas production is beyond twofold under thermophilic conditions contrasted with psychrophilic conditions. Organic nitrogen

degradation and phosphorus absorption rates also increases with temperature (Mao et al., 2015; C. Zhang et al., 2014).

2.3.2 pH

The functional pH essentially affects anaerobic digestion (AD). The ideal pH range for AD is accounted for to be 6.8-7.4. pH impacts microbial development, with various species flourishing at explicit pH levels. Controlling pH is significant for ideal microorganism development and decreasing ammonium toxicity. The composition of volatile fatty acids (VFA) and the hydrolysis rate are likewise altogether impacted by pH. Methanogenesis is generally effective at pH 6.5-8.2, while acidogenesis has an ideal pH scope of 5.5-6.5 (Mao et al., 2015; C. Zhang et al., 2014).

2.3.3 C/N Ratio

The C/N ratio plays a critical part in anaerobic digestion processes, influencing nutrient levels and system performance. A high C/N proportion can lessen protein solubilization rate and lead to low groupings of total ammonia nitrogen (TAN) and free ammonia (FA), while an unnecessarily low proportion can cause ammonia inhibition. Enhancing the C/N ratio within the range of 20-30, with 25 being regularly utilized, is significant for boosting biogas production. Co-digestion of agricultural waste with excrement can give positive synergistic impacts and dilute toxic compounds. Nonetheless, the economic sustainability of changing the C/N ratio with added substances, for example, urea or glucose in large digesters is a subject of concern. In general, keeping a proper C/N ratio is pivotal for proficient anaerobic digestion and biogas production (Mao et al., 2015; C. Zhang et al., 2014).

2.3.4 Volatile Fatty Acid (VFA)

Volatile fatty acids (VFAs) are significant intermediate substances in the anaerobic digestion (AD) of organic wastes, including acetic acid, propionic acid, butyric acid, and valeric acid. VFAs can be changed into methane (CH_4) and carbon dioxide (CO_2) by syntrophic acetogens and methanogenic microbes. Acetic and propionic acids assume a prevailing part in biogas production, and their concentrations can show AD performance. An exorbitant propionic acid to acetic acid proportion or high acetic acid concentration can lead to AD failure. VFAs likewise impact pH, which is a basic parameter influencing AD. Different pH ranges are

expected for the development of fermentative microbes and methanogens (Mao et al., 2015; C. Zhang et al., 2014).

2.3.5 Organic Loading Rate (OLR)

The organic loading rate (OLR) plays a huge part in anaerobic digestion processes. Increasing the OLR can prompt higher biogas yield but can also interrupt the digestion process. A high OLR can suppress bacterial activity, especially methanogenesis, and lead to increased volatile fatty acid (VFA) production, prompting irreversible acidification. The pH of the digester diminishes, disturbing hydrolysis and reducing methane conversion. Ideal OLR values have been determined for different substrates and circumstances, helping to maintain a stable and effective digestion process. Understanding and controlling OLR is significant for enhancing biogas production and keeping up with stable anaerobic digestion (Mao et al., 2015; C. Zhang et al., 2014).

2.4 Direct Interspecies Electron Transfer (DIET) Process in AD

Direct interspecies electron transfer (DIET) is a significant system for direct electron transfer between syntrophic microorganisms engaged in anaerobic degradation, working with quicker and more proficient electron exchange compared with hydrogen or formate-based pathways. Advancing the DIET process can improve methanogenic execution in anaerobic digestion (Dubé and Guiot, 2015). The primary constraint of the indirect interspecies electron transfer (IIET) component in AD is the accumulation of VFAs, which are poisonous to methanogens. DIET, then again, is thermodynamically beneficial as it bypasses the requirement for complex enzymatic steps and redox mediators, bringing about more productive energy transfer and quicker propionate degradation. DIET additionally shows higher external electron rates rates compared with hydrogen-based IET Figure 2.2 (Baek et al., 2018).

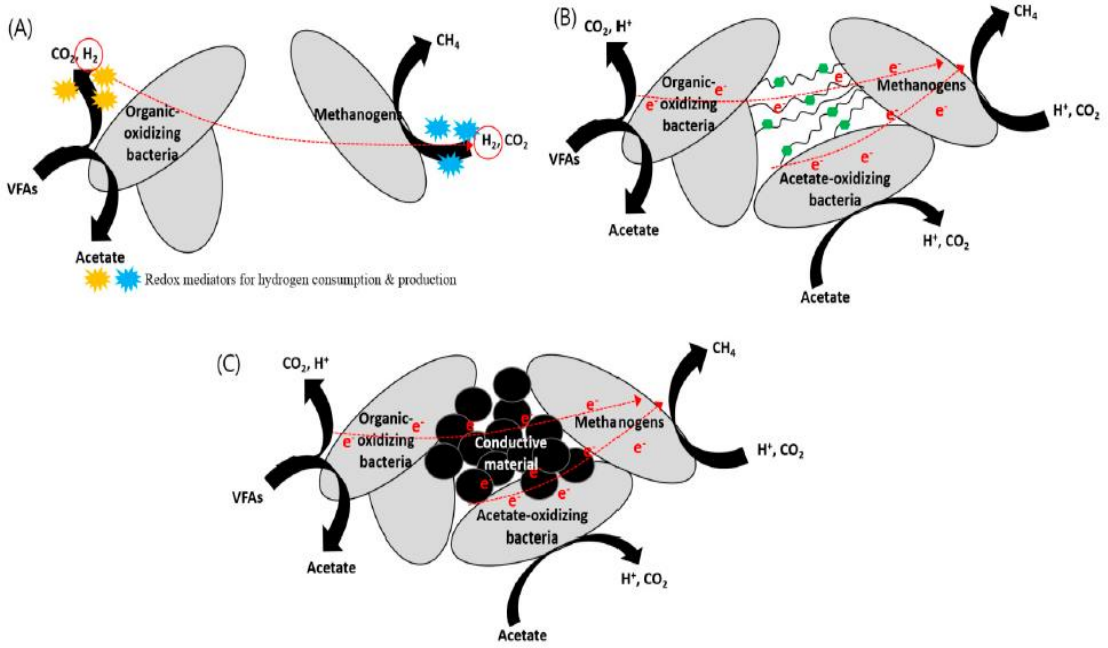


Figure 2.2: Mechanism of (A) IIET, (B) DIET, (C) DIET via conductive material (Baek et al., 2018).

2.5 Biochar

Biochar is a carbon-rich substance derived from a range of organic waste sources, including agricultural residues and municipal sewage sludge. It has gained growing interest because of its distinctive qualities, such as its high carbon content, capacity for cation exchange, extensive surface area, and stable composition (J. Wang & Wang, 2019).

2.5.1 Production of Biochar

There are different methods available in the literature for the production of biochar. Among the several methods, pyrolysis is the most effective and efficient way to produce the biochar. Pyrolysis is a thermochemical process in which the organic material is thermally degraded in the absence of air, along with this it offers less pollution as compared to other processes (Xiao et al., 2010). Pyrolysis can be divided into different classes depending on the operating conditions. Table 2.1 summarizes the different types of pyrolysis with operating conditions.

Table 2.1: Different types of pyrolysis with the operating condition (Tripathi et al., 2016).

Process	Temperature (ºC)	Heating rate (ºC/s)	Residence time (s)	Pressure (MPa)	Particle size (nm)
Slow pyrolysis	550-950	0.1-1	300-550	0.1	5-50
Intermediate pyrolysis	500-650	1-10	0.5-20	0.1	1-5
Fast pyrolysis	850-1250	10-200	0.5-10	0.1	<1
Flash pyrolysis	900-1200	>1000	<1	0.1	<0.5

2.5.2 Physiochemical Properties of Biochar

The properties of the biochar is related to the composition of the feedstock. The elemental composition of the biochar is determined by the composition of the feedstock (Stefaniuk & Oleszczuk, 2015). The feedstock that contains fixed carbon and high lignin are conducive during the production of biochar with the properties like high SSA and aromatic structures (Pan et al., 2019). Another parameter that influenced the physiochemical properties of biochar are temperature, heating rate and residence time during thermochemical conversion into pyrolysis product (Dudek et al., 2019). The physiochemical properties of biochar like elemental composition, functional groups, cation exchange capacity, SSA, PV, pH, EC and density as shown in Figure 2.3 are of primary concern due to its application and functionality (Tang et al., 2020).

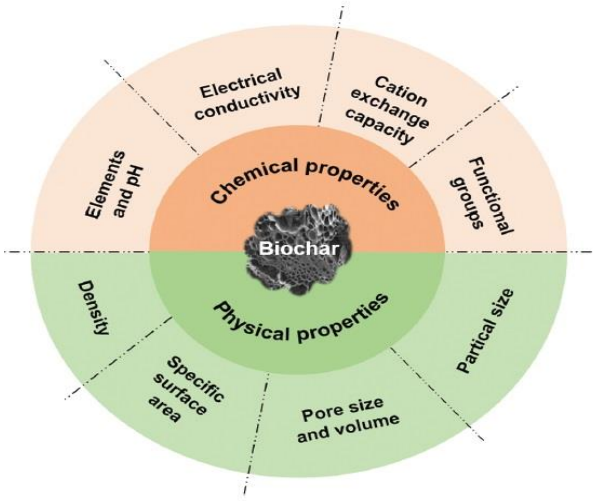


Figure 2.3: Physiochemical properties of biochar (Tang et al., 2020).

2.5.3 Biochar for Promoting AD

Several studies showed that biochar improved methane production during anaerobic digestion of waste-activated sludge by promoting hydrolysis, acidogenesis, and acetate-driven methanogenesis (C. Lü et al., 2020). Biochar improves biogas production, methane content, and process stability in anaerobic digestion. It absorbs CO_2, enhances microbial activity, and promotes methane production (Cruz Viggi et al., 2017; Qiu et al., 2019). The findings of the study by (G. Wang et al., 2018) showed that the addition of biochar to the UASB reactors improved anaerobic sludge granulation, shortened the methanogenesis lag time, and increased the COD removal rate. Biochar stimulates direct interspecies electron transfer (DIET) in methanogenic co-cultures, enabling the conversion of ethanol to methane. Biochar enhances electron transfer and promotes methane production (Chen et al., 2014). Figure 2.4 shows the application of biochar for the promotion of AD.

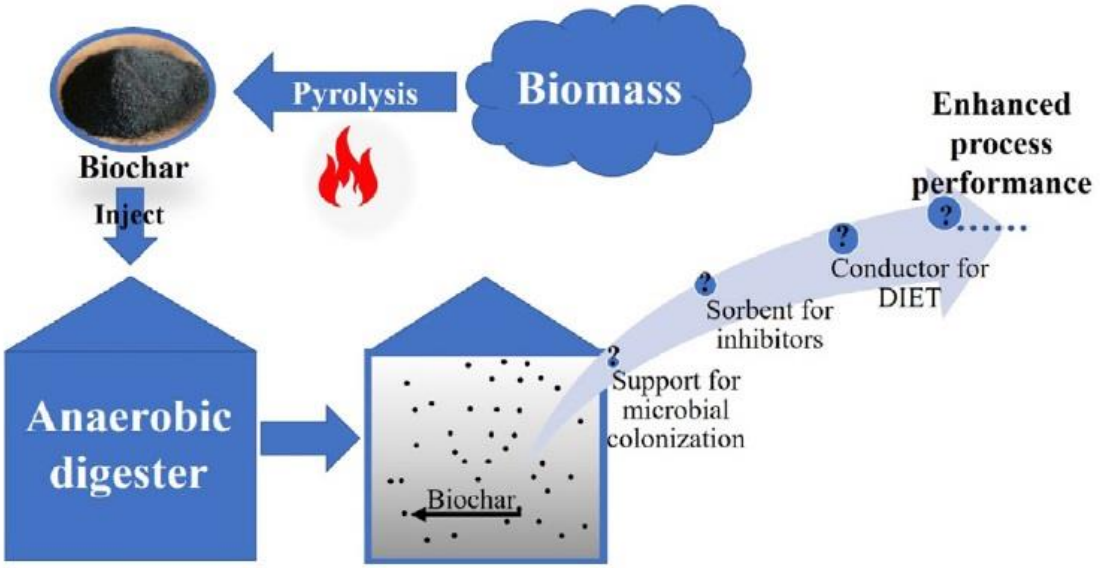


Figure 2.4: Biochar for promoting AD (Pan et al., 2019).

2.5.4 Biochar for Overcoming Inhibition in AD

Biochar addition improves anaerobic digestion (AD) by reducing ammonia inhibition and promoting the growth of beneficial microorganisms. It adsorbs ammonia, alleviates ammonia-related inhibition, and enhances methane production (Qiu et al., 2019). Biochar can reduce TAN concentrations and stabilize pH during anaerobic digestion, but its impact on total dissolved nitrogen and ammonium concentration varies (Pan et al., 2019). The adsorption capacity of biochar can enhance anaerobic digestion (AD) by alleviating acids and ammonia

inhibition via adsorption of volatile fatty acids (VFAs) and ammonium (NH_4-N) (Tang et al., 2020). The study carried out by (Giwa et al., 2019) suggests, the addition of biochar in anaerobic digestion reactors effectively mitigated the inhibitory effects of high total ammonium nitrogen (TAN) concentrations. In the study by (M. Zhang et al., 2019), biochar supplementation in anaerobic digestion facilitates ammonia alleviation and further develops methane production. The research by (Cheng et al., 2020) recommends that the addition of biochar be found to relieve ammonia stress, increment in biogas yield, and improve anaerobic digestion considerably under high ammonium stress.

2.5.5 Loading Rate of Biochar

Rice husk biochar pyrolyzed at 550ºC with a biochar dosage of 10 g/L effectively alleviated ammonia inhibition, resulting in an increase in methane production (Yu et al., 2021). Fruitwood biochar pyrolysed at 800°C to 900°C with a dosage of 10 g/L showed that there was an increase in methane production rate, and ammonium stress was mitigated (F. Lü et al., 2016). In a study conducted by (Cheng et al., 2020), rice straw biochar pyrolysed at 600°C with a dosage ranged from 2 g/L to 15 g/L, found that biochar application effectively improved anaerobic digestion (AD) performance, particularly under conditions of high ammonium stress with concentrations ranging from 900 mg/L to 3500 mg/L. In a study conducted by (Kaur et al., 2020), wheat straw biochar pyrolysed at 550°C and 700°C at a dosage of 10 g/L indicated that the use of wheat straw biochar led to the production of higher methane concentrations and improved volatile solids (VS) removal efficiency.

The aforementioned studies on biochar indicate that the optimal dosage of biochar for enhancing the methane production is 10 g/L. Therefore, for the current research, a biochar dosage of 15 g/L was chosen to observe the effect of biochar on methane production.

3 Materials and Methods

This section offers a summary of the materials and techniques employed in the research, encompassing the experimental configuration and the procedures for analyzing data. Three biochar materials were used for the enhancement of methane production in an anaerobic digestion.

3.1 Source of Inoculum

The inoculum used in the study was sourced from an anaerobic digester at Lindum AS in Drammen, Norway. This digester processed residual waste from the thermal hydrolysis of sewage sludge and food waste. The operational conditions included a hydraulic retention time of 19 days and a mesophilic temperature of 37 °C (Lindum, n.d.). The inoculum received from the Lindum AS was sieved using a sieve of 2 mm to remove larger and fibrous material and kept in an incubator at 37^{o}C for 3 weeks to degas (Kassegn et al., 2021). Following the degassing process, a 210 ml portion of inoculum and 90 ml of salt solution were transferred to bioprocess control automatic methane potential test reactor. The composition of the salt solution is shown in the Table 3.1.

Table 3.1: Ingredients and concentration of the salt solution (Aryal et al., 2023).

Ingredients	Concentration (g/L)
KH_2PO_4	2.72
$Na_2HPO_4 \cdot 2H_2O$	3.55
NH_4Cl	0.28
$CaCl_2 \cdot 2H_2O$	0.0076
$MgSO_4 \cdot 7H_2O$	0.001
$MgCl_2 \cdot 6H_2O$	0.09

3.2 Selection of Conductive Materials

In this study, biochar was chosen as the conductive material, with three distinct types of biochar supplied by Scanship AS being employed. The description of the biochar is shown in the Table 3.2.

Table 3.2: Description of different types of biochar used in the experiment (Flatabø et al., 2023).

Types of biochar	Abbreviation	Pyrolysis temperature	Description
Garden waste	GW	800º C	Garden trash from commercial and private establishments. Fraction consists of some sand or gravel, leaves, and twigs.
Digested sewage sludge	DSS	600º C	Sewage sludge from anaerobic digester
Waste timber	WT	600º C	Throw away wood items and goods from commercial buildings, private homes, and building and demolition projects

3.2.1 Preparation of Biochar

The biochar, obtained from Scanship AS and classified into three different types, underwent a process of grinding and sieving to achieve a particle size ranging from 0.5 to 1 mm. Subsequently, the ground biochar samples were rinsed with distilled water and then subjected to overnight drying at 105℃ in an oven (F. Lü et al., 2016). Figures 3.1 and 3.2 display the sieve used for biochar sieving and biochar sample in the particle size, respectively.

Figure 3.1: Sieve used for biochar sieving.

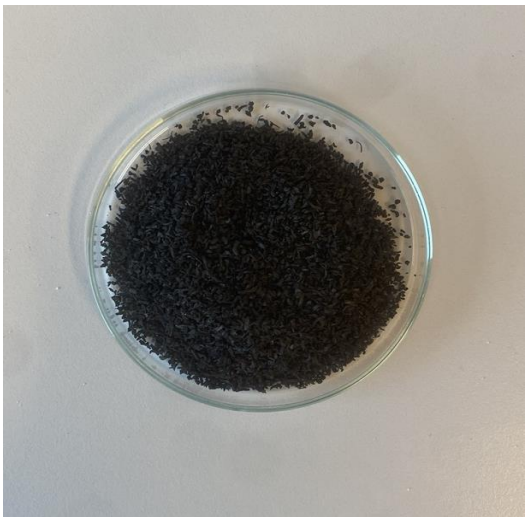


Figure 3.2: Biochar in the particle size after sieving.

3.3 Experimental Methods

Biochemical methane potential tests were conducted using the Bioprocess Control AMPTS II apparatus, consisting of three main components: (i) a digester, (ii) a CO_2 capture unit, and (iii) a gas collection unit. This system operates in batch mode, where it is configured and allowed to run until the digestion process is finished. A 500 ml digester, with an effective working

volume of 300 ml (60%) and a headspace of 200 ml (40%), was employed for biogas generation. The process temperature was maintained at a constant mesophilic level of 37°C (Maile et al., 2016). The digester was linked to a 100 ml bottle utilized as a scrubber, containing 80 ml of a 3M NaOH solution. The gas that emerged from the CO_2 capture unit was directed into the flow cell (used for gas collection), where the quantity of biomethane was measured, as depicted in Figure 3.3.

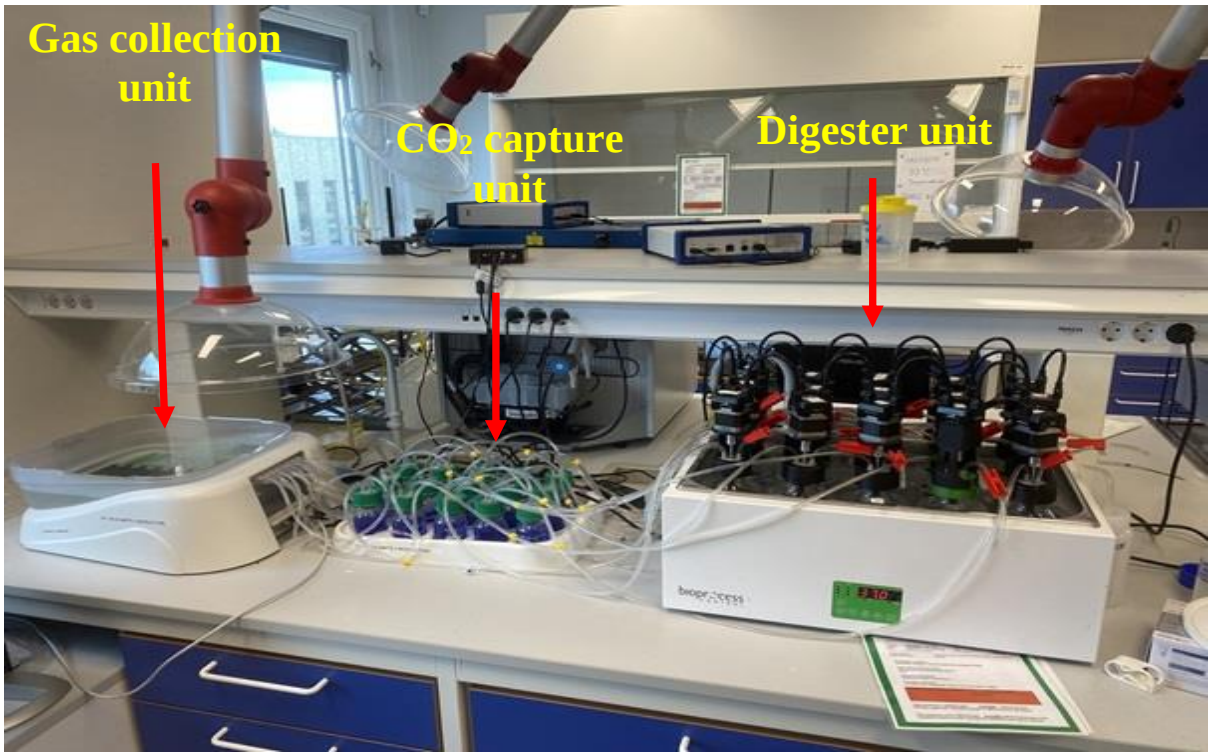


Figure 3.3: AMPTS setup used for the experiment.

3.3.1 BMP Test with Propionic Acid as Carbon Source

In the first batch, the degassed inoculum and the salt solution in the ratio of 1:1 were mixed and 1 g/L of propionic acid is added in a 1 litre bottle keeping 50% headspace and kept in the incubator for 2 weeks for adoption of the microbes (Aryal et al., 2023). After 2 weeks this incubated inoculum was transferred to the AMPTS reactor as the same experimental setup as shown in Table 3.3. But there was not any production of gas from the reactors and the experiment was stopped on the third day.

In the second batch, the degassed inoculum was directly used in the reactor. In this study, five samples were analyzed, each with three replicates. The ammonium concentration of the inoculum was initially 1375 mg/L, and to induce ammonium stress at a concentration of 3000 mg/L, the inoculum received an additional 1625 mg/L of ammonium chloride. Additionally,

15 g/L of biochar and 3 g/L of propionic acid were added as a carbon source. A detailed composition for each sample is presented in Table 3.3.

Table 3.3: Composition of the sample of the second batch in the BMP test.

Name	Number of parallels	Inoculum + salt solution (ml)	Propionic acid ($g/L_{inoculum}$)	Ammonium chloride ($g/L_{inoculum}$)	Biochar ($g/L_{inoculum}$)	Status
Blank	3	300	-	-	-	Negative control
Propionic acid	3	300	0.9	0.48	-	Positive control
GW biochar	3	300	0.9	0.48	4.5	Positive biochar
DSS biochar	3	300	0.9	0.48	4.5	Positive biochar
WT biochar	3	300	0.9	0.48	4.5	Positive biochar

3.3.2 BMP Test with Acetic and Propionic Acid as Carbon Source

The second batch experiment has to be stopped because of the potential inhibition from the ammonia and propionic acid. There was not much of methane production from the second batch also. In the third batch experiment, five samples were analyzed, each with three replicates as in the second batch. In the third batch another degassed inoculum sourced from Lindum As with ammonium concentration of 3600 mg/L was used. In this experiment no additional dose of ammonium chloride was given. Additionally, 15 g/L of biochar and 3 g/L (1.5 g/L acetic acid & 1.5 g/L propionic acid) were added as a carbon source. A detailed composition for each sample is presented in Table 3.4.

Table 3.4: Composition of the sample of the third batch in BMP test.

Name	Number of parallels	Inoculum + salt solution (ml)	Propionic acid ($g/L_{inoculum}$)	Acetic acid ($g/L_{inoculum}$)	Biochar ($g/L_{inoculum}$)	Status
Blank	3	300	-	-	-	Negative control
Propionic + Acetic acid	3	300	0.45	0.45	-	Positive control
GW biochar	3	300	0.45	0.45	4.5	Positive biochar
DSS biochar	3	300	0.45	0.45	4.5	Positive biochar
WT biochar	3	300	0.45	0.45	4.5	Positive biochar

3.4 Sample Analysis and Measurement

3.4.1 Measuring Total Solids and Volatile Solids

The total solids (TS) in wastewater are made up of all solids, including suspended and soluble organics and inorganics, as well as biodegradable and non-biodegradable materials. The volatile solids (VS) in wastewater are made up exclusively of biodegradable organics that are soluble in suspension. The American standard approach APHA 2540 B is followed in the measurement process for these solids (*Standard Methods for the Examination of Water and Wastewater*, 1999).

Initially, a crucible made of clean porcelain was picked, dried in an oven at 105°C, and afterward permitted to cool to room temperature. Then, the Sartorius model, an exceptionally precise analytical balance, was utilized to determine its weight (W1). Consequently, 30 mL of a completely blended sample was brought into the crucible, and its mass was noted as W2. The sample-containing crucible was next placed in an oven at 105°C. The sample and crucible were put inside a desiccator to cool to room temperature after being removed from the oven. At last, the total solids in the sample were determined using equation (3.1).

$$TS\ (g/L) = \frac{W3\ (g) - W1\ (g)}{V\ (L)} \tag{3.1}$$

The recently procured dried sample was set in a muffle furnace and heated to a temperature of 550°C for 20 minutes. The sample was then permitted to cool to room temperature in a desiccator. The sample-containing crucible was then reweighed with a similar analytical balance as before, and the recorded weight was demonstrated as W4. Equation (3.2) were utilized to calculate the volatile solids concentration of the sample.

$$VS\ (g/L) = \frac{W3\ (g) - W4\ (g)}{V\ (L)} \tag{3.2}$$

3.4.2 Organic Matter Measurement in Terms of TCOD and SCOD

The total organics present in the extracted sample are quantified using TCOD. A suitable sample was saved in case the results required to be cross-checked. The measurement process adheres to US standard 5220 D (*Standard Methods for the Examination of Water and Wastewater*, 1999). It was determined using the protocols outlined in the Spectroquant prove

300 instruction manual (Merck, 2017). The method number for measuring COD (500-10000 mg/L) according to US standards is 114555.

SCOD quantifies the amount of soluble organics in an extracted sample. In the instance of SCOD, the extracted sample was centrifuged at 12500 rpm for 15 minutes in a ThermoFisher Scientific Heraeus Megafuge 16 centrifuge. The centrifuge sample was then filtered by Acrodisc PSF 0.45 m GxF multi-layered filters. The filtered sample was then measured using the same process and instructions as the TCOD sample. Figure 3.4 depicts the use of spectrophotometer to measure the concentration of TCOD and SCOD.

Figure 3.4: Spectrophotometer used for measuring COD and ammonium concentration.

3.4.3 Ammonium Concentration

The extracted material was centrifuged and filtered in the same way that the SCOD sample was. Following filtration, the ammonium was measured using the procedure outlined in the Spectroquant prove 300 instruction handbook (Merck, 2017). The method number for measuring ammonium (4-80 mg/L) according to US standards is 114559.

3.4.4 Volatile Fatty Acids (VFA) Analysis

The distinct volatile fatty acids and their amounts were identified using a THERMO Scientific TRACE^{TM} 1300 Gas Chromatograph.

The sample collected from each reactor was diluted with distilled water at a 1:10 ratio. The diluted mixture was then be centrifuged to separate the fluid according to density. The centrifuged mixture was filtered via GxF/Glass and wwPTFE syringe filters. To determine the volatile fatty acids, 150 µL of formic acid was mixed with 1.35 mL of the diluted and filtered mixture in vials. Figure 3.5 depicts the use of a Gas Chromatograph to determine the concentration of various volatile fatty acids (Manni & Caron, 1995).

Figure 3.5: Gas chromatograph used for determining the concentration of VFA.

3.4.5 Statistical Analysis

To study the correlation between characteristics of biochar and methane yield, correlation analysis was done by Spearman rank correlation. To perform correlation analysis, and draw the scatter diagram MS Excel was used. One way analysis of variance (ANOVA) was performed to determine the significance between the samples. The ANOVA was performed in SPSS (Qin et al., 2020).

4 Results

All the results obtained from the second batch and third batch experiment are presented in this section with bar and line charts. These graphs are plotted on the basis of the results obtained from the automatic methane potential test system (AMPTS) and the data obtained from laboratory analysis. For calculation of data and graphical plot MS-excel was used.

4.1 Characterization of Biochar

The biochar used in this experiment was characterized by the elemental properties, specific surface area and pore volume, pH and electrical conductivity.

4.1.1 Elemental Properties of the Biochar

The elemental distribution of particles present in the GW, DSS and WT biochar is shown in the Figure 4.1. GW biochar is composed of carbon predominantly, followed by ash, hydrogen and then nitrogen. Carbon constitutes of 67.3% by weight in this biochar, followed by ash of 30.82%, hydrogen of 1.07% and then nitrogen of 0.81%.

DSS biochar is composed of ash predominantly, followed by carbon, oxygen, hydrogen and then nitrogen. Ash constitutes of 74.76% by weight in this biochar, followed by carbon of 13.1%, oxygen of 9.43%, hydrogen of 1.58% and then nitrogen of 1.13%.

WT biochar is composed of carbon predominantly, followed by ash, hydrogen and then nitrogen. Carbon constitutes of 79.6% by weight in this biochar, followed by ash of 15.77%, hydrogen of 2.82% and then nitrogen of 1.81%.

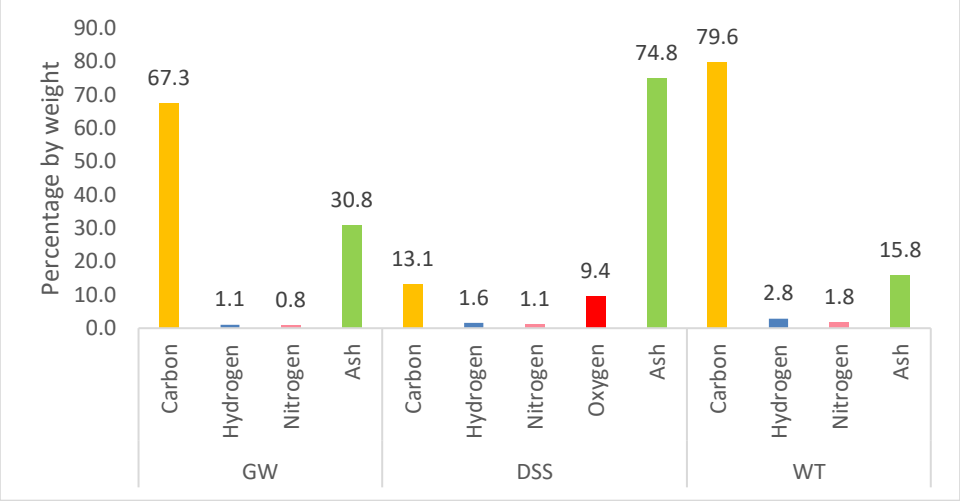


Figure 4.1: Elemental distribution of particles present in GW, DSS and WT biochar (Flatabø et al., 2023).

4.1.2 pH and Electrical Conductivity of Biochar

The Figure 4.2 represents pH and EC of biochar derived from different sources. The GW biochar is highly alkaline in nature and exhibits a pH of 12.1. The WT biochar is moderately alkaline in nature exhibiting and pH of 9.4 and DSS biochar exhibits a slightly alkaline exhibiting a pH of 8.1.

WT biochar has exceptionally higher conductivity of 881 µS/cm, while GW and DSS biochar exhibits significantly lower conductivity of 6.7 µS/cm and 2.5 µS/cm respectively.

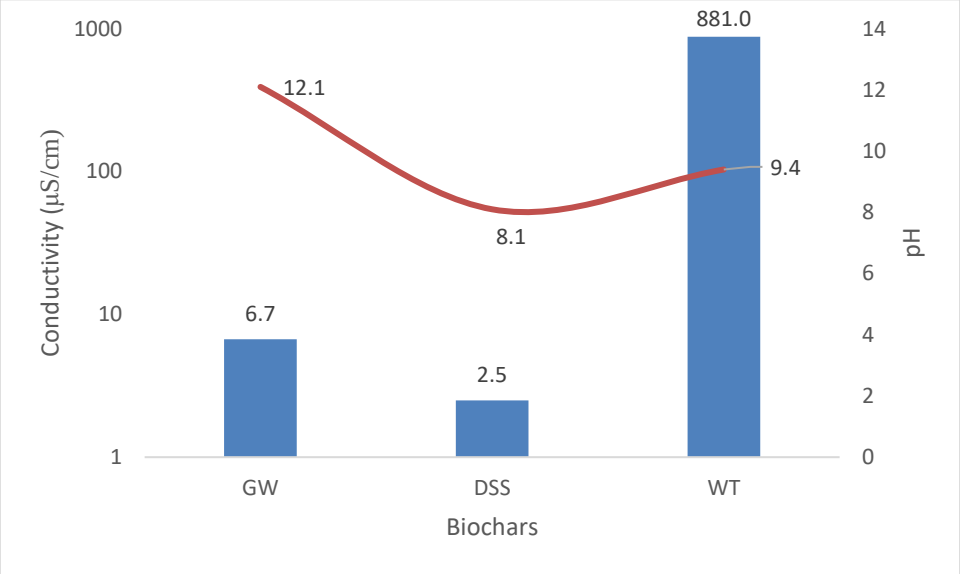


Figure 4.2: pH and EC of biochar samples derived from different sources (Sørmo et al., 2023).

4.1.3 Specific Surface Area and Pore Volume of Biochar

The Figure 4.3 represents the specific surface area and pore volume of the biochars derived from different sources. The WT biochar exhibits higher SSA with a value of 204 m^2/g, followed by the GW biochar of 146 m^2/g and DSS biochar of 133 m^2/g.

The DSS biochar has higher pore volume of 0.122 cc/g compared to other biochar. GW biochar has the pore volume of 0.042 cc/g and WT biochar has the lowest pore volume of 0.025 cc/g.

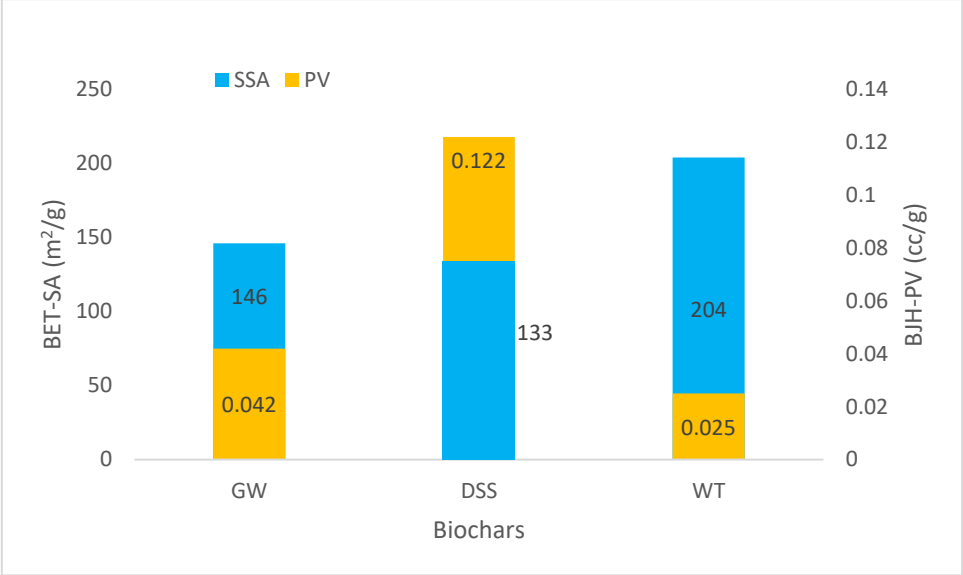


Figure 4.3: SSA and PV of biochar samples derived from different sources (Sørmo et al., 2023).

4.1.4 Correlation between Methane Yield and Biochar Characteristics

The correlation between the methane yield with SSA, EC and carbon content of biochar is shown in the Figure 4.4. The results from the correlation suggest that there is a very strong correlation between the methane yield with biochar SSA, EC and carbon content. A positive correlation coefficient of 1 indicates that with the increase in biochar SSA, EC and carbon content, the methane yield also increases in a linear approach.

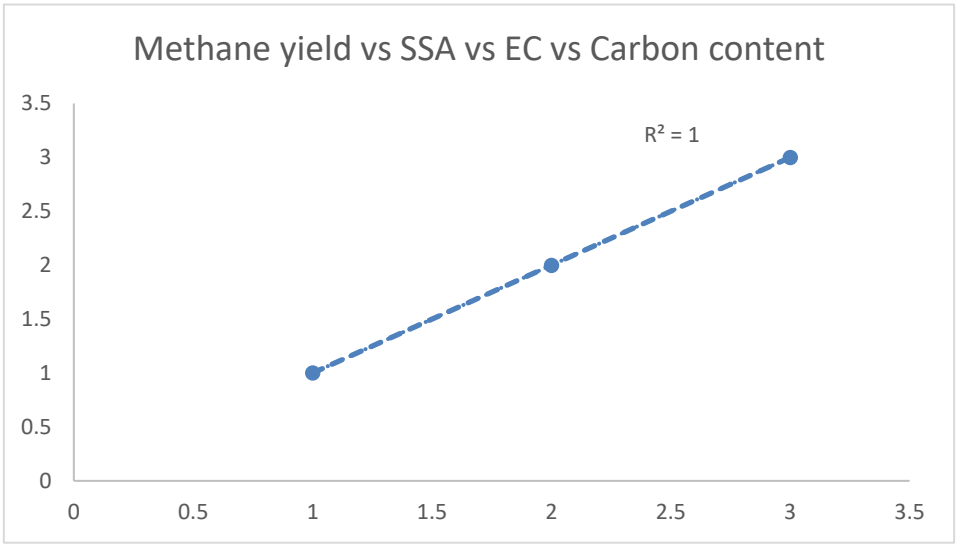


Figure 4.4: Correlation of methane yield with SSA, EC and carbon content of biochar.

The correlation between the methane yield and pH of the biochar is shown in the Figure 4.5. The results from the correlation suggest that there is medium correlation between the methane

yield and pH of biochar. A correlation coefficient of 0.25 indicates that the pH of the biochar doesn't have much influence on the production of methane.

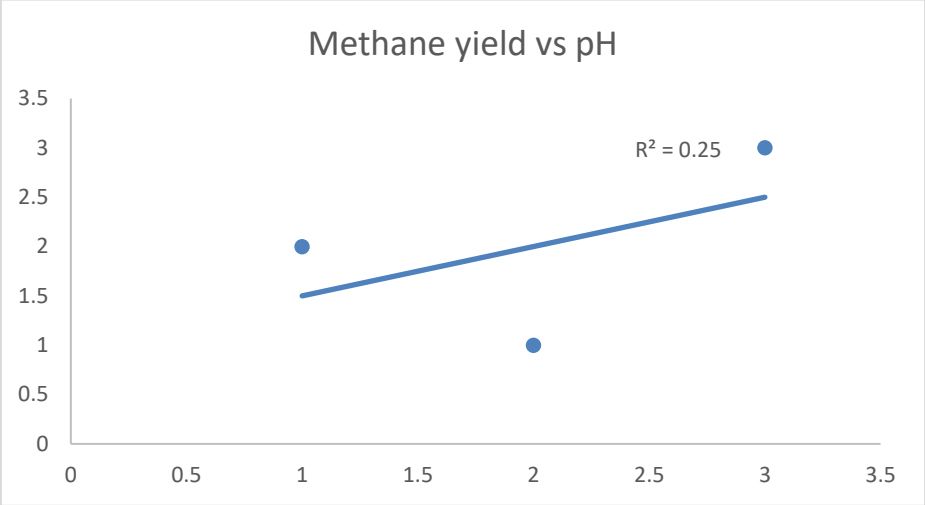


Figure 4.5: Correlation of methane yield with pH of biochar.

The correlation between the methane yield and pore volume of the biochar is shown in Figure 4.6. The results from the correlation suggests that there is a perfect negative linear relation between methane yield and pore volume of biochar. A correlation coefficient of -1 indicates the inverse relationship between methane yield and pore volume of biochar. As the pore volume of biochar decreases, there is tendency to increase in methane production, and as the pore volume of biochar increases, the methane yield decreases.

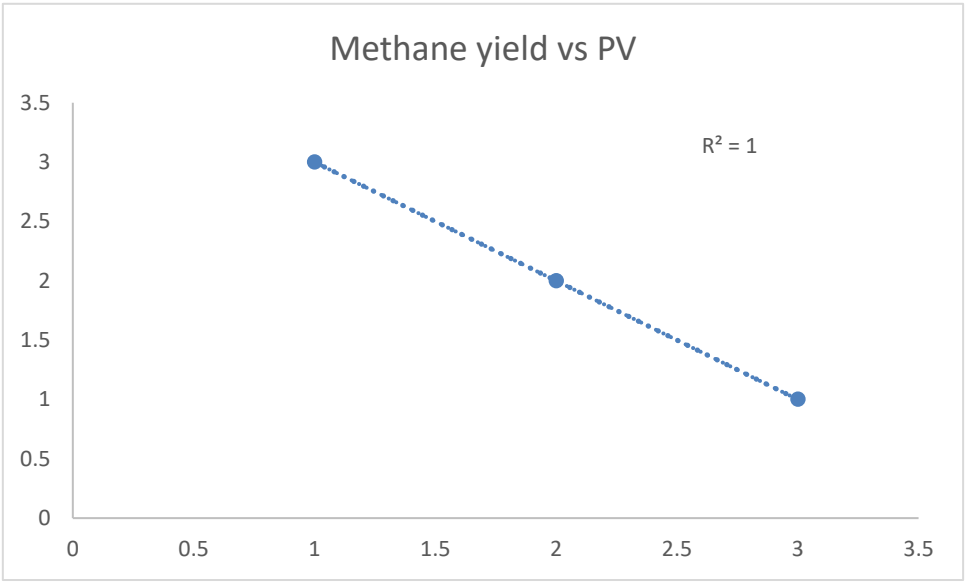


Figure 4.6: Correlation of methane yield with pore volume of biochar.

4.2 Effect of Biochar on AD in Second Run

This section summarises the anaerobic digestion parameters that were measured in the second batch experiment during the automatic methane potential system (AMPTS) test.

4.2.1 Analysis of Inoculum

The physical and chemical properties of the inoculum is presented in the Table 4.1.

Table 4.1: Concentration of the TCOD, SCOD, ammonium, TS and VS of the inoculum of the first and second run.

TCOD (g/L)	SCOD (g/L)	Ammonium (g/L)	pH	TS (g/L)	VS (g/L)	VS/TS
6.8	4.7	1.4	7.7	15.7±2.1	9.3±1.2	0.6

4.2.2 Cumulative Methane Production

The cumulative methane production during the period of 12 days is shown in Figure 4.7. Only the one reactor from DSS and WT biochar didn't work in this experiment, other reactor produces gas till 12th day of the experiment. Despite this, the experiment continued until 22nd day; since there was no methane production from the 12th day onward, the experiment was stopped.

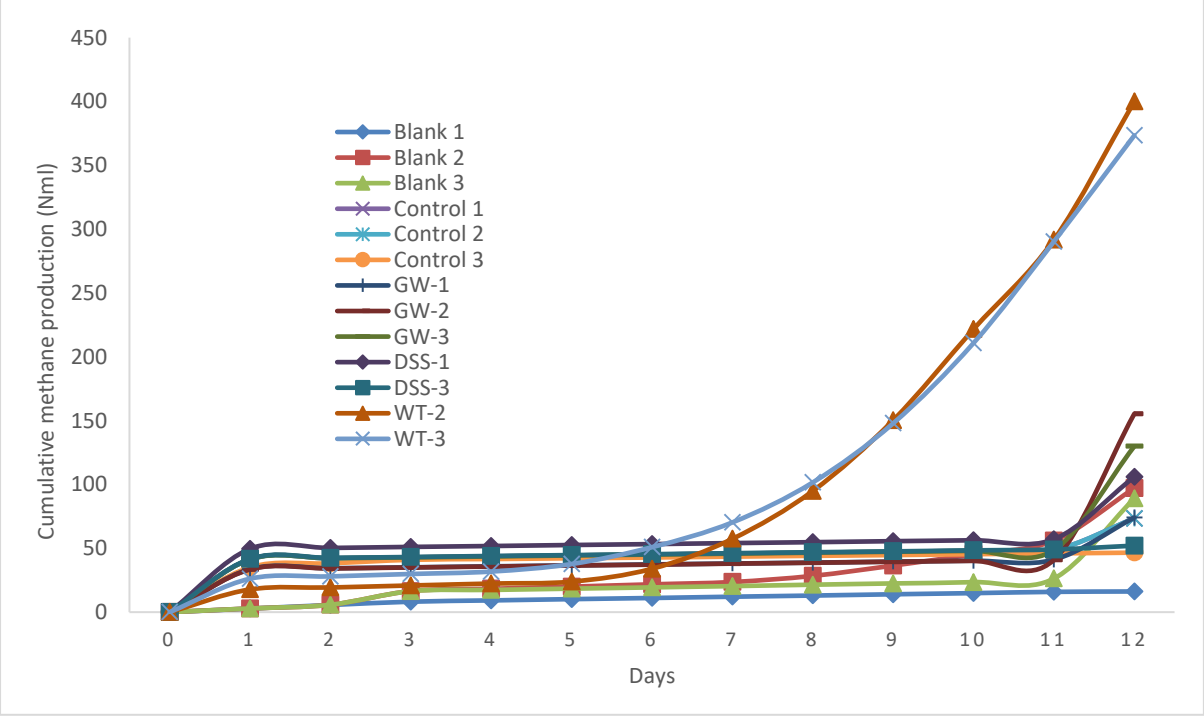


Figure 4.7: Cumulative methane production in the second run.

The average amount of cumulative methane produced by the five sample is shown in the Figure 4.8 and Figure 4.9. WT biochar attained the maximum volume of methane production (386.7 Nml) over a 12 day period. The methane production in the blank is relatively low throughout the 12 day period, with a significant rise on the 12th day reaching 67.5 Nml. The control also shows the relatively low methane production during the 12 day period, with a significant rise on the 12th day reaching 64.5 Nml. GW and DSS biochar shows slightly more methane production than the blank and control, with GW biochar producing slightly more methane than the DSS biochar. The GW biochar sample produces 119.8 Nml of cumulative methane while DSS biochar sample produces 79.1 Nml methane.

After the 12th day of the experiment, the methane production completely stopped in all the reactors. This results suggest that there might be inhibition from accumulation of VFA and ammonia that inhibit the methanogenic activity.

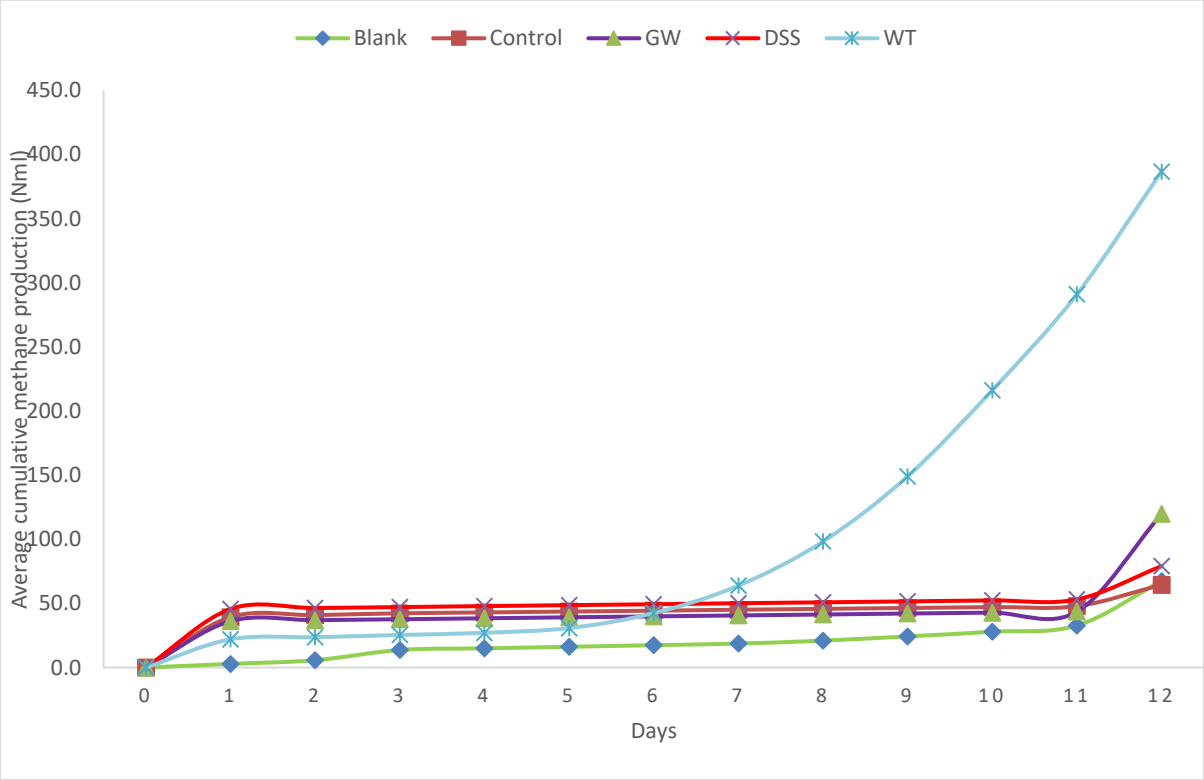


Figure 4.8: Average cumulative methane production in the second run.

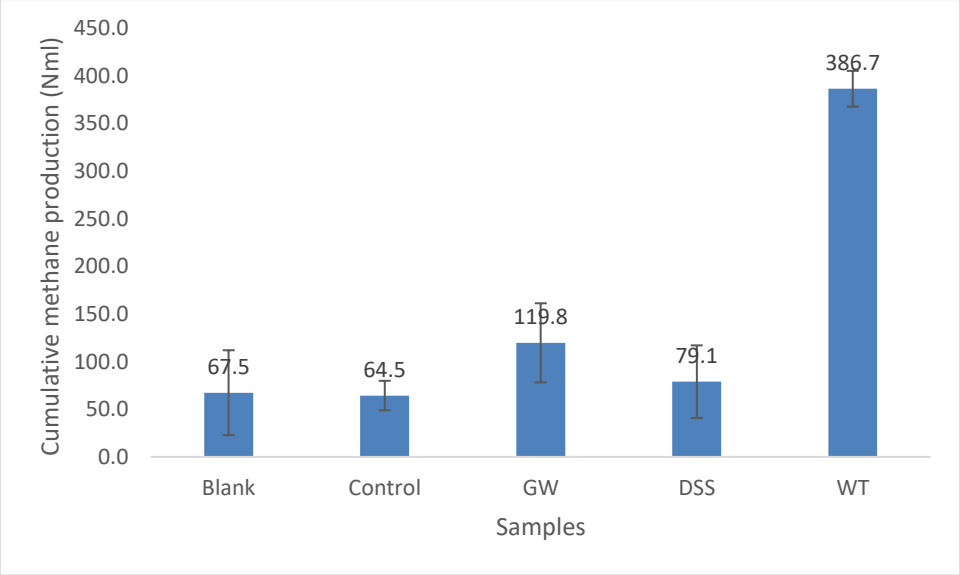


Figure 4.9: Average cumulative methane production and their standard deviation in bars in second run.

The average amount of methane produced by the four sample after removing the methane production from blank is shown in the Figure 4.10 and Figure 4.11. The methane production from the control gradually decreases and reaches a value of -3 Nml on 12th day of the experiment. This negative value indicates reduction in methane production compared to the blank. Both GW and DSS biochar sample show consistent decline in methane production over the 12 day period, but on the 12th day GW biochar shows slightly increase in methane production than DSS biochar. WT biochar shows significant increase in methane production from 6th day to till 12th day. By the 12th day WT biochar records the highest methane production of 319.2 Nml, which is higher than the others.

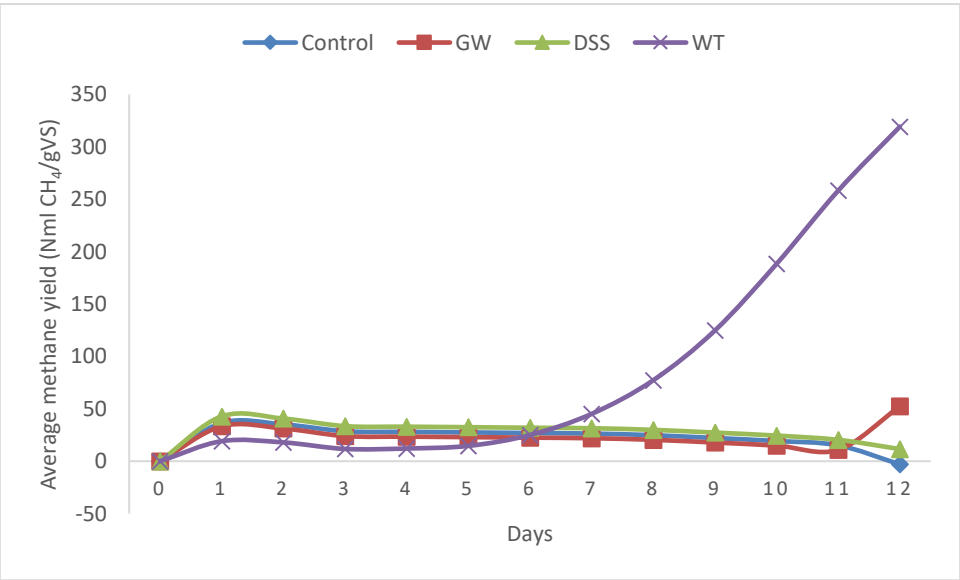


Figure 4.10: Methane yield after removing the blank in second run.

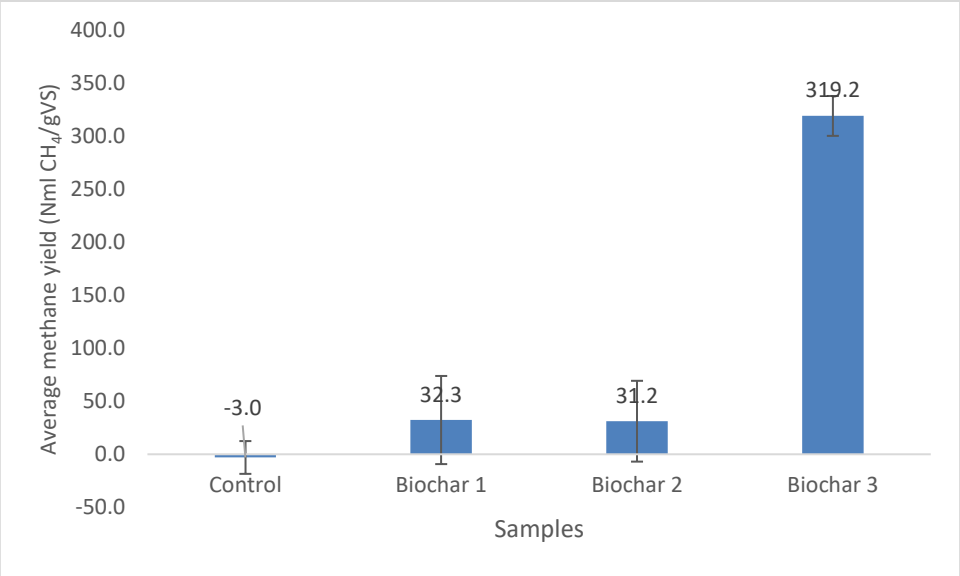


Figure 4.11: Methane yield with error bar after removing the blank in the second run.

4.2.3 COD

The COD concentration of the sample from digestate is shown in Figure 4.12. The sample from the GW biochar has highest TCOD value of 6.1 g/L and control sample has the highest value of SCOD of 4.505 g/L. The blank sample has the lower TCOD and SCOD value of 3.75 g/L and 2.215 g/L respectively. The sample from the DSS biochar has the TCOD of 5.25 g/L and SCOD of 3.795 g/L. Similarly WT biochar sample has the TCOD of 4.5 g/L and SCOD of 4.23 g/L.

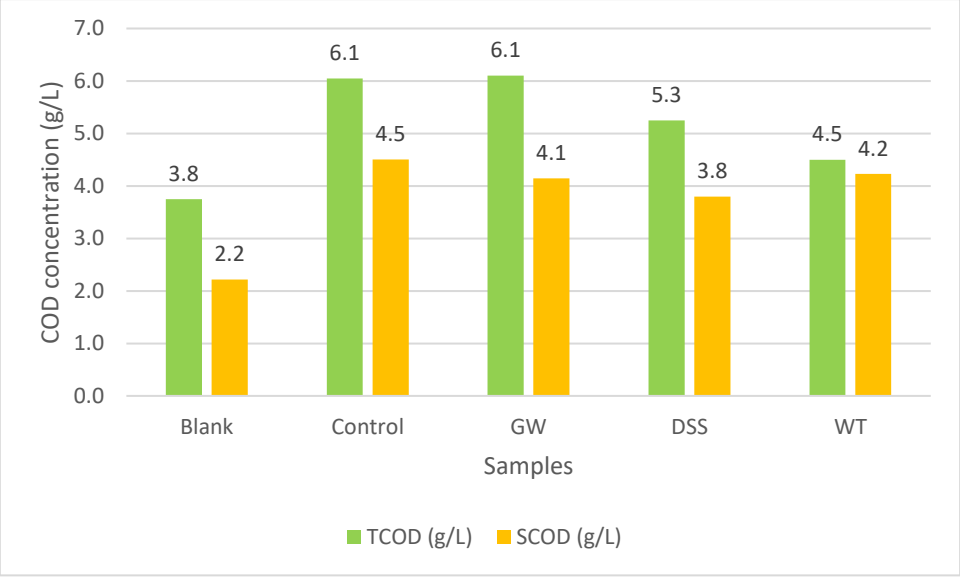


Figure 4.12: COD concentration of digestate in the second run.

4.2.4 Ammonium Concentration

The ammonium concentration of the sample from the digestate is shown in the Figure 4.13. The blank sample has the lowest ammonium concentration among all the samples, with a value of 1.2 g/L. The WT biochar sample has the highest ammonium concentration among all the samples, with a value of 2.41 g/L. GW and DSS biochar sample has the ammonium concentration of 2.15 g/L and 2.18 g/L respectively. The differences in ammonium concentration are relatively small, there is slightly increase in ammonium concentration from blank to the control and further to the biochar samples. The initial concentration of the ammonium in the inoculum was 1.375 g/L. Further 1.625 g/L of ammonium dose is added in the control and biochar samples to induce the ammonium stress at 3 g/L.

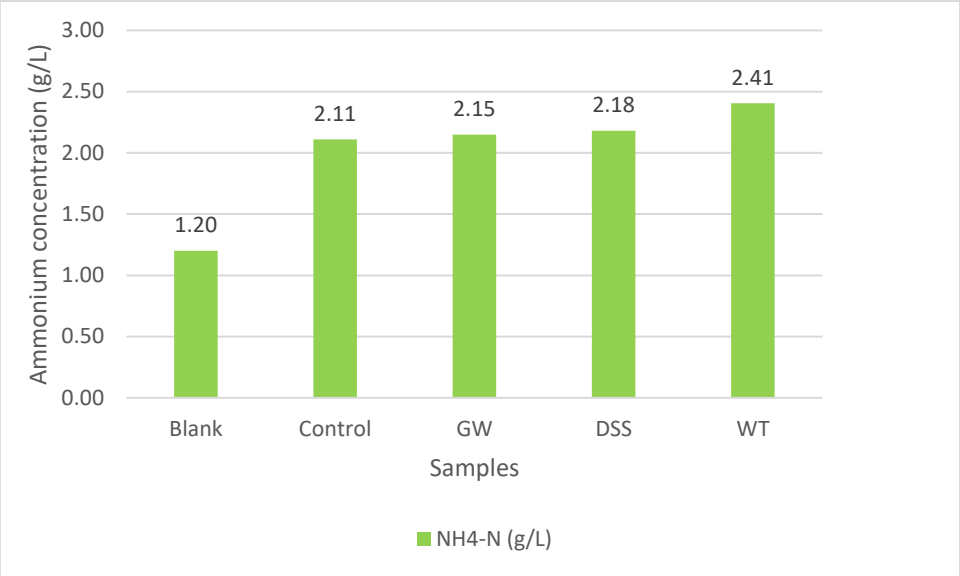


Figure 4.13: Ammonium concentration of digestate in the second run.

4.2.5 Volatile Fatty Acids (VFA)

The concentration of different volatile fatty acid (VFA) of the digestate samples is shown in the Figure 4.14. The concentration of propionic acid is higher in almost all the samples. GW biochar sample has the highest propionic acid concentration of 4625 mg/L followed by the sample of DSS biochar of 3736 mg/L and WT biochar of 3717 mg/L. The isobutyric acid concentration is comparatively lower than the concentration of acetic acid. GW biochar sample has the highest concentration of acetic acid of 1319 mg/L followed by DSS biochar of 822 mg/L, control of 490.61 mg/L and WT biochar of 209 mg/L. Blank has the lowest concentration of acetic and propionic acid. The concentration of isovaleric and isocaporic acid is relatively low in all the samples, the isocaporic acid is absent in the blank sample.

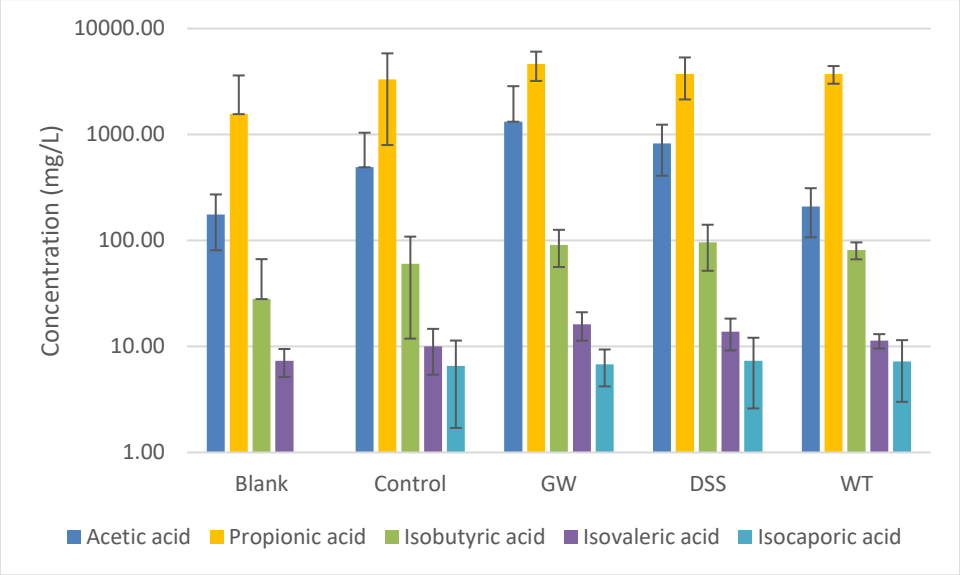


Figure 4.14: Total volatile fatty acid (VFA) concentration of the digestate with error bars in the second run.

4.3 Effect of Biochar on AD in Third Run

This section summarises the anaerobic digestion parameters that were measured in the third batch experiment during the automatic methane potential test system (AMPTS).

4.3.1 Analysis of Inoculum

In the third run another inoculum sourced from Lindum AS was used. The physical and chemical properties of the inoculum is provided in Table 4.2.

Table 4.2: Concentration of the TCOD, SCOD, ammonium, TS and VS of the inoculum of third run.

TCOD (g/L)	SCOD (g/L)	Ammonium (g/L)	pH	TS (g/L)	VS (g/L)	VS/TS
6.05	4.35	3.15	7.8	40.1±5.0	19.4±2.7	0.5

4.3.2 Cumulative Methane Production

The cumulative methane production during the period in the third run is shown in Figure 4.15. Only the one reactor from the WT biochar didn't work in this experiment, other reactors produces the methane till the end of the experiment.

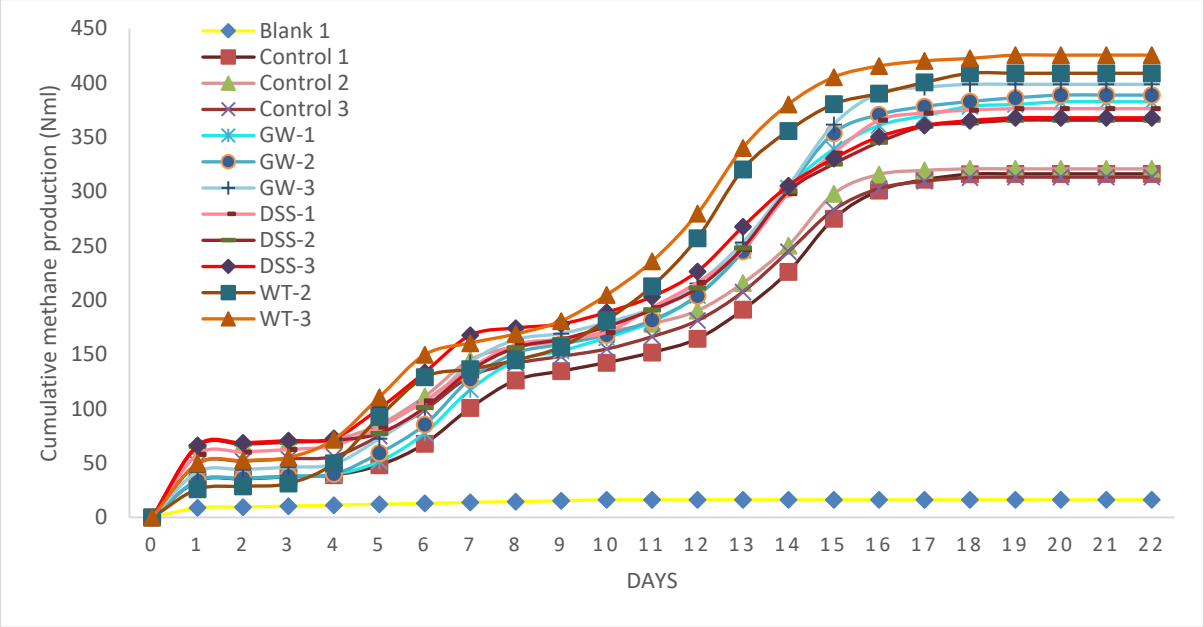


Figure 4.15: Cumulative methane production in the third run.

The average cumulative methane production in the third run is shown in the Figure 4.16 and 4.17.

The control and biochar reactors showed an increasing trend in methane production during 22 day period. All the biochar reactors showed the increased methane production as compared to the control one. Amongst the reactors, WT biochar reactor showed the highest level of methane production (417.2 Nml) followed by GW biochar reactor (390 Nml) and then DSS biochar reactor (369.9 Nml). The control reactors showed the methane production of 316.7 Nml and blank reactor showed the lowest methane production of 16.2 Nml.

From the day 6 there is an increase in methane production for all the reactors, particularly for the biochar reactors. The methane production from the control reactor continued to increase but at a lower rate compared to biochar reactors. From the 13th day there is significant rise in methane production from the control and biochar reactors till the 17th day. After the 17th day, the methane production rate decreases and remains relatively constant till 22nd day.

The WT biochar reactor produces 100.5 Nml more methane than the control reactor that is 31.75% higher than the control reactor. The GW biochar produces 73.3 Nml more methane than the control reactor that is 23.13% higher than the control reactor, while DSS biochar produces 53.2 Nml more methane that is 16.81% higher than the control reactor.

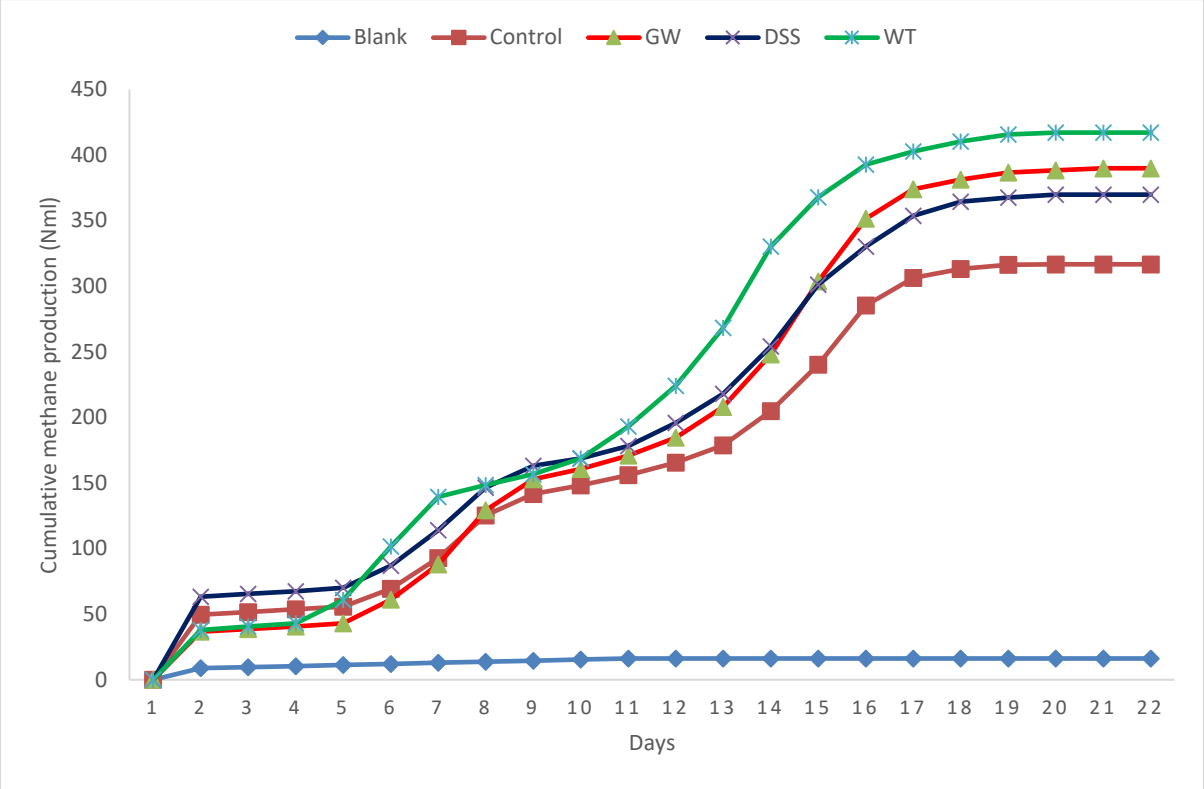


Figure 4.16: Average cumulative methane production in the third run.

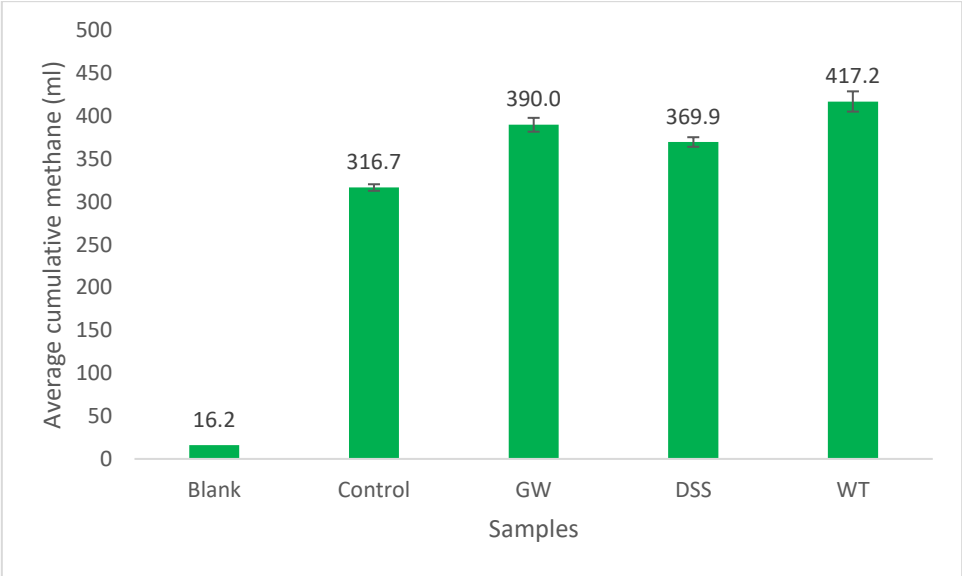


Figure 4.17: Average cumulative methane production with error bar in the third run.

The average amount of methane produced by the four sample after removing the methane production from blank during the third run is shown in the Figure 4.18 and Figure 4.19. From the figure, it suggests that all the biochar reactors enhances the methane production compared to the control reactor. WT biochar is the most effective in producing methane of 401 Nml g^{-}

$^{1}VS^{-1}$, followed by GW of 373.8 Nml $g^{-1}VS^{-1}$ and DSS biochar of 353.7 Nml $g^{-1}VS^{-1}$ for a period of 22 days.

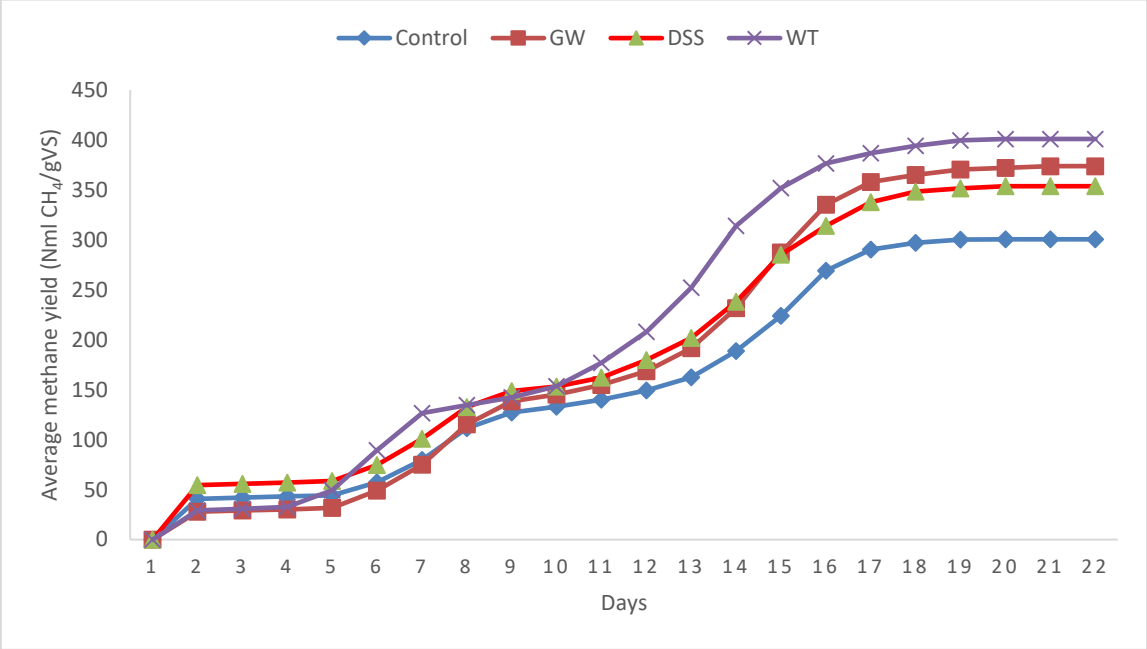


Figure 4.18: Average methane yield after removing blank in the third run.

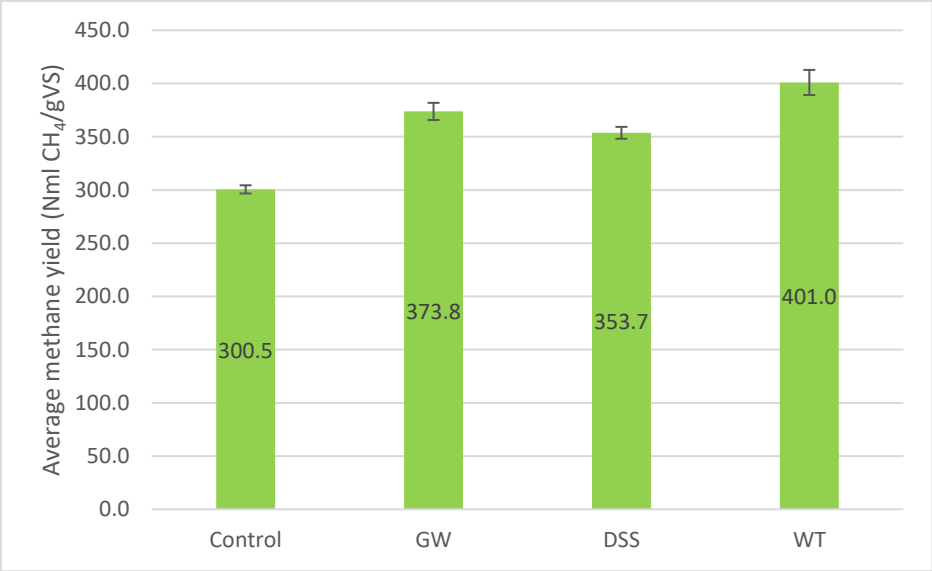


Figure 4.19: Average methane yield with error bar after removing the blank in the third run.

The four groups of control, GW, DSS and WT samples were tested for amounts of cumulative methane production (NmL). The dataset is normally distributed as there was not statistically significant ($p < 0.05$) variance variation between the four groups, as per the results obtained from Levene's test for homogeneity of variance. The mean methane production of the four samples was compared using ANOVA test to determine whether there were any significant differences. The ANOVA test findings revealed a statistically significant variation

in methane production across the samples. The p = 0.05 significance level with a 95% confidence level indicates that there is sufficient evidence to reject the null hypothesis, demonstrating that at least one of the sample has a different mean value of the methane production than the others. The results of the test is illustrated in Figure 4.20 with average values and standard deviation.

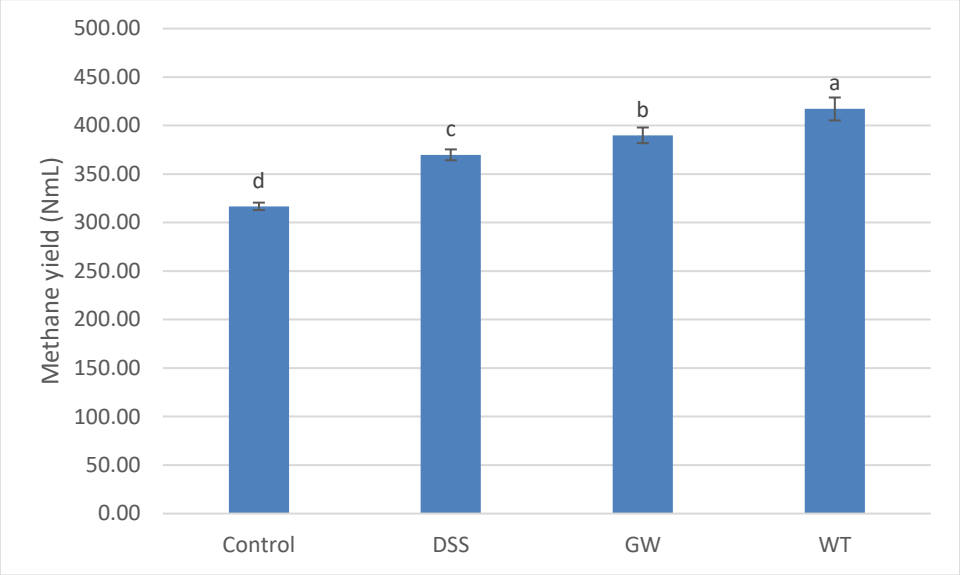


Figure 4.20: Cumulative methane yield from the different samples.

*Error bar indicates standard deviation and samples with different superscript letters differ significantly at $p < 0.05$.

4.3.3 pH Variation

The pH variation of the samples from the inoculum and the digestate of all the reactors from the third run is shown in Figure 4.21. The pH value of all the samples are within the range of 7.6 to 8, indicating relatively neutral to slightly alkaline environment. The control samples have pH values ranging from 7.8 to 8, indicating alkaline in nature compared to other samples. The majority of the samples from the blank and biochar have pH values within the range of 7.6 to 7.8.

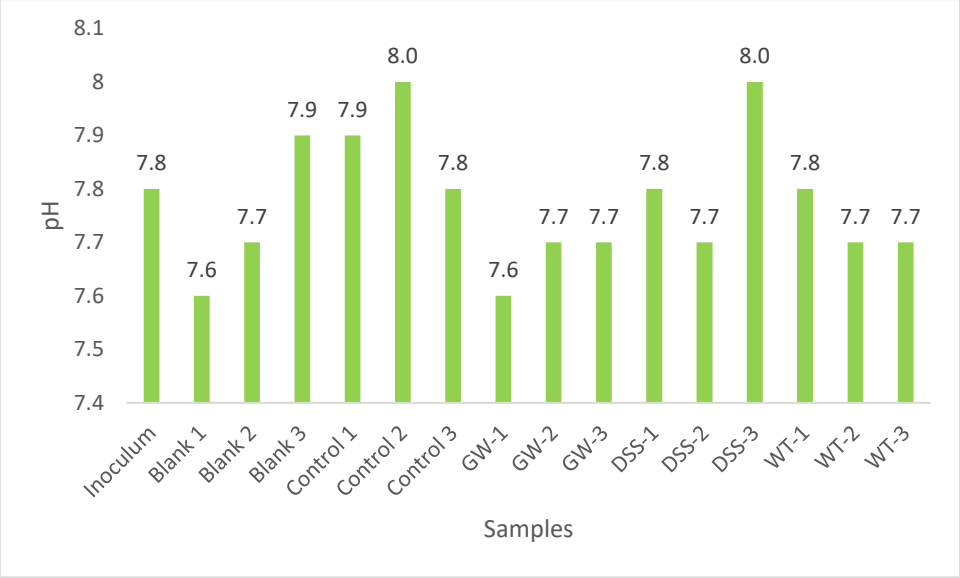


Figure 4.21: pH variation of the inoculum and digestate in the third run.

4.3.4 COD Removal

The COD concentration of the sample from the digestate and percentage of TCOD and SCOD removal is shown in the Figure 4.22. The control sample has the highest TCOD concentration of 5 g/L, while the GW biochar sample has the lowest TCOD concentration of 4.1 g/L. The blank sample has the highest SCOD concentration of 3.8 g/L, while the DSS biochar sample has the lowest SCOD concentration of 2.9 g/L.

The GW biochar sample has the highest TCOD removal percentage of 33%, followed by WT biochar sample of TCOD removal of 29% and DSS biochar sample of TCOD removal of 27%. The DSS biochar sample has the highest SCOD removal percentage of 34%, followed by WT biochar sample of SCOD removal of 28% and GW biochar sample of SCOD removal of 22%. Control sample has the lowest TCOD removal of 17%, while blank sample has the lowest SCOD removal of 13%.

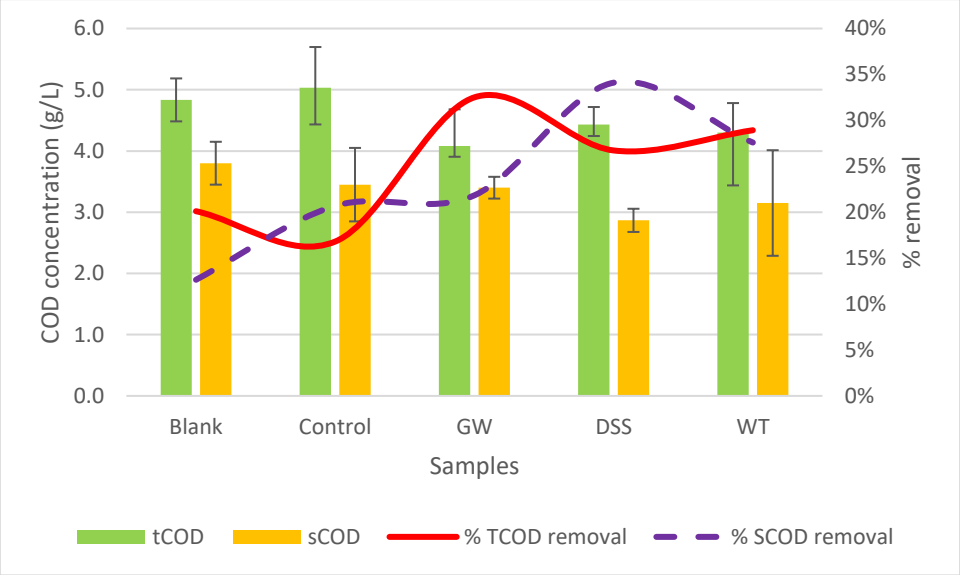


Figure 4.22: COD concentration and removal efficiency with error bars in the third run.

4.3.5 Ammonium Concentration

The ammonium concentration of the sample from the digestate is shown in the Figure 4.23. GW biochar sample has the highest ammonium concentration of 2.14 g/L, while DSS biochar has the lowest ammonium concentration of 0.99 g/L. WT biochar sample has the ammonium concentration of 1.87 g/L, while control has the ammonium concentration of 1.89 g/L.

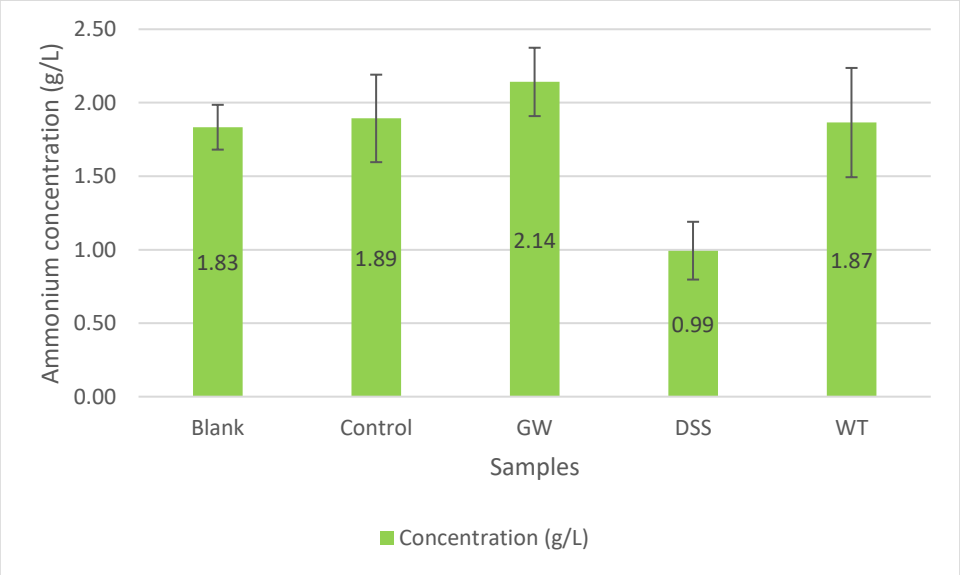


Figure 4.23: Ammonium concentration of the digestate with error bars in the third run.

4.3.6 Volatile Fatty Acids (VFA)

The concentration of different VFA of the digestate sample is shown in the Figure 4.24. Control sample exhibit a higher concentration of VFA of 17 mg/L, followed by the blank sample

exhibiting 14 mg/L. The biochar samples has lowest concentration of VFA as compared to the control sample. WT biochar has the lowest concentration of VFA of 2 mg/L, followed by the GW biochar of 4 mg/L and DSS biochar of 8 mg/L.

Among the different VFA acids, isovaleric acid concentration is higher in control, blank and DSS biochar sample. Isocaporic and propionic acid concentration is higher in blank and control sample, while acetic acid concentration is similar in all the samples.

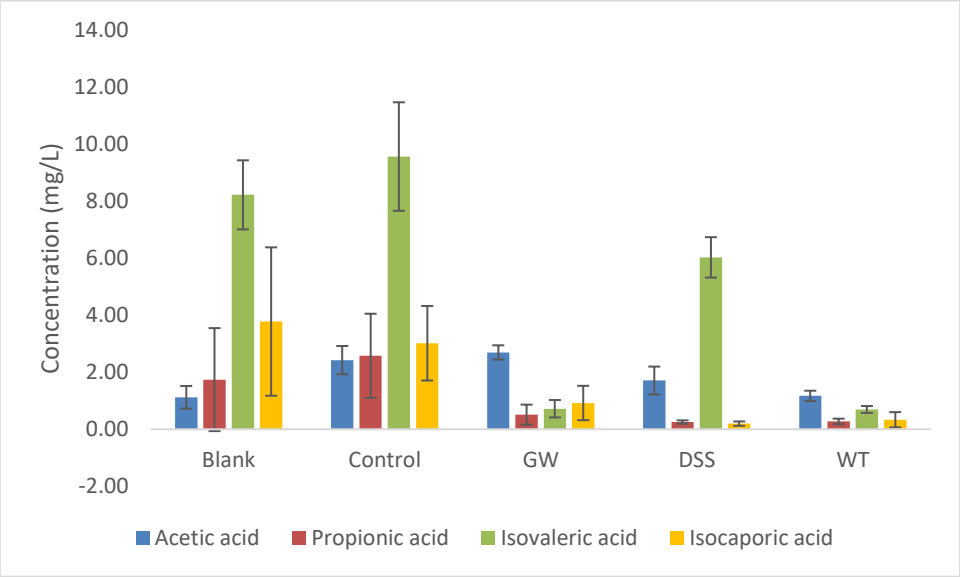


Figure 4.24: VFA concentration of the digestate with error bars in the third run.

5 Discussion

The results obtained from chapter 4 are discussed in this section under different sub-section; Analyzing the failures of the second run, biochar characteristics, effect of biochar on methane production.

5.1 Analyzing the Failures of the Second Run

In the second run only the WT biochar sample produced the methane which was in exponential phase, other samples did not produce the enough methane as shown in the Figure 4.7. After the 12th day of the experiment, there was no production of methane in all the reactors. Despite this, the experiment continued until 22nd day; since there was no methane production from the 12th day onward, the experiment was stopped.

There might be multiple factors that inhibit the production of methane in the second run. The contributing factors that inhibit the methane production are discussed in this section.

5.1.1 Accumulation of VFA

The high concentration of VFA shows an inhibitory effect to methanogens. When methanogens are unable to use hydrogen and VFAs as produced by acidogens and acetogens, there is high accumulation of VFA that led to process deterioration (Yuan & Zhu, 2016). In the second run there is high accumulation of VFA as shown in Figure 4.14. This high concentration of VFA might have potentially shown the inhibitory effects on the methanogic microbes. Specifically, when methanogens face the high level of hydrogen and VFA, they might struggle to efficiently degrade these organic matters. Such an imbalance in the anaerobic digestion process might have leads to the process deterioration in this experiment.

The next contributing factor for the accumulation of VFA might be the use of propionic acid as a substrate. This substrate might have introduced significant challenges that have triggered the inhibitory effects in the anaerobic digestion system. The data on accumulation of VFA as shown in the Figure 4.14, highlights the higher accumulation of VFA from propionic acid which might have inhibit the methanogenic activity leading to ending of degradation process. Propionic acid when used as a substrate, show the severe inhibition that leads to system failure. The high VFA accumulation of propionic acid inhibit the methanogenic microbes leading to ending of degradation process (Wong et al., 2008).

The higher dose of propionate impact the degradation rate of VFA leading to accumulation of VFA. After the slow degradation, VFA accumulation exceeded the acidogenic bacteria tolerance, which further suppressed the activity making the entire culture unfit for microbial growth leading to ending of methane production (Y. Wang et al., 2009). The concentration of propionic acid used in the second run was 3 g/L. The higher dose of propionic acid might have substantial impact for the degradation of VFA within the system. Specifically, the higher propionate levels might slowdown the degradation of VFA, leading to the accumulation of VFA over time. As the accumulation of VFA in the system increases, it likely exceed the tolerance level of acidogenic bacteria. This additional accumulation of VFA might have further supress the microbial activity leading to the ending of production of methane.

5.1.2 Inhibition from Ammonia

The initial ammonium concentration in the inoculum was 1375 mg/L, and to induce ammonium stress at a concentration of 3000 mg/L, the inoculum received additional 1625 mg/L dose of ammonium chloride. This additional stress of ammonium chloride might have inhibited the performance of the anaerobic digestion.

Many studies have suggested that FA is more prone to inhibition, which results in decline in methanogenic activity and AD performance. This reduced methanogenic activity results in the accumulation of VFA (Rajagopal et al., 2013). The use of salt for nutrient supplement and additional ammonium chloride dose might have some inhibition in the bacterial activity which affect the production of methane and accumulation of VFA (McCarty; & McKinney, 1961).

When FA inhibition occurs, it significantly decreased the methanogenic activity. The decreased microbial activity might have decreased the capacity of methanogens to breakdown the organic substrates, leading to an increased accumulation of VFA in the system. This accumulation of VFA signifies the disturbance of the anaerobic digestion process, leading to the decrease in production of methane.

5.2 Biochar Characteristics

5.2.1 Elemental Properties of Biochar

The high carbon content in the Figure 4.1, indicates the high loss of oxygen and hydrogen during pyrolysis. Low oxygen content or absence of oxygen in the biochar shows that the

oxygenated functional group might have been removed making the biochar more alkaline (Kim et al., 2011).

The ash content in the biochar indicates the presence of alkali and alkali earth metal. These presence of metals in the biochar are responsible for the alkalinity of biochar. This alkalinity of biochar might contribute to the buffering capacity of anaerobic digestion against the inhibition from VFA (Jang et al., 2018; J. Zhang et al., 2018).

The ratio of hydrogen to carbon elements in the biochar represents the degree of carbonization and aromatization. The ratio of oxygen to carbon indicates the oxygenated functional group. The lower the H/C ratio, the higher the degree of carbonization and aromatization (Sun et al., 2022). The Table 5.1 represents the H/C and O/C ratio of the biochars used in this experiment.

Table 5.1: H/C and O/C ratio of the biochars used in the experiment.

Biochar	**H/C**	**O/C**
Garden waste	0.02	-
Digested sewage sludge	0.12	0.72
Waste timber	0.04	-

Biochar when introduced in an anaerobic digester, it serves as a carbonaceous material which might have increased microbial growth and stability. It leads to a degradation of organic material and enhance the production of methane (Amalina et al., 2022). The biochars used in this experiment has low H/C ratio indicating the higher degree of carbonization and aromatization which might have resulted the higher methane production in the third run.

5.2.2 Electrical Conductivity of Biochar

The electrical conductivity of the biochar is related to the aromatic structures of the group. Due to the high aromaticity of the biochar, the AD with biochar shows higher electrical conductivity than without biochar. When butyrate is oxidized to acetate by butyrate-oxidizing bacteria, the DIET process would occur, where biochar works as electron acceptor on the metabolic activity of inactive methanogens (X. Li et al., 2013; G. Wang et al., 2018).

As shown in Figure 4.2, WT biochar exhibit higher electrical conductivity. This higher electrical conductivity might have facilitated the electron transfer process. Additionally, this biochar might have provided a conducive environment for the microbes working as an electron acceptor. This activity might have increased the metabolic activity of the methanogens, showing the highest methane production in this experiment with the WT biochar.

Biochar with the comparatively low electrical conductivity and a variety of redox-active organic functional group demonstrate a better electron accepting/donating capability (J. Wang et al., 2021). The GW and DSS biochar has significantly low electrical conductivity compared to the WT biochar. Despite of the lower electrical conductivity, these biochars might have unique redox-active organic functional group demonstrating the electron accepting/donating capability. This specific characteristics of these biochars might have enhanced the production of methane than the control sample.

5.2.3 Specific Surface Area of Biochar

The specific surface area of the biochar refers to the total surface area per unit mass, providing more space for microbial attachment and interaction. The higher surface area of biochar provides a conducive environment that can promote the immobilization of microbes. This immobilization of microbes is not only a physical attachment, it represents the syntrophic metabolic activities between the microbes where biochar act as a substrate allowing microbes to function optimally (Sun et al., 2022).

As shown in Figure 4.3, the WT biochar exhibits the larger surface area, the larger surface area of biochar might have acted like a catalyst for enhancing the microbial activity. The SSA of WT biochar might have offered more spaces and opportunities for the colonization of microbes. Consequently, the syntrophic effects of acetogens and methanogens for the colonization of WT biochar might have accelerated the production of methane.

Furthermore, the strong adsorption and immobilization ability of biochar due to its higher SSA represents the better interactions between biochar samples and microbes, leading to increase in production of methane, which is the evident for the higher production of methane in the biochar samples in this experiment.

5.3 Effect of Biochar on Methane Production

5.3.1 Cumulative Methane Yield

The biochar samples showed an increase in cumulative methane production than the control as shown in Figure 4.15. WT biochar exhibit 24% more methane production than the control, followed by GW biochar of 18% more methane production and DSS biochar of 13% more methane production than control as shown in Figure 5.1. Biochar has influenced the methane

production, where it would have acted as a conductive material to activate methanogenesis by promoting DIET among methanogens (Chen et al., 2014). It has been determined that the DIET is a more rapid and focused substitute for interspecies transfer between methanogens and bacteria. Consequently, it would have been expected that the increased DIET would increase microbial activity, speeding up the efficiency of methane generation in the biochar-containing samples. Because of the large surface area and porous structure of biochar, microbial colonisation and biofilm growth are likely the other possible explanation for the efficient methane generation with biochar (Sunyoto et al., 2016).

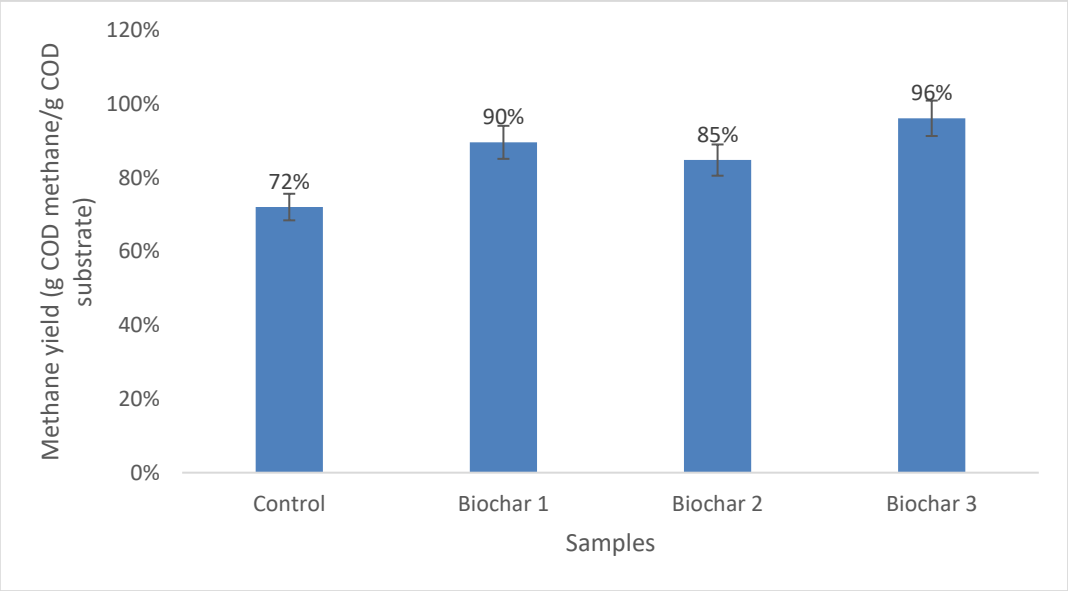


Figure 5.1: Methane yield in the third run and their standard deviation in bars.

The fine pore structure and electrical conductivity of biochar might have impact on the degradation of organic material which increase the cell concentration. Biochar might disrupt the cell wall of insoluble matter, increasing the availability of macromolecular organics which in turn enrich the hydrolytic bacteria promoting hydrolysis (Duan et al., 2019; Luo et al., 2015).

Biochar capacity to increase the DIET process might have encourage the selective colonisation of methanogens for improved methanogenesis efficiency. The time needed for new syntropic bacteria to adapt new consumption of substrate is relatively long in the absence of conductive material (Pan et al., 2019). During DIET process, the abundant transfers among microorganisms might be effectively substituted with biochar, which shortens the lag period during AD, which results in the increased methane production.

The adsorption capacity of biochar might influenced the methane production. Biochar has the capability to adsorb the VFA and other inhibitory compounds, resulting in enhanced methane production with the reduced lag phase than the control samples (Shen et al., 2016). By adsorbing the inhibitory substances, biochar promotes the growth of the methanogens ultimately enhancing the methane production. This adsorption capacity of biochar captures the potential inhibitors from the substrate, leading to an increased methane yield.

The characteristics of the biochar like specific surface area is considered to be a key factor that might have increased the methane production. The high specific surface area and porous structure of the biochar can promote the immobilization of microbes (Jaafar et al., 2015). The porosity of biochar provides appropriate habitat for microbial growth which ultimately promotes the growth of methanogens enhancing the production of methane. Furthermore microbial biofilm might have been formed on the surface of the biochar, which might have degraded the organic matter resulting in the enhanced methane production.

5.3.2 Degradation of VFA

Previous study have suggested that biochar can promote the production of VFA. Accelerating the breakdown of VFAs is equally important for maintaining the balance between acidogenesis-acetogenesis and methanogenesis. In general, the use of biochar resulted in both increased VFA generation and faster VFA breakdown (Watanabe et al., 2013). With the addition of biochar the propionate degradation might have been stimulated which can be observed in the VFA results of third run in Figure 4.24. Furthermore the alkalinity of biochar might have acted as a buffering capacity in the reactors against inhibition of VFA, resulting in lower VFA concentration in the biochar samples than the control sample. The degradation of VFA resulted in more methane production in biochar samples.

6 Conclusion

In conclusion, this thesis investigated the use of biochar obtained from different feedstock to enhance the production of methane in anaerobic digestion. There was no significant amount of methane production during first and second run. The high accumulation of VFA and use of propionic acid showed the inhibitory effect for the production of methane. Further the additional dose of ammonium chloride disrupt the activity of microbes, suppressing the production of methane with increased accumulation of VFA that hindered the process efficiency of anaerobic digestion.

The first research question was the biochar impact in the methane yield during AD. In the third run, biochar showed positive impacts for the enhancement of methane in an anaerobic digestion. All the biochar sample showed higher methane yield as compared to the control. Specifically, cumulative methane production of the WT biochar showed highest methane yield of 24% more than the control, answering the initial research question.

The second research question was the correlation of biochar characteristics with the production of methane, the unique characteristics of biochar like high carbon content, electrical conductivity and specific surface area has a very strong correlation with methane production. The role of biochar for degradation of VFA, and providing a conducive environment for methanogens are evident from the results. Furthermore, the adsorption capacity of biochar for adsorbing potential inhibitors and its ability to act as a buffering agent due to the alkaline nature showed benefits of biochar for enhancing the production of methane. The above findings collectively answers the second research question.

Finally, biochar can be a valuable additive in anaerobic digestion for enhancing the production of methane. Biochar potential to mitigate the challenges faced during AD emphasizes its potential role in the sustainable waste management and renewable energy solutions. Further research is necessary to harness the full potential of biochar for improving the challenges faced during AD.

7 Recommendation

- This study is limited to the measurement of methane production, however the study of microbial community analysis by the addition of the biochar is necessary to further investigate the microbial activity inside the reactor.
- This batch experiment is limited to the lab scale. For scaling up biochar amended AD, the study should be more focused on the large scale production of biochar and post treatment of the used biochar.

References

Amalina, F., Razak, A. S. A., Krishnan, S., Sulaiman, H., Zularisam, A. W., & Nasrullah, M. (2022). Biochar production techniques utilizing biomass waste-derived materials and environmental applications – A review. *Journal of Hazardous Materials Advances*, *7*(July), 100134. https://doi.org/10.1016/j.hazadv.2022.100134

Angelidaki, I., Ellegaard, L., Birgitte, ·, & Ahring, K. (n.d.). *Applications of the Anaerobic Digestion Process*.

Aryal, N., Feng, L., Wang, S., & Chen, X. (2023). Surface-modified activated carbon for anaerobic digestion to optimize the microbe-material interaction. *Science of the Total Environment*, *886*(March), 163985. https://doi.org/10.1016/j.scitotenv.2023.163985

Aryal, N., Kvist, T., Ammam, F., Pant, D., & Ottosen, L. D. M. (2018). An overview of microbial biogas enrichment. In *Bioresource Technology* (Vol. 264, pp. 359–369). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2018.06.013

Baek, G., Kim, J., Kim, J., & Lee, C. (2018). Role and potential of direct interspecies electron transfer in anaerobic digestion. In *Energies* (Vol. 11, Issue 1). MDPI AG. https://doi.org/10.3390/en11010107

Bajpai, P. (2017). Basics of anaerobic digestion process. In *SpringerBriefs in Applied Sciences and Technology* (Issue 9789811041297, pp. 7–12). Springer Verlag. https://doi.org/10.1007/978-981-10-4130-3_2

Bharathiraja, B., Sudharsana, T., Jayamuthunagai, J., Praveenkumar, R., Chozhavendhan, S., & Iyyappan, J. (2018). Biogas production – A review on composition, fuel properties, feed stock and principles of anaerobic digestion. In *Renewable and Sustainable Energy Reviews* (Vol. 90, pp. 570–582). Elsevier Ltd. https://doi.org/10.1016/j.rser.2018.03.093

Chen, S., Rotaru, A. E., Shrestha, P. M., Malvankar, N. S., Liu, F., Fan, W., Nevin, K. P., & Lovley, D. R. (2014). Promoting interspecies electron transfer with biochar. *Scientific Reports*, *4*. https://doi.org/10.1038/srep05019

Cheng, Q., Xu, C., Huang, W., Jiang, M., Yan, J., Fan, G., Zhang, J., Chen, K., Xiao, B., & Song, G. (2020). Improving anaerobic digestion of piggery wastewater by alleviating stress of ammonia using biochar derived from rice straw. *Environmental Technology and Innovation*, *19*, 100948. https://doi.org/10.1016/j.eti.2020.100948

Cruz Viggi, C., Simonetti, S., Palma, E., Pagliaccia, P., Braguglia, C., Fazi, S., Baronti, S., Navarra, M. A., Pettiti, I., Koch, C., Harnisch, F., & Aulenta, F. (2017). Enhancing methane production from food waste fermentate using biochar: The added value of electrochemical testing in pre-selecting the most effective type of biochar. *Biotechnology for Biofuels*, *10*(1). https://doi.org/10.1186/s13068-017-0994-7

Duan, X., Chen, Y., Yan, Y., Feng, L., Chen, Y., & Zhou, Q. (2019). New method for algae comprehensive utilization: Algae-derived biochar enhances algae anaerobic fermentation for short-chain fatty acids production. *Bioresource Technology*, *289*(April), 121637. https://doi.org/10.1016/j.biortech.2019.121637

Dudek, M., Świechowski, K., Manczarski, P., Koziel, J. A., & Białowiec, A. (2019). The effect of biochar addition on the biogas production kinetics from the anaerobic digestion of brewers' spent grain. *Energies*, *12*(8), 1–22. https://doi.org/10.3390/en12081518

References

Erlend Sørmo, Gabrielle Dublet-Adli, Gladys Menlah, Gudny Øyre Flatabø, Valentina Zivanovic, Per Carlsson, Åsgeir Almås, G. C. (2023). Fate of Heavy Metals in Industrially Relevant Pyrolysis of Diverse Contaminated Organic Wastes: Phase Partitioning and Ph-Dependent Leaching [Preprint]. *Social Science Research Network*, 1–33. https://ssrn.com/abstract=4581337

Flatabø, G. Ø., Cornelissen, G., Carlsson, P., Nilsen, P. J., Tapasvi, D., Bergland, W. H., & Sørmo, E. (2023). Industrially relevant pyrolysis of diverse contaminated organic wastes: Gas compositions and emissions to air. *Journal of Cleaner Production*, *423*(June). https://doi.org/10.1016/j.jclepro.2023.138777

Gahlot, P., Ahmed, B., Tiwari, S. B., Aryal, N., Khursheed, A., Kazmi, A. A., & Tyagi, V. K. (2020). Conductive material engineered direct interspecies electron transfer (DIET) in anaerobic digestion: Mechanism and application. In *Environmental Technology and Innovation* (Vol. 20). Elsevier B.V. https://doi.org/10.1016/j.eti.2020.101056

Giwa, A. S., Xu, H., Chang, F., Wu, J., Li, Y., Ali, N., Ding, S., & Wang, K. (2019). Effect of biochar on reactor performance and methane generation during the anaerobic digestion of food waste treatment at long-run operations. *Journal of Environmental Chemical Engineering*, *7*(4). https://doi.org/10.1016/j.jece.2019.103067

Jaafar, N. M., Clode, P. L., & Abbott, L. K. (2015). Soil Microbial Responses to Biochars Varying in Particle Size, Surface and Pore Properties. *Pedosphere*, *25*(5), 770–780. https://doi.org/10.1016/S1002-0160(15)30058-8

Jang, H. M., Choi, Y. K., & Kan, E. (2018). Effects of dairy manure-derived biochar on psychrophilic, mesophilic and thermophilic anaerobic digestions of dairy manure. *Bioresource Technology*, *250*(October 2017), 927–931. https://doi.org/10.1016/j.biortech.2017.11.074

Kapoor, R., Ghosh, P., Kumar, M., & Vijay, V. K. (2019). Evaluation of biogas upgrading technologies and future perspectives: a review. In *Environmental Science and Pollution Research*. Springer Verlag. https://doi.org/10.1007/s11356-019-04767-1

Kassegn, E., Desplentere, F., Berhanu, T., & Buffel, B. (2021). Study on Effects of Process Parameters on Mechanical Behaviors of Injection Molded Glass Fiber Reinforced Polypropylene Matrix Composite. In *Lecture Notes of the Institute for Computer Sciences, Social-Informatics and Telecommunications Engineering, LNICST* (Vol. 385). https://doi.org/10.1007/978-3-030-80618-7_32

Kaur, G., Johnravindar, D., & Wong, J. W. C. (2020). Enhanced volatile fatty acid degradation and methane production efficiency by biochar addition in food waste-sludge co-digestion: A step towards increased organic loading efficiency in co-digestion. *Bioresource Technology*, *308*(January), 123250. https://doi.org/10.1016/j.biortech.2020.123250

Kim, P., Johnson, A., Edmunds, C. W., Radosevich, M., Vogt, F., Rials, T. G., & Labbé, N. (2011). Surface functionality and carbon structures in lignocellulosic-derived biochars produced by fast pyrolysis. *Energy and Fuels*, *25*(10), 4693–4703. https://doi.org/10.1021/ef200915s

Li, X., Shen, Q., Zhang, D., Mei, X., Ran, W., Xu, Y., & Yu, G. (2013). Functional Groups Determine Biochar Properties (pH and EC) as Studied by Two-Dimensional 13C NMR Correlation Spectroscopy. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0065949

References

Li, Y., Chen, Y., & Wu, J. (2019). Enhancement of methane production in anaerobic digestion process: A review. In *Applied Energy* (Vol. 240, pp. 120–137). Elsevier Ltd. https://doi.org/10.1016/j.apenergy.2019.01.243

Lindum | The country's largest processor of food waste and sewage sludge. (n.d.). Retrieved October 2, 2023, from https://lindum.no/tjenester/matavfall-avløpsslam-og-biologiske-ressurser#Ressursertilbiogassproduksjon

Lü, C., Shen, Y., Li, C., Zhu, N., & Yuan, H. (2020). Redox-Active Biochar and Conductive Graphite Stimulate Methanogenic Metabolism in Anaerobic Digestion of Waste-Activated Sludge: Beyond Direct Interspecies Electron Transfer. *ACS Sustainable Chemistry and Engineering*, *8*(33), 12626–12636. https://doi.org/10.1021/acssuschemeng.0c04109

Lü, F., Luo, C., Shao, L., & He, P. (2016). Biochar alleviates combined stress of ammonium and acids by firstly enriching Methanosaeta and then Methanosarcina. *Water Research*, *90*, 34–43. https://doi.org/10.1016/j.watres.2015.12.029

Luo, C., Lü, F., Shao, L., & He, P. (2015). Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Research*, *68*, 710–718. https://doi.org/10.1016/j.watres.2014.10.052

Maile, I., Muzenda, E., & Mbohwa, C. (2016). Optimization of Biogas Production through Anaerobic Digestion of Fruit and Vegetable Waste: A Review. *2016 7th International Conference on Biology, Environment and Chemistry*, *98*, 9.

Manni, G., & Caron, F. (1995). Calibration and determination of volatile fatty acids in waste leachates by gas chromatography. In *Journal of Chromatography A* (Vol. 690).

Mao, C., Feng, Y., Wang, X., & Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. In *Renewable and Sustainable Energy Reviews* (Vol. 45, pp. 540–555). Elsevier Ltd. https://doi.org/10.1016/j.rser.2015.02.032

McCarty;, P. L., & McKinney, R. E. (1961). Salt toxicity in anaerobic digestion. *Journal of the Water Pollution Control Federation*, *33*(4), 399–415.

Merck. (2017). Analytical Procedures Appendices. *Supelco*. https://www.merckmillipore.com/Web-MY-Site/en_US/-/USD/ShowDocument-File?ProductSKU=MDA_CHEM-173018&DocumentId=201511.057.ProNet&DocumentUID=23600378&DocumentType=PI&Language=EN&Country=NF&Origin=PDP

Mulu, E., M'Arimi, M. M., & Ramkat, R. C. (2021). A review of recent developments in application of low cost natural materials in purification and upgrade of biogas. In *Renewable and Sustainable Energy Reviews* (Vol. 145). Elsevier Ltd. https://doi.org/10.1016/j.rser.2021.111081

Náthia-Neves, G., Berni, M., Dragone, G., Mussatto, S. I., & Forster-Carneiro, T. (2018). Anaerobic digestion process: technological aspects and recent developments. In *International Journal of Environmental Science and Technology* (Vol. 15, Issue 9, pp. 2033–2046). Center for Environmental and Energy Research and Studies. https://doi.org/10.1007/s13762-018-1682-2

Obileke, K. C., Nwokolo, N., Makaka, G., Mukumba, P., & Onyeaka, H. (2021). Anaerobic digestion: Technology for biogas production as a source of renewable energy—A review.

In *Energy and Environment* (Vol. 32, Issue 2, pp. 191–225). SAGE Publications Inc. https://doi.org/10.1177/0958305X20923117

Pan, J., Ma, J., Zhai, L., Luo, T., Mei, Z., & Liu, H. (2019). Achievements of biochar application for enhanced anaerobic digestion: A review. *Bioresource Technology*, *292*(August), 122058. https://doi.org/10.1016/j.biortech.2019.122058

Qazi, A., Hussain, F., Rahim, N. A. B. D., Hardaker, G., Alghazzawi, D., Shaban, K., & Haruna, K. (2019). Towards Sustainable Energy: A Systematic Review of Renewable Energy Sources, Technologies, and Public Opinions. *IEEE Access*, *7*, 63837–63851. https://doi.org/10.1109/ACCESS.2019.2906402

Qin, Y., Yin, X., Xu, X., Yan, X., Bi, F., & Wu, W. (2020). Specific surface area and electron donating capacity determine biochar's role in methane production during anaerobic digestion. *Bioresource Technology*, *303*(January), 122919. https://doi.org/10.1016/j.biortech.2020.122919

Qiu, L., Deng, Y. F., Wang, F., Davaritouchaee, M., & Yao, Y. Q. (2019). A review on biochar-mediated anaerobic digestion with enhanced methane recovery. In *Renewable and Sustainable Energy Reviews* (Vol. 115). Elsevier Ltd. https://doi.org/10.1016/j.rser.2019.109373

Rajagopal, R., Massé, D. I., & Singh, G. (2013). A critical review on inhibition of anaerobic digestion process by excess ammonia. In *Bioresource Technology* (Vol. 143, pp. 632–641). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2013.06.030

Sawyerr, N., Trois, C., Workneh, T., & Okudoh, V. (2019). An overview of biogas production: Fundamentals, applications and future research. *International Journal of Energy Economics and Policy*, *9*(2), 105–116. https://doi.org/10.32479/ijeep.7375

Scarlat, N., Dallemand, J. F., & Fahl, F. (2018). Biogas: Developments and perspectives in Europe. In *Renewable Energy* (Vol. 129, pp. 457–472). Elsevier Ltd. https://doi.org/10.1016/j.renene.2018.03.006

Schnürer, A. (2016). Biogas production: Microbiology and technology. In *Advances in Biochemical Engineering/Biotechnology* (Vol. 156, pp. 195–234). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/10_2016_5

Shen, Y., Linville, J. L., Ignacio-de Leon, P. A. A., Schoene, R. P., & Urgun-Demirtas, M. (2016). Towards a sustainable paradigm of waste-to-energy process: Enhanced anaerobic digestion of sludge with woody biochar. *Journal of Cleaner Production*, *135*, 1054–1064. https://doi.org/10.1016/j.jclepro.2016.06.144

Standard Methods for the Examination of Water and Wastewater. (1999).

Stefaniuk, M., & Oleszczuk, P. (2015). Characterization of biochars produced from residues from biogas production. *Journal of Analytical and Applied Pyrolysis*, *115*, 157–165. https://doi.org/10.1016/j.jaap.2015.07.011

Sun, Z., Feng, L., Li, Y., Han, Y., Zhou, H., & Pan, J. (2022). The role of electrochemical properties of biochar to promote methane production in anaerobic digestion. *Journal of Cleaner Production*, *362*(March), 132296. https://doi.org/10.1016/j.jclepro.2022.132296

Sunyoto, N. M. S., Zhu, M., Zhang, Z., & Zhang, D. (2016). Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresource Technology*, *219*, 29–36.

https://doi.org/10.1016/j.biortech.2016.07.089

Tang, S., Wang, Z., Liu, Z., Zhang, Y., & Si, B. (2020). The role of biochar to enhance anaerobic digestion: A review. In *Journal of Renewable Materials* (Vol. 8, Issue 9, pp. 1033–1052). Tech Science Press. https://doi.org/10.32604/jrm.2020.011887

Tripathi, M., Sahu, J. N., & Ganesan, P. (2016). Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review. *Renewable and Sustainable Energy Reviews*, *55*, 467–481. https://doi.org/10.1016/j.rser.2015.10.122

Wang, G., Li, Q., Gao, X., & Wang, X. C. (2018). Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: Performance and associated mechanisms. *Bioresource Technology*, *250*, 812–820. https://doi.org/10.1016/j.biortech.2017.12.004

Wang, J., & Wang, S. (2019). Preparation, modification and environmental application of biochar: A review. *Journal of Cleaner Production*, *227*, 1002–1022. https://doi.org/10.1016/j.jclepro.2019.04.282

Wang, J., Zhao, Z., & Zhang, Y. (2021). Enhancing anaerobic digestion of kitchen wastes with biochar: Link between different properties and critical mechanisms of promoting interspecies electron transfer. *Renewable Energy*, *167*, 791–799. https://doi.org/10.1016/j.renene.2020.11.153

Wang, Y., Zhang, Y., Wang, J., & Meng, L. (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy*, *33*(5), 848–853. https://doi.org/10.1016/j.biombioe.2009.01.007

Watanabe, R., Tada, C., Baba, Y., Fukuda, Y., & Nakai, Y. (2013). Enhancing methane production during the anaerobic digestion of crude glycerol using Japanese cedar charcoal. *Bioresource Technology*, *150*, 387–392. https://doi.org/10.1016/j.biortech.2013.10.030

Weiland, P. (2010). Biogas production: Current state and perspectives. In *Applied Microbiology and Biotechnology* (Vol. 85, Issue 4, pp. 849–860). Springer Verlag. https://doi.org/10.1007/s00253-009-2246-7

Werkneh, A. A. (2022). Biogas impurities: environmental and health implications, removal technologies and future perspectives. In *Heliyon* (Vol. 8, Issue 10). Elsevier Ltd. https://doi.org/10.1016/j.heliyon.2022.e10929

Wong, B. T., Show, K. Y., Su, A., Wong, R. J., & Lee, D. J. (2008). Effect of volatile fatty acid composition on upflow anaerobic sludge blanket (UASB) performance. *Energy and Fuels*, *22*(1), 108–112. https://doi.org/10.1021/ef700282r

Xiao, R., Chen, X., Wang, F., & Yu, G. (2010). Pyrolysis pretreatment of biomass for entrained-flow gasification. *Applied Energy*, *87*(1), 149–155. https://doi.org/10.1016/j.apenergy.2009.06.025

Yu, Q., Sun, C., Liu, R., Yellezuome, D., Zhu, X., Bai, R., Liu, M., & Sun, M. (2021). Anaerobic co-digestion of corn stover and chicken manure using continuous stirred tank reactor: The effect of biochar addition and urea pretreatment. *Bioresource Technology*, *319*(August 2020), 124197. https://doi.org/10.1016/j.biortech.2020.124197

Yuan, H., & Zhu, N. (2016). Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. *Renewable and*

Sustainable Energy Reviews, *58*, 429–438. https://doi.org/10.1016/j.rser.2015.12.261

Zhang, C., Su, H., Baeyens, J., & Tan, T. (2014). Reviewing the anaerobic digestion of food waste for biogas production. In *Renewable and Sustainable Energy Reviews* (Vol. 38, pp. 383–392). Elsevier Ltd. https://doi.org/10.1016/j.rser.2014.05.038

Zhang, J., Zhao, W., Zhang, H., Wang, Z., Fan, C., & Zang, L. (2018). Recent achievements in enhancing anaerobic digestion with carbon- based functional materials. *Bioresource Technology*, *266*(May), 555–567. https://doi.org/10.1016/j.biortech.2018.07.076

Zhang, M., Li, J., Wang, Y., & Yang, C. (2019). Impacts of different biochar types on the anaerobic digestion of sewage sludge. *RSC Advances*, *9*(72), 42375–42386. https://doi.org/10.1039/c9ra08700a

Appendices

Appendix A: Task Description

University of South-Eastern Norway

Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: The Role of Pyrolysis Product Biochar in Advancing Anaerobic Digestion Efficiency

USN supervisor: Thea Indrebø, Nabin Aryal

External partner: Lindum AS, Scanship AS

Task background:

Anaerobic digestion (AD) is an effective technology for treating organic wastes and producing biogas and energy. Despite the high ability of AD, the problems related to low methane yield, VFA accumulation, and process instability often occurs. The addition of biochar to enhance the production of methane in AD have been proposed.

Supplementing biochar has the benefit of promoting the growth and abundance of syntrophic acetic acid oxidizing bacteria (SAOB), further enhancing the DIET pathway. Biochar creates a favorable environment for microorganisms involved in DIET, supporting their functions and facilitating their proliferation.

Overall, biochar serves different capabilities in the AD process. It upholds rate-restricting hydrolysis, keeps a consistent pH, and encourages microbial growth. These approaches help to overcome the potential inhibition and the enhancement of the AD processes.

Task description:

This proposed Master thesis aims to explore the impact of biochar obtained from different substrate on the enhancement of methane production in the anaerobic digestion process. An automatic BMP test will be conducted to measure the production of methane during anaerobic digestion. Biochar obtained from a local company, Scanship AS, Norway (0.5-1mm sieved and dried at 105 degrees), will be employed as a conductive material. In addition, sludge from a mesophilic anaerobic digester from Lindum AS, and a synthetic carbon source acetic and propionic acid will be utilized.The study will assess the diverse effects of conductive materials on microbial methanogenesis, considering their composition. Additionally, key parameters in anaerobic digestion, including volatile fatty acids (VFAs) concentration, electrical conductivity, and redox potential of the medium, will be investigated to understand the influence of biochar addition.

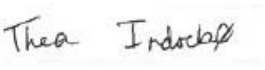
Student category: Exchange Student from KU, Nepal (Ashish Dutta Bhatta)

The task is suitable for students not present at the campus (e.g. online students): No

Practical arrangements:

The necessary accessories and instruments will be provided. The supervisor will provide the experiment set-up and operational training at USN, Porsgrunn Campus.

Signatures:

Supervisor (Date and Signature): Thea Indrebø 25.08.23 

Students (write clearly in all capitalized letters): ASHISH DUTTA BHATTA

Student (Date and Signature): 25.08.2023 

Appendix B: Correlation and ANNOVA Calculation

Correlation matrix between methane yield and biochar characteristics						
	Methane yield	*SSA*	*Carbon content*	*EC*	*pH*	*PV*
Methane yield	1					
Specific Surface area	0.965264009	1				
Carbon content	0.905371381	0.762979036	1			
EC (micro S/cm)	0.907710655	0.985811162	0.643645855	1		
pH	0.235529689	-0.026577685	0.625916664	-0.193999415	1	
PV	-0.902524221	-0.758658286	-0.999977836	-0.63853612	-0.63109524	1

Tests of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Values	Based on Mean	2.052	3	7	.195
	Based on Median	1.086	3	7	.415
	Based on Median and with adjusted df	1.086	3	4.817	.438
	Based on trimmed mean	1.979	3	7	.206

ANOVA					
Values					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14151.042	3	4717.014	91.277	<.001
Within Groups	361.745	7	51.678		
Total	14512.787	10			

Values							
			Subset for alpha = 0.05				
	Groups	N	1	2	3	4	
Duncan[a,b]	Control	3	316.7333				d
	DSS	3		369.8667			c
	GW	3			389.9667		b
	WT	2				417.1500	a
	Sig.		1.000	1.000	1.000	1.000	