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**Investigating the Product Spectrum of an Anaerobic
Biorefinery and Optimisation of Process Parameters**

MSc THESIS DISSERTATION

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Declaration

I **Primrose Paidamoyo Magama** declare that this research report is my own unaided work. It is being submitted to the degree of Master of Science in Engineering to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination in any other University.

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ABSTRACT

This study investigated the various products that can be generated from the anaerobic digestion of fruit and vegetable waste, and the downstream treatment of the FVW digestate using hydrothermal carbonisation. The aim of the study was to explore the concept of biorefineries, i.e. multiple product generation, for anaerobic digestion processes. Additionally, the study examined the potential of anaerobic biorefineries as alternative, environmentally friendly, waste management methods for treating organic waste such as fruit and vegetable waste. In Experiment 1 (Chapter 3), the study investigated the production of biohydrogen and volatile fatty acids using FVW as feedstock, via anaerobic digestion, in a process known as dark fermentation. To enhance biohydrogen production, the study applied seed inoculum pre-treatment including heat, alkali, acid, and heat-alkali. Acid pre-treatment resulted in the highest hydrogen yield (142, 74 Nml/g VS), and hydrogen content (54%). Heat pre-treatment, on the other hand, generated the lowest hydrogen yield (0, 90 Nml/g VS). The hydrogen yields of the pre-treatments tested were substantially different ($p < 0.001$). Valeric acid was the main VFA produced under all pre-treatments as well as the control. Butyric, propionic, and acetic acid were observed in small amounts only in the control, heat, and alkaline pre-treatments.

Experiment 2 (Chapter 4), focused on methane and volatile fatty acids production. Firstly, the study investigated how three factors, namely, pH, co-digestion and ultrasonification affect methane and VFA production. The effect of initial pH was investigated at pH (4, 6, 8, 10, 12), while for ultrasonic pre-treatment, specific energy from 2 500 up to 17 500 (kJ /kg TS) was applied. Fruit and vegetable waste to pig manure co-digestion ratios of (100/0; 0/100; 75/25; 50; 50; 25/75) were studied. Secondly, Response Surface Methodology, utilising Central Composite Design was applied to find the optimal conditions and interactive effects of the three studied process parameters. The maximum methane yield (497 Nml/g VS) was obtained at pH8 (control) and the minimum yield at pH 4 (11 Nml/g VS). Specific energy of 17 500kJ/kg TS resulted in the maximum methane yield (312 Nml/g VS). A FVW/pig manure (PM) co-digestion ratio of 75/25 gave the max methane yield (434 Nml/g VS). The maximum yield predicted via RSM model was 692.82 mL/g VS at the optimal conditions of pH 8 and the co-digestion ratio of 60 % fruit and vegetable waste: 40 % pig manure. The pH was found to be the only factor to significantly affect methane yield.

Experiment 3 (Chapter 5), investigated the production of hydro-char via hydrothermal carbonization of Fruit and Vegetable Waste (FVW) digestate. The Hydrothermal carbonization

(HTC) experiments were carried at temperatures of 180 °C and 200 °C for 2 hours in a 50ml stainless steel batch reactor, which was externally heated. The proximate composition, elemental composition, heating values, and thermal stability of the HTC solids were characterised. The study results show that there was an improvement in the Higher Heating Value (HHV) of the hydro-chars compared to the digestate. The highest energy yield (85, 26 %) and HHT (10, 75 MJ/kg) was obtained from the digestate treated at 200 °C for 2 hours.

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1. INTRODUCTION

1.1 Background

The South African fruit and the vegetable industry produce millions of tonnes of products annually. With a total export value of close to R22 billion, the fruit industry is the main contributor, by value, to the country's agricultural exports (Uys, 2016). However, the fruit and vegetable supply chain (production, processing, retail, and consumption) generates large amounts of solid and liquid waste streams, the disposal of which is increasingly becoming problematic (GFS, 2017; Gustavsson, *et al.*, 2011). While there is consensus among researchers that the amount of fruit and vegetable waste generated is huge (Gustavsson, *et al.*, 2011; Khan, *et al.*, 2015; GFS, 2017), data quantifying the waste generated along the Fruit and Vegetable supply chain is scarce. Van Dyk, *et al.*, (2013), reports that up to 20 to 50 % of the processed fruits or vegetables end up as solid waste. Given over 400 000 and 200 000 tons of fruit and vegetables are processed annually respectively (Palm, 2015), this translates to between 120 000 and 300 000 tons of solid waste generated per year which needs to be disposed of.

Open -dumping, landfilling, incineration, and composting are the most common solid waste disposal methods used for fruit and vegetable waste (FVW). These methods are, however, problematic due to environmental pollution, greenhouse gas emissions, and space limitations (Kumar & Samadder, 2017). Additionally, there is the opportunity cost of producing value-added products (valorisation) from the waste. Current treatment methods do not fully exploit the value of waste before disposal (Khan, *et al.*, 2015). The aforementioned limitations of conventional fruit and vegetable waste treatment methods have prompted research into alternative waste treatment methods such as Waste to Energy (WtE) technologies which are viewed as sustainable and environmentally friendly methods (Kumar & Samadder, 2017). WtE refers to the recovery of energy embedded in waste materials in the form of heat, electricity or fuel. WtE technologies include incineration, gasification, pyrolysis, sanitary landfill, and anaerobic digestion (Kumar & Samadder, 2017). Anaerobic digestion (AD) has emerged as a preferred WtE technology for the treatment of organic waste as evidenced by the number of biogas plants installed compared to other WtE plants. As of 2015, Europe had over 17 000 biogas plants installed (EBA, 2015) whereas there were very few pyrolyses or gasification plants amounting to less than 500 (Kumar & Samadder, 2017).

1.2 Problem Statement

Although anaerobic digestion is a promising WtE technology that is capable of treating a diverse range of organic, waste substrates while generating renewable biogas energy and organic fertilizer (Sawatdeenarunat, *et al.*, 2016), its economic viability and competitiveness are debatable. Surendra, *et al.*, (2014) and Coma, *et al.*, (2017) state that the production of biogas alone might not be economically competitive because of market volatility and the price indexes of fossil fuels. Additionally, Kaparaju, *et al.*, (2009), Surendra, *et al.*, (2014), Martinez-Gutierrez, (2018) , and many other researchers have pointed out that for complex feedstock like lignocellulosic materials the substrate conversion efficiency to biogas is low. In summary, the conventional AD single low-value product approach has numerous drawbacks. Chiefly, the poor economic performance of biogas plants, as the biogas energy generated is only marginally cheaper or even more expensive compared to fossil energy (Pfau, *et al.*, 2017)).

Additionally, the conventional use of digestate as an organic fertilizer or soil amendment has challenges (Garlapalli, *et al.*, 2016). Such challenges include an oversupply of the digestate (supply exceeding the land area available), over-application leading to leaching, potential GHG emissions, and the volatilisation of ammonia during storage and on the field and high transport costs (Monalu, *et al.*, 2015; Garlapalli, *et al.*, 2016). In South Africa in particular, there is no real monetary value placed on the digestate and the market for digestate is not fully established. In some cases, biogas plant owners have to give the digestate away for free (Personal Communication). This ensures that biogas plant owners are unable to benefit from the digestate, further jeopardizing the biogas plants' financial viability.

One way of overcoming the challenges of the economic viability of AD plants, low substrate utilisation and digestate application is to investigate the concurrent production of alternative products/chemicals with biogas through adopting a biorefinery approach (Shamsul, *et al.*, 2016). The International Energy Agency (IEA) Bioenergy Task 42 defines a biorefinery as the sustainable processing of biomass into a spectrum of marketable bio-products (chemicals and materials) and energy (IEA, 2010). A biorefinery aims to maximise resource utilisation by fully exploiting the biomass feedstock to produce multiple products using a single facility that integrates various biomass conversion (thermal/thermochemical/ biochemical/chemical) routes and equipment (Coma, *et al.*, 2017). A number of studies focusing on biorefineries based on thermochemical conversion routes such as gasification and pyrolysis (Libra, *et al.*, 2011; Kambo & Dutta, 2015; Maity, 2015) or on biochemical routes (Mussato & Bikaki, 2016) have

been conducted. There is, however, limited research primarily centred on anaerobic biorefineries as the main conversion route (Sawatdeenarunat, *et al.*, 2016). This study, therefore, explores the concept of an anaerobic biorefinery using fruit and vegetable waste.

1.3 Project Objectives

The overall project objective is to enhance the sustainability of biogas plants by adopting a biorefinery approach through investigating the product spectrum of an anaerobic biorefinery and optimisation of process parameters to maximise production of target products.

The specific project objectives are:

1. To investigate the product yield and composition of products that can be produced via AD of mixed fruit and vegetable waste
2. To investigate the effects of process parameters on the AD product of selected target products
3. To maximise product yield of selected products through optimisation of process parameters
4. To investigate the products of the thermo- chemical valorisation of anaerobic digestate

1.4 Thesis Outline

This dissertation is structured as follows:

Chapter 1: Gives the background of the study, including the problem statement, and the objectives of the study.

Chapter 2: Outlines the general literature, reviewing the various researches on FVW as feedstock for a biorefinery, causes and magnitude of FVW, and conventional and emerging FVW management methods. The review also examines how process parameters influence the yield, and composition of AD products, and describes various techniques for optimising process parameters.

Chapter 3: Presents the experimental investigation of the simultaneous production of biohydrogen and volatile fatty acids via anaerobic digestion of fruit and vegetable waste.

Chapter 4: Outlines the experimental procedure, and the results obtained from investigating how three process parameters (pH, co-digestion and ultrasonification) affect the methane yield and volatile fatty acid yield of anaerobically digested FVW and optimisation, via RSM, of the process parameters.

Chapter 5: Describes the experimental procedure ,and the results obtained from applying hydrothermal carbonisation to FVW digestate.

Chapter 6: Gives the conclusion and recommendations of the study.

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2. LITERATURE REVIEW

2.1 Introduction

This chapter reviews the various researches on FVW as feedstock for a biorefinery concept that aims to produce a spectrum of bio-products. Section 2.2 describes the causes and magnitude of FVW, while Section 2.3 looks at conventional and emerging FVW management methods. Section 2.4 introduces the concept of waste based bio - refineries including the product spectra of anaerobic biorefineries and Section 2.5 focuses on how process parameters influence the yield and composition of AD products. Lastly, Section 2.6 reviews methods for process parameter optimisation.

2.2 Fruit and Vegetable Waste

Fruits and vegetables are the most commonly consumed horticultural crop in the world by volume (Sagar, *et al.*, 2018), as they form a critical part of daily human diets due to their high nutrient value and beneficial health compounds (Singh, *et al.*, 2012; Saini, *et al.*, 2019). The production of fruits and vegetables has increased significantly in order to meet the increasing demand for fresh produce owing to population growth and a push towards healthier eating habits (Sagar, *et al.*, 2018). Global fruit production more than doubled from 400 million metric tons in 1990 to 865 million metric tons in 2017 (Statista, 2018a). As regarding vegetable production, yields rose from around 680 million metric tons in 2000 to close to 1 000 million metric tons in 2017, an increase of over 60 % (Statista, 2018b).

However, substantial wastage and losses have accompanied the increase in fruit and vegetable production. In a 2019 study by the Food and Agriculture Organization of the United Nations (FAO, 2019), global fruit and vegetable post-harvest losses reached up to 20 %, second only to roots, tubers and oil bearing crops which stood at 25 %. In Sub-Saharan Africa, fruit and vegetables post-harvest losses are much higher than the global average, falling in the range of 40 to 50 % (FRA, 2019). South Africa in particular, FVW accounts for 44 % of total food waste generated annually, followed by grains (26 %), meat (15 %) and roots, tubers and oilseeds (13 %) (WWF-SA, 2017). De Laurentiis, *et al.*, (2018) and FAO, (2019), forwarded possible reasons why high fruit and vegetable wastages occur. Firstly, fruit and vegetables inherently have inedible components that require discarding; secondly, fruit and vegetables are highly

perishable and therefore more likely to spoil before consumption. Lastly, because FVWs are relatively cheap commodities (compared to meat and fish), consumers pay less attention to prevent their spoilage.

2.2.1 Global FVW extent and causes

FVW generation occurs throughout the food supply chain (Dora, *et al.*, 2019; Girotto, *et al.*, 2015) including at harvest, storage, transportation and distribution, processing as well as at the retail and consumer level, due to physiological reasons and poor handling (Dora, *et al.*, 2019; Ghosh, *et al.*, 2016; Singh, *et al.*, 2012; Panda, *et al.*, 2016). The type and causes of losses differ at each stage of the food supply chain (FSC) depending on crop type, degree of economic development as well as socio-cultural attitudes in a region (Campbell & Munden-Dixon, 2018; Sagar, *et al.*, 2018; Dora, *et al.*, 2019; Jimenez-Moreno, *et al.*, 2019). For example, FVW at the farm level is comprised mainly of culled fruit and vegetables (rotten/damaged/low quality) and crop residues as shown in Figure 2.1 (Goldernberg, 2016; Ghosh, *et al.*, 2016)). While losses at processing stages mainly consist of inedible portions such as peels (Figure 2.2), seeds, stones, and pulp as well as processed products rejected due to not meeting quality standards (Sharma, *et al.*, 2016).



Figure 2.1 Not fit for market watermelons dumped to be used as cattle feed on a farm (Goldernberg, 2016)



Figure 2.2 Citrus peels from a citrus juice factory (Wadhwa, *et al.*, 2020)

There are also significant differences between developed and developing countries in terms of where the losses occur along the FSC. A recent study by FAO, (2019) reports that in developed countries fruit and vegetable losses primarily occur at the agricultural production stage (sorting and grading) mainly due to rejection of produce not satisfying the high quality standards required by retailers. Significant FVW also occurs at the end of the FSC in developed regions, with 15-30% of purchases by volume discarded by consumers. In contrast, processing-related losses in developing countries are more dominant accounting for 14-21 % of losses. Harvest-related losses are also high in developing nations accounting for more than 10 % of total wastage (Plazzotta, *et al.*, 2017). Comparing processing-related losses in developing countries to that of developed countries one can see that process losses (14 - 21 %) are much higher in developing countries than in developed regions (less than 2%) (Rezaei & Liu, 2017; Moreno, 2016; Plazzotta, *et al.*, 2017). Fig 2.3 shows fruit and vegetable losses at various stages of the FSC in different regions of the globe as reported in (FAO, 2019).

Quantitative data on the amount of fruit and vegetable waste globally is limited, however, there is some data available indicating a magnitude of losses at the country level. Peng, *et al.*, (2019) reports that China discards up to 100 billion tons of FVW every year and that India generates 5.6 million tons of FVW annually. In Europe, fresh FVW accounts for roughly 50 % of the 88 million tonnes of food waste generated annually (Roberts, 2018). A WWF 2017 report (WWF-SA, 2017), states that South Africa generates roughly 10 million tons of food

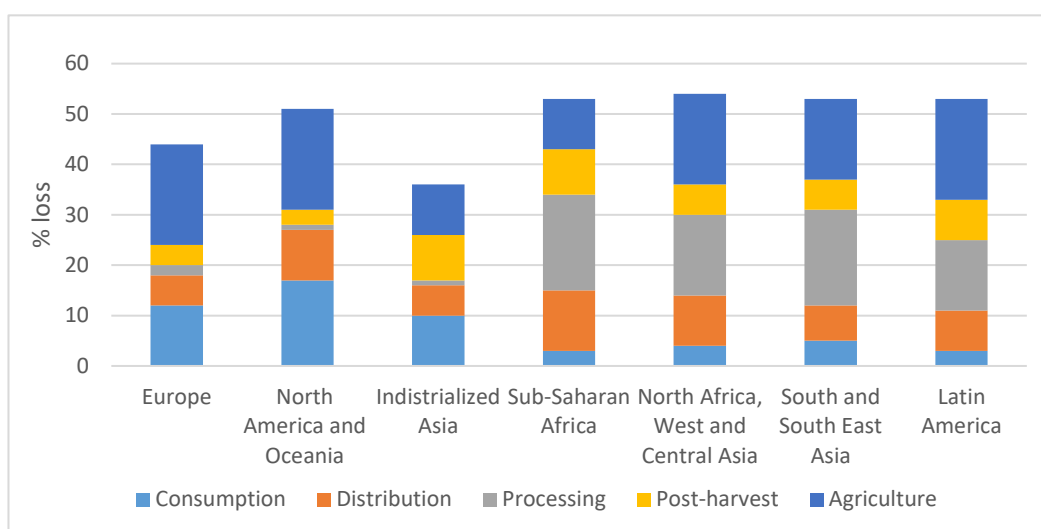


Figure 2.3 Percentage FVW loss across the FSC, by commodity groups and region

Source: (FAO, 2019)

waste per annum and that fruit and vegetable waste accounts for 44 % of this waste, translating to 4.4 million tons of FVW generated per year.

2.2.2 Composition of FVW

FVW streams are generally characterised in terms of their physical, chemical, and biological characteristics in order to determine their potential as feedstock for a range of transformations (Singh, *et al.*, 2012). Waste composition analysis is of paramount importance as it determines the suitability of the waste for application for various processes. Physical parameters such as moisture, ash, total and volatile solids are frequently investigated characteristics. Biological parameters (pathogens and organisms) as well as chemical parameters including cellulose, hemicellulose, starch, protein, fat, total organic carbon, phosphorus, nitrogen, biological oxygen demand pH and toxic metals, although important, are less frequently measured (Saini, *et al.*, 2019; Sharma, *et al.*, 2016).

Researchers like Sharma, *et al.*, (2016) and Edwigs, *et al.*, (2018), report that FVW typically has a high moisture content (80–90%), are high in biodegradable organic compounds, and are rich sources of carbohydrates, starch, cellulose, soluble sugars, minerals, and organic acids depending on their origin (Kaur, *et al.*, 2019). Furthermore, (Diaz, *et al.*, (2017) states that carbohydrates are the primary component of fruits and vegetables and account for 70 – 90 % of their dry weight. FVWs also contain considerable quantities of proteins, fats, and other nutrients like vitamins, minerals, and fibres.

2.3 Fruit and Vegetable Waste Management and Treatment Methods

FVW management methods fall under conventional and alternative/emerging methods. Conventional methods include landfilling; open field dumping, burning, incineration, and composting. Literature extensively discusses the drawbacks of traditional waste methods. For example, slow degradation rates, emission of toxic gases (dioxins), odours, leachates, water pollution, eutrophication, environmental and health problems are some drawbacks mentioned by (Shamsul, *et al.*, 2016; Panda, *et al.*, 2016; Paritosh, *et al.*, 2017; Singhania, *et al.*, 2017; Shanthia, *et al.*, 2018). Other scholars, Ji, *et al.*, (2017), Plazzotta, *et al.*, (2017) and Masebinu, *et al.*, (2018) also mention that conventional treatment methods are unsuitable for FVW management owing to characteristics such as high moisture content, high biodegradability, and rich organic content.

To address the limitations of conventional FVW treatment methods, the development of alternative FVW management methods that are more efficient, low cost and, environmentally friendly has been a study area of interest for many researchers. Waste valorisation, in particular, defined as the process of transforming waste materials into value-added products such as chemicals, materials, and fuels, has received much research attention (Gumisiriza, *et al.*, 2017). A study by (Khan, *et al.*, 2015) discussed potential valorisation routes for agro-industrial wastes in South Africa particularly focusing on fruit waste. These include densification, extraction methods and substrate for microbial growth as shown in Figure 2.4. Owing to its chemical composition such as high moisture and organic matter content and high polysaccharide fraction, FVW has great potential for conversion into various high value-added products such as biocolors, bio- pesticides, fragrances, animal feed, fuels, chemicals, and enzymes (Diaz, *et al.*, 2017; Panda, *et al.*, 2017).

While the aforementioned methods are viable, valorisation routes for FVW management having their own merits and demerits, this review will direct its focus on Waste to Energy (WtE) routes for FVW management. This is because there is growing interest in WtE technologies due to expected energy supply shortages in the future and climate change worries surrounding fossil-fuel energy.

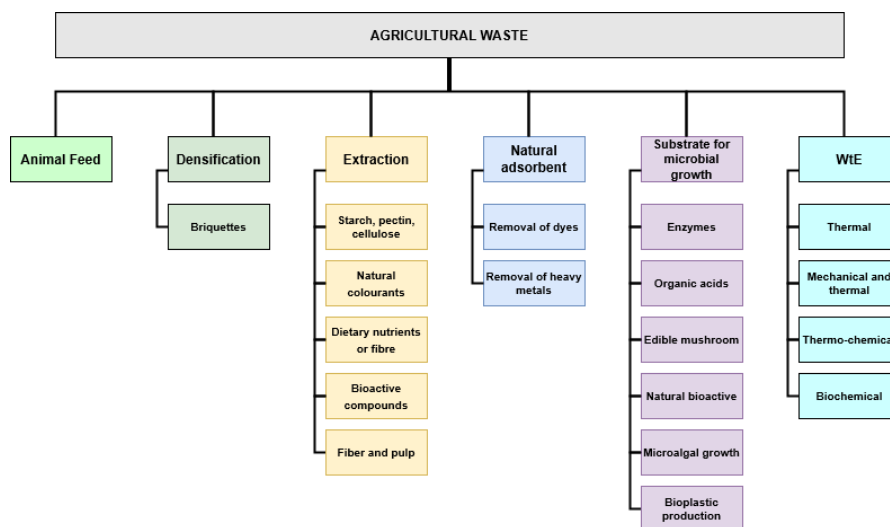


Figure 2.4 Valorisation options for agro-industrial wastes and possible end products

2.3.1 Fruit and vegetable waste to energy conversion technologies

Gumisiriza, *et al.*, (2017) defined Waste to Energy (WtE) technologies as the process of transforming waste into energy including heat, electricity, and biofuels. WtE technologies offer dual benefits of waste management and energy generation. WtE technologies broadly fall under three main categories, namely (i) thermochemical, (ii) biochemical and, (iii) mechanical and thermal (Figure 2.5) Thermochemical conversion routes of FVW

Thermochemical conversion routes use thermal energy to convert organic matter into either heat energy, fuel oil, or gas. Thermochemical conversion routes are typically best suited for dry waste, with low moisture content and low percentage of biodegradable matter, making most thermochemical routes less suitable for FVW management due to the high moisture content of most fruits and vegetables (Tsui & Wong, 2019). Incineration, gasification, and pyrolysis are the principal thermochemical technologies used for WtE.

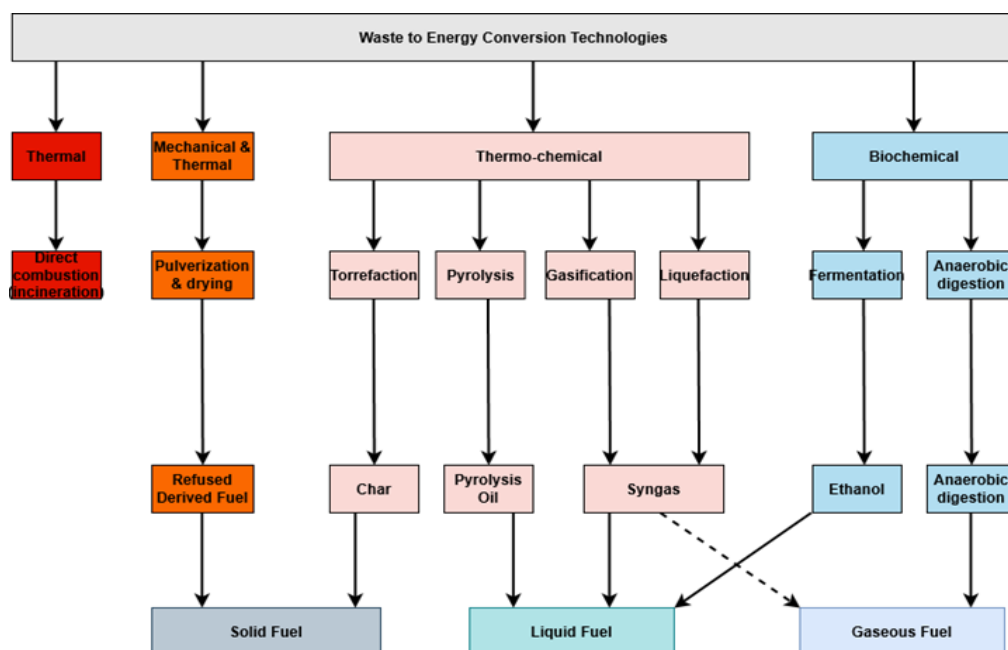


Figure 2.5 Waste to Energy Technologies

Source: Adapted from (Gumisiriza, et al., 2017)

Incineration, which is the controlled combustion of waste in excess air at high temperatures ($> 850^{\circ}\text{C}$), is the most widely applied WtE treatment (Tsui & Wong, 2019). In contrast, the other thermochemical technologies (gasification, pyrolysis, and liquefaction) operate in reduced or no oxygen conditions at temperatures as low as 300°C for pyrolysis and above 650°C for gasification (Pieta, *et al.*, 2018). Generally, incineration converts waste into heat while gasification and pyrolysis routes first transform the waste into secondary energy carriers such as syngas and bio-oil, which can subsequently be converted into other energy forms e.g. electricity, heat (Gumisiriza, *et al.*, 2017; Tsui & Wong, 2019).

Another thermochemical technology, hydrothermal carbonization (HTC), is regarded as more suited for substrates with high moisture content. HTC is a thermochemical process that converts wet biomass into hydro-char, small amounts of water-soluble organic compounds, and gas as shown in Figure 2.6 (Garlapalli, *et al.*, 2016; Correa, *et al.*, 2017). The hydro-char has chemical properties similar to fossil coals and has potential applications as a soil amendment, solid energy fuel, low-cost adsorbents, and medical uses (Monalu, *et al.*, 2015). HTC is carried out under aqueous conditions in a pressurized reactor (1-5 MPa) at temperatures of around $180 - 260^{\circ}\text{C}$ and time of 2 -20 h (Garlapalli, *et al.*, 2016; Breulmann, *et al.*, 2017).

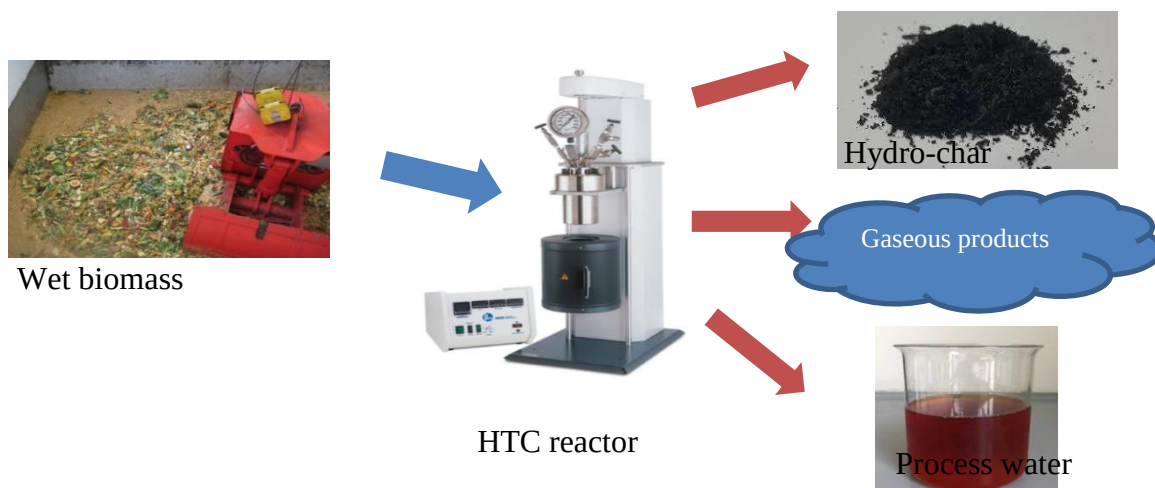


Figure 2.6 Products of Hydrothermal carbonization of wet waste

Source: Adapted from: (Langone & Basso, 2020)

Scholars Pieta, *et al.*, (2018), compared the reaction conditions and products of incineration, gasification, pyrolysis and HTC, revealing that the major difference in the four technologies are operating temperature, pressure, and oxygen as well as the end products (Table 2.2.1).

Table 2.2.1 Typical reaction conditions and products of thermo-conversion processes adapted from (Pieta, *et al.*, 2018)

	INCINERATION	GASIFICATION	PYROLYSIS	HTC
TEMPERATURE [°C]	>850	>650	300 -850	180-260
PRESSURE [BAR]	1	1 -45	1	1-15
REACTION ENVIRONMENT	Excess oxygen	Low oxygen, H ₂ O	Zero oxygen	Aqueous conditions
GAS PRODUCTS	CO ₂ and H ₂ O	CO, H ₂ , (Syngas)	CO, H ₂ , CO ₂ , H ₂ O, CH ₄	
SOLID PRODUCTS	Ash, slag	Ash, slag	Ash, coke	Hydro-char
LIQUID PRODUCTS			Pyrolysis oil (bio-oil) and water	Small quantities of soluble organic compounds

2.3.1.1 Biochemical conversion routes

Biochemical technologies utilise microorganisms such as fungi, yeast and bacteria to harness the energy stored in biomass (Beyene, *et al.*, 2018; Lee, *et al.*, 2019). Biochemical conversion routes are generally more eco-friendlier than thermochemical techniques and more suited for wet waste that is rich in organic matter (Kumar & Samadder, 2017; Beyene, *et al.*, 2018). The most popular biochemical conversion routes are AD and fermentation.

(i) Fermentation

Gautam, *et al.*, (2019) describes fermentation as the conversion of organic waste into acid or alcohols (mainly ethanol) and a nutrient-rich residue using yeast, fungi, or bacteria in zero oxygen conditions. According to (Gumisiriza, *et al.*, (2017), fermentation is most suited for high sugar-content crops such as sugarcane, sugar beets, and fruits as they primarily contain simple sugars such as glucose and fructose, which easily convert into ethanol by alcohol fermenting microorganisms. Fig 2.7 summarises the production of ethanol in four main steps. Step 1: substrate pre-treatment via physical, chemical, physiochemical or biological methods, to breakdown the lignocellulose into its components. Step 2: hydrolysis or saccharification by specific hydrolytic enzymes to obtain a hydrolysate consisting of fermentable monomeric sugars. Step 3: fermentation using yeast to convert monomeric sugars (e.g. glucose) into ethanol solution. Lastly, Step 4: distillation to separate and purify the ethanol (Anyanwu, *et al.*, 2018).

Figure 2.7 Schematic of laboratory bioethanol production using fruit and vegetable

Researchers (Arapoglou, *et al.*, 2010; Rodriguez, *et al.*, 2010; Oberoi, *et al.*, 2011) cited by Pham, *et al.*, (2014) successfully produced bioethanol from FVW, including banana peel, sugar beet pulp, and potato peels using laboratory reactors. Beyene, *et al.*, (2018) concur that ethanol fermentation is a viable technology for treating food waste, but stressed the importance of further research to achieve sustainability and reduce the price of ethanol derived from food waste.

(i) AD

AD is a microbial catalysed process involving the decomposition of organic matter by a complex mixture of microbial organisms in the absence of oxygen into predominately biogas and nutrient-rich digestate. AD technique is generally considered best suited for treating waste that is easily biodegradable with high moisture content (Beyene, *et al.*, 2018). As such, many regard AD as a promising solution for managing fruit and vegetable waste and simultaneously assisting in climate change mitigation and adaptation (Ji, *et al.*, 2017; Maile & Muzenda, 2014).

In recent years, various researchers have investigated AD of FVW to generate energy using either single substrate or co-substrate digestion (Ji, *et al.*, 2017; Scano, *et al.*, 2014; Maile & Muzenda, 2014). While these studies confirmed the feasibility of AD to treat fruit and vegetable waste, they also highlighted the challenges of using FVW as a single substrate. For example, a study by Sitorus, *et al.*, (2013) discovered that rapid acidification due to relatively low pH of FVW and production of volatile fatty acids (VFAs) inhibit the AD process. Similarly, Maile & Muzenda (2014) observed that high VFA concentrations lead to a decrease in pH values resulting in gas production inhibition.

2.3.1.2 Emerging/novel technologies for Waste to energy

Ongoing research in the field of Waste to Energy technology is leading to the development of new and innovative technology for the production of other high-value bio-commodities. In their review paper Beyene *et al.*, (2018), discussed some of the upcoming novel WtE technologies used for biological hydrogen production.

Hydrogen is a clean and renewable energy source, described as the energy of the future owing to its high-energy content and clean combustion (Barca, *et al.*, 2016; Khan, *et al.*, 2017). Although it is possible to produce hydrogen from various renewable resources such as biomass and water, the bulk of global hydrogen production (around 95 %) comes from fossil-based

methods such as oil steam reforming and coal gasification. However, fossil-based hydrogen production emits greenhouse gases, is expensive and energy extensive (Ghimire, *et al.*, 2016; Khan, *et al.*, 2017; Mahidhara, *et al.*, 2019), this has stimulated research in finding alternate sustainable hydrogen production methods including biological processes using waste. There are three major pathways in which the production of biological hydrogen is achieved, namely, fermentation, bio-photolysis, and bio-electrochemical systems (Osman, *et al.*, 2020). This can be further classified as light dependant or light- independent as shown in Fig 2.8 (Ghimire, *et al.*, 2015). This review will look at light- independent systems (dark fermentation and bio-electrochemical systems (BESs)) as their easier to integrate with AD.

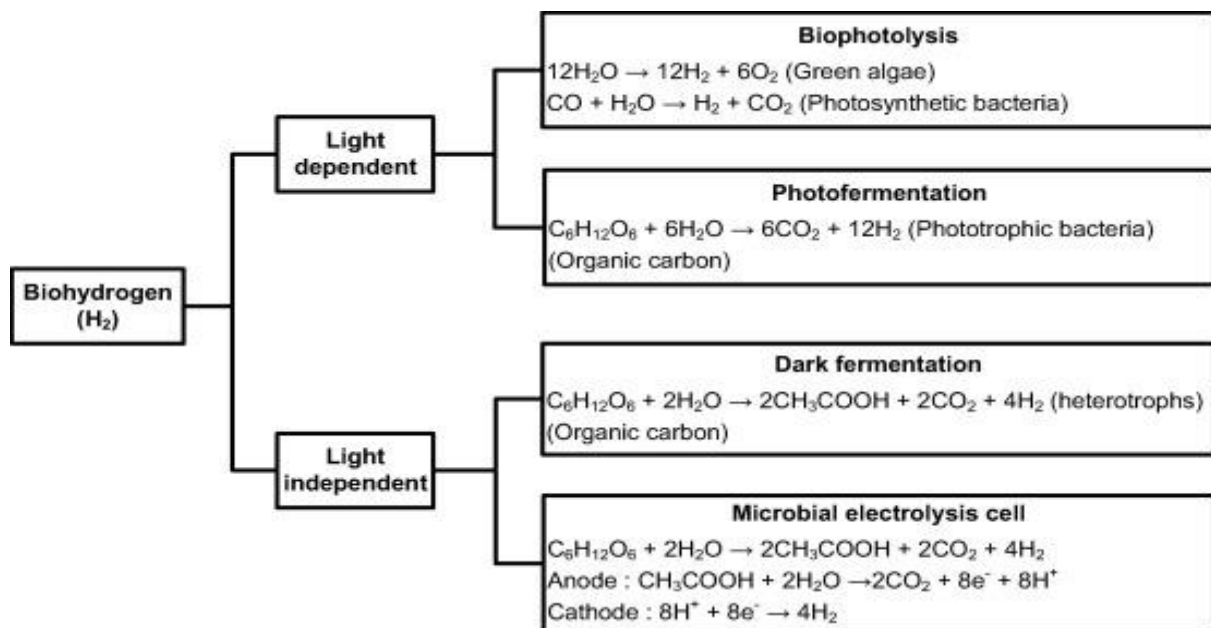


Figure 2.8 Classification of bio-hydrogen production methods (Ghimire, *et al.*, 2015)

(i) Dark fermentation

In dark fermentation, fermentative anaerobic bacteria produce hydrogen in the absence of light. This takes place during the first phase of AD in the acidogenesis and acetogenic steps (Nathoa, *et al.*, 2014; Khan, *et al.*, 2016b). Biological hydrogen production via dark fermentation employs mixed anaerobic cultures consisting of both hydrogen- producing bacteria (HPB) and hydrogen consuming bacteria (HCB) (Manunga, *et al.*, 2019). This presents a challenge, as the hydrogen generated by HPBs can be easily utilised by HCBs resulting in low hydrogen yields, as is in the case of conventional AD. To overcome this, the mixed anaerobic inoculum

is subjected to different kinds of pre-treatment to enhance hydrogen production while suppressing hydrogen eaters (Bundhoo & Hassan, 2015; Sittijunda & Pattra, 2016; Pachapur, *et al.*, 2019). The pre-treatment methods are classified as chemical or physical . Chemical pre-treatment includes acid, base, chloroform, sodium 2 bromethanesulfonate (BES), carbon dioxide, and iodopropane. Falling under physical methods are aeration, infrared drying, sonication, and heat pre-treatment (Manunga, *et al.*, 2019; Wang & Yin, 2017).

Yasin, *et al.*, (2013) illustrates the changes in the microbial community of a mixed culture inoculum due to inoculum pre-treatment and environmental conditions using Fig 2.9. The untreated mixed culture inoculum (a) consists of HPBs, methane- producing bacteria (MPBs) and acid-producing bacteria. After pre-treatment (b) the composition of the inoculum changes as fewer MPBs and acid producing bacteria are present. Again, Fig 1 (d) and (e) demonstrates the effect of process pH on inoculum population, wherein pH 5-6 promotes growth of HPB and suppresses MPB and acid –producing bacteria. Fig 1 (f) shows that non-pre-treatment of inoculum promotes germination of methane- producing bacteria while suppressing HPB.

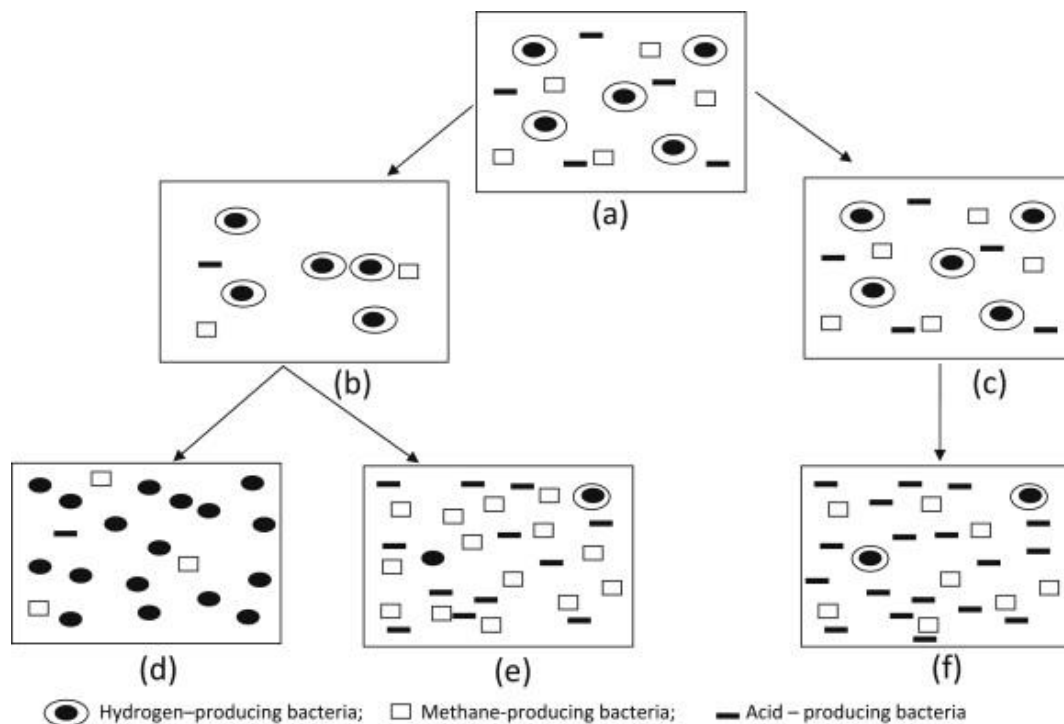


Figure 2.9 The effect of pre-treatment, pH and temperature on mixed culture inoculum (Yasin, *et al.*, 2013)

The working principle as well as relative advantages and disadvantages of some of the above-mentioned inoculum pre-treatment methods as elucidated by Wang & Yin, (2017) and Pachapur, *et al.*, (2019) are summarised in a comparative table in Table 2.2.2.

Table 2.2.2 Comparison of inoculum pre-treatment methods to enhance hydrogen and VFA production

PRE-TREATMENT	WORKING PRINCIPLE	ADVANTAGE	DISADVANTAGE
Heat shock Inoculum heated at temps 65 -100 °c for 30 - 60 min	Non-spore forming methanogens destroyed at high temperatures.	Simple, inexpensive, easy to control	Large energy inputs, Can inhibit activity of some hydrogen producing bacteria
Acid/alkali chloroform, iodopropane, sulphuric acid	Activity of methanogens and other non-sporulating bacteria cannot survive at pH below 6.3 or above 7.8	Ease of implementation Effective	Expensive to use at large scale
Aeration	Methanogens are strict anaerobes while hydrogen producers are facultative	Easier control Aeration blowers easily available	Energy consuming process

(i) Bioelectrical Chemical Systems (BESs)

Microbial transformation of waste via novel bioremediation strategies such as BESs are emerging as a promising alternative to conventional waste treatment approaches. BESs, as explained by Escapa, *et al.*, (2014), Srikantha, *et al.*, (2016) and Ziara *et al.*, (2018), utilise microorganisms to biochemically catalyse organic substrates such as FVW into electricity or hydrogen or value-added chemicals depending on the configuration. Of particular interest are microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) which have been extensively researched for bioelectricity and bio-hydrogen generation respectively (Ramanjaneyulu & Reddy, 2019).

MECs are a class of BESs that combine bacterial metabolism and electrochemistry to produce H₂ and by-products from a wide range of organic substrates using an external power source (Mohan, 2014; Ziara, *et al.*, 2018; Lamb & Lien, 2020). A MEC (Fig 2.10) is a promising technology for producing renewable bio-hydrogen gas and waste treatment (Mohan, 2014). MECs work similarly to the water electrolysis process (Mohan, 2014)), wherein anode

respiring microbes attached at the anode in the anodic chamber degrade organic substrates (ethanol, butyrate, lactate, acetate, and propionate) into carbon dioxide, electrons (e^-), and protons (H^+) (Lamb & Lien, 2020). Mohan (2014) and Mohan & Pandey, (2019) explain that the negatively charged electrons (e^-) travel through an external circuit, while the positively charged H^+ ions migrate to the cathode via a membrane. The H^+ ions receive electrons (e^-) at the cathode and are reduced to form H_2 . Adding also that an external power supply is required to overcome the endothermic barrier to form H_2 gas; which presents the biggest challenge of bio-hydrogen production via MECs (Lama, *et al.*, 2012).

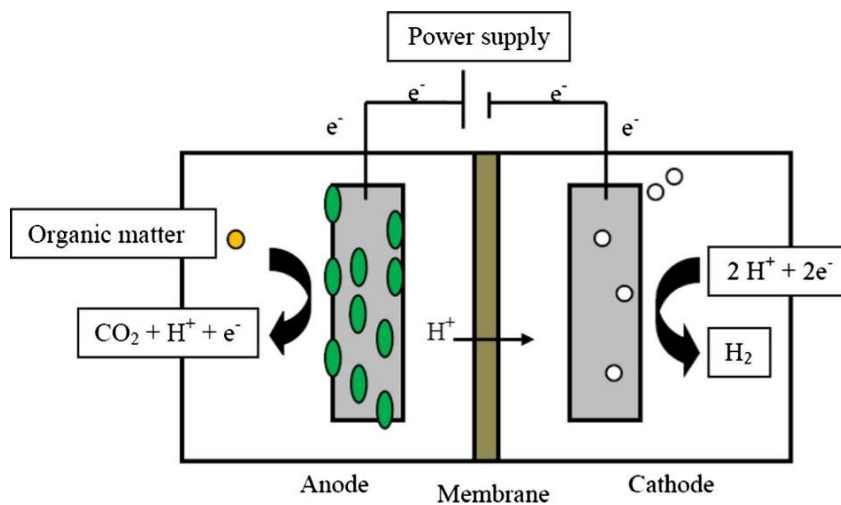


Figure 2.10 Schematic of two chamber MEC operation (Kadier, *et al.*, 2016)

Compared to conventional water electrolysis and improved substrate degradation over dark fermentation, the low energy consumption of MECs makes MECs a potentially better alternative for biohydrogen production. Although dark fermentation can degrade various organic substrates to generate hydrogen at a low energy demand, low H_2 recovery rates of roughly 23%–25% are experienced due to thermodynamic limitations. To increase efficiency, MECs can be coupled to dark fermentation, by degrading complex organic substrates via dark fermentation followed by MEC treatment of the digestate effluent as in the case of Sciarria, *et al.*, (2018) and Cristiani, *et al.*, (2020) who coupled MEC to anaerobic digestion (AD-MEC) as a post-treatment to recover nutrients and produce bio-hydrogen from anaerobic digestate. In contrast, Huang, *et al.*, (2020) and Hassanein, *et al.*, (2017) used MEC as a pre-step to anaerobic digestion (MEC-AD) in order to produce bio-hydrogen and increase biogas production.

On the other hand, microbial fuel cells (Fig 2.11) generate electricity by utilising electrons released by bacterial oxidation of substrates in the anode chamber. Similar to MECs, electrons and protons are produced at the anode, however, the cathode reactions differ (Ramanjaneyulu & Reddy, 2019). The electrons flow via a conductive material to the positive cathode chamber where they combine with oxygen (pumped into the system) and protons that diffused through a proton exchange membrane to produce water (Kyazze, 2020; Kadier, *et al.*, 2016). Researchers such as (Khan & Obaid, 2015; Logrono, *et al.*, 2015; Kamau, *et al.*, 2018; Kalagbor, *et al.*, 2019; Flores, *et al.*, 2020) have successfully demonstrated production of bioelectricity from fruit and vegetable waste using MFCs.

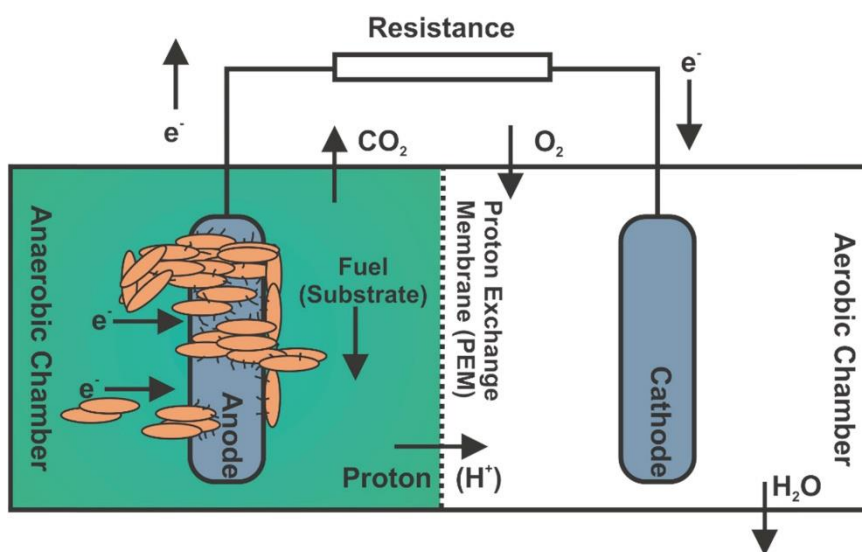


Figure 2.11 Schematic of two chamber MFC displaying (Kadier, *et al.*, 2016)

2.3.2 Comparison of Waste to Energy Technologies

Table 2.2.3 compares ten different Waste to Energy technologies with a focus on the advantages, disadvantages, and primary products. The comparison gives a detailed energy system analysis of the biochemical and potential future emerging system with a large share of combined microbial and novel waste management. The comparison shows the potential of using waste for the production of transport fuels such as hydrogen.

Table 2.2.3 Comparison of WtE technologies

	TECHNOLOGY	ADVANTAGES	DISADVANTAGES	PRIMARY PRODUCTS	APPLICATION
Thermochemical	Incineration	Reduces volume and mass	High capital maintenance and operation costs	Heat	Electricity and steam/heat generation
	Pyrolysis	Produce high quality fuel, reduces flue gas treatment, suited for carbonious waste	Produces harmful pollutants and solid residues	Char, bio-oil and syngas	Electricity generation, chemical and solvent production
	Gasification	Production of fuel gas/oil which can be used for various purpose		Syngas producer gas	Electricity generation and chemicals
BIOCHEMICAL	Anaerobic	Low cost natural resources recycled to the soil	Soil and groundwater pollution	Biogas and digestate	Electricity, organic fertilizer and food bio refinery
	Landfill	Preferred for biomass with high water content, higher methane content and lower CO ₂	Need to fill this in		
	Ethanol Fermentation	Does not contribute to increase in CO ₂ emissions		Bioethanol	Fuel, agricultural biorefinery
NOVEL WtE	Photo-biological process	A wide spectra of energy can be used by photosynthetic bacteria		H ₂ and CO ₂ gas, organic acids	Bioelectricity
	Dark fermentation	Wide range of biodegradable substrates, No light requirement		H ₂ gas	Bioelectricity
	MFC	Effective for electricity generation and odour removal	High temperatures required as microbial reactions are slow at low temperatures	H ₂ gas	Bioelectricity, bio-hydrogen production, WWT treatment
	MEC	High product recovery (H ₂) compared to photo/, dark fermentation /MFC, low energy requirement	Yields affected by substrate composition, high capital costs, dense architecture	H ₂ gas, CH ₄ , acetate, hydrogen peroxide and formic acid	Electricity generation and immediate wastewater treatment

Source: Adapted from (Beyene, et al., 2018)

2.4 Biorefineries for Waste Management

Coma and co-workers (Coma, *et al.*, 2017), noted that the majority of sustainable waste management methods used employ single biomass technologies that primarily generate a single high-value product. In general, these processes are inefficient as they transform only a fraction of the organics into bioenergy and other low-value by-products or waste. To overcome this limitation Ismail & Nizami, (2016) suggested a biorefinery approach to waste management. Such waste-based biorefineries can integrate several WtE and other valorisation technologies to produce fuels and other high value-added bio-commodities (Ismail & Nizami, 2016; Cristóbal, *et al.*, 2018). The biorefinery concept incorporates a wide range of biomass technologies including thermochemical, biochemical, and mechanical to produce a spectrum of marketable products: biochemical, biomaterials, and energy (De Jong & Jungmeier, 2015). A biorefinery is analogous to a petroleum refinery, which converts crude oil into multiple products such as fuels and intermediates used as raw materials for the petrochemical industry (Vea, *et al.*, 2018; De Jong & Jungmeier, 2015).

Globally there are growing calls to adopt the concept of biomass cascading and to shift towards a circular, bio-based economy with the goal of increasing resource efficiency, reduce utilisation of fresh materials, improve eco-efficiency and lower the greenhouse gas footprint (Carus, 2017). Such calls are driving research into waste-based biorefineries that exploit utilisation of waste to produce bioenergy and multiple bio-products (Rehan, *et al.*, 2019). According to Moncadaa, *et al.*, (2014), biorefinery feedstock falls into three main categories: first-generation (1G) which utilise crops; second-generation (2G) that makes use of agro-industrial residues, and non-edible crops (non-edible crops are considered fourth-generation by some authors), while third-generation (3G) apply algae as feedstock. Based on the aforementioned classification waste biorefineries are second-generation biorefineries (Esteban & Ladero, 2018).

A number of valorisation pathways for food waste-based (which include fruit and vegetable waste) biorefineries have been investigated. These pathways employ different valorisation techniques (e.g. extraction, Solid-State Fermentation (SSF), thermochemical and biochemical) and have different product portfolios. For example, Nguyen, *et al.*, (2017) integrated enzymatic hydrolysis and fermentation processes to produce D-mannose and bioethanol from coffee

residues. Research to evaluate the potential for a potato biorefinery based on SSF route to produce protein, citric acid, and organic compounds as well as animal feed from potato peels have been reported (Pathak, *et al.*, 2018). Similarly, Torres, *et al.*, (2020) and Matharu, *et al.*, (2016) investigated a potato biorefinery to recover starch and bioactive compounds. Food waste biorefineries incorporating AD technology has received considerable attention in recent years as evidenced by the increase in research publications (Begum, *et al.*, 2016; Nizamia, *et al.*, 2017; Bastidas-Oyanedel & Schmidt, 2018; Miguel Carmona-Cabello, *et al.*, 2018; Demichelis, *et al.*, 2018; Strazzera, *et al.*, 2018). However, the majority of these food waste biorefineries (incorporating AD) have by in large focused on methane production as the only valuable digestion product and have ignored valorisation of other by-product streams such as the digestate.

2.4.1 Anaerobic bio refinery

An anaerobic biorefinery is a biochemical platform in which an anaerobic reactor/digester is the principal bioconversion system of transforming low-value feedstock into various high-value products and intermediates with concurrent waste treatment (Sawatdeenarunat, *et al.*, 2016). In an anaerobic-based biorefinery, AD functions as a centrepiece to generate high value, but low quantity products to boost economic viability (e.g. chemicals and drop in biofuels) as well as high-volume but low value products (i.e., heat, electricity, and vehicular biofuels) to attain energy security.

Unlike conventional AD plants designed to harvest a single product (biogas), anaerobic biorefinery targets multiple product generation by extracting intermediate products generated at the different stages of AD (Coma, *et al.*, 2017). In line with the concept of an anaerobic, Zhao, *et al.*, (2017); Liu, *et al.*, (2018); Tampio, *et al.*, (2018); Wainaina, *et al.*, (2019); and Yeshanew, *et al.*, (2018) achieved simultaneous production of methane and hydrogen or methane and VFAs or hydrogen and VFAs from FVWs.

Additionally, AD can be coupled with other biomass conversion technologies for further conversion of the initial product outputs e.g. pyrolysis of the digestate (Kambo & Dutta, 2015; Garlapalli, *et al.*, 2016; Pecchi & Baratieri, 2019). Like in the case of (Pecchi & Baratieri, (2019); Pamar, (2019); Zhang, *et al.*, (2019), who integrated AD with HTC producing hydro-char, bio-oil, and aqueous HTC in addition to biogas. On the other hand, (Moreno, 2016; Zeppillia, *et al.*, 2017; Sciarria, *et al.*, 2018) integrated AD with MEC or MFC to generate

methane, electricity, hydrogen, and recovered nutrients, from the digestate. A schematic model of anaerobic biorefinery using FVW as feedstock is shown in Figure 2.12.

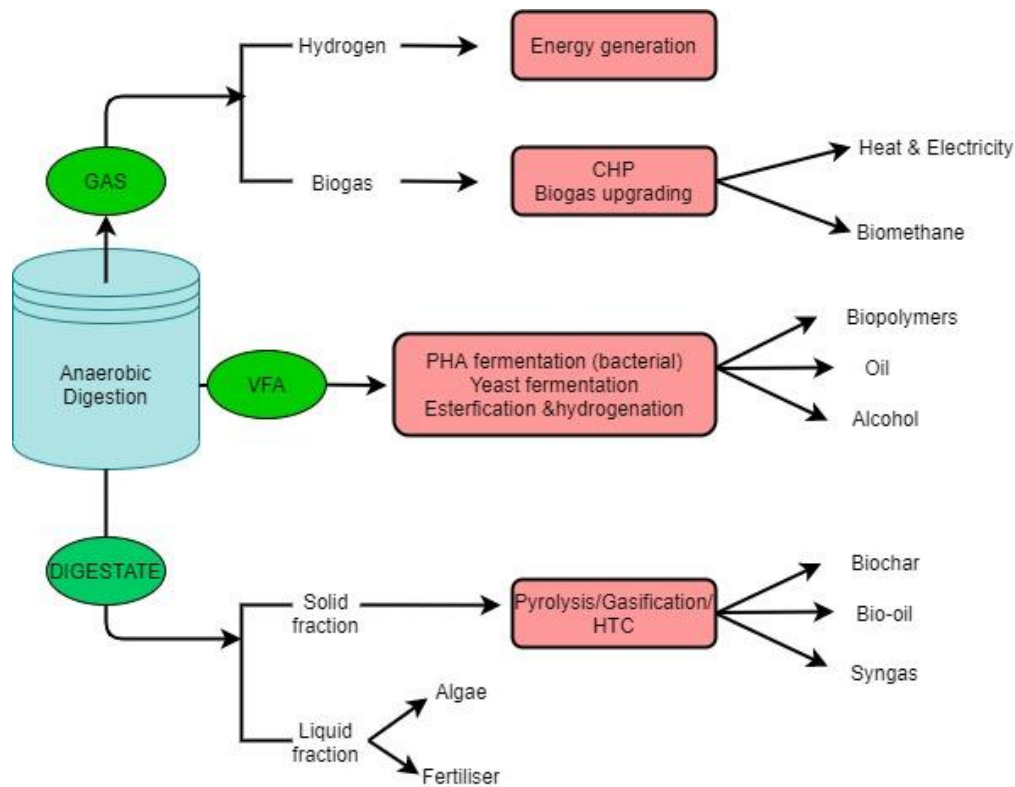


Figure 2.12 Model anaerobic biorefinery including possible valorisation pathways Source: Adapted from (Sawatdeenarunat, *et al.*, 2016)

2.4.2 Anaerobic biorefinery product spectrum

Since AD is a complex biochemical process that involves numerous physicochemical and biochemical reactions, there are several simultaneous reactions occurring in sequence or in parallel (Bong, *et al.*, 2017). The process occurs in four different stages, namely hydrolysis, acidogenesis, acetogenesis and lastly methanogenesis. Figure 2.13 illustrates the key process stages of AD with the first stage of the digestion process being hydrolysis.

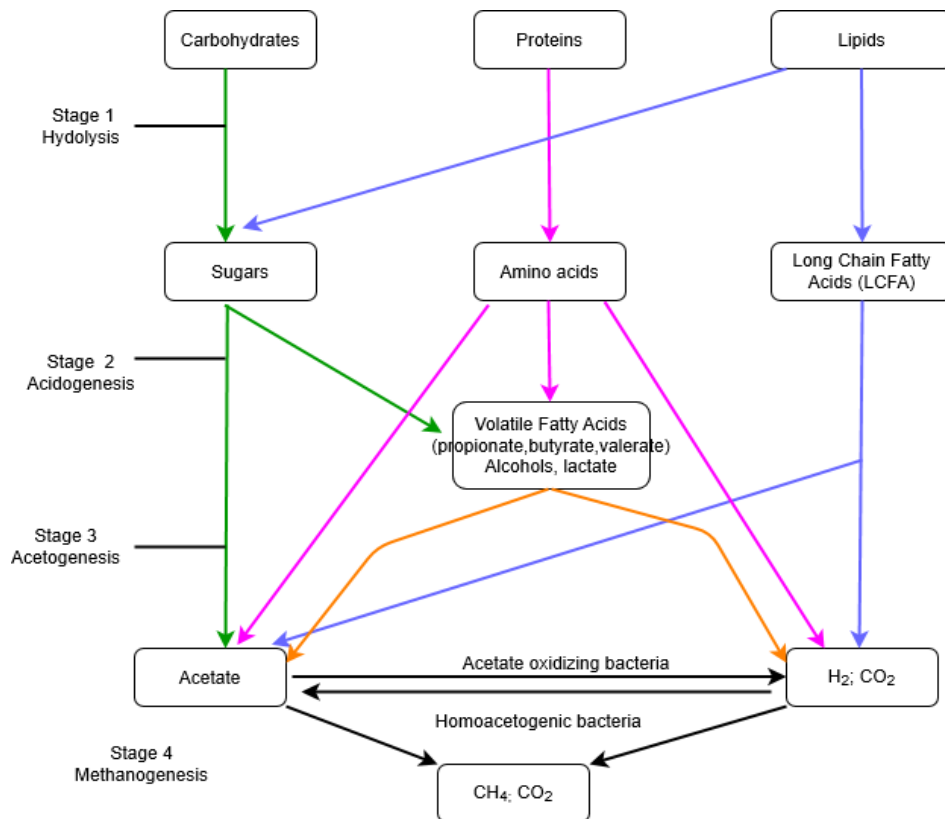


Figure 2.13 Key steps and pathways of AD process

Source: Modified from (Jha, *et al.*, 2011)

The four different stages of AD produce different intermediary products, mainly, alcohols, carboxylic acids, and hydrogen as well as the products taking the form of gases including methane and carbon dioxide. In the first stage, known as hydrolysis, insoluble polymers (carbohydrates, proteins and fats) are broken down into simpler soluble molecules (monomers) by hydrolytic bacteria (Khan, *et al.*, 2017; Mir, *et al.*, 2016). During the second stage, also referred to as acidogenesis or dark fermentation, acid-forming bacteria (acidogens) convert monomers into short-chain volatile fatty acids, hydrogen, carbon dioxide, acetate and alcohols (Khan, *et al.*, 2017; Mir, *et al.*, 2016). In the third stage (acetogenesis), facultative and obligate

acetate bacteria (acid formers) convert the products of hydrolysis and acidogenesis into acetic acid, hydrogen, and carbon dioxide (Mir, *et al.*, 2016). In the final stage of AD, the acetotrophic group of methanogens converts acetate (acetic acid) into methane and carbon dioxide while the hydrogenotrophic reduce hydrogen and carbon dioxide into methane (Khan, *et al.*, 2017). Anaerobic digestate is a by-product of the overall digestion process and is collected after digestion is complete. The various products, by-products, or intermediates of AD are discussed below:

2.4.2.1 Volatile fatty acids

VFAs are a subgroup of carboxylic acids containing between 2 to 8 carbons in their chains including acetic (C2), propionic (C3), butyric (C4), valeric (C5), caproic (C6), heptanoic (C7) and octanoic (C8) acids (Murali, *et al.*, 2017). The VFAs are produced via the acidogenic /dark fermentation stages of AD are a mixture comprising mainly of acetic, propionic, butyric, and valeric (Ramos-Suarez, *et al.*, 2021). Because VFA production via dark fermentation utilises mixed culture bacteria (as opposed to pure cultures), a spectrum of VFAs in varying fractions is generated. Studies by Wainaina, *et al.*, (2019); Khan, *et al.*, (2016a); Strazzera, *et al.*, (2018), show that process parameters (pH, temperature, etc.) remarkably influence the VFA product yield and spectrum. Numerous studies also reveal that changing process parameter setting, influence the metabolic pathways that occur and that some pathways favour the production of certain VFAs (Cheah, *et al.*, 2019). Researchers Ramos-Suarez, *et al.*, (2021) emphasize understanding the VFAs of choice during the design process as this strongly influences the design parameters and subsequent optimisation.

Figure 2.14 shows potential conversion pathways for the mixed VFAs including physical separation, chemical and biological methods as well as the option of products. Substitution of bio-VFAs for petro-chemicals would be an ideal application, however, physical separation of the mixed VFAs into individual VFAs is largely problematic due to the similar properties of the VFAs, low concentration yields (1 – 100g/L), and possible formation of aqueous-acid azeotropes (Ramos-Suarez, *et al.*, 2021). An alternative route is the chemical conversion of the mixed VFA into esters (Wallis, *et al.*, 2017; Placido & Zhang, 2018) or polymerization for the manufacture of plastics and fibrous materials such as polyvinyl acetate (Straathof, 2014). The application of mixed VFAs as a biological conversion feedstock is another applicable conversion route, providing chemical building blocks for producing polyhydroxyalkanoates

(PHA) or medium-chain fatty acids; substrate for bioenergy production and for biological nutrient removal (Kleerebezem, *et al.*, 2015; Colombo, *et al.*, 2017; Bhatia & Yang, 2017).

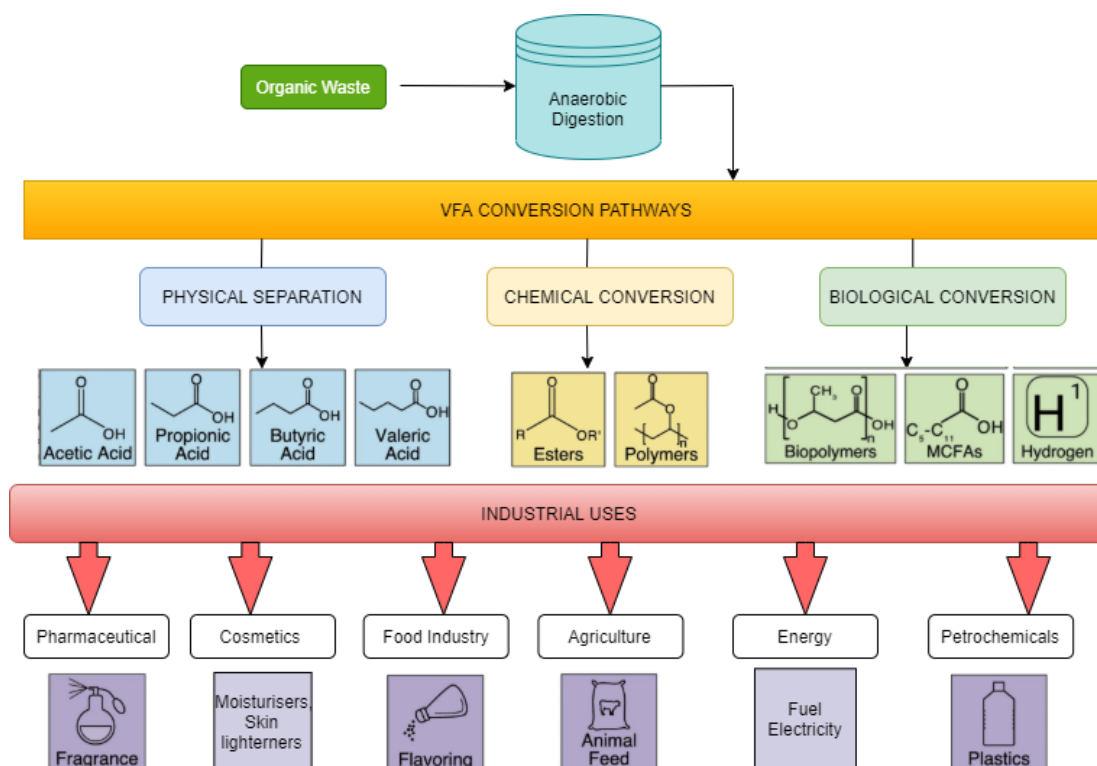


Figure 2.14 Possible VFA conversion pathways and potential industrial applications

Source: Adapted from (Zacharof & Lovitt, 2013; Ramos-Suarez, *et al.*, 2021)

2.4.2.2 Alcohols

The acid-forming stage (stage 2) of AD produces alcohols alongside carboxylic acids, hydrogen and carbon dioxide. However, few studies have explored the production of bio-alcohols from AD. Geradi, (2013) listed the major alcohols produced as methanol, ethanol, propanol and butanol. Kuruti, *et al.*, (2015), investigated the production of bioethanol and VFAs from press mud using mixed microbial consortia. Additionally, Shamsul, *et al.*, (2016), produced bio-alcohol from co-digestion of agricultural and animal waste, obtaining maximum yields of 19.08 g/L bio-methanol and 51.30g /L bio- ethanol.

2.4.2.3 Biogas

Currently, the main product of AD is biogas, which is a mixture of gases consisting mainly of carbon dioxide (30 -50 %) and methane (50-70 %). Trace amounts of hydrogen sulphide, water

vapour, nitrogen, oxygen, carbon monoxide and ammonia are also present in quantities varying from 0 % to 10 % (Angelidaki, *et al.*, 2018; Kapoor, *et al.*, 2019). Biogas is convertible into multiple forms of energy including heat, electricity and vehicular fuel. The market for biogas is mainly for the cooking, power, heat and transport industry and is forecast to increase to 29.5 GW by 2022 (Kapoor, *et al.*, 2019). A report by Grand View Research (GRC, 2020), valued the global biogas market at USD 55.1 billion in 2019 and forecasted growth at a CAGR of 4.48% over the period 2020-2027 driven by rising environmental concern.

Biogas is mainly utilised directly to produce heat and electricity using a combined heat and power (CHP) plant. Upgrading biogas to enrich the methane content by removing water vapour and carbon dioxide and other impurities is also possible (Angelidaki, *et al.*, 2018; Kapoor, *et al.*, 2019). Upgraded biogas (bio-methane) is comparable to natural gas in terms of composition hence injection into the natural gas pipe network is possible (Kleerebezem, *et al.*, 2015). Another popular application of bio-methane is vehicular fuel, many European countries like Sweden, Germany, Norway, Iceland and Finland use bio-methane as a transport fuel. As of 2015, the European Union had the highest usage of bio-methane as a transport fuel, consuming a combined 160 million m³ of biomethane. Similarly, in South Africa, the City of Cape Town and City of Johannesburg have plans to run some of their buses on biomethane (Deklerk, 2017; Liedtke, 2017). The carbon dioxide removed during the purification process can also be captured and used for industrial purposes as is the case with the proposed New Horizon Biogas plant based in Cape Town, South Africa (Cloete, 2017).

2.4.2.4 Hydrogen

Dark fermentation produces hydrogen using fermentative anaerobic bacteria in the absence of light during the acidogenesis and acetogenic steps of AD (Khan, *et al.*, 2016b). Hydrogen is a clean (water is the only by-product of hydrogen combustion) and renewable energy source. Hydrogen has a high-energy content (142 kJ/g) which is about 2.75 times higher than that of other fossil fuels. Furthermore, the energy content of hydrogen is the highest of any known gaseous fuel (Ghimire, *et al.*, 2015; De Gioannis, *et al.*, 2017). The production of hydrogen is an attractive option as they are a wide range of industrial applications that utilise hydrogen including ammonia synthesis, hydrogenation of edible oils, fertilizer and diesel refining (Guo, *et al.*, 2010; Ghimire, *et al.*, 2015; De Gioannis, *et al.*, 2017). Hydrogen is also useful for direct combustion in engines or for electricity production using fuel cell technology (Ghimire, *et al.*, 2015).

2.4.2.5 Bio-digestate

Digestate also termed effluent slurry is a wet solid residue collected from the bioreactor after the digestion process is complete (Garlapalli, *et al.*, 2016). The digestate can be mechanically separated into solid and liquid fractions. Currently, the main use of digestate is for land application as a fertilizer or soil amendment owing to its rich organic matter and nutrient content (Garlapalli, *et al.*, 2016; Wisniewski, *et al.*, 2015). Land application of bio-digestate has limitations which include oversupply of digestate, GHG emissions during storage, environmental pollution and high transportation costs (Garlapalli, *et al.*, 2016; Wisniewski, *et al.*, 2015; Monalu, *et al.*, 2015). For these reasons, alternative valorisation routes for bio-digestate are being explored such as coupling AD with thermochemical processes like hydrothermal carbonization (HTC), pyrolysis and gasification (Garlapalli, *et al.*, 2016; Kambo & Dutta, 2015).

2.4.3 Comparative Market Value of AD products

A study by Bastidas-Oyanedel & Schmidt (2018) compared the market value (product price /tonne) of the various products of AD. As illustrated in Figure 2.15, digestate and methane had the lowest market value with price ranges of USD 0 to 10 and USD 100 to 600/tonne for digestate and methane, respectively. Hydrogen had the widest price range of all the products analysed from as low as USD 600 up to 1800 USD/tonne. Butyric and Caproic acid had the highest product price range (USD 2 000 to USD 2 500) while the market price of the other volatile fatty acids ranged between USD 400 and USD2 200.

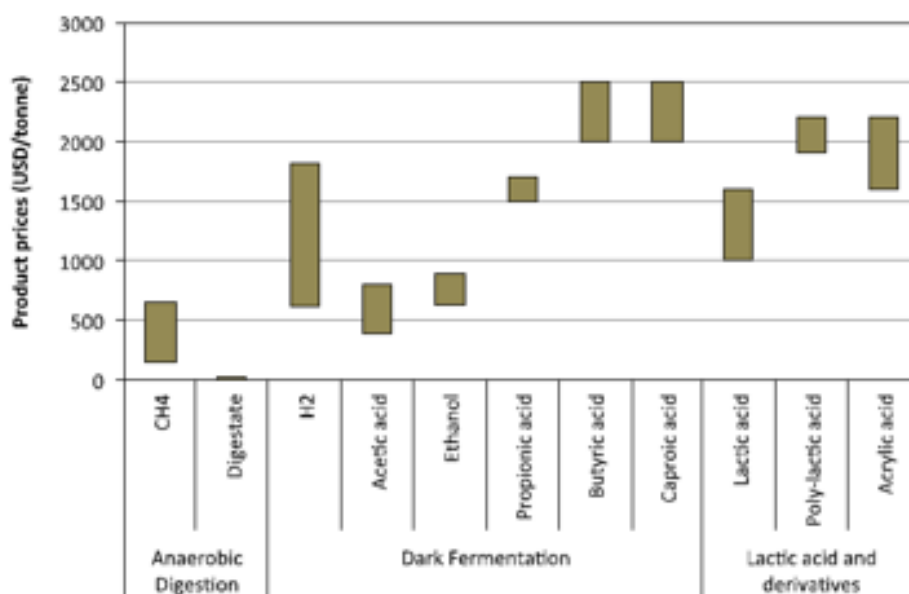


Figure 2.15 Market price ranges of main AD based products

Source: (Bastidas-Oyanedel & Schmidt, 2018)

2.5 Factors Influencing the Anaerobic Biorefinery Product Spectrum

This section discusses the factors that affect AD and their influence on the yield and relative distribution of the main products (biogas, hydrogen, VFAs) produced. Ongoing research shows that operating conditions within a biogas reactor affects the product spectrum as well as the yield of each product, producing either biogas or hydrogen as the main gaseous product and a group of VFAs whose relative distribution also depends on the operating conditions. Insight into how various process factors affect the product spectrum is thus important for maximising yields and for selecting desired final products.

2.5.1 Effect of Temperature on product spectrum

The temperature has a great influence on AD as it affects the rate of reaction, metabolic activities, process kinetics, process stability, and product yield. AD can take place under different temperature ranges, psychrophilic (10-25°C), mesophilic (30-39°C,) and thermophilic (50-64°C), (Mir, *et al.*, 2016). Generally, biogas production increases as temperature increases, with higher biogas yields obtained at thermophilic than mesophilic range. However, lower temperatures exhibit better process stability (Dhanalakshmi & Ramanujam, 2012; Babaee & Shayegan, 2011).

Numerous studies agree that increasing temperature increases hydrogen yields, however, beyond certain levels, further temperature increases result in a decrease in gas production (Ghimire, *et al.*, 2015). This is because high temperatures elevate the activity of enzymatic hydrogenase and inhibit the activities of hydrogen consumers leading to increased hydrogen production. On the other hand, too high temperatures induce the thermal breakdown of proteins and critical enzymes resulting in reduced hydrogen production (Ghimire, *et al.*, 2015; Khan, *et al.*, 2017).

Various study results show that temperature significantly influences VFA production rate and yield (Khan, *et al.*, 2016a). Mesophilic conditions are normally preferred and viewed as the most efficient and economical production route, as they demonstrate higher yields or yields very close to those obtained under thermophilic conditions (Zhou, *et al.*, 2017). Authors

including Yuan, *et al.*, (2011) and Zhuo, *et al.*, (2012) demonstrated that production rates and yield of VFAs increase as temperature increases within the psychrophilic and mesophilic ranges. Khan, *et al.*, (2016a) attributes this to an increase in solubility of carbohydrates and proteins at high temperatures and an upsurge in hydrolysis rates.

2.5.2 Effect of pH on product spectrum

Operational pH influences AD in many ways, including digestion rate, microbial population, metabolic pathways as well as product yield and composition (Mohammadi, *et al.*, 2012; Ghimire, *et al.*, 2016). Previous studies reveal that the optimal pH varies for each process stage of AD, for example, the ideal pH for methanogenesis lies between 6.5 and 8.5, while for hydrolysis and acidogenesis it is 5.5 and 6.5 respectively (Mao, *et al.*, 2015; Mir, *et al.*, 2016; Sawyerr, *et al.*, 2019). The optimum range reported for methane production lies between 6.8 and 7.4 (Mao, *et al.*, 2015). However, the optimal pH values reported by (Lama, *et al.*, 2012; Mondal & Biswas, 2012; Dhanalakshmi & Ramanujam, 2012) for AD of vegetable waste are in the range of 5.48 to 7.5.

The operational pH in AD process either drive metabolic pathways towards hydrogen production or shift towards hydrogen consumption reactions (Ahn Y, 2005). According to Khan, *et al.*, (2017), the optimal pH for hydrogen production in AD process is pH 5 - 7, and that below pH 5 inhibition of hydrogen production occurs. This range likely favours the activity of hydrogenases and is ideal for increased bacterial growth.

The pH is a critical parameter that controls the production of VFAs during acidogenic fermentation. Extreme acidic (< pH 3) or alkaline (> pH 12) conditions are known to inhibit acidogenic activities (Zhou, *et al.*, 2017). The optimal pH for VFA production is dependent on substrate type and operational conditions hence a wide range (pH 5.25 - 11) of optimal values are seen for different substrates (Zhao, *et al.*, 2017; Khan, *et al.*, 2016a). Author Zheng, and co-workers (2015) investigated the effects of pH (4-6) on FVW fermentation type, they observed that the VFA production varied with pH values. For example, at pH 4-4.5, the dominant fermentation route was ethanol and at pH 5.5-6.5, it was butyric type. Cheah, *et al.*, (2019) also demonstrated that VFA yield and composition vary with pH.

2.5.3 Effect of Hydraulic Retention time (HRT) on product spectrum

The (HRT) describes the average amount of time that the substrate and microbial biomass stays in a digester (Wainaina, *et al.*, 2019). The retention time chemically links to microbial growth rates and is a function of temperature, organic loading rate and substrate type (Khan, *et al.*, 2017). In general, a relatively longer hydraulic retention time favour higher biogas productivity. Nevertheless, shorter HRTs are desirable as they reduce required reactor sizes and consequently capital costs.

The variation in growth rates of hydrogen consumers and producers enables the use of HRT as a control parameter to increase hydrogen production by suppressing the growth of hydrogen consumers. However, Ghimire, *et al.*, (2015) and Khan, *et al.*, (2016a), states that HRT control alone is not sufficient to suppress methanogenic activities. Numerous studies concur that low HRTs favour hydrogen production; however, too short an HRT can also lead to biomass washout (Akinbomi & J, 2015; Khan, *et al.*, 2017; Li, *et al.*, 2017).

Increasing HRT increases VFA yield up to a certain limit, beyond this point any further increase reduces yields or causes process failure (Khan, *et al.*, 2016a). In their study, Khardenavis, *et al.*, (2013), observed efficient acidogenic fermentation of mixed food and fruit waste at an HRT of 1–3 days yielding a VFA composition of 50–75% acetic acid. Vegetable waste alone required an HRT of 9 days with a VFA composition of 45% acetic acid. Acidogenic fermentation of FVW by (Wu, *et al.*, 2015) using an HRT of 3 days and pH of 4, yielded 10–20 g/L of lactic acid. Research results by (Bolaji & Dionisi, 2017), suggested that HRT influences both VFA yields and the product spectrum of FVW.

2.5.4 Effect of Organic loading rate (OLR) on product spectrum

The OLR represents the amount of volatile solids that can be fed to a digester daily and is expressed in terms of mass per unit of volume and time e.g. (kg volatile solids (VS)/m³d) (Mir, *et al.*, 2016). A high OLR enhances different microbial species, decreases reactor volume, and reduces the energy required for heating. However, too high an OLR (overloading) can lead to accumulation of VFAs and ethanol, low pH, poor heat transfer, and inhibit digestion leading to low biogas yields or process failure (Mir, *et al.*, 2016; Li, *et al.*, 2017).

In their study (Shin & Young, 2005), observed that hydrogen production from food waste increased as OLR increased up to an OLR of 8 kg VS/m³d, but decreased considerably at 10 kg VS/m³d under thermophilic conditions. Under mesophilic digestion of fruit waste, (Liu, *et al.*, 2018)) found that the hydrogen yield increased with increasing OLR within the range of 2.5 to 10 kg VS/m³d. Another study observed that increasing the OLR (from 21.0 to 59.8 kg TVS/m³d) coupled with increasing the food waste (FW): organic fraction of municipal solid waste (OFMSW) proportion from 20:80 to 50:50 improved hydrogen yield (Campoy, *et al.*, 2017). However, increasing OLRs above 70.3 kg TVS/m³d and food waste (FW) percentage above 50 resulted in process destabilization due to an imbalance of the hydrolytic phase.

Several researches have shown that increasing OLR increases VFA yield up to a certain point beyond which the system becomes unstable (Jiang, *et al.*, 2013; Khan, *et al.*, 2016b; Khan, *et al.*, 2016a; Zhou, *et al.*, 2017). Production of VFAs increased with improving levels of OLR from 5 to 13 kg/m³d during a study conducted by (Lim, *et al.*, 2008), nevertheless, above 13 kg/ m³d the system became unstable. Similarly, (Jiang, *et al.*, 2013), observed an increase in VFA concentration from OLR of 5 to 11 kg total solid (TS)/m³d, however, at 16 kg TS/m³d the reactor was unstable. The fraction of acetate and butyrate increased with increasing OLR while the fraction of butyrate and propionate reduced at higher OLRs.

2.5.5 Effect of Feedstock Composition on Product spectrum

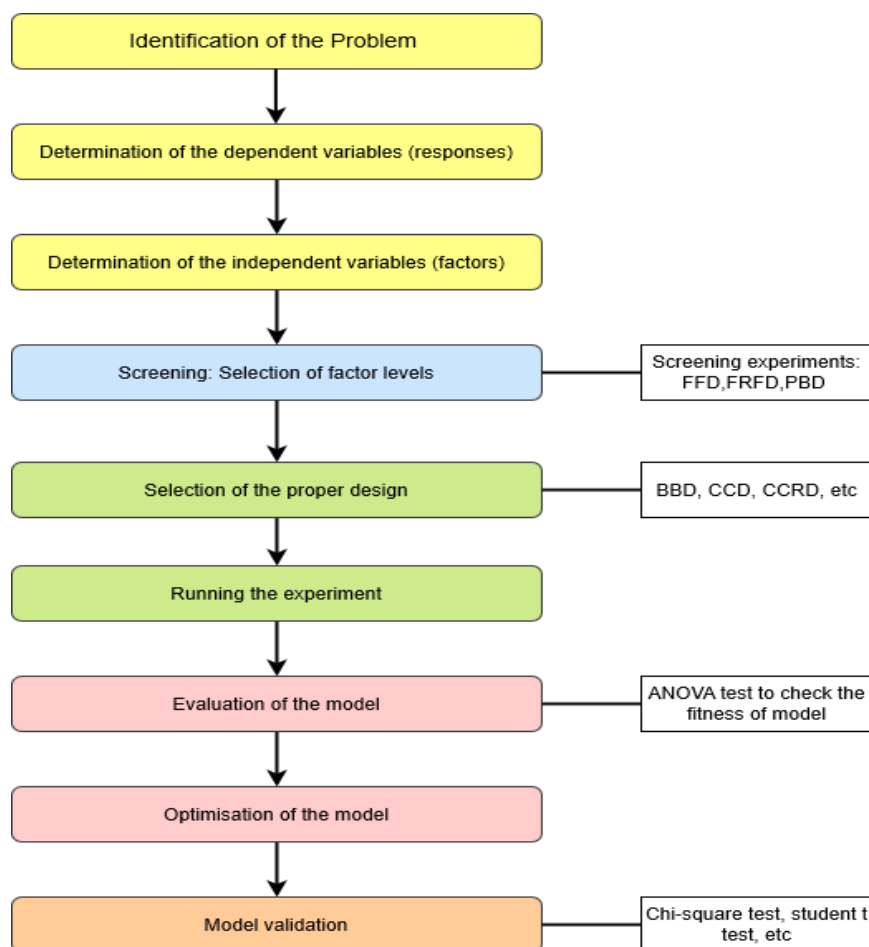
OFC affects the product yield, biodegradability and process stability of AD process. For instance, lipids have been seen to offer higher biogas potential (1, 43 g/L) compared to proteins (0, 92 g/L) and carbohydrates (0.83 g/L) (Das, *et al.*, 2019). However, the AD of lipid-rich substrates often leads to the accumulation of long-chain fatty acids resulting in process inhibition or failure (Das, *et al.*, 2019). Protein and carbohydrate-rich substrates, on the other hand, favour the production of VFAs instead of biogas and hydrogen. However, protein-rich substrates are also prone to ammonia inhibition, a phenomenon associated with the accumulation of chemicals, among which are ammonia (NH₃) and ammonium ion (NH₄⁺) (Atasoya, *et al.*, 2018). Biogas production from lignocellulosic biomass is limited due to the feedstock having low biodegradability necessitating feedstock pre-treatment. The organic fractions also tend to direct the metabolic pathways of the fermentation processes (Altamira, *et al.*, 2008). For example, carbohydrate –rich substrates, support the production of propionic

and butyric acid while protein-rich substrates favour valeric and iso-valeric production (Atasoya, *et al.*, 2018). Pre-treatment is one of the most important strategies employed to improve methane or hydrogen yields from substrates. Pre-treatment or fractionation is an important step in AD and is required to modify the structural composition of the lignocellulosic biomass to make the cellulose accessible to enzymes that transform it into fermentable sugars (Kratky L., 2015).

2.6 Optimisation via Response Surface Methodology

Optimisation of bioprocesses, often involves maximising the yield, product quality, or profits over the process conditions (Sekoai, *et al.*, 2017). It thus includes identifying key parameters that influence a process, the interactions between these parameters and finding the optimal set points for those key parameters (Balaji, *et al.*, 2018). There are several optimisation techniques available including One-Factor-at-a-Time (OFAT), Artificial neural network (ANN) and Response Surface Methodology. However, the review focused mainly on RSM as it is the most frequently applied experimental design for optimisation. This is because it allows for the evaluation of the effects of multiple parameters and their interactions, on one or more response variables (Aydar, 2018). Additionally, RSM models the system mathematically, saves time and money, as it reduces the number of experimental runs required (Aydar, 2018).

Researchers Balaji, *et al.*, (2018), Aydar, (2018), Uhoraningoga, *et al.*, (2018) summarised typical steps involved in process optimisation (Figure 2.16). Step 1 involves defining the problem and determining the dependent (responses) and independent (factors) variables. Step 2, the screening step, identifies variables with significant influence. In Step 3, the optimisation phase, significant variables identified in Step 2 are optimised *via* a polynomial model. Step 4 evaluates the polynomial model developed by testing the goodness of fit between the experimental data and the model. Step 5 validates the model by running experiments using the optimum conditions predicted by the model



Source: Adapted from (Aydar, 2018)

2.6.1 Step 1: Problem identification

Many optimisation designs begin with a specific research question (Step 1) in mind and a field of study to identify knowledge gaps and to find solutions that interest the researcher. Part of the current study reviewed some of the existing knowledge on factors that affect anaerobic digestion and product formation effect of process parameters on AD in order to identify possible relationships between process parameters (input variable) and the formation of several products and intermediates. In short, herein the experimental inputs and identify the response is done.

2.6.2 Step 2: Screening

Screening studies (Step 2) occur during the initial stages of optimisation in order to identify the factors that significantly influence the response variable from a large pool of potentially

significant factors. Screening designs study a huge number of factors in a minimal number of experimental runs (Jiju, 2014; Das & Dewanjee, 2018). Screening designs identify the main effects as well as the interaction effects of input variables. According to (Rahman, 2019), main effects refers to the effect of one independent variable on the response variable. While, an interaction effect, is the combined effect of two (low order interactions) or more (high order interactions) independent variables on a response. Screening designs commonly used include One Factor at a Time (OFAT) and Design of Experiments.

2.6.2.1 OFAT

OFAT approach is a traditional experimental approach commonly applied by researchers. Despite criticisms, scientists and engineers have often performed experiments involve changing only one factor at a time and holding all other factors constant. Critics of OFAT point out that the OFAT method does not study interactions between factors and is not ideal for numerous factors, as it is time-consuming (Abou-Taleb & Galal, 2018). Instead, statistically designed experiments that vary several factors simultaneously are more efficient when studying two or more factors of AD process (Uhoraningoga, *et al.*, 2018).

2.6.2.2 Design of experiment (DOE)

DOE is a multipurpose statistical tool used for planning, conducting, analysing and interpretation of experimental data (Bower, 2019; Jiju, 2014). Researchers use standard DOE screening designs to study the effect of multiple input variables on one or more response outputs in order to identify which input parameters and interactions significantly influence the response output (Rahman, 2019). The most widely applied DOE screening designs include full factorial, fractional factorial, Placket-Burman and Taguchi (Gundogdu, *et al.*, 2014). Compared to OFAT, DOE is more efficient, requires fewer tests and resources to obtain valid results. In addition, OFAT only detects main effects while DOE detects both main effects and interactions (Uhoraningoga, *et al.*, 2018; Das & Dewanjee, 2018).

2.6.3 Step 3 Model development using statistically designed experiments

A more in-depth optimisation design occurs after identifying the independent variables that are most significant during screening. In comparison to the screening design, the factor level ranges in optimisation experiments are narrower resulting in a smaller experimental domain. Several experimental designs are available for optimisation experiments, of which Box Behnken design

(BBD) and central composite design (CCD) are two of the main popular design methods applied in Bioprocessing research due to efficiency and cost savings (Zhu, *et al.*, 2010).

2.6.3.1 CCD

Developed by Box-Wilson, CCDs are a popular response surface design for building second-order polynomials (quadratic models) (Sahoo & Barman, 2012). They are based on 2-level factorial or fractional factorial design with centre points that are augmented with a group of axial points (also known as star points) that allow for estimation of curvature and generating the quadratic terms (Das & Dewanjee, 2018). Typically, a CCD contains three design points, namely, factorial, central and axial points. The factorial points represent the low and high levels of each factor, coded as -1, and +1, respectively, while the central point is coded 0 and represents the medium value (Adekele, *et al.*, 2019).

A cube can be used to represent a CCD (Figure 2.18) with the factorial points forming the corners of the cube consisting of factor level combinations coded -1 (min) and +1 (max). The central points, located at the centre of the cube (design space) representing replicate terms provide a good estimate of the experimental error. Axial points, having symmetry about the central point are located at a distance $\pm\alpha$ from the design centre, lie along the axes of the cube. Axial points introduce new extreme values for the max and min level of each factor (Sahoo & Barman, 2012; Ahn, 2015; Adekele, *et al.*, 2019).

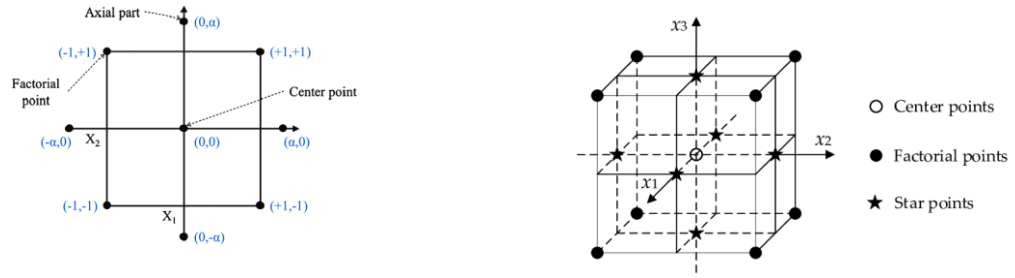


Figure 2.16 A two factor and three-factor layout for Central Composite Design

Source: (Wang, *et al.*, 2018)

The total number of experimental runs (N) needed to examine k factors for a CCD is determined using the equation (Adekele, *et al.*, 2019).

$$N = 2k + 2k + 1$$

Experiments of the full factorial design ($2k$) are situated at levels -1 and $+1$, those of the star design at levels $-\alpha$ or $+\alpha$ for each factor and the centre point at levels 0 . A CCD always contains twice as many star points ($2k$) as there are factors in the design (Sahoo & Barman, 2012). To achieve rotatability, the value of alpha (α) is determined from a number of runs the factorial.

2.6.3.2 BBD

BBD are a special class of three-level fractional factorial designs that are rotatable or close to rotatable, which enable modelling of 1^{st} and 2^{nd} order response surfaces (Gundogdu, *et al.*, 2014). Similar to central composite design, the Box Behnken design can adapt to the full quadratic model of the response surface design (Das & Dewanjee, 2018). However, unlike CCD, BB designs do not include any embedded factorial or fractional factor designs. The Box Behnken design ignores all the corner points, and the star points, which are extreme points in the design space. Instead, the treatment combinations for BBD are at the midpoints of the edges of the cube and at the centre (Figure 2.19) (Das & Dewanjee, 2018); (PennState, 2020)

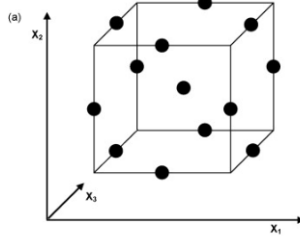


Figure 2.17 Box Behnken Design

Source: (Wang, *et al.*, 2018)

The number of experiments required for BBD is given by:

$$N = 2k(k-1) + C_0$$

Where k is the number of factors and C_0 . This design requires a minimum of three factors. It is also applicable for more than three factors; however, the design becomes more complicated. Additionally, BB designs require three levels per factor that are coded as -1 (low), 0 (middle) and $+1$ (high) (Gundogdu, *et al.*, 2014). For the same number of factors, a BB design requires less runs than a CCD to generate a response surface. Box Behnken design are generally less time consuming and economical compared to CCD as they require less runs than a CCD to generate a response surface (Wagner, *et al.*, 2014; Gundogdu, *et al.*, 2014).

2.6.4 Step 4: Model fitting

Data obtained from the optimisation experimental runs is used for model fitting through mathematical and statistical methods. Model fitting entails developing a mathematical model that describes the relationship between the output response (Y) and the input variables using a polynomial equation. The polynomial equations frequently used for modelling relationships between the response variable (Y) and input factors include linear, second-order, and the quadratic model. A linear model (Equation 2.1) is the simplest polynomial model containing only linear terms describing linear relationships between input factors and response outputs. A second order model (Equation 2.2) consists of a first order model with an additional term to describe interactions between factors, if any exist. Lastly, the quadratic model or second-order polynomial includes linear terms for all factors, squared terms for all factors, and products of all pairs of factors. The quadratic model captures main effects (coefficient of the terms in the first order), interaction effects (product of linear terms) and quadratic effects (coefficient of square of linear terms (Das & Dewanjee, 2018).

$$Y = b_o + \sum_{i=1}^k b_i X_i + \varepsilon \quad (2.1)$$

$$Y = b_o + \sum_{i=1}^k b_i X_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} X_i X_j + \varepsilon \quad (2.2)$$

$$Y = b_o + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} X_i X_j + \varepsilon \quad (2.3)$$

Equations (2.1-2.3) are multiple linear regression models with k independent variables (Wu, 2013). Where Y , is the dependant output response, b_o the model intercept, i is the factor number ($1 \dots k$), and X_i and X_j are independent input variables. The terms b_o , b_i , b_{ii} , and b_{ij} are the regression coefficients for intercept, linear, quadratic, as well as interaction terms, respectively (Das & Dewanjee, 2018). The error term, ε represents a statistical error having a normal distribution with zero mean and variance σ^2 (Wu, 2013).

From the experimental response surface values (Y_{exp}), obtained from the optimisation experimental runs, using the experimental variables ($X_{i\text{exp}}$) as inputs, the coefficients of regression and intercept (b_o , b_i , b_{ii} , and b_{ij}) are estimated using regression techniques such as least-squares technique (Lekou, 2013; Arora, 2012).

2.6.5 Step 5: Evaluation of the model

Model evaluation involves checking the significance and adequacy of the model using conventional statistical approaches such as analysis of variance (ANOVA) (Elazazy, 2017). A common method used to check model accuracy is the coefficient of determination (R^2) which is the ratio of the regression sum of squares SSR to the total sum of squares (SSY). The coefficient of determination ranges between 0 and 1, where 1 generally represents a good model. Alternatively, the adjusted coefficient of determination (R^2_{adjusted}) is applied, given by: $R^2_{\text{adjusted}} = 1 - \text{MSE} (\text{SSY}_n - 1)$, where n signifies the number of data points and MSE denotes the mean square error. The best model corresponds to the one with the highest R^2_{adjusted} (Jamshidnezhad, 2015). The least-squares technique determines the model (linear or second-order) that best represents the relationship between the input variables and the output response by minimizing the residual error obtained from the sum of squared deviations between the actual and the estimated responses (Jamshidnezhad, 2015).

2.6.6 Step 6: Model Validation

The response surface method generates a theoretical model that represents the relationship between dependant and independent variables. Commonly, model development seeks to replace experimentation with model predictions; as such, model predictions require some degree of accuracy. Thus, it is necessary to conduct experiments in order to verify the reliability of the theoretically developed models under optimum conditions. Commonly applied tests to determine accuracy include Chi-Square, F-tests, and T-tests. Alternatively, the experimental error can be determined between theoretical and experimental values (Aydar, 2018).

2.7 Discussion and Conclusion

The literature review revealed that globally, vast amounts of fruit and vegetable waste are generated annually, showing also and that FVW occurs along the entire food supply chain (FSC). The review also evidenced that waste management will pose serious challenges in the near future, particularly in urban areas where space is limited and the population is rapidly growing, driving the urgent need to devise sustainable waste management strategies.

A considerable amount of research effort has gone into developing innovative waste technologies to overcome the limitations of conventional waste management including environmental degradation, GHG emissions as well as water, ground and air pollution. At the heart of the waste management technologies currently under development is valorisation/beneficiation of waste, i.e. viewing waste as a valuable resource and striving to generate value added products from it. Of the waste valorisation options available e.g. animal feed, extraction of specific compounds and substrate for microbial growth, this study elected to focus on Waste to Energy Technologies (WtEs) due to their ability to simultaneously produce renewable energy, another currently trending topic owing to the forecasted global energy crisis. Three main WtE conversion pathways were identified namely, thermochemical, biochemical and electrochemical routes, with the former two pathways being more established. The conversion routes most suited for FVW treatment owing to the composition of the substrate (high moisture and organic matter content) are biochemical and electrochemical routes. Hydrothermal carbonization was identified as the thermochemical route most suited for treatment of wet waste. The review illuminated that various conversion pathways can be used to generate a wide spectrum of bio-products including liquid products (bioethanol, volatile

fatty acids,) gaseous products methane and hydrogen and solid products (digestate and hydro-char) from fruit and vegetable waste.

Anaerobic digestion, in particular, can generate a wide array of products on its own (methane, hydrogen, VFAs and digestate), making AD technology an ideal centrepiece for biorefineries. A look at the market value of the AD products showed that hydrogen and VFAs are of more economic value than methane. However, low hydrogen yields and challenges in extracting the VFAs presents challenges to scaling up production, thus more research is required before commercial production becomes viable. It is also possible to couple AD technology with the other conversion routes to produce more value added products and increase resource utilisation. This study selected coupling AD technology to hydrothermal carbonization to (i) demonstrate different conversion routes, i.e. biochemical and thermochemical and (ii) diversify the product range (solid, liquid and gas).

There is strong evidence that process parameters affect the product yield and composition of products generated by AD and that optimisation of process parameters leads to enhanced product yield.

2.8 References

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3. Investigating the Product Spectra of Anaerobic Biorefinery of FVW: Bio-hydrogen and VFA production via Dark Fermentation

Abstract

This research used inoculum pre-treatment to investigate the development of hydrogen and volatile fatty acids through AD (dark fermentation) of mixed fruit and vegetable waste. The effects of four inoculum pre-treatment methods, including heat, base, acid, and heat-base, on hydrogen and VFA development, were studied. The results show that the pre-treatment process used affects hydrogen and VFA production, as well as the fermentation pathway. Acid pre-treatment produced the maximum hydrogen yield (142,74 Nml/g VS) and hydrogen content (54 %), while a minimum hydrogen yield of 0,90 Nml/ gVS was observed under heat pre-treatment. The hydrogen yields of the studied pre-treatments differed significantly ($p < 0.001$). Acid>heat-alkali>alkali>heat was the order of pre-treatment efficacy in terms of hydrogen yield enhancement, from highest to lowest. The results also show that acid, alkaline, and heat-alkali pre-treatments are successful in increasing hydrogen production, though heat pre-treatment was only effective for a limited time in enriching hydrogen production. Only acid and heat-alkali pre-treatment successfully inhibited methane production while small amounts of methane were detected in the other pre-treatments. Valeric acid was the main VFA produced under all pre-treatments as well as the control. The highest valeric acid fraction observed was 86,1% under acid pre-treatment, an increase of 65 % compared to the control. The results show that the valeric acid fermentation pathway favoured hydrogen production. Butyric, propionic, and acetic acid were observed in small amounts only in the control, heat, and alkaline pre- treatments.

3.1 Introduction

Although AD technology is a more sustainable and environmentally responsible alternative to landfilling for organic waste management, there are a few environmental and economic issues with traditional anaerobic digestion plants, which mainly generate low-value products like methane and digestate (Bhatt, *et al.*, 2020) . Recently, science has moved away from methanogenesis in favour of developing other intermediate metabolites such as hydrogen, alcohols, and carboxylic acids, including lactic, succinic, and volatile fatty acids, which are in greater demand and have a higher economic benefit than methane-rich biogas (Kleerebezem,

et al., 2015; Wainaina, *et al.*, 2019; Bhatt, *et al.*, 2020) . For example, volatile fatty acids are well-known precursors with a broad range of industrial uses, including biofuels, industrial biochemicals, and pharmaceuticals (Ramos-Suarez, *et al.*, 2021) . In reality, Righetti, *et al.*, (2020) argue that the processing of bio-based molecules from agro-waste has the potential to be more cost-effective than biogas.

Because of the multi-step nature of anaerobic digestion, in which the four stages of AD, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, occur in sequential and parallel pathways, the harvesting of intermediate products produced at the various stages of AD allows for the development of various products (Coma, *et al.*, 2017; Bhatt, *et al.*, 2020) . Stage 2 produces short-chain volatile fatty acids and alcohols, for example, while Stage 3 produces acetic acid, hydrogen, and carbon dioxide (Figure 3.1). The final stage, methanogenesis, releases the more well-known components of AD processes, methane, and carbon dioxide.

The use of anaerobic digestion to generate bio-hydrogen and VFAs through dark fermentation is gaining popularity (Stage 1-3 of AD). Via various mechanisms such as pre-treatment of bacterial inoculum and modification of operational parameters such as pH, temperature, and organic loading volume, the synthesis of methane is inhibited and the reaction proceeds only up to Stage 3 as shown in Fig 3.1, thus favouring bio- hydrogen formation (Kothari, *et al.*, 2017; Srisowmeya, *et al.*, 2020) . Since VFAs and hydrogen are used as precursors for methane production in traditional AD processes and are consumed during methane production, selective inhibition of methanogens is essential to avoid VFA and hydrogen depletion (Wainaina, *et al.*, 2019). Although various studies achieved successful production of bio-hydrogen via dark fermentation using different inoculum pre-treatment methods including aeration, heat shock, ultrasonification, alkali and acid pre-treatment to inhibit methane production, there is no consensus on the best inoculum pre-treatment method (Kothari, *et al.*, 2017; Srisowmeya, *et al.*, 2020) . According to Cappai, *et al.*, (2018), this is because the efficacy of an inoculum pre-treatment method is affected by numerous physical, chemical and biological factors including substrate type, type of inoculum, and operating conditions. Pachapur, *et al.*, (2019), further stresses that the best pre-treatment method for enriching hydrogen-producing bacteria varies for different inoculum sources, probably because different inoculums have their own unique bacterial structures and fermentation pathways.

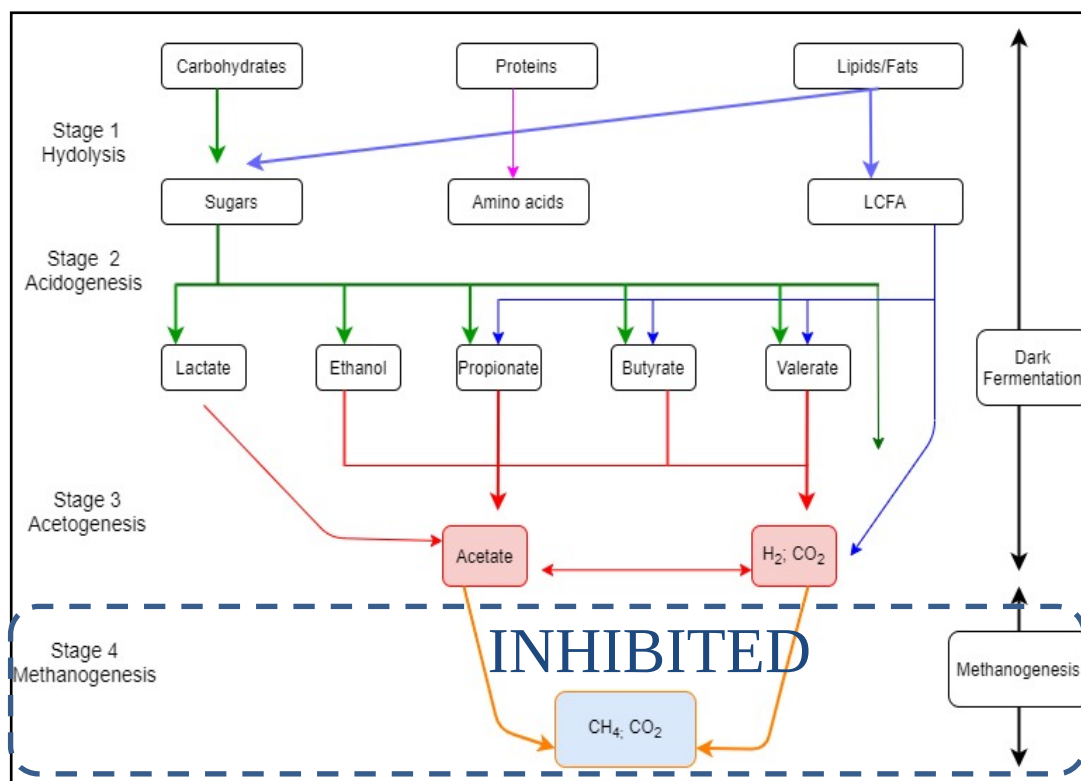


Figure 3.1 Products formed at the different stages of AD and inhibition of methanogenesis

Source: (adapted and modified from (Ghimire, *et al.*, 2015))

Thus, the overall objective of this study was to investigate the effectiveness of four different inoculum pre-treatment methods for enhancing hydrogen and VFA production via dark fermentation of mixed fruit and vegetable waste. The specific study objectives were to:

- (i) Investigate the influence of inoculum pre-treatment on biogas composition and hydrogen content of FVW under dark fermentation
- (ii) Investigate the influence of inoculum pre-treatment on hydrogen yield
- (iii) Investigate the influence of inoculum pre-treatment on VFA yield and composition
- (iv) Compare the effectiveness of the different pre-treatment methods to enhance hydrogen production.

3.2 Materials and Methods

Laboratory anaerobic batch tests were conducted to investigate hydrogen and VFA production through dark fermentation of fruit and vegetable waste using different inoculum pre-treatments. This section describes the research materials and methods used in conducting the experiments.

3.2.1 Research materials

The research materials utilised in conducting the anaerobic batch experiments are described here, including the raw materials, chemical reagents, and experimental instruments.

3.2.1.1 Feedstock

Mixed fruit and vegetable waste collected from a waste bin at Silverton Fruit Sop (25°44'02.2"S; 28°18'55.5"E), a fruit and vegetable shop located in Pretoria was used as feedstock for the dark fermentation tests. The mixed FVW (Figure 3.2) consisted mainly of peels, seeds, and trimmings of fresh fruit and vegetables such as pineapples, lettuce, tomatoes, peppers, apples, onions and oranges leftover during the preparation of pre-cut vegetable and fruit salads. The FVW was blended using a household electric food blender (Kambrook, 500W Glass Jug Blender) to reduce particle size and homogenize the mixture (Figure 3.3) and thereafter stored in a freezer at -4°C until time of use.



Figure 3.2 FVW after blending



Figure 3.3 Collected FVW

3.2.1.2 Inoculum

Liquid digestate (Fig 3.4) was collected from a commercial biogas plant situated in Bronkhorstspuit, Pretoria, to inoculate the AD batch tests. The inoculum was collected in a 20

L bucket two days before running each experiment, and kept in a fridge at -4 °C until the time of use.



Figure 3.4 Liquid digestate used as inoculum source for the experiments

3.2.1.3 Feedstock and inoculum characterisation

Since anaerobic reactors are fed based on the TS or VS content of the inoculum and substrate, the TS and VS content of the FVW substrate and inoculum was determined according to EPA method 1684: Total, Fixed, and Volatile Solids in Water, Solids, and biosolids (EPA, 2001). The pH of the substrate and inoculum was also measured using the HACH 1682 pH probe. Table 3.1 summarises the characteristics of the FVW and inoculum.

Table 3.1 Mixed Fruit and Vegetable Waste and Inoculum Characteristics

Parameter	Unit	FVW	Inoculum
TS	%	11,42	2,48
VS	%	1,45	10,25
VS/TS	%	58,76	89,75
pH		6,6	8

3.2.1.4 Experimental device automated methane potential test system (AMPTS) (II)

The study employed the Bioprocess Control AMPTS II), an anaerobic batch testing instrument used widely for measuring the bio-methane potential (BMP) or bio-hydrogen potential (BHP) of organic substrates (BioprocessControl, n.d.). The system consists of 15 x 500 mL glass bottle reactors housed in a heating water bath (A) as shown in Fig 3.5. The 15 glass bottle reactors connect to a carbon dioxide absorption unit (scrubber) (B), which consists of 15 x 100 mL glass bottles filled with a blue solution made up of 80 mL of 3 M NaOH and 0.4 % Thymolphthalein pH indicator. The absorption unit absorbs carbon dioxide from the biogas produced leaving purified gas consisting of methane or hydrogen, measured at the gas counter. The flow cell gas counter (C) uses liquid displacement to measure the volume of methane/hydrogen gas produced in each reactor bottle. The gas counter connects to a laptop equipped with the Bioprocess Control software that records the gas production data and controls the experiment (BioprocessControl, n.d.).

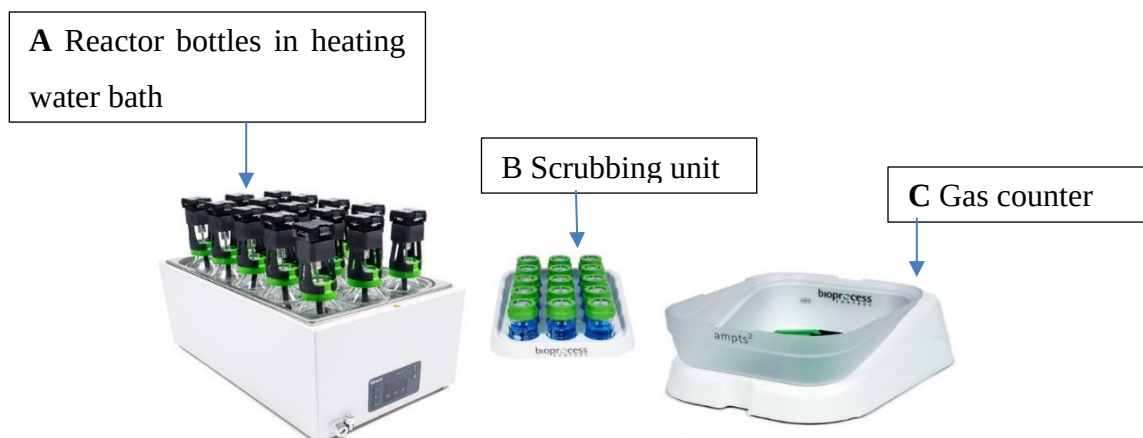


Figure 3.5 Automated Methane Potential Test used for dark fermentation batch tests

3.2.2 Experimental methods

Anaerobic dark fermentation batch tests using four different inoculum pre-treatment methods were conducted at a mesophilic temperature of 37 °C and ran for 15 days. The experimental methods employed including the experimental design and procedure are described below.

3.2.2.1 Experimental design

The study employed Randomized Complete Block Design (RCBD) for the experimental batch dark fermentation tests. The experimental design consisted of three blocks (representing replicates) and four treatments denoted T1-T4, and the control (C) (Table 3.2). Each block consisted of five AD batch tests with different treatments applied to them, so in total 15 batch tests were set up. Table 3.2 and Fig 3.6 show that the four treatments (T1-T4) were randomly assigned within each block, with each block having its own unique randomizations. Secondly, no treatment was repeated within a block.

Table 3.2 Experimental layout based on RCBD

BLOCK	TREATMENT				
1	C	T4	T3	T2	T1
2	T3	T2	C	T1	T4
3	T1	C	T4	T3	T2

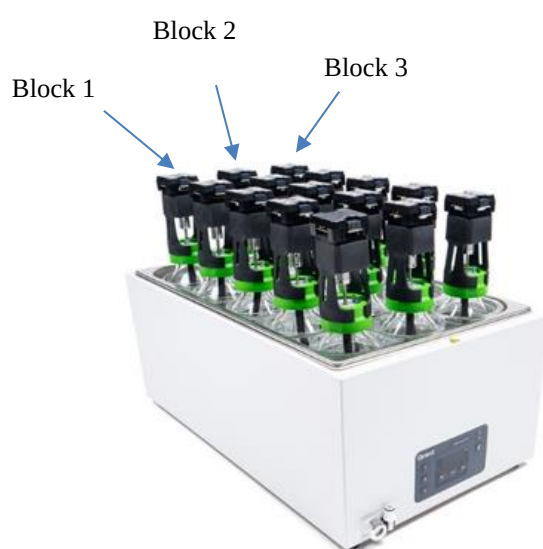


Figure 3.6 Pictorial view of the experimental set up on the AMPTSII

3.2.2.2 Experimental procedure

The experimental procedure is summarised in the 5 steps below:

Step 1: Inoculum pre-treatment

Firstly, different pre- treatments (T1-T4) were applied to the parent inoculum before inoculation of the substrate to examine the shift in product spectrum caused by manipulating the bacterial inoculum. The four pre-treatments are explained in Table 3.3.

Table 3.3 Description of inoculum pre-treatment methods applied

TREATMENT	TREATMENT PROCEDURE	REFERENCE
T1(HEAT)	Inoculum was heated at a constant temperature of 90 °C for 30 min using a pre-heated water bath	Ghasemian, <i>et al.</i> , (2019) and Zagrodnik & Seifert (2020),
T2 (BASE)	The pH of the inoculum was increased to pH 12 using 4 M NaOH and maintained for 24 h. After 24 hours, the initial pH was readjusted to pH 6.5 using H ₂ SO ₄ (98 %).	Rafieenia, <i>et al.</i> , (2018); Amin, <i>et al.</i> , (2019)
T3 (ACID)	The pH of the inoculum was lowered to pH 4 using H ₂ SO ₄ (98%) and maintained for 24 h following. The pH of the inoculum was lowered to pH 4 using H ₂ SO ₄ (98%) and maintained for 24 h following which the pH was increased to pH 6.5 by adding 4 M NaOH	(Amin, <i>et al.</i> , 2019; Wongthanate, <i>et al.</i> , 2014)
T4 (HEAT-BASE)	The inoculum was subjected to alkali pre-treatment (pH 12) followed by heat treatment (90 ⁰ C for 30 min) as described previously	Ghasemian, <i>et al.</i> , (2019) and Rafieenia, <i>et al.</i> , (2018)

Step 2: Loading reactor bottles

Step 2 was to load the 15 x 500 ml reactor bottles with 400g (or 400ml assuming density of 1g/mL) of reactant consisting of the pre-treated inoculum and substrate (FVW). Bioprocess Control (2016) recommends a maximum loading of 400g to avoid foaming problems. This created a working volume of 400 ml and a 200 ml headspace made up of 100 ml reactor volume headspace plus 100 ml volume of the connecting tubes. Using Equation 3.1- 3.4, the amount of inoculum (M_i) and FVW substrate (M_s) added to each reactor was calculated using an inoculum volatile solid (VS_i) to substrate volatile solid (VS_s) ratio (I/S) of 2:1 and total reactant amount of 400 g.

$\frac{M_i \times VS_i}{M_s \times VS_s} = 2$3.1	
$M_i + M_s = 400$3.2	
$M_i = \frac{800 \times VS_s}{VS_i + 2VS_s}$3.3	
$M_s = 400 - M_i$3.4	

Using the above equations, the AMPTS II Software calculates the amount of inoculum (M_i) and substrate (M_s) to be loaded to each reactor as well as the amount of volatile solid added. The results of the calculations are shown in Table 3.4 showing the amount of inoculum and substrate loaded in the reactors.

Table 3.4 Contents of reactor bottles

Condition	<i>Dark fermentation experimental conditions</i>				
<i>Factor level</i>	Control	Heat	Base	Acid	Heat-Bas
<i>Temp</i>	37 °C				
<i>I/S</i>	2				
<i>Inoculum added</i>	5,52 g VS/373,07 g				
<i>Substrate added</i>	2,76 g VS/26,93 g				

- *Step 3: Connecting the reactors*

After loading the glass reactor bottles were placed in the heating water bath. Each reactor was then closed with a gas-tight rubber stopper which had three outlet ports. An aluminium cap mounted with power supply cables was used to seal the reactor.

- *Step 4: Purging*

In Step 4, the headspace of each reactor was flushed with a carbon dioxide and nitrogen (20/80) gas mixture for a period of one minute at a flow rate of 1 m³/min at a pressure of 0.1 bar to purge oxygen and thus create an anaerobic environment within the reactors.

- *Step 5: Start Up*

After purging, the water bath temperature was set to 37° C (mesophilic) and the motor speed was set to 180 rpm using the AMPTS software. Once the desired temperature inside the water bath was reached the experiment was started using the AMPTS II software.

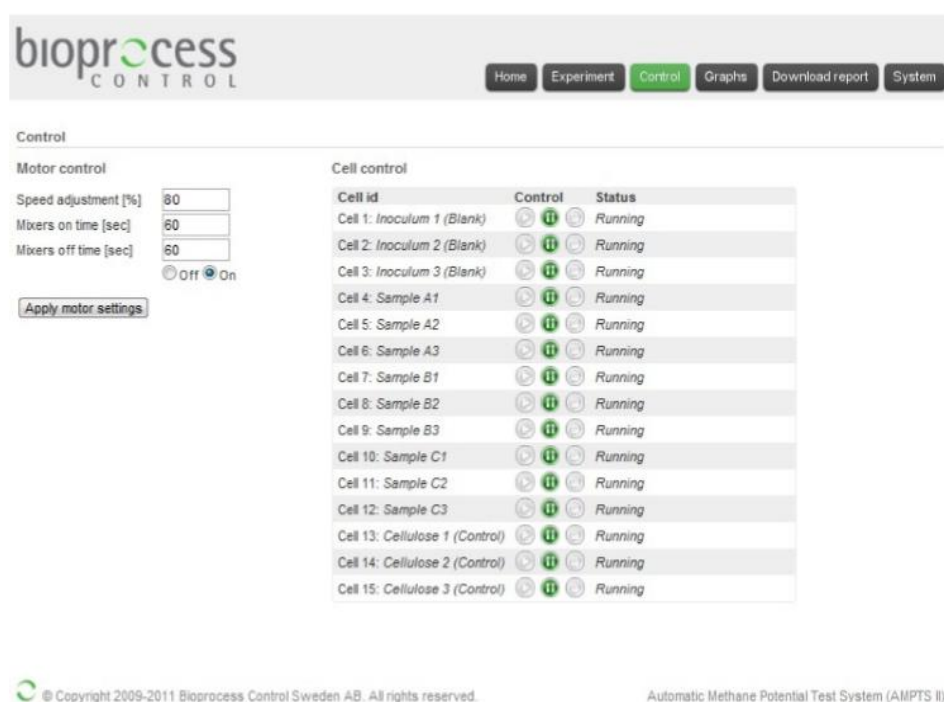


Figure 3.7 Bioprocess Control Start- up Window

3.3 Analytical Methods

3.3.1 Gas analysis

In-line biogas composition was carried out every second day using the GeoTech Biogas 5000 biogas analyser coupled with a hydrogen sensor. The biogas analyser measured the percentage of methane (CH₄), carbon dioxide (CO₂), oxygen (O₂), and other trace gases (ammonia, carbon monoxide, and hydrogen sulphide) in the biogas while the hydrogen sensor detected the presence of hydrogen (H₂). However, since the hydrogen sensor only detected hydrogen up to

a maximum threshold of 2 000 ppm, the hydrogen content was calculated by difference: $H_2 \% = 100 \% - CH_4\% - CO_2\% - O_2\% - \text{trace gases } \%$.



Figure 3.8 Geotech Biogas 5000 analyser used for gas analysis

3.3.2 VFA analysis

On Day 15, the last day of the experiment, 15 mL aliquots of the reactor broth were withdrawn for each pre-treatment T1-T4 and control (i.e. 5 samples were taken, one sample each for every pre-treatment applied). After sampling, the samples were stored inside a freezer at -4°C until time for analysis. The HACH TitraLab AT1000 Series analyser measured the pH of the reaction broth.

The volatile fatty acids and alcohols present were analysed using an HPLC (Agilent, n.d.). The HPLC analyses were conducted following procedures described by Vasconcelos de Sa, et al., (2011). A temperature of 55 °C under isocratic conditions. The mobile phase was made up of 0.005 mol/L solution of H₂SO₄. The mobile phase solution was filtered using a 0.45 µm membrane and degassed for 10 minutes before use. The samples were also filtered with a 0.20 µm membrane before injection. An injection volume of 20 µL and flow rate of 1.0 mL/min were used and the VFAs were detected using UV–Vis detector at 210 nm.

3.3.3 Statistical analysis

Statistical analysis of the collected experimental data was conducted using IBM SPSS Statistics software package. A one-way analysis of variance test (ANOVA) set at a 95% confidence level ($p < 0.05$) was used to analyse the statistical significance of treatments.

3.4 Results and Discussion

The section presents the results obtained from the experimental investigation on the effects of inoculum pre-treatment on hydrogen and VFA production from dark fermentation of fruit and vegetable waste. The study investigated the effect of heat, alkali, acid, and combined alkaline-heat pre-treatment on biogas production and composition, hydrogen production as well as the effectiveness of the pre-treatment methods to enhance hydrogen production. Data on biogas and hydrogen production is attached in Appendix A1.

3.4.1 Effect of inoculum pre-treatment on biogas production

The time profile of cumulative biogas yield over the 15-day experimental period for all the four pre-treatment methods including the control is shown in Figure 3.9.

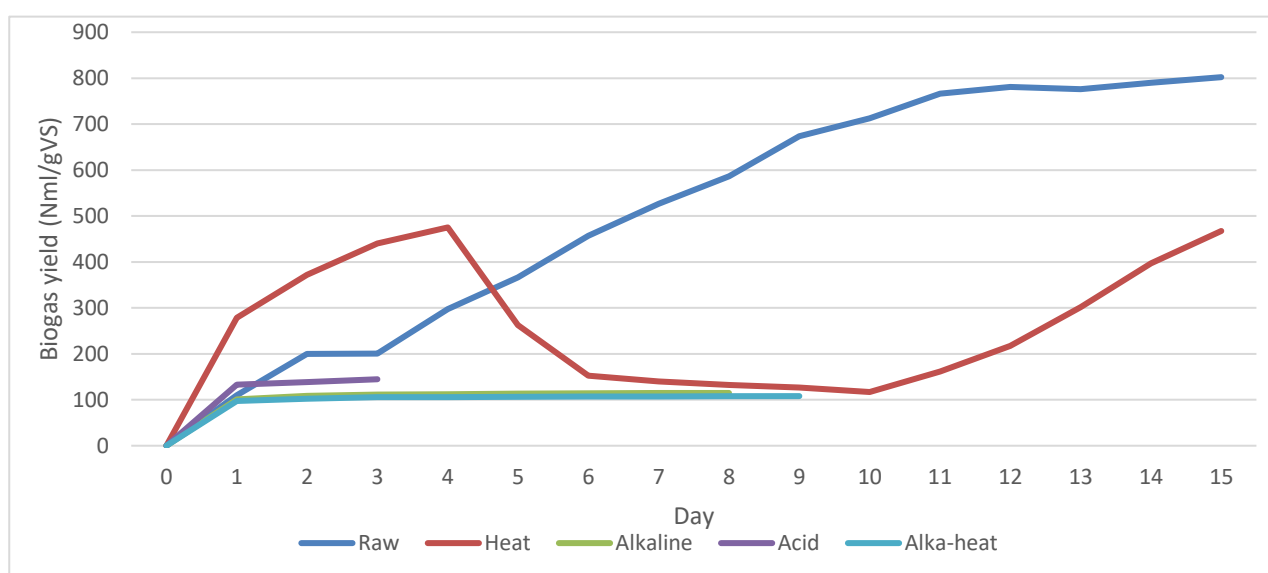


Fig 3.9 Cumulative biogas yield on Day 15 under different inoculum pre-treatments

The cumulative biogas yield graphs of Fig 3.9, is evidence that inoculum pre-treatment influenced the amount of biogas produced. A maximum biogas yield of 802 Nml/gVS was

obtained from the control and the lowest yield (107, 54 Nml/gVS) was observed for the acid pre-treatment. All the cumulative biogas yield profiles, except heat treatment, showed a steady increase in gas production until reaching a maximum. However, the biogas production curve for heat pre-treatment showed fluctuations in gas production throughout the run period, similar to the observations made by Manunga, *et al.*, (2019) , who reported a zig-zag decrease in biogas production curves after applying chemical and heat pre-treatment to the inoculum. A possible explanation for the drop in gas production after Day 4 is the consumption of intermediary gaseous products (H_2 and CO_2) formed during the acidogenesis stage and its subsequent conversion to methane and carbon dioxide, giving the increase in gas production seen from Day 10. This also points to likely changes in the active dominant microbial population from hydrogen producers to hydrogen consumers'/methane producers as suggested by (Manunga, *et al.*, 2019).

3.4.2 Effect of inoculum pre-treatment on biogas composition and hydrogen content

The biogas composition was analysed over the 15-day experimental period (Figure 3.10a-e) on every second day (Raw data attached in Appendix A2). The graphs show that the biogas composition for all treatments varied with time. For the control, the biogas was mainly composed of methane and carbon dioxide. The methane content increased with time, a common feature seen in biogas experiments, rising from 40 % to a maximum of 72 %. The methane percentages observed fall within the ranges reported in the literature for AD of fruit and vegetable waste (Bouallagui, *et al.*, 2003; Edwigs, *et al.*, 2018; Kell, 2019; Maile & Muzenda, 2014). No or little hydrogen was detectable over the 15-day period as expected.

There was great variation in the biogas composition of heat pre-treated inoculum. During the first six days of reaction, the biogas produced had a high carbon dioxide content (66 -88 %), a hydrogen content of 7.7 to 16.7 % , and negligible methane production. However, from Day 8, the carbon dioxide and hydrogen content decreased while the methane content increased from 13 % to 76.8 %, the highest methane content observed for all pre-treatments. By Day 15 no hydrogen was detectable, indicating that inhibition of hydrogen consuming methanogens had ceased, the metabolic pathway was now fully proceeding to Stage 4 (bio-methanation) instead of stopping at Stage 3 (Dark fermentation). This means heat pre-treatment had short-term positive effects on hydrogen production, a shortcoming of inoculum pre-treatment pointed out by Abubackar, *et al.*, (2019) who reported that pre-treating inoculum to inhibit hydrogen

consumers is not effective in the long term. Additionally, Abubackar, *et al.*, (2019) suggested that repeated pre-treatment is necessary to achieve sustained bio-hydrogen production. Scholars Luo, *et al.*, (2010), also support the view that inhibition of hydrogen consuming bacteria can be temporal, adding that after some time, spore-forming hydrogen consuming bacteria can reactivate and new stable microbial communities, adapted to the environment can form. This helps explain the gas fluctuations observed in the heat pre-treated biogas yield curves of Figure 3.9, and the shift in gas composition from hydrogen production in the first few days (up to Day 6) of the experiment, to detection of methane gas from Day 10.

The biogas composition for alkaline pre-treatment showed little variation in gas content, consisting of mainly carbon dioxide and hydrogen, which is consistent with product formation at the acetogenesis stage (stage 3) as seen in Fig 3.1. The carbon dioxide content dropped from a maximum of 85% on Day 2 to 37.75 % on Day 8. The hydrogen content gradually increased from 3.6 % up to a maximum of 48% on Day 8, after which gas production ceased. Small amounts of methane (7.5 - 13 %) were detected throughout the 8-day run period, evidence that methanogens were not fully destroyed by alkaline pre-treatment. Luo, *et al.*, (2010), also detected small amounts of methane in alkaline pre-treated inoculum when producing hydrogen from dark fermentation of cassava under thermophilic conditions .

Similar to alkaline pre-treatment, the biogas generated under acid pre-treatment consisted mainly of carbon dioxide and hydrogen. However, a lower carbon dioxide content was observed (40-50 %) and the reaction stopped after only 3 days. The hydrogen content was also higher, ranging between 46 and 54 %, the highest hydrogen content observed of all the pre-treatments investigated. Additionally, no or little methane was detected indicating that acid pre-treatment had fully inhibited the activity of methanogens. Alkali-heat pre-treatment produced biogas consisting mainly of carbon dioxide and hydrogen with no methane being detected during the 9- day run period. The carbon content decreased from a maximum of 84 % to 60 %, and the hydrogen content was between 11 and 34 %.

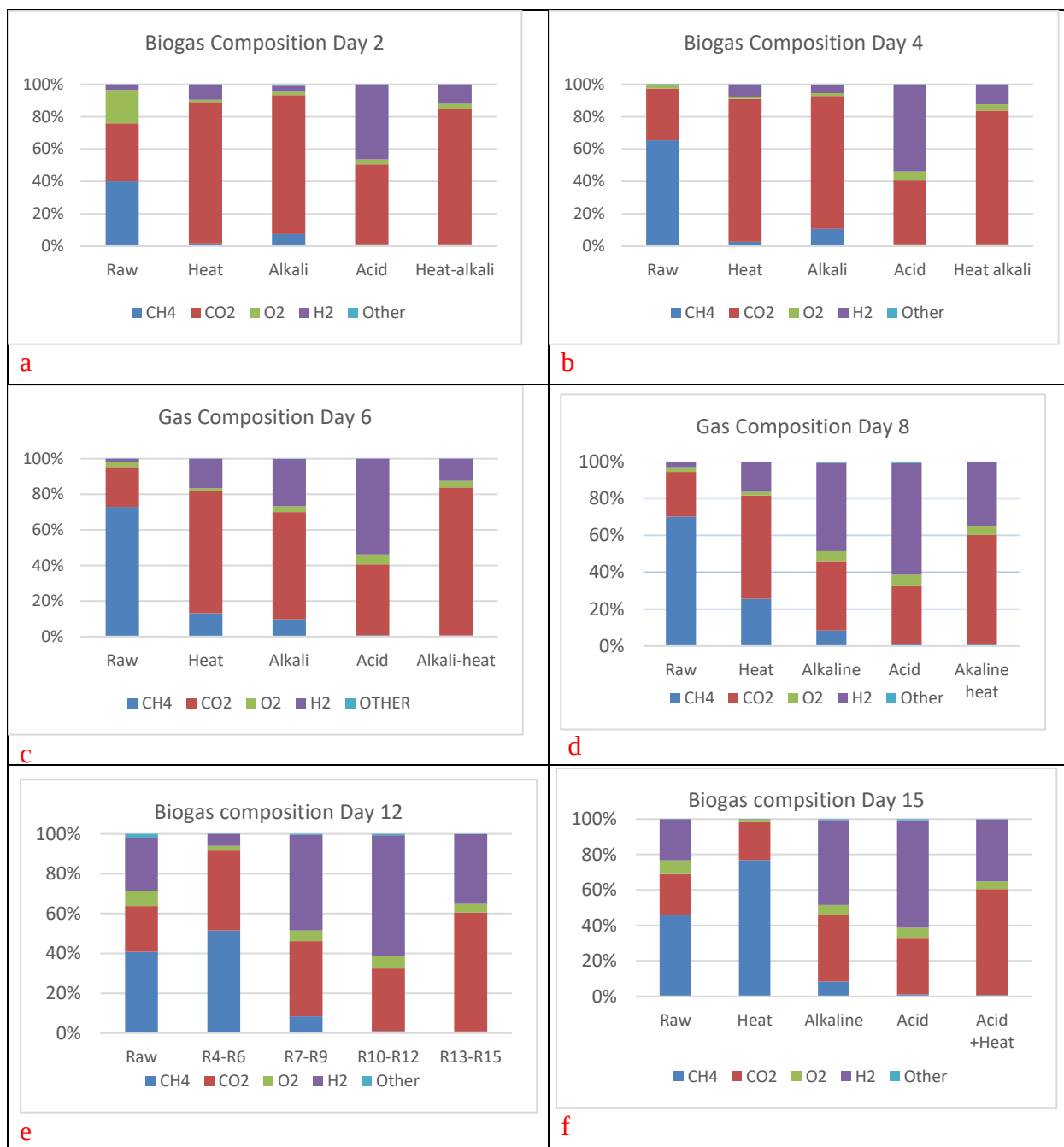


Fig 3.10 Variation in biogas composition under different inoculm pre-treatments from Day 2- Day 15

3.4.3 Effect of inoculum pre-treatment on hydrogen yield and content

The cumulative hydrogen yield time profile and mean hydrogen yield bar chart is shown in Fig 3.11 and Fig 3.12 respectively.

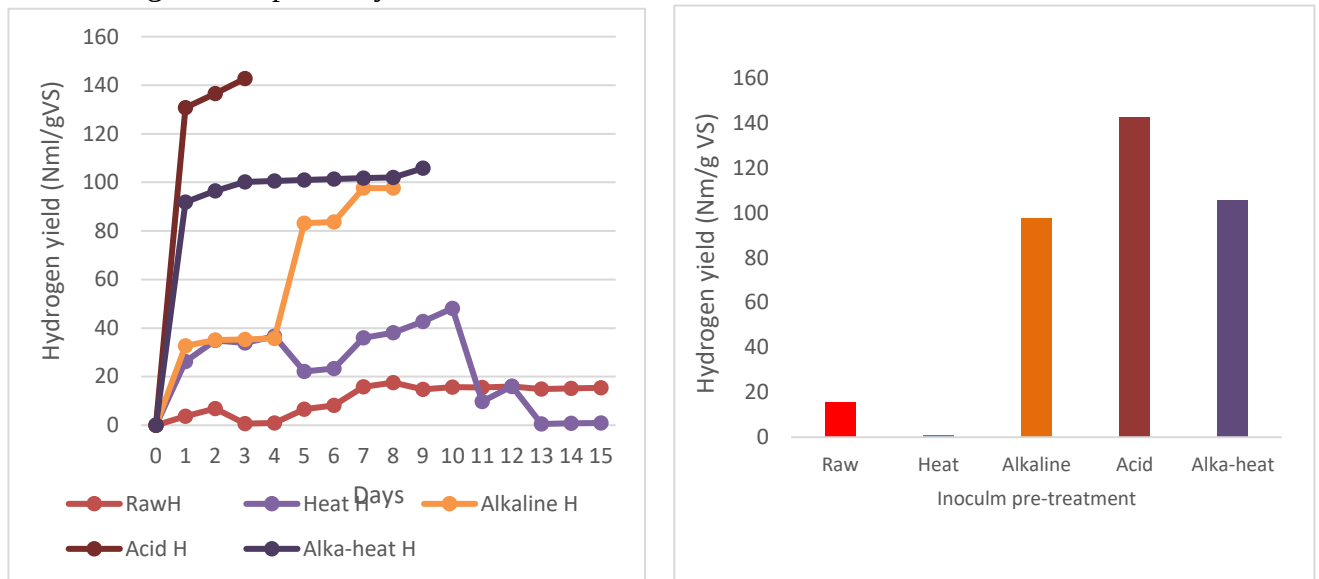


Fig 3.11 Time profile cumulative hydrogen yield as a function of pre-treatment

Fig 3.12 Total hydrogen yield on day 15

The results show that compared to the control, inoculum pre-treatment had a positive effect on hydrogen production, indicating that pre-treatment can effectively inhibit hydrogen-consuming microbes. This concurs with other research findings that concluded that chemical and physical pre-treatment of the inoculum enhances hydrogen production (Abubackar, *et al.*, 2019; Pascualone, *et al.*, 2019; Pecorini, *et al.*, 2019; Mockaitis, *et al.*, 2020). Fig 3.12 clearly shows that the different inoculum pre-treatments had a varied effect on hydrogen production as similarly reported by Rafieenia, *et al.*, (2018); Primasari, *et al.*, (2019) ; Magrini, *et al.*, (2020) who observed statistical differences amongst hydrogen yields between treatment groups.

Acid pre-treatment generated the highest hydrogen yield (142, 74 Nml/ gVS) amongst the investigated pre-treatment methods, while heat pre-treatment produced the lowest hydrogen yield (0,90 Nml/ gVS). Researchers Mockaitis, *et al.*, (2020) also found that acidic pre-treatment performed better than heat pre-treatment as did Chang, *et al.*, (2011). In contrast, (Luo, *et al.*, 2010) report that alkaline and heat pre-treatment increased hydrogen production better than acid pre-treatment. Zhenhong, *et al.*, (2010) observed that heat pre-treatment was the best method to enhance H₂ yields from the organic fraction of municipal waste. As did

Mohammadi, *et al.*, (2012a) and Argun & Kargi, (2009), who reported maximum hydrogen yield from heat pre-treatment of waste ground wheat and palm oil mill effluent, respectively.

Compared to the control, acid pre-treatment increased hydrogen production by more than 850 %, while the other two chemical treatments increased hydrogen production by less than 600 %. Only heat pre-treatment had lower hydrogen production compared to the control. Similarly, (Wang, *et al.*, 2011; Bagchehsaraee, *et al.*, 2008) obtained a lower hydrogen yield after 95°C heat pre-treatment compared to the control. Although several studies have reported increased hydrogen production due to heat pre-treatment, literature also alludes to the occurrence of reduced hydrogen yields when temperatures above 80 °C are applied, as observed in this study. Short fermentation times (3-9 days) were observed for the treatments with high hydrogen production, suggesting that acidogenic fermentation was the dominant pathway. According to Dwivedi, *et al.*, 2020 and Rafieenia, *et al.*, (2018) hydrogen microbes have low solid retention times (3 days) compared to that of methanogens (15 days).

The maximum hydrogen yield (143 Nml/gVS) and hydrogen content (50 %) obtained from this study are comparable to data reported in the literature which also used fruit and vegetable waste as a substrate for dark fermentation batch tests (Table 3.5). From the wide range of maximum hydrogen yields (4.9 to 129.2 ml/ gVS) and hydrogen content 28 -98.8 % reported, it can be concluded that the inoculum type, substrate type, pre-treatment applied, and operating temperature affects the hydrogen yield. Although the production of hydrogen via dark fermentation using waste biomass has been widely investigated, few researchers have studied dark fermentation of fruit and vegetable waste. These include researchers Abubackar, *et al.*, (2019) who obtained yields of 20.81-27.19 Nml H₂/g VS added. In other studies, Cieciora-Włoch, *et al.*, (2020) achieved a maximum yield of 52mL/gVS_{added} while Akinbomi & J, (2015) and Cahyari, *et al.*, (2017) obtained higher yields of 352 and 160.3 mL H₂/g VS _{added} respectively. Similarly, fruit and vegetable waste bio- hydrogen productivity data collated by Turhal, *et al.*, (2019), from numerous studies displayed a wide range of values (23 - 351.12 mLH₂/L reactor.h⁻¹). This is likely due to the different FVW substrates, inoculum, and operating temperature applied in the various studies.

Table 3.5 Comparison of fruit and vegetable bio-hydrogen yields (BY) reported in literature

<i>Substrate</i>	<i>Inoculum</i>	<i>Pre-treatment</i>	<i>T</i> (°C)	<i>pH</i>	<i>BY</i> (Nml/gVS)	<i>H₂ %</i>	<i>Reference</i>
<i>Mixed fruit and</i>	Anaerobic	Acid	37		143	54	Present study
	sludge						
<i>Veggie waste</i>	No inoculum	NA	37	6.8	19	32	(Marone, <i>et al.</i> , 2014)
<i>+potato peels</i>							
<i>Vegetable</i>	cow dung	Heat preated	37		4.9	98.8	(Bansal, <i>et al.</i> , 2013)
<i>waste</i>							
<i>Autoclaved</i>	Anaerobic	Heat shock,	55	5.5-	27.19	41	(Abubackar, <i>et</i>
<i>FVW</i>	sludge	105 °C for		6.75			<i>al.</i> , 2019)
		10 min					
<i>FVW</i>	Anaerobic	None	55	7	76	28	(Keskin, <i>et al.</i> , 2018)
	sludge						
<i>Heat pre-</i>	Vermicompost	Heat shock	35	5.5	129.2	31	(Pascualone, <i>et</i>
<i>treated FVW</i>		100°C, 15 min					<i>al.</i> , 2019)

3.4.4 Influence of inoculum pre-treatment on VFA composition

Numerous research studies have confirmed that hydrogen production is accompanied by the production of VFA and other lower molecular weight compounds. Research also shows that the distribution of the VFAs is strongly dependant on experimental conditions as well as the active microbial population (Pascualone, *et al.*, 2019). Figure 3.13 shows the VFA distribution, by percentage, generated during dark fermentation of FVW.

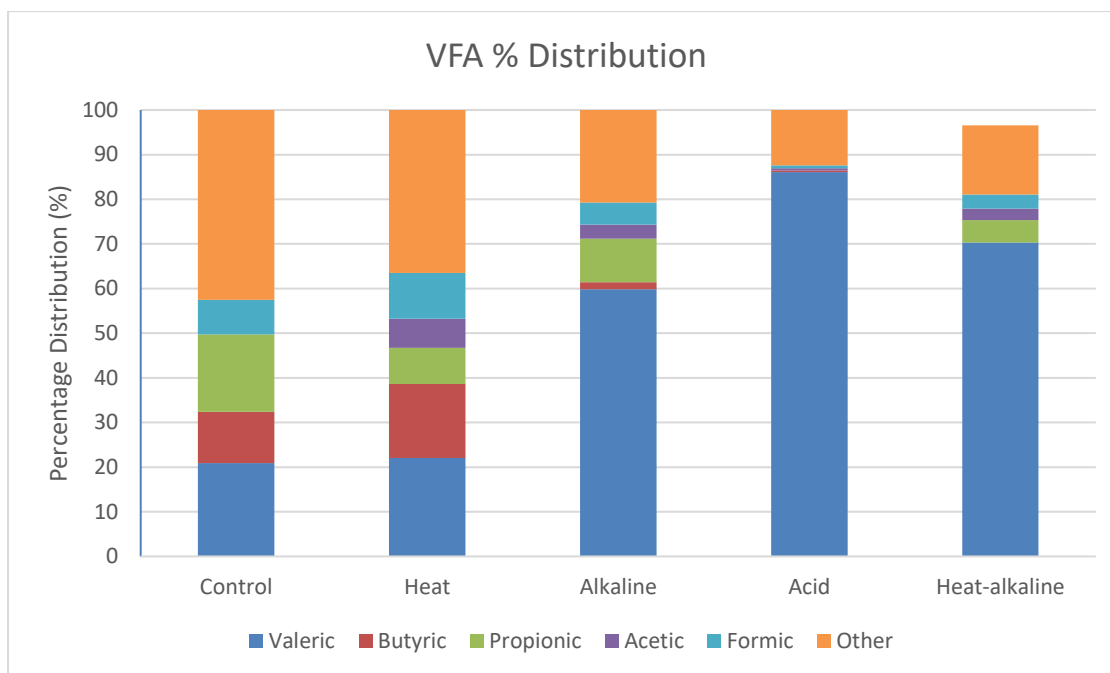


Fig 3.13 Distributions of VFA produced by various pre-treatment methods on mixed inoculum

The VFAs generated included acetic, butyric, propionic, and valeric. Of the VFAs produced, valeric acid was the principal VFA produced for all pre-treatments including the control. The highest VFA fraction (86, 1 %) was observed under acid pre-treatment, indicating that valeric type fermentation was the favoured hydrogen-producing metabolic pathway as this pre-treatment coincided with highest hydrogen production recorded (Section 3.4.3). Further supporting the assertion that the valeric route was the preferred hydrogen-generating pathway, was that high valeric amounts (above 60%) were detected in pre-treatments in which hydrogen was detected (alkaline and heat alkaline). In contrast, pre-treatments with negligible hydrogen production recorded low valeric acid distribution (~ 20 %).

Of particular interest was that no or little propionic acid was detected in the pre-treatments linked with hydrogen production (alkali/acid/heat-alkali). This is in agreement with (Magrini, *et al.*, 2020; Pascualone, *et al.*, 2019) who report that propionic acid production does not favour hydrogen formation since 2 moles of hydrogen are consumed to form one mole of propionic acid (Equation 3.4) resulting in reduced hydrogen yield. In this study as well, low hydrogen formation was accompanied by propionic production.



The results of this study are in stark contrast to other research studies that report acetic/propionic/butyric acid as the main metabolites formed under dark fermentation. Butyric and acetic are commonly associated with high hydrogen yields. Sittijunda & Pattra, (2016) states that there is a positive correlation between hydrogen production and acetate and butyrate formation owing to the metabolic pathways involved in their formation (Equation 3.5 and 3.6). However, the same authors and Rafieenia, *et al.*, (2018) note that acetic production can be due to consumption of hydrogen via homoacetogenesis.



Alcohols were not detected in this study, possibly due to deficiencies in the detection method as similarly alluded by Magrini, *et al.*, (2020).

3.4.5 Comparison of pre- treatments methods

To compare the effectiveness of the investigated inoculum pre-treatment methods for methanogenesis inhibition and enhancing hydrogen production, the hydrogen yield and methane content were considered. Additionally, one-way ANOVA was applied.

3.4.5.1 Hydrogen yield

Based on the mean hydrogen yields obtained from this study, the effectiveness of inoculum pre-treatment, ranked in descending order is: Acid >Alkaline-heat>Alkaline>Heat. Comparing to the effectiveness of inoculum pre-treatment results obtained in other studies (Table 3.6), no conclusive answer can be given on the best pre-treatment method for enhancing hydrogen production from FVW. This is because the effectiveness of inoculum pre-treatment is affected by substrate composition, operating conditions, and inoculum type amongst other factors (Rafieenia, *et al.*, 2018; Salem, *et al.*, 2018). In their works, Rafieenia, *et al.*, (2018) gave further insight on the variation in results of the best inoculum pre-treatment methods, explaining that the type of substrate affects the effectiveness of the inoculum pre-treatment owing to variances in substrate biodegradability. Moreover, differences in composition of the parent inoculum also influence the effectiveness of a pre-treatment method, further contributing to the contradiction in results between studies. This is because the microbial

communities present in the parent inocula display different levels of tolerance towards changes in environmental conditions, thus the inocula respond differently to the harsh conditions they are subjected to by pre-treatments (Rafieenia, *et al.*, 2018).

However, this study notes that the majority of studies in Table 3.5 ranked heat pre-treatment as the most effective method. Similarly, a review paper by Bundhoo & Hassan, (2015) concluded that heat and acid pre-treatment are the most frequently investigated pre-treatments and most effective among the treatment methods they reviewed. Dessi, *et al.*, (2018) echoed similar views, mentioning that heat and acid-base pre-treatments are commonly applied because they lead to a prevalence of hydrogen-producing bacteria by eliminating zero spore forming hydrogen consuming bacteria and promote propagation of HPB spores, which are abler to adjust to harsh environmental conditions (high temperature and acidic or basic pH). However, other studies have found other pre-treatment methods such as sonication, BESA, or LA addition being more effective (Bundhoo & Hassan, 2015). Furthermore, upcoming technologies such as infrared and ionizing irradiation are also evidencing to be effective for inoculum pre-treatment (Dessi, *et al.*, 2018).

While there is no consensus on the best inoculum pre-treatment method, this study and other previous research confirm that inoculum pre-treatment enhances hydrogen production.

Table 3.6 Comparison of literature inoculum pre-treatment ranking with study results

Inoculum	Investigated pre-treatment	Most effective pre-treatment	Ranking	Ref
Anaerobic sludge	Acid, base, heat, heat-base	Acid	Acid>heat-base>base>control >heat	This study
Activated sludge	Acid, base, BES heat, aeration, chloroform	Acid	Acid>base>heat>aeration>chloroform>BES	(Chang, <i>et al.</i> , 2011)
Anaerobic granulated sludge	Chloroform, acid, base, heat, freeze thaw	Heat (100°C, 1h)	Heat>base>chloro>acid>freeze thaw>control	(Mohammad i, <i>et al.</i> , 2012a)
Anaerobic sludge	Acid, base, chloroform, heat, load shocking	Untreated sludge(control)	Control>load shock>base>heat>acid>chloroform	(Luo, <i>et al.</i> , 2010)
Anaerobic mixed microflora	Acid, base, heat	Heat (80 °C, 30 min)	Heat> base >acid	(Wang, <i>et al.</i> , 2011)
Digested sludge	Aeration, Acid, base, chloroform, heat	Heat (100, 15 min)	Heat >base>aeration>acid>chloroform>control	(Wang & Wan, 2008)

3.4.5.2 Hydrogen and methane content

Another method employed by this study to compare the effectiveness of the inoculum pre-treatment was looking at how well the inoculum pre-treatment selectively enriched hydrogen-producing bacteria and inhibited activity of methanogens using hydrogen and methane content of the biogas at Day 15 of the experiment as indicators (Fig 3.13).

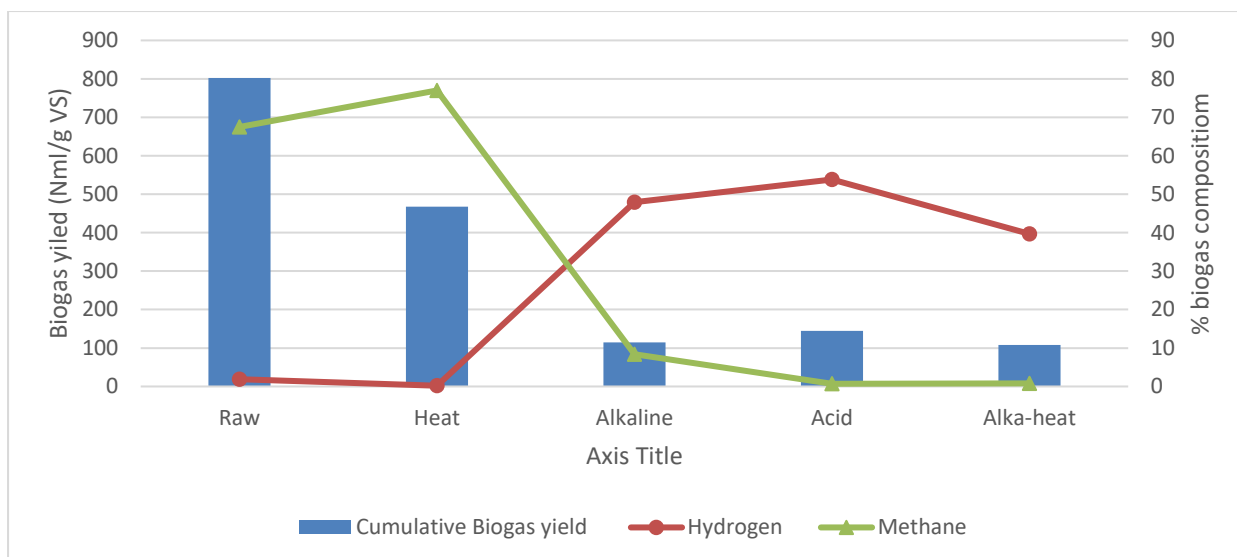


Fig 3.14 Hydrogen and methane content of different inoculum pre-treatments biogas on Day 15

The hydrogen and methane content graph shows that acid and alkaline-heat pre-treatment were the most effective in inhibiting methanogenesis and enhancing hydrogen content, as the methane content recorded was low $< 0, 5\%$. Comparable to this study, Wang, *et al.*, (2011) hardly detected methane after acid pre-treatment, similarly concluding that methane-producing bacteria had been efficiently inhibited by acidification pre-treatment. Wang, *et al.*, (2011) backed their findings with microbiology theory, mentioning that methane-producing bacteria becomes inactivated if the culture conditions are too acidic or alkaline, with acidic conditions resulting in more severe inhibition. For the alkaline pre-treatment the hydrogen content (47,9 %) was comparable to that of acid pre-treatment (53 %), however, methane was also detected. Indicating that alkaline pre-treatment had not fully inactivated methanogens.

Heat pre-treatment was the least effective because on Day 15 the hydrogen content was negligible (less than 0, 2 %) while the methane content was high (77 %). Bundhoo & Hassan, (2015) argue that it is possible for a pre-treatment to also suppress non-spore forming hydrogen-producing bacteria while some sporulating hydrogen-consuming bacteria (methane producers) manage to survive the harsh pre-treatment conditions. For example, Yuan, *et al.*, (2011) state that suppression of non-spore forming HPB from the genus *Enterobacter* can occur while sporulating HCB like *Clostridium aceticum* and *Clostridium thermoautotrophicum* can withstand pre-treatment. This can possibly lead to what was observed in this study, wherein hydrogen content dropped while methane content increased.

3.4.5.3 ANOVA

ANOVA analysis (Table 3.7) showed significant differences in the mean hydrogen yield between different pre-treatment groups ($p < .001$). The differences were also correlated with variances in metabolic products (VFAs) observed, which could be explained by the diversity of microbial populations.

Table 3.7 ANOVA results comparing mean hydrogen yields between pre-treatment groups

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45180.633	4	11295.158	85.750	<.001
Within Groups	1317.217	10	131.722		
Total	46497.849	14			

3.5 Conclusion

The batch dark fermentation results of this study proved that it is possible to produce hydrogen and volatile fatty acids from fruit and vegetable waste via mixed culture AD by pre-treating the inocula to selectively enhance hydrogen- producing bacteria while suppressing hydrogen-consuming methanogens. Furthermore, the experimental results support the proposition of this study, which is that the AD process can be used to produce a range of other value- added products separately or simultaneously with methane. As such, AD can form an integral part of waste biorefineries of the future.

The pre-treatment methods (heat, alkali, acid, heat-alkali) applied for selective enhancement of hydrogen- producing bacteria had a significant influence on hydrogen yield and VFA fermentation pathways. However, not all pre-treatment methods positively influenced H_2 yield, as heat pre-treatment generated lower hydrogen yields compared to the control. Of the various inoculum pre-treatments investigated, the present study concludes that acid and combined heat-acid were the most effective method to increase hydrogen yield for the FVW substrate investigated. Other studies, however, reached different conclusions on the best pre-treatment method to apply. Demonstrating that selection of the best pre-treatment is influenced by many factors including the substrate type, inoculum type, and reaction conditions.

The study also showed the feasibility of simultaneously producing bio-hydrogen and volatile fatty acids via anaerobic digestion. Inoculum pre-treatment showed to have effect on the VFA produced, to further enhance the production of the identified volatile fatty acids optimisation of other operation parameters e.g. temperature , organic loading rate, and pH is recommended.

While the present study revealed acid pre-treatment to be the most effective method to enhance hydrogen production for the FVW substrate investigated, other considerations must be taken into account when selecting a pre-treatment method to utilise. This includes conducting a cost-benefit analysis to investigate the input costs versus bio-hydrogen production. For example, energy costs associated with heat pre-treatment or cost of chemicals for acid/alkali pre-treatment need to be calculated and compared against the extra bio-hydrogen produced. Additionally, a continuous process is recommended for industrial- scale production of hydrogen, therefore research on the long -term effectiveness of inoculum pre-treatment for continuous processes is necessary.

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4. Optimisation of VFA and methane production from FVW using RSM

Abstract

The purpose of the study was to determine the optimal FVW/pig manure mix ratio, pH, and sonication conditions for enhancing methane and VFA production from anaerobic batch digestion of fruit vegetable waste. Response surface methodology utilising Central Composite Design was applied to find the optimal conditions and interactive effects of process parameters. Factor screening using OFAT anaerobic batch tests was first conducted to analyse the effect of each parameter on methane and VFA yield and identify factor levels that produce maximum and minimum yields. Five levels were investigated for each parameter. The effect of initial pH was investigated at pH (4, 6, 8, 10, 12), while for ultrasonic pre-treatment, specific energy from 2 500 up to 17 500 (kJ /kg TS) was applied. Fruit and vegetable waste to pig manure co-digestion ratios of (100/0; 0/100; 75/25; 50; 50; 25/75) were studied. For the effects of initial pH investigations, the maximum methane yield (497 Nml/gVS) was obtained at pH8 (control) and the minimum yield at pH 4 (11 Nml/gVS). Specific energy of 17 500kJ/kg TS resulted in the maximum methane yield (312 Nml/gVS). A FVW/pig manure (PM) co-digestion ratio of 75/25 gave the max methane yield (434 Nml/gvs). The maximum yield predicted via RSM model was 692.82 mL/g VS at the optimal conditions of pH 8 and the co-digestion ratio of 60 % fruit and vegetable waste: 40 % pig manure. The pH was found to be the only factor to significantly affect methane yield.

4.1 Introduction

AD can generate volatile fatty acids, bio-hydrogen, and/or bio-methane independently or concurrently, according to research (Khan, *et al.*, 2016a). The conversion of anaerobic digesters into biorefineries could be aided by the processing of volatile fatty acids during the acidogenic fermentation stage of AD (Strazzera, *et al.*, 2018; Wainaina, *et al.*, 2019). Acetate (C2), propionic acid (C3), butyric acid (C4), and valeric acid (C5), chemical compounds that are essential precursors for the synthesis of several industrial chemicals and bioenergy, may be generated using the volatile fatty acid platform (Kleerebezem, *et al.*, 2015). The product delivery formation (VFA/bio-hydrogen/bio-methane/combination of ingredients) of AD is highly influenced by bioreactor operational parameters such as pH, HRT, OLR, and temperature (Kleerebezem, *et al.*, 2015; Khan, *et al.*, 2016a); hence operational parameters play a key role in selecting the end products produced. The role of process parameters in

deciding the yield and composition of AD products has been illustrated in a number of studies. For example, in the case of VFA synthesis, the dominant metabolic pathway (acetate-ethanol type/propionate type/ butyrate type/ lactate type/mixed acid type), changes under different operational conditions because operational parameters have a regulatory effect on the synthesis and distribution of VFA (Zhou, *et al.*, 2017).

As a result, understanding how operating parameters affect the different metabolic pathways and, as a result, the substance formation distribution of AD is crucial (Zaki, 2016). This would allow operators to manipulate process parameters in order to facilitate metabolic pathways that favour the synthesis of their preferred products, allowing them to monitor the final products generated. Furthermore, defining the ideal conditions for increasing the yield of the target items is critical.

Although previous research has shown a correlation between process parameters (such as pH, temperature, and hydraulic retention time) and VFA and methane production, the best conditions for VFA and methane production vary depending on substrate type and other factors. According to Khan, *et al.*, (2016a), the optimum pH for VFA generation from kitchen waste is pH 7, while the pH for wastewater treatment is in the range of 5.25 to 6. In contrast, Cheah, *et al.*, (2019) discovered that alkaline conditions of pH 9.5 -10 improved VFA production from food waste, while acidic pH (pH6) yielded higher VFA production from organic fraction municipal solid waste. This emphasizes the importance of ongoing studies to determine the best conditions for various substrates and reactor configurations.

This research used OFAT experiments to see how process parameters like pH, ultrasonification pre-treatment, and co-digestion influence the VFA and methane yield of mixed fruit and vegetable waste. The OFAT experiments also acted as a screening stage before the method parameters were optimised. Following that, process conditions were optimised using Response Surface Methodology and CCD.

4.2 Materials and Methods

This section describes the research materials and methods utilised in conducting optimisation of process parameters to maximise methane and volatile fatty acid production via Response Surface Methodology.

4.2.1 Research materials

The research materials including feedstock, inoculum, and experimental device are described forthwith.

4.2.1.1 Feedstock

All experiments under this section were performed using the same mixed fruit and vegetable waste (FVW) collected from Silverton Fruit Stop as described in Section 3.2.1.1).

4.2.1.2 Inoculum

Similar to Section 3.2.1.2 digestate collected from a commercial biogas plant situated in Brokunspruit, Pretoria was utilised as inoculum.

4.2.1.3 Feedstock and inoculum characterisation

Before each experimental test, the TS and VS content of the feedstock and inoculum were re-measured following procedures set out in Section 3.2.1.3. The feedstock and inoculum characterisation for each run is in Appendix B1.

4.2.1.4 Experimental device

The optimisation experiments were conducted out using the AMPTS (II) equipment, which is described in detail in Section 3.2.1.4.

4.3 Experimental Methods

RSM was used to optimise methane and VFA performance. To improve product yields, three variables were optimised: pH, ultrasonification, and co-digestion, with a focus on their interactions. The experiments were carried out using a Central Composite template. The key steps in carrying out the optimisation are summarised in Figure 4.1. The study used RSM to optimise methane and VFA performance. To improve product yields, three variables were optimised: pH, ultrasonification, and co-digestion, with a focus on their interactions. The experiments were carried out using a Central Composite template. The key steps in carrying out the optimisation are summarised in Figure 4.1.

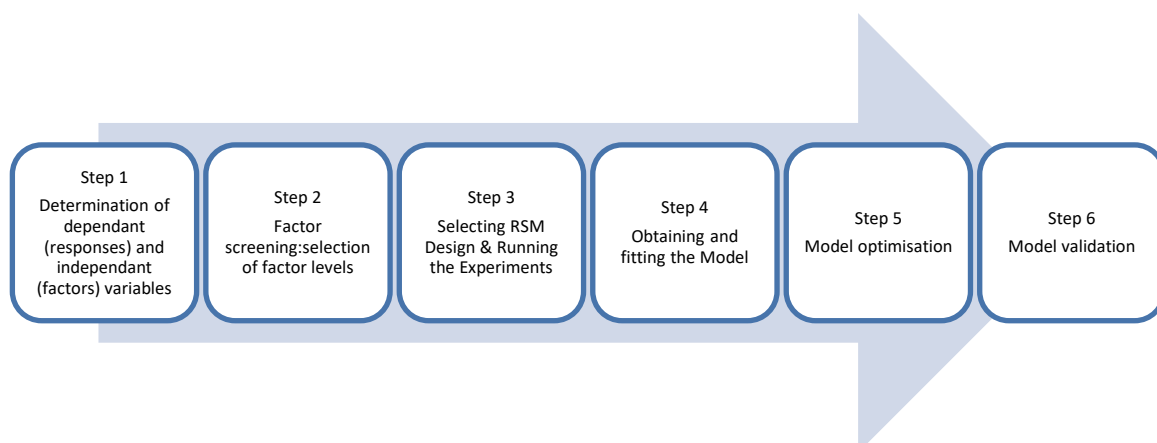


Fig 4.1 Overview of the main steps undertaken for optimisation using RSM

4.3.1 Factor Screening

The purpose for factor screening was to evaluate the influence of the three selected factors (pH, ultrasonification and co-digestion) on methane and VFA production as well as to select factor levels (high and low value) to use for the CCD experiments.

4.3.1.1 Experimental design

A series of batch AD tests (Table 4.1), using OFAT were conducted. For each factor investigated, all other factors were held constant while the factor under consideration was running at 5 levels to determine (i) how the factor levels influence methane and VFA production, and (ii) identify which factor level generated the maximum VFA/methane yield. Since each of the 5 levels in a series was run in triplicates, a total of 15 batch tests were run per Series.

Table 4.1 Experimental Design for OFAT experiments Factor screening

<i>Series</i>	<i>Factor Investigated</i>	<i>Factor Levels</i>				
		Level 1	Level 2	Level 3	Level 4	Level 5
1	pH	Control	pH 2	pH4	pH 10	pH12
2	Ultrasonification	Control	US 2 500	US 7 500	US12 500	US17 500
3	Co-digestion	Control	PM100%	FW75%	FW50%	FW25%

4.3.1.2 Experimental procedure

Batch AD tests using the AMPTS II were carried out at a mesophilic temperature of 37 °C. The effect of each process parameter on methane and VFA production was determined by measuring the volume of methane and VFA produced as well as the VFA composition over the 15 day experimental period. Consequently, for each parameter, the factor levels corresponding to maximum and minimum methane and VFA yield were identified for application in the CCD experiments. The experimental procedure for the Factor Screening experiments (S1-S3) is summarised in Figure 4.2, which shows the five main steps for conducting the experiments.

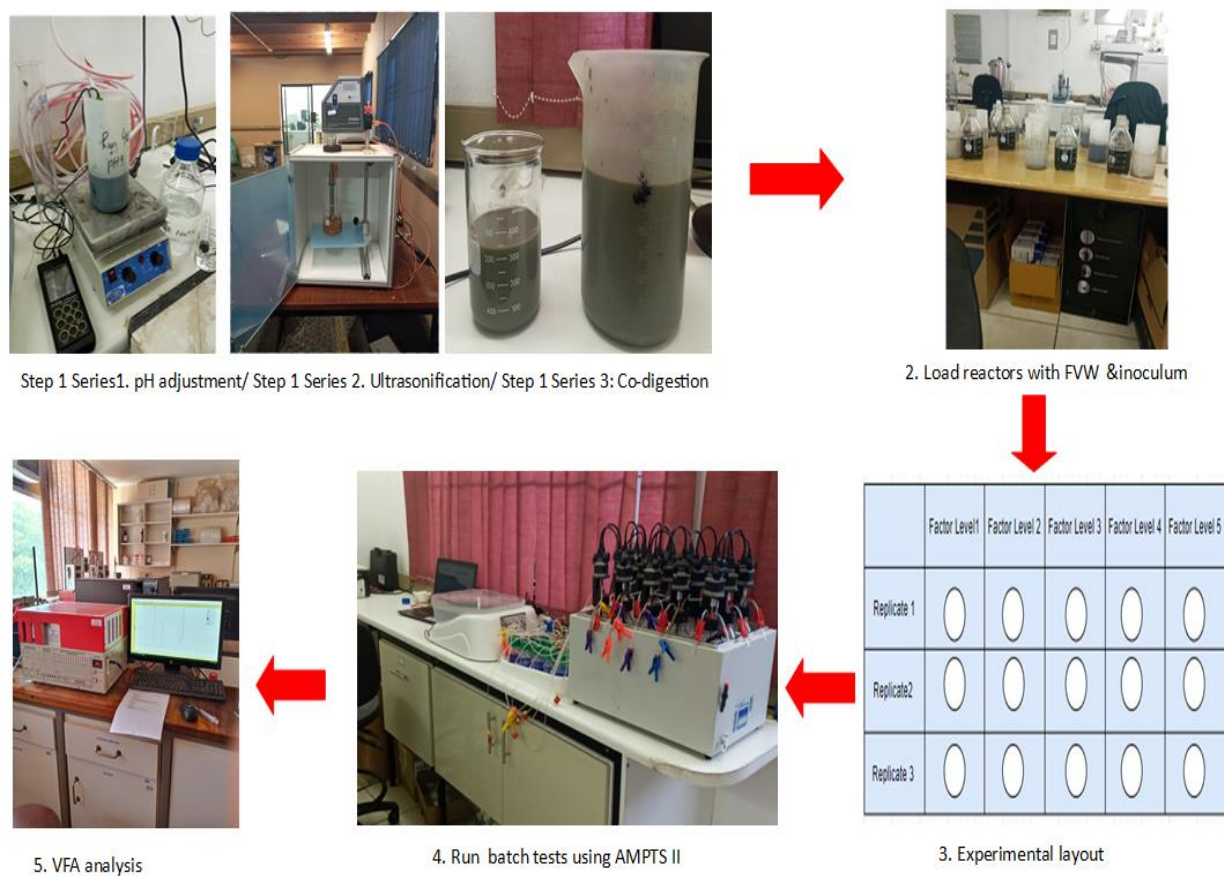


Fig 4.2 Step by step procedure for running Factor Screening experiments

Step 1, which differed for each Series, was to apply pH adjustment or ultrasonification or co-digestion depending on which factor was being investigated. Following this, in Step 2, the inoculum and substrate were weighed and loaded into 15 x 500 ml reactor bottles at an inoculum (I) to substrate (S) ratio (I/S) of 2 and a working volume of 400 ml. Step 3, was placing the reactor bottles in a heating water bath set at 37 °C using a 5 x 3 experimental layout.

Each factor level was given its own row (5 rows = 5 factors), and each level was run three times. The experiment was started using the AMPTS (II) start-up procedures, which were previously defined in Section 1.3.21. The experiment lasted 15 days (Step 4) and the amount of methane produced was measured daily. On Day 15, samples of liquid broth were analysed for VFA structure and concentration (last day of the experiment). The first step in each Series is outlined in detail below.

(i) **Step 1 for Series 1: Factor screening for initial pH**

To screen the effect of initial pH on VFA and methane production of anaerobically digested FVW at a mesophilic temperature of 37 °C, five initial pH levels, the control (pH 8), acidic (pH 4 & 6), and alkaline (pH10 & 12) conditions were tested. For acidic pH, concentrated sulphuric acid (98 % solution) (H_2SO_4) was added to adjust the initial pH of the inoculum and FVW substrate mixture from initial pH 8 to pH 4 or pH 6. To increase the pH to 10 or 12, a 4 M sodium hydroxide solution (NaOH) was added. A portable HACH pH meter (*HACH HQ11D53000000, Thermo Fischer*) was used to check if the desired pH had been achieved (Figure 4.3). Table 4.2 summarises the experimental conditions.



Fig 4.3 Initial pH adjustment using acid/base solution and pH meter for Series 1 experiments

Table 4.2 Experimental conditions for (Series 1) pH factor screening

Condition	<i>pH Experimental conditions</i>				
<i>Factor level values</i>	Control	pH 2	pH4	pH 10	pH12
<i>Temp</i>	37 °C				
<i>I/S</i>	2				
<i>Inoculum added</i>	7,56 g VS/370,52 g				
<i>Substrate added</i>	3,78 g VS/24,48 g				

(ii) Series 2: Factor screening for ultrasonification

To investigate the effect of ultrasonic pre-treatment on VFA and methane production of anaerobically digested FVW, a Hielscher UP400tS sonicator with a maximum power output of 400 W operating at a frequency of 20 kHz was utilised. A 38 mm diameter sonotrode probe was placed in a 500 mL glass beaker containing 250 mL of fruit and vegetable waste suspension (174 g fruit and vegetable waste mixed with 76 ml of water giving a TS content of 8%) at half the depth of the suspension (Figure 4.4). The ultra-sonication pre-treatment was applied at a constant frequency of 20 kHz, power input of 250 W, 40 % amplitude, and pre-treatment times ranging from 12 to 99 minutes, delivering specific energy of between 2500 to 17500 kJ kg/TS (Table 4.3). The temperature during ultra-sonication was controlled by an ice bath.

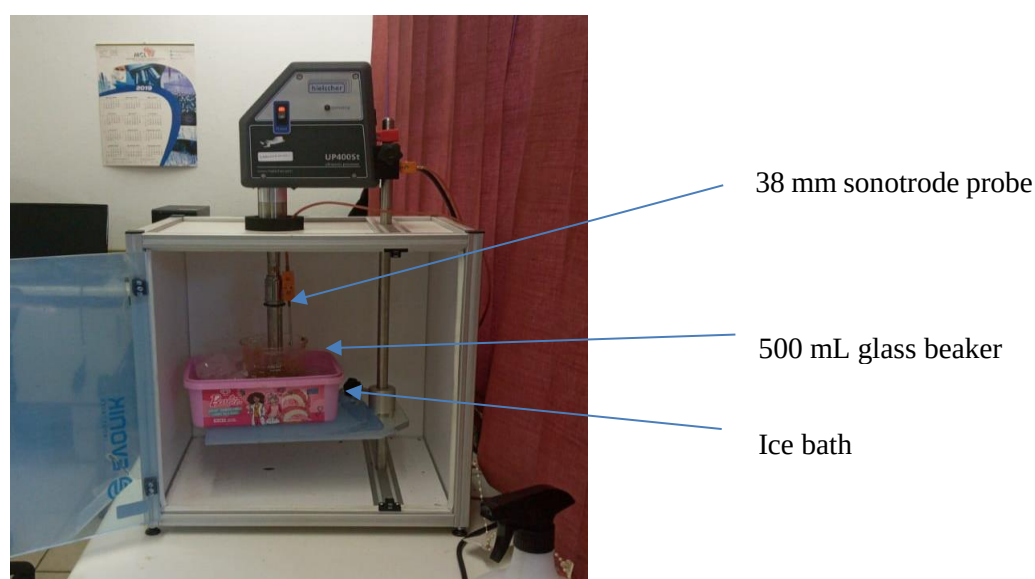


Figure 4.4 Ultrasonication pre-treatment of FVW using Hielscher UP400tS sonicator

The Specific energy input (SE) in kJ /kg TS is obtained from Eq. 4.1:

$$SE(kJ/kgTS) = \frac{P \times t}{V \times TS_0}$$

Where P is the ultrasonic power in kW; t is the ultra-sonication treatment time in (s); V is the volume (L) of the ultasonified sample; and TS_0 is the initial total solid concentration (kg. L⁻¹). To achieve the required specific energies of 0- 17 500 (kJ/ kg TS), the amount of input energy ($P \times t$) in kJ required, was determined by rearranging Equation 4.2.

$$P \times t (kJ) = SE \times V \times TS_0 \dots\dots\dots \text{Equation 4.2}$$

The Hieslscher UP400St has a function that measures the cumulative energy input ($P \times t$) of the sonicator with time recorded in Wh, hence sonication was running until the Hieslscher UP400St input power reading equaled the required energy input as calculated using Equation 4.3. Equation 4.3 calculated the required input energy in Wh.

$$P \times t (kJ) = (SE \times V \times TS_0 \times 1000)/3600 \dots\dots\dots \text{Equation 4.3}$$

Table 4.3 shows the specific energies applied (2 500 – 17 500), the calculated energy input needed, the actual energy supplied as recorded by the Hieslscher UP400St and the time taken to reach the energy input. Table 4.4 on the other hand summarises the experimental test conditions.

Table 4.3 Ultrasonic pre-treatment applied to fruit and vegetable waste

SE	VOLUME	ENERGY REQUIRED	ENERGY APPLIED	TIME TAKEN	AMPLITUDE
KJ/KG TS	(L)	CALCULATED EPN 4.3	(READING)	(READING)	%
		WH	WH	MIN	
2500	0,25	13 889	13,906	12 min 17 sec	40
7500	0,25	41 667	41,682	37 min 20 sec	40
12 500	0,25	69 444	69,455	65 min	40
17 500	0,25	97 222	97,228	99 min	40

Table 4.4 Experimental conditions for (Series2) ultrasonic pre-treatment factor screening

Condition	<i>Ultrasonification experimental conditions</i>				
<i>Factor level values</i>	Control	2500	7500	12500	17500
<i>Temp</i>	37 °C				
<i>I/S</i>	2				
<i>Inoculum added</i>	4,09 gVS/ 371,41g				
<i>Substrate added</i>	2,04 gVS/28,59g				

(iii) Series 3: Factor screening for co-digestion

To investigate the effects of co-digestion on AD of fruit and vegetable waste (FVW) and PM were incubated at a mesophilic temperature of 37 °C at various mixing ratios on a VS matter basis (FVW/PM: 100:0, 75:25, 50:50, 25:75, and 0:100). Table 4.5 details the experimental conditions for the batch tests for Series 3.

Table 4.5 Experimental conditions for (Series 3) co-digestion factor screening

Condition	Co-digestion experimental conditions				
<i>Factor level values</i>	FVW100	PM100	FVW75:PM25	FVW50:PM50	FVW25:PM75
<i>Temp</i>	37 °C				
<i>I/S</i>	2				
<i>Inoculum added</i>	8,23 gVS/ 354,64g				
<i>Substrate added</i>	4,11 gVS/45,36g				

4.3.2 Central Composite Design

Central Composite Design was used to plan and conduct the statistical experiments necessary to develop second -order polynomial equations that model the relationship between the three input variables (pH, ultrasonification and co-digestion) and the two output responses (methane and VFA yield). The quadratic model is important for (i) identifying the interactive effects of the three input variables on the methane and volatile fatty acid yield and, (ii) optimisation of the variables and responses using RSM.

4.3.2.1 Experimental design

Design-Expert Software version 13.0.3 was used to generate a five- level-three –factor CCD and for data analysis (Design-Expert, 2021). The three independent variables: initial pH, ultrasonication and co-digestion were coded (X_1), (X_2), and (X_3) respectively, and assigned five levels each, namely, High Level (+1), Low Level (-1), Mean (0) and 2 outer points Min (-A) and Max (A) corresponding to ($\pm\alpha$) which are shown in Table 4.6. The high level (+1) and low level (-1) value for each variable was informed by the Factor Screening experiments.

Table 4.6 CCD input variables and the respective 5 factor levels

VARIABLE	UNIT	CODED	MIN	MAX	LOW	HIGH	MEAN	STD. DEV
			(-A)	(+A)	(-1)	(+1)	(0)	
CO-DIGEST	% FWW	A	41,48	83,52	50	75	62,50	10,60
INITIAL PH	pH	B	6,32	9,68	7	9	8,00	0,8478
SPECIFIC ENERGY	kJ/g VS	C	9 943,2	22 556,7	12 500	20000	16250	2119,53

The Design-Expert Software generated an experimental design matrix consisting of 20 runs according to Equation 4.4:

$$N = 2^k + 2k + C_p \dots \dots \dots \text{Equation 4.4}$$

Consisting of (i) $2^k = 8$ factorial points situated at Low and High levels (-1) and $(+1)$, (ii) $2k = 6$ axial points or Min/Max corresponding to levels $(-\alpha)$ or $(+\alpha)$ and, (iii) 6 replicate number of central points (C_p /Mean). The C_p represents the estimation of experimental errors and the lack of fit (LOF).

4.3.2.2 Experimental procedure

The Bioprocess Control AMPTS II was again used to conduct the 20 batch tests for the CCD. Each experimental run received a specific co-digestion, pH, and ultrasonification treatment combination. The treatment level combination for each run, i.e. co-digestion %, pH or ultrasonification (specific energy applied), was obtained from the experimental design matrix generated by Design-Expert Software (Table 4.6). The treatment application was sequential for each batch test as depicted in Fig 4.5.

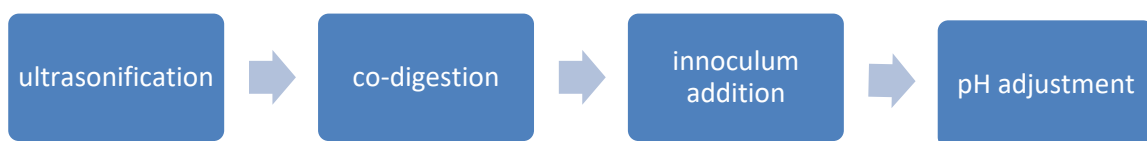


Fig 4.5 Sequential treatment applied to substrate and inoculum for CCD runs

The FVW and pig manure were ultrasonified separately at specific energies ranging from 12 500 to 22 500 kJ/kg TS. Following ultrasonification, the FVW and PM were co-digested according to the mix ratio for that run on a volatile matter basis. After that, inoculum (I) was added at a 2:1 ratio to the FVW/PM substrate (S) mixture to make up a working volume of 400ml. Finally, the initial pH levels of the inoculum and substrate mixture were adjusted to be between 4 and 9, which corresponded to the chosen pH for that run. The batch experiments were carried out for 15 days at 37°C. composition and concentration on Day 15 (last day of the experiment).

4.3.3 Model fitting

Design-Expert Software Version 13 was utilised for data calculation, graphing, and modelling. The ANOVA was performed to investigate the effect of variables X_1 , X_2 , X_3 on output responses (experimental methane yield (EMY)) as well as evaluate the adequacy of the generated model. The statistical significance of each variable was checked using regression analysis at level of $p = 0.05$. The regression coefficient (R_2), adjusted regression value (R_{adj2}) and LOF explained the statistical significance of the model.

4.3.4 Effect of variables on responses

Graphical 2D contour plots and 3D response surface plots were used to describe the effect of the individual variables as well as their interactive effects on methane and VFA yield.

4.3.5 Model optimisation

Design-Expert 13 software applies the desirability approach, an inbuilt function in the software, for optimisation. The approach searches for a combination of factor levels that simultaneously meet the targets placed on each one of the input factors and the output for methane yield (Alfarjani, 2012). The optimisation process can be performed using either the graphic or the numeric function. The latter was applied for this study.

4.3.6 Model validation

To validate the model, three more experiments (three each for MY) were conducted using the optimal conditions given by the model. The MY_{EXP} values obtained from running the experiments were compared against the MY_M predicted values by the model.

4.4 Analytical Methods

4.4.1 Methane production

The AMPTS flow cell array measured and recorded the volume of gas produced after removal of carbon dioxide for each reactor bottle.

4.4.2 VFA analysis

Sampling and analysis of the liquid broth was carried out as described in Section 4.4.2.

4.5 Results and Discussion

The results of the screening experiments and the optimisation stages are described in the following sections.

4.5.1 Factor Screening

This section presents the results of the screening experiments conducted to investigate the effects of the factors (pH, ultra-sonication and co-digestion) on methane and VFA yield as well as to identify the factor levels that result in maximum methane and VFA yields. Effect of pH on methane yield

4.5.1.1 Effect of pH on methane yield

The effect of initial pH (4, 6, 8, 10, and 12) on methane and volatile fatty acid production under mesophilic (37 °C) AD was studied over a 15-day period. The cumulative methane yield time profile and methane

The effect of initial pH on the methane yield obtained from FVW is easily noticeable from the cumulative yield graphs of Fig 4.6 and 4.7

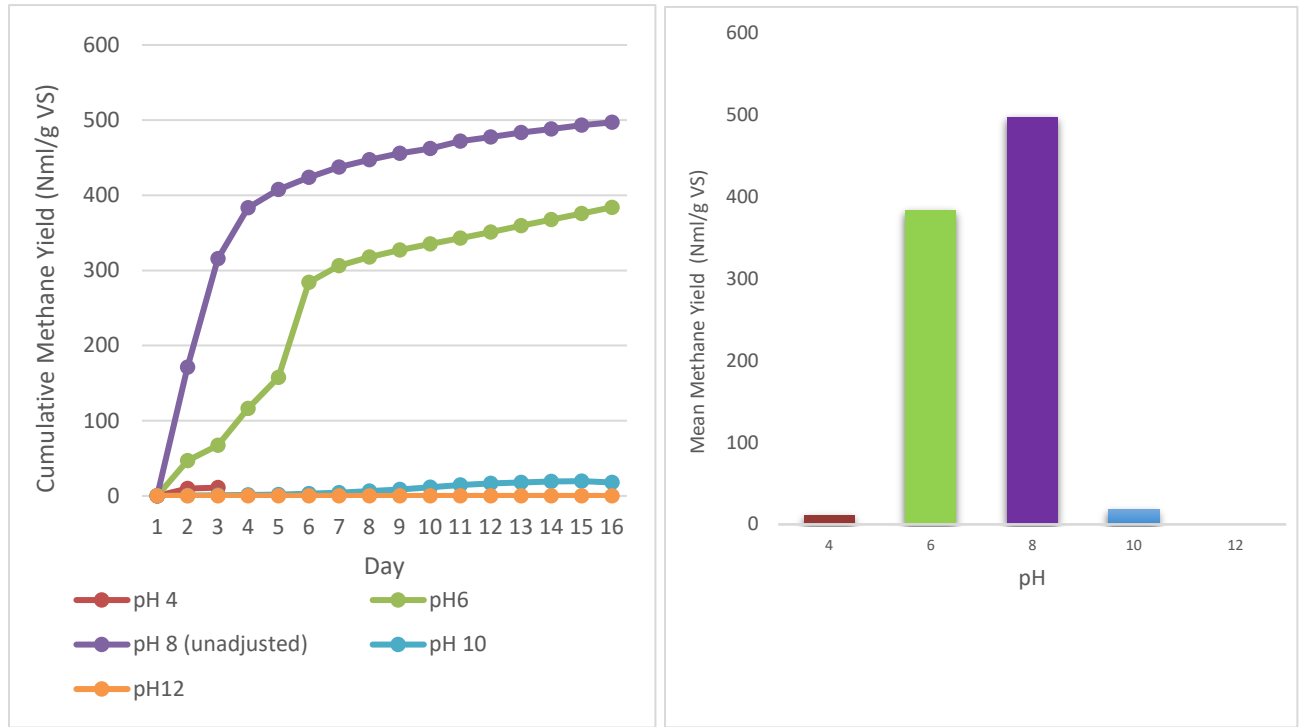


Fig 4.6 Time profile cumulative methane yield at various initial pH

Fig 4.7 Cumulative methane yield at various initial pH

Figures 4.6 & 4.7 show that the control (pH8) resulted in the maximum cumulative methane yield (497 Nml/g VS) followed by pH 6 (384 Nml/g VS). This falls within the tolerable pH ranges of 6.5 to 8 for biogas production reported in the literature with 6.8 – 7.2 being the optimum (Cioabala, *et al.*, 2012); (Vivas, *et al.*, 2019); (Ali, *et al.*, 2019). The methane production was 22 % higher at pH 8 compared to pH 6. This was consistent with other studies like Zhai, *et al.*, (2015), (Grzelak, *et al.*, 2018), (Yin, *et al.*, 2019) who obtained higher methane yields at pH 8 than pH 6. Considering the effect of initial pH, it was found that organic degradation increased with higher initial pH, as indicated by a significantly higher methane production rate.

However, methane production fell drastically at below pH 4 and above pH 8, while no gas was produced at pH 12, confirming that methanogens cannot withstand extreme acidic or alkali conditions (Fig. 4.7). The results of this study are comparable to investigations by Moreno-Davila, *et al.*, (2011) who yielded no gas at initial pH of 4 and 4.5 although observing methane

production in the range pH 5 – 11. The researcher and co-workers attributed the lack of gas production at low pH to complete inhibition of methanogenic activity a view also supported by Ali, *et al.*, (2019). According to Reungsang, *et al.*, (2012), excessively alkaline environments can cause the breakdown of microbial granules and subsequent collapse of the digestion process. Ali, *et al.*, (2019) also mentions that high pH poses the risk of ammonia inhibition due to the increase in free ammonia.

4.5.1.2 Effect of pH on VFA production and composition

Operating pH is one of the key parameters well-known to influence anaerobic fermentation including the microbial community and metabolic pathways thereby affecting fermentation products (Wang & Lee, 2021). The effect of initial pH on VFA composition of anaerobic digested FVW is recorded in Table 4.7.

Table 4.7 VFA composition of anaerobic digested FVW at different initial pH levels

VFA composition (%)	Valeric	Butyric	Propionic	Acetic	Formic	Other
pH4	75,12	5,61	0	0,67	0,54	18,06
pH 6	71,44	0	0	2,39	0,70	25,47
pH 8	11,7	0	0	6,09	9,43	72,79
pH 10	8,77	0	0	6,65	0,63	83,96
pH 12	2,80	0	0	4,85	8,54	83,81

Valeric and acetic acid were the main VFAs formed for all initial pH values. The highest valeric content (71-75 %) was observed under acidic conditions while VFA production decreased with increasing pH. The lowest valeric content was observed at pH12. This is opposite to the findings of Lin, *et al.*, (2013); Atasoya, *et al.*, (2018) and Grzelak, *et al.*, (2018) who reported maximum VFA content under alkaline conditions of pH 10 -12. No butyric or propionic acid was detected in any of the pH treatments, except at pH4. The majority of studies that investigated the effect of initial pH on VFA production commonly reported butyric, propionic and acetic acids as the main acids formed.

There was no major shift in the VFA composition in terms of dominant product type with varying initial pH, for all initial pH values valeric acid was the main VFA formed except for pH12 where formic acid was the major acid formed. In other studies, Jankowski (2017) observed that under pH 5.5 the dominant VFA was acetic acid while butyric acid dominated at pH8. Similarly, (Atasoya, et al., 2018) found that at pH 5 acetic ($35 \pm 8\%$) and butyric acid ($37 \pm 4\%$) were the main VFAs formed ($60 \pm 12\%$), while acetic was the main VFA under alkali conditions ($60 \pm 12\%$).

The contrast in the results of this study to the findings of other research findings can be explained by difference in operating conditions such as substrates utilised, inoculum source, organic loading rate and inoculum to substrate ratio (Grzelak, *et al.*, 2018; Eryildiz, *et al.*, 2020). Lin *et al.*, (2013) expressed the importance of substrate composition on the VFA product formation, revealing that acetic, propionic and butyric acid are directly formed from soluble proteins, carbohydrates and lipids. On the other hand, valeric formation is via protein degradation. The time of sampling can also be a factor contributing to the differences in the VFAs observed. In their study Eryildiz, *et al.*, (2020) that the dominant VFA produced from anaerobic digestion of fruit waste varied with reaction time, at Day 6 Butyric acid was the main VFA formed while on Day 14 the main VFA had shifted to Caproic acid.

4.5.1.3 Effect of ultrasonic pre-treatment on methane yield

Four ultrasonic (US) pre-treatments with specific energy input between SE2500 and SE17500 kJ/kg TS were applied to mixed fruit and vegetable waste to investigate the effects of ultrasonic pre-treatment on methane and VFA production over a 15 day period. The results are presented in Fig 4.8 and 4.9.

In general, as the specific energy value increased, there was an increase in the methane yield. The US treatment of SE 17500 generated the highest methane yield (312 mL/g VS) and SE2500 the lowest yield (171 mL/ g VS_{added}). Comparing the maximum methane yield obtained (312 mL/ g VS) to that of the control (219 mL/ g VS), sonication improved methane yield by 42 %. The percentage increase was lower than that of 80, and 195 % reported by Zeynali *et al.* (2017) and Shanti, *et al.*, (2019) respectively for fruit and vegetable waste, despite using much lower sonication energies of 2 380 kJ/kg TS and 5 400 kJ/kg TS. Lizama *et al.* (2017) also reported a wide range of methane yield improvements (27 - 97 %) for sonicated waste-activated sludge from the literature.

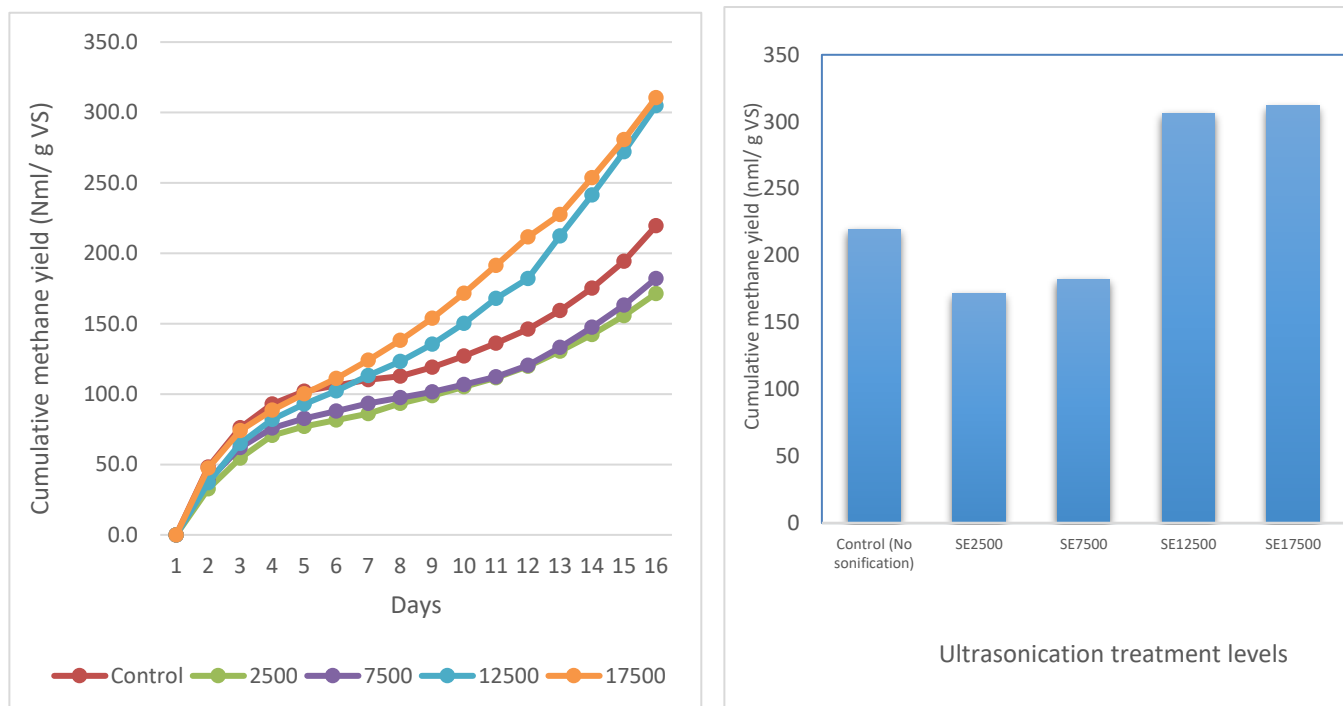


Fig 4.8 Time profile cumulative methane yield at various ultrasonic pre-treatment levels

Fig 4.9 Cumulative methane yield at various ultrasonic pre-treatment levels

The variances in the cited yield improvements can allude to differences in substrate composition such as the total solid content and treatment conditions applied. A study by Rasapoor, *et al.*, (2016) carried out sonification (1 200-18 000kJ/TS) on the OFMSW at different solid contents (6 – 10 %) and concluded that biogas yields were much improved at lower TS content (6 & 8 %) than at TS of 10 %. Lizima *et al.*, (2017) also mention that the total solid content limits effectiveness of ultrasonification as does the type of ultrasonic equipment used. Ultrasonic pre-treatment of substrate reportedly enhances biogas and methane yield by improving the solubility of organic matter.

The results of this study also confirm that in general, as the levels of SE treatment increase the methane yield also increases, a trend reported also by Lizama, *et al.*, (2017), who observed increased cumulative methane production from 544 to 616 ml in the range 5 000 – 35 000 kJ/kg TS. Likewise, Boni, *et al.*, (2016), reported higher cumulative methane yield with increasing US energy in the range SE 6000 to SE 50 000. However, Zeynali *et al.* (2017) and Li, *et al.*, (2018) report that above a certain threshold, any further increase in SE does not lead to any significant improvement in the methane yield or can even cause a drop in the methane yield.

This study also observed that at low ultrasonic pre-treatment levels (SE2 500 and SE7 500) the methane yield generated was close to that of the control (219 mL/g VS_{added}) suggesting that low specific energies have no effect on methane yield, a finding also made by Li, *et al.*, (2018) and Boni, *et al.*, (2016).

4.5.1.4 Effect of ultrasonification on VFA composition

Liu, *et al.*, (2018) reports that ultrasonic pre-treatment can be applied to enhance the hydrolysis step of fruit and vegetable waste AD. This is because sonication improves solubilisation of organic matter and subsequently increases VFA production (Jiang, et al., 2014; Kong, et al., 2021). The results of applying different sonication energies on VFA composition are displayed in Table 4.8.

Table 4.8 VFA composition of anaerobic digested FVW at different sonication levels

VFA	Valeric	Butyric	Propionic	Acetic	Formic	Other
Control	9.5799	9.25	11.348	6.035	9.86	53.93
US2500	0.00	0	22.1688	0.00	6.68	71.15
US7500	9.5418	0	0	0.00	10.41	80.05
US12500	10.32	0	0	0	11.26	78.42
US17500	12.11	3.6004	0	6.99	9.70	67.60

US is ultrasonification energy density (kJ/kg TS)

At low ultra-sonication energies (0 to 2 500 kJ/ kg TS) propionic, and formic acid were the main VFAs generated. As higher ultrasonification energies, formic, and valeric acid were the main VFAs detected. There were really no detectable trends on the effects of ultra-sonication on VFA composition, save that valeric acid content slightly increased with increasing ultra-sonication energy. The results of the study were not in line with the expected results as ultra-sonication was expected to influence VFA composition. Other studies reported the positive effects of ultra-sonication on improving volatile fatty acid production and composition (Guo, et al., 2014; Liu, et al., 2018; Jiang, et al., 2014)

4.5.1.5 Effect of co-digestion on methane yield

Batch tests were carried out to investigate the effect of co-digestion of fruit and vegetable waste and pig manure on methane and VFA composition and yield. Co-digestion is known to enhance nutrient supply, diluting potentially inhibitory compounds and promoting the synergic effect of microorganisms (Nghiem *et al.*, 2017). Fruit and vegetable waste to pig manure ratios (FVW:PM), on a VS matter basis, of 100:0; 75:25; 50:50; 25:75; 100:0 were studied. Figures 4.10 and 4.11 display the effects of co-digestion on methane yield. The methane yields obtained for this study from the co-digestion of FVW waste and pig manure was in the range of 318 – 434 Nml /g VS_{added}. Figure 4.10 and 4.11 shows that a FVW: PM co-digestion ratio of 75:25 generated the highest methane yield (434 Nml /g VS_{added}) while FVW25:PM75 gave the lowest yield. Comparing the methane yield of FVW alone to that of FVW75:PM25, co-digestion enhanced methane yield by 20 %. Similar results have been reported by Velmurugan, *et al.*,

2010), who found that a 75:25 mixtures of fruit and vegetable waste: primary sewage sludge produced the maximum methane yield compared to 25:75 and 50:50 mixture ratios.

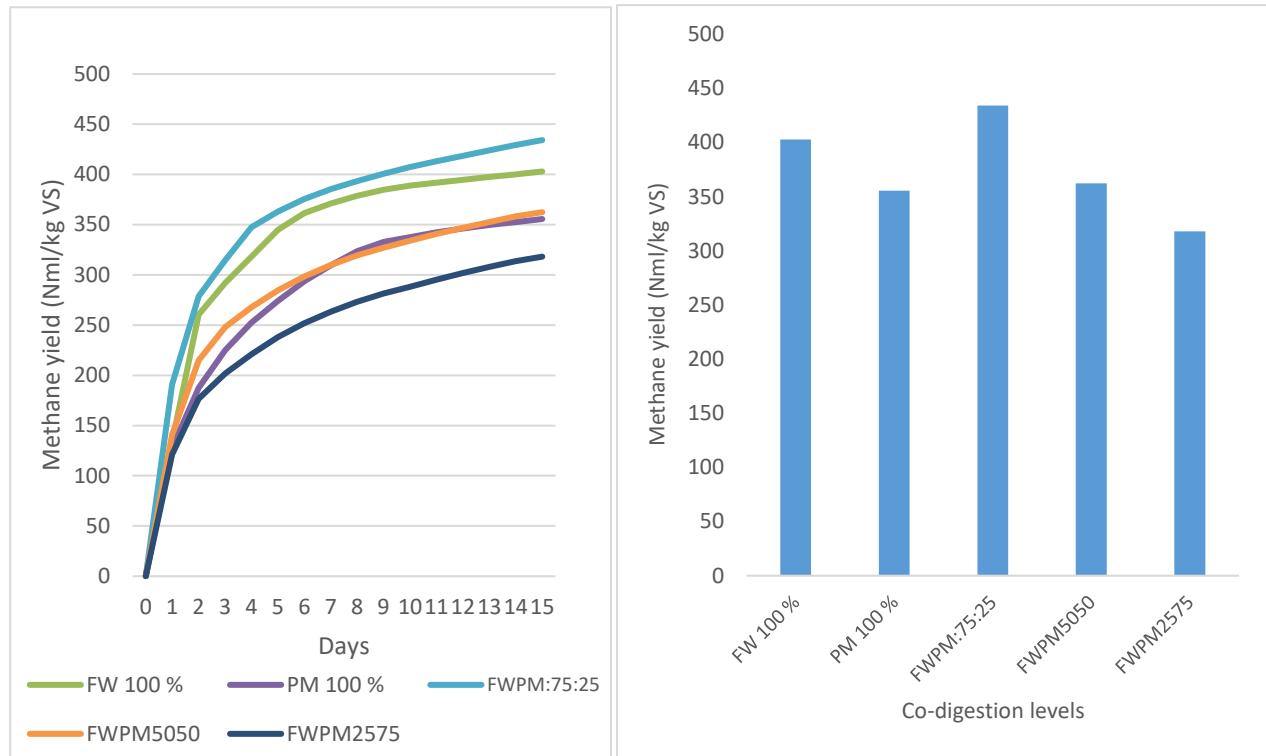


Figure 4.10 and 4.11 Effect of ultrasonic-pretreatment on cumulative methane yield

Focusing on co-digestion of FVW and livestock manure studies, Dennehy, *et al.*, (2017) looked at co-digestion of PM and FW at varying percentages (85/15; 63/37 and 40/60) and concluded that a higher percentage of FW led to a significant increase in methane yields. Also Cárdenas-Cleves, *et al.*, (2018) studied different FVW:PM ratios (100:0; 80:20; 60:40; 50:50; 40:60; 20:80; 0:100) and obtained maximum methane yield of 72.87 mL/ g VS at a FW:PM of 60:40 an increase of 27 % compared to mono digestion of FVW. Other studies reported higher methane yields from mixtures having higher livestock manure fractions than FVW. For example, Hubenov, *et al.*, (2015), reported an optimal PM:FVW mix ratio of 70:30 generated the maximum methane yield. Abebe, (2017) investigated co-digestion of fruit and vegetable waste with cow dung and reported that the highest methane yield was obtained from cow dung alone while fruit and vegetable waste alone generated the lowest methane yield, opposite to the findings of this study.

In general, for co-digestion of FVW and livestock manure, literature results point to an increase in methane yield as the FVW fraction increases up to a certain point, usually 60 -75 %. Beyond this critical point, a decrease in methane yields is observed. Effect of co-digestion on VFA composition.

4.5.1.6 Effect of co-digestion on VFA composition

The VFA distribution due to co-digestion of FVW with pig manure is shown in Figure 4.12. The results are presented in graphic form to best show how there was little variation in the VFA distribution under varying co-digestion ratios. A mixture of VFAs consisting of acetic, propionic, butyric, valeric, and formic acid were generated in more or less equal amounts, signifying that the fermentation pathway was a mixed type. The results of this study concur with the findings of Owusu-Agyeman, *et al.*, (2020) who observed mixed VFA production from co-digestion of sewage sludge and organic waste as did Kaur, *et al.*, (2020) produced a mixture of VFAs from co-digestion of food waste and sewage sludge.

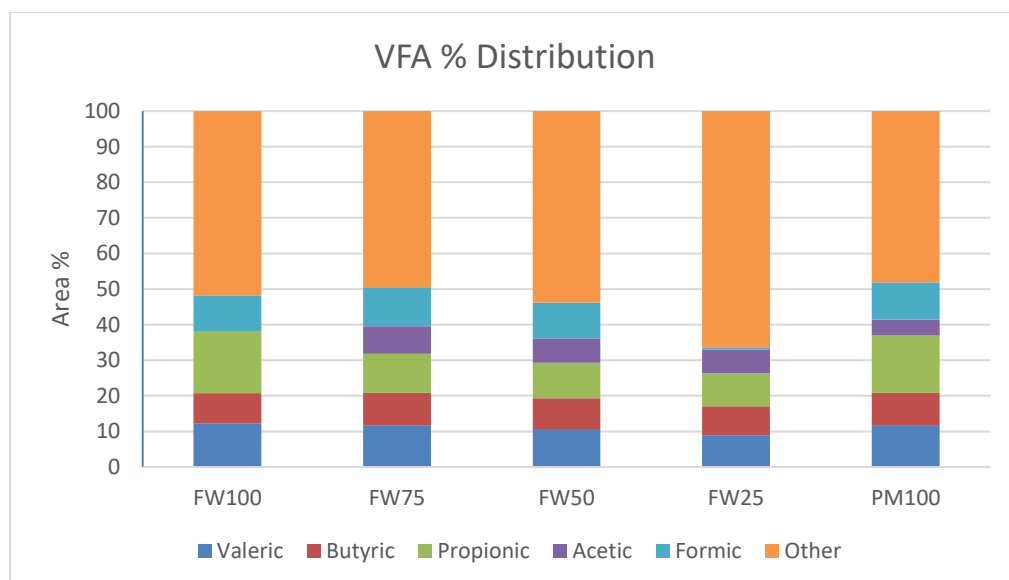


Figure 4.12 VFA composition of anaerobic digested FVW at different co-digestion levels

4.5.2 CCD experimental runs

From the screening experiments, the effect of process parameters on VFA composition was not clear and the content of VFAs was low, therefore optimisation was conducted only for methane.

The CCD with the experimental results obtained from the 20 runs as well as the predicted values is shown in Table 4.9 .

Table 4.9 Experimental Methane and VFA yields and predicted values for the CCD

Std	Run	Factor 1 A:Co- digestion	Factor 2 pH	B: Factor 3:Ultrasonification (KJ/ Kg VS)	Response Methane gVS	1 Nml/ Methane Nml/gVS	Predicted Methane Nml/gVS
15	1	62,5		8	16 250	574,57	590.39
11	2	62,5	6,31821		16 250	348,37	254.55
19	3	62,5		8	16 250	653,02	590.33
3	4	50		9	12 500	533,01	592.45
9	5	41,4776		8	16 250	555,33	542.03
18	6	62,5		8	16 250	558,88	590.39
16	7	62,5		8	16 250	637,33	590.39
17	8	62,5		8	16 250	543,19	590.39
2	9	75		7	12 500	242,80	305.70
5	10	50		7	20 000	465,12	511.77
20	11	62,5		8	16 250	668,71	590.39
1	12	50		7	12 500	406,83	418.12
6	13	75		7	20 000	269,50	402.38
8	14	75		9	20 000	245,28	384.53
10	15	83,5224		8	16 250	533,27	583.66
13	16	62,5		8	9 943,28	520,94	403.69
12	17	62,5	9,68179		16 250	471,93	496.03
7	18	50		9	20 000	479,38	418.12
14	19	62,5		8	22 556,7	700,69	597.11
4	20	75		9	12 500	524,33	498.80

4.5.3 Model fitting and Analysis

Using multiple regression analysis, the EMY data were fitted to a second –order polynomial equation relating co-digestion, ultrasonification, and pH to yield. The first polynomial equation

generated via multiple regression analysis was not significant according to the ANOVA carried out (Appendix C1), henceforward regression was applied, following Alfarjani (2014) methodology. Alffarjani (2014) explains that commonly the full model equation generated via multiple linear regression consists of insignificant model terms that need require to be eliminated and that the elimination can be achieved manually or automatically, further listing the three automatic procedures available as forward, backward or multi-step method. The final reduced model consists only of significant terms and the terms required to maintain hierarchically. In this case, the forward regression method was applied to automatically eliminate the insignificant model terms, generating a reduced quadratic model according to coded values (Equation 4.4):

$$\text{Methane yield} = 590.39 - 46.83A + 44.34B + 4C - 52.21BC - 44.94A^2 - 92.37B^2 \dots\dots\dots \text{Equation 4.4}$$

The adequacy and statistical significance of the reduced quadratic model were determined using ANOVA (Table 4.10).

Table 4.10 ANOVA of the model for methane production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.227E+05	6	37109.60	3,90	0,0188	Significant
A-Co-digestion	29945.75	1	29945.75	3.15	0,0993	
B-pH	26849.63	1	26849.63	2.82	0.1167	
C-Ultrasonification	218.47	1	218.47	0.0230	0.8818	
BC	21807.07	1	21807.07	2.29	0.1538	
A ²	29393.40	1	29393.40	3.09	0.1022	
B ²	1.242E+05	1	1.242E+05	13.06	0.0031	
Residual	1.236E+05	13	9505.58			
Lack of Fit	1.093E+05	8	13661.79	4,17	0.0507	not significant
Pure Error	14278.21	5	2855.64			
Cor Total	3.462E+05	19				

$R^2 = 0.6431$; Adjusted $R^2 = 0.4784$; Predicted $R^2 = -0.2344$; Adeq Precision

A Model F-value of 3.90 indicates that the model is significant. There was a low chance (1.88 %) of obtaining such a large F due to noise. Liu, *et al.*, (2018) and Mkruqulwa (2018) explain that a model term is deemed significant if the P –value is less than 0.0500, while P values

greater than 0.100 indicate insignificant model terms. However, the linear model terms (ABC), the quadratic model terms (A^2 , B^2) and the interactive model term BC were insignificant ($P > 0.05$). The “Lack of Fit F-value” of 4.78 implies the lack of fit not significant. There is a 5.07 % chance that a large LOF F-value was obtained was due to noise, a probability $>10\%$ is more desirable (Design-Expert, 2021). Adeq precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of this research is 5.9390, which indicates an adequate signal and that the model can be applied to navigate the design space (Design-Expert, 2021).

A good statistical model ideally has "Pred R-Squared" in the range of 0.75-1 (Mkruqulwa, 2018). The Predicted R^2 value for the model is -0.2344, this implies that the overall mean might be a better predictor of the methane response than the current model (Design-Expert, 2021).

Figure 4.13 is a plot of predicted values vs actual experimental data. A good fit is represented by experimental data points lined linearly along the 45 degree line, showing that the model predicted data is are close to the actual experimental data (Madondo, 2017). The experimental methane yield points are well distributed along the 45° line, confirming the validity of the model to describe methane yield production.

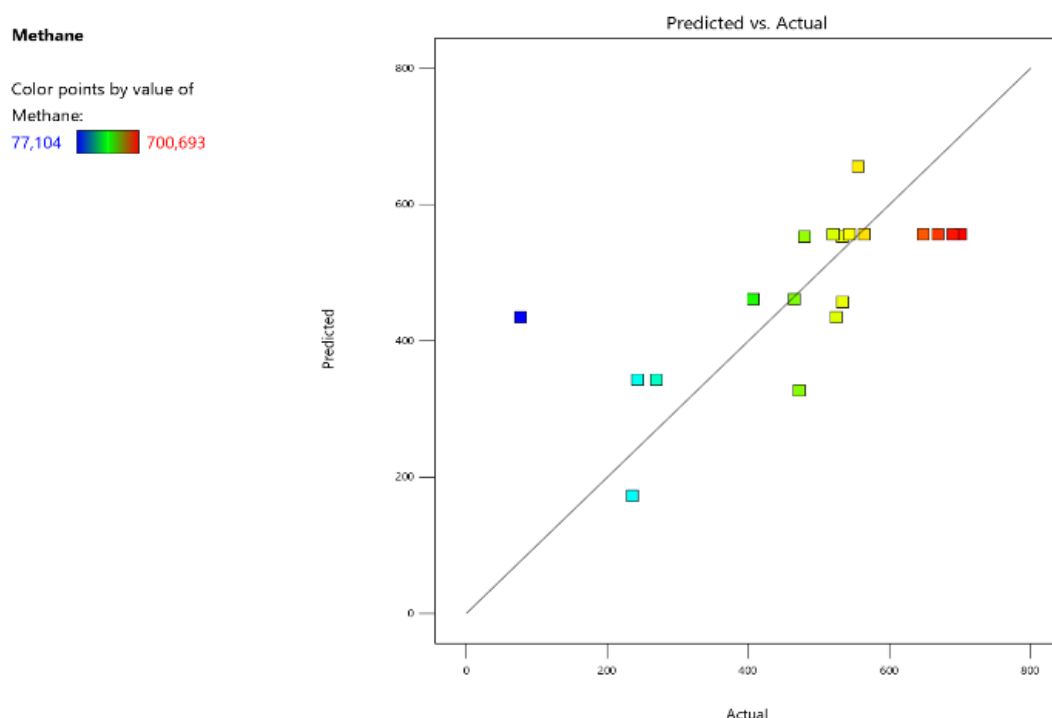


Figure 4.13 Predicted versus experimental methane yields

Further diagnostic analysis were conducted to assess and validate the model fit including Normal Plot of Residuals, Residuals vs Predicted, Box-Cox Plot for Power Transforms, and

Cook's Distance following procedures described by (Madondo, 2017). The diagnosis analysis and results are attached to Appendix C2. In summary, the diagnosis showed that no response transformation was required for any of the experimental runs for the methane yields and that the methane yield values predicted by the model were close to the actual experimental data.

4.5.4 Response Surface Analysis and interactions among factors

The optimum level of each of the three factors (co-digestion, pH, ultra-sonication) for methane yield and the interaction effects of the three variables were determined using 3D response surfaces and contour plots.

(i) Interactive effects of co-digestion and pH on methane yield

The relationship between co-digestion and pH was represented was using a contour plot and matching 3D response surface (Fig 4.14). The C variable (ultrasonification) was kept at the central level of 16 250 kJ/kg TS the plot while the other variables (A & B) were varied inside the estimated experimental range. The 3D surface shows that the methane yield was at a minimum at the lowest pH value (7) and highest FVW co-digestion ratio (75%). The methane yield increased with increasing pH up to pH8 for all co-digestion ratios, beyond pH 8, the methane yield decreased. This is in agreement with previous research findings ((Zhao, *et al.*, 2017; Khan, *et al.*, 2016a) that report that optimum methane production occurs within a narrow range pH range, usually pH of 6.8 -7.2. In the case of this study, the optimum pH occurred around pH 8, possibly because the pH of the inoculum used was pH 8, suggesting that for the biogas plant which the inoculum was taken, the biogas process runs at pH 8 and the microbial population is acclimatised to that pH. Similarly, methane yield increased with increasing FVW co-digestion ratio up to a certain limit, beyond this limit, methane yield decreased. The increase in methane yield due to changes in co-digestion ratios was much smaller compared to changes due to pH variation, supporting the ANOVA results that co-digestion did not significantly affect methane yield.

(ii) Interactive effects of pH and ultrasonification on methane yield

Figure 4.15 shows the interaction between pH and ultrasonification. None of the factors pH and ultrasonification showed significant impact on the methane yield ($p > 0.05$), their interactive effect was also statistically insignificant ($p > 0.05$). The lowest methane yield occurred at lowest pH and ultrasonification values. Methane yield increased with increasing

pH for all ultrasonification values, until reaching a maximum at pH 8, after this the methane yield decreased with increasing pH. The methane yield increased with increasing ultrasonification energy for all pH values, however, the increase was minimal, again confirming the ANOVA results that ultrasonification (factor C) has no significant effect on methane yield.

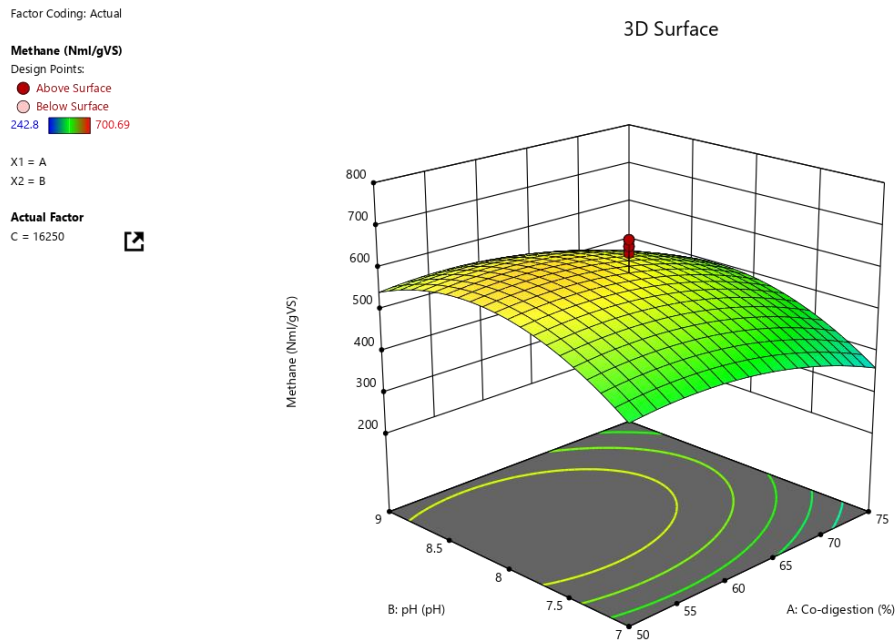


Figure 4.14 3D surface, interactive effects of pH and co-digestion on methane yield

Factor Coding: Actual

Methane (Nm/gVS)

Design Points:

● Above Surface

○ Below Surface

242.8 700.69

X1 = B

X2 = C

Actual Factor

A = 62.5

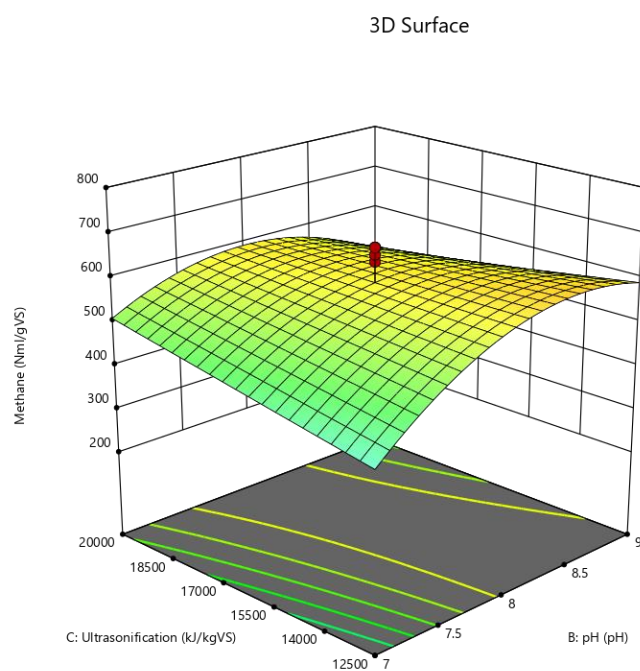


Figure 4.15 3D surface, interactive effects of pH and ultrasonification on methane yield

(iii) Interactive effects of co-digestion and ultrasonification on methane yield

The interactive effects of co-digestion and ultrasonification are depicted in Figure 4.16. The graph shows that for low FVW co-digestion ratios (50-62,5%) the methane yield was at its highest and remained constant for all ultrasonification values. However, for FVW co-digestion ratios above 62, 5 % the methane yield dropped. Ultrasonification appears not to have had an effect on the methane yield, as the methane yields stayed constant despite an increase in ultrasonification energy applied for all FVW co-digestion ratios. Although varying the FVW co-digestion ratio resulted in changes in the methane yield, the changes were small. Implying that both ultrasonification and co-digestion had no significant influence on methane yield as shown in Table 4.10.

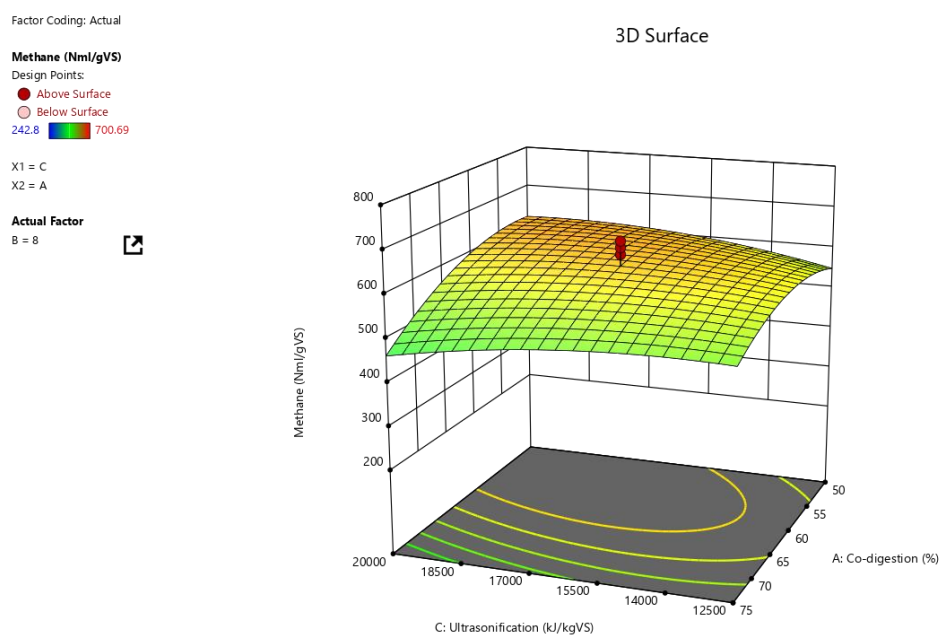


Figure 4.16 3D surface, interactive effects of pH and ultrasonification on methane yield

4.5.5 Optimisation and model validation

The criteria set for each factor and response as well as the importance level allocated for each criterion are recorded in Table 4.11. Ultrasonication was set to goal of equal to 0 since analysis of data showed that ultrasonication had little influence on methane yield.

Table 4.11 Criteria set optimisation of methane yield

Factor	Goal	Importance
Co-Digestion	In Range (60-65)	Level 3 (Software Selected)
pH	In Range (8-8.5)	Level 3 (Software Selected)
Ultrasonication	Equal To 0	Level 3
Methane Yield	Maximise	Level 4

Two solutions were generated; the solution with the highest desirability value (0.886) was selected. This solution gave optimum conditions of co-digestion = 60 %; pH = 5 and a predicted maximum yield of 698.82 ml/g VS. For the model validation experiment a yield of 637,36 N/ml was obtained (Figure 4.17) (Appendix C2).

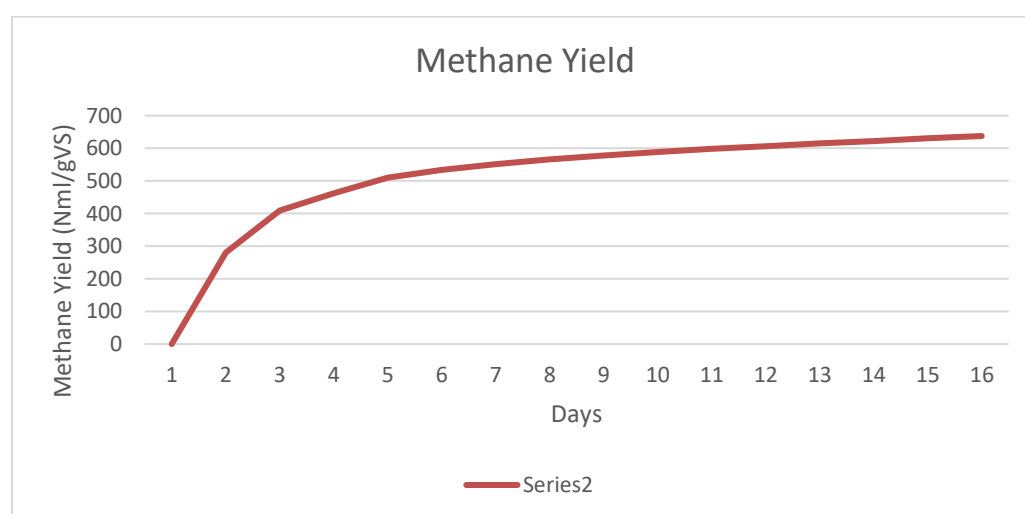


Figure 4.17 3D Methane yield for model validation

4.6 Conclusion and Recommendations

The findings of this study support previous research findings that concluded that process parameters influence the anaerobic digestion process, which influences product yield and composition. The tests have shown the importance of maintaining digestion parameters within a specific range, as deviations from this range will result in lower yields or even process failure. For example, in this analysis, pH levels lower than 6 or higher than 8 resulted in no gas production. Co-digestion is a more possible and simpler technique to use to increase product yield than the other ones investigated (pH, ultra-sonification, and co-digestion), as are many agricultural waste sources readily available for use. While pH was found to be the most important factor influencing methane production, controlling it at a large-scale plant can be expensive due to high chemical costs. As a result, there is a need for research into cost-effective pH management strategies, which may include the use of other readily available organic waste materials.

One of the goals of this research was to look at the simultaneous development of VFA and methane using one-stage reactors with a focus on process parameters to boost VFA production. The research was unable to produce adequate quantities of VFAs using this approach, nor to specifically distinguish how the process parameters selected influence volatile fatty acid delivery. A safer way to generate both methane and VFAs is to use two stages of anaerobic digestion, or to look at other considerations like HRT, organic loading, and various substrates. The research was effective in using RSM to optimize process parameters in order to increase methane yield.

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5. Investigating the product spectra of Anaerobic Biorefinery of FVW: Hydro-char production via Hydrothermal Carbonization

Abstract

Anaerobically digested Fruit and Vegetable Waste (FVW) digestate were subjected to Hydrothermal Carbonization as a downstream treatment process to generate energy- rich hydro-char. The Hydrothermal carbonization (HTC) experiments were carried at temperatures of 180 °C and 200 °C for 2 hours in a 50ml stainless steel batch reactor, which was externally heated. The proximate composition, elemental composition, heating values, and thermal stability of the HTC solids were characterised. There was an increase in the carbon content of both hydro-chars compared to the digestate (27, 65 %). The hydro-char that received HTC treatment of 200 °C for 2 hours had the highest heating value of 10, 75 MJ/kg, an increase of 46.66 % compared to the digestate. The highest hydro-char yield and energy yield was observed for the digestate treated at 200 °C for 2 hours. The study shows that valuable products can be generated successfully from anaerobic digestate using hydrothermal carbonization.

5.1 Introduction

AD produces large quantities of digestate, a nutrient-rich substrate consisting of partially digested organic matter, microbial biomass and inorganic compounds (Cesaro, 2021), in addition to biofuels (methane & hydrogen) and biochemicals (e.g. volatile fatty acids). Traditionally, digestate valorisation receives little attention, with land application being the preferred option owing to the high agronomic value of digestate (Wang & Lee, 2021). However, there is rising concern over land application of digestate, including ammonia volatilization, biological contamination, greenhouse gas emissions and high transport and storage costs as described by Cesaro, (2021) and Selvaraj, *et al.*, (2021), who also noted that poor digestate management negatively affects the sustainability of AD plants. The same researchers also advocated for the application of circular economy principles for digestate treatment. The anaerobic biorefinery platform is a promising avenue to create value added products from digestate in line with the circular economy approach (Wang & Lee, 2021). Although research on digestate valorisation has occurred in recent years, Peng & Pivato, (2019)

and Perez, *et al.*, (2020) mention that the biorefinery process for digestate management is underdeveloped underpinning the need for further studies in digestate valorisation and system integration. Researchers like Akarsu, *et al.*, (2019); Bernardo, *et al.*, (2020); and Singlitico, *et al.*, (2020), studied the integration of AD with thermochemical conversion process such as gasification, pyrolysis, and hydrothermal carbonization producing value - added products such as syngas, bio-char, hydro-char and bio-oils in addition to biogas.

Hydrothermal carbonization (HTC) in particular, has gained attention as it treats wet digestate negating the need for drying the digestate prior to treatment. An additional benefit of HTC according to (Kambo, *et al.*, 2018), is that it utilises water as a reaction medium that is inexpensive, environmentally friendly and non-toxic. HTC requires moderate temperatures (180-350⁰ C), pressures (2 -10 MPa) and time (2 - 20 h) unlike gasification and pyrolysis (Selvaraj, *et al.*, 2021; Pamar, 2019). Under such conditions, Ischia & Fiori, (2020), and Langone & Basso, (2020) explain that liquid water serves as a catalyst, reactant and a solvent medium for the biomass, resulting in biomass degradation via heterogeneous chemical reactions, such as dehydration, hydrolysis, aromatization, and polymerization.

The resulting products are a carbon-rich solid phase (hydro-char) as well as liquid (process water) and gaseous by-products. Hydro-char has multiple applications in agriculture such as a solid amendment, energy generation and as a building block for advanced carbon materials (Pamar, 2019; Parmar & Roos, 2019). Previous research on HTC of organic biomass (Correa, *et al.*, 2017; Stutzenstein, *et al.*, 2018; Cao, *et al.*, 2019; Zaccariello, *et al.*, 2019) shows that process conditions including temperature, pressure and residence time significantly influence the yield, composition, and properties of products generated. This study investigated the solid products generated from hydrothermal carbonization of mixed fruit and vegetable waste digestate under different operating conditions looking at the yield and properties of the generated products.

5.2 Materials and Methods

5.2.1 Feedstock for HTC and its characteristics

The feedstock used for the HTC experiments was digestate collected from the control run (i.e. untreated inoculum and FVW) of the bio-hydrogen and VFA production experiments of Section 3.1 (Fig 5.1). The digestate was utilised directly as a slurry without dewatering. Proximate and ultimate analysis was conducted on the digestate, the properties which are recorded in Table 5.1.



Figure 5.1 Digestae used as feedstcok for HTC experiments

5.2.2 Hydrothermal Carbonization Experimental set up

The HTC was conducted in an unstirred batch 50 mL PTFE (polytetrafluoroethylene) lined, 304 grade stainless steel, and BAOSHISHAN hydrothermal synthesis reactor (Figure 5.2). A laboratory biomass oven was used to heat the stainless steel reactor.



Figure 5.2 50 ml PTFE lined stainless steel hydrosynthesis reactor

5.2.3 Experimental procedure

The experimental procedure is shown in Fig 5.3 -5.7. For each run, 30g (30 mL) of digestate slurry was measured and loaded into the PTFE liner (Fig 5.3) and placed inside the stainless steel reactor. Afterwards the reactor was closed tightly, and placed in an electric muffle furnace (Fig 5.4). The HTC was heated to target temperatures of 180 °C and 200 °C following procedures outlined by (Suwelack, *et al.*, 2016; Manyuchi, *et al.*, 2018; Sharma, *et al.*, 2020). The ramp time to reach the target operating temperatures ranged from 20 to 25 minutes. The reactor was kept at the target temperature for a stipulated time of 2 hours (Table 5.1).

Table 5.1 HTC experimental conditions

Run No	Temperature °C	Holding Time (hours)
1	180	2
2	200	2

After the reaction was complete, the oven was turned off and the reactor allowed to cool down up to 50 °C after which the reactor was placed in an ice water bath to cool down rapidly as described by (Suwelack, *et al.*, 2016; Huang, *et al.*, 2018). After cooling was complete, the PTFE liner and contents were weighed. The solid and liquid fractions were separated via filtration using a buchner funnel (Fig 5.5). Subsequently the solid fraction was oven dried at a

temperature of 105 °C for 5 hours (Fig 5.6) before analysis of the solid hydro-char (Velebil, *et al.*, 2016; Saetea & Tippayawong, 2013). No further analysis was conducted on the HTC process water /filtrate. After drying, the dried samples (hydro-char) were ground into a powder in preparation of ultimate analysis (Martin-Batista, *et al.*, 2020; Deng, *et al.*, 2020) .The hydro-chars (HC) were labelled HC-T_t according to the temperature (T) and holding time (t) applied.



Figure 5.4 Measuring 30 g of digestate



Figure 5.3 HTC reactor placed in oven



Figure 5.6 Filtering



Figure 5.5 Drying the hydro-char

5.3 Analytical Methods

5.3.1 Proximate and ultimate analysis

Proximate and ultimate analysis was done to characterize the produced hydro-char. Proximate analysis was conducted to measure moisture content, ash content, and volatile matter content using EPA method 1684. For ultimate analysis, a Flash 2 000 elemental analyser measured the C, H, N, S content. The oxygen content was determined indirectly by difference:

$O \% = 100 \% - C \% - H \% - N \% - S \%$. In the same way, fixed carbon was calculated by difference: $100 \% - \text{Volatile Solids} \% - \text{Ash} \% - \text{Moisture} \%$ (Saetea & Tippayawong, 2013) .

5.3.2 Hydro-char yield

Using proximate analysis data, the Hydro-char mass yield (HY) and Fuel ratio (FR) was determined according to Equation 5.1 and 5.2 by:

$$HY (\%) = \frac{\text{weight of hydrochar (g)}}{\text{weight of digestate (g)}} \times 100 \dots\dots\dots \text{Equation 5.1}$$

$$\text{Fuel Ratio (FR)} = \frac{\text{Fixed Carbon}}{\text{Volatile Solids}} \dots\dots\dots \text{Equation 5.2}$$

5.3.3 Energy properties of hydro-char

Mass and energy calculations were performed using equations outlined by Deng, et al., (2020). The Higher Heating Value (HHV) of the hydro-char and digestate was determined using Dulong's equation (Equation 5.3):

$$HHV \text{ (kJ/ kgVS)} = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151 N - 0.0211\text{Ash}$$

Equation 5.4 and 5.5 calculated the Energy Densification (ED), an indicator of the energy retention capacity of the hydro-char, and the Energy Yield (EY):

$$\text{Energy Densification (ED)} = \frac{HHV \text{ hydrochar}}{HHV \text{ feedstock}} \dots\dots\dots \text{Equation 5.4}$$

$$(EY)\% = \text{Hydrochar yield} \times \text{Energy Densification} \dots\dots\dots \text{Equation 5.5}$$

5.4 Results and Discussion

5.4.1 Proximate and ultimate characterisation of hydro-char and feedstock

The hydro-chars formed (HTC-180₂ and HTC-200₂) are depicted in Figures 5.7 and 5.8 , respectively. The two hydro-chars have no discernible variations in shape.



Figure 5.7 HTC-200₂ hydro-char



Figure 5.8 HTC-180₂ hydro-char

The results of the proximate and ultimate study performed on the two hydro-chars generated under the various experimental conditions, as well as the digestate feedstock, are mentioned in Table 5.2.

Table 5.2 Proximate and ultimate analysis of digestate and hydro-char

Property	Unit	Digestate	HTC-180 ₂	HTC-200 ₂
VS	%	34,96	43,74	40,0
Ash	%	53,74	45,46	47,02
Fixed carbon	%	11,30	10,80	13,20
C	%	27,65	34,13	35,20
H	%	3,88	3,97	4,40
O	%	64,49	58,025	56,325
N	%	2,94	2,64	2,91
S	%	1,05	1,24	1,18

According to the proximate and ultimate analysis conducted, hydrothermal pre-treatment did not cause a major change to the elemental composition of the digestate. An increase in carbon content was observed similar to the findings of Sharma, *et al.*, (2020), who reported an increase in carbon content after applying HTC treatment at 180 °C and 200 °C for 6 hours.

5.4.2 Hydro-char yield

Table 5.3 compared the hydro-char yields obtained from this study to values obtained in the literature. The solid yields obtained in this study are lower than those observed in other studies. A possible cause of this is the different digestate substrates used as well as the reactor type including longer holding times. Additionally, this study employed a batch HTC reactor with external heating while other studies have used self- heating pressurised reactors where the temperatures and pressure are better controlled. This experiment recorded a higher hydro-char yield at a higher temperature of 200⁰C compared to a low one. In contrast, Sharma, *et al.*, (2020) and Cao, *et al.*, (2019) obtained maximum solid yield at a lower temperature . However, a study by Manyuchi, et al., (2018) reported that hydro –char yield increased with increasing temperature up to a certain point, beyond this point, the yield dropped.

Table 5.3 comparison of the hydro-char yields obtained from this study to values obtained in literature.

HTC temp (°C)	HTC holding time (hr)	Feedstock	Hydro-char yield (%)	Reference
180	2	FVW Digestate	42,7	This study
200	2	FVW Digestate	58,2	This study
180	6	Digestate Yard waste digestate	68,18	(Sharma, <i>et al.</i> , 2020)
200	6	Yard waste digestate	65,62	(Sharma, <i>et al.</i> , 2020)
190	2	Digestate	80,4	(Cao, <i>et al.</i> , 2019)
210	2	Digestate	72,2	(Cao, <i>et al.</i> , 2019)

200	2	Brewery spent grain	70	(Manyuchi, et al., 2018)
300	2	Brewery spent grain	40	(Manyuchi, et al., 2018)

5.4.3 Energy properties of the Hydro-char

The HHV or calorific value is a critical parameter for hydro-char purposed for use as a fuel (Sharma, *et al.*, 2020). The energy properties of the hydro-chars are tabulated in Table 5.4 . There was an improvement in the HHV of the hydro-chars compared to the digestate, this can be attributed to an increased value of fixed carbon content (Sharma, *et al.*, 2020). The highest energy yield was obtained from the HTC-200₂, the increase in energy yield at higher temps can be linked to the higher hydro-char yield and carbon content obtained (Sharma et al., 2020).

Table 5. 4 Energy properties of the produced hydro-chars and digestate

Property	Unit	Digestate	HTC-180 ₂	HTC-200 ₂
HHV	MJ/kg	7,33	9,74	10,75
Fuel ratio		0,79	0,78	0,88
Energy densification		--	1,33	1,47
Hydro-char yield	%		42,7	58,2
Energy yield	%		56,8	85,26

The HHVs achieved by this study are low compared to the results of other studies: 19-25 MJ/kg (Xu, *et al.*, 2013); 26, 1 MJ/kg (Manyuchi, *et al.*, 2018); 11.9-26 MJ/kg (Cao, et al., 2019) and 15-23 Mk/kg (Sharma *et al.*, 2020). There is room to improve the process through, for example, investigating longer holding times, higher temperatures and, perhaps even employing catalysts. In order for hydro-chars to compete with solid fuels like coal, it is important that the HHV value be equal or better than that of the solid fuels it must displace. In the case of coal, the HHV of the produced hydro-chars must exceed 24,7 MJ/kg (Manyuchi, *et al.*, 2018).

5.5 Conclusion and Recommendation

Hydrothermal carbonisation can be coupled to anaerobic digestion as a digestate post-treatment method. Hydrothermal carbonisation presents an opportunity to address the challenges currently faced with digestate storage and application as it reduces the bulk density of the digestate, making it easier to store and transport to another site for use if the hydro-char is not needed on site. However, there is a need to factor in the costs of HTC treatment as heat energy is an important input, which might make the process not viable financially. Although this study did not investigate the liquid fraction produced via the HTC process, the chemicals in the liquid fraction are worthwhile investigating as they are reported to be valuable.

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6. CONCLUSION AND RECOMMENDATIONS

This study looks at the concept of an anaerobic waste biorefinery that generates several products from fruit and vegetable waste, mainly through the anaerobic digestion process with digestate downstream processing. The aim of the study was to show how anaerobic digestion is a versatile Waste to Energy process that can be used to manage agricultural waste, produce green energy, and other high-demand bio-chemicals.

The circular and bio-economy theories, which advocate for resource management, resource recovery, and biodiversity, are well-suited to the idea of a waste biorefinery. The circular economy proponents imagine a bio-based economy in which renewable resources substitute fossil-based fuels, commodities, and chemicals as the foundation for economic growth and sustainability. The current research contributes to the body of ongoing scientific research aimed at developing novel methods for producing value-added bio-products. More specifically, this study, as well as the several other studies cited in this thesis, has shown how waste can be used as a resource to create a variety of goods of economic benefit, providing a safer alternative to waste disposal.

The literature reviewed in Chapters 1 and 2 of the Thesis showed that vast quantities of organic waste are produced by any operation along the food supply chain, and that the causes and types of waste differ at each phase of the FSC. The disposal of this waste, while not actually a big problem, will become one in the future as the world's population grows and urbanisation accelerates. The study also revealed the flaws in traditional waste management schemes, such as their detrimental impacts on the environment, climate change, and possible health risks. The study also looked at new and emerging Waste-to-Energy systems that could be used to treat fruit and vegetable waste. Biochemical processing methods such as bioethanol and anaerobic digestion were used, as well as thermochemical conversion methods such as pyrolysis, gasification, and hydrothermal carbonisation. In addition, new bio-electrical chemical systems, such as microbial fuel cells, are being created. The literature reviews also revealed how different stages of the anaerobic digestion process produce various intermediate products (hydrogen, VFAs, and alcohols), as well as the impact of process parameters on the formation of these intermediates and the end product (methane).

The experimental studies in Chapters 3 and 4 looked at the range of products that can be produced by anaerobic digestion using various anaerobic digestion manipulation strategies.

The research demonstrated in Chapter 3 that pre-treatment of the parent inoculum (acid, alkali, heat, and heat-alkali) can produce hydrogen and volatile fatty acids. The numerous pre-treatments have differing effects on enhancing hydrogen production and affecting the VFA composition, with acid pre-treatment being the most successful, according to Chapter 3.

In Chapter 4, we looked at the processing of methane and volatile fatty acids, modifying process conditions to produce various products and increase yields. Screening experiments revealed that the chosen research parameters (pH, co-digestion, and ultra-sonication) influence methane yield but not the VFA composition. The use of RSM with a CCD prototype was effective in optimizing process parameters to increase methane yield.

Finally, Chapter 5 used a different WtE technique to produce hydro-char from anaerobic digestate: hydrothermal carbonisation, a thermo-chemical conversion method.

The following are recommendations of the study:

- Investigate how inoculum pre-treatment affects the microbial community of the parent inoculum
- Investigate the effects of inoculum pre-treatment combined with varying process parameters on the hydrogen and methane yield
- Investigate and develop affordable VFA extraction methods or investigating other conversion routes to produce bio-materials and chemicals from the produced VFA.
- Exploring other anaerobic based biorefineries by coupling anaerobic digestion with e.g. micro-fuel cells.

APPENDICES

6.1 Appendix A1: Raw Data Biogas and Hydrogen Production

1. Combined methane and hydrogen production as recorded by AMPTS														
Flow Cell nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	15
Name	Raw Inoculum 1	Raw Inoculum 2	Raw Inoculum 3	Heat 1	Heat 2	Heat 3	Alkaline 1	Alkaline 2	Alkaline 3	Acid 1	Acid 2	Acid 3	Alka Heat 1	Alka Heat 3
Substrate VS/COD amount [g]	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76	2,76
Inoculum VS/COD amount [g]	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52	5,52
Type of unit [VS/COD]	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS
Headspace volume [ml]	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Assumed CH4 content [%]	65	65	65	65	65	65	65	65	65	65	65	65	65	65
Day	Raw Inoculum 1 Volume [Nml]	Raw Inoculum 2 Volume [Nml]	Raw Inoculum 3 Volume [Nml]	Heat 1 Volume [Nml]	Heat 2 Volume [Nml]	Heat 3 Volume [Nml]	Alkaline 1 Volume [Nml]	Alkaline 2 Volume [Nml]	Alkaline 3 Volume [Nml]	Acid 1 Volume [Nml]	Acid 2 Volume [Nml]	Acid 3 Volume [Nml]	Alka Heat 1 Volume [Nml]	Alka Heat 3 Volume [Nml]
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	30,7	228,5	136,08	78,3	89,9	87,5	255,5	330,3	248,8	417,4	303,6	378,525	289,695	275,9
2	128,4	342,1	247,0125	104,8	118,1	118,1	276,7	357,4	263,4	430,8	322,2	395,325	308,595	293,9
3	235,5	480	375,6375	118,3	132,1	126,2	289	360,9	269,3	447,1	338,5	411,625	318,885	303,7
4	381,5	678,2	556,3425	125,8	142	138,9	292,5	364,4	272,8				319,935	304,7
5	574,3	910,1	779,31	129,3	150,3	142,7	294,8	366,7	275,1				320,985	305,7
6	771	1082,6	973,14	133	155,9	156,6	297	368,9	277,3				322,035	306,7
7	915,9	1178	1099,2975	139,4	163	167,5	299,3	371,2	279,6				323,085	307,7
8	1019,9	1311,4	1223,9325	149,2	173,7	175,2	299,3	371,2	279,6				324,135	308,7
9	1138,3	1429,4	1348,0425	178,2	185,1	194,4							325,185	309,7
10	1241,9	1474,7	1426,215	229,2	201,6	197,7								
11	1305,3	1502,8	1474,2525	341,9	226,4	201,1								
12	1333,3	1529,6	1503,0225	569,6	276	422,4								
13	1366	1557	1534,575	848,3	357,6	714,1								
14	1394,2	1583,2	1563,135	1085,6	518,4	928,4								
15	1414,9	1607,6	1586,8125	1269,2	776,2	934,8								

2. Average cumulative methane/hydrogen production

Day	Raw	Heat	Alkaline	Acid	Alka-heat
0	0,00	0,00	0	0	0,00
1	131,76	85,23	278,2	366,51	282,80
2	239,17	113,67	299,17	382,78	301,25
3	363,71	125,53	306,4	399,08	311,29
4	538,68	135,57	309,9		312,32
5	754,57	140,77	312,2		313,34
6	942,25	148,50	314,4		314,37
7	1064,40	156,63	316,7		315,39
8	1185,08	166,03	316,7		316,42
9	1305,25	185,90			317,44
10	1380,94	209,50			
11	1427,45	256,47			
12	1455,31	422,67			
13	1485,86	640,00			
14	1513,51	844,13			
15	1536,44	993,40			

The average yield for each treatment was calculated by adding the daily hydrogen production data for the three triplicates and dividing by 3.

E.g. Heat pre-treatment Day 1

Average methane /hydrogen produced =

$$(73,9+89,9+87,5)/3 = 85,23$$

3. Mean cumulative biogas yield calculated using biogas composition data					
	Raw	Heat	Alkaline	Acid	Alka-heat
	0,0	0,0	0	0,0	0,0
1	1,0	110,0	278,7696979	100,8	132,8
2	2,0	199,7	371,7656119	108,4	138,7
3	3,0	200,6	439,9787034	111,0	144,6
4	4,0	297,1	475,1442875	112,2826087	
5	5,0	366,0	262,6811594	113,115942	
6	6,0	457,0	152,173913	113,9130435	
7	7,0	526,9	139,7163636	114,7463768	
8	8,0	586,6	132,4404394	114,7463768	
9	9,0	673,7	126,8115942		
10	10,0	712,7	116,9380004		
11	11,0	766,3	161,3532651		
12	12,0	781,2	217,3913043		
13	13,0	775,7	301,1751157		
14	14,0	790,2	397,2374286		
15	15,0	802,1	467,4802498		

4. Cumulative Hydrogen yield calculated using measured gas composition					
Day	Raw H	Heat H	Alkaline H	Acid H	Alkali-heat H
0	0	0	0	0	0
1	3,739931877	26,23548088	32,64768006	130,7274956	91,85497818
2	6,788726652	34,98748135	35,10818698	136,5295481	96,47115506
3	0,601734663	33,89698593	35,26635559	142,7362313	100,2391337
4	0,891206461	36,60622458	35,66920234		100,6061819

5	6,587829579	22,08625329	83,13689013		100,9732302
6	8,226354694	23,29961309	83,72273625		101,3402785
7	15,76599052	35,97230341	97,63896895		101,7073267
8	17,55349047	38,13110093	97,63896895		102,0568965
9	14,81869891	42,72013371			105,7943415
10	15,67802994	48,14345354			
11	15,60469331	9,718204964			
12	15,9092185	16,01596555			
13	14,89408525	0,581569148			
14	15,17127931	0,767065475			
15	15,40108541	0,902704362			

6.2 Appendix A2: Biogas Composition

Day 2	CH4	CO2	O2	NH3	CO	H2S	BAL	H2	
Recorded biogas composition									
R1	0,8	0,2	20,6	1	0	12	78,4	112	
R2	0,7	0,1	20,6	1	0	13	78,6	110	
R3	40	66,8	2,2	12	0	55	0	68	Correct R1
R4	1,3	87,6	1,5	33	<<<	699	10,4	180	
R5	2,1	90,3	1,1	41	42	947	6,8	906	
R6	1,6	84,4	1,6	44	60	748	9,1	524	
R7	11	85,8	1,6	219	>>>>	>>>>>	1,7	-----	
R8	7,7	75,8	3,3	472	1040	>>>>	0	-----	
R9	3,7	95,9	1,6	665	447	8575	>>>>>	-----	-
R10	0,7	53,5	2,3	402	>>>>>	>>>>>>>	41,3	-----	
R11	0,7	50,7	2,2	450	>>>>>>	>>>>>	45,4	-----	
R12	0,8	45,2	4,5	332	>>>>>	>>>>>	51,4	-----	
R13	0,8	80,7	1,3	342	>>>>	>>>>	3,8	-----	
R14	0,6	87	4,1	377	>>>>>>	>>>>>>>>	7,4	-----	
R15	0,8	85,4	3,3	398	>>>>>	>>>>>	8	-----	
Calculated Average Biogas Composition									
	CH4	CO2	O2	Other	H2				
Raw	40	36	20,6	0,0013	3,3987				
Heat	1,666667	87,43333	1,4	0,088833	9,411167				
Alkali	7,466667	85,83333	2,166667	0,95635	3,576983				
Acid	0,733333	49,8	3	0,050567	46,4161				
Heat-alkali	0,733333	84,36667	2,9	0,037233	11,96277				

Day 4	CH4	CO2	O2	NH3	CO	H2S	BAL	H2			
Recorded Biogas Composition											
R1	65,4	39,7	2,3	17	0	86	0	0			
R2	0,8	0,2	19,7	5	0	53	79,4	0			
R3	2	0,8	18,8	7	0	50	78,2	0			
R4	1,4	89,2	1,1	55	<<<	236	9,7	134			
R5	5	88,5	1,4	62	<<<<<	244	5,1	906			
R6	1,5	87,2	1,5	72	>>>>>	204	10,2	0	Error msg over 6 000ppm		
R7	16	79,3	1,7	920	>>>>	2124	0,9	328	Error Exceed 9500 ppm		
R8	12,3	81,8	1,7	>>>	>>>>	6001	3,7	-----	Error Exceed 9500 ppm		
R9	3,5	85,6	1,8	>>>>>	976	>>>>>	9,2	-----	Error Exceed 9500 ppm		
R10	0,7	41	6,9	533	>>>>>	>>>>>>	50,9	-----	Error Exceed 9500 ppm		
R11	0,7	45	5,1	345	>>>>>>	>>>>>	48,3	-----			
R12	0,7	33,8	4,5	250	>>>>>	>>>>>	60,8	-----			
R13	0,8	76,5	1,5	864	>>>>	>>>>	2,1	-----			
R14	0,6	87	4,1	377	>>>>>>	>>>>>>>	7,4	-----			
R15	0,6	85,8	5,7	633	>>>>>	>>>>>	8,6	-----			
Calculated Average Biogas Composition											
	CH4	CO2	O2	Other	H2						
Raw	65,4	32	2,3	0,007267	0,292733	0,292733		100			
Heat	2,633333	88,3	1,333333	0,0291	7,704233	7,704233		100			
Alkali	10,6	82,23333	1,733333	0,49825	4,935083	4,935083		100			
Acid	0,7	39,93333	5,5	0,0376	53,82907	53,82907		100			
Heat alkali	0,666667	83,1	3,766667	0,062467	12,4042	12,4042		100			

Day 6	CH4	CO2	O2	NH3	CO	H2S	BAL	H2	pH** D5			
Recorded Biogas Composition												
R1	75,9	24,5	2,8	2	185	32	0	0				
R2	1	0,2	20,1	1	242	29	78,8	0				
R3	2	0,8	18,8	7	0	50	78,2	0				
R4	9	72,8	1,6	50	210	103	17,5	0**				
R5	18,9	72	1,1	48	341	112	9,3	0**	Error msg over 6 000ppm			
R6	11,1	61,7	1,7	27	573	77	26,6	0	7,55	Error msg over 6 000ppm		
R7	16,3	65,2	2,1	>>>>	764	2493	17,3	0		Error msg over 6 000ppm		
R8	9,5	66,3	2,6	852	474	3128	22,7	-----				
R9	3,1	49,4	4,9	>>>>>	761	>>>>>	43	----- -	6,71	Error msg over 6 000ppm		
R10	0,7	41	6,9	533	>>>>>	>>>>>>	50,9	-----				
R11	0,7	45	5,1	345	>>>>>>	>>>>>	48,3	-----				
R12	0,7	33,8	4,5	250	>>>>>	>>>>>	60,8	-----	5,38			
R13	0,8	76,5	1,5	864	>>>>	>>>>	2,1	-----				
R14	0,6	87	4,1	377	>>>>>>	>>>>>>>	7,4	-----				
R15	0,6	85,8	5,7	633	>>>>>	>>>>>	8,6	-----	6,5			
Calculated Average Biogas Composition												
	CH4	CO2	O2	OTHER	H2							
Raw	72,9	22,5	2,8	0,0219	1,7781							
Heat	13	68,83333	1,466667	0,023633	16,67637							
Alkali	9,633333	60,3	3,2	0,151833	26,71483							
Acid	0,7	39,93333	5,5	0,0376	53,82907							
Alkali- heat	0,666667	83,1	3,766667	0,062467	12,4042							

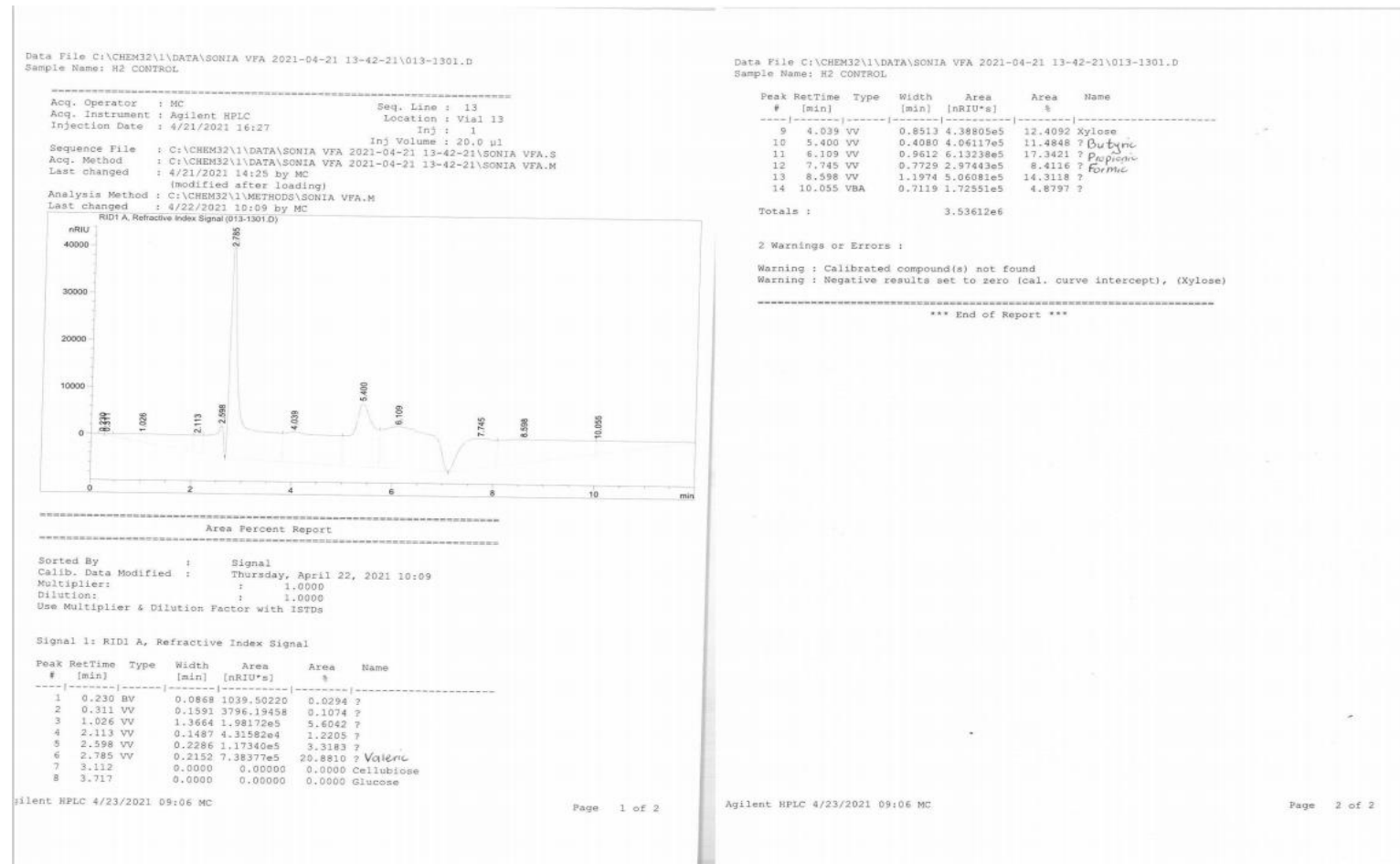
Day 8	CH4	CO2	O2	NH3	CO	H2S	BAL	H2	pH** 5
Recorded Biogas Composition									
R1	70,2	24,3	2,5	7	45	26	0	0	
R2	0,9	0,2	20,3	3	74	17	78,8	0	
R3	0,9	0,2	20,2	2	59	13	78,7	0	
R4	26,8	52,7	3,1	34	97	62	18,3	0**	
R5	23,2	63,6	1,4	41	136	79	13,1	0**	Error msg over 6000ppm
R6	27	52	1,3	43	195	79	20,4	0	7,55
R7	14,3	57,1	2,1	>>>>	353	>>>>>	31,9	0	
R8	9,6	56,9	2,1	860	462	4503	31,3	-----	
R9	1,3	18,6	11,8	>>>>>	329	>>>>>	69	_____ -	6,71
R10	1,2	26,3	4	379	>>>>>	4638	66,8	-----	
R11	0,8	33	3	210	>>>>>>	5246	63,2	-----	
R12	0,7	24,6	3,5	174	1946	2033	72,9	_____	5,38
R13	0,9	73,7	3,8	>>>>	370	>>>>	22	_____	
R14	0,8	52,5	5,9	>>>>	>>>>>>	>>>>>>>>	40,4	_____	
R15	0,8	52,6	3,6	683	563	>>>>	43,1	_____	6,5
Calculated Average Biogas Composition									
	CH4	CO2	O2	Other	H2				
Raw	70,2	24,3	2,5	0,0078	2,9922	100			
Heat	25,66667	56,1	1,933333	0,018933	16,28107	100			
Alkaline	8,4	37,75	5,333333	0,574433	47,94223	100			
Acid	0,9	27,96667	5,5	0,617267	53,8291	88,81303			
Akaline heat	0,833333	59,6	4,433333	0,1616	34,97173	100			

Day 12	CH4	CO2	O2	NH3	CO	H2S	BAL	H2	pH** 5		
Recorded Biogas Composition											
R1	40,9	22,9	7,7	4	2	31	29,2	0			
R2	0,9	0,2	20,3	3	74	17	78,8	0			
R3	2	0,6	20,1	1	6	27	77,3	0			
R4	63,5	34,2	2,7	13	9	62	0	0**			
R5	32	56,8	2	24	1,9	93	9,5	0**	Error msg over 6 000ppm		
R6	59,2	29,4	2,1	16	29	64	10,2	0	7,55		
R7	13,5	44,5	1,7	>>>>	48	>>>>>	40,1	0			
R8	10	47,9	2,7	762	71	6075	39,6	-----			
R9	0,7	0,4	20,1	251	75	1221	78,8	----- -	6,71		
R10	0,7	23,4	4,2	167	6654	1759	71,4	-----			
R11	0,7	24,5	5,9	149	>>>>>	1633	68,7	-----			
R12	0,9	19,3	4,5	132	39	469	74,9	-----	5,38		
R13	0,9	73,7	3,8	>>>>	370	>>>>	22	-----			
R14	0,7	40,9	4	>>>>	>>>>>	>>>>>>>	54,8	-----			
R15	0,8	52,6	3,6	683	563	>>>>	43,1	-----	6,5		
Calculated average biogas composition											
	CH4	CO2	O2	Other	H2						
Raw	40,9	22,9	7,7	2,0035	26,4965						
R4-R6	51,56667	40,13333	2,266667	0,010397	6,022937						
R7-R9	8,4	37,75	5,333333	0,574433	47,94223						
R10-R12	0,9	27,96667	5,5	0,617267	53,8291						
R13-R15	0,833333	59,6	4,433333	0,1616	34,97173						

Day 15	CH4	CO2	O2	NH3	CO	H2S	BAL	H2	pH** 5		
Recorded Biogas Composition											
R1	46,1	22,9	7,7	4	2	31	29,2	0			
R2	0,9	0,2	20,3	3	74	17	78,8	0			
R3	2	0,6	20,1	1	6	27	77,3	0			
R4	77,1	20,8	1,3	14	76	38	0	0**			
R5	76	19,6	2	8	66	34	0	0**	Error msg over 6 000ppm		
R6	77,3	24,2	1,1	7	36	36	0	0	7,55		
R7	10	35,1	1,4	>>>>	113	>>>>	52,8	0			
R8	10,6	36,9	2,4	>>>>	133	>>>>	50,8	-----			
R9	2,2	2,7	17,7	>>>>	144	>>>>>	79,2	_____ -	6,71		
R10	1,3	20,1	2	272	1694	1978	71,4	-----			
R11	1	20,9	2,1	217	399	1327	76	-----			
R12	0,8	14,7	3,6	292	65	1218	80,6	_____	5,38		
R13	0,9	73,7	3,8	>>>>	370	>>>>	22	_____			
R14	0,7	40,9	4	>>>>	>>>>>	>>>>>>	54,8	_____			
R15	0,8	52,6	3,6	683	563	>>>>	43,1	_____	6,5		
Calculated average biogas composition											
	CH4	CO2	O2	Other	H2						
Raw	46,1	22,9	7,7	0,0037	23,2994						
Heat	76,8	21,53333	1,466667	0,0105	0,1931						
Alkaline	8,4	37,75	5,333333	0,574433	47,94223						
Acid	0,9	27,96667	5,5	0,617267	53,8291						
Acid +Heat	0,833333	59,6	4,433333	0,1616	34,97173						

6.3 Appendix A3: Volatile Fatty Acids Chromatograms

Sample: Hydrogen Control



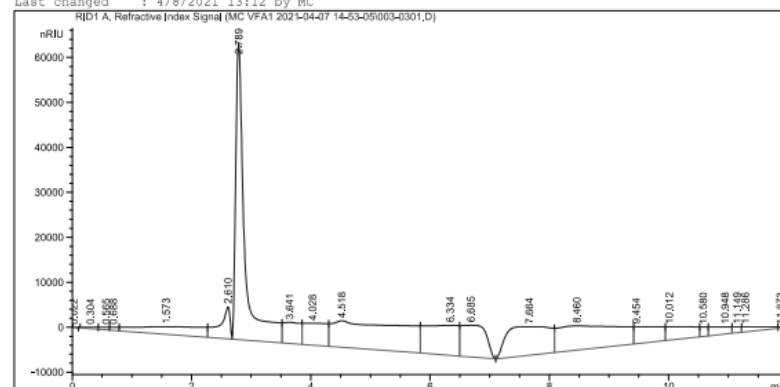
Sample treatment: Heat (Hydrogen production)

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\003-0301.D
Sample Name: S2 H2 HEAT

=====

Acq. Operator : MC	Seq. Line : 3
Acq. Instrument : Agilent HPLC	Location : Vial 3
Injection Date : 4/7/2021 15:20	Inj : 1
	Inj Volume : 20.0 µl

Acq. Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M
Last changed : 4/6/2021 07:08 by MC
Analysis Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M
Last changed : 4/8/2021 13:12 by MC



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	0.022	BB	0.1711	298.51617	21.43922	9.526e-3
2	0.304	BV	0.2154	5145.88135	306.32181	0.1642
3	0.565	VV	0.1355	5838.94775	555.85248	0.1863
4	0.688	VV	0.1296	6895.79883	677.45227	0.2201
5	1.573	VV	0.9675	1.39267e5	1698.38269	4.4444
6	2.610	VV	0.1604	8.50391e4	7146.82373	2.7138
7	2.789	VV	0.1506	6.91239e5	6.58451e4	22.0592
8	3.641	VV	0.2656	9.34737e4	4719.36182	2.9830
9	4.028	VV	0.3645	1.29670e5	4871.72705	4.1381
10	4.518	VV	1.0741	5.18824e5	5908.91943	16.5570
11	6.334	VV	0.4965	2.54780e5	6685.20801	8.1306

Agilent HPLC 4/8/2021 13:13 MC

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\003-0301.D
Sample Name: S2 H2 HEAT

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
12	6.685	VV	0.4573	2.03342e5	7053.78857	6.4892
13	7.664	VV	0.6038	3.20663e5	6331.53223	10.2331
14	8.460	VV	0.9325	3.81767e5	5359.67822	12.1831
15	9.454	VV	0.3640	1.09332e5	3748.77295	3.4890
16	10.012	VV	0.3734	8.91300e4	2920.25952	2.8444
17	10.580	VV	0.1089	1.83365e4	2089.01196	0.5852
18	10.948	VV	0.3129	4.00155e4	1567.61218	1.2770
19	11.149	VV	0.1161	1.22517e4	1279.29065	0.3910
20	11.286	VV	0.2959	2.66682e4	1083.12244	0.8510
21	11.873	VBA	0.0909	1593.28552	220.21013	0.0508

Totals : 3.13357e6 1.30090e5

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 13:13 MC

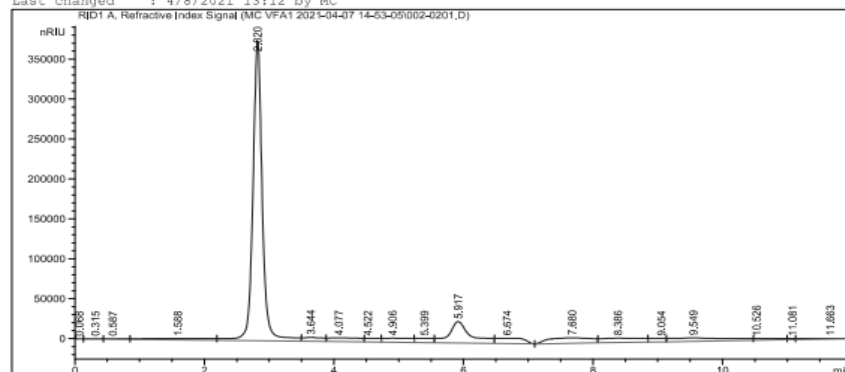
Page 2 of 2

Sample treatment: Alkali (Hydrogen production)

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\002-0201.D
Sample Name: S3 H2 BASE

=====

Acq. Operator : MC	Seq. Line : 2
Acq. Instrument : Agilent HPLC	Location : Vial 2
Injection Date : 4/7/2021 15:07	Inj : 1
	Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M	
Last changed : 4/6/2021 07:08 by MC	
Analysis Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M	
Last changed : 4/8/2021 13:12 by MC	



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	0.068	BV	0.1004	484.75510	63.12643	7.689e-3
2	0.315	VV	0.2111	4871.24414	296.51303	0.0773
3	0.587	VV	0.3260	1.48524e4	546.00830	0.2356
4	1.588	VV	0.8927	1.16686e5	1574.52759	1.8509
5	2.820	VV	0.1476	3.77232e6	3.75095e5	59.8388
6	3.644	VV	0.2844	1.03137e5	4901.18359	1.6360
7	4.077	VV	0.4584	1.62900e5	4750.57080	2.5840
8	4.522	VV	0.2209	7.43627e4	4711.54346	1.1796
9	4.906	VV	0.4021	1.54804e5	5093.34668	2.4556
10	5.399	VV	0.2543	9.92644e4	5376.74023	1.5746
11	5.917	VV	0.3218	6.15149e5	2.67983e4	9.7578

Agilent HPLC 4/8/2021 13:12 MC

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\002-0201.D
Sample Name: S3 H2 BASE

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
12	6.674	VV	0.4749	1.99649e5	6704.49268	3.1669
13	7.680	VV	0.6112	3.13247e5	6796.05664	4.9689
14	8.386	VV	0.5884	2.33291e5	5421.28027	3.7006
15	9.054	VV	0.2357	7.32467e4	4159.83447	1.1619
16	9.549	VV	0.8384	2.62807e5	4124.43506	4.1688
17	10.526	VV	0.3266	5.47998e4	2052.40845	0.8693
18	11.081	VV	0.1026	1.04643e4	1299.71313	0.1660
19	11.663	VBA	0.7362	3.78072e4	672.05481	0.5997

Totals : 6.30415e6 4.60437e5

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 13:12 MC

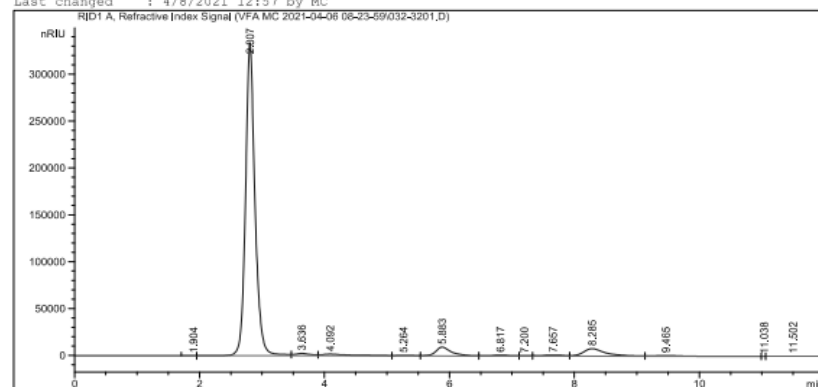
Page 2 of 2

Sample treatment: Acid (Hydrogen production)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\032-3201.D
Sample Name: S4 H2 ACID

=====

Acq. Operator : MC	Seq. Line : 32
Acq. Instrument : Agilent HPLC	Location : Vial 32
Injection Date : 4/6/2021 15:17	Inj : 1
	Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M	
Last changed : 4/6/2021 07:08 by MC	
Analysis Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M	
Last changed : 4/8/2021 12:57 by MC	



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 08, 2021 11:38
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	1.904	BV	0.1199	159.28140	3.904e-3	?
2	2.807	VV	0.1576	3.51401e6	86.1234	Valeric
3	3.636	VV	0.2406	4.51646e4	1.1069	?
4	4.092	VV	0.4667	6.73012e4	1.6495	?
5	4.748		0.0000	0.00000	0.0000	lactic
6	5.264	VV	0.3617	1.27706e4	0.3130	Butyric
7	5.883	VV	0.2563	1.67796e5	4.1124	?
8	6.163		0.0000	0.00000	0.0000	Propionic
9	6.817	VV	0.3178	2.14693e4	0.5262	Acetic
10	7.200	VV	0.1858	4446.57373	0.1090	?

Agilent HPLC 4/8/2021 13:09 MC

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\032-3201.D
Sample Name: S4 H2 ACID

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	7.657	VV	0.3455	2.56282e4	0.6281	Formic
12	8.285	VV	0.3615	1.97752e5	4.8466	?
13	9.465	VV	0.4693	1.88427e4	0.4618	?
14	11.038	VV	0.0429	6.88099	1.686e-4	?
15	11.502	VBA	0.3972	4854.45801	0.1190	?

Totals : 4.08021e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Formic)

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 13:09 MC

Page 2 of 2

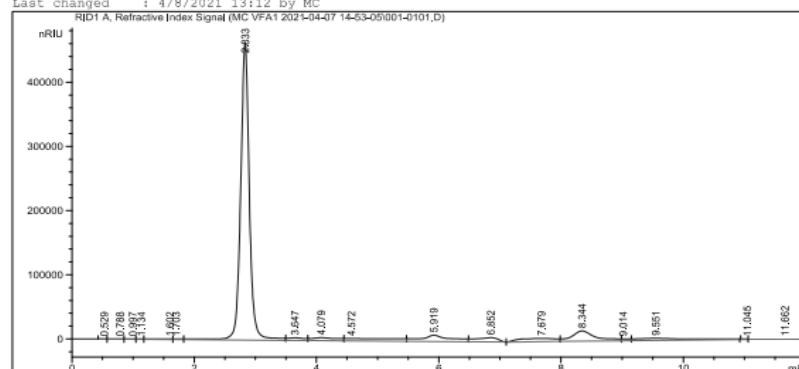
Sample treatment: Alkali-heat (Hydrogen production)

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\001-0101.D
Sample Name: S5 H2 HEAD BOO

=====

Acq. Operator : MC	Seq. Line : 1
Acq. Instrument : Agilent HPLC	Location : Vial 1
Injection Date : 4/7/2021 14:54	Inj : 1
	Inj Volume : 20.0 µl

Acq. Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M
Last changed : 4/6/2021 07:08 by MC
Analysis Method : C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\MC VFA.M
Last changed : 4/8/2021 13:12 by MC



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	0.529	BV	0.0769	463.36362	74.48272	7.072e-3
2	0.788	VV	0.1666	3465.41309	259.46704	0.0529
3	0.997	VV	0.1330	4247.59375	405.61499	0.0648
4	1.134	VV	0.1073	3883.89404	503.36325	0.0593
5	1.602	VV	0.2882	2.00183e4	842.00177	0.3055
6	1.703	VV	0.1392	1.00385e4	912.00458	0.1532
7	2.833	VV	0.1484	4.60876e6	4.63558e5	70.3396
8	3.647	VV	0.2520	7.83827e4	4205.88086	1.1963
9	4.079	VV	0.3973	1.33716e5	4544.01953	2.0408
10	4.572	VV	0.7778	2.25205e5	3555.50122	3.4371
11	5.919	VV	0.4453	3.29409e5	9713.94727	5.0275

Agilent HPLC 4/8/2021 13:12 MC

Data File C:\CHEM32\1\DATA\MC VFA1 2021-04-07 14-53-05\001-0101.D
Sample Name: S5 H2 HEAD BOO

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
12	6.852	VV	0.3674	1.65945e5	6490.10645	2.5327
13	7.679	VV	0.5530	2.10163e5	5066.70068	3.2075
14	8.344	VV	0.3893	4.35940e5	1.59907e4	6.6534
15	9.014	VV	0.1158	3.01265e4	3153.16138	0.4598
16	9.551	VB	0.8761	2.41914e5	3574.09668	3.6921
17	11.045	BV	0.1046	8337.05078	1061.48657	0.1272
18	11.662	VBA	0.7009	4.21426e4	811.50928	0.6432

Totals : 6.55216e6 5.24722e5

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 13:12 MC

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6.4 Appendix B1: TS and VS calculations

Experiment	Effect of pH (Series 1)					
	Inoculum			FVW FRUITSTOP		
	1	2	3	4	5	6
	Measured values					
Mass of Tray	24,604	25,686	25,72	21,773	23,445	27,304
Wet sample and tray (g)	42,749	44,998	45,889	47,616	49,847	54,643
Dry sample ₃ and tray (g) after 1 hours	25,053	26,172	26,211	24,692	26,571	30,345
	Calculated values					
Moisture content	17,696	18,826	19,678	22,924	23,276	24,298
Moisture content %	97,52548912	97,48343	97,56557	88,70487	88,15999	88,8767
MC AVERAGE	97,52			88,58		
Total solid g(dry sample ₃ -tray)	0,449	0,486	0,491	2,919	3,126	3,041
Total solid (%)	2,474510885	2,51657	2,434429	11,29513	11,84001	11,1233
TS AVEARAGE %	2,48			11,42		
CHECK	100,00			100		
	WET SAMPLE =MC+TS					
	TS =VS+ASH					
	VOLATILE SOLIDS					
	Inoculum			FVW		
	1	2	3	4	5	6
	Measured values					
Dry sample+ tray ₁	25,053	26,172	26,211	24,692	26,571	30,345
Ashed sample+ tray ₂	24,791	25,883	25,924	22,066	23,772	27,616
Ashed sample+ tray _s	24,791	25,883	25,924	22,066	23,772	27,616

	Calculated values					
ASH	0,187	0,197	0,204	0,293	0,327	0,312
ASH %	41,6481069	40,53498	41,54786	10,03768	10,46065	10,25978
ASH AVERAGE %	41,24364928			10,25270657		
Volatile solid amount	0,262	0,289	0,287	2,626	2,799	2,729
VS (%) (% of TS)	58,3518931	59,46502	58,45214	89,96232	89,53935	89,74022
VS average (%)	58,76			89,75		
VS (% of Wet sample)	1,443923946	1,496479	1,422976	10,16136	10,60147	9,982077
Average VS (Wet Basis)	1,45			10,25		

Experiment	Effect of ultra-sonication (Series 2)					
	FVW SUBSTRATE			INNOCULUM		
	1	2	3	4	5	6
	Measured values					
Mass of Tray	26,4123	28,978	0	26,1707	22,9976	22,9241
Wet sample and tray (g)	52,732	54,5916	0	52,4478	42,6735	42,6327
Dry sample ₃ and tray (g) after 1 hours	29,5062	31,8622	0	26,444	23,1902	23,2658
	Calculated values					
Wet sample g(without tray)	26,4123	28,978	0	26,2771	19,6759	19,7086
Moisture content	23,2258	22,7294	0	26,0038	19,4833	19,3669
Moisture content %	88,2449268	88,73958	0	98,95993	99,02114	98,26624
MC AVERAGE	88,49			98,75		
Total solid g(dry sample ₃ -tray)	3,0939	2,8842	0	0,2733	0,1926	0,3417
Total solid (%)	11,7550732	11,26042	0	1,040069	0,978862	1,733761
TS AVEARAGE %	11,51			1,25		
CHECK	100,00			100		

	WET SAMPLE =MC+TS					
	TS =VS+ASH					
	FVW			Inoculum		
	1	2	3	4	5	6
	Measured values					
Mass of Tray	26,4123	28,978	0	26,1707	22,9976	22,9241
Dry sample	3,0939	2,8842	0	0,2733	0,1926	0,3417
Ash sample + tray	26,737	29,288	0	26,2771	23,028	23,0993
Ash	0,3247	0,31	0	0,1064	0,0304	0,1752
ASH %	10,49484469	10,74821	0	38,93158	15,78401	51,27305
ASH AVERAGE %	10,62152955			35,32954395		
Volatile solid amount	2,7692	2,5742	0	0,1669	0,1622	0,1665
VS (%) (% of TS)	89,50515531	89,25179	0	61,06842	84,21599	48,72695
Average VS (Wet Basis)	10,29			0,77		

Experiment Name	Effect of co-digestion								
							TOTAL SOLIDS		
	Inoculum			FVW FRUITSTOP			MAROWA PIG MANURE		
	1	2	3	4	5	6	7	8	9
	Measured values								
Mass of Tray	27,2965	22,9774	26,1237	27,2945	23,4315	26,1407	25,7118	26,1334	23,4272
Wet sample and tray (g)	42,4705	38,3506	40,9584	48,8343	44,3944	47,2613	36,4563	41,3522	38,7936
Dry sample ₃ and tray (g) after 1 hours	27,8552	23,5418	26,661	29,0854	25,737	28,3657	29,3064	31,5159	28,8395
	Calculated values								
Moisture content	14,6153	14,8088	14,2974	19,7489	18,6574	18,8956	7,1499	9,8363	9,9541
Moisture content %	96,31804402	96,32868	96,37809	91,68562	89,002	89,46526	66,54474	64,63256	64,77835
MC AVERAGE	96,34			90,05			65,32		
Total solid g(dry sample ₃ -tray)	0,5587	0,5644	0,5373	1,7909	2,3055	2,225	3,5946	5,3825	5,4123
Total solid (%)	3,681955977	3,671324	3,621913	8,314376	10,998	10,53474	33,45526	35,36744	35,22165
TS AVEARAGE %	3,66			9,95			34,68		
CHECK	100,00			100			100,00		
	WET SAMPLE =MC+TS								
	TS =VS+ASH								
	Inoculum			FVW			Marowa Pig Manure		
	1	2	3	4	5	6	7	8	9
	Measured Values								
Mass of Tray	27,2965	22,9774	26,1237	27,2945	23,4315	26,1407	25,7118	26,1334	23,4272

Ashed sample+ tray ₂	27,5243	23,209	26,3466	27,4534	23,6281	26,3463	27,2015	28,6486	25,9261
Ashed sample+ tray _s	27,5243	23,209	26,3466	27,4534	23,6281	26,3463	27,2015	28,6486	25,9261
	Calculated Values								
ASH	0,23	0,23	0,22	0,16	0,20	0,21	1,49	2,52	2,50
ASH %	40,77	41,03	41,49	8,87	8,53	9,24	41,44	46,73	46,17
ASH AVERAGE %	41,10			8,88			44,78		
Volatile solid amount	0,3309	0,3328	0,3144	1,632	2,1089	2,0194	2,1049	2,8673	2,9134
VS (%) (% of TS)	59,22677645	58,9652 7	58,5148	91,1273 7	91,4725 7	90,7595 5	58,5572 8	53,2707 8	53,8292 4
VS average (%)	58,90			91,12			55,22		
VS (% of Wet sample)	2,180703836	2,16480 6	2,11935 5	7,57667 2	10,0601 5	9,56128 1	19,5904 9	18,8405 1	18,9595 5
Average VS (% of Wet sample)	2,15			9,07			19,13		

6.5 Appendix B2 Methane Yield

Methane yield: Effect of pH

CO2 concentration [%] of flush gas	25															
Process temperature [Celsius]	37															
Mixer on time [sec]	90															
Mixer off time [sec]	90															
Mixer speed adjustment [%]	40															
Eliminate overestimation	No															
Flow Cell nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Name	Control 1	Control 2	Control 3	pH4(1)	pH4(2)	pH4 (3)	pH6 (1)	pH6(2)	pH6(3)	pH12(1)	pH12(2)	pH12(3)	pH10(1)	pH10(2)	pH10(3)	
Substrate VS/COD amount [g]	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	3,78	
Inoculum VS/COD amount [g]	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	7,56	
Type of unit [VS/COD]	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	
Headspace volume [ml]	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200	
Assumed CH4 content [%]	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	
Day	Control 1 Volume [Nml]	Control 2 Volume [Nml]	Control 3 Volume [Nml]	pH4(1) Volume [Nml]	pH4(2) Volume [Nml]	pH4 (3) Volume [Nml]	pH6 (1) Volume [Nml]	pH6(2) Volume [Nml]	pH6(3) Volume [Nml]	pH12(1) Volume [Nml]	pH12(2) Volume [Nml]	pH12(3) Volume [Nml]	pH10(1) Volume [Nml]	pH10(2) Volume [Nml]	pH10(3) Volume [Nml]	
0	0	0	0	0	0	0	0	0	0					0	0	
1	632	658,3	653,5	34,9	40,8	34,8	182,8	99,8	250,7					1,8	1,2	
2	1172,2	1208,4	1201		41,6		216,3	117	427,5					3,6	2,5	
3	1435,3	1447	1463,8				260,8	373,6	686,6					5,4	3,7	
4	1529,5	1532	1562,7				263,2		927,1					8,2	5	
5	1587,7	1589,7	1628						1073,2					15,2	6,2	
6	1637,5	1641,4	1683,7						1157,3					22	9,9	
7	1673,1	1676,9	1724,1						1201,7					29,7	17,3	
8	1703	1705,3	1759,1						1235,9					38,4	25,2	
9	1720,6	1730,6	1790,2						1266,8					49,9	36,8	
10		1751,7	1817,1						1296,4					63,6	44,9	
11		1771,2	1840,3						1327,1					72,9	51,6	
12		1790,6	1864,1						1358,2					78,2	57,5	
13		1808	1884,1						1389,1					81,4	62,8	
14		1823,3	1904,5						1420,5					83,5	65,2	
15		1835,9	1921,9						1451,5						67,6	
16		1840	1932,7						1470,9						69,4	

Effect of pH Daily Average Methane Production (Nml/day)					
Day	pH 4	pH6	pH 8 (unadjusted)	pH 10	pH12
0	0	0	0	0	0
1	36,83	177,77	647,93	1,5	0
2	41,60	253,6	1193,87	3,05	0
3		440,33	1448,70	4,55	0
4		595,15	1541,40	6,6	0
5		1073,20	1601,80	10,7	0
6		1157,30	1654,20	15,95	0
7		1201,70	1691,37	23,5	0
8		1235,90	1722,47	31,8	0
9		1266,80	1747,13	43,35	0
10		1296,40	1784,40	54,25	0
11		1327,10	1805,75	62,25	0
12		1358,20	1827,35	67,85	0
13		1389,10	1846,05	72,1	0
14		1420,50	1863,90	74,35	0
15	41,60	1451,50	1878,90	67,6	0
Bar	pH 4	pH6	pH 8 (unadjusted)	pH 10	pH12
	42	1452	1879	68	0
Average Methane yield (Effect of pH) (Nml/ g VS) : Methane production/VS added (Effect of pH)					
Day	pH 4	pH6	pH 8 (unadjusted)	pH 10	pH12
0	0	0	0	0	0
1	9,744268078	47,02821869	171,4109347	0,396825397	0
2	11,00529101	67,08994709	315,8377425	0,806878307	0

3		116,4902998	383,2539683	1,203703704	0
4		157,4470899	407,7777778	1,746031746	0
5		283,9153439	423,7566138	2,830687831	0
6		306,1640212	437,6190476	4,21957672	0
7		317,9100529	447,4514991	6,216931217	0
8		326,957672	455,6790123	8,412698413	0
9		335,1322751	462,2045855	11,46825397	0
10		342,962963	472,0634921	14,35185185	0
11		351,0846561	477,7116402	16,46825397	0
12		359,3121693	483,4259259	17,94973545	0
13		367,4867725	488,3730159	19,07407407	0
14		375,7936508	493,0952381	19,66931217	0
15		383,994709	497,0634921	17,88359788	0

Methane yield: Effect of ultra-sonication

CO2 concentration [%] of flush gas	25														
Process temperature [Celsius]	37														
Mixer on time [sec]	90														
Mixer off time [sec]	90														
Mixer speed adjustment [%]	49														
Eliminate overestimation	No														
Flow Cell nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name	Control 1	Control 2	Control 3	SE2500 1	SE 2500 2	SE 2500 3	SE7500 1	SE 7500 2	SE7500 3	SE12500 1	SE12500 2	SE12500 3	SE17500 1	SE17500 2	SE17500 3
Substrate VS/COD amount [g]	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04	2,04
Inoculum VS/COD amount [g]	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09	4,09
Type of unit [VS/COD]	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS
Headspace volume [ml]	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Assumed CH4 content [%]	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65
Day	Control 1	Control 2	Control 3	SE2500 1	SE 2500 2	SE 2500 3	SE7500 1	SE 7500 2	SE7500 3	SE12500 1	SE12500 2	SE12500 3	SE17500 1	SE17500 2	SE17500 3
	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]	Volume [NmL]
0		0	0	0	0	0	0	0	0	0	0	0		0	0
1		100,7	95,5	69,2	63,9	67,1	73	80,7	82,8	88,2	87,3	51,6		97,6	97,3
2		159,3	151,5	115,3	107	110,9	121,1	129,5	128,2	154,8	144,2	100,2		151,6	152,3
3		195,9	183,4	146,9	140,9	144,5	149,2	158,4	156	189,7	179,5	135,2		181,9	181,2
4		213,7	202,5	160,6	153	157,4	160,5	173,9	171	213,4	202,9	154,4		205,3	205,7
5		222,6	210,6	171,8	160,6	166,3	170,2	185,2	182,3	235,9	221,8	170,7		226,8	228,8
6		229,9	219,3	184,6	166,8	174,8	181,5	194,5	195,1	262,7	244,4	189,2		251,6	257,4
7			229,8	196,9		184,4	191,5	196	208,9	292,4	262,3	202,9		275,4	291,1
8			243	209		194,4	201,4	197,5	223	324,6	285,8	222,3		306,5	324,3
9			259,3	222,7		206,4	214,4	199	239,7	363	314,2	246		340,7	362,4
10			277,5	236,8		218,5	228,4	200,4	258,7	408	349,3	275,6		377,5	406,7
11			298	254,3		233,9	243,7	212,1	281,8	460,3	353,5	305		410,7	457,2
12			325	275,9		255,8	265,7	239,2	309,6	524,5		345,8		415,7	517,1
13			357,2	300,3		280,5	292,4	269,4	340,3	596,5		392,8		456,5	583,8
14			396,5	327,6		307,9	323,5	301,7	374	671,3		444,2		495,6	655
15			447,7	360		339,5	360,8	340,1	413,7	747,6		501,7		542,2	730,8
		447,7			349,75			371,5333333			624,65			636,5	

Effect of US Daily Average Methane Production (Nml/day)					
Day	Control (No sonification)	SE2500	SE7500	SE12500	SE17500
0	0	0	0	0	0
1	98,1	66,7	78,8	75,7	97,45
2	155,4	111,1	126,3	133,1	151,95
3	189,65	144,1	154,5	168,1	181,55
4	208,1	157	168,5	190,2	205,5
5	216,6	166,2	179,2	209,5	227,8
6	224,6	175,4	190,4	232,1	254,5
7	229,8	190,7	198,8	252,5	283,25
8	243	201,7	207,3	277,6	315,4
9	259,3	214,6	217,7	307,7	351,55
10	277,5	227,7	229,2	344,3	392,1
11	298	244,1	245,9	372,9	433,95
12	325	265,9	271,5	435,2	466,4
13	357,2	290,4	300,7	494,7	520,15
14	396,5	317,8	333,1	557,8	575,3
15	447,7	349,8	371,5	624,7	636,5
Effect of US Daily Average Methane Yield (Nml/g VS)					
Day	Control (No sonification)	SE2500	SE7500	SE12500	SE17500
0	0	0	0	0	0
1	48,08823529	32,7	38,6	37,1	47,76960784
2	76,17647059	54,4	61,9	65,2	74,48529412
3	92,96568627	70,6	75,8	82,4	88,99509804
4	102,0098039	77,0	82,6	93,3	100,7352941
5	106,1764706	81,5	87,9	102,7	111,6666667
6	110,0980392	86,0	93,3	113,8	124,754902

7	112,6470588	93,5	97,5	123,8	138,8480392
8	119,1176471	98,9	101,6	136,1	154,6078431
9	127,1078431	105,2	106,7	150,8	172,3284314
10	136,0294118	111,6	112,3	168,8	192,2058824
11	146,0784314	119,7	120,5	182,8	212,7205882
12	159,3137255	130,3	133,1	213,3	228,627451
13	175,0980392	142,4	147,4	242,5	254,9754902
14	194,3627451	155,8	163,3	273,4	282,0098039
15	219,4607843	171,4	182,1	306,2	312,0098039

Methane yield: Effect of co-digestion

CO2 concentration [%] of flush gas	25														
Process temperature [Celsius]	37														
Mixer on time [sec]	90														
Mixer off time [sec]	90														
Mixer speed adjustment [%]	49														
Eliminate overestimation	No														
Flow Cell nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name	Control 1 CD IN	Control 2 CD IN	Control 3 CD FW	Control 4 CD FW	Control 5 CD PMD	ontrol 6 CD PMD	FW:PDM 75:25 1	FW:PDM 75:25 2	FW:PDM 75:25 3	FW:PDM 50:50 1	FW:PDM 50:50 2	FW:PDM 50:50 3	FW:PDM 25:75 1	FW:PMD 25:75 2	FW:PMD 25:75 3
Substrate VS/COD amount [g]	0	0	4,11	4,11	4,55	4,55	4,11	4,11	4,11	4,11	4,11	4,11	4,11	4,11	4,11
Inoculum VS/COD amount [g]	9,28	9,28	8,23	8,23	9,1	9,1	8,23	8,23	8,23	8,23	8,23	8,23	8,23	8,23	8,23
Type of unit [VS/COD]	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS	VS
Headspace volume [ml]	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Assumed CH4 content [%]	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65
Day	Control 1 CD IN Volume [Nml]	Control 2 CD IN Volume [Nml]	Control 3 CD FW Volume [Nml]	Control 4 CD FW Volume [Nml]	Control 5 CD PMD Volume [Nml]	Control 6 CD PMD Volume [Nml]	FW:PDM 75:25 1 Volume [Nml]	FW:PDM 75:25 2 Volume [Nml]	FW:PDM 75:25 3 Volume [Nml]	FW:PDM 50:50 1 Volume [Nml]	FW:PDM 50:50 2 Volume [Nml]	FW:PDM 50:50 3 Volume [Nml]	FW:PDM 25:75 1 Volume [Nml]	FW:PMD 25:75 2 Volume [Nml]	FW:PMD 25:75 3 Volume [Nml]
0			0	0			0	0	0	0			0	0	0
1			243	858,5			580,9	777	788,8	790,6	666,2		492,2	511,3	509,4
2			744,9	1396,5			853,3	1121,2	1146,5	1166,4	953,8		813,4	763	734,9
3			876,2	1523,4			1023,7	1270,3	1291	1318,8	1081,8		958,1	878,4	835,5
4			990,8	1625			1149,5	1406,8	1418,2	1458,3	1159		1042,6	969,5	910,1
5			1102,5	1731,9			1247,6	1470,1	1477	1531	1229		1110,9	1047,6	978,1
6			1162,2	1809,2			1335,7	1523,9	1524,3	1582,1	1285,7		1167,6	1110,4	1031,4
7			1203,3	1847,2			1408,9	1566,7	1561,9	1622,2	1335,3		1212,6	1163,5	1075,9
8			1233,4	1878,7			1471,6	1603,2	1593	1654,7	1373,8		1251,5	1207,9	1116,2
9			1256,6	1905,3			1514,1	1637,9	1620,4	1683,8	1407,7		1281,7	1242,4	1150,2
10			1268,2	1929,3			1536,8	1666,7	1645,1	1710,5	1437,3		1309,8	1263,3	1180
11			1269,9	1951,9			1557,5	1692,6	1667,7	1734,3	1464		1336,8	1295,9	1206
12			1271,7	1973,4			1575,4	1717,4	1688,8	1756,6	1488,3		1361,7	1327,4	1231,5
13			1273,5	1993,6			1590,7	1740,4	1709,1	1777,9	1512,5		1386,7	1357,1	1254,4
14			1275,9	2013,7			1604,9	1764,5	1729,4	1800	1534,7		1410,3	1386,3	1275,9
15			1279,9	2032,7			1618,1	1784,7	1747,3	1821,8	1553,2		1425,9	1406,3	1293,4

Effect of co-digest Daily Average Methane Production (Nml/day)						
Day	Control Inoculum	FW 100 %	PM 100 %	FWPM:75:25	FWPM5050	FWPM2575
0	0	0	0	0	0	0
1	0	550,75	580,9	785,47	579,20	497,77
2	0	1070,7	853,3	1144,70	883,60	726,17
3	0	1199,8	1023,7	1293,37	1019,95	828,97
4	0	1307,9	1149,5	1427,77	1100,80	908,27
5	0	1417,2	1247,6	1492,70	1169,95	978,70
6	0	1485,7	1335,7	1543,43	1226,65	1034,60
7	0	1525,25	1408,9	1583,60	1273,95	1081,90
8	0	1556,05	1471,6	1616,97	1312,65	1123,40
9	0	1580,95	1514,1	1647,37	1344,70	1157,57
10	0	1598,75	1536,8	1674,10	1373,55	1184,40
11	0	1610,9	1557,5	1698,20	1400,40	1212,87
12	0	1622,55	1575,4	1720,93	1425,00	1240,40
13	0	1633,55	1590,7	1742,47	1449,60	1265,23
14	0	1644,8	1604,9	1764,63	1472,50	1288,87
15	0	1656,3	1618,1	1784,60	1489,55	1307,67
Effect of US Daily Average Methane Yield (Nml/g VS)						
Day	Control	FW 100 %	PM 100 %	FWPM:75:25	FWPM5050	FWPM2575
0	0	0	0	0	0	0
1	0,09	134	128	191	141	121
2	0,17	261	188	279	215	177
3	0,26	292	225	315	248	202
4	0,34	318	253	347	268	221
5	0,43	345	274	363	285	238
6	0,51	361	294	376	298	252
7	0,59	371	310	385	310	263

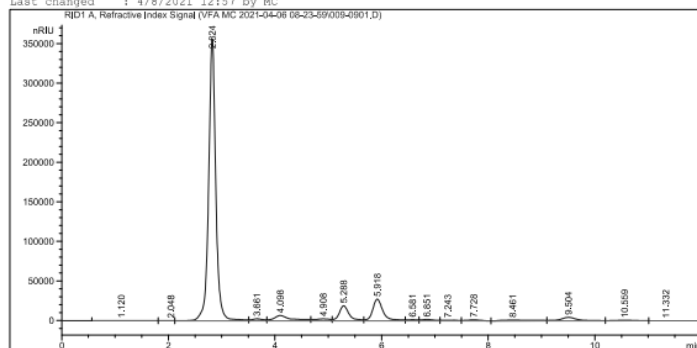
8	0,68	379	323	393	319	273
9	0,77	385	333	401	327	282
10	2,27	389	338	407	334	288
11	4,29	392	342	413	341	295
12	6,13	395	346	419	347	302
13	7,79	397	350	424	353	308
14	9,52	400	353	429	358	314
15	11,25	403	356	434	362	318

6.6 Appendix B3: Volatile Fatty Acids Chromatograms

Sample: Effect of pH (pH4)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\009-0901.D
Sample Name: PH4 PF2

Acq. Operator : MC Seq. Line : 9
Acq. Instrument : Agilent HPLC Location : Vial 9
Injection Date : 4/6/2021 10:11 Inj : 1
Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/6/2021 07:08 by MC
Analysis Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/8/2021 12:57 by MC



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 08, 2021 11:38
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	1.120	VV	0.6237	3539.28125	0.0806 ?	
2	2.048	VV	0.2020	1023.65100	0.0233 ?	
3	2.824	VV	0.1362	3.29795e6	75.1186	Valeric
4	3.661	VV	0.1957	3.46570e4	0.7894 ?	
5	4.098	VV	0.3062	1.44354e5	3.2880 ?	
6	4.748	VV	0.0000	0.00000	0.0000	lactic
7	4.908	VV	0.2770	4.77415e4	1.0874 ?	
8	5.288	VV	0.1946	2.46363e5	5.6115	Butyric
9	5.918	VV	0.2009	3.69549e5	8.4173 ?	
10	6.163	VV	0.0000	0.00000	0.0000	Propionic

Agilent HPLC 4/8/2021 12:59 MC

Page 1 of 2 Agilent HPLC 4/8/2021 12:59 MC

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\009-0901.D
Sample Name: PH4 PF2

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	6.581	VV	0.2026	2.05647e4	0.4684 ?	
12	6.851	VV	0.2447	2.95229e4	0.6725	Acetic
13	7.243	VV	0.3014	1.56534e4	0.3565 ?	
14	7.728	VV	0.2673	2.38205e4	0.5426	Formic
15	8.461	VV	0.6278	4.35303e4	0.9915 ?	
16	9.504	VV	0.3176	9.26093e4	2.1094 ?	
17	10.559	VV	0.4061	1.50309e4	0.3424 ?	
18	11.332	VBA	0.4836	4412.93652	0.1005 ?	

Totals : 4.39032e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found

Warning : Negative results set to zero (cal. curve intercept), (Formic)

*** End of Report ***

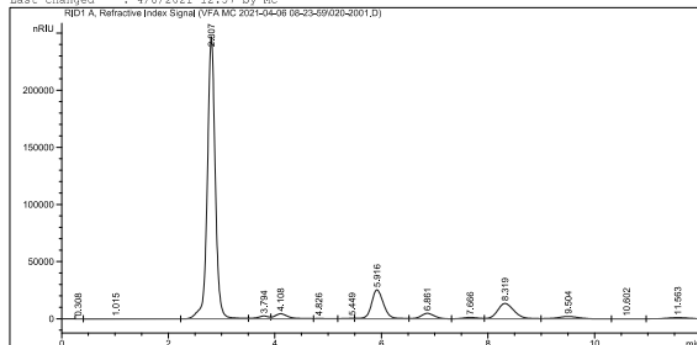
Page 2 of 2

Sample: Effect of pH (pH6)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\020-2001.D
Sample Name: PP3 PH6

=====

Acq. Operator	: MC	Seq. Line	: 20
Acq. Instrument	: Agilent HPLC	Location	: Vial 20
Injection Date	: 4/6/2021 12:37	Inj	: 1
		Inj Volume	: 20.0 µl
Acq. Method	: C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M		
Last changed	: 4/6/2021 07:08 by MC		
Analysis Method	: C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M		
Last changed	: 4/8/2021 12:57 by MC		



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 08, 2021 11:38
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.308	UV	0.0824	35.73748	1.003e-3	?
2	1.015	VV	0.5829	2831.25415	0.0794	?
3	2.807	VV	0.1573	2.54619e6	71.4433	Valeric
4	3.794	VV	0.2110	3.58933e4	1.0071	?
5	4.108	VV	0.2611	7.85783e4	2.2048	?
6	4.826	VV	0.3334	7365.64941	0.2067	Lactic
7	5.274		0.0000	0.00000	0.0000	Butyric
8	5.449	VV	0.2179	6500.76855	0.1824	?
9	5.916	VV	0.2390	3.97187e5	11.1446	?
10	6.163		0.0000	0.00000	0.0000	Propionic

%Agilent HPLC 4/8/2021 13:04 MC

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\020-2001.D
Sample Name: PP3 PH6

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	6.861	VV	0.2713	8.51235e4	2.3885	Acetic
12	7.666	VV	0.3013	2.47827e4	0.6954	Formic
13	8.319	VV	0.3313	2.90381e5	8.1478	?
14	9.504	VV	0.4178	5.79421e4	1.6258	?
15	10.602	VV	0.3202	2493.42920	0.0700	?
16	11.563	VBA	0.4233	2.86282e4	0.8033	?

Totals : 3.56393e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Formic)

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 13:04 MC

Page 2 of 2

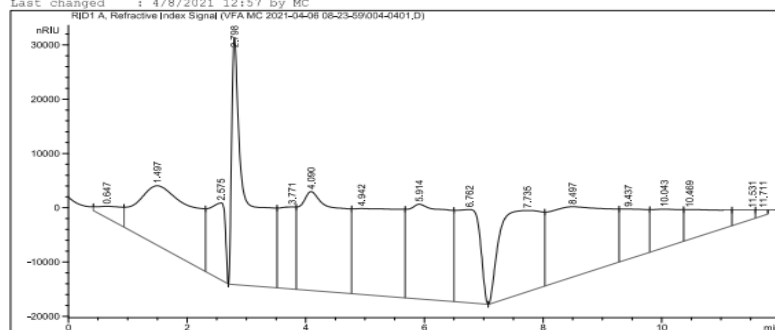
Sample: Effect of pH (pH8, control)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\004-0401.D
Sample Name: PH CONT PP1

=====

Acq. Operator	: MC	Seq. Line	: 4
Acq. Instrument	: Agilent HPLC	Location	: Vial 4
Injection Date	: 4/6/2021 09:04	Inj	: 1
		Inj Volume	: 20.0 µl

Acq. Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/6/2021 07:08 by MC
Analysis Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/8/2021 12:57 by MC



Area Percent Report

Sorted By : Signal
Calib. Date Modified : Thursday, April 08, 2021 11:38
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.647	BV	0.4722	6.91161e4	0.8832	?
2	1.497	VV	0.9532	7.65470e5	9.7821	?
3	2.575	VV	0.2848	2.73124e5	3.4903	?
4	2.798	VV	0.2636	9.15467e5	11.6989	Valeric
5	3.771	VV	0.2480	2.87965e5	3.6800	?
6	4.090	VV	0.6398	9.07883e5	11.6020	?
7	4.748		0.0000	0.00000	0.0000	lactic
8	4.942	VV	0.6893	8.67117e5	11.0810	?
9	5.274		0.0000	0.00000	0.0000	Butyric
10	5.914	VV	0.6088	8.32942e5	10.6443	?

Agilent HPLC 4/8/2021 12:57 MC

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\004-0401.D
Sample Name: PH CONT PP1

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	6.163		0.0000	0.00000	0.0000	Propionic
12	6.762	VV	0.4036	4.76236e5	6.0859	Acetic
13	7.735	VV	0.8024	7.37762e5	9.4280	Formic
14	8.497	VV	0.9219	9.07032e5	11.5911	?
15	9.437	VV	0.3835	2.72353e5	3.4804	?
16	10.043	VV	0.4488	2.34754e5	3.0000	?
17	10.469	VV	0.4718	2.12011e5	2.7093	?
18	11.531	VV	0.3757	5.19351e4	0.6637	?
19	11.711	VV	0.1653	1.40646e4	0.1797	?

Totals : 7.82523e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Formic)

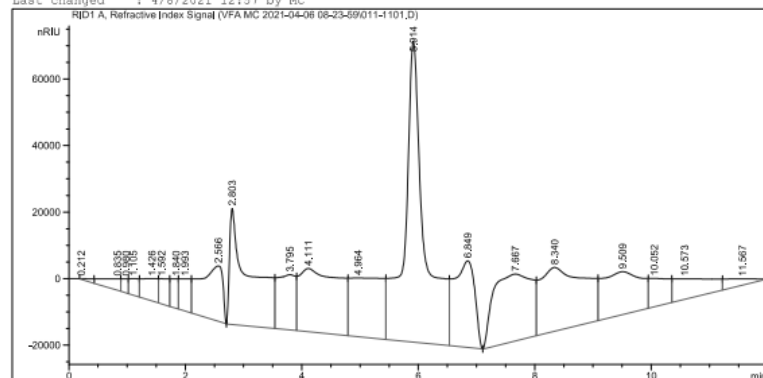
*** End of Report ***

Sample: Effect of pH (pH10)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\011-1101.D
Sample Name: PH10 PP4

=====

Acq. Operator : MC	Seq. Line : 11
Acq. Instrument : Agilent HPLC	Location : Vial 11
Injection Date : 4/6/2021 10:38	Inj : 1
	Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M	
Last changed : 4/6/2021 07:08 by MC	
Analysis Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M	
Last changed : 4/8/2021 12:57 by MC	



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 08, 2021 11:38
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.212	BV	0.6920	1.01103e4	0.1041 ?	
2	0.835	VV	0.2514	7.03944e4	0.7251 ?	
3	0.980	VV	0.0942	3.22415e4	0.3321 ?	
4	1.105	VV	0.1444	5.77312e4	0.5947 ?	
5	1.426	VV	0.2427	1.25393e5	1.2917 ?	
6	1.592	VV	0.1531	9.09419e4	0.9368 ?	
7	1.840	VV	0.1171	7.77377e4	0.8008 ?	
8	1.993	VV	0.1774	1.31666e5	1.3563 ?	
9	2.566	VV	0.3759	4.40165e5	4.5342 ?	
10	2.803	VV	0.3112	8.51491e5	8.7714	Valeric

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\011-1101.D
Sample Name: PH10 PP4

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	3.795	VV	0.2854	3.57500e5	3.6827 ?	
12	4.111	VV	0.6253	9.28195e5	9.5615 ?	
13	4.748	VV	0.0000	0.00000	0.0000	lactic
14	4.964	VV	0.5007	6.95213e5	7.1615 ?	
15	5.274	VV	0.0000	0.00000	0.0000	Butyric
16	5.914	VV	0.3211	2.12118e6	21.8508 ?	
17	6.163	VV	0.0000	0.00000	0.0000	Propionic
18	6.849	VV	0.3602	6.45112e5	6.6454	Acetic
19	7.667	VV	0.6014	9.24008e5	9.5184	Formic
20	8.340	VV	0.6811	1.00566e6	10.3595 ?	
21	9.509	VV	0.6248	5.99424e5	6.1748 ?	
22	10.052	VV	0.3258	1.94763e5	2.0063 ?	
23	10.573	VV	0.5631	2.72099e5	2.8030 ?	
24	11.567	VV	0.5261	7.65555e4	0.7886 ?	

Totals : 9.70759e6

2 Warnings or Errors :

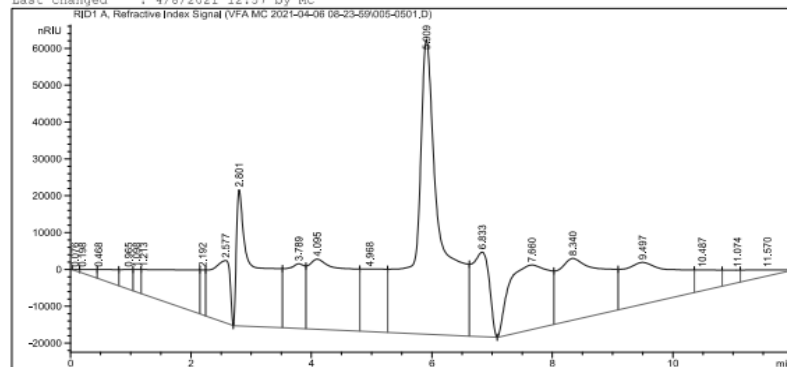
Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Formic)

*** End of Report ***

Sample: Effect of pH (pH12)

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\005-0501.D
Sample Name: PH12 PP5

Acq. Operator : MC Seq. Line : 5
Acq. Instrument : Agilent HPLC Location : Vial 5
Injection Date : 4/6/2021 09:18 Inj : 1
Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/6/2021 07:08 by MC
Analysis Method : C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\MC VFA.M
Last changed : 4/8/2021 12:57 by MC



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 08, 2021 11:38
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.076	BV	0.1267	2604.49292	0.0277 ?	
2	0.198	VV	0.3348	2.65003e4	0.2821 ?	
3	0.468	VV	0.3452	7.18763e4	0.7651 ?	
4	0.965	VV	0.1726	7.24432e4	0.7711 ?	
5	1.098	VV	0.1102	4.93002e4	0.5248 ?	
6	1.213	VV	0.9490	5.36814e5	5.7141 ?	
7	2.192	VV	0.0832	7.12790e4	0.7587 ?	
8	2.577	VV	0.3232	3.74526e5	3.9866 ?	
9	2.801	VV	0.3115	8.97225e5	9.5504 Valeric	
10	3.789	VV	0.2964	3.90415e5	4.1557 ?	

Agilent HPLC 4/8/2021 12:58 MC

Data File C:\CHEM32\1\DATA\VFA MC 2021-04-06 08-23-59\005-0501.D
Sample Name: PH12 PP5

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
11	4.095	VV	0.6323	9.35606e5	9.9590 ?	
12	4.748		0.0000	0.00000	0.0000	lactic
13	4.968	VV	0.3656	4.70171e5	5.0047 ?	
14	5.274		0.0000	0.00000	0.0000	Butyric
15	5.909	VV	0.3945	2.36042e6	25.1253 ?	
16	6.163		0.0000	0.00000	0.0000	Propionic
17	6.833	VV	0.2991	4.55886e5	4.8526	Acetic
18	7.660	VV	0.6069	8.02116e5	8.5381	Formic
19	8.340	VV	0.6965	8.85890e5	9.4298 ?	
20	9.497	VV	0.7972	6.90901e5	7.3542 ?	
21	10.487	VV	0.3299	1.45364e5	1.5473 ?	
22	11.074	VV	0.2395	6.83383e4	0.7274 ?	
23	11.570	VBA	0.6626	8.69179e4	0.9252 ?	

Totals : 9.39460e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Formic)

*** End of Report ***

Page 1 of 2 Agilent HPLC 4/8/2021 12:58 MC

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Sample: Effect of co-digestion (FW 25: PM 75)

Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\005-0501.D

Sample Name: FW 25%

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
10	3.639	VV	0.2356	2.03128e5	2.5254	Glucose
11	4.034	VV	0.5779	7.53644e5	9.3698	Xylose
12	4.898	VV	0.3512	3.90706e5	4.8575	? Lactic
13	5.387	VV	0.3462	7.31645e5	9.0963	? Butyric
14	5.906	VV	0.8778	1.55227e6	19.2989	? Propionic
15	7.808	VV	0.8176	8.64709e5	10.7506	? Formic
16	8.395	VV	0.4649	4.90334e5	6.0961	?
17	8.813	VV	0.5109	5.18631e5	6.4480	?
18	9.472	VV	0.4354	3.83367e5	4.7663	?
19	10.105	VV	0.3016	1.78352e5	2.2174	?
20	10.527	VV	0.7381	3.71420e5	4.6177	?

Totals : 8.04333e6

1 Warnings or Errors :

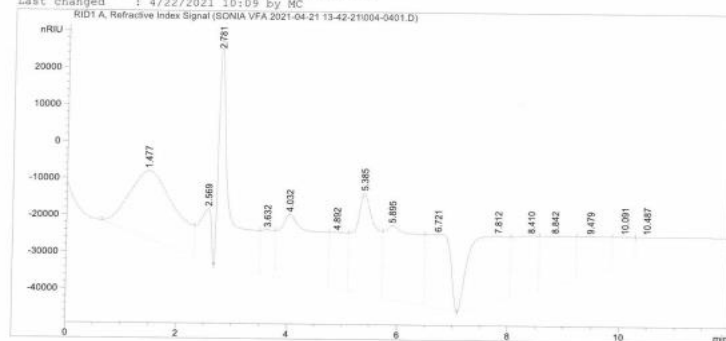
Warning : Calibrated compound(s) not found

=====
*** End of Report ***

Sample: Effect of co-digestion (FW 50:PM75)

Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\004-0401.D
Sample Name: FW 50

Acq. Operator : MC
Acq. Instrument : Agilent HPLC
Injection Date : 4/23/2021 14:27
Seq. Line : 4
Location : Vial 4
Inj : 1
Inj Volume : 20.0 µl
Acq. Method : C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\SONIA VFA.M
Last changed : 4/21/2021 14:25 by MC
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\SONIA VFA.M
Last changed : 4/22/2021 10:09 by MC



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 22, 2021 10:09
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RI1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	1.477	UV	0.8597	1.07877e6	12.3335	?
2	2.569	VV	0.2275	2.24289e5	2.5643	?
3	2.781	VV	0.2074	9.35965e5	10.7008	? Valeric
4	3.112		0.0000	0.00000	0.0000	Cellulobiose
5	3.632	VV	0.2310	2.06209e5	2.3576	Glucose
6	4.032	VV	0.6265	8.70466e5	9.9520	Xylose
7	4.892	VV	0.2950	3.21836e5	3.6795	? Lactic
8	5.385	VV	0.3730	7.57715e5	8.6629	? Butyric
9	5.895	VV	0.5563	8.70830e5	9.9562	? Propionic

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Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\004-0401.D
Sample Name: FW 50

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
10	6.721	VV	0.4726	5.92840e5	6.7779	?
11	7.812	VV	0.8144	8.85488e5	10.1237	? Formic
12	8.410	VV	0.4667	4.86846e5	5.5661	?
13	8.842	VV	0.5131	5.35899e5	6.1269	?
14	9.479	VV	0.4406	3.96505e5	4.5332	?
15	10.091	VV	0.3211	2.03284e5	2.3241	?
16	10.487	VBA	0.7006	3.79704e5	4.3411	?

Totals : 8.74665e6

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

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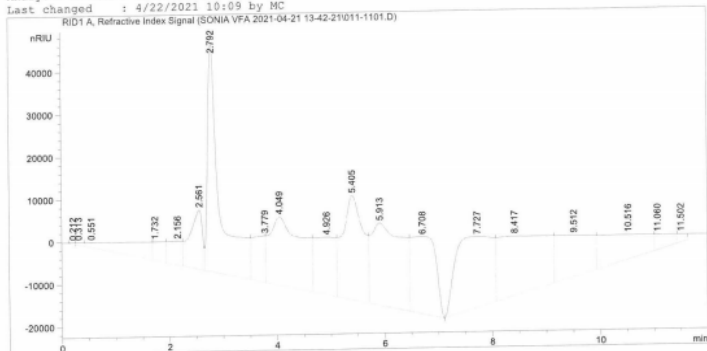
Sample: Effect of co-digestion (FW 75:PM25)

Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\011-1101.D
Sample Name: FW 75%

=====

Acq. Operator : MC	Seq. Line : 11
Acq. Instrument : Agilent HPLC	Location : Vial 11
Injection Date : 4/21/2021 16:00	Inj : 1
	Inj Volume : 20.0 µl

Acq. Method : C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\SONIA VFA.M
Last changed : 4/21/2021 14:25 by MC
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\SONIA VFA.M
Last changed : 4/22/2021 10:09 by MC



Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 22, 2021 10:09
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.212	BV	0.0894	1438.42114	0.0197 ?	
2	0.313	VV	0.1442	5466.01611	0.0749 ?	
3	0.551	VV	1.9501	1.90030e5	2.6043 ?	
4	1.732	VV	0.1997	7.03098e4	0.9636 ?	
5	2.156	VV	0.2451	1.00792e5	1.3813 ?	
6	2.561	VV	0.2372	2.26734e5	3.1074 ?	
7	2.792	VV	0.2237	8.59928e5	11.7852 ?	Valeric
8	3.112		0.0000	0.00000	0.0000	Cellulbiose
9	3.779	VV	0.2143	1.77159e5	2.4279	Glucose

Agilent HPLC 4/23/2021 09:10 MC

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Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\011-1101.D
Sample Name: FW 75%

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
10	4.049	VV	0.5501	6.82494e5	9.3535	Xylose
11	4.926	VV	0.3590	3.64371e5	4.9937 ?	Lactic
12	5.405	VV	0.3708	6.62249e5	9.0761 ?	Butyric
13	5.913	VV	0.5399	7.98539e5	10.9439 ?	Propionic
14	6.708	VV	0.5010	5.67261e5	7.7743 ?	Acetic
15	7.727	VV	0.7752	7.99146e5	10.9522 ?	Formic
16	8.417	VV	0.7838	8.54690e5	11.7134 ?	
17	9.512	VV	0.6144	4.55547e5	6.2432 ?	
18	10.516	VV	0.7818	3.80536e5	5.2152 ?	
19	11.060	VV	0.2736	7.85086e4	1.0760 ?	
20	11.502	VV	0.1401	2.14573e4	0.2941 ?	

Totals : 7.29666e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Xylose)

*** End of Report ***

Agilent HPLC 4/23/2021 09:10 MC

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Sample: Effect of co-digestion (FW 100)

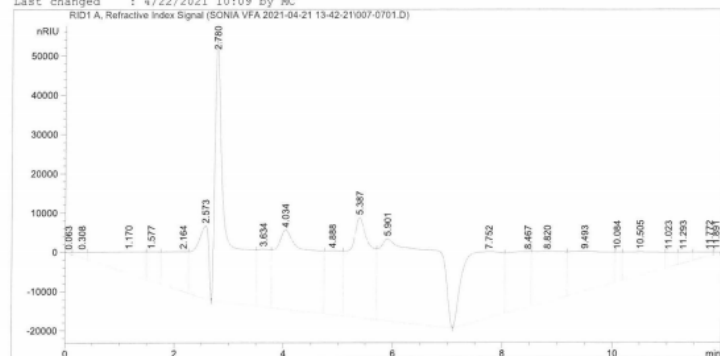
Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\007-0701.D
Sample Name: FW 100%

=====

Acq. Operator : MC Seq. Line : 7
Acq. Instrument : Agilent HPLC Location : Vial 7
Injection Date : 4/21/2021 15:07 Inj : 1
 Inj Volume : 20.0 µl

Acq. Method : C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\SONIA VFA.M
Last changed : 4/21/2021 14:25 by MC
 (modified after loading)

Analysis Method : C:\CHEM32\1\METHODS\SONIA VFA.M
Last changed : 4/22/2021 10:09 by MC



=====

Area Percent Report

=====

Sorted By : Signal
Calib. Data Modified : Thursday, April 22, 2021 10:09
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.063	BV	0.1112	2323.43896	0.0271 ?	
2	0.308	VV	0.1913	2.09626e4	0.2445 ?	
3	1.170	VV	0.6186	2.87196e5	3.3491 ?	
4	1.577	VV	0.2109	1.21139e5	1.4126 ?	
5	2.164	VV	0.3654	2.86411e5	3.3399 ?	
6	2.573	VV	0.2585	3.32496e5	3.8773 ?	
7	2.780	VV	0.2099	1.05067e6	12.2522 ?	Valeric
8	3.112		0.0000	0.00000	0.0000	Cellulobiose
9	3.634	VV	0.2222	2.38647e5	2.7829	Glucose

Agilent HPLC 4/23/2021 09:22 MC

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Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\007-0701.D
Sample Name: FW 100%

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
10	4.034	VV	0.6097	9.60533e5	11.2011	Xylose
11	4.888	VV	0.3000	3.43725e5	4.0083 ?	Lactic
12	5.387	VV	0.3801	7.32111e5	8.5374 ?	Butyric
13	5.901	VV	0.8800	1.49569e6	17.4418 ?	Propionic
14	7.752	VV	0.8207	8.45204e5	9.8562 ?	Formic
15	8.467	VV	0.3578	4.19891e5	4.8965 ?	
16	8.820	VV	0.5064	4.91193e5	5.7280 ?	
17	9.493	VV	0.6498	4.97331e5	5.7995 ?	
18	10.084	VV	0.1021	5.79391e4	0.6756 ?	
19	10.505	VV	0.5856	2.66830e5	3.1116 ?	
20	11.023	VV	0.1653	4.93696e4	0.5757 ?	
21	11.293	VV	0.1900	4.35277e4	0.5076 ?	
22	11.772	VV	0.3998	2.93969e4	0.3428 ?	
23	11.891	VV	0.0824	2765.32129	0.0322 ?	

Totals : 8.57535e6

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

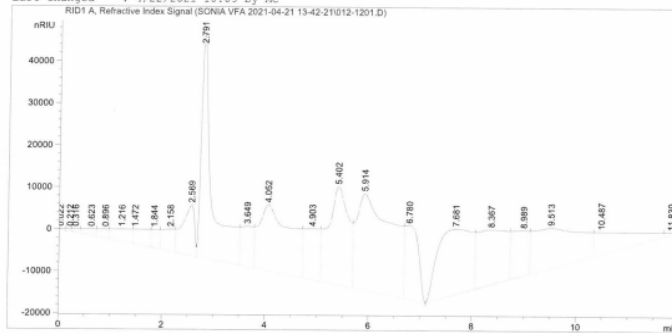
Agilent HPLC 4/23/2021 09:22 MC

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Sample: Effect of co-digestion (PM 100)

Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\012-1201.D
Sample Name: PM 100%

```
=====
Acq. Operator   : MC                               Seq. Line : 12
Acq. Instrument : Agilent HPLC                     Location  : Vial 12
Injection Date  : 4/21/2021 16:13                  Inj       : 1
                                                    Inj Volume: 20.0 µl
Acq. Method     : C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\SONIA VFA.M
Last changed    : 4/21/2021 14:25 by MC
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\SONIA VFA.M
Last changed    : 4/22/2021 10:09 by MC
=====
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Area Percent Report

Sorted By : Signal
Calib. Data Modified : Thursday, April 22, 2021 10:09
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	Ret[Time]	Type	Width [min]	Area [nRIU*s]	Area %	Name
1	0.022	BV	0.2875	1268.42175	0.0184	?
2	0.212	VV	0.0815	2900.63227	0.0407	?
3	0.316	VV	0.1492	8469.63379	0.1227	?
4	0.623	VV	0.2101	2.4847864	0.3600	?
5	0.896	VV	0.1697	2.9218864	0.4233	?
6	1.216	VV	0.3345	8.1500464	1.1808	?
7	1.472	VV	0.272	0.9558464	1.3672	?
8	1.844	VV	0.1326	4.7655764	6.905	?
9	2.158	VV	0.2376	9.0318664	1.3086	?

ilent HPLC 4/23/2021 09:12 MC

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Data File C:\CHEM32\1\DATA\SONIA VFA 2021-04-21 13-42-21\012-1201.D
Sample Name: PM 100t

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Area %	Name
10	2.569	VV	0.2385	1.98367e5	2.8740	?
11	2.791	VV	0.2095	9.97484e5	11.5538	? Valeric
12	3.121	VV	0.0000	0.00000	0.0000	Cellulbiose
13	3.391	VV	0.1631	1.63135e5	2.3636	Glyucose
14	4.052	VV	0.5620	6.78712e5	9.8335	Xylose
15	4.903	VV	0.2935	2.58600e5	3.7467	?
16	5.402	VV	0.3751	6.43863e5	9.3287	? Butyric
17	5.914	VV	0.6177	1.10613e6	16.0261	? Propionic
18	6.780	VV	0.2873	3.14906e5	4.5625	? Acetic
19	7.681	VV	0.7533	7.16132e5	10.3757	? Formic
20	8.367	VV	0.5945	1.13945e5	7.4463	?
21	8.989	VV	0.3224	2.50212e5	3.6252	?
22	9.513	VV	0.8399	6.09808e5	8.8363	?
23	10.487	VV	0.6178	2.76134e5	4.0008	?
24	11.830	VV	0.1761	7816.75684	0.1133	?

Totals : 6.90202e6

2 Warnings or Errors :

Warning : Calibrated compound(s) not found
Warning : Negative results set to zero (cal. curve intercept), (Xylose)

*** End of Report ***

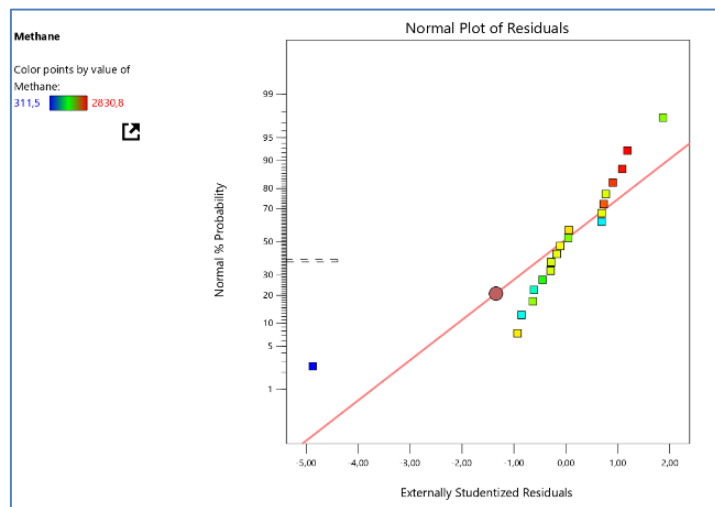
Agilent HPLC 4/23/2021 09:12 MC

Page 2 of 2

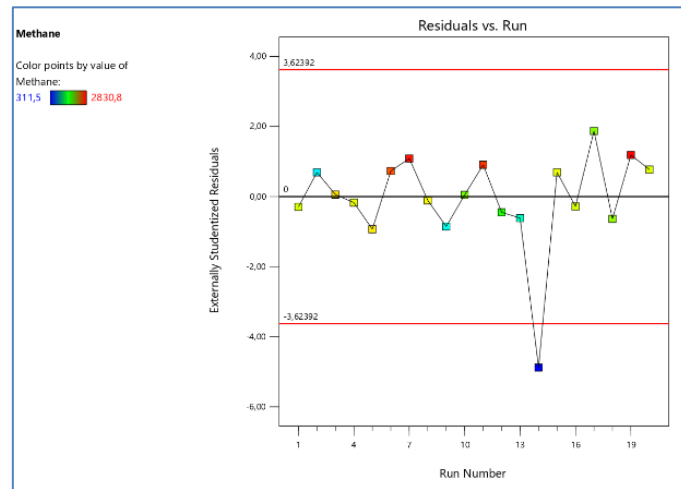
6.7 Appendix C1: Model diagnostics for methane yield

Further diagnostic analysis were conducted to assess and validate the model fit including Normal Plot of Residuals, Residuals vs Predicted, Box-Cox Plot for Power Transforms and Cook's Distance following procedures outlined by (Madondo 2018).

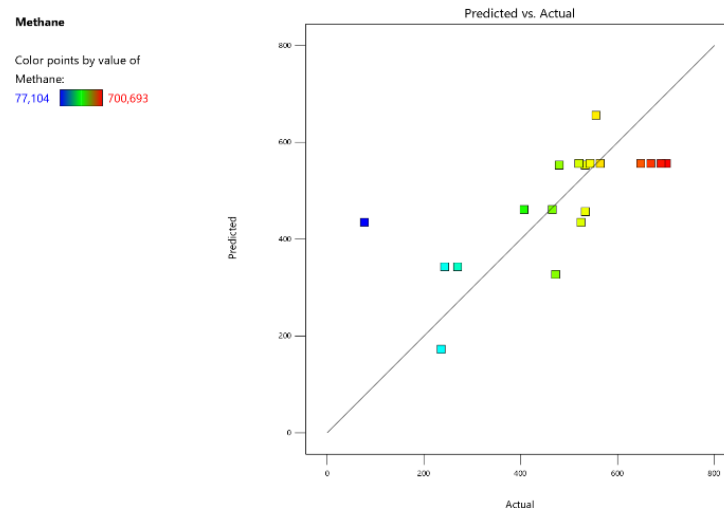
The first diagnostic analysis was normal plot of residuals (Fig 4.6), a good fit is characterised by all data points sitting on a straight line (or close as possible) which indicates a normality in the error term. A straight line shows that a transformation is not required for the methane yield responses.



The “Residual versus Run” graph was also utilised as diagnostic tool to validate the model. Figure 4.6, the residual versus run plot for methane yield, shows the residual limits are ± 3.62 . None of the experimental data points lay beyond the residual points, *indicating that no response transformation was required for any of the experimental runs for methane yields*. Furthermore, the graph displays a random scatter, signifying minimal possibility of variable lurking in the background that is time-linked.



The other diagnostics analysis used was the “Predicted versus Actual” plot. A good fit is represented by experimental data points lined linearly along the 45 degree line, showing that the model predicted data is close to the actual experimental data (Madondo, 2018). The experimental methane yield points are well distributed along the 45° line.



Another powerful diagnostic tool used was the “Box-Cox Plot for Power Transforms” graph. Figure P23 and Figure P24 show the Box-Cox plots for power transformations for biogas and methane yields, respectively. The Box-Cox plot recommended no transformation was necessary signifying that the current quadratic transformation was ok.

6.8 Appendix C2: Methane yield model validation

CO2 concentration [%] of flush gas	25
Process temperature [Celsius]	37
Mixer on time [sec]	90
Mixer off time [sec]	90
Mixer speed adjustment [%]	49
Eliminate overestimation	No

Flow Cell nr.	1	2	3
	FW:PDM 60:40	FW:PDM 60:40	FW:PDM 60:40
Name	1	2	3
Substrate VS/COD amount [g]	5,6	5,6	5,6
Inoculum VS/COD amount [g]	2.8	2,8	2,8
Type of unit [VS/COD]	VS	VS	VS
Headspace volume [ml]	200	200	200
Assumed CH4 content [%]	65	65	65

	Control 1 CD IN Volume [Nml]	Control 2 CD IN Volume [Nml]	Control 3 CD FW Volume [Nml]
Day			
0	0	0	0
1	777	788,8	790,6
2	1121,2	1146,5	1166,4
3	1270,3	1291	1318,8
4	1406,8	1418,2	1458,3
5	1470,1	1477	1531
6	1523,9	1524,3	1582,1
7	1566,7	1561,9	1622,2
8	1603,2	1593	1654,7
9	1637,9	1620,4	1683,8

10	1666,7	1645,1	1710,5
11	1692,6	1667,7	1734,3
12	1717,4	1688,8	1756,6
13	1740,4	1709,1	1777,9
14	1764,5	1729,4	1800
15	1784,7	1747,3	1821,8

Day	FWPM:75:25
0	0
1	785,466667
2	1144,7
3	1293,366667
4	1427,766667
5	1492,7
6	1543,433333
7	1583,6
8	1616,966667
9	1647,366667
10	1674,1
11	1698,2
12	1720,933333
13	1742,466667
14	1764,633333
15	1784,6

Methane Yield	
VS	2,8
Day	
0	0
1	280,5238095
2	408,8214286
3	461,9166667
4	509,9166667
5	533,1071429
6	551,2261905
7	565,5714286
8	577,4880952
9	588,3452381
10	597,8928571
11	606,5
12	614,6190476
13	622,3095238
14	630,2261905
15	637,3571429