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Title Page for Master's Thesis
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Optimization of the Inoculum-to-Substrate Ratio in Solid-State Spent Coffee Grounds Anaerobic Digestion Biorefineries Using Anaerobic Sludge for Maximal Bio-CH_4 Production.

Master's Thesis

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Specialization in Water Science and Technology

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Stavanger, Norway

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I know my fate. One day my name will be associated with the memory of something tremendous. A crisis without equal on this earth. The most profound collision of consciousness. A decision that was conjured up against everything that was ever said, thought, believed, understood, and demanded. I am no human. I am dynamite.

3aziz 3awayes

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Abstract

The optimization of bio-CH_4 production from spent coffee grounds (SCGs) using anaerobic sludge (AS) under different inoculum-to-substrate ratios (ISRs) using solid-state batch-fed anaerobic digestion (AD) reactors was tested. All reactors were operated under mesophilic conditions (37 °C) and run at least in duplicates. The working volume was fixed at 400 ± 20 mL. The initial moisture content of the SCGs was measured at 58.4%. The tested ISRs were **0.5, 1, 2, 3, 4, 5, and 6** on a volatile solids (VS) basis (g VS_{AS} /g VS_{SCGs}). The biomethane potential (BMP) was determined after subtracting the blank AS BMP. Reactors with an ISR of 2 (8g VS_{AS} $4g^{-1}$ VS_{SCGs}) showed the highest cumulative BMP of **1401 ± 137 NmL CH_4**. However, reactors with an ISR of 6 (8g VS_{AS} $1.33g^{-1}$ VS_{SCGs}) exhibited the highest specific BMP of **533 ± 22 NmL CH_4 g^{-1} VS_{SCGs}**. The lowest was attributed to reactors with an ISR of 0.5 with a BMP of **271 ± 12 NmL CH_4 g^{-1} VS_{SCGs},** where after 41 days, anaerobic bioprocesses had not yet ceased. Placed in descending order of specific BMP, it follows **ISR 6 > ISR 4 > ISR 3 > ISR 5 > ISR 2 > ISR 1 > ISR 0.5**. Linear regression showed a clear trend between the achievable BMP and the ISR used. No pretreatments were applied to enhance biomethanation. Microbial growth was modelled using the modified Gompertz equation and showed a near perfect fit to the model (**R^2 = 0.98 to 0.99**). The hydrolysis constant (k_h) at ISR 0.5 was revealed to be as low as **0.02 $days^{-1}$**, and assumed to be inhibited by high VFA concentrations, especially propionic acid. The **VS reduction was 76.2 ± 12.6%** in ISR 0.5 reactors. The **COD removal efficiency was 61.6 ± 3.2%** in ISR 1 reactors. **A total of 5881 kgs of SCG were produced in 2018 at the University of Stavanger (UiS).** This can yield an average of **39088 MJ** of heat and **369 kW_e** of power yearly. This enables SCGs bioprocessing in sustainable AD biorefineries whilst maintaining the integrity of their circular bioeconomy.

Keywords

Anaerobic Digestion. Biomethane. Bio-CH_4. BMP. Spent Coffee Grounds.

Inoculum Substrate Ratio. Lignocellulosic Biomass. Pretreatment. Biorefinery.

Dedications and Acknowledgements

This work bears the ultimate fruit of an extensive voyage of evolving and ripening. With that in mind, these acknowledgments should be remarked.

This thesis is dedicated to Ralph Naim Azar. May your soul rest in the heavens where you are now an angel amongst us.

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Georgeio Georges Semaan

Stavanger, Norway

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List of Abbreviations

IPCC	–	Intergovernmental Panel on Climate Change
AD	–	Anaerobic Digestion
SWM	–	Solid Waste Management
MSW	–	Municipal Solid Waste
SCG	–	Spent Coffee Grounds
LCA	–	Life Cycle Assessment
VAP	–	Value Added Product
GHG	–	Greenhouse Gas
$mtCO_2e$	–	Million Tons CO_2 Equivalent
AR5	–	Assessment Report 5
CCS/U	–	Carbon Capture and Storage/Utilization
FW	–	Food Waste
ISR	–	Inoculum to Substrate Ratio
SIR	–	Substrate to Inoculum Ratio
AMPTS	–	Automatic Methane Potential Test System
BPC AB	–	BioProcess Control AB
IVAR	–	Interkommunalt Vann Avløp og Renovasjon
ATP	–	Adenosine Triphosphate
BMP	–	Biomethane Potential
ORP	–	Oxidation Reduction Potential
CAPEX	–	Capital Expenses
OPEX	–	Operational Expenses
WWTP	–	Wastewater Treatment Plant
bn	–	Billion
CAGR	–	Compounded Annual Growth Rate
COD	–	Chemical Oxygen Demand
BOD	–	Biological Oxygen Demand
5-HMF	–	5-hydroxymethylfurfural
PLS	–	Partial Least Squares
VFA	–	Volatile Fatty Acid
LCFA	–	Long Chain Fatty Acid
NGV	–	Natural Gas-Powered Vehicle
PHA	–	Polyhydroxyalkanoates
FAME	–	Fatty Acid Methyl Ester
FFA	–	Free Fatty Acid
OLR	–	Organic Loading Rate
NREL	–	National Renewable Energy Laboratory
AnMBR	–	Anaerobic Membrane Bioreactor
STP	–	Standard Temperature and Pressure
TPAD	–	Temperature Phased Anaerobic Digestion
TAN	–	Total Ammonia Nitrogen
UiS	–	Universitetet i Stavanger
SiS	–	Studensamskipnaden i Stavanger
LCH	–	Lignin, Cellulose, and Hemicellulose

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§1 – Introduction

This section briefly touches upon and introduces some of the elements of this thesis, giving a general overview as to why this master's thesis is conducted and what it aims to achieve.

§1.1 – Comprehensive and Brief Overview

The world is changing at an alarming rate. Global population increase, climate change, the exhaustion of natural resources, and the accessible need for energy, fuel, and resources are only a few of the challenges we are faced with today. Scientists are exploring appropriate solutions for problems in the fields of SWM and green/renewable fuel and energy. A proposed method for achieving and implementing solutions is by examination of the circular bioeconomy and LCA of such wastes. [1] clarifies how a circular economy differs from a '*linear economy*' such that wastes from resources are viewed and treated as resources in of themselves and can be utilized rather than discarded unethically. According to [1], circular economies are often referred to as "*Resources Circulated Economies*" which inflict no adverse effect on the environment, or in some cases, can reverse adverse environmental effects.

It is projected by [2] that the world is looking at an astonishing 2.2 bn tons of MSW per year at a quote of 1.42 kg MSW capita^{-1} day^{-1} by 2025. This comes at an approximate 120% increase from just 2002. It is forecasted that these numbers will continue to aggressively increase past 2025 if no coherent policies are applied to subdue them. Household, industry, business, and even agricultural solid wastes end up being disposed of in incorrect ways, such as incineration and/or landfilling which wildly produce uncontrolled GHGs. Only some waste ends up being composted, recycled, or utilized in ways that promote environmental and sustainable biorefineries. MSWs can be separated and exploited as a driver in the shift towards a sustainable circular bioeconomy to produce a plethora of demanded products. According to [2], countries such as China, Nigeria, and India, specified as low to middle income, will be hit the hardest in their fight against waste. But these countries also have the highest potential for using their waste as a resource. The issue of SWM ties closely to that of water pollution, poverty, air pollution and even branches into a country's social condition. An estimated 1,460 mtCO_2e of GHGs come directly from the disposal of MSW worldwide. While the majority of the world is in an uptrend, Europe is in a downtrend. The EU's numbers fell from 69 to 32 mtCO_2e from 1990 to 2007 [2], which is most probably attributed to the optimistic '*glass half full*' approach they have embraced in fighting global carbon reduction and climate change through implementing and monitoring effective policies.

GHGs and global climate change have become major news headlines worldwide. Over the course of the 20th and 21st century, the heated debate over climate change has made way into our daily lives. Some people acknowledge it, some do not. Nonetheless, here are the facts according to the IPCC AR5. [3] clearly expresses that anthropogenic GHG emissions are at their sharpest in accounted history and that this can weigh heavily on natural systems and societies. The AR5 shows a substantial increase in CH_4, N_2O, and CO_2 from the 1950s onwards and a collective increase in global ocean and land temperatures. The rise in CO_2 alone attributes to a pH increase of 0.1 across all open ocean surfaces, *(a massive increase bearing in mind the combined volume of the oceans).* The sectors with the highest GHG emissions are the electricity and heat, transportation, and agricultural or land-use sectors summing up at 30870 $MtCO_2e$ in 2010 alone [3]. However nowadays scientists and researchers are working coherently on technologies such as oceanic and biochar CCS, CCU, as well as waste biomass valorisation to biofuels and VAPs in anticipation of pushing us further away from our dependence on crude oil and petrochemical refineries and inching closer to sustainability through biorefinery novelties.

[4] defines biorefineries as the practical approach for applying a sequence of biotechnologies, ideally to produce biofuels, biopower, biomaterials, and biochemicals through series of chemical and physical conversions of waste biomass. Simply put, biorefineries function similarly to petrochemical refineries with the exception of using biomass as a feedstock instead of crude oil. With a worldwide adopted positive attitude and an abundance of biomass and municipal organic waste, development in the field of biorefineries green chemistry has excelled in order to shift us away from premature linear economies and adopt newer more robust approaches [4]. Some of the basic biorefinery processes and systems found today are pyrolysis for the production of bio-oil, biochar and syngas, fermentation for the production of ethanol, and finally AD for the production of bio-CH_4 and bio-H_2. These are but a few pathways for a biorefinery approach to valorize biomass to biofuels or VAPs. There is however more complex methods for more selective bioproduction processes.

SCGs are a biomass. They can be found in substantially large quantities around households, cafes, hotels, factories, and businesses due to the high demand which has infiltrated our lives worldwide. To the full extent of the authors knowledge, SCGs are not qualitatively separated as an independent waste stream on the grander scale until now. Research on the valorisation of SCGs have been proposed and documented by [5], [6] and [7]. SCGs can be converted into biofuels (biogas, biohydrogen, biodiesel, and bioethanol), biomaterials/biochemicals (biochar, biopolymers, compost, and bioactive compounds) and more specialized VAPs [7]. SCGs contain a fusion of over 1000 compounds [5] which can function as feedstocks for individual biorefineries. Certain chemicals are extremely resistant to breakdown and require large

amounts of O_2 and time to degrade, even then their residuals can be highly toxic to the environment they leach into. Essentially, SCGs are a prime resource of carbohydrates, proteins, lipids, phenolics, and minerals [6] which make them a prime candidate for biorefinery research options such as AD.

AD is a relatively old technology which utilizes natural occurring phenomena. It is established with known fundamentals in the areas of research and industry [8]. The current scientific stance on AD is to utilize its underlying theory and diverge research into combining/orienting numerous process parameters for increased process efficiency, optimization, and stability depending on the substrate or co-substrates used [8]. With the developing paradigm shift of the 21st century and the ever-growing problem of waste and energy, scientists are pushing forward waste-to-wealth technologies such as AD to overcome [9].

The research surrounding SCGs AD dates back to 1983, when it was first considered as a substrate for bio-CH_4 production by [10]. Research has come a long way suggesting the AD of SCGs to aid in the mitigation of this waste which can firstly provide biobased fuel resources, and secondly limit the damage such waste has on the environment. This innately cranks the shaft that propels us onto a circular renewable platform for the biorefinery of SCGs biomass.

§1.2 – Research Objectives

A total of four (4) research objectives are to be achieved by this research (as listed below).

a) Determine and calculate the BMP of SCGs in batch mode AD using the AMPTS II system for optimized bio-CH_4 production by adjusting the ISR.
b) Evaluate the system COD mass balance and determine the VS and COD reduction as well as the degree of biodegradability at the selected ISRs.
c) Use the modified Gompertz model and the first order hydrolysis model to model BMP and degradation kinetics at the selected ISRs.
d) Evaluate the theoretical annual CH_4 energy output by the AD of SCGs produced at UiS campus Ullandhaug, Stavanger, Norway.

§1.3 – Word Cloud

The word cloud below (see figure 1.1) represents some of the issue being addressed in this research and can be considered as a broad generalization of key words associated with this thesis and corresponding subjects.

Figure 1.1 – Word Cloud associated with this research thesis.

§2 – Literature Review and Theoretical Background

This section will describe, investigate, and explain the underlying theories and terms featured in the introductory word cloud; figure 1.1. This section intends to act as a focused hub of information on previous research and theories around AD, SCGs, and more. More importantly, this literature review acts as a reference point throughout this thesis to describe and tessellate the results and discussion section.

§2.1 – Anaerobic Digestion

A biological waste treatment approach, AD is the title given to the controlled biochemical process by which a consortia of anaerobic microorganisms break down biodegradable organics in an O_2 deprived environment [8], [11]. The fate of electrons in anaerobic systems differ from that of aerobic systems primarily in two ways. First, anaerobic respiration by *Clostridium*, *Pseudomonas* and *Streptococcus* species (*to name a few*) [12], utilize electron acceptors such as nitrate (NO_3^-), sulfate (SO_4^{-2}), and ferric iron (Fe^{3+}) instead of O_2 [13]. Second, anaerobic respiration produces considerably less ATP as opposed to aerobic respiration due to a lower ORP [13], which is why aerobic systems produce more sludge than anaerobic systems as the produced ATP is primarily funneled into microorganism growth and respiration [14] (see figure 2.1). Table 2.1 expresses the main advantages and disadvantages of AD operations.

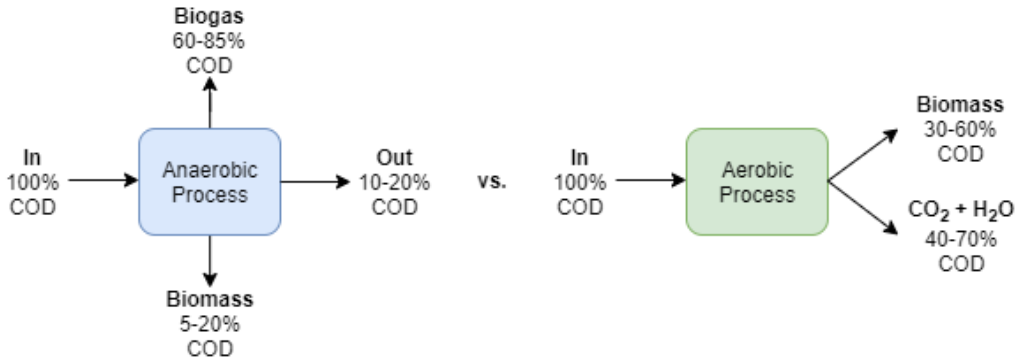

Figure 2.1 – Distinctions Between Aerobic and Anaerobic Processes (COD Balance Shown) [14], [15].

Table 2.1 – Advantages and Disadvantages of AD Processes.

Advantages	Disadvantages
• Reduction of organic-based wastes [16]. • Waste can be co-digested. Offers higher BMP and enhanced quality of CH_4 [17].	• High CAPEX and OPEX [16]. • Heavy metals, ammonia, and other inhibitors can decrease the BMP and lead to reactor failure [18].

• AD coupling for two-stage processes which can produce biohythane (CH_4 and H_2) as products [19]. • Circular bioeconomy through biorefinery/bioenergy option. Reduces GHGs [16]. • Upgraded biogas can serve as natural gas and be injected into natural gas grids. Digestate can be separated into H_2O and soil fertilizer [9].	• Requires a stable/constant flow of feedstock into digestors to ensure process stability [16], [9]. • AD facilities can decrease real estate properties value due to fouls odors of digestion [17]. • Processing costs for post-treatment of digestate. Processing costs for pre-treatment of feedstock/substrates. Post-processing costs to upgrade biogas. [9].

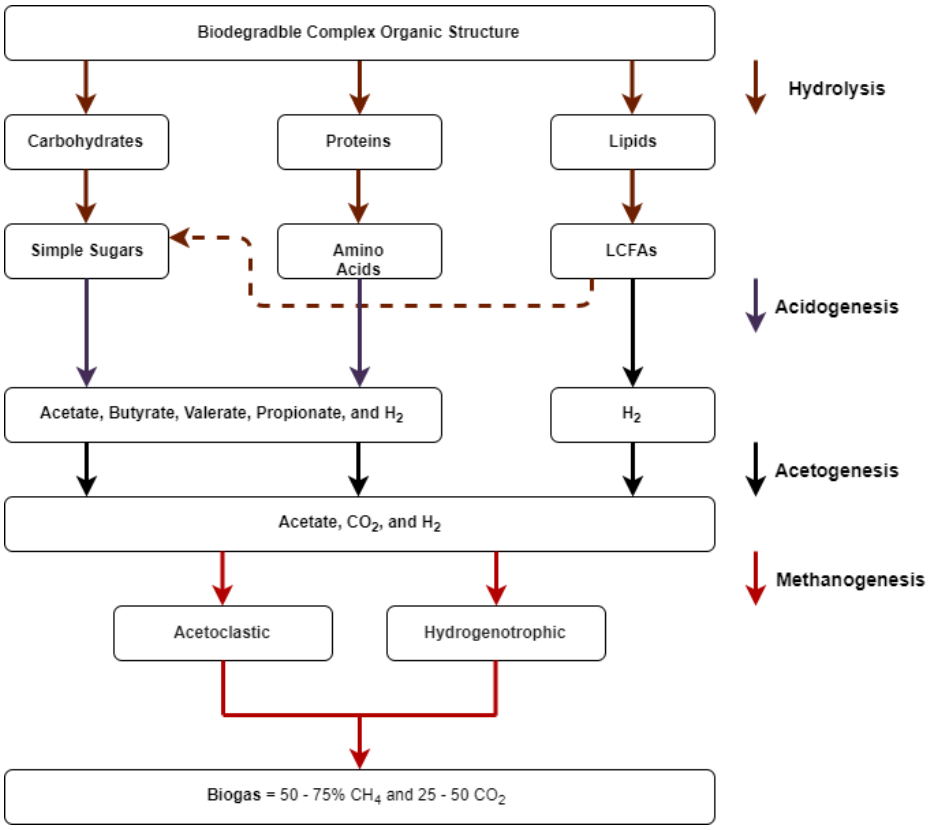

Figure 2.2 – Overview of the Main Biochemical Reactions Involved in AD [20].

Essentially the idea is to inoculate certain feedstocks with anaerobic biomass under certain process parameters and conditions (see sections §2.4 and §2.5) to maximize COD elimination and CH_4 recovery. AD is used mainly to reduce feedstock undesirable effects, by reducing their COD and second, bio-converting this COD into biogas which can be upgraded in gas processing facilities to yield bio-CH_4. Principle components such as carbohydrates, proteins and lipids and others are eventually oxidized by a sequence of biochemical conversions (see sections §2.1.2 to §2.1.5) (see figure 2.2) into subsequent biogas, which can be collected, processed and used as biofuel (see section §2.1.6). Using a mixed cultured inoculum is more reliable as it is somewhat already acclimatized to a certain limit of inhibitory compounds without the

need for pure culture adaptation strategies [32]. The complete and theoretical degradation of feedstocks can be chemically calculated by the Buswell and Boyle equation (1952) (see equation 1) [21].

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S$$

Equation 1 – Buswell and Boyle Equation for Complete Anaerobic Degradation (Including N and S) [21].

The future of AD and biogas is optimistic. Initially, AD has been investigated to primarily treat organic sewage sludge from WWTPs and animal manure. Recently the utilization shift to treat other sources of waste such MSW, lignocellulosic biomass, and other organics has increased [22], [23]. AD is being explored as an appropriate solution to the ever-growing problem of environmental wellbeing. The USA and China dominate research in AD-related endeavors. Between 2016 and 2017, these 2 superpowers contributed 11.8% and 29% to research outcome, respectively [24]. Market research suggests that the global biogas market is on an uptrend and will be sized at 110 bn USD by 2025. A 40 bn USD increase with a 7.0% CAGR between 2018 and 2025 [25]. Similarly, the European market share for AD is expected to increase with a CAGR of 7.8% to reach over 75 bn USD by 2026 [26]. Both CAGR rates and global market shares are expected to continue increasing after their forecasted periods. Linking such opportunistic trends with the increase in AD research, we can somewhat envision the role AD plays in the near to long term future. [24] calls for additional research related to the pretreatment of biomass (lignocellulosic), kinetic modelling, LCAs, case studies, and the optimization and monitoring of processes to incentivize policy makers and investors to consider AD biorefineries for a circular bioeconomy [27].

§2.1.1 – BMP, Feedstocks, and Pretreatments

BMP experiments are aimed at quantifying the degree of anaerobic biodegradability of a specific substrate or co-substrates (with known compositions), depending on parameters such as carbohydrates, proteins, lipids, and fibers within the waste [28]. Therefore, a precise BMP is crucial to determine design, financial, and management issues in feasibility studies of a new AD plant [29]. The incoming substrate to an anerobic digestor reactor is called a '*feedstock*'. Issues such as reactor souring, BMP, degradation kinetics, biogas composition, COD reduction, and energy balances are dependent on the feedstock being fed into AD reactors and reason the overall feasibility (*and lifetime*) of an AD reactor. Before building industrial scale operations, laboratory BMP tests for substrates have to be assessed to determine any and all inhibitory effects that will eventually lead to reactor failure. Then, pilot scale models are built and

operated to judge if the process is feasible at even larger scales. On top of that, BMP test trails are essential for LCAs to determine whether a feedstock is at all appropriate for AD or better suited for other bioprocesses/biotreatments in their circular bioeconomies.

Almost any organic substrate can be degraded under AD, but this should be taken with a grain of salt. Some feedstocks serve as excellent substrates with high a biodegradability whilst other are more tedious and expensive to work with. Substrates classified as lignocellulosic (see section §2.1.1.1) can be quite problematic due to their sluggish degree of biomethanation i.e. (low BMP) and their recalcitrant nature (low biodegradability) [30]. This means that they normally require pretreatments, which is a separate added process, implying added cost and energy into the overall picture.

For the purposes of this experimental thesis, SCGs were employed as the sole substrate for their laboratory scale batch AD using the AMPTS II system provided by BPC, Lund, Sweden. SCGs are a solid FW that have a lignocellulosic nature. The results from these BMP tests were used to draft up a preliminary heat and power survey for the AD of SCGs generated at UiS campus Ullandhaug (see section §2.6).

§2.1.1.1 – Lignocellulosic Biomass

Scientists have agreed on the importance of lignocellulosic biomass for a broad variety of VAPs obtained by direct or indirect biorefinery options. Lignocellulosic biomass is the world's most abundant natural material. It can be found in agricultural residues, crops, tree wood, and MSW fractions [31]. SCGs too are of a lignocellulosic nature. Lignocellulosic materials are exploited for their carbohydrate content, mainly for bioethanol and biogas manufacture [32]. Lignin shields holocellulosic fractions (cellulose and hemicellulose) rendering them inaccessible for processing. Therefore, pretreatment methods may be applied to retain the maximum potential from these biomass. The main '*take home concept*' is that pretreatments are used to make alterations in the structure of lignin thereby releasing cellulose and hemicellulose portions. Thereafter, hemicellulose can dissolve and cellulose can be hydrolyzed more effectively by hydrolytic enzymes generating high sugar yields [32]. Fractions of lignin, hemicellulose and cellulose widely vary between species. LCH intertwined structures are oriented as shown in figure 2.3.

Lignin stems from a class of phenolic organic polymers which is vital for plant health. It provides the support and structural rigidity in their cell walls, which tightly forms a shield layer around internal plant tissue from outsider influence such as microbial infiltration, O_2, and H_2O [32]. Lignin content varies but is within the range of 15 to 40% dry weight [33]. The higher the lignin content in a feedstock the more opposed it is to hydrolyze. Lignin begins solubilizing at temperatures of 160 to 180°C and breaks down into its principle phenolic monomers. Phenolic lignin monomers greatly inhibit the activity of methanogenic

archaea or bacteria, hence negatively affecting the BMP [32]. A double-edged sword argument raises itself here; how do we pretreat lignocellulosic biomass enough to release their components yet at the same time hold the structural integrity and limit the formation of inhibitors. This is why [24] calls for research in the field of feedstock pretreatment to better formulate and understand such events in order to improve the quality of pretreatment methods.

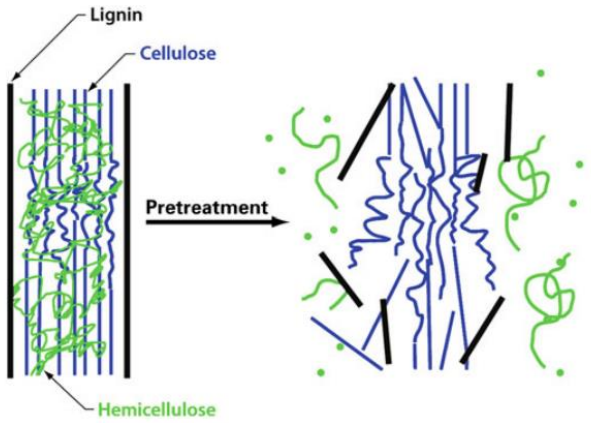

Figure 2.3 – Demonstrational schematic of lignocellulosic biomass before and after pretreatment [30].

Hemicellulose is a plant cell wall polysaccharide which acts a medium of connection between biomass lignin and cellulose segments. Hemicellulose covers approximately 25 to 35% dry weight of all heterogenous polysaccharides in a lignocellulosic biomass [34]. Hemicellulose is comprised of primarily C5 (xylose and arabinose) and C6 (galactose, glucose, and mannose) oligosaccharides alongside a minimal concentration of organic acids [32]. They exhibit in polysaccharides *viz.* xylans, xyloglucans, β-glucans and mannans [35]. Hemicellulose consists of shorter biopolymers hence embedding in it the ability to be easily hydrolyzed [32]. Upon thermal treatment of hemicelluloses, the organic acids detach, solubilize, and acts as a catalyst for further polysaccharide hydrolysis. This acidic environment is more prone to be infested with inhibitors of the solubilization of hemicellulose and lignin. A powerful pretreatment can lead to the formation of furfurals, 5-HMF, vanillins, and other phenolic and heterocyclic inhibitors [32], [36]. Inhibitory compounds can lead to severe negative consequences on subsequent downstream processes, such as low BMP [36], low CH_4 concentrations, and can even end CH_4 production [32]. The solubilization of hemicellulose has proved in of itself an aid to further hydrolyze cellulose. Hemicellulose research is still in its young stages but indicates to be of value for the production of biobased VAPs from lignocellulosic biomass [34]. [37] validates that concentrations of 0.6 g 5-HMF L^{-1} was enough to discontinue biohydrogen production from galactose and enough to restructure the microbial community composition completely.

The cellulose content in biomass is the most variable, and the most unpredictable. Records of 9 to 80% cellulose by composition have been recorded [38]. Cellulose has the empirical formula $(C_6H_{10}O_5)_n$. It is

an arrangement of α-D-glucose monomers connected by β-1,4 glycosidic bonds. Glucose molecules are naturally arranged in crystalline/organized structures interwoven into amorphous/non-organized structures. The degree of arrangement between the organized to non-organized monomers gives the overall crystallinity of cellulose in a biomass [32]. The tough or recalcitrance nature of lignocellulosic biomass is attributed to a high degree of crystallinity of cellulose within the lignin-hemicellulose-cellulose matrix [36]. Reduction of cellulose crystallinity to the amorphous form allows for a more coherent degradation by enzymatic hydrolysis [36]. Enzymatic hydrolysis, by cellulases, promote the conversion of cellulose into monomeric glucose which in turn are biochemically converted much quicker and more efficiently by bacterial fermentation into biogas and other bioproducts [32].

Due to the variability in the composition of the main building blocks in biomass *viz.* researchers have been able to use data sets and statistical modelling to predict the influence of varying LCH matrix compositions on the biodegradability, solubility, and BMP via mathematical correlations [39], [40], [41]. [39] demonstrates this by using a PLS factors prediction model. The BMP of a particular substrate or co-substrate can be predicted to a high relative degree of accuracy given the lignin and BOD parameters. [41] proved, by the use of statistical analysis, that the BMP and biodegradability potential of a substrate can be predicted from the lignin content as an independent variable. [41] also suggested that cellulose and lignin can be both used as independent variables to determine BMP and biodegradability due to the high R^2 of their model. [40] validates this too by using a canonical linear mixture model. The predicted BMP of a substrate (pBMP) can be forecasted using the composition of cellulose, hemicellulose, lignin, and residuals as x_C, x_H , x_L and x_R respectively, where x_C, x_H , x_L and x_R are equal to 1, (see equation 2). The lignin fraction (x_L) is hindering the BMP (due to the negative sign) as seen in the correlation proposed by [40].

The concentrations of lignin, hemicellulose, and cellulose in SCGs play a significant role in their digestion. By freeing up hemicellulose and hydrolyzing cellulose fractions, pretreatments will allow for an improved BMP as well as increased process kinetics. The key is to link optimized pretreatment conditions for the highest levels of bio-CH_4 conversion.

$$pBMP = 378x_C + 354x_H - 194x_L + 313x_R$$

Equation 2 – Predicted BMP Equation Proposed by the Canonical Linear Mixture Model [40].

§2.1.1.2 – Pretreatment Methods

Pretreatment continues to be a hot research topic in the field of SWM and lignocellulosic biomass, not only for AD but a wide variety of biorefinery approaches. Pretreatment can essentially be grouped into 3 main categories; physical, chemical, and biological as per [42]. In the case of AD, it is essential to combine

digestion with pretreatments of SCG substrates in order to evaluate the trade-off between the OPEX of processing and the overall BMP achieved, whilst maintaining a stable digestion. Listed below are some of the main pretreatment strategies available alongside their mechanism of action (table 2.2). Also, presented are some of the few pretreatment strategies employed by various researchers today to improve the BMP of their substrate (table 2.3). Table 2.3 reasons that pretreatments are important methods that can be utilized for boosting the BMP.

Table 2.2 – Some Lignocellulosic Biomass Pretreatment Strategies and Their Corresponding Mechanism of Action.

Pretreatment Method	Category	Mechanism of Action	References
Milling	Physical	Reduces particle size and increases surface area. Reduces degree of crystallinity in cellulose. More efficient when combined with other pretreatments.	[43], [44]
Microwave Irradiation	Physical	Improved enzymatic hydrolysis rate. Enhanced breakdown of the crystalline structure of cellulose into glucose. Solubilization of hemicellulose. Paired with acid or alkali pretreatments.	[42], [43]
Hydrothermal (Liquid Hot Water)	Physical	Pierces into the biomass and hydrating cellulose as well as solubilizing parts of lignin and hemicellulose. More efficient when combined with hydrolytic enzymes.	[42], [45]
Acid	Chemical	Near 100% saccharification and solubilization of hemicellulose. Cellulose is more prone to enzymatic hydrolysis and microbial degradation. Acid can be recovered.	[43], [46]
Alkali	Chemical	Cleavage of the lignin-holocellulose bonds. Disruption of the lignin structure and its polymerization degree. Increasing functional surface area by swelling.	[43], [47]
Steam Explosion	Chemical	Sudden venting of high pressure causes implosive decompressions in the biomass, which solubilizes hemicellulose and parts of lignin.	[43], [48]
Wet Oxidation	Chemical	Lignin undergoes oxidative cleavage by free radicals. Hemicellulose is broken into sugars and then to organic acids. Amorphous cellulose is hydrolyzed.	[43], [49]
Fungal	Biological	Heterotrophic fungi species degrade lignin and hemicellulose (as their C source) allowing cellulose to be accessible to hydrolytic enzymes.	[43]
Enzymatic	Biological	Cellulase and hemicellulase enzymes aim to increase the solubilization by increasing the hydrolytic activities of cellulose and hemicellulose.	[43]

Generally speaking, biological pretreatment methods are the more environmentally friendly but are not merely in competition with other pretreatments such as chemical or physical. The cost, retention time, selectivity, and efficiency of biological pretreatments methods weigh in heavily on their feasibility for

industrial scale AD of lignocellulosic biomass [43]. Usually, pretreatments work better when combined. Usually in the form of physical/chemical or physical/biological. Most pretreatments can be utilized at higher temperatures to increase reaction kinetics and pretreatment efficiency. However, this may lead to the formation of inhibitors such as free radicals, furfural, 5-HMF, vanillin, and so on. Biological pretreatments operate most efficiently at a certain temperature (usually mesophilic temperatures), but that depends on the microorganism used. High temperatures take a toll on microbial life (fungi, bacteria, archaea) with an elevated possibility of denaturing enzymes and stopping their metabolisms.

Table 2.3 – Using Pretreatment Methods to Improve BMP (Using the Prelisted Methods from Table 2.2).

Biomass Tested	Pretreatment	Notable parameters	BMP increase (%)	References
Banana Peelings	Milling	Decreased particle size from 6mm to 0.4mm	9	[50]
Microalgal Biomass Mixture	Microwave Irradiation	65,400 kJ/kg TS – 900W – 98 °C – 3 mins	78	[51]
Rice Straw	Hydrothermal	Saturated H_2O vapor – 200 °C – 1.55MPa – 10 mins	222	[52]
Sugarcane Bagasse	Acid	2% H_2SO_4 – 121 °C – 15 mins	166	[53]
Rice Straw	Alkali	9.8% $Ca(OH)_2$ – 25 °C – 6 days	74	[54]
Residual Manure Fibers	Steam Explosion	180 °C – 15 mins	29	[55]
Residual Manure Fibers	Wet Oxidation	O_2 deficient – 180 °C – 10 mins	136	[56]
Japanese Cedar Wood	Fungal	*C. subvermispora* ATCC 90467 – wheat bran as fungal supplement – 28 °C – 70% relative humidity – 8 weeks	25	[57]
Microalgal Biomass Mixture	Enzymatic	Enzyme mixture (cellulase, glucohydrolase, xylanse) 1% – 37 °C – 6 hours	15	[58]

§2.1.2 – Hydrolysis

The word hydrolysis reduces to 2 words, *hydro* and *lysis*, which involves the breaking of bonds (*lysis*) in the presence of water (*hydro*). Organic waste feedstocks are more commonly found in their complex form. Dense biopolymers serve no purpose to AD acidogenic microorganisms in their natural form. They must be exposed to physical fragmentation/disintegration and simplified into their corresponding oligomer and monomer counter parts [8]. This is known as the process of hydrolysis. 'Pretreatment' and 'hydrolysis'

are different concepts and should not be confused. However, the two are congruent and complementary. The objective of pretreatment is to alter the structure of lignocellulosic biomass in which improvements are made that microorganisms can capitalize on [43]. Yet during hydrolysis, the secretion of exocellular enzymes converts carbohydrates, proteins and lipids into sugars, amino acids, and long chain fatty acids, respectively (brown lines; see figure 2.2) [8]. In other words, hydrolysis occurs regardless of pretreatment, but pretreatment gravely enhances the rate of hydrolysis. As hydrolysis is primarily a biological process, it is highly sensitive to fluctuations in temperature and pH, with an optimum pH of 5 – 7 [8].

Various microorganisms such as *C. proteolyticum*, *P. anaerobicus*, *C. sporogenes,* and others secrete exocellular enzymes such as cellulases, amylases, gulcanases, proteases, and lipases which hydrolyze both soluble and particulate fractions of the complex biomass [14]. The kinetics of soluble fractions hydrolysis corresponds to the Michaelis Menten kinetic reaction. The hydrolysis of particulate fractions corresponds to the first order kinetics reaction; however, first order models have been too used to model hydrolysis as a whole. Both ways, it is shown that the amount of substrate to be hydrolyzed strongly influences the rate constant and conversion efficiency, which is where pretreatments serve their initial role. A successful hydrolysis conversion correlates to a successful biomethanation process [8]. Hydrolysis remains an interesting and hot topic of research in order to successfully optimize the breakdown of biopolymers for various VAP biorefining options [8], especially for a more fluid BMP process.

During the AD of lignocellulosic biomass, hydrolysis is assumed to be the rate limiting step due to the large polymerization degrees of cellulose and lignin [43]. The recalcitrance of crystalline cellulose and lignin as well as the operational surface area (active sites) available for enzymes were found to impact the rate of hydrolysis [36]. A sharp drop in the rate of hydrolysis is expected after the hydrolysis of amorphous cellulose fibrils is complete, if crystalline cellulose assumes form [42]. For easily biodegradable substrates, it is generally viewed that hydrolysis is the rate limiting step [59]. For more complex substrates, acidogenesis and even methanogenesis can be the rate limiting steps. However, it is argued that acidogenesis may be the rate limiting step when it comes to the AD of SCGs [60].

§2.1.3 – Acidogenesis

Acidogenesis is a natural biochemical fermentation process. It is exploited in biorefinery processes for the large array of biobased products produced mainly VFAs. Acidogenesis and hydrolysis are two distinct reactions, but they occur simultaneously. The products of hydrolysis are the reactants for acidogenesis. Acidogens metabolize hydrolysis by-products such as monosaccharides and amino acids to secrete intermediary VFAs viz. acetic, propionic, butyric acids typically in the ratios of 75:15:10 to 40:40:20

respectively [8], [20] (purple lines; see figure 2.2). Besides VFAs, acidogenic processes produce alcohols, formic and lactic acids, CO_2 and H_2, as well as NH_3 and H_2S (depending on the composition of the substrate and its hydrolysis by-products). Lipids hydrolyzed into monosaccharides undergo acidogenesis. Lipids hydrolyzed into LCFAs undergo a conversion via a separate pathway known as acetogenesis (see section §2.1.4). Acidogenic bacteria such as *Clostridium*, *Escherichia*, and *Lactobacillus* can be used as pure cultures, however it is best to conjure a mixed culture in order to adapt their encounter to a variety of hydrolysis by-products [14], [61].

The rate of acidogenesis involuntarily affects the methanogenesis rate and consequently influences the CH_4 production rate. Also, NH_3 and H_2S production have proved to affect the kinetics of VFA production and can lead to a lower BMP and/or reactor souring, especially in a protein potent feedstock [62], [63]. In general, acidogenesis is the fastest bioconversion process in AD (*with rate constants (day^{-1}) being at least 3 to 4 times more than hydrolysis*) [11] ,[63].

The accumulation of VFAs in a digestor can cause reactor acidification and retard both the CH_4 production rate as well as the cumulative BMP. Insufficient alkalinity (external or internal) can cause pH fluctuations terminating the activities of methanogens altogether at pH of 5 to 6.5 even if acidogens function at similar pHs [64].

§2.1.4 – Acetogenesis

Acetogenesis is a crucial intermediary step with a similar objective to acidogenesis; production of acetic acid/acetate (black lines; see figure 2.2). Intermediary products such as propionic, butyric, formic, lactic acids, and alcohols from acidogenesis are reduced further and thereby converted by acetogenic microorganisms to generate H_2 and acetate as end products [8]. Both H_2 and acetate are used by methanogens (see section §2.1.5) i.e. (acetoclastic and hydrogenotrophic methanogens), which secrete CH_4. To some degree, acetogens modulate reactor conditions against inhibition, VFA acidification, and H_2 partial pressure changes as the more complex VFAs have a higher acidity [65].

Unless lipidic LCFAs undergo hydrolysis to monosaccharides they primarily undergo breakdown by acetogenesis (dashed brown lines; see figure 2.2) [66]. Acetogens utilize LFCAs such as stearic and palmitic acids and break them down into propionate and then into acetate and H_2 via β-oxidation pathways [8]. Feedstocks with saturated fatty acid profiles enhance biogas production during their AD. This occurs due to stoichiometrical intricacies (densely packed C chains in lipids) leading the degradation of LCFAs into high concentrations of CO_2 and acetate and then CH_4. Overall, LCFAs produce much more acetate per mole LFCAs than other intermediates broken down by acetogens. More acetate implies more methanogenic

activity yielding more bio-CH_4. Fats produce more biogas per mole (1.2 – 1.6 m^3 kg fat^{-1}), more than proteins and carbohydrates [14], [67] and that is due to the increase acetate availability when acetogens digest LCFAs as opposed to simple sugars and amino acids.

§2.1.5 – Methanogenesis

Methanogenesis stems from 2 words, *methano* and *genesis*, implying the creation (*genesis*) of methane (*methano*) and indicates the final bioprocess in AD (red lines; see figure 2.2). *Acetoclastic methanogens* utilize the acetate raised up in the acetogenesis stage to produce CO_2 and CH_4. Simultaneously, *hydrogenotrophic methanogens* utilize H_2 and CO_2 to produce CH_4 (see equations 3 and 4). Out of all the microorganisms involved in AD, methanogens are by far the most sensitive, for example to changes in pH and exposure to O_2 [8]. When determining the BMP of a substrate, is its projected that the end of methanogenesis is concluded by the plateauing of the cumulative BMP profile [8].

Methanogenesis *can* also be considered a rate limiting step for two reasons: inhibitory effects and relatively slow kinetics. Generally speaking, methanogens have a low growth rate (sometimes up to 9 $days^{-1}$) which leads to the high retention time experienced by BMP tests and operating digestors. They typically convert the majority of the available COD whether it be acetate or H_2 with a low growth yield of 0.05 – 0.1 g VSS g COD^{-1} [68], [69]. They do not function too well under stress conditions. They survive between pHs of 5 to 8 but are rapidly rendered inactive under pHs of 7 [69]. Electron acceptors such as O_2 immediately disarm methanogenic *archaea* because of their obligations towards strict anaerobic environments [8], [70]. Two stage AD reactors proved to be extremely practical when dealing with low pH digestates. Methanogenesis can be treated as a standalone secondary process by retaining methanogens separately through HRT and SRT recirculation strategies. This produces a high conversion of COD into bio-CH_4 with less risks of reactor failure [71]. [72] also used two stage CSTR reactors to upgrade CO_2 from the biogas effluent with hydrogenotrophic methanogens by supplementing them with H_2 to further produce methane.

$$CH_3COOH \rightarrow CO_2 + CH_4$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

Equations 3 (Top) - Acetate to methane [73]
Equation 4 (Bottom) – Hydrogen to methane [73]

§2.1.6 – Biogas

As seen in sections §2.1.2 to §2.1.5, lignocellulosic substrates undertake a journey of biochemical conversions under microbial supervision to eventually become biogas. Issues such as pH change, kinetics,

inhibitory compounds, symbiotic effects between microorganisms, as well as compositional build of the feedstock to be digested [69] are all pressing factors that effectively improve/impair the BMP and the CH_4 concentration. The typical composition of biogas is shown (see table 2.4). It varies based on substrate and inoculum properties.

Table 2.4 – Typical composition of biogas (%) [74]

Gas Component	Agricultural Waste	Landfill	Industrial Waste
CH_4	50 – 80	50 – 80	50 – 70
CO_2	30 – 50	20 – 50	30 – 50
H_2S	0.7	0.1	0.8
N_2	0 – 1	0 – 3	0 – 1
H_2	0 – 2	0 – 5	0 – 2
O_2	0 – 1	0 – 1	0 – 1
CO	0 – 1	0 – 1	0 – 1
NH_3	Traces	Traces	Traces

Known for its fuel abilities in the technology and energy sectors, biogas, especially biomethane is of great importance in the advancements in circular bioeconomies and sustainability approaches. A rise in biogas purification and separation technologies are gaining traction to supplement natural gas grids, powerplants, industries, and homes as well as combustion engines (NGPVs) ultimately reaching a plethora of new costumers [23], [74], [75].

Chemical refinery plants cooperating with biogas AD plants are essential to modify effluent biogas into sales gas. However, in the EU only 4.4% of all natural gas use comes from biogas [23], which is still considered quite low as the EU is in the forefront of the sustainability picture. However, the European committee for standardization has drafted a technical group by the name TC-408. They are involved in formulating and drafting EU coherent policies for the further integration of upgraded biogas i.e. (high purity biomethane) for NGPVs and existing gas grids [23]. On top of that, low income households, for example in Africa and Asia, have increasingly been assembling decentralized home-made digestors and using the them for personal heating and cooking.

Removal of gaseous components such as H_2S, H_2O, and CO_2 is critical for the utilization of biogas. This raises both its market value (leading to a higher return on investment) and it also increases the heating values of the gas (HHV and LHV) [74], [75]. Eventually, upgraded biogas with similar compositions and parameters to that of natural gas can be blended and sold as a single gas stream.

H_2S removal is required for optimal engine and boiler operations. H_2O removal is required to avoid condensation and corrosion issues. CO_2 removal is required to increase heating values and sales revenues, also CO_2 removal is needed to avoid any corrosion from H_2CO_3 formation. Siloxanes removal is required to avoid the rapid deposit onto equipment effectively reducing heat and mass transfer. N_2, O_2, NH_3, and other alienated compound removal is required to purify biogas streams into biomethane utilizable fuel (95% v CH_4/v) [74].

The removal of the aforementioned impurities is achieved by various technologies. The use of selective membranes (for siloxanes and H_2O removal), gas scrubbing (for H_2O, H_2S, NH_3, and CO_2 removal), biologically by algae and other microorganism (for CO_2 and H_2S removal), glycol absorption (for H_2O removal), and SiO_2 adsorption (for H_2O removal) are some of the common technologies used to upgrade biogas [74], [76]. H_2 removal is not necessary as it can be utilized alongside bio-CH_4 as a fuel source known as bio-hythane. Bio-CH_4 injection into gas grids requires a maximum amount of processing (biogas upgrading) in order to satisfy CH_4 transportation regulations.

§2.2 – Substrates Used for Biogas Production by AD

This section will discuss parameters, processes, and concerns around SCGs, especially in terms of the literature available concerning their AD for biorefinery processes (see section §2.2.2). Some of the data presented in this section will be used to supplement gaps in the thesis. This is done because laboratory workflow was limited and suddenly cut due to the COVID – 19 crisis.

§2.2.1 – SCGs from the General Perspective

Coffee is the world's 2nd most traded commodity and the go-to beverage to the vast majority of the world's population [5]. Before any coffee is produced for human consumption it undergoes a series of processes to reach the final product, which we as consumers see and drink. Green coffee beans encapsulated within the coffee cherry are harvested, roasted (to a certain degree), and ground (to various sizes) until they become consumable. Around half of the fruit itself is non-edible exoskeletal layers (mucilage, hull, skin, pulp, pericarp, and silverskin) which house the green bean [6]. Only about 5 to 10% of the bean itself finds its way into coffee beverage (depending on the brewing process), the discarded solids are termed SCGs. Around 90 to 95% of the coffee fruit is wasted, either as excess skin layers or as brewed SCGs. SCGs and effluents from coffee processing/roasting plants constitute an eminent environmental concern. Their polluting potency is upheld by the large number of organic components present within them and their resistance to O_2 degradation [77]. SCGs are generally transferred to landfills or incinerators and

in some cases have reported to spontaneously combust leading to large fires [77]. It is estimated that 6 million tons of SCGs are generated worldwide every year [78]. Other than the sheer near costless volumes, their segregation from other FWs as an economically and industrially viable waste can be easily adopted with some optimistic policy changes. SCGs are non-edible. This eliminates the ethical food vs. fuel argument aimed at using edible feedstocks for fuel and energy production.

Table 2.5 – Coffee Consumption Rates for Various Countries and Regions for 2013 [81].

Country/Region	Consumption (tons)	Annual consumption per capita[3] (kg person^{-1} year^{-1})
Finland	65,700	**12.08**
Norway	45,780	**9.01**
Denmark	49,320	**8.75**
Sweden	70,500	**7.33**
EU[1]	2,139,900	**5.10**
EU[2]	2,495,100	**4.93**
USA	1,405,020	**4.44**
Japan	446,100	**3.48**
UK	169,680	**2.61**

[1] – Excluding Finland, Sweden, Denmark, and UK from EU as separate states for purposes of comparison.
[2] – Including Finland, Sweden, Denmark, and UK into the total EU-28 calculation for purposes of comparison.
[3] – Listed in descending order of consumption per capita per year (for the year 2013).

Green coffee production has been generally dominated by Brazil and Vietnam. Whilst Brazil managed to double its exports between 1990 and 2018, considering the same timeframe, Vietnam managed to increase their exports by a whopping factor of 20 [79], indicating the possible uses of SCGs in Asia. On the other hand, coffee consumption has been dominated by EEA states, specifically the Nordic countries (see table 2.5). Typically it is assumed that 91% of coffee grounds end up as wasted SCGs after the brewing process [80] which end up as FW and MSW. On top of that, soluble coffee (instant coffee) production generates about 1.125 kg of SCGs for every 1 kg of soluble coffee produced [77]. Adding on top of that contaminated water streams from the production process itself, we realize that we are dealing with massive scattered quantities of waste which need to be dealt with effectively.

It is inevitable that SCGs are produced in any coffee drinking society. As demonstrated by table 2.5, SCGs are predominantly a waste issue all over the world, especially in the EU and other high-income countries. EU consumption activities has remained relatively constant between 2013 and now.

FW in Norway is 32% of all waste generated. The more developed a nation becomes, the more waste it tends to produce. SCGs are included in this calculation of FW and are not considered an independent stream of waste [82]. According to [82], organic waste across Norway is subjected to the following: 16% is composted, 21% is used for energy utilization, 4% is incinerated without energy utilization and 23% is landfilled.

Exoskeletal coffee husks and coffee pulp layers (which make up at least half of all the waste associated with coffee harvesting) are excellent sources of carbohydrate which can be utilized in biorefineries for bioethanol and VAPs [77]. Coffee husks have been studied as a source of animal feed, biofuel, adsorption techniques, and bacterial fermentation but necessary detoxification requirements were essential to remove theophylline, theobromine, caffeine, and phenolics before bioconversion can take place [77].

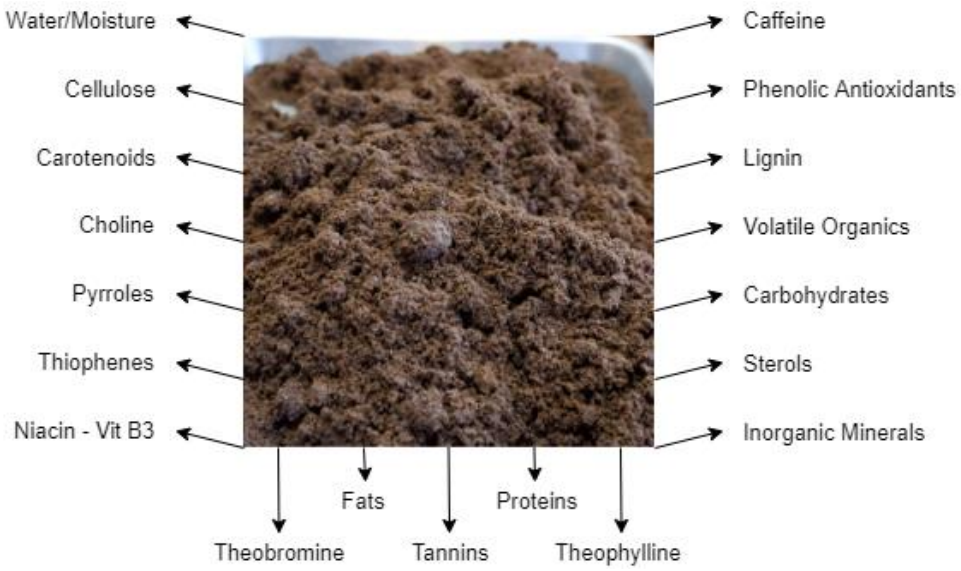

Figure 2.4 – SCG Waste and the Various Natural Chemicals That Can Be Exploited for Biorefineries [6], [83], [84].

SCGs are also a source of oil/lipids (known as SCG oil) and contains a large amount of carbohydrates bound within its fibrous lignocellulosic matrix. SCGs have been proposed by various researchers as animal feed, biofuel source, adsorbent, and as a source of bioactive compounds [7], [77]. SCGs can be exploited for an abundance of VAPs and biofuels. Updated literature on the use of SCGs in AD biorefinery studies can be found later in the thesis (see table 2.7), (crimson enclosure is AD; see figure 2.6). SCGs contain at least over 1000 individual compounds within it. Proteins carbohydrates, lipids, tannins, lignin, cellulose, cellulose, polyphenols, amino acids and fatty acids, and other volatile organics (see figure 2.4) [7]. These leach into the environment where which they are landfilled and cause serious environmental

damage, or they are incinerated where GHGs are produced without extracting further VAPs. Table 2.6 shows the LCH and CHNOS composition of SCGs as documented in literature today.

SCGs have been explored as source of solid fuel which can be directly utilized to generate heat and energy in industrial furnaces and boilers. SCGs have been reported to have a high calorific heat value of 5960 kcal kg^{-1} SCG [83], higher than that of some species of wood. However, the high moisture content in SCGs can be an issue in terms of combustibility and designing such boilers. Therefore, utilizing SCGs as a fuel in the form that it is seems to be counterproductive as the extraction of various valuable chemical VAPs can have more influence on generating a sustainable circular bioeconomy. After processing SCGs through biorefineries, one can pelletize the remnants for burning, or even better use them as agro-pellets[7].

Over 90% of all lipids remain in SCGs after their brewing and these can be exploited. Extraction of lipids by polar and non-polar solvents from SCGs have been tested for biodiesel and bioglycerin production. However, higher SCG oil extraction yields were observed when using non-polar solvents [85]. SCGs has been reported to contain between 7.9 – 26.4% oil *viz.* palmitic and linoleic acids which can be extracted [85], [83]. SCG oil can be converted into FAME biodiesel by direct transesterification using ethanol and methanol [83], [7]. The extraction of oil brings with it the extraction of phenols and phenolic derivatives which enhance the stabilization of biodiesel during periods of storage and transportation. However, the high concentration of FFAs in SCG oil negatively influences the esterification process limiting the achievable yield of biodiesel produced [85]. SCG oil has also been investigated in the cosmetic and pharmaceutical industries as it can aid in blocking ultraviolet irradiation as well boasting antimicrobial properties [85]. SCG oil has been employed as a C source for the production of PHAs [86], [87]. A degradable bacterial biopolymer acting as an alternative to petrochemically derived plastics. The high level of FFA supports bacterial growth and PHA production [85] in so called 'feast-famine strategies'. [86] and [87] obtained yields of 0.82 kg PHA kg^{-1} SCG oil and 0.77 kg PHA kg^{-1} SCG oil respectively. SCGs with elevated FFA concentrations of palmitic, oleic, and linoleic acids result in sustainable microbial cultures for PHA biopolymer production [6]. Increased lipid concentrations in may be inhibitory for AD methanogens and subsequently the BMP, however, the literature so far does not suggest that this is true for SCGs.

Physical and chemical hydrolysis of residual de-fatted SCGs can open up and yield high levels of carbohydrates which be utilized by various biorefinery processes due to the high concentration of hemicellulose in SCGs, specifically mannans and arabinogalactans [6]. Detoxification requirements are somewhat essential when dealing with microorganisms in order to establish successful grounds for fermentation. But, the direct use of SCGs and its hydrolysates without the removal of phenolics, caffeine, alkaloids, melanoidins, and FFA fractions has been known to inhibit enzymatic processes in bioethanol

fermentation [6], [85], [88]. Bioethanol markets are expected to reach 68.95 bn USD by 2022 with a CAGR greater than 5% between 2017 and 2022, where SCGs have the ability to be a primary contender [89].

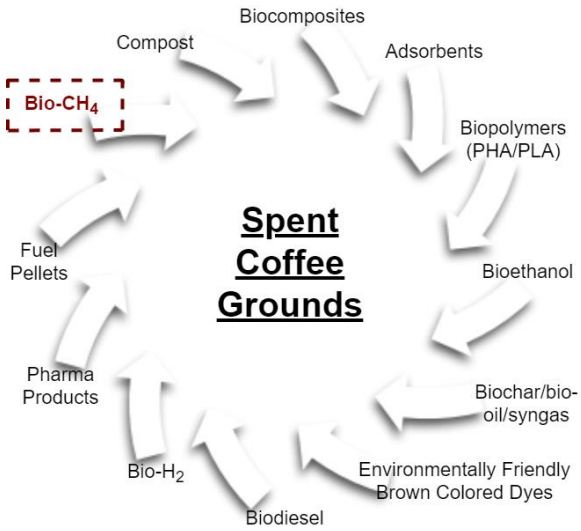

Figure 2.5 – Sustainable Biorefinery Approach for SCGs.

Table 2.6 – Composition of SCGs Determined by Numerous Researchers and Their Publications.

Component	Concentration	Reference
Lignin[a]	23.90[b] – 27.70	[91] – [92]
Hemicellulose	39.10[b] – 36.70[b]	[91] – [93]
Cellulose	12.40[b] – 8.60[b]	[91] – [93]
C – Carbon[c]	56.10 – 51.80 – 52.95	[94] – [95][e] – [96][e]
O – Oxygen[c]	34.00 – 39.57 – 38.07	[94] – [95][d, e] – [96][e]
H – Hydrogen[c]	7.20 – 6.40 – 6.76	[94] – [95][e] – [96][e]
N – Nitrogen[c]	2.40 – 2.08 – 2.10	[94] – [95][e] – [96][e]
S – Sulphur[c]	0.14 – 0.15 – 0.12	[94] – [95][e] – [96][e]

[a] – Soluble and insoluble lignin combined.
[b] – g/100 g dry SCGs.
[c] – As %.
[d] – Oxygen calculated by difference (from 100%)
[e] – Elemental analysis conducted on *Coffea arabica*.

SCGs enjoy exceptional adsorbent capabilities. They exhibit large surface area to volume ratios up to 1000 m^2 g^{-1} SCGs with a highly dense microporous structure [85]. They are initially finely ground for brewing purposes and need not further milling by physical means, which effectively cuts costs when using them as adsorbents. Adsorption of heavy metals viz. copper and lead, nitrobenzenes, phenols, cationic and organic dyes have all been successfully reported [6], [7], [83], [85]. Also, defatted SCG biochar produced by

pyrolysis has proved to be effective in its CCS properties as well as its ability to host essential microbial cultures revitalizing soil conditions [83]. [90] reports that biochar from SCGs can contain upwards of 73.4% C through its hydrothermal carbonization process.

Niche products from SCG biorefineries, such as bio-oil from pyrolysis, carotenoids, phenols, polyols, glycerin, and other bioactive compounds can also be obtained (see figure 2.6) as opposed to landfilling and/or incineration. Therefore, it is important to recognize the effects and options SCGs pose on a global scale. It is recommended to concoct adequate management and administrative plans for the separation of SCGs from FW so that they are exploited in regional and cross-national EU biorefineries. On top of that, studying LCAs to better connect the dots between the generation of SCGs and their final destinations will certainly aid in the implementation of green circular bioeconomy management options.

§2.2.2 – SCGs from the AD Perspective

SCGs contain combined carbohydrates (*mannose, galactose, arabinose, and glucose*), amino acids (*alanine, glycine, leucine, isoleucine, lysine, proline, valine, tyrosine, and glutamic acid*), and lipids (*sterols, terpenes, behenic, arachidic, linoleic, oleic, stearic, and palmitic acids*) amongst a cluster of even more organic classes [83]. These eventually are bio-converted into CH_4 through AD, although some may prove to be somewhat inhibitory. The BMP of de-fatted SCGs significantly different from that of raw SCGs. As lipids hold the highest methanation conversion ability by acetogenesis (see section §2.1.4) [14], however a too high lipid concentration would choke digestion and be problematic for methanogens as aetate overload [97]. Also, [98] verifies a strong negative correlation (R^2 = 0.95) between concentration of lignin and the achievable BMP in solid-state AD. The AD of SCGs and SCG related wastes has been investigated by [10], [60], [99]–[110]. Below are their collected findings. Table 2.7 is ordered in ascending order of year. AD literature on coffee pulp, husk, silverskin, and other processing wastes did not fit in the scope of this thesis and were excluded.

Table 2.7 – Literature on the AD of SCG and SCG related wastes between 1983 and 2018

Biomass studied/process	Notable parameters, observations, and results	Reference
SCGs	• Inoculum – Active digestive sludge from a municipal sewage digestor. • Mesophilic digestion. • 99% solids conversion. • Supplemented with N_2. • 56 to 63% CH_4 concentration. • BMP = 0.54 m^3 biogas kg^{-1} dry SCG fed.	[10]

SCGs	• Mesophilic and thermophilic digestion in batch reactors. • Thermophilic CH_4 production rate is approximately 1.5 times higher than mesophilic digestion. • Thermophilic hydrolysis rate is nearly double the mesophilic hydrolysis rate. • Acidogenesis is the rate limiting step (18 days lag phase) at thermophilic digestion, not hydrolysis. • A high propionate concentration due to a high lipid concentration. Led to a lowered BMP.	[60]
SCGs from instant coffee processing	• Thermophilic digestion in continuous mode. • Stable AD process up to an OLR of 4.7 g VS L^{-1} day^{-1}. • C:N ratio fixed at 30:1 using NH_4NO_3. • pH control was necessary. • High levels of VFAs. • 56% CH_4 concentration.	[99]
Wastewater containing SCGs	• Mesophilic and thermophilic digestion. • 87% lipid reduction in mesophilic mode. • 64% hemicellulose reduction in thermophilic mode. • Lignin component was not reduced in neither case. • Optimum OLR in mesophilic mode – 1.3 kg COD m^{-3} day^{-1}. • Optimum OLR in thermophilic mode – 1.6 kg COD m^{-3} day^{-1}. • 60% VS and COD conversion. • Inhibitory levels of VFAs in thermophilic digestors. • 60 – 65% CH_4 concentration(thermophilic). 65 – 70% concentration (mesophilic). • BMP = 0.23 L biogas L^{-1} reactor day^{-1} (thermophilic). BMP = 0.34 L biogas L^{-1} reactor day^{-1} (mesophilic).	[100]
Co-digestion with SCGs	• 45% SCGs – 32% Barley – 23% Chicory (co-substrate). BMP = 0.28 m^3 $CH_{4(STP)}$ kg^{-1} $VS_{initial}$. • Reducing SCGs content by 25% SCG decreased BMP by 0.03 m^3 $CH_{4(STP)}$ kg^{-1} VS. • 80% reduction in VS. • 85% biomethanation/COD conversion. • Hydrolysis constant (k_h) = 0.035 day^{-1}.	[101]
SCG co-digested with WAS	• AD connected with a submerged AnMBR (AnMBR 75 g L^{-1}). • Thermophilic digestion. • Phase I to IV – Insufficient micronutrient supply from sole SCGs AD. Leading to reactor failure. • Phase VI to IX – WAS (15%) mixed with SCGs (85%). • Phase IX – COD conversion of 67.4%. • Phase IX – OLR = 11.1 kg COD m^3 day^{-1} at an HRT and SRT of 20 days. • NH_4HCO_3 (0.12 g N g^{-1} TS_{in}) added as alkalinity. • Phase IX – 55% CH_4 concentration.	[102]
SCGs	• Mesophilic digestion in batch reactors. • BMP between 0.271 and 0.325 m^3 CH_4 kg^{-1} kg dry organic matter. • 60% CH_4 concentration.	[103]
SCGs co-digested with other waste feedstocks	• SCGs, FW, *Ulva*, WAS and Whey co-substrate at 0, 25, 50 and 70% mixing ratios. • SCG/WAS co-digestion unfavored due to low BMP and CH_4 production rate. • SCG:FW ratio of 1:4 showed the highest BMP of 0.308 ± 0.016 L CH_4 g^{-1} VS_{in} (co-digestion increased yield by 13.1%).	[104]

	• No significant variability in DDGE band patterns (microbial species dominance) between co-digestions. • Co-digestion with *Ulva* decrease the BMP of SCGs, but not as much as WAS did.	
SCG soluble liquid fraction	• Mesophilic digestion. • SIR of 1.5 (g $VS_{substrate}$ g^{-1} $VS_{inoculum}$ = 1.5). • Inoculated with fresh cow manure (100g). • C:N ratio of 22.25. • 53.7% CH_4 concentration. • BMP = 296 ml CH_4 g^{-1} VS. • Diluting fresh cow manure with 500 mL H_2O decreased CH_4 content to 36.7% CH_4 and decreased the total BMP by approximately 38%.	[105], [106]
Alkaline Pretreated SCGs	• Mesophilic digestion. • Pretreatment = NaOH (8% w/w) for 24 hours. • Lignin degradation increased by 24%. • SIR fixed at 2 (2 g $VS_{substrate}$ g^{-1} $VS_{inoculum}$). SCG concentration = 8 g VS L^{-1}. • VS reduction = 35.9 ± 0.8% at optimal conditions. • BMP = 392 ± 3.0 mL CH_4 g^{-1} VS.	[107]
SCG hydrochar	• SCG hydrochar produced by hydrothermal liquefication at 180, 220 and 250 °C for 1 hour. • Inoculated with cow dung. • 180 °C samples showed highest BMP = 491 mL CH_4 g^{-1} VS. • 70% CH_4 concentration. • Highest CH_4 production rate at 46 mL CH_4 g^{-1} VS day^{-1}.	[108]
SCGs co-digested with *Ulva*	• Varying OLR between 0.7 – 1.6 g COD L^{-1} day^{-1} between 5 phases. • 3:1 ratio by feedstock COD. SCG:*Ulva*. • Phase 4 highest COD conversion = 51.8% and VS removal = 44.54%. • Phase 4 BMP = 0.19 ± 0.01 L CH_4 g^{-1} COD_{in}. • Phase 4 operational OLR 1.5 – 1.71 g COD L^{-1} day^{-1}. • Phase 4 HRT 25 days. • *Ulva* improved biomethanation of SCGs and positively influenced various methanogens for increased CH_4 production rate.	[109]
Mild Thermo-Alkaline Pretreated SCGs	• Pretreatment = NaOH 0 – 0.2M between 60 – 90 °C. • Optimized degree of solubilization (36.4%) at 0.18M NaOH and 90 °C. • BMP = 163.31 mL g^{-1} COD_{added}. • Increasing NaOH concentrations effects methanogenic consortium in BMPs by concentrated salt stress.	[110]
Co-digestion SCGs with Cow Manure	• Measured the optimum ISR ratio for SCGs/cow manure co-substrate. • Highest BMP measured at ISR 3. Lowest BMP measured at ISR 0.5. • HAc concentration around 300 mg L^{-1}. • No acidification found. VFA/ALK showed a stable process. Only in ISR 0.5 reactors it was demonstrated that the reactors had failed.	[111]

AD co-digestion strategies between SCGs and other substrates [101], [102], [104], [109] have proved to be advantageous in increasing process feasibility and stability. Co-digestion allows for C:N ratio regulation, replenishing of essential trace elements and micronutrients, increasing buffering capacities, and disrupting inhibitors such as high VFA concentrations, furfural, and 5-HMF [60], [85].

Propionic acid inhibition was a major setback whenever SCGs digestion took place. COD reduction is relatively high due to the number of hydrolyzables (hemicellulose, parts of lignin and cellulose, as well as

proteins, fats, and other minute organics). The AD of SCGs can be achieved with a relatively high COD conversion as shown in table 2.7 (85 %). COD and stoichiometrical themes are presented in the subsequent section (see section §2.3). SCGs AD can effectively reduce its volume, increase its stability, and remove volatile toxic organics. Outlet sludge can then be further processed in a circular bioeconomy fashion yielding stabilized organic fertilizer or other post-digestion VAPs.

[112] conduced 9 various pretreatments (physical, chemical, and physiochemical) on SCGs to measure changes in hydrolysis, reducing sugar yield, and lignocellulosic fractions. 8 of the 9 pretreatments were single pretreatments and the last was a two-stage sequential pretreatment (concentrated H_3PO_4/$(CH_3)_2CO$ coupled with NH_3 fiber explosion). Reducing sugar yields increased by 170% when sequential pretreatment was conducted. On top of that, the secondary part of the pretreatment (NH_3 fiber explosion) managed to delignify SCGs by a factor of *more than half*. Such proposed pretreatments can undoubtedly increase the stability of SCGs AD with or without co-digestion at mesophilic temperatures without the need for increased HRTs and larger reactors.

One suggestions is to extract the high lipids content prior to pretreatment and AD. This may meddle with the achievable BMP, but a sustainable biorefinery model is set to gain from this. The antimicrobial properties of SCG oil can prompt it for use in pharmaceutical or cosmetic industries whilst defatted SCGs can be subject to pretreatment optimizations as suggested by [112].

[77] argues that research outcomes are still not abundant enough on SCGs biorefinery processes and that it should be further researched to bioconvert this waste stream into VAPs, biofuels, and more importantly, niche chemicals. [77] also maintains that current SCG biorefineries are deemed unviable due to technical or financial issues that precede and advocates for further research.

§2.3 – COD Balances and AD Stoichiometry

This section encompasses the concepts related to the overall AD stoichiometry and COD measurements, especially related to solid waste COD determinations. The COD mass balance is presented (see figure 2.7). It is used further in determining the process efficiency and degree of biodegradability of the AD of SCGs at the different experimental ISRs.

§2.3.1 – COD, COD Balance, and CH_4 Production

One of the first laws of science taught is that *mass is neither created nor destroyed*, it merely changes from (see equations 5 and 6). This is precisely what happens in AD, incoming substrates (classified as COD) are reoriented through biochemical processes and finally assume form in CH_4 and CO_2 [15]. AD is

a complex practice with multifaceted biochemical processes and conversions all working synergistically. COD is extensively used as a measure of digestor efficiency, health, and biodegradability. For this thesis, COD balances were used as a measure of the biodegradability of SCGs at different ISRs, by determining the changes in inlet COD, effluent COD, and gaseous COD (see figure 2.7). COD is a measure of the power of a pollutant if it was to be completely oxidized (*chemically*) and is given units of g COD L^{-1} or mg COD L^{-1}. COD measurements are one the most commonly used control tools that operators use to monitor and troubleshoot AD process. COD mass balance analysis is calculated to evaluate digestor performance as well as investigate COD loss at the selected ISRs.

Inlet COD is converted to CH_4 through anaerobic bacterial processes (see sections §2.1.2 to §2.1.5), but at the same time some is used to sustain the growth and health of the inoculum. Non-utilized COD does not change form and exits with the effluent. 100% COD conversion is the ultimate goal for a complete biomethanation process. However, COD for biomass growth and non-degradable COD limit 100% biomethanation. Nonetheless, COD mass balances with complete COD accountability is highly desired.

At STP conditions (0 °C and 1 bar), the theoretical CH_4 production can be estimated based on the COD_{inlet} supplied. 1 kg inlet COD can be biochemically converted to 0.35 m^3 CH_4 at STP (see equation 7) [14], [15]. However, in practice obtaining the maximum theoretical CH_4 production is not possible due to bacterial growth, indigestible COD, and foreign species presence (such as S and N). It is known that the presence of sulphate (SO_4^{-2}) will reduce the available COD for CH_4 production into H_2S causing toxicity complications for methanogens. At room temperatures (20 °C), the theoretical CH_4 production is higher than 0.35 $m^3\,kg^{-1}\,COD_{removed}$ (it is 0.37 $m^3\,kg^{-1}\,COD_{removed}$). [113] calls for a standardization of BMP tests at STP to avoid any faulty or inconsistent results.

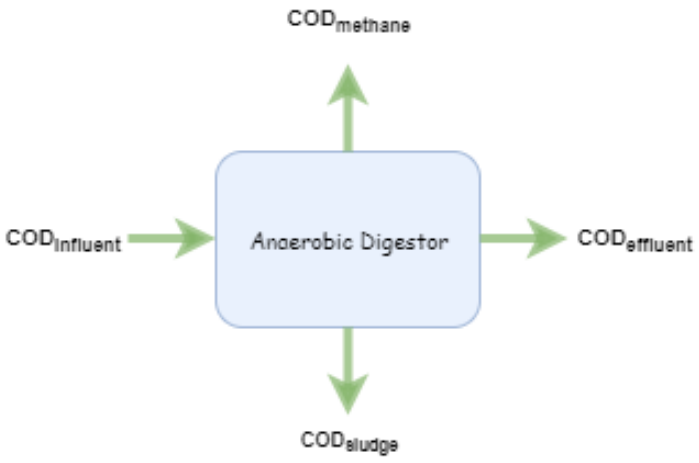

Figure 2.6 – COD Mass Balance.

$$COD_{in} = COD_{out}$$

$$COD_{influent} = COD_{methane} + COD_{sludge} + COD_{effluent}$$

Equations 5 and 6 – COD Mass Balance Equations.

$$V_{CH_4} = \frac{22.40 \frac{L\ CH_4}{mol\ CH_4}}{64 \frac{g\ COD}{mol\ CH_4}} = 0.35 \frac{L\ CH_4}{g\ COD} = 0.35 \frac{m^3 CH_4}{kg\ COD}$$

Equation 7 – Theoretical CH_4 Production at STP.

COD mass balances are used in conjunction with BMP test results allowing them to be compared. However the exactness of solid-state AD COD mass balances is compromised due to the inability in accurately measuring and monitoring the COD of solids practically with large variations of error [114]. There is a need for improvement here. The high number of oxidizable components in SCGs is precisely the reason behind its toxicity in landfills [5] and its inherent high COD. However, AD of SCGs have shown a COD conversion of up to 85% under optimized process parameters [101]. [15] suggests that a high COD methanation efficiency with a low CH_4 production rate can incur problems by incomplete accounting of COD mass balances and can possibly lead to digestor operational difficulties.

§2.3.2 – Solid Substrates, BMPs, and Biodegradability

Initially AD systems were employed to stabilize and treat effluents from WWTPs, which is primarily why most literature around AD is based on wastewater and organic sludge processing. However, recently the technology itself has been instigated towards optimization for organic solid waste substrates. Similarly, COD measurements for wastewater and sludge samples (*wet samples*) is and has been an established procedure for some time now. However, that changes for samples with a high solids content where sample dilution is not possible [115], [116]. Methods for accurately determining solid COD are not yet established. If they are established, they lack standardization and verification [114], [115]. This creates operational and technical issues. COD balances are underestimated and cannot be fully used as a way of validating results and operations. Optimistically, [116]–[119] are investigating new methods that are decisive, safe, accurate, and quick for determining COD in solids. Doing so reduces uncertainty in the results but more importantly allows for trustworthy solid-state AD COD mass balance which not only provides valuable information about the system but is also less time consuming. [119] found that drying solid waste samples triggered a loss of volatile organics which equated to a 10% error in determining their COD, even when using a new method. Regardless of the methods used, it is always desirable to standardize AD BMPs in terms of g $COD_{methane}$ g^{-1}

$COD_{substrate}$, but this will prove to be misleading if the measured substrate COD is over or underestimated depending on the method used.

Anaerobic biodegradability is a measure of how successful a substrate is in undergoing digestion to produce bio-CH_4 and is evaluated by a BMP test procedure (see section §3) [28]. The degree of biodegradability equation (see equation 8) is evaluated between 0 and 100%. Effectively it is the ratio between the BMP obtained and the theoretical BMP that can be obtained if all the feedstock COD was menthanized [115]. [115] too stresses that a higher content of recalcitrant lignin and crystalline cellulose will drastically affect the highest achievable degree of biodegradability of that substrate effectively limiting its bio-CH_4 producing ability and its position in AD biorefineries.

$$Degree\ of\ Biodegradability\ =\ BD\ (\%)\ =\ \frac{BMP\ (mL\ CH_{4,STP}/g\ VS)}{350 * COD_{substrate}\ (g\ COD/g\ VS)}$$

Equation 8 – Biodegradability Equation [115].

[120] argues that equation 8 can prove to be faulty as it does not account for new biomass generation and assumes the complete conversion of 1 g COD_{in} to 350 mL CH_4 to be true. Instead, [120] suggests to use a different method to calculate the degree of degradability (see equations 9 and 10). This removes the need to account for COD_{sludge} as substrate biodegradability is calculated based on BMP rather than COD. The subscripts (a – e) are assimilated to the subscripts present in equation 1 and the empirical formula can be calculated from the elemental composition provided (see table 4.2). BMP_{Th} denotes '*theoretical BMP*' and BMP_{Exp} denotes '*experimental BMP*'. The volume of one mole of gas at STP is denoted by the factor 22,400 (22,400 mL mol^{-1}) i.e. (***Avogadro's Law***).

$$BMP_{Th} = \frac{\left[\left(\frac{a}{2}\right) + \left(\frac{b}{8}\right) - \left(\frac{c}{4}\right) - \left(\frac{3d}{8}\right) - \left(\frac{e}{4}\right)\right] * 22{,}400}{(12a + b + 16c + 14d + 32e)}$$

$$Degree\ of\ Biodegradability\ =\ BD\ (\%)\ =\ \frac{BMP_{Exp}}{BMP_{Th}}$$

Equation 9 (Top) – BMP_{Th} of a Substrate Based on its Elemental Composition at STP [120].

Equation 10 (Bottom) – Calculating The Degree of Biodegradability from BMP_{Exp} and BMP_{Th} [120].

§2.4 – Process Parameters, Operation, and Conditioning

Designing and operating anaerobic digestors, especially solid-state digestors, is contingent upon understanding and critically evaluating the different process parameters and factors associated with

successful digestor performance. Understanding how these parameters and factors work and affect one another is imperative for both operators and designers.

§2.4.1 – SRT and HRT

SRT and HRT are amongst the most crucial AD process parameters. They effectively dominate the degree of performance of continuous AD through the regulation of anaerobic biochemical processes (see section §2.1.2 to §2.1.5). They regulate the contact time between biomass and feedstock. The SRT decides the type of microorganisms that grow. It dictates which biomass are included in the AD digestate and therefore controls the extent of the biochemical processes taking place. For example, methanogens require a much longer SRT than acidogens and so by controlling the SRT, the microbial structure can be complemented accordingly. By changing the SRT of the AD process, operators can control the biochemical process taking place depending on the end product needed whether it is complete methanation or VFA production only. The HRT determines the average length of which hydrolyzed compounds remain in the digestor before being wasted or recirculated. A shortened HRT will surely decrease the degree of COD conversion in a continuous AD system. Longer retention times unavoidably means higher CAPEX and OPEX [121]. For this thesis, HRT and SRT were not determined as the BMP methodology for batch assay AD is required to run to completion without wasting or recirculation of biomass.

§2.4.2 – OLR

The OLR is another important parameter affecting the quality and degree of digestion. Its name suggests exactly what it means, organic loading rate; designating the ideal quantity/rate of organics being loaded on an anaerobic reactor/digestor and is usually calculated as kg COD m^{-3} day^{-1} or kg VS m^{-3} day^{-1}. The OLR of a substrate is augmented for that specific substrate or co-substrate. Operators control the OLR for maximum bioconversion, without compromising quality by overloading or underloading. Operating above the OLR (overloading) can result in digestor failure and disproportionately increasing VFA concentrations leading to process souring (decreased pH) which can cease CH_4 production [121]. On the other hand, working below the OLR (underloading) implies an incomplete COD removal as well as increased CAPEX and OPEX [122].

The ratio of HRT to OLR is comparable to the food to microorganism ratio otherwise known as the SIR [114]. In continuous mode AD the OLR is used because the incoming substrate COD is fed intermittently into the digestor. In batch mode the SIR is used as all the COD is fed at the start without further sequential feeding. The inverse of SIR (g $VS_{substrate}$ g^{-1} $VS_{inoculum}$) is ISR (g $VS_{inoculum}$ g^{-1} $VS_{substrate}$). This thesis aims to

identify the optimum ISR with which SCGs can be digested by employing a batch process operation (see section §1.2 and §2.5).

§2.4.3 – Solid-state AD vs. Liquid-phase AD

As previously mentioned, the AD method was initially used to treat WWTP effluents (liquid-phase effluents) in order to further stabilize them, reduce their volume, COD potency, and environmental impact. Liquid-phase or solid-state AD are also known as wet or dry AD. The TS content of a feedstock determines whether it is treated by a wet or a dry AD process. The TS range for dry vs. wet digestion is somewhat unclear and disputed between scientists. However [123] best formulates this. A TS content of 15% and lower is considered wet AD and a TS content of 20% and higher is dry AD. When the TS content falls between 15 and 20% the process is denoted as semi-solid AD [123]. Solid-state AD systems offer a wide array of benefits such as decreased reactor size, lower CAPEX and OPEX, and a lower energy demand [124]. However, solid-state AD suffers from the ever-impending problem of inhibition. On top of that, the compositional complexity of solids wastes, extremely long retention times (up to 3 times that of wet AD), the large amounts of inoculum needed, VFA accumulation, as well as designing robust and powerful pumps able to transport feedstocks of a viscous/high solids nature [123] are some of the undesirable consequences. The low moisture content of SCGs may prove to be problematic. Decreasing the moisture content can lead to a lower BMP and decreased mass transfer between hydrolysates and microorganisms [125]. Also, a decreased moisture content can induce imbalances in alkalinity equilibria.

SCGs, as any other lignocellulosic wastes or MSW, have a high solids content. SCGs have reported to have a TS of between 32 and 39% [7], which drives their valorization to biofuels and VAPs by processes such as solid-state AD or solid-state fermentation, respectively. Increasing the TS % content resulted in a decreased hydrolysis rate constant, lower COD and VS removal, high acetate and propionate concentrations, and eventually a premature BMP and CH_4 production rate [123], [126]. Co-digestion for regulating the TS content of a feedstock is a heavily researched field aimed at bypassing digestor failure. [124] asserts that research in feedstock pretreatment, operational management, and mainly reactor design are vital to push forward solid-state AD technologies further into the market.

§2.4.4 – Single-stage AD vs. Multi-stage AD

Multi-stage AD is achieved through regulating the HRTs, SRTs, pHs, and temperatures autonomously. Doing so, one can split up the anaerobic bioprocesses (sections §2.1.2 to §2.1.5) into individual processes, finally combining then them together downstream (see figure 2.7). Multi-stage AD

decouples the processes from each other and allows for independent improvement for subprocess optimization and prompts operators and plants to control process kinetics more effectively. Two-stage AD splits hydrolysis and acidogenesis (acetogenesis is considered another form of acidogenesis) from methanogenesis whereas three-stage systems split hydrolysis, acidogenesis-acetogenesis, and methanogenesis apart. Single-stage AD combines them all in one reactor.

One of the significant advantages of multi-stage systems is their ability to be spread out in both space and time allowing for a more customized optimization. Also, [127] noticed that by using a two-stage AD system, a more selective and enriched microbial consortia followed, which improved buffering capacity and pH stability. Other than the fact that multi-stage systems can increase BMP and decrease the COD content of lignocellulosic biomass, they can also be operated for bio-H_2 production as part of the total COD mass balance [128]. However, H_2 fermentation so far is limited in terms of process performance and yield [128]. Integrated bio-CH_4 and bio-H_2 systems are called biohythane fermentation systems and are considered an encouraging approach to treat lignocellulosic and MSW streams in integrated biorefineries for a circular bioeconomy. Multi-stage AD requires more CAPEX and OPEX due to a more complex infrastructure for operating the processes, *but its rewards are grand*.

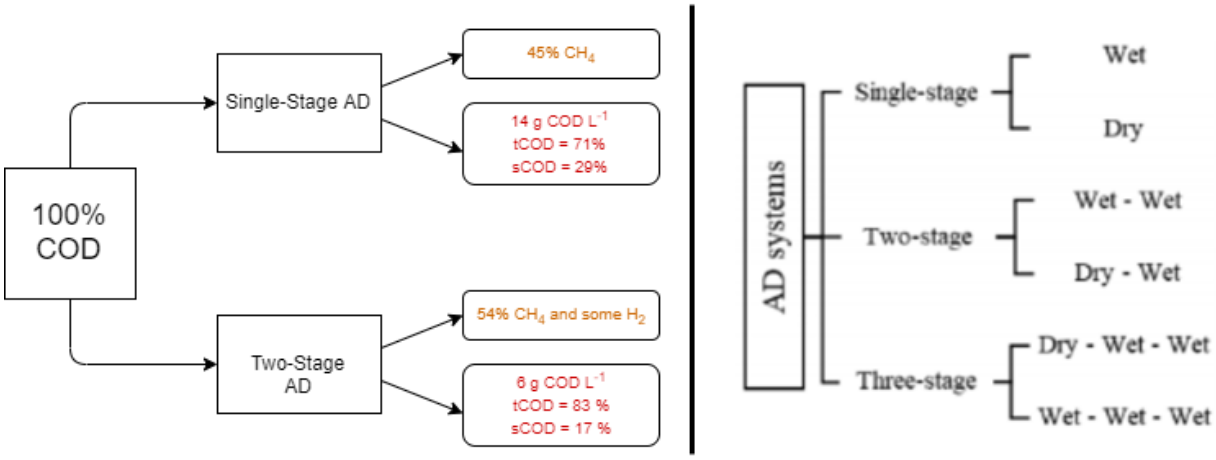

Figure 2.7 – Left: Two-Stage AD System (50% Decrease in Total COD, 20% Increase in CH_4 Content) [129].

Figure 2.7 – Right: Categorization of Multi-Stage AD Systems [130].

So far, SCGs have not been studied extensively in multi-stage systems. There are more studies around the AD of coffee husks, pulp, and mucilage in multi-stage AD biorefinery options rather than SCGs. However, studies on SCGs and SCG wastewater in multi-stage AD and have been conducted temporarily by [60], [100], [102].

§2.4.5 – Temperature

All biochemical processes have a certain temperature threshold where they optimally function within. Once temperatures fall outside of set thresholds, biochemical metabolisms start to change, and community growth rates rapidly follow. The three main temperature classifications are psychrophilic (optimum at 10 °C), mesophilic (optimum at 37 °C), and thermophilic (optimum at 55 °C). Microbial community changes, BMP, process stability, and kinetics are all affected by the selected temperature of the process. On top of that, enzymes render permanently denatured at high temperatures ceasing growth of microbial life [13].

Most AD facilities operate in the mesophilic range due to increased stability and favorable energy balances [125]. However, scientific attention has shifted towards TPAD and thermophilic digestion because these techniques allow for further reduced COD content and possible increases in BMP and CH_4 production kinetics [8].

TPAD is a physical pretreatment method as well as a multi-stage model of operation. Operating multi-stage operations in different temperature ranges allows better assimilation of hydrolysis, acidogenesis, and acetogenesis before methanogenesis commences. TPAD is a more realistic option to optimize biochemical processes in conjunction with pH, HRT, and OLR to accelerate hydrolysis and acidogenesis processes which are considered rate limiting steps in the AD of SCGs [60], [131].

According to authors knowledge, TPAD systems have been studied and investigated fundamentally for AD use with lignocellulosic biomass, let alone SCGs. The compositional complexity of SCGs has proved to be tough on bioprocesses such as hydrolysis, acidogenesis, and acetogenesis and TPAD can help untangle some of these issues. Also, psychrophilic AD of SCGs has not been studied, but the high level of COD in SCGs is not suited for psychrophilic AD. Mesophilic and thermophilic AD studies on SCGs are more prevalent and can be found earlier in this thesis (see table 2.7).

§2.4.6 – pH and Alkalinity

Together with temperature changes, pH and alkalinity changes also enable desired/undesired effects on microbial processes which can lead to total reactor failure if unmaintained. A neutral pH (6.5 – 7.5) is desired for maximum synergy between all the species of microorganisms in a digestor and maximizing the BMP [15], [125]. However, the optimization of hydrolysis/acidogenesis requires a much lower pH of 5.5 – 6 and sometimes even lower. This can completely halt methanogenic activity, which do not operate below a neutral pH of 7. This gives another reason as to why multi-stage digestors are gaining importance [127]. VFAs and dissolved H_2CO_3 can be inhibitory to microorganisms without sufficient

alkalinity to help equilibrate. Falling below neutral pHs suffocates the BMP and CH_4 production rate and excessively increase the potency of VFAs (especially propionic and valeric acid in the case of SCGs AD [60]). In consequence, propionate and valerate decreases the CH_4 production rate by at least a factor of 2 as opposed to acetate and butyrate [132]. Formic acid too is toxic to methanogenic archaea.

Alkalinity can be added to combat the sudden changes in pH, either by adding external regulating agents or by the inoculating with an inoculum with an inherently high alkalinity. Also, alkalinity can be produced in the form of $NH_4(HCO)_3$, if the substrate has a high concentration of proteins, as N biochemically rearranges after NH_3 production [14]. However, a too high protein concentrations can produce too much NH_3 and be inhibitory to AD systems altogether causing NH_4^+ inhibitions.

The VFA/ALK ratio is often used to monitor AD stability. A VFA/ALK of 0.8 and above is presumed to be unstable and a factor of 0.4 and below acknowledges stability [114]. Some argue that instability starts with a VFA/ALK ratio of 0.3 rather than 0.4.

§2.4.7 – Nutrients and Inhibition

This sub-section aims to demonstrate the various types of inhibition tied to AD processes and how their effect plays a role on the BMP of SCGs.

§2.4.7.1 – VFA Accumulation

As previously mentioned, VFA concentration and composition play a paramount role on methanogenic behavior, which can sprawl processes downhill quickly if not closely remediated. An increase in the COD concentration of VFAs *viz.* propionate and valerate (as SCGs are saturated in LCFAs) will decrease pH and lead to digestor failure if no proper alkalinity is present [14], [18], [60]. This calls for the optimization of the acetogenesis step. [63] acknowledges that different AD systems react differently to what is considered 'normal' levels of VFA based on substrate and microbial compositions. However, certain limits uphold to determine whether reactor failure is bound. [99] observed a surge of VFA concentration (a tad shy of 3 g VFAs L^{-1}) when digesting SCGs at thermophilic conditions at high OLRs of 3.4 g VS L^{-1} day^{-1} and even above 2 g VFAs L^{-1} when the OLR was cut by half suggesting that the OLR plays a relatively important role in minimizing VFA production when digesting SCGs and other lignocellulosic feedstocks. [133] claims that a propionic acid concentration of 0.9 g L^{-1} can be detrimental for methanogens, at the same time, concentrations up to 2.4 and 1.8 g L^{-1} for acetic and butyric acid respectively upheld and showed no inhibitory signs suggesting the nature of the VFA in question changes how AD systems react to it. [113]

proclaims quality benchmarks for a suitable AD inoculum; VFA concentrations should be less than 1 g $CH_3COOH\ L^{-1}$ and an alkalinity of 3 g $CaCO_3\ L^{-1}$ or more are considered good starting points.

§2.4.7.2 – C:N Ratio and TAN

Another important substrate-based factor that determines a reactors accomplishment is the C:N ratio. Especially when it comes to the AD of lignocellulosic biomass. Anaerobic microorganisms depend on carbon and nitrogen from the substrate as their food source, for activities such as growth, reproduction, and production of enzymes. The ideal C:N ratio is disputed amongst researchers but lies somewhere within the range of 20 to 35 C:N, but it varies between substrates used and the operating temperature [8], [125].

A low C:N ratio results in accumulation of VFAs which rapidly consume alkalinity and destabilize the pH integrity of a system and eventually inhibits methanogens and their metabolism [134]. A high C:N ratio manifests high nitrogen deficiency, returning lowered microbial growth rates and consequently decreased BMPs [135]. SCGs have also reported an array of C:N ratios which fall either short or towards the lower end of the proposed range. [7] summarizes the available C:N ratios found in literature (17:1, 23.7:1, 23.3: and 16.9 ± 0.1:1 as C:N). Balancing the C:N ratio in SCGs and other solid organic wastes can be overcome by co-digestion with higher C:N ratio wastes such as wastewater, activated sludge, and corn straw [125]. The C:N ratio of the SCGs used in this thesis is found in table 4.2.

TAN is the measure of the NH_3/NH_4^+ equilibrium in an AD system. Nitrogen is essential to AD bioprocesses. NH_3 is produced in AD processes by the biological degradation of the substrate (see equation 1) and assimilates into NH_4^+ at lower pHs. TAN inhibitions occur by changes in pH which heavily disrupts biochemical functions especially in methanogens [18]. NH_3 from the AD of SCGs comes either from proteins, non-protein nitrogenous compounds, or a combination of the two [83]. NH_3, usually referred to as free ammonia is the source of inhibition. NH_4^+ is not merely as dangerous. It is recognized that NH_3 concentration of about 100 mM NH_3 are completely inhibitory to methanogenic activity whereas roughly 100 mM NH_4^+ does 90% less damage [125].

The amounts of proteins and non-protein nitrogenous compounds in SCGs are considered somewhat limiting in terms of TAN supplementation. [102] reports that NH_3 levels were well below inhibitory thresholds and at some stages would require nitrogen boosting due to a relatively low C:N. Similar nitrogen boosting methods for the AD of SCGs were also reported by [10], [100]. None have reported severe inhibition caused solely by ammonia or other nitrogenous means.

Last but not least, figure 2.8 shows how the aforementioned process parameters and conditions (section §2.4) work in synergy to optimize the BMP of a substrate or co-substrate.

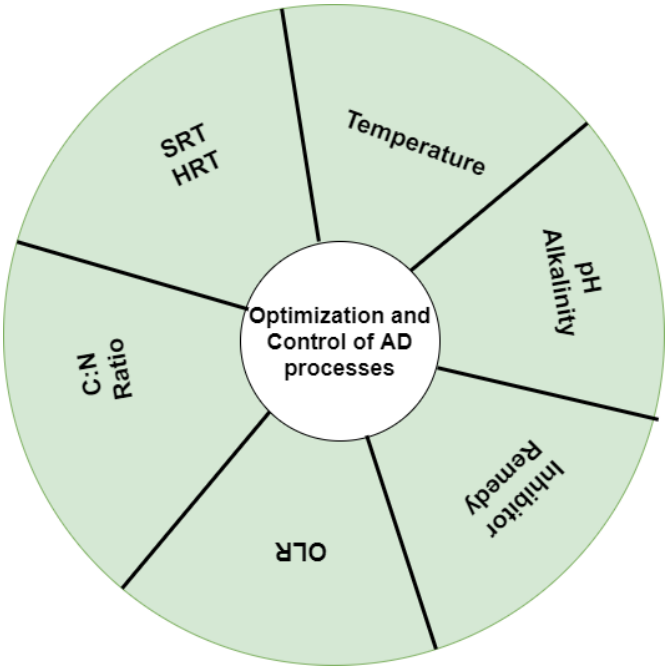

Figure 2.8 – Optimization and Conditioning of AD Process Parameters [136].

§2.5 – ISR

This section will go over what the ISR means and what it resembles. In fact, the ISR is a crucial process parameter touched upon previously (see section §2.4.2). Section §2.5.1 will give a brief overview of the ISR and the scientific outcomes surrounding it. Section §2.5.2 aims to identify the knowledge gap surrounding optimal ISR SCGs AD which is the driving force behind this thesis and one of the research objectives (see section §1.2).

§2.5.1 – Introducing the ISR (or SIR)

The ISR is a standard parameter which operators specify for AD bioreactor design and operation. Similar to organic loading, it affects and determines the maximum BMP and CH_4 production rate attainable in AD bioreactors [29]. The ISR depends on both inoculum and substrate characteristics and intrinsically changes between different operations. Microorganism biochemical reaction kinetics differ based on the ISR chosen [114]. As a baseline, the portion of inoculum in a reactor should be higher than the substrate as this regulates moisture levels, mass transfer rates, as well as supplementation of alkalinity, nutrients and essential elements for healthy growth and metabolism [113]. [137], [138] demonstrates how different inocula react differently to substrates. Researchers advise to use a mixed culture inoculum accustomed towards the substrate if possible.

For recalcitrant lignocellulosic substrates it is recommended that the ISR be lowered below 1 [113], [114]. This allows for the acclimatization of microorganisms to the substrate granting successful hydrolysis and acidogenesis conversions [139]. However, low ISRs take longer to establish a complete BMP profile. However, for an undiscovered substrate, a wide range of ISRs should be studied to establish a complete overview by monitoring any signals of reactor damage or overload [113]. Determining the optimum ISR for digestion or co-digestion sheds valuable insight on the limiting biodegradation kinetics as well as verifying the highest BMP with the highest CH_4 production rate. SIR is the inverse of ISR.

Table 2.8 – Literature Available on the AD of Various Substrates for ISR/SIR Optimization.

Biomass studied	Notable parameters and observations	Reference
Kitchen FW	• SIRs of 0.5, 1, 1.35, and 2.3 g VS g^{-1} VS tested using both granular and suspended sludge inocula. • Alkalinity adjustments of 37 mg $NaHCO_3$ g^{-1} COD was enough to keep granular sludge from VFA overload at all SIRs. Suspended sludge with 37 mg $NaHCO_3$ g^{-1} COD ended up with a pH of 5.5 at the three highest SIR. • Both sludges failed with low alkalinity except at SIR of 0.5. • The highest BMP was at SIR = 0.5 with highest biodegradability for both inocula. • Optimization of SIR is more important that the inocula source, although the inocula source does affect the BMP to some degree.	[137]
Co-digestion pig urine and rice straw	• Thermophilic solid-state digestion at SIRs 0.5, 1, 2, and 3 g VS g^{-1} VS. • 1.8x higher utilization efficiency at SIR 0.5 than 3. • BMP = 257 ± 5 m^3 t^{-1} VS without VFA or NH_3 inhibition. • Rice straw C:N is 75.2. Pig urine stabilizes co-substrate C:N at 23:1. • 83.3% in VS reduction at ISR 0.5, nearly 10% more than ISR 3.	[140]
Maize Bran	• Mesophilic digestion at ISRs 1, 1.5, 2, and 3 g VS g^{-1} VS. • Initial Alkalinity addition of 9100 mg $CaCO_3$ L^{-1} and VFA/ALK ratio was less than 0.4, i.e. (stable). • ISR 1 had the highest BMP = 233 ml $CH_{4,\,STP}$ g^{-1} VS, but deemed insignificant compared to the other ISRs. • Stable CH_4 concentration at 59% for ISR 1.	[139]
Solid Agro-industrial Waste	• Mesophilic digestion of 4 wastes (winery waste, cotton gin waste, olive pomace, and juice industry waste) with 3 inocula (anaerobic sludge, landfill leachate, and thickened anaerobic sludge) at SIRs 0.25, 0.5, 1, and 2. • Low SIR (0.25 and 0.5) gave the best BMP for all substrates. Higher SIR indicated AD inhibition. • Anerobic sludge was the favored inoculum. Landfill leachate and thickened anaerobic sludge lacked necessary nutrient and microbial consortia loads. • Kinetic modelling optimization. 3 parameter models for high SIR, and 2 parameter models for lower SIRs.	[138]
Microalgae mixtures	• Microalgal mixture A, B, and C tested at SIRs 0.5, 1, and 3 with thermal hydrolysis, ultrasound, and biological pretreatments at concentrations of 3, 10, and 20 g TS kg^{-1}. • The maximum BMP = 395 ± 11 ml CH_4 g^{-1} VS and BD was 70 ± 3% at SIR of 0.5 and 10 g TS kg^{-1} for mixture A. • A 4-day lag phase at ISR 3 due to VFA accumulation. No notable NH_4^+ inhibition.	[141]

	• Thermal hydrolysis on mixture B at 170 °C increase the BMP by 62% at ISR of 0.5.	
Reconstituted MSW	• Mesophilic digestion at ISRs 0.015, 0.03, 0.06, 0.12, 0.25, 1, 2, and 4 g VS g^{-1} VS. • Strongly correlated decreasing lag phase with increasing ISR. • Strongly correlated increasing CH_4 production rate with increasing ISR. • Weak positive correlation between an increased BMP and an increasing ISR. Possibly due to readily available hydrolysis by products.	[142]
Whole corn stillage residue from bioethanol plant	• Mesophilic digestion at ISRs 3.67, 1.83, 0.92, and 0.46 g VS g^{-1} VS. • Alkalinity addition of 4000 mg $CaCO_3$ L^{-1}. • Highest CH_4 concentrations of 65.4 %. • VS and COD removals are 83 ± 1 and 86 ± 3 %, respectively at ISR 0.46. • BMP = 458 ml $CH_{4, STP}$ g^{-1} VS at ISR 0.46.	[143]
Co-digestion SCGs with Cow Manure	• Measured the optimum ISR ratio. • Highest BMP measured at ISR 3. Lowest BMP measured at ISR 0.5. • Acetic acid concentration around 300 mg L^{-1}. • No acidification found. VFA/ALK showed a stable process except at ISR 0.5.	[111]

It is evident from table 2.8 that the ISR is a fluctuating parameter that is hard to identify without laboratory and pilot scale research. Nonetheless, it is an important parameter as it dictates the accountability of an AD process. Choosing the wrong ISR can influence biochemical reaction kinetics resulting in digestor failure.

§2.5.2 – Knowledge Gap/Research Ingenuity

As the world unfolds in wake of the impending paradigm shift towards integrated circular bioeconomies and biorefineries, the AD of lignocellulosic biomass can prove to be a useful bioprocess to minimize anthropogenic environmental impacts whilst simultaneously producing biofuels. The AD of SCGs has previously been previously studied (see table 2.7). However, the knowledge gap falls with regard to the optimal ISR for digestion of SCGs with the highest COD and VS reduction. This was conducted and published by [110] at the same time as it was conducted here at UiS. This was revealed during the COVID – 19 crisis. Ultimately, there are knowledge gaps with regard to the various pretreatments SCG have been subjected to for optimal bio-CH_4 production. Due to the COVID – 19 misfortune, only the first part (ISR optimization) was conducted. Part two of this thesis (pretreatment selection at the optimal ISR) and part 3 (pretreatment optimization) were not conducted. The chosen yet not completed pretreatments were:

1) Microwave assisted NaOH pretreatment.
2) Aqueous NH_3 soaking pretreatment.
3) Hydrothermal pretreatment.
4) Dilute HCl pretreatment.

§2.6 – Bio-CH_4 Evaluation for A Local SCG AD Facility

Biogas can generate bioelectricity and bioheat streams. Norway characterizes very well in terms of urban and regional planning. Cities and towns are well connected through a vast network of interworking roads and highways. Also, the vast areas of uninhabited space make it easier for centralized power generation and transmission. It also helps that Norwegian policy is oriented towards green sustainable circular bioeconomy solutions making it more applicable to implement centralized cost-effective biogas solutions. One can also inject bio-CH_4 into the existing regional or national gas grid. In Stavanger, waste management options and regulations are overlooked by the municipality. This simplified section alongside sections in chapters 3, 4, and 5 seek to consider the case study for the AD of SCGs produced at UiS, campus Ullandhaug.

§2.6.1 – Overall Concept

Considering the volume of SCGs per capita produced, Norway places second with 9.01 kg SCGs per person per year. Various social, economics, and environmental benefits can be derived from the AD of SCGs considering the volumes produced nationally. Waste reduction through means other than incineration or landfills improve soil fertility, renewable energy generation, removal of detrimental COD fractions, environmental conditioning, as well as job creation. These trump cards in the hands of local and national authorities have the ability to incentivize further research, project funding and investment schemes.

The evaluations for CH_4 energy and heat estimation comes from the proposed equations in [144]. However, [143] assumes a linear connection between BMP and field operation which may result in the overestimation of the total CH_4 volume and energy potentials. This case study also assumes a constant flow of SCG feedstock being supplied from UiS, which is not the case due to winter, summer, and Easter breaks. Equations 11 – 13 focus on the theoretical description of how the AD of UiS produced SCGs can generate heat and electrical energy potentials [144]. The definitions of the variables in equations 11 – 13 is as such:

- $\underline{vCH_4}$ – Total potential volume of CH_4 produced (dam^3).
- $\underline{E_{AD}}$ – Annual electrical energy potential (MW_e $year^{-1}$).
- $\underline{H_{AD}}$ – Heating potential (TJ $year^{-1}$).

- q – Available amount of SCGs to be digested (tons).
- f_{vs} – Ratio of VS to VS i.e. VS/TS or VS_{TS} (unitless).
- b – VS reduction for the proposed process (unitless).

- g – The BMP achievable by the suggested BMP test of SCGs (see chapter 4) ($dam^3\ CH_4\ ton^{-1}\ VS$).
- cCH_4 – Achievable CH_4 concentration in biogas ($m^3\ m^{-3}$ or %).
- qCH_4 – Heating potential of CH_4 ($MJ\ m^{-3}$).
- η_e – Engine generator efficiency (unitless).

$$vCH_4 = q * f_{vs} * b * g * cCH_4$$

$$E_{AD} = \frac{1}{3600} * vCH_4 * qCH_4 * \eta_e$$

$$H_{AD} = \frac{1}{1000} * vCH_4 * qCH_4$$

Equation 11 (Top) – Estimation of the Total Volume of CH_4 Produced from the AD of SCGs [144].

Equation 12 (Middle) – Estimation of the AD Electrical Energy Potential [144].

Equation 13 (Bottom) – Estimation of the AD Heating Potential [144].

§3 – Materials and Methods

This chapter presents detailed descriptions of the various analytical experiments and methods used for determining the BMP of SCGs at the chosen ISRs. This encompasses the characterization of the substrate and inoculum used, setting up of the batch reactors within the AMPTS II equipment, BMP calculations, modified Gompertz fitting, hydrolysis modelling, and the initial and final characterization of reactor COD and VS. All laboratory works during this study were performed at the UiS campus Ullandhaug, Kjølv Egelands hus, Laboratories A and C wing, unless stated otherwise. Two separate experiments were conducted, termed '*batch 1*' and '*batch 2*'. The COVID – 19 lockdown took place in the middle of batch 2. All results are given as ***average ± standard deviation***.

§3.1 – Substrate and Inoculum

SCGs were donated by SiS Bokkafeen (UiSs largest on-campus café) for this thesis. The coffee species studied was *Coffea arabica* and was harvested in Brazil. *Solberg & Hansen AS* is responsible for roasting, processing, packaging, and distributing the beans within Norway. The coffee beans were roasted for espresso brewing purposes, which highly disrupts the intercellular structure of the beans as opposed to lighter roasting techniques [84]. A high loss in organic nitrogen is seen in espresso roasted beans. During brewing, espresso beans are ground extra finely and tightly packed in a filter cake while superheated water (up to 90 °C and 9 bar) [84] extracts as sugars, proteins, fats, caffeine, and carotenoids. The remnants are the collected SCGs. The SCGs were further grinded by a standard mortar and pestle and sieved through a 25 µm sieve manufactured by VWR®. Later, they were dried at (103 °C for 24 hours) until the moisture fell below 10% wt%.

Anaerobic sludge was obtained from the WWTP (Sentralrenseanlegg Nord Jæren) operated by IVAR at Randaberg, Norway. The WWTP treats influent wastewater for 400000 person equivalents and the produced sludge is then transferred to the AD section of the facility to further accommodate biogas production and COD removal. IVAR also receives FW, fish sludge and waste, and septic wastes which they co-digest alongside primary and secondary sludge. Anaerobic sludge was obtained from reactor 3. AD reactor 3 operates at 17 to 18 days of HRT and SRT without the recirculation. The inoculum used in the BMP test assays was not preincubated beforehand.

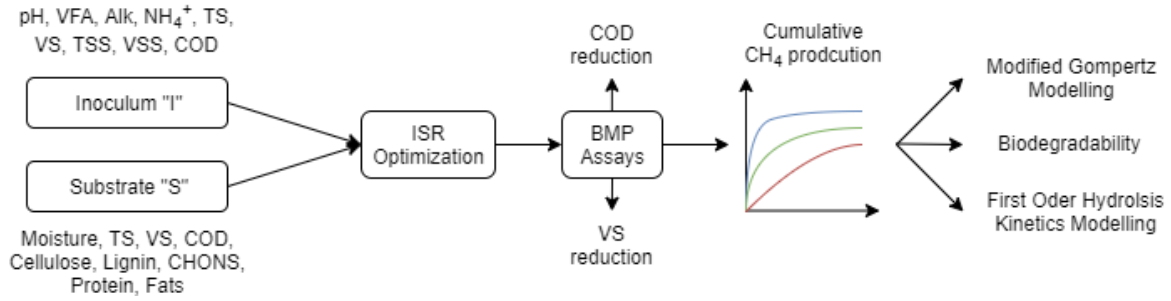

Figure 3.1 – Experimental Flow Diagram

Both the inoculum and the substrate were stored in a walk-in fridge kept at 4 °C away from moisture and were subjected to standard characterization (TS, VS, pH, VFA, ALK, NH_4^+, COD, TSS, and VSS) as proposed by [29], [113], [114], [120]. The above schematics demonstrates the experimental flow chart.

§3.2 – AMPTS and BMP assays

This section covers the equipment (AMPTS) used in determining the BMP of SCGs at the selected ISR. Also, records of sample preparation and control as well as data handling is demonstrated in this section.

§3.2.1 – AMPTS and BMP

The BMP method is a laboratory test which provides important information about the biomethanation of a substrate or co substrate as well as the rate of bio-CH_4 or biogas production and its biodegradability potential [113], [114]. This information can prove to be extremely valuable in industrial scale AD bioprocesses as they can be used to track process performance [114]. Varying experimental conditions allows for a general understanding of the trends different parameters have on the process. In this case the ISR of SCGs AD was varied between 0.5, 1, 2, 3, 4, 5, 6. All other conditions and parameters was kept the same.

The AMPTS II apparatus is manufactured and distributed by BPC AB in Lund, Sweden. It takes on a modern automized approach for determining the BMP of substrates on a laboratory scale with a high degree of precision and accuracy [145], without the need for bulk and complicated equipment. The AMPTS can also be used in determining the specific methanogenic activity (SMA) or the residual gas potential (RGP) of sludges but was restricted to the BMP methodology for this thesis. The experimental setup can be seen below (figure 3.2). The AMPTS can be coupled with a GC-FID or GC-TCD to determine the quality of bio-CH_4 in the outlet biogas, however that was not done in this thesis due to limited laboratory facilities.

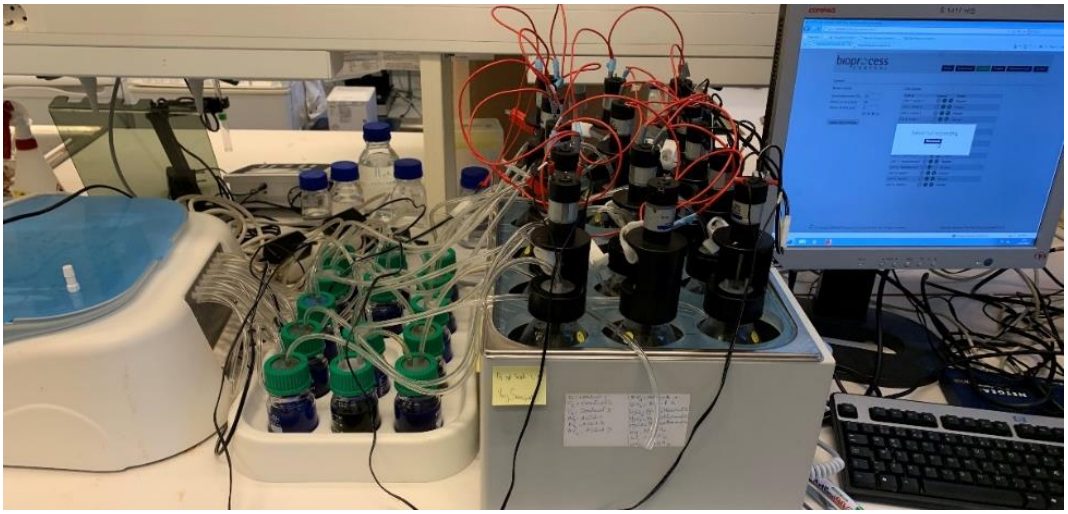

Figure 3.2 – AMPTS II. From Left to Right: Gas Volume Counter, CO_2 and Acid Gas Absorption, Reactor Incubation Unit, AMPTS II Software

Moving from right to left, figure 3.2 shows the sample incubation unit that holds room for 15 500 mL reactors to hold the amalgam with a rotating motor head to ensure mixing of the digestate. Biogas is produced continuously until the AD biochemical reactions cease.

Consequently, biogas passes to the gas absorber unit through Tygon® tubing where the removal of CO_2 takes place. The CO_2 absorber is an alkaline solution (3M NaOH, 80 mL) used to absorb acid gases in the biogas and works similarly to a gas scrubber. The blue color comes from adding the pH indicator *thymolphthalein*. The procedure for the preparation for the blue NaOH solution can be found in the AMPTS manual [145]. The absorber units can remove up to 2.9 L equivalent of CO_2 [145]. They change color from dark blue to colorless upon saturation. CO_2 is the main removed fraction of biogas but the NaOH can absorb other acid gases such as H_2S.

Finally, the gas volume counter device is connected to the software on the provided computer and records the amount of CH_4 which leaves the absorption unit. The gas counter is pre-calibrated by BPC AB. The efficiency of the gas absorber is high [145] but some CO_2 gases escape it and end up being assumed as CH_4 as the gas volume counter does not distinguish between gas species and assumes everything that passes through it to be CH_4. Also, the potential for other gases such as N_2, H_2S, and NH_3 to escape the process and be assumed as CH_4 is also high giving room for the overestimation of the BMP of SCGs [146] [147]. This issue can be resolved by using a GC-FID or GC-TCD. An integrated data collecting system is consistent with the gas volume measuring device and communicates remotely to the computer which records and displays the results. The data can be downloaded from the software into a Microsoft Excel™ CSV file for further analysis.

§3.2.2 – SCG Batch BMP assays

This section will describe the sample preparation process as well as the usage of the AMPTS equipment. The experiments were carried out mainly from January to mid-March 2020 until the COVID – 19 pandemic where laboratory access was heavily restricted.

§3.2.2.1 – Sample Preparation

Before any BMP testing can commence, a primary characterization of the TS and VS of the substrate and the inoculum is required. This determines the necessary mass and volume of SCGs, and anaerobic sludge needed. The ISR is achieved by varying the VS concentrations of sludge and substrate within the reactor at a specified amount in terms of g $VS_{inoculum}$ g^{-1} VS_{SCGs}. 6 different tests were used to determine the density of sludge by weighing the mass a specific volume of sludge using a 10 mL adjustable pipette. This method is more susceptible to random error though. After concluding the mass and volume characteristics, it was a matter of weighing in the required masses of homogenized AS for a specific VS concentration.

§3.2.2.2 – SCGs Batch Assays

A total of 2 batches were investigated. Each batch contained 15 reactors (5 triplicates). Batch 1 was conducted whilst fixing the VS of SCGs in the reactor and alternating the VS of inoculum to attain the required ISRs (0.5, 1, and 2). In batch 2, the VS of the inoculum was fixed and the VS of SCGs was alternated to reach the required ISRs (3, 4, 5, and 6). Both BMP assays were ran to completion such that it satisfies the criteria proposed by [113]. Batch 1 ran for 41 days with the exception of ISR 0.5 which took too long to start. The need to stay on schedule and run batch 2 saw the premature removal of ISR 0.5 from batch 1. Batch 2 ran for 23 days. The composition and ISRs for batches 1 and 2 can be seen in the tables below (tables 3.1 and 3.2). The *experimental ISR* is calculated based on the concentrations of reactants used, it serves as a reference to the *theoretical ISR*. 5 reactors were a positive control. Another 4 reactors were blank references. Starch was used as a control for batch 1. D-glucose was used as a control in batch 2. Both batches used the same AS inoculum. Dried SCGs were used a substrate rather than wet SCGs. Handling of the substrate can be seen in section §3.1.

Both batches fixed the working volume at 400 mL based on the 615 mL VWR® borosilicate glass bottle reactors that housed the digestion process. The headspace volume (215 mL) is compliant with similar authors [113], [145]. Distilled water was added to raise the final working volume to 400 mL when it was needed.

Before starting up the experiment, each reactor was flushed with pure N_2 gas for at least 5 minutes.

This allowed for an anaerobic environment to be established within the reactor. Although [29], [113] support using CO_2/N_2 (20/80% v/v) as a flush gas, that mixture was not available. Adding CO_2 into the flush gas allows for a replenished alkalinity prior to start up. However, [120] claims that using a CO_2/N_2 flush gas showed no significant difference in the BMP results obtained as opposed to flushing with N_2 alone.

The AS inoculum used came from a mesophilic AD reactor and so the batch process temperature was set at 37 °C. The temperature was checked daily. The water bath was filled every other day to insure it against drying out. The water in the water bath was added until the water level was above the liquid level inside the reactors to confirm homogenous mesophilic conditions and optimal heat transfer.

The brushless motors produced by BPC AB allowed for the same mixing regime over all the reactors in question, as they were connected to the same motor controller. The mixing strategy chosen was 600 seconds of mixing at half speed i.e. (100 RPM) [145] and 60 seconds resting. This strategy was chosen to enable maximum contact (maximum mass transfer) between the SCGs and its hydrolysis products and the microorganisms as SCGs have a much higher specific gravity and settle rather quickly.

The CO_2 gas absorbers were checked daily and were not allowed to become saturated (colorless) as that would severely tamper with the actuator inside the gas measuring device giving an extremely false reading of the ultimate BMP. Extra NaOH/indicator bottles were prepared and stored such that it was easy to change the absorption unit immediately as the color changed from dark blue to light blue avoiding the event of saturation (colorless).

Table 3.1 – Composition of the Reactors in Batch 1 (ISR 0.5, 1, and 2).

Batch 1							
Cell	Name	Inoculum		Substrate		Experimental ISR	Theoretical ISR
		g VS	mL	g VS	g		
1	Blank 1	4	182	-	-	-	-
2	Blank 2	4	184	-	-		
3	Blank 3	4	183	-	-		
4	Control S1	4	186	4.87	4.87	-	-
5	Control S2	4	184	4.86	4.86		
6	Control S3	4	185	4.87	4.87		
7	ISR0.5x1	2	91	4	4.43	0.50	0.5
8	ISR0.5x2	2	91	4	4.42	0.50	
9	ISR0.5x3	2	91	4	4.42	0.50	
10	ISR1x1	4	182	4	4.42	0.99	1
11	ISR1x2	4	184	4	4.42	1.00	
12	ISR1x3	4	186	4	4.43	1.01	
13	ISR2x1	8	369	4	4.42	2.01	2
14	ISR2x2	8	367	4	4.44	1.99	
15	ISR2x3	8	370	4	4.43	2.01	

Table 3.2 – Composition of the Reactors in Batch 2 (ISR 3, 4, 5, and 6).

Batch 2							
Cell	Name	Inoculum		Substrate		Experimental ISR	Theoretical ISR
		g VS	mL	g VS	g		
1	Blank 4	8	368	-	-	-	-
2	Control G1	8	369	2.02	2.02	-	-
3	Control G2	8	368	4.01	4.01		
4	ISR3x1	8	371	2.67	2.95	3.04	3
5	ISR3x2	8	372	2.67	2.96	3.04	
6	ISR3x3	8	368	2.67	2.94	3.02	
7	ISR4x1	8	370	2	2.21	4.03	4
8	ISR4x2	8	371	2	2.23	4.02	
9	ISR4x3	8	373	2	2.22	4.05	
10	ISR5x1	8	371	1.6	1.77	5.07	5
11	ISR5x2	8	372	1.6	1.77	5.05	
12	ISR5x3	8	372	1.6	1.75	5.12	
13	ISR6x1	8	372	1.33	1.49	6.05	6
14	ISR6x2	8	371	1.33	1.47	6.08	
15	ISR6x3	8	370	1.33	1.47	6.06	

All SCGs used was pre-dried and no pretreatment was applied. Parts 2 and 3 of this thesis were focused towards pretreatment, but due to COVID, lab access was restricted. No alkalinity was added to the reactors. Glucose was used as it is easily biodegradable. Starch was used to check the inoculum activity.

§3.3 – BMP Calculations

$$BMP = \frac{vCH_{4\ SCGs} - vCH_{4\ blank}}{m_{VS\ SCGs}}$$

$$BMP_{SCGs} = BMP_{avg\ SCGs} \pm \sqrt[2]{(SD_{blank})^2 + (SD_{SCGs})^2}$$

Equation 14 (Top) – Determining the BMP (NmL CH_4 g^{-1} $VS_{SCGs\ added}$) from AMPTS Assay Data [145].
Equation 15 (Bottom) – Displaying the BMP as Average ± Standard Deviation [113].

BMP test results can be expressed in a variety of ways. Most commonly in the units of mL CH_4 g^{-1} VS_{added} however g COD CH_4 g^{-1} COD_{added} is also viable and interesting because it alternates between 0 and 1 (0 being low BMP and 1 being the highest BMP achievable). The BMP is be calculated by equation 14 and is presented as shown in equation 15. $vCH_{4\ SCGs}$ is the cumulative CH_4 of each reactor and $vCH_{4\ blank}$ is the cumulative CH_4 yield from the blank reactors. $m_{VS\ SCGs}$ is the concentration of VS used in the BMP assay and can be obtained from tables 3.1 and 3.2. Biodegradability assessments at different ISRs can be compared to the theoretical BMP (see section §2.3.3).

§3.4 – VS and COD Reduction

Measuring VS and COD reduction for solid substrates is quite tricky. Solids in the AD digestate fluid tend to settle rather quickly and so sampling techniques are not adequate enough to cover human and random error. Nevertheless, when sampling for COD and VS, it was made sure the digestate was as homogenous as can be by pipetting whilst stirring both the liquid and solid contents. This technique is certainly liable to give deviated results as solid-state AD is a relatively new area of research. Equation 16 is used to calculate VS and COD removal efficiencies. Both COD and VS removal efficiencies were standardized against the amount of inoculum they held as shown in equation 17. The batch nature of the BMP means only initial and final measurements are taken, denoted by the subscripts $_i$ and $_f$, respectively. I stands for inoculum and S for substrate.

$$COD\ or\ VS\ Removal\ Efficiency\ (\%)\ = \left(\frac{COD\ or\ VS_i - \ COD\ or\ VS_f}{COD\ or\ VS_i}\right) * 100$$

$$COD\ or\ VS\ =\ COD\ or\ VS_{I+S} - \ COD\ or\ VS_I$$

Equation 16 (Top) – Removal efficiency from COD or VS based on initial and final characterization.
Equation 17 (Bottom) – Standardizing the removal efficiency against blank reactors.

§3.5 – Kinetic Modelling

Two types of kinetic modelling were performed. The non-linear 'modified Gompertz' regression was done on SigmaPlot V10.0 for Windows by SyStat Inc. First order hydrolysis modelling was completed using the solver tool in Microsoft Excel™ for Windows. Equations 18 and 19 demonstrate the models applied. For the modified Gompertz modelling, a group of representative points over the whole data set (whole reaction) were used. For the first order hydrolysis model, only the first 5 days of the experimental results were used [29]. Hydrolysis is the first biochemical reaction to start and the first to end, and usually lasts between 5 to 7 days.

The modified Gompertz model's significance is that it sheds information about the microbial concentration changes (cell growth and decay) over the AD process. The modified Gompertz model allows the BMP data to be modelled for optimal bio-CH_4 production kinetics in batch reactors as there is a pre-assumed relationship between microbial growth and decay and the BMP achievable [148]. BMP_t is the cumulative bio-CH_4 yield. BMP_∞ is the maximum bio-CH_4 attainable. R_{max} is the maximum bio-CH_4 production rate. e is Euler's number (2.71828). λ is the lag phase measured in days.

The first order hydrolysis kinetic model uses linear regression to determine the hydrolysis constant (k_h) from the linear part of the BMP curve over the first 5 days. The aim is to demonstrate the differences

in hydrolysis changes of SCGs based on their selected ISR regime only.

$$BMP_t = BMP_\infty * \exp\left\{-\exp\left[\left(\frac{R_{max}}{BMP_\infty}\right) * (\lambda - t) * e + 1\right]\right\}$$

$$\ln\left(\frac{BMP_\infty - BMP_t}{BMP_\infty}\right) = -k_h * t$$

Equation 18 (Top) – Modified Gompertz Kinetic Model for Microbial Growth [60], [105].
Equation 19 (Bottom) – First Order Hydrolysis Kinetic Model for Enzymatic Hydrolysis [29].

§3.6 – Analytical Procedures

The analytical techniques used to monitor changes during the experimental process are discussed here. No sacrifice reactors were prepared and so all reactors were characterized at initial and final digestion stages.

§3.6.1 – pH and Conductivity

The pH of the anaerobic sludge was taken as soon as it arrived at the laboratory from IVARs sentralrenseanlegg at Nord Jæren. Also, prior to incubation and N_2 flushing, pH was adjusted to 7 in each reactor using 3M HCl acid. pH was taken immediately when the reactors were opened after gas production plateaued. pH measurements were also taken to determine VFA and ALK (see section §3.6.4).

The pH probe was calibrated whenever it was used, using pH buffers 4 and 7. The pH electrode used was the SenTix 41 manufactured by WTW GmbH. Another pH electrode was used for measuring VFA and alkalinity). That pH electrode was inbuilt with the TitroLine® 5000 titrator. Conductivity measurements were used to determine the total amount of dissolved solids. The probe used was the Tetra Con® 325 manufactured also by WTW GmbH.

§3.6.2 – COD

Both total and soluble COD measurements were taken before and after digestion. Soluble COD (sCOD) was done by centrifuging the contents of the digestor at 13000 RPM for 15 minutes at 4 °C by a Heraeus SepaTech Biofuge 17R centrifuge. Samples from the supernatant were pipetted and diluted into a known volume of distilled water. Total COD (tCOD) mesasurments were taken by directly pipetting a set volume of digestate into a set volume of distilled water without centrifugation. COD absorbance measurements were standardized against the COD of various glucose concentrations. Calculating the COD reduction for batch 2 was no possible due to the COVID pandemic.

All COD measurements were done using the Spectroquant® COD Cell Test kits as well as the Spectroquant® Pharo 300 spectrophotometer. To determine COD, digestion took place in a specialized heater at 148 °C for 2 hours. Measurement was conducted when the vials cooled down to room temperature. SCGs COD was measured using the test kit #1.14555.0001 (500 – 10000 mg COD L^{-1}). Five replicates were conducted by weighing the difference of the vials three times to determine the amount of sole SCGs in the vials. 1 mL of distilled water was added to these tests as per the accompanying manual #1.14555.0001. The rest of the COD tests conducted in this research used the test kit #1.09773.0001 (100 – 1500 mg COD L^{-1}). 2 mL was pipetted into these vials. All tests were conducted at least in triplicate, some tests were conducted more often.

§3.6.3 – TS and VS

SCGs, anaerobic sludge, and AD digestate were all characterized for TS and VS. All tests conducted were at least in triplicates. Biogas is generated from the VS content in SCGs, so knowing the VS decrease of the process is favored. VS reduction from ISRs 3, 4, 5, and 6 was not taken due to the COVID pandemic.

TS measurements were taken by weighing the sample in a porcelain dish and heating it in an oven at 105 °C for 24 hours. After cooling the sample in a desiccator, it was weighed again and ignited in a muffle furnace at 550 °C for 2 hours where it was cooled in a desiccator and weighed again. Volatile organic matter can get burned off in the oven or while transporting the sample to the desiccator giving an erroneous reading, especially in solid substrates such as SCGs.

$$TS\ (\%) = \frac{m_{total} - m_{dish}}{m_{sample} - m_{dish}} * 100$$

$$VS\ (\%) = \frac{m_{total} - m_{volatile}}{m_{total} - m_{dish}} * 100$$

Equation 20 (Top) – Calculating TS % by Weight Difference [149].
Equation 21 (Bottom) – Calculating VS % by Weight Difference [149].

TSS and VSS tests were conducted using a 0.45 µm filter and a known volume of inoculum, according to the standard procedure. TSS and VSS measurements were only conducted on the anaerobic sludge. Using a pipette, a certain volume of sludge was filtered through a vacuum filter. Afterwards, heating at 105 °C for 24 hours, and burning at 550 °C for 2 hours was done, as stated above. The tests were conducted in triplicate. The control tests were empty filter papers (3) that were combusted at 550 °C, to determine the fraction of volatile matter combusted from the filter as opposed to the sample. Equations 22 and 23 show the TSS and VSS calculation. Masses are in mg, volumes are in mL.

$$TSS\ (mg\ L^{-1}) = \frac{(m_{filter+solids} - m_{filter}) * 1000}{V_{filtered}}$$

$$VSS\ (mg\ L^{-1}) = \frac{(m_{filter+solids} - m_{filter+solids\ burned}) * 1000}{V_{filtered}}$$

Equations 22 (Top) – Calculating TSS by Weight Difference.
Equation 23 (Bottom) – Calculating VSS by Weight Difference.

§3.6.4 – VFA and ALK

VFA and ALK measurements were conducted using the TitroLine ® 5000 automatic titrator. The VFA and ALK of all samples was determined based on a 5-point titration method proposed by [150] with the supplementary software "*TITRA 5*". Although [150] filtered his digestate before titration, in this experiment the digestate was not filtered as solid SCGs do not contribute to VFA and ALK. The TITRA 5 program automatically calculates the VFA and ALK concentrations after specifying the required variables such as pH, conductivity, volume, and temperature. VFA and ALK concentrations were calculated as mg $CH_3COOH\ L^{-1}$ and mg $CaCO_3\ L^{-1}$, respectively. All VFA and ALK tests were done in triplicates. The VFA and ALK of ISRs 3, 4, 5, and 6, were not determined due to the COVID pandemic.

Using 0.1M HCl, a defined volume of acid was pipetted into a beaker with a known volume of digestate diluted with distilled water. The solution was stirred using a magnetic stirrer at low RPM to minimize CO_2 transfer into the solution. The titration commenced and the volumes of acid used at pHs 6.7, 5.9, 5.2, and 4.3 were noted. The conductivity was also noted using the undiluted volumes of digestate.

§3.6.5 – NH_4^+

Triplicate NH_4^+ measurements were conducted on the inoculum only. This was done to check it against the requirements proposed by [113]. The Spectroquant® Ammonium Cell Test kits #1.14544.0001 (0.5 – 16.0 mg NH_4–N L^{-1}) in conjunction with the Spectroquant® Pharo 3000 spectrophotometer were used. NH_4^+ testing was done when the anaerobic sludge was brought to the laboratory and was still warm. Distilled water samples were used as blanks to calibrate and standardize NH_4^+ absorbance measurements.

0.5 mL of filtered diluted anaerobic sludge sample was pipetted into the vial. One dose of NH_4 – 1K was dosed into the vial and shook. The vial rested for 15 minutes before it was measured.

§3.6.6 – Elemental Compositional Analysis

Dry SCGs samples were sent to Yonsei University, Seoul, South Korea. Glucose, xylose, lignin, proteins, fats, and elemental analysis tests were conducted at the Department of Civil and Environmental

Engineering.

Elemental Analysis was conducted using the Thermo Fisher Scientific™ FLASH 2000 CHNS elemental analyzer. Oxygen concentrations were calculated as the difference between 100 and CHNS concentrations as %. The data is shown in table 4.2. The data is to be used in conjunction with data presented in table 2.6 and experimental data from experiments conducted at UiS. Carbohydrate content (glucose and xylose) was measured using an HPLC–RI with a 300 x 7.8 mm Aminex HPX–87P ion exclusion column using deionized water as eluent at 85 °C.

Acid soluble and insoluble lignin, proteins, lipids, and fats measurements were conducted according to the "Chemical Analysis and Testing Laboratory Analytical Procedures" manual composed by the NREL.

§3.7 – Energy and Power Assessment for the AD of SCGs at UiS

The two main coffee receiving bodies at UiS Campus Ullandhaug are SiS and UiS. SiS distributes its coffee to SiS Bokkafeen and the on-campus canteens such as 'SiS Optimisten' and others. UiS receives coffee for its faculties, administrative buildings, and staff. SCGs produced by student organizations at the student house "StOr" were not considered in this case study. Emails were sent to SiS and UiS asking for their coffee consumption numbers and both complied willingly to provide such numbers by email. The heat and energy production estimation was evaluated by using the equations in section §2.6.1 provided by [144] and is displayed in section §4.6.

SiS provided information about the quantities of coffee it receives in a single academic year. Using these values and the generalized relationship of 91% discarded SCGs in the brewing process [80], it can give an understanding of the amount of SCGs SiS produces annually.

UiS however could not provide us with consumption numbers, but rather the cost of all coffee purchases in one year. Contact with the seller "WaterLogic Norge AS" confirmed the price of 1 kg of coffee to be sold at a rate 120 Norweigian Kroner to UiS. Given their price per kg, and the total purchased over a single academic year, as well as the relationship derived by [80], it becomes a simple calculation to calculate the SCGs production at UiS facilities.

The academic year considered for this study is 2018. It is important to note this metholodogy is quite open for errors however it is a start. Not all the coffee used in the form of beans, it can be instant coffee/capsules too. Not all the coffee is bought at 120 NOK kg^{-1}. Also, this study assumes that all coffee purchased is used within that same year, which is not the case, some coffee is not used immediately and is stored.

§4 – Results and Discussion

This chapter is broken down into, a) substrate and inoculum characterization; b) bio-CH_4 production performance for ISRs studied; c) kinetic models and biodegradability; d) COD and VS removal efficiency and COD mass balance analysis; e) pH, VFA, and ALK; and finally f) energy and power assessment for SCGs generated at UiS. Further after, discussion of the data takes place.

The results for this thesis are divided into two parts, those prior to the COVID – 19 pandemic with complete numerical support, and those that were half complete without numerical support as lab access was stopped. Results are displayed as either initial or final.

§4.1 – Solid and Inoculum Characterization

Table 4.1 – Initial SCGs and Inoculum Characterization.

Parameter	Units	SCGs – Substrate	Anaerobic Sludge – Inoculum
TS	%	92.1 ± 0.1	3.2 ± 0.09
VS	%	90.4 ± 0.05	2.2 ± 0.05
VS_{TS}	%	98.2 ± 0.03	69.1 ± 0.5
tCOD	-	1.49 ± 0.07[a]	32699 ± 2637[b]
sCOD	mg COD L^{-1}	N.A.	2520 ± 308
tCOD/VS	g COD g^{-1} VS	1.65 ± 0.07	1.51 ± 0.13
TSS	g TSS L^{-1}	N.A.	12.50 ± 2.16
VSS	g VSS L^{-1}	N.A.	9.46 ± 1.29
pH	-	N.A.	7.5 ± 0.07
ALK	as mg $CaCO_3$ L^{-1}	N.A.	2229 ± 241
VFA	as mg CH_3COOH	N.A.	212 ± 7.9
NH_4^+	mg NH_4–N L^{-1}	N.A.	1537 ± 38.9

[a] – g COD g^{-1} SCGs.
[b] – mg COD L^{-1}.

Table 4.2 – SCGs Characterization Conducted at Yonsei University, South Korea

Parameter	Unit	SCGs value
C – H – N – S – O	% – % – % – % – % (dry mass)	**52.35 – 7.04 – 2.27 – 0.09 – 38.25**
Empirical Formula	-	**$C_{1553}H_{2488}O_{852}N_{58}S$**
C:N Ratio	mol C/mol N	**26.89:1**
Glucose	%	17.8 ± 3.1
Xylose	%	1.3 ± 0.4

Acid Soluble Lignin	%	4.4 ± 0.6
Acid Insoluble Lignin	%	21.1 ± 1.8
Proteins	%	24 ± 2.7
Other (fats, lipids, extractives)	%	28.3 ± 3.2

The physicochemical results can be seen (tables 4.1 and 4.2). The SCGs used contain an incredibly high VS_{TS} ratio and consequently a low ash content of 1.8 ± 0.03%. The combination of high VS/low ash makes SCGs a suitable substrate to be used in AD bioprocesses, although some issues may be encountered due to insufficient micronutrient supplementation [151]. 5 runs for quantifying the inoculum density were done, to determine the VS parameter of the sludge in terms of mass rather than volume, at 982 g_{sludge} L^{-1}_{sludge}. Table 2.6 is to be used in conjunction with table 4.2. This way the theoretical BMP can be calculated via the concentration of LCH fractions and CHNOS fractions from literature as well as CHNOS fractions for the specific SCGs used in this study (sent to South Korea).

It is important to note that the SCGs used in this AD BMP study were pre-dried to a moisture content of less than 10% which resembles the high VS in table 4.1. However, the wet SCGs obtained from SiS Bokkafeen initially have a TS of 37.2 ± 0.2%, a VS of 36.5 ± 0.2%, and a moisture content of 58.4 ± 1.1%, but these SCGs were not used in the batch tests. The AS inoculum used comprised of a high concentration of water (light inoculum), hence the low solid content. Elevated moisture levels harbor a secure environment in which microorganisms interact maximally to produce bio-CH_4, which is why there was no sever inhibitory signs for ISRs 1 and above. Finding the most favorable ISR for SCGs digestion relies heavily on a secure healthy environment in which microorganisms flourish without VFA, NH_4^+, other inhibitors, and maximal mass transfer.

The initial characterization of SCGs (tables 4.1 and 4.2) fit the descriptions proposed by [7], [83] in terms of TS, VS, CHNOS, proteins, and fats. The COD of SCGs was measured to be **1.49 ± 0.07 g COD g^{-1} SCGs**. This is measured as conveyed in section §3.6.2. The COD of the feedstock (especially when it is a solid), is an essential parameter and must be measured successfully. The COD of solid substrates has proven to be somewhat troublesome due to the rigidity in their lignin superstructure. However, measuring the '***complete***' oxidation of SCGs using the proposed Spectroquant® vials method is incorrect for two reasons. Spectroquant® vials are designed for liquids such as wastewater, algae cultures, or hydrolysates. Secondly, the solid nature of SCGs will give a false reading due to the extremely high solids content (92 %). [152] uses a similar (COD Spectroquant® vials), yet adjusted method to the one used in this thesis to determine the COD of a solid substrate. The method described by [152] can be used to monitor dry AD processes. It is important to note that the standard deviation measured is low (**0.07 g COD g^{-1} SCGs**). Nonetheless, the

method requires further standardization to be considered complete.

The C:N ratio obtained from the substrate matches other SCGs tested (see section §2.4.7.2). 26.89:1 falls between 20:1 and 35:1, which is the optimum C:N ratio for microbial bioprocess. A moderate ratio of 26.89:1 allows SCGs to have a stable digestion with adequate of N supplementation to microbial life [153]. Also, it allows it to be used as a co-substrate to bioaugment and alter low or high C:N ratio feedstocks such as rice flour and wheat straw for a more coherent digestion and successful growth of the different microbial groups (especially methanogens) [153]. The elemental analysis of the inoculum was not conducted and so the empirical formula was not calculated. However, the average empirical formula of the inoculum (and newly grown biomass) is standardized to be $C_5H_7O_2N$ by [14].

Through personal communication with IVAR, the inoculum used matches very well the characteristics from the IVAR laboratory. The VFA/ALK ratio of the inoculum is 0.1 ± 0.01, which is considered to be a stable non-inhibiting inoculum as per [114]. The pH of the inoculum measured at IVAR remains relatively stable until it reaches the UiS laboratories where the pH is measured again at 7.5 ± 0.07. The pH is also in accordance with [113] thresholds. The NH_4^+ concentration is well below the required limit and proves the inoculum to be non-inhibiting [113]. The moderate concentration of fats and lipids (table 4.2) deem SCGs a valid feedstock for AD.

§4.2 – BMP Batch Assays and CH_4 production

The figures below show the total bio-CH_4 production over the course of the BMP test (figures 4.1 and 4.2). Figure 4.1 shows the cumulative bio-CH_4 production whereas figure 4.2 shows the specific bio-CH_4 production per g VS_{SCGs} loaded. Residual CH_4 from the inoculum blanks were subtracted from the cumulative and specific BMPs. Blank and positive control reactors to measure the inoculum activity were held but are not displayed to keep figures 4.1, 4.2, 4.5, and 4.6 from getting overly congested. The ISR BMP curves are plotted as triplicate averages with their standard deviation error bars.

The daily bio-CH_4 production is presented, (figures 4.5 and 4.6). A correlation curve between the ISR of SCGs AD and their respective BMPs is drafted, (figures 4.3 and 4.4). Lastly, table 4.3 gives the numerous presentations of the BMP at the different ISRs, as well as the normalization of the BMP at STP as called for by [29]. The data is processed and presented as proposed by equations 14 and 15. An important factor to consider is the flush gas concentration. Using a pure N_2 flush gas to fashion an anaerobic environment will somewhat overestimate the overall BMP of a substrate as it destabilizes the carbonate equilibrium and gives a slightly erroneous reading [113], [145].

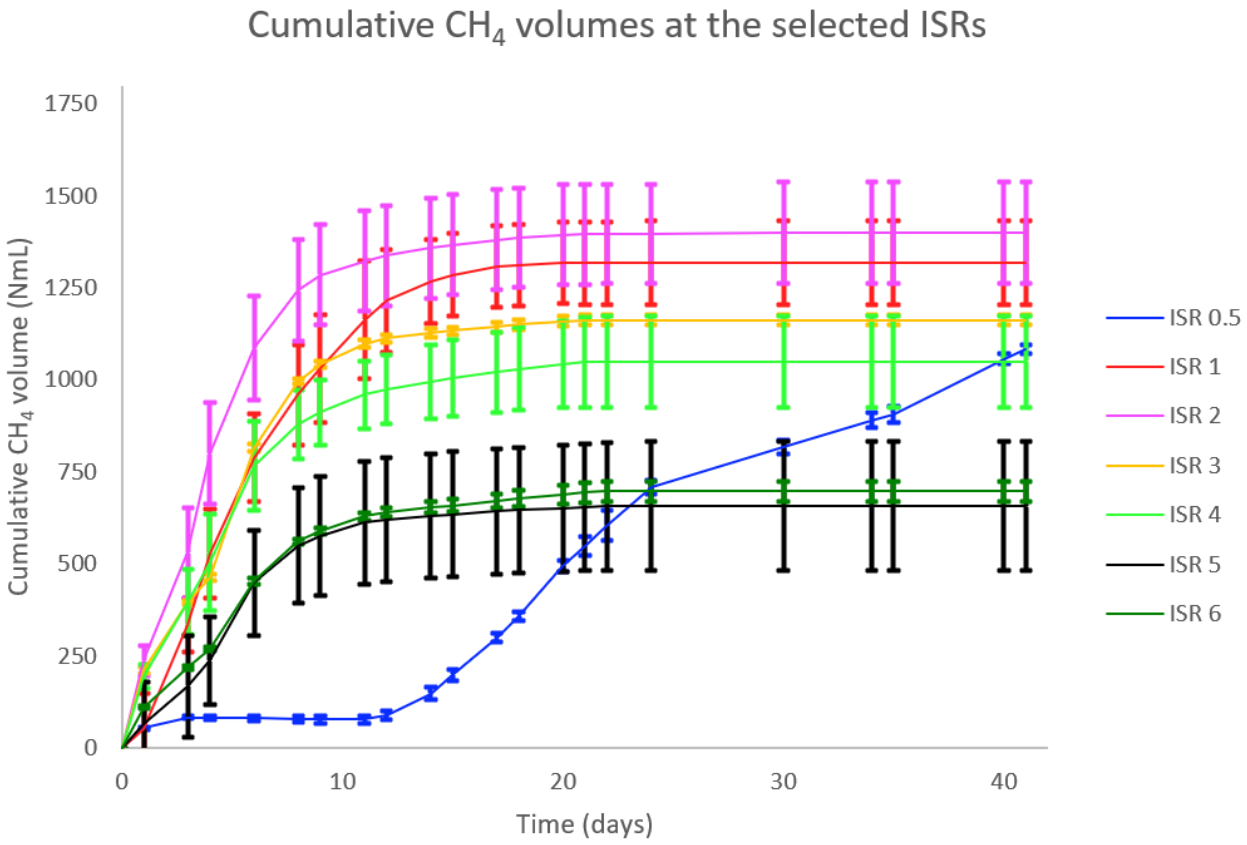

Figure 4.1 – Cumulative CH_4 Production from SCGs at the Selected ISRs.

As shown in figure 4.1, the different reactors produced different volumes of bio-CH_4 over the course of the study. Most notably is ISR 2 (8 g $VS_{inoculum}$ 4 g^{-1} VS_{SCGs}), producing a total CH_4 volume of 1401 ± 137 NmL CH_4. Reactors with ISR 0.5 (2 g $VS_{inoculum}$ 4 g^{-1} VS_{SCGs}) took approximately 10 days to start and had a low level of CH_4 production throughout the whole 41 days of study compared to the rest. Hence, its curve does not plateau because the process was deemed too slow and shut down prematurely before ending leading us to consider slow kinetics and/or inhibitory parameters. More importantly is figure 4.2 which gives insight on the specific BMP based on the amount of substrate initially loaded. According to table 3.1, ISR 2 showed the highest concentration of SCGs in relation to other ISRs chosen. This boosted the breakdown of the substrate and allowed for the increase in total bio-CH_4 production. Inversely, table 4.3 signifies the relationship between the SCGs concentration and BMP achievable. Loading more substrate is not necessarily better for optimizing bio-CH_4 yields as seen by ISR 0.5.

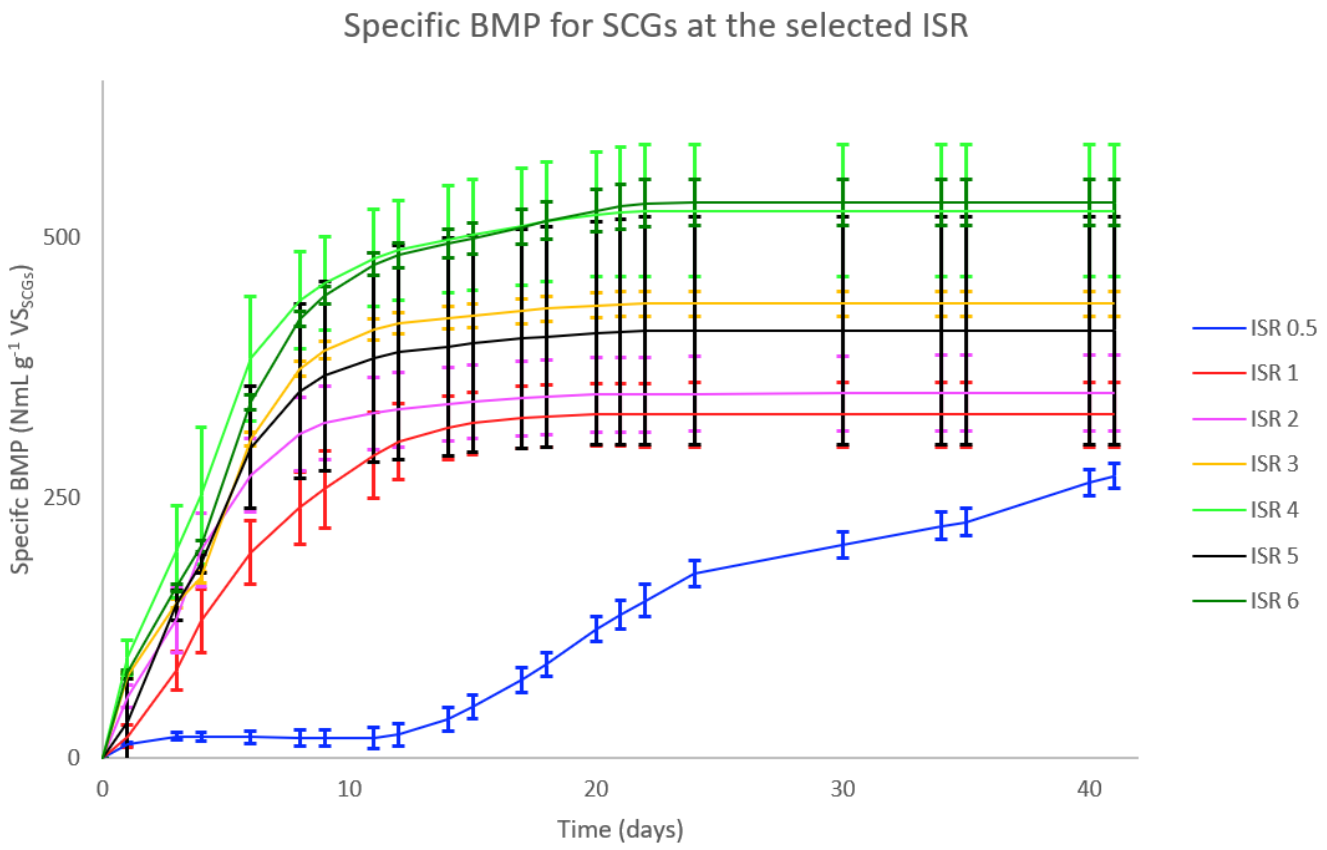

Figure 4.2 – Specific CH_4 Production (BMP) at the Selected ISRs Based on the Loaded Substrate (As g VS_{SCGs}).

The difference between figure 4.1 and 4.2 is that figure 4.2 layers the BMP curves in terms of the amount of loaded substrate. We can then clearly see that ISR 6 is the most CH_4 producing unit. ISR 6 tops CH_4 production **at 533 ± 22 NmL CH_4 g^{-1} VS_{SCGs}**. The least producing is ISR 0.5 as the reaction was incomplete. ISR 0.5 produced up to **271 ± 12 NmL CH_4 g^{-1} VS_{SCGs}** by day 41. ISR 5 produced less than ISR 4, but with the largest sources of error (see figures 4.3 and 4.4). According to [111], digesting SCGs yielded a propionic acid concentration of 3366 mg L^{-1} which explains the limitation of the BMP achieved even after 41 days of digestion in ISR 0.5 reactors. This occurs due to the high load of SCGs on an inoculum which cannot accept such high loading and is unable to convert them successfully. This is shown by the stagnant lag phase needed for acclimating to the substrate. This leads to a destabilized process ecology and premature bio-CH_4 production as seen in figure 4.2. This can be confirmed using an HPLC to test VFA concentration at ISR 0.5, but that was unavailable.

Figure 4.3 shows how the ISR affects both cumulative CH_4 yields as well as specific yields based on dosage. Linear plot fittings show a weak downtrend (R^2 = 0.644) between increasing the ISR and the cumulative volume of CH_4 produced (green lines, right axis). On the contrary, a weak uptrend (R^2 = 0.754), is observed for the specific BMP for its respected ISRs (red lines, left axis). Figure 4.4 shows the same data

as figure 4.3 but uses a 2nd degree polynomial to obtain a correlation between BMP and ISR. The 2nd degree polynomial gives a better correlation (R^2 = 0.795) as opposed to a linear correlation (R^2 = 0.754). **According to the correlation in figure 4.4, the optimum ISR was calculated to be 6.5 (by differentiation of 2nd degree polynomials) and then falls beyond that**. However, this might not be the case when using a different inoculating agent.

The large concave engulfment around ISR 5 occurs due to a failure in one of the reactors. The AMPTS apparatus did not respond to CH_4 production signals, giving less data to work with. Hence, the lower bound of ISR 5 aligns with that of ISR 2 which is theoretically and practically incorrect. Also, one of the ISR 2 reactors experienced failed gas production due to undetermined reasons. Surely, this disturbs the correlation and their R^2 values as calculated in figures 4.3 and 4.4. However, it is clear that there is a connection between ISR and BMP for SCGs AD.

It is projected that ISR above 6 will also yield below 500 mL $CH_{4,\ STP}$ g^{-1} VS. This occurs due to the diminishing concentration of substrate with increasing ISR which lowers the BMP drastically as demonstrated by the quadratic relationship in figure 4.4.

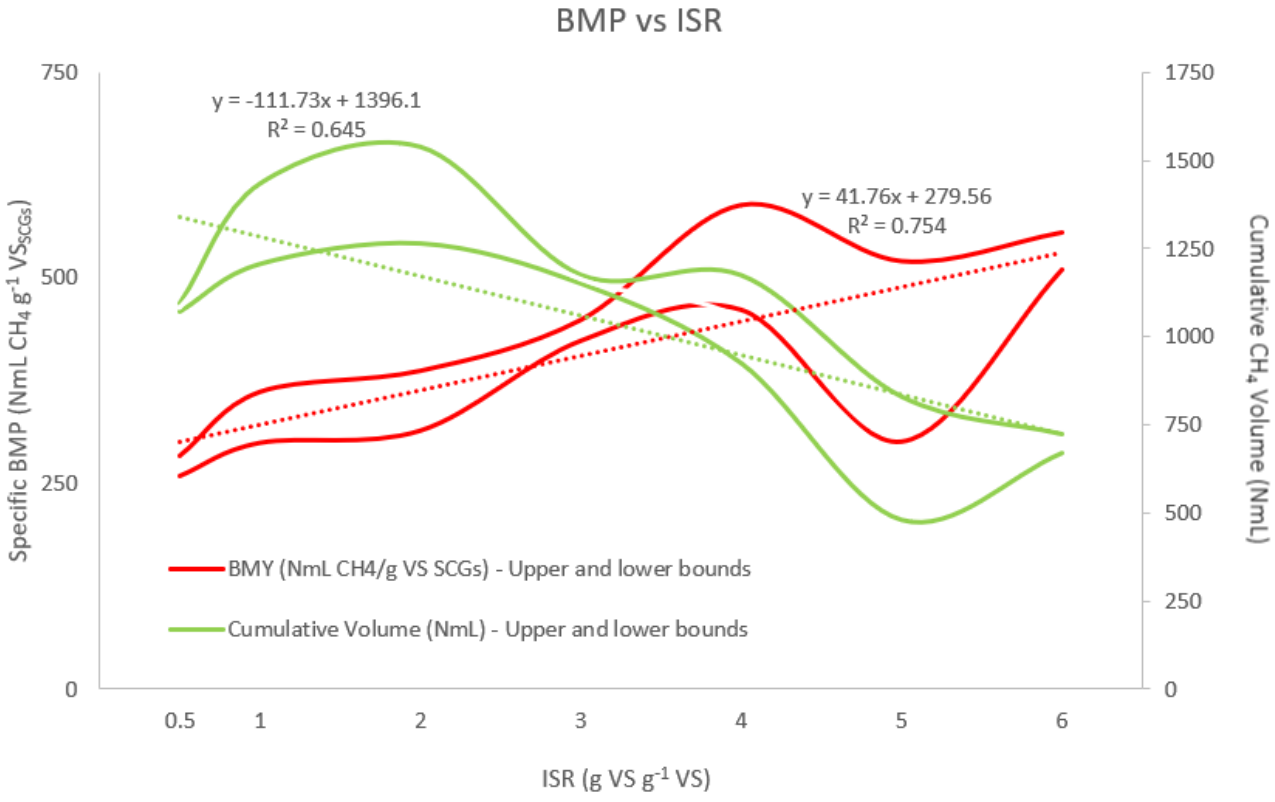

Figure 4.3 – The Correlation Between Bio-CH_4 Production from SCGs and the ISR Dosed (Linear).

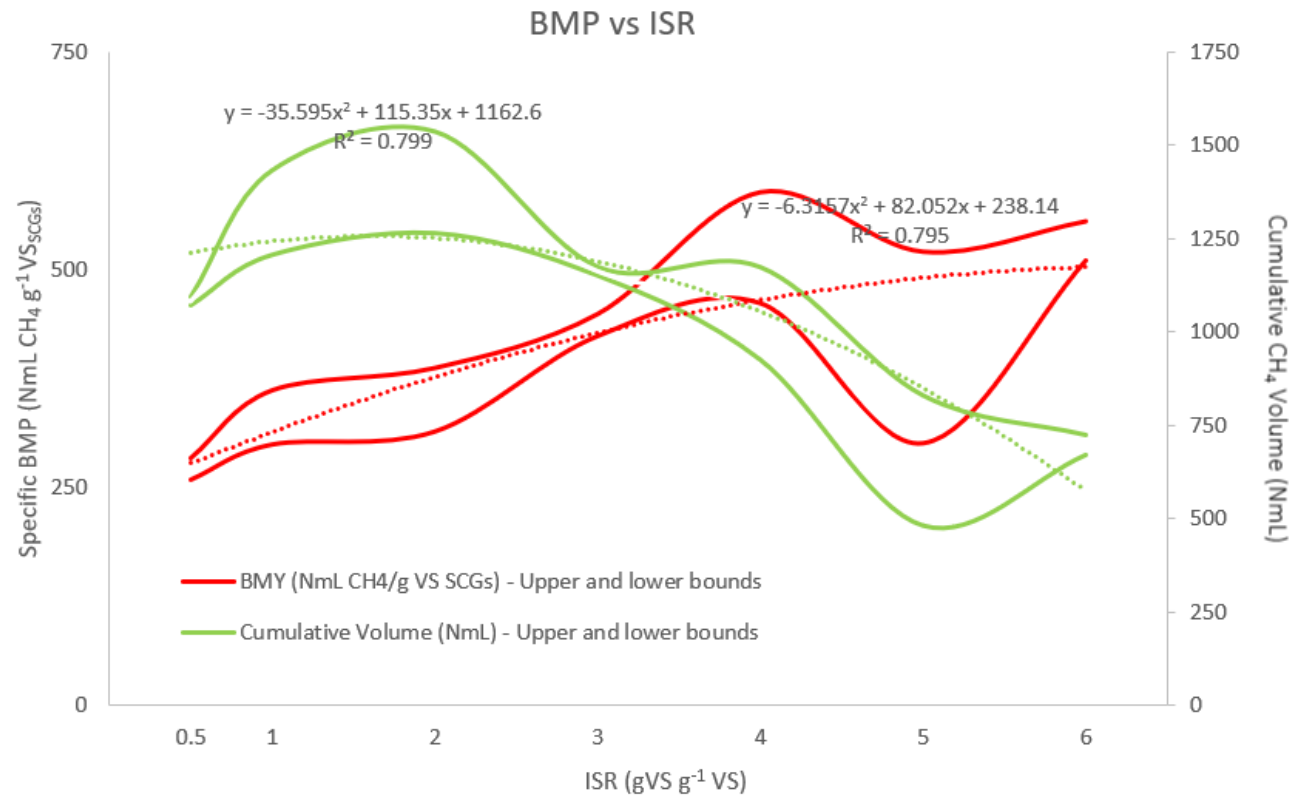

Figure 4.4 – The Correlation Between Bio-CH_4 Production from SCGs and the ISR Dosed (2nd Degree Polynomial).

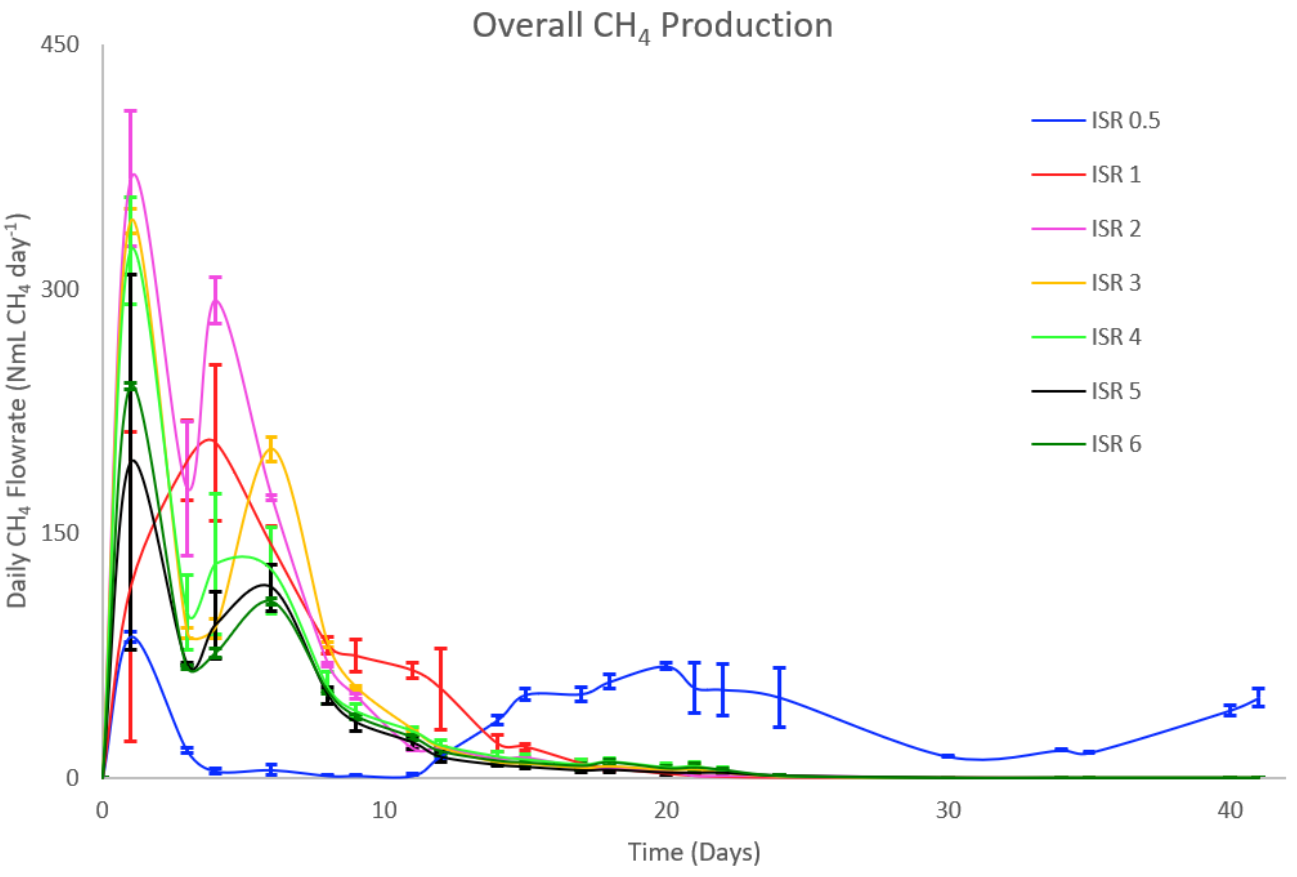

Figure 4.5 – Total Bio-CH_4 Production Rate Profile at the Selected ISRs.

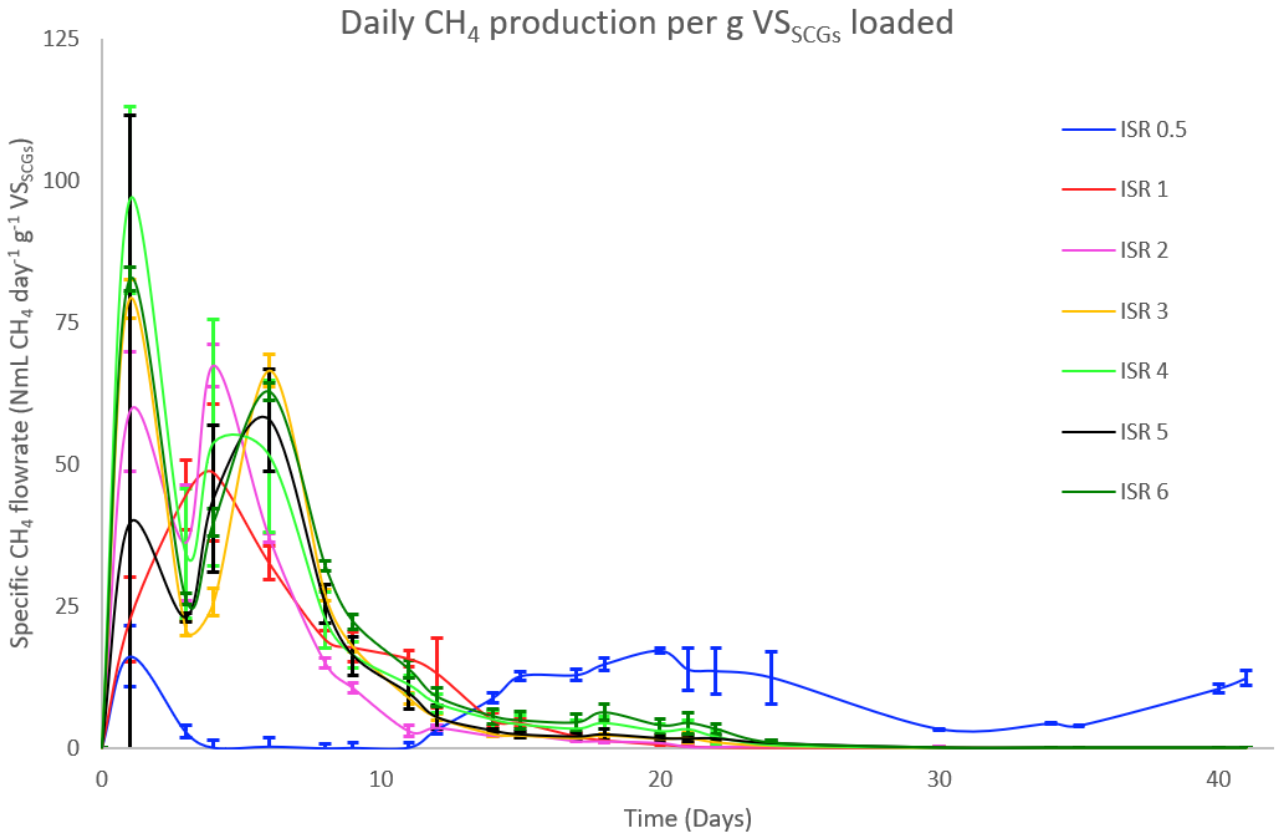

Figure 4.6 – Specific Bio-CH_4 Production Rate Profiles Relative to the g VS_{SCGs} Initially Loaded.

Figures 4.5 and 4.6 show the CH_4 flowrate profiles. Tallying up the results of figures 4.5 and 4.6 yields the data in figures 4.1 and 4.2, respectively. The highest daily production rate of **367 ± 41 NmL CH_4 day^{-1}** is produced by ISR 2 around day 2/3 (see figure 4.5). However, based on the g VS_{SCGs} loaded, it is observed from figure 4.6 that ISR 4 has a production rate of **97 ± 16 NmL CH_4 day^{-1} g^{-1} VS_{SCGs}** also at day 2/3. All BMP profiles showed 2 intensified bio-CH_4 production surges (shown as 2 successive humps). The first surge, which happened almost immediately (day 2/3), can be attributed to the conversion of readily available COD. The high concentration of FFA and LCFAs from SCGs can result in a delayed bio-CH_4 production surge as observed between days 3 and 8, where hydrolysis slows down. However, for ISR 0.5, the delayed gas production can be seen between days 10 and 30 implying some form of inhibition or inactivity. Also, ISR 1 did not demonstrate any sharp increase or decrease in CH_4 production which can be attributed to an increased VFA concentration. Although HPLC analysis would shed more light on the matter. All reactors with ISR above 1 are considered to have a high resistance towards failing. ISR 1 is borderline. ISR 0.5 failed.

Normal conditions are measured at 1 atm and 20 °C whereas STP is measured at 1 bar and 0 °C, accounting for the removal of water vapor to form dry bio-CH_4.

Table 4.3 – Maximum BMP Parameters Achieved for the Selected ISRs.

Reactor	BMP – mL $CH_{4,\ STP}$ g^{-1} COD	BMP – NmL CH_4 g^{-1} VS	BMP – mL $CH_{4,\ STP}$ g^{-1} VS	BMP – g COD g^{-1} COD
ISR 0.5	0.15 ± 0.01	271 ± 12	256 ± 11	0.44 ± 0.03
ISR 1	0.19 ± 0.02	330 ± 31	312 ± 29	0.54 ± 0.06
ISR 2	0.20 ± 0.02	350 ± 36	330 ± 34	0.57 ± 0.06
ISR 3	0.25 ± 0.01	436 ± 12	412 ± 11	0.71 ± 0.04
ISR 4	0.30 ± 0.04	525 ± 63	496 ± 59	0.86 ± 0.04
ISR 5	0.23 ± 0.06	410 ± 110	387 ± 104	0.67 ± 0.18
ISR 6	0.30 ± 0.02	533 ± 22	503 ± 21	0.87 ± 0.05

§4.3 – Biodegradability and Process Kinetics

In this thesis, biodegradability is calculated based on two methods. First, using the lignocellulosic composition of SCGs available in literature (see table 2.6). Second, using the compositional analysis from the average values of CHNSO found in literature (see table 2.6). Lastly, comparing the results from CHNSO literature studies to the CHNSO experiments conducted at Yonsei University, South Korea (see table 4.2). Table 4.4 below summarizes the main parameters. Equations 1, 2, 9, and 10 are applied here to calculate the theoretical BMP (BMP_{Th}). All biodegradability calculations above 100% are considered incorrect. The parameter of interest is $CHNSO_{Yonsei}$. CHNSO and LCH (top 2 rows) serve as a reference and comparison for the experimental outcomes.

Table 4.4 – Theoretical BMP based on LCH or CHNSO compositions

Name	Composition (%)	BMP_{Th} (mL $CH_{4,\ STP}$ g^{-1} VS_{SCGs})	Equations Used
LCH – (Table 2.6)	L (25.8) – C (10.5) – H (37.9) – Residual (25.8)	226.3	Equation 2 - [40]
CHNSO – (Table 2.6)	C (53.61) – H (6.78) – N (2.19) – S (0.14) – O (37.21)	592.3	Equation 9 - [120]
$CHNSO_{Yonsei}$	C (52.35) – H (7.04) – N (2.27) – S (0.09) – O (38.25)	593.4	Equation 9 - [120]

[40] uses lignin, cellulose, and hemicellulose (LCH) data to calculate the BMP_{Th} of substrates. However the model proposed by [40] (equation 2) is subject to very large ranges of error sometimes ranging up to 150%. It is evident from column 1 in table 4.5 (BD LCH %) that the model does not fit for SCGs, as biodegradability thresholds go over 100 % and even reach 200%. However, using the compositional characteristics of SCGs we can see that CHNSO data from this study of previous studies fit extremely well to demonstrate the final biodegradability of the AD of SCGs in anaerobic sludge inoculum. The highest biodegradability was that of ISR 6 and, however ISR 4 closely followed. ISR 5 showed a large

deviation. However, it is thought that ISR 5 is similar to ISR 4 and 6 but would require further justification.

BD CHNSO is quite close to BD $CHNSO_{Yonsei}$. This is due to the fact that CHNSO analysis of previous SCGs display similar compositions as the ones used in this thesis. This shown by their BMP_{Th}, which differs by only 1.1 mL $CH_{4,\ STP}$ g^{-1} VS_{SCGs} only.

Table 4.5 – Biodegradability for the ISRs. Based on the Selected Method for Determining BMP_{Th} i.e. (Table 4.4).

Reactor	BD LCH (%)	BD CHNSO (%)	BD $CHNSO_{Yonsei}$ (%)
ISR 0.5	113 ± 5	43 ± 2	43 ± 2
ISR 1	138 ± 18	53 ± 7	53 ± 7
ISR 2	146 ± 21	56 ± 8	56 ± 8
ISR 3	182 ± 7	70 ± 3	69 ± 3
ISR 4	219 ± 37	84 ± 14	84 ± 14
ISR 5	171 ± 65	65 ± 25	65 ± 25
ISR 6	222 ± 13	85 ± 5	85 ± 5

Lastly, as per section §3.5, microbial and process kinetics are modeled via the modified Gompertz model over 41 days, and the enzymatic degradation hydrolysis model using the first 5 days. Both models employ the results from figure 4.2. The lag phase (λ) for ISR 4 was modeled as negative indicating an active inoculum producing gas prior to incubation.

The modified Gompertz parameters resemble a tight fitting towards microbial growth curve parameters returning R^2s of 0.98 and above. Modified Gompertz parameters shed light on how microbial biomass interact with the substrate in question and relates feedback on how to sustain continuous AD operation of SCGs at their respective ISRs.

The hydrolysis constant was calculated according to initial CH_4 production in the first 5 consecutive days. Most notably, is the k_h for ISR 0.5 which is significantly lower than any other ISR. This suggests reactor overloading which resulted in a decreased BMP, increased VFA concentrations (see table 4.9) failing these reactors. ISR 2 had the highest hydrolysis rate constant, allowing it to produce bio-CH_4 the quickest. On the other hand, ISR 6 had the highest BMP at 0.87 ± 0.05 g COD g^{-1} COD with a lower k_h than ISR 2 indicating that the ISR plays a major role in deciding the biodegradability, hydrolysis kinetics, and BMP of SCGs.

Table 4.6 – Modified Gompertz Model and First Order Hydrolysis Model Kinetic Parameters.

Reactor	BMP_∞ (NmL CH_4 g^{-1} VS)	R_{max} (NmL CH_4 g^{-1} VS day^{-1})	λ (day)	R^2	k_h (day^{-1})
ISR 0.5	266 ± 9	12.5 ± 0.5	10.5 ± 0.2	0.98	0.02 ± 0.00
ISR 1	330 ± 28	38.1 ± 5.1	0.9 ± 0.3	0.99	0.14 ± 0.02

ISR 2	348 ± 34	52.3 ± 6.9	0.3 ± 0.2	0.99	0.22 ± 0.02
ISR 3	436 ± 1	54.9 ± 0.6	0.2 ± 0.0	0.99	0.16 ± 0.01
ISR 4	522 ± 59	64.4 ± 6.6	**- 0.1 ± 0.3**	0.99	0.20 ± 0.04
ISR 5	408 ± 110	59.2 ± 4.9	0.5 ± 0.3	0.99	0.20 ± 0.06
ISR 6	530 ± 14	57.7 ± 1.4	0.1 ± 0.1	0.99	0.15 ± 0.00

§4.4 – COD Mass Balance and COD/VS Reduction

COD mass balances illustrate the distribution of organic fractions in AD systems. A well accounted COD mass balance can prove to be vital for design and operation of large-scale energy efficient AD systems. COD and VS removal efficiencies for batch 1 (ISRs 0.5, 1, and 2) were identified and calculated based on the initial and final characterization of COD and VS concentrations during the 41-day test period. Due to the COVID – 19 pandemic and imposed laboratory restrictions, COD and VS removal efficiencies for ISRs 3, 4, 5, and 6 (batch 2) were not identified. Only initial characterization was conducted for batch 2. These are not showed below. The COD mass balance is shown for batch 1.

§4.4.1 – COD and VS Removal Efficiency

Testing and calculation for COD and VS reduction is done according to sections §3.4, §3.6.2 and §3.6.3. Table 4.7 shows the results for batch 1. Figure 4.7 shows initial and final COD characterization for batch 1. **A clear correlation between ISR and COD/VS reduction remains inconclusive due to the lack of results at higher ISRs.** Figure 4.7 shows the different COD fractions measured (as a percentage of $tCOD_{in}$). A clear increase in $sCOD_{in}$ and $sCOD_{eff}$ is seen as the ISR is increased from 0.5 to 2, due to the increase of SCGs available in the reactors. However, $tCOD_{eff}$ seems to decrease and then increase which is erroneous and needs further standardization. Both COD and VS removal efficiencies were controlled against the COD and VS of the inoculum (see equation 17).

Table 4.7 – COD and VS Removal Efficiencies. Taken as Triplicate Average and Corrected for the Inoculum.

Reactor	COD reduction (%)	VS reduction (%)
ISR 0.5	49 ± 16.2	76.2 ± 12.6
ISR 1	61.6 ± 3.2	68 ± 4.3
ISR 2	46.8 ± 7.8	56.5 ± 3

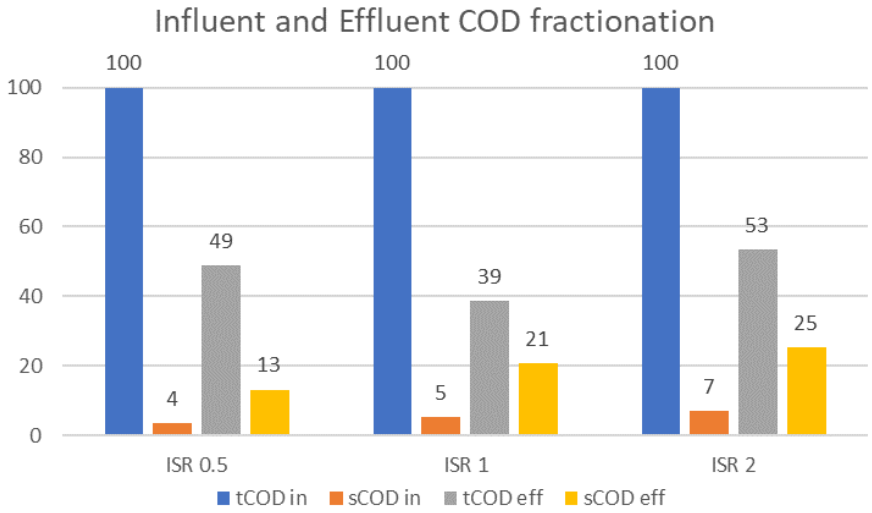

Figure 4.7 – Influent and Effluent COD Characterization. Taken as Triplicate Average and Corrected for Inoculum COD.

§4.4.2 – COD Mass Balance

As per equation 6, $COD_{influent}$ at t = 0 was measured and $COD_{effluent}$ at t = 41 days was also measured. The COD measurements taken were diluted 30 to 50 times to facilitate the COD vial concentration range. COD_{gas} i.e. bio-CH_4 COD was theoretically calculated using equation 7 and the experimental values obtained are in table 4.3. Table 4.8 shows the breakdown of the COD fractions. COD mass balances for ISR 3, 4, 5, and 6 were not available due to COVID – 19. $COD_{biomass}$ or COD_{sludge} was not measured in this study but is assumed be between 5 – 20% for microbial growth. The COD balance column in table 4.8 is calculated based on equation 24 below, which comes as a modification to equations 6 and 7.

$$COD\ balance\ (\%) = \frac{COD_{gas} + COD_{effluent}}{COD_{influent}} = \frac{\frac{Q_{gas}}{350\frac{mL\ CH_{4,\ STP}}{g\ COD}} + COD_{effluent}}{COD_{influent}}$$

Equation 24 – COD Mass Balance Equation Used in this Thesis

Table 4.8 – COD Mass Balance at the Selected ISRs

Reactor	$COD_{influent}$ (mg COD)	$COD_{effluent}$ (mg COD)	COD_{gas} (mg COD)	COD reduction (%)	COD balance (%)
ISR 0.5	5488 ± 1096	2680 ± 418	2926 ± 126	49 ± 16.2	102 ± 22
ISR 1	8727 ± 859	3373 ± 620	3566 ± 331	61.6 ± 3.2	80 ± 11
ISR 2	13739 ± 635	7333 ± 1281	3771 ± 389	46.8 ± 7.8	81 ± 10

Influent COD for ISRs 3, 4, 5, and 6 were measured however effluent COD was not. $COD_{influent}$ was **8752 ± 815**, **4976 ± 45**, **3920 ± 1131**, and **2832 ± 815** mg COD for ISRs **3**, **4**, **5**, and **6**, respectively showing a clear decrease in $COD_{influent}$ with increasing ISR. ISR 1 and 2 mass balances show a maximum of 80 and 81% respectively. This is solidified by assuming that COD_{sludge} contributed from 2 to 20% for microbial growth [14], [15]. This allows for a somewhat complete (100%) COD mass balance. However, ISR 0.5 does not account for COD_{sludge} and yet the COD mass balance is overestimated at 102%. This can be due to an incomplete process and/or the assumption of elevated VFA concentrations.

§4.5 – pH, VFA, and ALK

Table 4.9 shows pH changes across all batch 1 reactors. Also, table 4.9 shows VFA and ALK concentrations in the digestate in order to determine plausible indications of reactor failure. The addition of coffee lowered the pH of the inoculum from 7.50 ± 0.07 (table 4.1) to pH (column 2, table 4.9). The higher the ISR, the lower the difference between the two pHs. This indicates that SCGs have a pH lowering effect when they come in contact with the inoculum. ISR 0.5 reactors has the lowest start pH due to the highest concentration of SCGs solids present. The pH column (column 2) indicates the pH when SCGs and the inoculum were mixed before adjusting the pH to 7. $pH_{initial}$ (column 3) indicates the pH after adjustment to 7. pH_{final} (column 4) is the pH when the reactors were finally opened on day 41. As discussed before, pH, VFA, and ALK measurements for batch 2 were not completed.

Table 4.9 – pH, VFA, and ALK Results.

Reactor	pH	$pH_{initial}$	pH_{final}	VFA (mg HAc L^{-1})	ALK (mg $CaCO_3$ L^{-1})	VFA/ALK
ISR 0.5	7.01 ± 0.02	7.01 ± 0.01	6.83 ± 0.13	280 ± 27	982 ± 68	0.29 ± 0.05
ISR 1	7.14 ± 0.01	7.00 ± 0.01	7.59 ± 0.02	284 ± 50	2371 ± 301	0.12 ± 0.03
ISR 2	7.21 ± 0.01	7.00 ± 0.01	7.66 ± 0.07	507 ± 88	4206 ± 283	0.12 ± 0.03

pH_{final} was measured for ISR 3, 4, 5, and 6. However VFA and ALK concentrations were not. pH_{final} was **7.66 ± 0.01**, **7.65 ± 0.03**, **7.71 ± 0.02**, and **7.60 ± 0.21** for ISR **3**, **4**, **5**, and **6**, respectively. We can see that pH_{final} had stabilized at 7.66 for ISRs 1 and above, indicating a successful digestion and little to no NH_3 inhibition.

It is assumed that SCGs have little to no alkalinity embedded within their solid structure. Hence, the inoculum used plays a crucial role in stabilizing the pH and minimizing VFA overwhelming. It can be seen that the VFA/ALK values for ISR 0.5 are at least double that of ISR 1 and 2 which is attributed to the rapid consumption of inoculum alkalinity by the heavy production of VFAs at this low ISR [111], [113]. Technically,

the VFA/ALK ratio of ISR 0.5 is 25% well below the attributed value (0.4) which determines failure at this ISR. However, it is also argued that a VFA/ALK of 0.3 is considered failed or near failed. It is decisive though to assume the reaction failed completely without running ISR 0.5 reactors to completion again. The lowered CH_4 yield and production rate as well as the delayed lag phase argues that ISR 0.5 is not best for CH_4 production from SCGs. The pH in ISR 0.5 reactors was significantly lower than all other reactors in batch 1 and 2. This indicates the overload in VFA concentration, relating to the extended hydrolysis as well as the reduced alkalinity to 982 mg HAc L^{-1}. ISR 0.5 reactors showed a pH lower than 7 when they were opened, which indicates methanogenic inhibition. Finally, ISR 2 reactors had noticeably more VFAs present but were backed by even more alkalinity (**4206 ± 283 mg $CaCO_3$ L^{-1}**).

§4.6 – Energy and Power Calculations for SCGs at UiS

This section aims to quantify the overall energy and heat production output from an AD unit digesting solely SCGs from UiS campus Ullandhaug within the characteristics obtained in this study. The method proposed by [144], is able to demonstrate both the heat and electrical potential of the bioproduced CH_4. Information associated with this section is found in §2.6.1 and §3.7. Equation 11 estimates the bio-CH_4 volume by digesting SCGs at ISR of 6 (the highest producing ISR). Equation 12 evaluates the potential electrical energy that can be derived from SCGs digestion at ISR 6 in MW_e $year^{-1}$. Equation 13 demonstrates the heating potential at SCG digestion at ISR 6 in TJ $year^{-1}$. H_{AD} and E_{AD} for ISR 1 are given to show the comparison in heat and energy potential by simply optimizing the ISR.

Through private communication with SiS and UiS personnel, it is demonstrated that SCGs used at UiS campus Ullandhaug amounted to **5881 kg coffee in 2018** (based on consumption statistics from SiS and purchasing statistics from UiS). This amounts to an average of 16.1 kg coffee consumed per day on campus.

If the value column is split, the left side indicates ISR 1 values and the right side indicates ISR 6 values. cCH_4 was not measured in this study. The value of cCH_4 is obtained from average values in table 2.7.

Table 4.10 – Parameters to Calculate vCH_4, H_{AD}, and E_{AD}.

Parameter	Value		Reference
q – tons	5.352[1]		This study – Section §4.6
f_{VS} – unitless	0.982		This study – Table 4.1
b – unitless	0.68	0.68[2]	This study – Table 4.7
g – dam^3 $tons^{-1}$ VS	0.312	0.503	This study – Table 4.3
cCH_4 – m^3 m^{-3}	0.599[3]		This study – Table 2.7

η_e – %	34	[144]
qCH_4 – MJ m^{-3}	36.3	[144]

[1] – 0.91 kg SCGs produced from 1 kg coffee consumed [80]. Based on 5881 kg coffee consumed in 2018.

[2] – No documented values for VS reduction at ISR 6 due to COVID related issues. Instead, VS reduction of ISR 6 was taken to be equal to ISR reduction of ISR 1. However, VS reduction for ISR 6 should be higher than ISR 1.

[3] – Taken as average bio-CH_4 concentration from SCG produced biogas. [10], [99], [100], [102], [103], [105], [106], [108].

Table 4.11 – H_{AD} and E_{AD} (Heat and Energy) Production at ISRs 1 and 6.

Reactor	vCH_4 (m^3 CH_4 2018^{-1})	H_{AD} (MJ 2018^{-1})	E_{AD} (kW_e 2018^{-1})
ISR 1	668	24245	229
ISR 6	1077	39088	369

For 2018 alone, theoretically digesting SCGs produced at UiS campus Ullandhaug would have yielded 369.2 kW_e of electrical power and 39088 MJ of heat energy at ISR 6. We can see that the ISR plays a crucial role in the quantity of bio-CH_4 producible (668 m^3 vs. 1077 m^3). By simply shifting the operational ISR from 1 to 6, a 61 % increase in heat and power production can be observed.

From an investments and operational perspective, understanding how the ISR plays a role in deciding the maximum BMP achievable is crucial to maximizing bio-CH_4 production and profits while simultaneously reducing the organic strength (COD) of the waste. The construction and operation of an on-campus AD biorefinery seems unreasonable due to noise, odor, CAPEX, and energy generation issues. However, this does not rule out the need to review and critically asses the cost vs. benefits to draft up suitable alternatives for minimizing SCGs waste on campus for regional biomaterial, biopolymer, or bio-CH_4 production [8], [111]. CAPEX and OPEX feasibility studies for building and maintaining a digester solely for SCGs (or any sole lignocellulosic biomass) has yet to be determine the success and profitability of these digestors. Putting numbers into perspective, SCGs from UiS are only 0.013 % of Norwegian produced SCGs. A rough estimate for the AD of all Norwegian based SCGs (see table 2.5), yields approximately 2873 MW_e (2873000 kW_e) if they were digested at ISR 6. Given this, the complete digestion of SCGs all over Norway at ISR 6 still falls quite shy of the overall power consumption statistics for Norway. Let alone digesting SCGs for a 11,000-student university appears to be an unsustainable method for utilizing these SCGs. SCGs produced on campus can surely be used in other biorefinery process and are not limited to AD, however further research and feasibility assessments are to be considered here too for enhancing SCGs biorefinery approaches for a regional and national circular bioeconomy.

§5 – Conclusion

Coffee is a renowned beverage worldwide. Phenomenal volumes of SCGs are generated from all levels of the distribution and supply chain. The predominantly organic nature of SCGs leaves them as prime candidates for solid-state AD for biogas and bio-CH_4. The BMP for digesting SCGs at incremental ISRs has been tested using anaerobic sludge as the inoculum using the AMPTS II biogas measurement system. It was shown that the ISR plays a critical role in optimizing bio-CH_4 production from SCGs as the concentration of SCGs and its components can be inhibitory towards anaerobic microorganisms at low operational ISRs.

Although some of the results appear to be erroneous, more repetitions are required to concisely conclude over the data. Especially, after COVID – 19 affected batch 2 results. Nonetheless, the experiments conducted were successful in answering the research questions posed in section §1.2.

The following conclusions are made.

- ISR 2 (g $VS_{inoculum}$ g^{-1} VS_{SCGs}) produced the most bio-CH_4 at **1401 ± 137 NmL CH_4**. However, the specific BMP results showed that ISR 6 produced the most bio-CH_4 per g VS of loaded substrate at **503 ± 21 mL $CH_{4,\ STP}$ g^{-1} VS_{SCGs} i.e. (0.87 ± 0.05 g COD g^{-1} COD)** and this is confirmed in its biodegradability potential. **Moreover, the 2nd degree polynomial correlation between the ISR and the attainable BMP suggests that a higher ISR of 6.5 is able to produce more bio-CH_4 under the experimental conditions given in this thesis**.
- COD measurements were wildly varied due to the solid nature of SCGs. **The highest VS reduction was ISR 0.5 at 76.2 ± 12.6%. The highest COD reduction was attributed to ISR 1at 61.6 ± 3.2%.** The COD mass balances for ISR 1 and 2 were considered complete and accounted for all parameters. However, VS/COD reduction and COD mass balances was not calculated for batch 2. **Biodegradability according to CHNSO analysis proved to be highest for ISR 6 at 85 ± 5% with a BMP_{Th} of 593.4 mL $CH_{4,\ STP}$ g^{-1} VS.**
- All modified Gompertz modelling parameters fit optimally with the digestion process returning a regression of 98% and higher, and it reassured that the highest producing reactors were ISR 6. On the other hand, hydrolysis rate modelling showed relative similarity of **k_h in the range of 0.14 to 0.22 day^{-1} for ISRs 1 to 6**. **ISR 0.5 showed a much lower k_h at 0.02 day^{-1} and a VFA/ALK ratio of 0.29 ± 0.05 indicating imminent failure at ISR 0.5 and hence a reduced BMP of 256 ± 11 mL $CH_{4,\ STP}$ g^{-1} VS_{SCGs}.**

- Theoretically, the annual energy production from bio-CH_4 using SCGs produced by UiS and SiS is estimated to be **39088 MJ of heat and 369 kW_e of electrical power for all SCGs digested in 2018.** These numbers are expected to remain relatively stable going forward. **Digestion at ISR 6 over ISR 1 gave a 61% increase in energy output.**

Intrinsically, the biorefinery option is enabled for the bioconversion of SCGs to bio-CH_4 biofuel. Hence, reducing the effect of SCG wastes on the environment and enabling their circular bioeconomy. This comes into play effectively to generate investment through new refined technologies for a more sustainable environment. SCGs are an interesting organic waste with ways to be used other than AD. Researchers should continue investigating this waste for other biorefinery options, other than solid-state AD.

§6 – Future Works/Recommendations

The following recommendations/follow ups for this thesis and its surrounding works are presented.

1. Repetition of ISR 5 to conclusively determine the trend between ISR and BMP. Also, repetition of entire batch 2 reactors to assess COD/VS reduction as well as their COD mass balance.
2. AD of SCGs under continuous reactor conditions to deduce the favorable OLR and HRT.
3. Refining the model proposed by [40], to better deduce BMP_{Th} of lignocellulosic substrates based on their lignin, hemicellulose, and cellulose (LCH) fractions.
4. TPAD of SCGs to enhance process kinetics and limit the generation of inhibitors at low ISRs.
5. Testing various pretreatments on SCGs to allow for a maximum biodegradability and COD conversion to bio-CH_4. ***(This was the next phase in this thesis but was unfortunately cut short due to COVID – 19 related issues).***
6. Optimizing process parameters for a select pretreatment at ISR 6 to further develop and model the changes in BMP according to set pretreatment conditions. ***(This was the next phase in this thesis but was unfortunately cut short due to COVID – 19 related issues).***
7. SWOT analysis/techno-economic analysis/reactor design for generating biogas through SCGs AD biorefineries at UiS or the Stavanger area.

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