Biogas Production by Psychrophilic Anaerobic Digestion and Biogas-to-Hydrogen through Methane Reforming: Experimental Study and Process Simulation

By

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ABSTRACT

This research investigated the early stage of the culture's adaptation during psychrophilic anaerobic digestion (20 ºC) of complex substrates (dairy manure and grass silage) at increasing organic loading rate (OLR; 1-5 g VS/L.d) and total solids content (TS; 7-10%) in batch reactors. The methane yield from mono-digestion of dairy manure was higher at lower OLRs, while in co-digestion of the dairy manure and grass silage, the trend was the opposite. Similarly, the reactors with lower TS content showed higher methane yield in mono-digestion of dairy manure. Introducing grass silage to the bioreactors enhanced the methane yield in all experiments. The substrate degradation by inoculum was confirmed by three kinetic models (first-order, Cone, and modified-Gompertz). The microbiome analysis revealed that the *Bacteroidetes* phylum was dominant, indicating the inoculum's capability to degrade and ferment the organic matter in the complex substrates. An integrated psychrophilic anaerobic digestion with a dry methane reforming plant for green hydrogen production was rigorously simulated using Aspen Plus V11. The results indicated that 48.07 kg/h biogas could produce 8.11 kg/h hydrogen. In addition, the proposed process reduced the CO_2 emission by 398,736 tonnes/year compared to the direct use of biogas for electricity production. The current research provides an assessment of the on-farm biogas plants potential in cold environments. In addition, the developed process simulation platform could be employed to design and optimize the biogas plants.

Keywords: Anaerobic digestion, Psychrophilic, Biogas, Kinetic modeling, Microbiome analysis, Hydrogen, Dry methane reforming, CO_2 emissions

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NOMENCLATURE

Acronyms

ABR	Anaerobic baffle reactor
AD	Anaerobic digestion
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ADM1	Anaerobic digestion model no. 1
AFBR	Anaerobic fluidized bed reactor
ALB	Airlift bioreactor
ARD	Average relative deviation
ATR	Auto-thermal reforming
BG	Biogas
BMP	Bio-methane potential
BR	Batch reactor
C/N	Carbon to Nitrogen
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DEA	Diethanolamine
DMR	Dry methane reforming
GC	Gas chromatograph
GHG	Greenhouse gas
GL	Glucose
HAc	Acetic acid
HE	Heat exchanger
HPr	Propionic acid
HRT	Hydraulic retention time
IN	Inoculum
IPCC	Intergovernmental panel on climate change
ISR	Inoculum to substrate ratio

LCFA	Long-chain fatty acid
LCV	Lower calorific value
LDF	Lester's dairy farm
LHHW	Langmuir-Hinshelwood-Hougen-Watson
MDEA	Methyl-diethanolamine
MEA	Monoethanolamine
MPR	Methane production rate
MX	Mixer
NDF	Neutral detergent fiber
NL	Newfoundland and Labrador
NMP	N-methylpyrrolidone
NRTL	Non-random two liquids
OC	Original culture
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
OTU	Operational taxonomic unit
PC	Principal component
PCA	Principal component analysis
PEG	Polyethylene glycol ethers
PFR	Plug flow reactor
PSA	Pressure swing adsorption
PSM	Process simulation model
RA	Relative abundance
REC	Renewable energy certificate
RIN	Renewable identification number
RWGS	Reverse water-gas shift
RWTF	Riverhead wastewater treatment facility
SEP	Separator
SMR	Steam methane reforming
SMY	Specific methane yield
SP	Splitter

SRT	Solid retention time
STP	Standard temperature and pressure
STR	Stirred tank reactor
TAN	Total ammonia nitrogen
TCOD	Total chemical oxygen demand
TS	Total solids
UAFBD	Up-flow anaerobic fixed-bed digester
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acid
VS	Volatile solids
VOC	Volatile organic compounds
WGS	Water-gas shift

Variables/Letters

$B_{(t)}$	Specific methane yield, NL CH_4/kg VS_{added}
f_d	Maximum specific methane yield, NL CH_4/kg VS_{added}
E_A	Activation energy, J/mol
E_i	Activation energy, J/mol
$F(pH)$	pH growth-modulating function
H	Enthalpy, kJ/mol
k_{0i}	Pre-exponential factor
k_{hyd}	Hydrolysis kinetic constant, 1/d
k_i	Kinetic parameter
K_i	Inhibition constant
K_s	Half-saturation constant
K_{s,NH_3}	Half-saturation constant for total ammonia
LHV	Lower heating value, MJ/kg
$\dot{m}$	Mass flow rate, kg/h
n	Shape factor
P_i	Partial pressure of component i, kPa

P	Pressure, kPa
$\dot{Q}$	Heat flow, MW
R	Universal gas constant, J/mol.K
R_m	Maximum methane production rate, NL CH_4/kg VS_{added}.d
r_A	Reaction rate
t	Time or Hydraulic retention time, d
T	Temperature, K
W	Work, MW

Greek Letters

η	Energy efficiency
λ	Lag-phase duration, d
μ	Biological reaction rate
$\mu_{max}(T)$	Temperature-dependent maximum specific growth rate of microorganisms

Subscripts

BG	Biogas
C	Compressor
DMR	Dry methane reforming reactor
E	Electric
est	Estimation
exp	Experimental
P	Pump
th	Thermal
WGS	Water-gas shift reactor

Chapter One

INTRODUCTION

1.1. Background

Climate change due to environmental pollution is increasing the demand for renewable energy sources. The shortage of fossil fuels, on the one hand, and the environmental concerns related to fossil fuels combustion, on the other hand, push governments, industrial managers, and researchers to look for alternative sources of energy. According to the British Petroleum Annual Statistical Review, the global primary energy consumption grew by 2.9% in 2018, the highest rate since 2010 (British Petroleum, 2019). Consequently, the carbon emission rate has experienced its highest growth rate (33890.8 million tonnes) in the last seven years and increased by 2% in 2018 (British Petroleum, 2019). Figure 1.1 illustrates the share of global primary energy sources over 25 years (British Petroleum, 2020). Despite that oil and coal remained as the primary energy sources, their share decreased over the past decades. Oil and coal provided about 66% of the total energy in 1994, while their contribution to energy consumption decreased to 60% in 2019. On the other hand, natural gas contribution to the global primary energy sources increased from 21% in 1994 to 24% in 2019.

Renewable energy sources include hydro, wind, tidal, solar, geothermal, and biomass. Among the renewable energy sources, the proportion of hydroelectricity contribution in the energy consumption remained relatively constant over the past years, while that of nuclear energy decreased. The renewable sources' contribution to energy consumption showed the most increasing trend; it grew from less than 1% in 1994 to more than 5% in 2019. These facts indicate a constant

effort to use clean energy sources such as natural gas and renewable energies. Renewable energy sources can compensate for the depletion of fossil fuels and alleviate their environmental impacts.

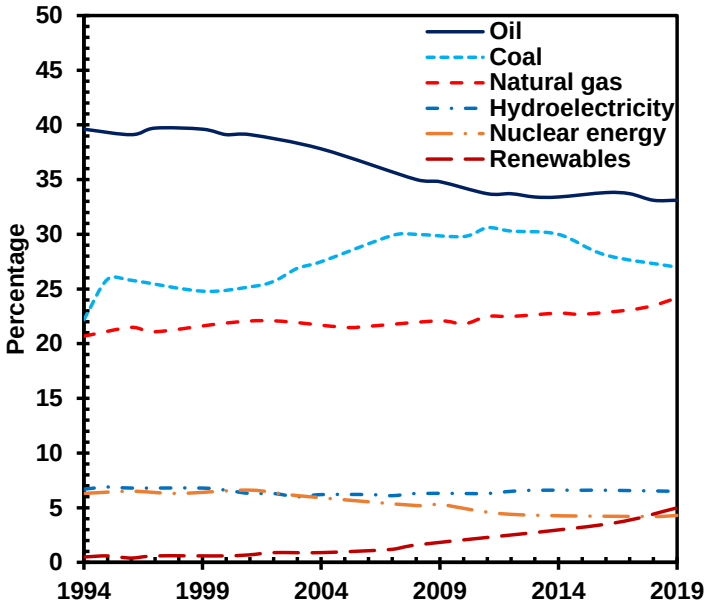

Figure 1.1. Share of various sources in the global primary energy in the period 1994-2019 (British Petroleum, 2020)

Furthermore, the large amount of waste produced by agricultural activities imposes several environmental issues such as soil-, air-, and water pollution, odor nuisance, low crop yields, and degradation of soil quality. Thus, various organic wastes treatments have been employed to reduce the negative impacts of waste disposal. Statistics (Figure 1.2) show that 44% of the global waste generated is food and green wastes (Kaza et al., 2018). This large amount of food and green wastes production implies that waste treatment is essential. About 40% of the waste is disposed of in landfills (Figure 1.3). In comparison, about 19% is processed for material recovery using composting and recycling (Kaza et al., 2018).

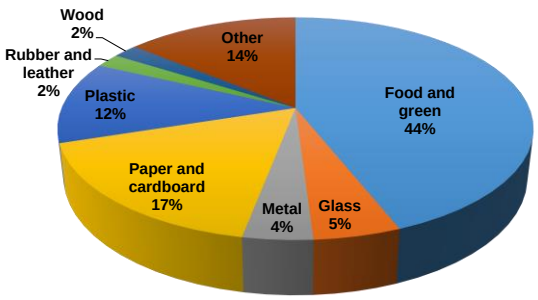

Figure 1.2. Global waste composition by source (Kaza et al., 2018)

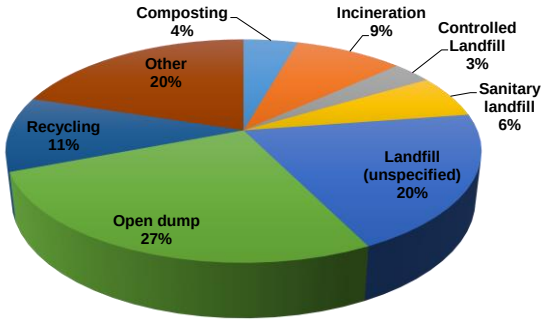

Figure 1.3. Global waste treatment and disposal methods (Kaza et al., 2018)

Figure 1.4 shows the total amount of waste disposal in Canada by provinces and territories. According to Statistics Canada, Newfoundland and Labrador (NL) produced 407,728 tonnes of waste in 2006 (Statistics Canada, 2009) and grew to 499,038 tonnes in 2014 (Dillon Consulting, 2014). Knowing that the amount of waste is increasing incessantly, developing sustainable treatment methods and reuse seems even more important than ever.

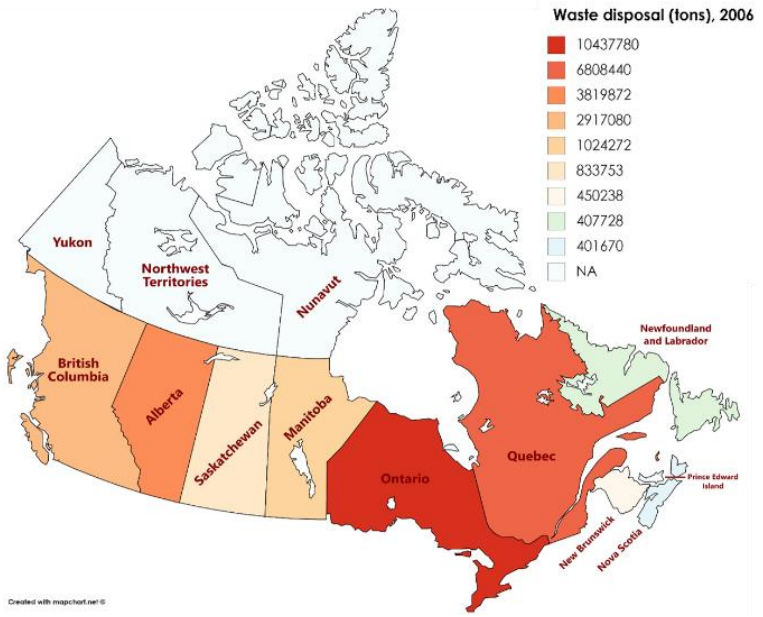

Figure 1.4. Waste disposal in Canada by province and territories (Statistics Canada, 2009)

Anaerobic digestion (AD) is a promising method for renewable energy production, in the form of biomethane, from organic wastes (Petropoulos et al., 2017). Energy production, environmental protection, and nutrients recovery are motives for developing various methods of reusing organic waste (Sadugh et al., 2009; Zabaleta and Rodic, 2015). Carbon, nitrogen, and phosphorus are the main elements that should be recovered (Kjerstadius et al., 2015; Reijnders, 2014; Theobald et al., 2016). Reusing organic wastes is more appealing because of the rising costs of nutrients and the decrease in their global availability.

1.2. Research Problems

The recoverable manure from the livestock sectors in the Atlantic Provinces is around 7,000 tonnes/day; it could generate about 3,325 GJ/day by anaerobic digestion. The electrical energy potential would be 260 MW h/day. If biogas is used to replace heating oil, it could cut 1.2 million tonnes of CO_2 emissions annually from the Canadian emissions. Simultaneously, large titles of methane, one of the most damaging GHGs, could be eliminated from being released into the atmosphere (Helwig et al., 2002).

In Newfoundland, the dairy and poultry farms form the largest farming operations. Compared to other farms, dairy and poultry farms produce the largest amount of manure in NL (Butler et al., 2017). According to the Department of Natural Resources (2013), around 39 dairy producers exist in the province. However, Butler et al. (2017) indicated that NL has only 27 active dairy producers. National statistics indicate that NL has around 5,700 dairy cows. In the Avalon region, about 141 m^3 of dairy manure is produced daily (Dillon Consulting, 2014); other manure types are also produced. For example, the Avalon region also generates about 16 tonnes/day of

poultry manure and 106 m^3/month of mink manure, which could be co-digested with the dairy manure.

Although there is a huge potential of producing biogas, generating electricity, and eliminating carbon emissions from manure storage tanks in NL dairy farms, some obstacles need to be addressed. In this regard, this study addressed several research problems/questions:

- As NL is located in a cold region and the temperature is low, the microorganisms' performance will be diminished if not using an acclimated culture with a sufficient hydraulic retention time. The lower activity of microorganisms leads to lower biogas production. To what extent the low temperature will affect the performance of the anaerobic digestion system in terms of biogas production?
- The performance of the anaerobic digester is directly and strongly connected to the ability of microorganisms to convert organic wastes to desired products. To this end, the microorganisms need to be adapted to the operating conditions of the anaerobic digester. What is the consequence of using an unacclimated inoculum? Are the groups of microorganisms able to hydrolyze the organic waste and produce CH_4?
- The first step of each anaerobic digestion system is to adapt microorganisms. Microorganism adaptation has the most crucial role in methane yield. Microorganisms are adapted based on the specific substrates, and if they are used for another substrate, it is normal to see a significant reduction in the methane yield. The microorganism adaptation process takes long times, months and years. The early stages (early cycles) of the culture adaptation is imperative to continue inoculum acclimation and attain an acclimated culture. What groups of microorganisms present in the culture during the early stages of the culture adaptation?

- Kinetic modeling is a mathematical approach to correlate the experimental data and determine the meaningful parameters of the models. What are the kinetic parameters for psychrophilic anaerobic digestion at NL conditions?
- What is the real potential of NL dairy farms in biogas production and electricity generation?

1.3. The Knowledge Gap and Novelty of this Study

Inoculum adaptation is a crucial step before commissioning and operating a biogas plant. Several factors should be considered in the adaptation of inoculum: (1) source of inoculum (Saady et al., 2012; Wojcieszak et al., 2017); (2) operating parameters (De Vrieze et al., 2015; Ho et al., 2014; Kundu et al., 2014); (3) type of substrate (Agabo-García et al., 2020; Zahedi et al., 2016); and (4) type of process (batch vs. continuous and wet vs. dry) (Güelfo et al., 2010; Kakuk et al., 2017). The inoculum source is an essential factor as each source has a specific community in which specific microorganisms are dominant (Liu et al., 2017b). The inoculum requires adaptation to the operation parameters such as temperature (Ho et al., 2014), organic loading rate (OLR) (Kundu et al., 2014), and concentrations of volatile fatty acids (VFAs) and ammonia (De Vrieze et al., 2015). The effect of the substrate in the inoculum adaptation is also important. When the inoculum is fed a new substrate different from the substrate to which the inoculum is adapted, low biogas production is a consequence. Each substrate has its molecular structure, which needs specific microorganisms to access its digestible portion. The type of process (wet vs. dry) is also important; increasing the solids content to an inoculum adapted to wet anaerobic digestion can negatively affect the process performance.

The temperature affects the digester performance; decreasing the temperature decreases the rate of substrate consumption (i.e., substrate turnover), microbial growth rates, and methane production rate. At psychrophilic temperatures, the rate of hydrolysis decreases, indicating a reduction in the substrate utilization rate and a lower removal of the influent chemical oxygen demand (COD) (Petropoulos et al., 2017; Saady and Massé, 2015). Thermodynamically, a decrease in temperature causes an increase in the energy required to convert propionate and butyrate to acetate since their reactions are endothermic (Lettinga et al., 2001). Moreover, temperature influences methanogenesis and alters the microbial community structure and the degradation pathway of organic matter (Chin and Conrad, 1995; Kotsyurbenko, 2005; Nozhevnikova et al., 2007). Bowen et al. (2014) observed that using mesophilic anaerobic microorganisms in a psychrophilic (<8 ºC) digester lowers the rate of methanogenesis. Psychrotolerant microbial communities could adapt, but they need adequate time; otherwise, it can cause digester failure. Enhanced digester performance would be attained by using an acclimated inoculum. Arikan et al. (2015) showed that operating a digester at 28 ºC could be as effective as a mesophilic temperature (i.e., 35 ºC) using an acclimated inoculum. However, reducing the temperature to 22 ºC decreased the biogas production rate by 30%. Although the inoculum adaptation has a vital impact on the digester performance, it is rarely considered in quantitative studies. The early-stage (the first incubation cycle or HRT) of culture's adaptation is of great importance; there is a lack of information in the accessible literature on the early-stage adaptation of inoculums to the psychrophilic AD of complex substrates.

In addition to experimental analysis, the modeling of the AD process is helpful as it provides useful information on designing and analyzing AD systems. The most common mathematical models developed for AD systems are the Comprehensive Model by Angelidaki et al. (1999) and

the Anaerobic Digestion Model No. 1 (ADM1) by Batstone et al. (2002). These models are mathematical and theoretical and has been widely applied to the AD systems (Jurado et al., 2016; Page et al., 2008; Rivas-García et al., 2013). Implementing these models within simulation tools will provide a comprehensive platform, which is capable of simulating the AD process integrated with other processes (i.e., biogas upgrading and utilization processes). Peris Serrano (2010) and Rajendran et al. (2014) used the concepts of AD from earlier studies and developed the process simulation framework in Aspen Plus simulation tool. However, the previous simulation studies are valid for the mesophilic and thermophilic range of temperatures. The psychrophilic anaerobic digestion process simulation has not been reported yet.

1.4. Scope and Objectives

Dairy farms are important in NL. For example, Lester's Dairy Farm (LDF), St. John's, houses 500-600 cows and generates 10.5 tonnes of wet manure daily. This amount of manure production provides an opportunity for biogas production. An on-farm simple technology for manure-to-biogas conversion will respond to the immediate need of these farms to manage their farm waste in an environmentally friendly manner and reduce their energy bills. In addition, such technology will reduce the current greenhouse gas (GHG) emissions from the open-top manure storage tank and manure spreading on land.

The objective of this study is to investigate the early stage of inoculum adaptation during psychrophilic AD of dairy manure and grass silage. In order to have a successful biogas industry, it is necessary to provide the anaerobic digesters with an acclimated inoculum. The acclimated culture could be obtained from an anaerobic digester operating at the same operating conditions as the proposed AD system. In other words, the AD from which the inoculum is obtained should be

fed with the same feedstock(s), work at the same temperature, and operate at the same range of OLR and TS. It is more feasible and less costly to adapt an unacclimated culture than importing adapted inoculum from other province. This case is considered for remote locations in which no similar operating AD could be found nearby.

This study is for developing a real biogas plant in Eastern Newfoundland. Notice that Newfoundland is an island and that adapted microbial culture is not accessible. Thus, the first step of establishing the biogas plant is inoculum adaptation. The inoculum seed could be obtained from the wastewater treatment facilities, which exist in most urban areas. In this regard, this study used inoculum obtained from a wastewater treatment facility in St. John's. This research aims to assess the potential of biogas production from dairy manure and grass silage using psychrophilic (20 ºC) on-farm wet anaerobic digestion process to convert the organic waste (dairy manure and grass silage) into biogas in laboratory-scale experiments. The early-stage adaptation of the inoculum was investigated and monitored during two cycles of experiments. The first cycle of experiments was conducted at increasing organic loading rate (OLR) to explore the effect of this parameter on the methane yield and the performance of microorganisms. Kinetic modeling and microbial community analysis were studied to explain the initial adaptation stages. The second cycle of experiments was aimed at investigating the effect of TS on the process performance in terms of methane yield and kinetic models. The project focuses on the inoculum adaptation and kinetics of biogas production during the initial stage of the start-up phase.

The research objectives are:

- To develop, adapt, and characterize anaerobic mixed microbial culture to operate at psychrophilic condition (20 ºC) and digest dairy manure and grass silage

- To investigate the effect of digestion of dairy manure alone and co-digestion of dairy manure and grass silage on biogas production
- To evaluate the impact of increasing the organic loading rate (OLR, the mass of volatile solids fed to the digester daily) on biogas yield and microbiome performance
- To evaluate the impact of increasing the total solid content (TS) of the dairy manure and mixture of dairy manure and grass silage on biogas yield and microbiome performance
- To assess the kinetics of biogas production at low temperature through three different kinetic models
- To analyze microbiome during the process to find out the dominant groups of microorganisms which indicate the adaptation of inoculum
- To perform a preliminary analysis on the economics of a proposed biogas plant at Lester's Dairy Farm
- To simulate the anaerobic digestion process using Aspen Plus, involving the kinetic parameters of the fermentation reactions applicable at a wide range of temperatures (10 to 65 ºC)
- To evaluate the potential of hydrogen production from biogas using process simulation of the dry methane reforming process

1.5. Structure of the Thesis

This thesis includes seven chapters. Chapter one contains an introduction, scope and objectives, and thesis organization. Chapter two is a comprehensive literature review and presents

the different aspects of the anaerobic digestion relevant to biogas production from dairy manure. Chapter three presents an experimental study on methane production under increasing OLR and its kinetic and microbiome analyses. Chapter four includes the results of anaerobic digestion experiments for increasing the TS followed by the kinetic analysis. Chapter five presents the economic assessment of biogas production plants using dairy manure in Newfoundland. Chapter six includes the assessment of hydrogen production from biomass through integration of anaerobic digestion and dry methane reforming processes. Finally, Chapter seven provides the conclusions and recommendations.

1.6. Contributions from this Thesis

This thesis contributes to both scientific and practical aspects of the anaerobic digestion systems. On the scientific side, the early stage of inoculum adaptation has rarely been investigated. This study explored the response of the microbial community during the adaptation process by increasing the organic loading rate and the total solids content of the bioreactors. In terms of practical aspects, the results of this study will be used to find the feasibility of constructing an on-farm biogas plant at a typical farm in St. John's. The contribution of the thesis are presented below:

- Abdollah Hajizadeh, Noori M. Cata Saady, Sohrab Zendehboudi (2020). "Evaluating Biogas Potential of Dairy Manure from a Typical Dairy Farm in St. John's Area Using Psychrophilic Anaerobic Digestion," an unpublished project report (pp. 157) to the Department of Fisheries and Land Resources, Government of Newfoundland and Labrador through the Canadian Agriculture Partnership Program.
- Abdollah Hajizadeh, Noori M. Cata Saady, Sohrab Zendehboudi (2020). "Biogas Production Potential of Dairy Manure and Agricultural Wastes in a Typical Dairy

Farm in Newfoundland and Labrador," oral presentation at 70th Canadian Chemical Engineering Conference, 26-30 Oct, Ottawa, Canada.

- Abdollah Hajizadeh, Noori M. Cata Saady, Sohrab Zendehboudi (2020). "Cost Analysis of On-Farm Biogas Plants for Dairy Farms," oral presentation at 1st Virtual Eastern Canadian Symposium on Water Quality Research, 6 Nov, Canada.
- Abdollah Hajizadeh, Noori M. Cata Saady, Sohrab Zendehboudi (2021). "Evaluation of Biogas Production Potential from Dairy Manure and Grass Silage at Low Temperature," oral presentation at 22nd Aldrich Interdisciplinary Conference, 16-25 Aug, Memorial University, Canada.
- Abdollah Hajizadeh, Noori M. Cata Saady, Sohrab Zendehboudi, Andrew S. Lang (2020). "The Early Stage of Culture Adaptation to Psychrophilic Anaerobic Digestion: Kinetics and Microbiome Dynamics", (submitted).

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Chapter Two

LITERATURE REVIEW

2.1. Introduction

This chapter presents a literature review of biogas production's scientific and engineering aspects from biomass through anaerobic digestion. It reviews the background and basics of anaerobic digestion, biogas production, various types of anaerobic digesters, and the inhibition phenomena in anaerobic digestion. The microbiology analysis is also presented to provide the biological aspect of the process. In the end, the biogas upgrading has been briefly reviewed.

2.2. Anaerobic Digestion

Some signs are showing that the Assyrian and Persian people were using biogas to warm bathing water in the 10th century BCE (Lusk, 1998). Later on, the production of flammable gas in lakes, originating from the degradation of organic materials, was observed by Jean Baptiste van Helmont in 1662 (Bond and Templeton, 2011). However, the first research attempts to produce biogas were carried out by Alessandro Volta in 1776; he experimentally found a direct relationship between biomass degradation and gas production (Ferry, 2012). Methane production from cattle manure was first observed by Humphry Davy in 1808, where he confirmed methane production potential from cattle manure through anaerobic digestion (Lusk, 1998).

Anaerobic digestion (AD) is a biochemical process in which the organic materials are degraded in an oxygen-free (anaerobic) environment. It is a well-known process for treating wastewater and industrial organic waste (Lettinga, 1995). It is also used to produce bioenergy from animal manure and agricultural residue (Chynoweth et al., 2001). Among various biodegradable

organic materials, agricultural residue, energy crops, silage, cow manure, and sewage are the most common feedstock for biogas production (Gunaseelan, 1997). Biogas is produced as an end product of the AD process and can be used directly to generate heat energy or electricity. Biogas usually contains around 60% methane (CH_4), 40% carbon dioxide (CO_2), and traces of other gases such as nitrogen, hydrogen, ammonia, and hydrogen sulfide (Chynoweth et al., 2001). Also, it is favored to remove CO_2 from the biogas to produce bio-methane, which can be used for heating purposes or compressed as a vehicular fuel (Holm-Nielsen et al., 2009).

The AD process uses organic matter as feedstock or substrate and produces biogas. This process is performed by a set of microorganisms that convert organic matters biologically into biogas. The microorganisms have a crucial role in the degradation of organic materials and the production of methane/carbon dioxide (Sonakya et al., 2001). The AD process is generally carried out through four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Yuan and Zhu, 2016). Figure 2.1 gives a schematic of the anaerobic digestion process.

2.2.1. Hydrolysis

Hydrolysis is the first step in the anaerobic digestion process. It involves the physical conversion of large organic materials (polymers) such as carbohydrates, lipids, and proteins into monomers and small substances (Adekunle and Okolie, 2015). As a result, carbohydrates are broken down into simple sugars, proteins are degraded into amino acids, and lipids are hydrolyzed to long-chain fatty acids (LCFAs) (Figure 2.1). Hydrolytic bacteria secrete extracellular enzymes to catalyze these parallel degradation processes. The bacteria directly use the soluble products. The hydrolysis step is the most important step when the substrate is complex or hard, such as lignocellulosic biomass, because it controls the rate of the process (rate-limiting step) (Pavlostathis

and Giraldo-Gomez, 1991). Therefore, the hydrolysis step dramatically affects biogas production (El Mashad, 2003; Shrestha et al., 2017). In the hydrolysis step, enzymes play the main role. The enzymes such as cellulase, amylase, protease, and lipase break down large molecules into small molecules.

2.2.2. Acidogenesis

During the hydrolysis step, some large molecules are directly converted to hydrogen and acetate, which methanogens (the methane-producing microorganisms) can directly use to produce methane. However, most of the hydrolysis products are still relatively large and require further conversion to smaller molecules such as acetic acid. In acidogenesis, acidogenic bacteria convert the products of the hydrolysis step to forms usable by methanogens. In this step, simple sugars, amino acids, and fatty acids are degraded to produce acetate, carbon dioxide, and hydrogen. In addition, short-chain volatile fatty acids (VFAs) and alcohols are produced. The acidogenic reactions are given in Table 2.1.

2.2.3. Acetogenesis

Acetogenesis follows acidogenesis; acetogenic bacteria convert the previous step's acidic products (such as propionic acid, butyric acid, and valeric acid) to acetic acid, hydrogen, and carbon dioxide; substrates that methanogens (the methane-producing microorganisms) consume to produce methane. The acetogenic reactions are given in Table 2.1.

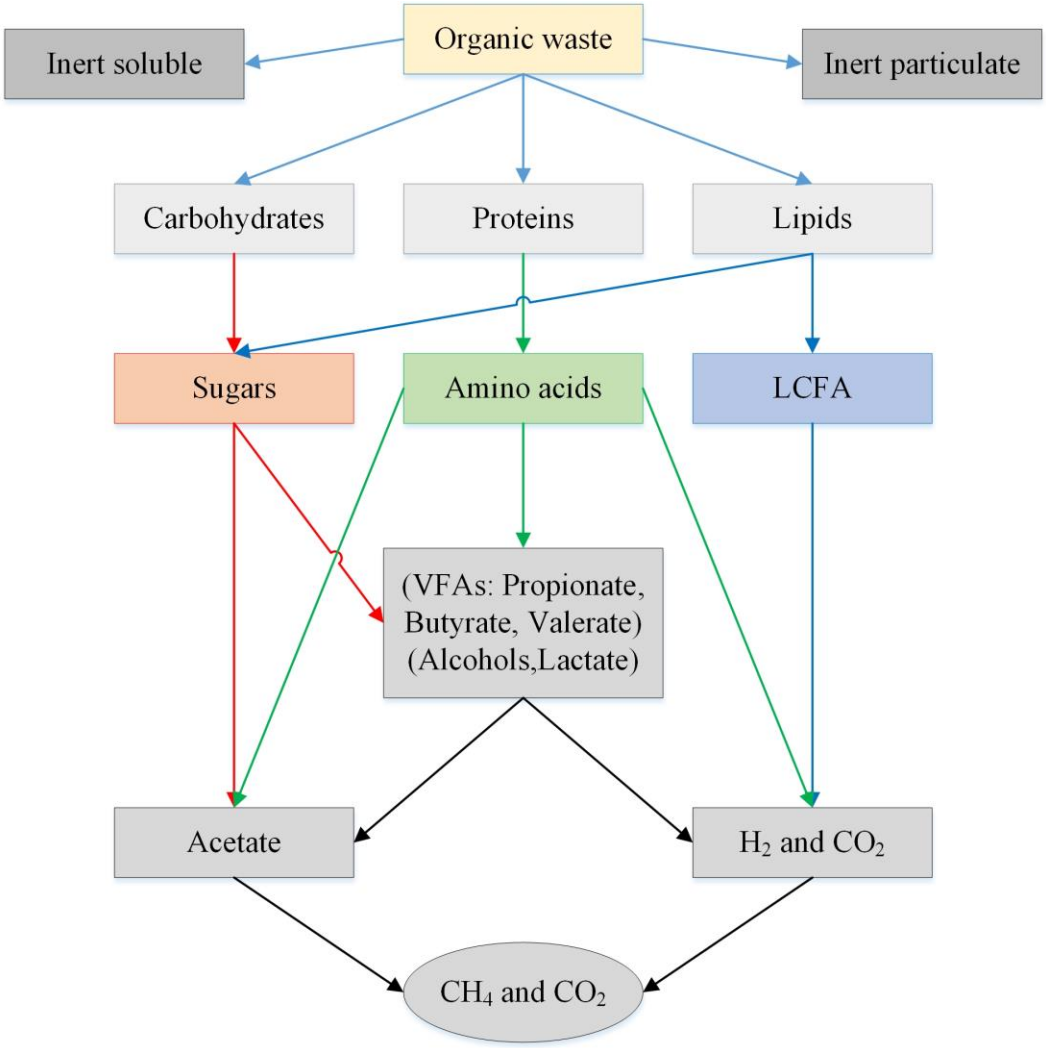

Figure 2.1. Schematic diagram of the anaerobic digestion process (Jha et al., 2011)

Table 2.1. The biochemical reactions involved in anaerobic digestion

Step	**Biochemical reaction**
Acidogenesis	$C_6H_6O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$
Acetogenesis	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$ $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$ $2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$
Methanogenesis	$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COOH + CH_4$ $CH_3COOH \rightarrow CH_4 + CO_2$ $CH_3OH \rightarrow CH_4 + H_2O$ $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ $CH_3COO^- + SO_4^{2-} + H^+ \rightarrow 2HCO_3^- + H_2S$ $CH_3COO^- + NO^- + H_2O + H^+ \rightarrow 2HCO_3^- + NH_4^+$

2.2.4. Methanogenesis

Methanogenesis is the final stage of AD where methane (CH_4) is generated either from VFAs or directly from hydrogen and carbon dioxide. Methane, a completely reduced carbon molecule reduced (i.e., rich in electrons that can be extracted as energy), is an ideal waste product for AD as it is sparingly soluble in water. Once it is generated, it leaves the aqueous phase as gas and does not further affect the microbial ecology. The methanogenic reactions are given in Table 2.1.

2.3. Biogas Production

Anaerobic digestion (AD) produces biogas that can be used to generate energy. Livestock farmers use AD to offset their electricity bills and reduce their farms' carbon footprint. The main products of AD are biogas and digestate. Biogas is used in an engine to produce electricity and heat, whereas the digestate is usually used as a fertilizer, soil amendment, and/or bedding source. A wide range of organic wastes can be fed to the anaerobic digester for biogas production. Lignocellulosic wastes from agricultural and municipal activities are collected in large quantities every day. Animal manure and slurry, sewage sludge and municipal solid waste, and food waste are the most typical wastes used for biogas production. Table 2.2 gives the typical amount of energy production from different sources of waste.

2.4. Overview of Operational Parameters

The performance of AD depends parameters that should be taken into account in the design and operation of an anaerobic digester. The most important parameters involving in the anaerobic digestion process are total solids content, volatile solids content (it refers to the amount of organic matter), and Carbon-to-Nitrogen (C/N) ratio of the substrate, pH and alkalinity, temperature,

hydraulic retention time, organic loading rate, and inoculum-to-substrate ratio. These parameters are described in the following sections.

2.4.1. pH and alkalinity

The pH has a strong impact on the performance of microorganisms inside the digester. Methanogens are highly sensitive to the low pH while high pH leads to the formation of free ammonia (or other toxic agents) (Appels et al., 2008). The pH inside the digester is dynamic; i.e., it is continuously changing by the steps of the AD process.

Table 2.2. Selected researches on the biogas production from various substrates

Substrate	Biogas yield (m^3 CH_4/kg VS)	Reference
Municipal and industrial		
Paper	0.08-0.37	(Owens and Chynoweth, 1993)
Household waste	0.49	(Khoshnevisan et al., 2018)
Municipal solid waste	0.12-0.20	(Benbelkacem et al., 2015)
Organic fraction of municipal solid waste	0.27	(Qian et al., 2016)
Industrial and commercial waste		
Fruit and vegetable waste	0.16-0.35	(Labatut et al., 2011)
Kitchen waste	0.501	(Jiang et al., 2018)
Food waste	0.545	(Li et al., 2018a)
Molasses	0.31	(Angelidaki and Ellegaard, 2003)
Agricultural waste		
Swine manure	0.30-0.51	(Ahring et al., 1992; Møller et al., 2004)
Cow manure	0.15-0.30	(Angelidaki and Ellegaard, 2003)
Poultry manure	0.30	(Braun et al., 2003)
Wheat straw	0.197	(Menardo et al., 2012)
Rice straw	0.182	(Menardo et al., 2012)
Animal and slaughterhouse waste		
Slaughterhouse wastewater	0.25-0.3	(Jensen et al., 2014)
Stomach and gut contents	0.40-0.46	(Ahring et al., 1992)

Hydrolytic bacteria, acidogens, and methanogens have different ranges of optimal pH in which they maintain their maximum rate of reaction. Hydrolytic and acidogenic bacteria can operate in a wide range of pH of 4-8.5, while methanogens maintain their activity in a narrow range of pH from 6.5 to 7.2 (Appels et al., 2008).

Alkalinity is the equilibrium of CO_2 and bicarbonate ions; it provides the medium's resistance against change in pH. Assessing digester imbalance based on alkalinity is more reliable than direct pH measurement. Volatile fatty acids (VFAs) production during acidogenesis decreases the pH. Methanogens produce alkalinity in the form of CO_2 and bicarbonate, which neutralizes this reduction in pH. The CO_2 concentration in the gas phase and bicarbonate concentration in the liquid phase determine the pH value inside the digester. Low alkalinity can be relieved by reducing the organic loading rate (OLR), salt addition to convert CO_2 to bicarbonate, or by direct addition of bicarbonate. Generally, it is recommended to keep alkalinity between 1000 and 5000 mg $CaCO_3$/L for optimum methane production (Metcalf, 2003).

2.4.2. Temperature

Temperature is a critical variable that affects many parameters in the AD process. The temperature affects the microbial growth rate, diversity of microorganisms (Jain et al., 2015), thermodynamic equilibrium, stability, process kinetics (Fernández-Rodríguez et al., 2013), and methane yield (Gil et al., 2018). The anaerobic digester can operate in the temperature range of 20 ºC to 60 ºC. Based on the operation temperature, AD process is called psychrophilic (20 ºC), mesophilic (35 ºC), and thermophilic (60 ºC). Table 2.3 gives the advantages and disadvantages of each temperature range. One more configuration in AD is introduced to use the advantages of each temperature range which is called temperature-phased AD (Fuess et al., 2018).

2.4.3. Carbon to Nitrogen (C/N) ratio

Carbon and Nitrogen contents of the substrate impact the AD process. Carbon provides the required energy source for the anaerobic microorganisms, while nitrogen is responsible for increasing the microbial population. The C/N ratio indicates the amount of total ammonia nitrogen (TAN) released, accumulation of volatile fatty acids (VFA) inside the digester, and nutrient level of feedstock. The C/N ratio is recommended to be between 20:1 to 30:1, while a ratio of 25:1 is the optimum for bacterial growth in the AD process (Khalid et al., 2011). To adjust the C/N ratio of a specific substrate, another substrate is added; this is called co-digestion (Piñas et al., 2018).

Table 2.3. Comparison of various operation temperatures in the AD process

Temperature range	Advantages	Disadvantages
Psychrophilic (5-20 ºC)	• No energy requirement	• Low reaction rate
Mesophilic (30-45 ºC)	• Less energy requirement than thermophilic • Higher process stability • Better biogas quality	• Energy consumption
Thermophilic (45-65 ºC)	• High reaction rate • High growth rate of microbes • Low hydraulic retention time • High biogas production • High pathogens removal	• High energy requirement • Accumulation of propionic acid inhibits the methanogens • Process instability

2.4.4. Total solids content

Total solids (TS) content shows the amount of moisture in the substrate. Water is an important parameter in the AD process because it contributes to the diffusion of soluble nutrients and substrates into the microbial cells. AD process is divided into two types based on the total solids content of the substrate: 1) dry (15-40% total solid); and 2) wet AD (10-15% total solid).

Dry AD has the advantage of smaller reactor volume, less energy and water consumption, and fewer moving parts compared to wet AD. Thus, more than 60% of total installed AD in Europe in 2005 are dry AD (De Baere, 2008). However, in terms of specific methane production and process kinetics, wet AD is more efficient (Zhang and Banks, 2013).

2.4.5. Hydraulic retention time

Hydraulic retention time (HRT) is the average time a water particle takes to pass the distance between the inlet and outlet of the digester. Besides HRT, solid retention time (SRT) is another retention time defined as the average time that bacteria spend in the digester. The retention time is an important parameter in the AD process; it directly influences the number of microorganisms. For instance, methanogens double after 2-4 days (Jain et al., 2015). Process kinetics, substrate type, temperature, and OLR are the most important parameters that must be optimized to have an effective SRT (Zhang and Banks, 2013). Low HRT increases the risk of biomass washout from the reactor which may negatively affect the stability of the entire process. Low SRT has the same problem as low HRT plus VFA accumulation and increasing alkalinity.

2.5. Pretreatments

The first and the limiting step in the anaerobic digestion process is hydrolysis. Different techniques (pretreatment methods) have been developed to facilitate the hydrolysis during the AD. The pretreatment is to make the organic material more available and accessible to the microorganisms. The pretreatment methods could be mechanical, thermal, chemical, and biological. In addition, it is possible to combine these methods to bring the advantages of each method together. A good pretreatment method should: a) retain the organic contents in the waste

biomass; b) improve and facilitate the hydrolysis; c) not produce any toxic material or impose inhibition; d) be environmental-friendly; and e) be economically realistic (Choi et al., 2019; Derman et al., 2018). A brief description of each type of pre-treatment method is provided in the following paragraphs.

2.5.1. Mechanical Pretreatment

The mechanical pretreatment involves changing the physical structure of the substrate. In other words, there is no change in the chemical composition of the substrate during mechanical pretreatment processes. Mechanical pretreatment has the advantage of simple technology; it keeps the substrate in a safe condition because there are no by-products and/or toxic materials produced during the disintegration step (Rodriguez et al., 2018; Tsapekos et al., 2015). The mechanical pretreatment techniques increase the biological kinetic rates (Hansen et al., 2007), reduce the particle size, polymerization, and crystallization, increase the ratio of surface area to substrate volume and pore volume (Jain et al., 2015).

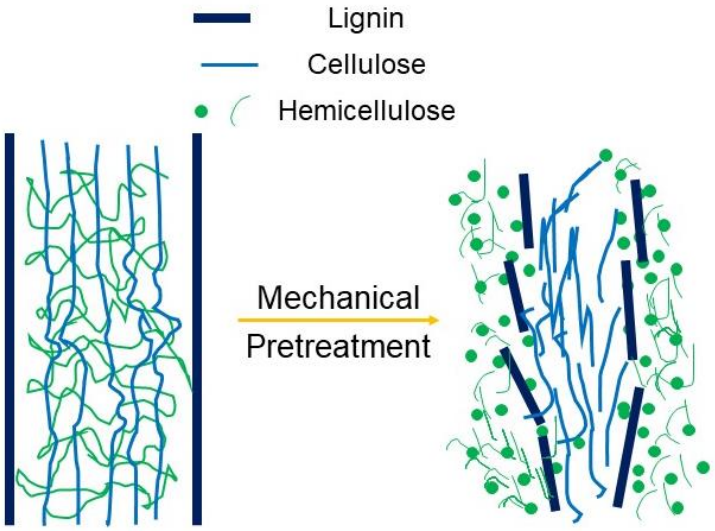

Figure 2.2. Schematic of the effect of mechanical pretreatment on the substrate (Kumar et al., 2009)

2.5.2. Thermal Pretreatment

Thermal pretreatment is the process of heating the feedstock to a specific temperature for a specific time. The thermal pre-treatment provides the feedstock with the following characteristics: making refractory particles soluble (Ariunbaatar et al., 2015; Liu et al., 2012), deflocculating large molecules and dewaterability improvement (Jin et al., 2016; Wang et al., 2010), sterilizing which helps with disinfecting (Bazargan et al., 2015), and decreasing exogenous pollution. It also inactivates methanogenic bacteria in the feedstock.

2.5.3. Chemical Pretreatment

The chemical pretreatment methods use chemical substances to change the molecular structure of the biomass. They include acid-, alkaline-, and oxidative pretreatment, which are described below.

Acid pretreatment: Acid pre-treatment is widely used recommended for substrates rich in lignocellulosic (Mancini et al., 2018). During the acid pretreatment, the hydrogen bonds and van der Waals forces are disrupted by acid. This results in solubilizing hemicellulose, reducing cellulose, and hydrolyzing hemicellulose to produce monosaccharides (Sarto et al., 2019). Dilute acid pretreatment can be used for different agricultural wastes such as agricultural residues, wood chips, crop waste, and paper waste (Mosier et al., 2005). The acids employed for dilute acid pretreatment are H_2SO_4, HNO_3, HCL, H_3PO_4, $C_2H_4O_3$, and $C_2H_2O_4$. The most convenient acid is H_2SO_4 for its availability and low cost.

Alkaline pretreatment: The alkaline pretreatment improves the hydrolytic enzymes accessibility to cellulose and hemicellulose by removing acetyl groups and substituting uronic acid. Besides, the internal surface area will increase, the lignin will disrupt, and bonds between

lignin and carbohydrates will break by alkaline pretreatment. Alkaline reagents such as NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$, and NH_4OH have been used in previous studies (Bazargan et al., 2015). Among these basic materials, NaOH is known to be the most efficient based on COD stabilization and followed by KOH, $Mg(OH)_2$, and $Ca(OH)_2$ for WAS (Kim et al., 2006). Alkaline pretreatment by NaOH decreases the crystallinity and degree of polymerization and increases the biodegradability of the substrate. This advancement is caused by an increase in the substrate's internal surface area during pretreatment by NaOH. On the contrary, using NaOH increases the cost of the process and leads to an inhibition of the AD process because of the high concentration of Na^+ (Neves et al., 2006b). Compared to NaOH, $Ca(OH)_2$ is cheaper, safer, and easier to recover by CO_2 from the AD process.

Oxidative pre-treatment: Applying the oxidative pretreatment results in higher reaction rates in the AD process. It involves exposing the substrate to oxygen or air at high pressure and temperature (10 MPa and 260 ºC, respectively). The most conventional methods for oxidative pretreatment are Fenton, peroxymonosulfate, dimethyldioxyrane, and the activated oxidation process (AOP) (Morone et al., 2018).

2.5.4. Biological Pretreatment

Biological pretreatment is usually used to improve the digestibility of complex substrates. As a result, it breaks the bonds between hemicellulose and lignin and increases the particulate's surface area. The biological pretreatment method is suitable for a wide range of substrates, including grass, wood, paper, lignocellulosic materials, agricultural wastes, and hardwood. Despite being slow and costly, the biological pretreatment methods are environmentally friendly because they do not use chemicals.

2.5.5. Hybrid Pretreatment

Each pre-treatment method highly affects a particulate type of substrate. For example, chemical pretreatment with NaOH influences lignin instead of hemicellulose. Thus, using hybrid pretreatment methods combines the advantages of each method and improves the process of pretreatment. Therefore, the pretreated substrate is at its highest readiness for the digestion process. The more likely advantages are high methane yield, low pretreatment problems, and improved substrate digestion. However, hybrid pretreatment may increase the costs of the process such that an economic feasibility study is required (Abudi et al., 2016).

2.6. Co-digestion

Digesting a single substrate (mono-digestion) has some disadvantages (Mata-Alvarez et al., 2014), which are directly related to the substrate properties. For example, substrates of high nitrogen content release ammonia upon their degradation; a high ammonia concentration inhibits the microorganisms. This issue could be solved by co-digesting another substrate that has low nitrogen content. Co-digestion improves the biogas production rate. The co-digestion can also be categorized as a pre-treatment method because it balances the primary substrate's properties.

The anaerobic digestion of dairy manure alone has shown to be less attractive as it’s biogas production potential per unit mass is low (Zhang et al., 2007b). Thus, dairy manure is usually co-digested with another substrate to enhance digestion process and increase the biogas yield. Generally, the methane yield potential of feedstocks with higher proportion of fats and lipids is more than that of feedstocks containing more proteins and sugars (Neves et al., 2009). Feeding dairy manure as the main substrate in co-digestion helps with mitigating GHG emission and energy production. In addition, dairy manure is a unique organic compound for anaerobic co-digestion

due to its abundance, buffering capacity, and water content (Li et al., 2009c). Ma et al. (2020) carried out a statistical analysis on the effect of co-digesting dairy manure with other feedstocks. Using data from 160 batch experiments, they found that the co-digestion of dairy manure with various feedstocks significantly increases the CH_4 yield (204.1 L CH_4/kg VS in co-digestion of dairy manure and other feedstocks compared to 147.4 L CH_4/kg VS in mono-digestion of dairy manure). The selected studies of co-digestion of dairy manure and other substrates are provided in Table 2.4.

2.7. Anaerobic Digesters

The type of anaerobic digester (reactor) choice depends on the substrate's characteristics, the cost, and the outcome power. The operation of anaerobic digestion can be categorized as a batch or continuous process. Batch reactors are used in the batch processes. On the other hand, various types of reactors are used in a continuous process. The continuous stirred tank reactor (CSTR), plug flow reactor (PFR), and up-flow anaerobic sludge blanket (USAB) are the most convenient continuous reactors. A brief description of each technology is provided in the following sections.

2.7.1. Batch reactor (BR)

The batch reactor (BR) (Figure 2.3) is operated by loading the reactor once at the beginning of the process and the digestate is emptied at the end of the process. The BR can be operated as a sequence batch reactor where the digestate is recirculated, i.e., it is reused in the next cycle as inoculum.

Table 2.4. Selected studies on co-digestion of dairy manure with various feedstocks

Co-substrate	**Reactor type**	**Substrate ratio (dairy or cattle manure : co-substrate)**	**Temp. (°C)**	**TS (%)**	**OLR (g VS/L.d)**	**CH_4 yield (m^3 CH_4/kg VS)**	**Reference**
Grass silage	CSTR (4 L)	70:30 VS	35	4.7	2	0.268	(Lehtomäki et al., 2007)
Meat and bone meal	Batch (1 L)	90:10 VS basis	38	6.74	0.5	0.25	(Andriamanohiarisoamanana et al., 2017)
Food waste	Batch (1 L)	68:32 VS basis	35	NA	NA	0.282	(El-Mashad and Zhang, 2010)
Wheat straw	Batch (1 L)	97.3:2.7 VS basis	35	NA	NA	0.211	(Wang et al., 2012)
Switchgrass	Batch	3:1 TS basis	37	6	NA	0.134	(Zheng et al., 2015)
Glycerine	Continuous	96:4 w basis	35	NA	NA	0.235	(Castrillón et al., 2011)
Aloe peel waste	Batch	3:1 w basis	36	9	NA	0.195	(Huang et al., 2016)
Rice straw	Batch (2.5 L)	2:1 VS basis	37	NA	NA	0.181	(Li et al., 2015a)
Lignocellulosic biomass	Batch (0.54 L)	80:20 VS basis	37	NA	NA	0.189	(Awais et al., 2018)

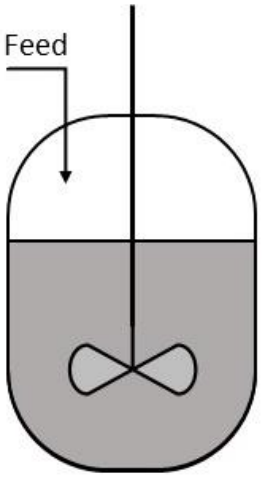

Figure 2.3. A typical stirred batch bioreactor for anaerobic digestion process (Ahmad et al., 2019)

In this process, the BR is filled with microbial culture. Then, the feedstock or substrate is fed to the microorganisms. The reactor acts as a closed system until a specific retention time. The batch reactor consists of a single vessel, which is partly drained at the end of the retention time, and then is filled with fresh feedstock for the next batch. During this reaction period, the amount (concentration) of cells, substrates, nutrients, vitamins, and products vary with time. The fermentation is allowed to run for a determined time, and the products are collected at the end (Carberry, 2001). In addition, due to the different speeds of reactions during the retention time, the gas production peaks at around 50% of the retention time. The residual sludge formed during the previous operation becomes the inoculum for the next batch as this fill-and-draw operation is repeated at intervals (Mao et al., 2015); this operation is called a sequence batch reactor. It is suitable to process a relatively small volume of feed though the operation can be adjusted for different strengths of waste (Zupančič and Jemec, 2010). This type of reactor is used at a laboratory scale for bio-methane potential (BMP) tests.

The anaerobic mixed culture in a batch reactor passes through several growth phases (

Figure 2.4) in response to the substrate and nutrients availability. The phases are:

- **Lag phase:** A delay in the activity (growth, substrate consumption and product generation) of the microorganisms occurs at the beginning of the batch incubation. It is perceived as an adaptation period for the culture to the new conditions.
- **Linear or accelerated growth phase:** During which the growth of the microorganisms proceeds according to straight-line rate kinetics.
- **Log or exponential phase:** In this phase, the microbial cell numbers double per unit time, and their growth curve attains a constantly increasing slope.
- **Stationary phase:** There is no net increase or reduction in cell number in this phase. The cell functions such as energy metabolism and some biosynthetic processes go on.
- **Death phase:** The cells may start to die if the incubation is continued after the bacterial population attains the stationary phase. The cells may die due to starvation or cell lysis.

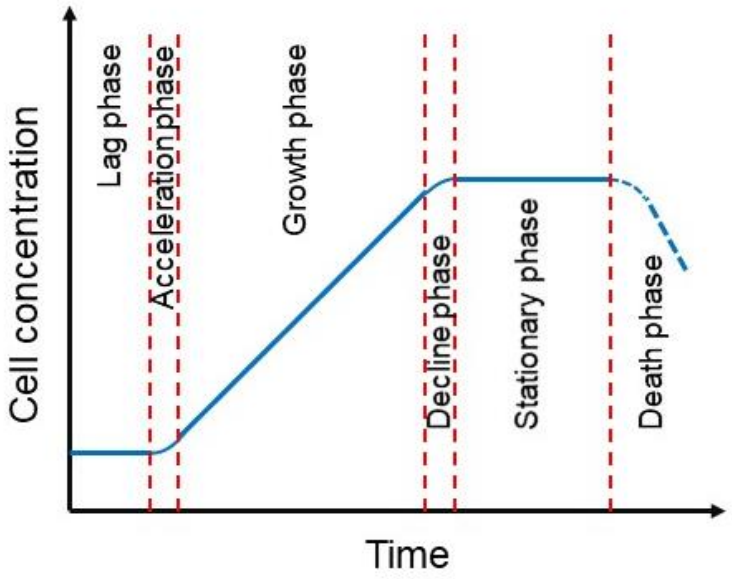

Figure 2.4. Growth phases of the mixed anaerobic culture in batch reactors (Doran, 2013)

The tank is called stirred tank reactor (STR) if it is equipped with an agitator/mixer to mix the reactants (Figure 2.3). Moreover, the reactor could be equipped with a heating and cooling system. These vessels may vary in capacity from less than 1 liter to more than 2,000 cubic meters. The processing capacity depends on the number of feeding- retention-emptying cycles that can be completed in a given period of time (Dague, 1993). The produced gases during the process will discharge through connections in the top. In general, the liquid can be removed from the bottom. The impeller in the STRs is connected to an external motor, which drives the stirrer system. The impellers contribute to mixing and maximizing the interfacial area between the gaseous and aqueous phases (Garcia-Ochoa and Gomez, 2009; Martín et al., 2008). The design of the impeller blades, speed of agitation, and the depth of liquid determine the effectiveness of agitation. The important variables that affect mixing and mass transfer rates are the number and types of the stirrer, speed of stirrer, and gas flow rate. Improved mixing during anaerobic operation enhances performance (Maurina et al., 2014). Batch reactors require inexpensive equipment and are the easiest to operate with little attention since they are fed with feedstock and left for a longer period before being emptied (Khalid et al., 2011). Methane production is initially high and decreases toward the end of the process as the substrate is depleted.

2.7.2. Continuous stirred tank reactor (CSTR)

Continuous stirred tank reactors (CSTRs) (Figure 2.5) are the most commonly used type of reactors in biogas production. In practice, mechanical or hydraulic agitation is required to achieve uniform composition and temperature (Martín et al., 2008). The reactor consists of a rectangular or cylindrical tank with one or more mechanical stirrers, and the reactants are well mixed in a continuous stirred-tank reactor (Mao et al., 2015). However, this type of operation is only possible

for substrates that can be pumped for continuous feeding. Otherwise, a semi-continuous process is applied with a discrete amount of feed several times a day.

Typically, the CSTRs are used to process slurries of < 10% total solids (TS) (Browne et al., 2013). Hence, substrates with total solids > 10% are typically diluted with fresh or recirculated process water or will be co-digested with co-substrates of lower TS content. Consequently, the substrates used in dry digestion have high solid content (25–40% TS). Hence, a different approach regarding waste handling and treatment is needed. The high viscosity in the dry digestion systems makes for inadequate heat and nutrient transfer, which is not the case in wet processes; therefore, mixing is essential to prevent local overloading and acidification (Luning et al., 2003; Wellinger et al., 1993).

Mechanical mixing maintains good contact between the microorganisms and the waste material. However, since conventional mechanical mixers are not appropriate for solid-state processes, recirculation of the waste or re-injection of the produced biogas is usually used to resolve this type of mixing problem (Luning et al., 2003). Sufficient mixing is crucial to prevent accumulation of volatile fatty acids, resulting in souring or acidification of the reactor. Acidification inhibits biogas production (Liao et al., 2006; Ozgun et al., 2013). Mixing also affects microbial flocs' formation, structure, and metabolic efficacy (Jiang et al., 2016). Mixing may be continuous, or intermittent and excessive mixing can reduce the generation of the biogas.

For continuous bioreactor, fresh feedstock is continuously fed, and the products with the culture are removed simultaneously to maintain constant concentrations of nutrients and cells throughout the process (Abbott et al., 2013). A continuous process is used for high-volume production, reactions using gas, liquid, or soluble solid substrates, and processes involving microorganisms with high mutation-stability (Williams, 2002). A chemostat is a typical example

of a continuous reactor. The chemostat is a bioreactor to which fresh feedstock is continuously added, while culture liquid containing leftover nutrients, metabolic end products, and microorganisms are continually removed at the same rate to keep the culture volume constant.

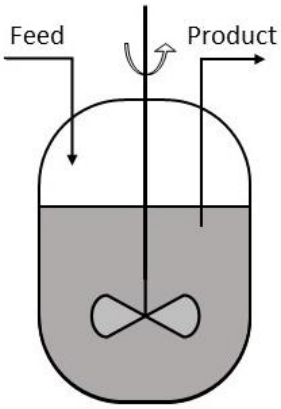

Figure 2.5. A typical scheme of the continuous stirred batch reactor (CSTR) (Doran, 2013)

The CSTR design is often used in a single-stage system where the reactor operates in conditions favorable to both acidogenic and methanogenic microorganisms. Single-stage systems are easy to operate and have lower capital and operating costs (Vandevivere et al., 2003). However, a sequence of biochemical reactions perform the conversion of organic matter to biogas; these reactions do not necessarily have the same optimal environmental conditions. This led to the development of two and multi-stage systems to provide optimal conditions for the different groups of microorganisms participating in the degradation process, leading to higher reaction rates and higher biogas yields (Ghosh et al., 2000). In two-stage reactors (Figure 2.6), hydrolysis/acidification and acetogenesis/methanogenesis are separated. Therefore, the first stage can operate at lower pH, which is more favorable for the growth of hydrolytic and acidogenic microorganisms, whereas the second stage is optimized to favor the growth of methane-forming microorganisms (Ince, 1998).

The CSTR requires more energy than some other types of reactors because of the long retention times, dilute nature of the digesting matrix, mixing, and heating. This is a major disadvantage, but a high concentration of active biomass in the reactor improves the substrate conversion and shortens the required retention time (Wu et al., 2008).

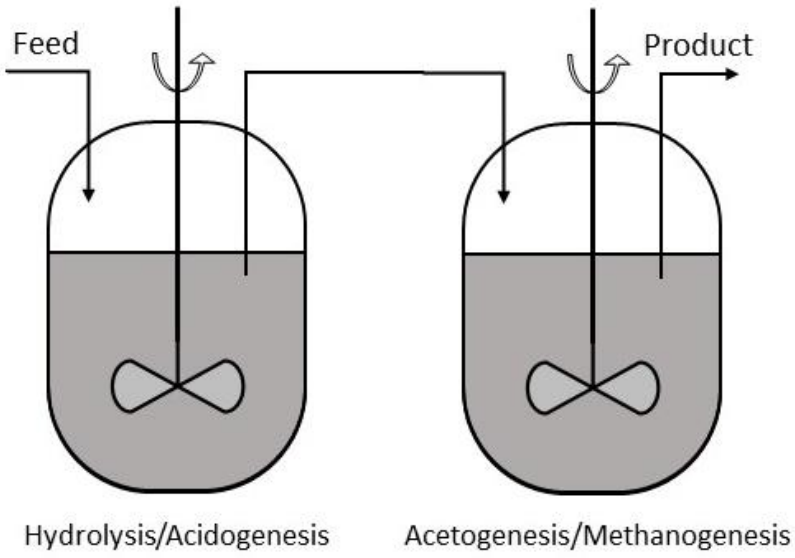

Figure 2.6. A two-stage continuous stirred tank reactor (updated from (Doran, 2013))

2.7.3. Plug flow reactor (PFR)

Plug-flow reactors (PFRs) (Figure 2.7) are typically long rectangular or cylindrical vessels with the substrate entering continuously at one end, flowing in unidirection, and leaving at the another end in a steady-state system. They are also called tubular or piston-flow reactors. In an ideal tubular reactor, the fluids flow as if they were solid plugs or pistons, and reaction time is the same for all flowing material at any given tube cross-section. In PFRs, there is little mixing in the direction of flow. The tanks, or channels, are generally placed above ground, and the concentration of substrates and microorganisms vary throughout the reactor length (Doran, 1995). Tubular

reactors and batch reactors function similarly as they provide high driving force initially with gradual reduction as the reaction continues along the tubes.

The PFR can be used in both mesophilic and thermophilic operations (Kim et al., 2003). Flow in small diameter tubes could be laminar for highly viscous liquids and turbulent for gases with turbulent flow providing better mixing and heat distribution. The heat transfer rate can also be optimized using tubes with larger or smaller diameters arranged in parallel. However, temperature and heat control can result in undesirable temperature gradients, which is expensive to maintain (Purohit, 2012).

PFRs are used for treating various organic wastes, including slurries of animal manure, distillery wastewater, and the organic fraction of municipal solid waste (Rajeshwari et al., 2000; Sharma et al., 2000). The PFRs, when compared to single-stage CSTRs, are generally more efficient in converting the substrate to biogas and are more stable to operate (Mao et al., 2015).

In principle, plug flow configuration can provide local environmental conditions favored by different steps of anaerobic digestions in different parts of the reactor. For instance, hydrolysis may be predominant in the reactor's entry zone, whereas methanogenesis may be the dominant activity near the exit (Pal, 2017).

Furthermore, microbial sludge builds up along the length of the rector due to growth and a high overall sludge content explains both the better efficiency and stability of PFR (Mao et al., 2015). Plug flow reactors with agitators are used to improve local mixing while minimizing mixing in the direction of flow. PFRs are also simple to build and maintain (Lansing et al., 2008).

2.7.4. Up-flow anaerobic sludge blanket (UASB)

An up-flow anaerobic sludge blanket (UASB) reactor (Figure 2.8) consists of a rectangular or cylindrical unmixed tank fed with the waste stream near its bottom. It is characterized by a bed of dense granular sludge confined mainly to the reactor's lower zone where the wastewater enters (Mao et al., 2015).

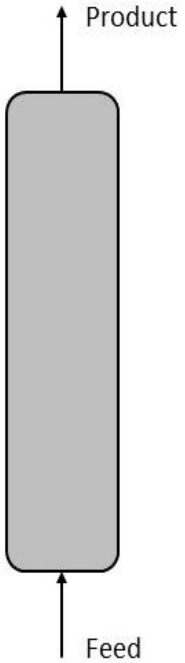

Figure 2.7. A vertical plug flow bioreactor (Doran, 2013)

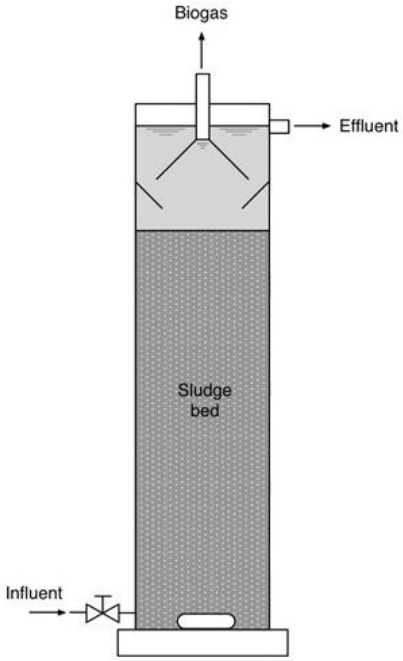

Figure 2.8. Schematic of up-flow anaerobic sludge blanket (Tervahauta et al., 2014)

The up-flow of wastewater and the rising bubbles of biogas keep the granular sludge (culture) suspended (Jiang et al., 2014). The top of the reactor may be expanded or modified to facilitate retention of the granular sludge by the action of gravity. The reactor's performance depends critically on the development and retention of the granular sludge formed through the self-immobilization of microorganisms (Schmidt and Ahring, 1996).

The UASBs can handle high organic loading rates (OLR), especially when compared to the anaerobic fluidized bed reactor (AFBR), but are unable to achieve very high percent COD removal as the anaerobic baffle reactor (ABR), an improved septic tank with series of baffles under which the wastewater is forced to flow (Tilley, 2014). A high sludge load enhances the efficiency and results in short hydraulic retention times (HRT) and high permissible OLR (Ahmad et al., 2011). Start-up periods can be long because of the slow development of granular sludge (Schmidt and Ahring, 1996). Variations in hydraulic loadings can be accommodated within narrow limits, as the sludge granules need to remain suspended without being washed out (Liu and Tay, 2004). Both the quality of the sludge granules formed and the rate of their formation depend on the type of waste being treated (Bhatti et al., 1995).

2.7.5. Airlift bioreactor (ALB)

In the airlift bioreactor (ALB) (Figure 2.9), air flows up the riser tube, forming bubbles, and exhaust gas is released from the top of the column, and the fluid volume is divided by an inner draft tube. This improves circulation and equalizes shear forces in the reactor (Veera and Joshi, 1999). The degassed liquid then flows through the down comer and the product is emptied from the bottom of the tank. The down comer tube can be designed to serve as an internal heat exchanger, or a heat exchanger can be added to an internal circulation loop (Chisti, 1998).

Airlift bioreactor, also known as a tower reactor, uses the expansion of compressed gas for mixing and can be used for both free and immobilized cells. In the absence of agitation, the reactor requires low energy, thereby making it energy efficient.

2.7.6. Anaerobic fluidized bed reactor (AFBR)

Anaerobic fluidized bed reactors (AFBR) (Figure 2.10) are conceptually similar to the expanded granular sludge blanket reactor which permit improved mixing and mass transfer between the sludge granules and the surrounding liquid. However, instead of granular sludge, the AFBR uses relatively heavy small inert particles (e.g., fine sand or alumina) supporting a self-immobilized microbial biofilm (Zhang et al., 2008).

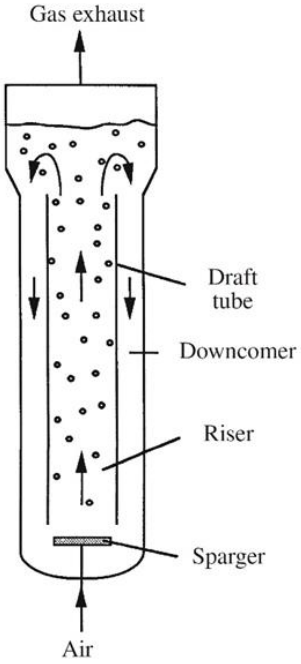

Figure 2.9. A typical scheme of air lift bioreactor (Doran, 2013)

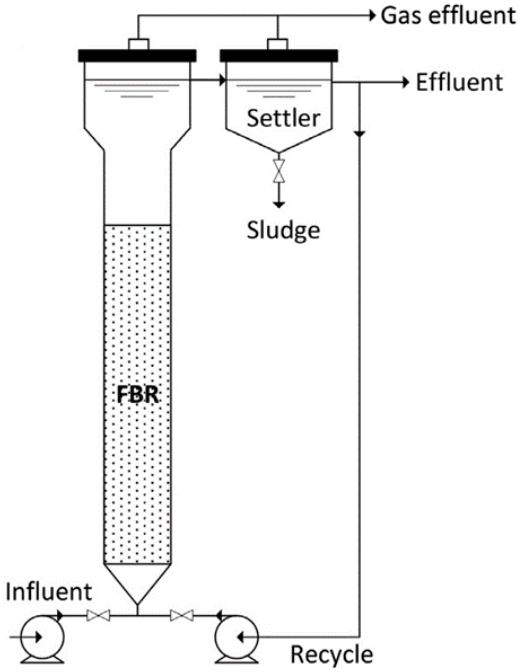

Figure 2.10. Anaerobic fluidized bed bioreactor scheme (Özkaya et al., 2019)

These reactors operate in a continuous state with uniform particle mixing and temperature gradients as particles remain sustained in suspension by a constant up-flow of the wastewater (Mao et al., 2015). They constitute a packed bed with smaller size particles. Thus, the problems of clogging, high liquid pressure drop, channeling, and bed compaction are easily prevented. Good mass transfer of organics to the biofilm is achieved through good mixing and the relatively high

velocity between suspended solids and liquids. Consequently, due to the high biomass loading and good biodegradation activity, the reactors can handle a high organics load and better tolerate inhibitory chemicals (Karadag et al., 2015). Some undesirable properties of AFBRs include increased reactor vessel size, pumping requirements, pressure drop, particle entrainment, erosion of internal components, pressure loss, etc.

The application of various reactors for anaerobic digestion of cow manure and co-digestion of cow manure is presented in Table 2.5.

2.8. Inhibition in Anaerobic Digestion

Process instability and low methane yield are among the major problems experienced in anaerobic digestion, thus preventing this technology's wide acceptability. Various inhibitors have been observed to cause anaerobic digester failure. This is because they are present in a relatively high concentration in the wastes used as feedstock. Appreciable research efforts are presently being made to address these issues and identify the mechanism and the controlling factors of inhibition (Chen et al., 2008). In particular, acclimation and pH can have a strong effect on the inhibitory effects of chemicals at different concentrations (Chen et al., 2008). A gradual increase in inhibitor concentration allows greater time for microorganisms to acclimate than a sudden spike in concentration. The pH can affect the ionization of the inhibitor, thereby affecting the 'active' concentration, which can produce inhibitory effects on the microbiological community.

Table 2.5. Operational and performance data for different bioreactors applied for cow manure

Reactor type and volume	Feed	Temp. (°C)	OLR[a]	HRT (day)	VS or COD removal (%)	CH_4 yield[b]	CH_4 (%)	Reference
Batch (1 L)	CM[c]	35	NA	14	31.1% VS	0.15	NR	(Callaghan et al., 1999)
	CM + Fish offal				47.3% VS	0.37		
Mixed and unmixed	Beef	NR	NR	NR	7.3% unmixed, 9.6% mixed	0.2	NR	(Sadaka and Engler, 2000)
CSTR (3 L)	CM	55, 65	3	15	NR	0.202, 0.165	NR	(Ahring et al., 2001)
UASB (8 L)	CM	37	2.35	22.5	75.5% COD	0.2-0.39	64	(MarañóN et al., 2001)
Batch	CM slurry	21-23	NA	14	36.1% VS	NR	55	(Kalia and Singh, 2001)
UASB (9 L)	CM	55	NR	7.3-22.5	79.7% COD	NR	67.7	(Castrillon et al., 2002)
TPAD system (20 L + 30 L)	Dairy manure	55/35	5.8	14	41% VS	0.21	60	(Harikishan and Sung, 2003)
CSTR (3 L)	CM + Lipid	37	3	15	37% VS	0.224	NR	(Mladenovska et al., 2003)
CSTR (8 L)	CM	50, 60	NR	20, 10	NR	NR	NR	(El-Mashad et al., 2004)
2-phase (0.6 L + 2.4 L)	CM	55, 68	3	3, 12	47.1% VS	0.26	NR	(Nielsen et al., 2004)
1-phase (2 L)	DM[d]	36	5	20	52% VS	NR	NR	(Demirer and Chen, 2005)
2-phase (0.4 L + 1.6 L)			5-6	10	68% VS		50	
Batch (60000 L)	DM	55	6.75	13	NR	NR	56	(Aoki et al., 2006)
USAB (9 L)	CM	37	1.5-3.7e	14	85% COD	NR	NR	(Marañón et al., 2006)
Batch (1 L)	DM	37	NA	NA	NR	0.166	NR	(Amon et al., 2007)
Bench scale	CM	35	1.9	13.8	63% VS	NR	NR	(Karim et al., 2007)
Batch (10 L)	CM	53	NA	17	78% VS	0.184	65	(Omar et al., 2008)

Table 2.5. Continued

Reactor type and volume	**Feed**	**Temp. (ºC)**	**OLR[a]**	**HRT (day)**	**VS or COD removal (%)**	**CH_4 yield[b]**	**CH_4 (%)**	**Reference**
Batch	CM	37	NA	NR	NR	0.168	56	(Dubrovskis et al., 2009)
Batch (1 L)	CM + KW[f]	35	NA	10, 20	52.5, 65.8	0.298, 0.31	NR	(Li et al., 2009b)
Pilot scale batch (128 L)	CM + Whey mix	35	NA	56	74% COD	0.211	51.4	(Comino et al., 2009)
Batch (1 L)	CM + KW	35	NA	12	61% VS	0.297	50.9	(Li et al., 2009a)
Semi-cont. (1.05 L)	CM + A. residue	20, 35	3	20-30	33-51% VS	NR	46.9-56.7	(Alkaya et al., 2010)
Pilot CSTR (1500 L)	DM	37	4.5, 2.3	10, 20	38% VS, 46% VS	NR	67, 70	(Rico et al., 2011)
Batch (1 L)	DM	20		31, 63, 94		0.145, 0.179, 0.238	53, 50, 66	(Saady and Massé, 2013)

a. OLR is in kg VS/m^3.day; b. CH_4 yield in m^3/kg VS; c. CM=Cow manure; d. DM=Dairy manure; e. in kg COD/m^3.day; f. KW=Kitchen waste

The degradation of nitrogenous material found in urea and proteins produces ammonia. Methanogenic populations are the most likely to be damaged by ammonia inhibition (Kayhanian, 1994). Several mechanisms for this inhibition have been proposed, including changes in intracellular pH, inhibition of specific enzyme reactions, or increase of the energy required for cell maintenance (Yenigün and Demirel, 2013). The ammonia concentrations in the anaerobic digestion process are accepted as less than 200 mg/L to avoid ammonia inhibition while providing enough nitrogen as an essential nutrient for the microorganisms (Liu and Sung, 2002). However, at higher concentrations, inhibition occurs. Several studies reported a wide range of inhibitory concentrations ranging from 1.7 to 14 g/L, causing a 50% reduction in methane production (Angelidaki and Ahring, 1994; Bujoczek et al., 2000; Hashimoto, 1986; Kroeker et al., 1979; Sung and Liu, 2003). The inhibitory concentration is affected by pH, temperature, the presence of cations such as sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), and the speed at which ammonia concentration has increased, allowing acclimation of the micro-organisms as mentioned above (Chen et al., 2008).

2.9. Microbiology of Anaerobic Digestion

A consortium of microorganisms catalyzes the bioreactions involved in anaerobic digestion. The microbial culture could be flocculent, suspended, granular, or biofilm. However, the different anaerobic microorganisms interact mutualistically and degrade the complex organic substances to basic products: methane and carbon dioxide. Four distinct types of microorganisms (fermentative, syntrophic, acetogenic, and methanogenic microorganisms) collaborate to convert complex natural organics to CH_4 and CO_2. They produce a balanced intermediatory mix of acidic by-products (e.g.,

acetic acid, propionic acid, etc.), which are ultimately converted to CH_4 and CO_2, and new bacterial cells (CH_7O_2N) (Gerardi, 2003).

2.9.1. Symbiotic relationship among anaerobic microorganisms

Acetogens (microorganisms that produce acetate) collaborate with methanogens (methane-producing microorganisms). Acetate is used as a substrate by methanogens. For example, when ethanol (CH_3CH_2OH) is fed to acetogenic microorganisms, carbon dioxide is utilized, and acetate and hydrogen are produced (Equation (2-1)). The production of hydrogen accompanies acetate production as in Equation (2-1). There is a type of methanogens that uses hydrogen and carbon dioxide to produce methane as in Equation (2-2).

$$CH_3CH_2OH + CO_2 \rightarrow CH_3COOH + 2H_2 \quad (2\text{-}1)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2\text{-}2)$$

2.9.2. Sulfate-reducing Bacteria (SRB)

If sulfates are available, sulfate-reducing microorganisms (SBR), for example, *desulfovibrio desulfuricans*, consume hydrogen and acetate; thus, they compete with methanogens and decrease the methane yield. Sulfate is converted to hydrogen sulfide (H_2S). At substrate-to-sulfate mass ratio < 2, sulfate-reducing bacteria out-weight methanogens for hydrogen. At substrate-to-sulfate proportions of 2 to 3, the competition is between SBR and methanogens. At substrate-to-sulfate proportions > 3, methanogens are favored. The H_2S produced by SBR imposes a more prominent inhibitory impact on methanogens and acetogens.

Methanogens are divided into two groups: hydrogenotrophic methanogens and acetoclastic methanogens: The hydrogenotrophic methanogens use hydrogen and carbon dioxide to produce methane (Equation (2-4)). The acetoclastic methanogens split acetate into methane and carbon dioxide. Hydrogenotrophic methanogens might use the carbon dioxide resulting from acetate splitting to produce methane. Most of the methane in an anaerobic digester is produced from acetate (70%) and hydrogen (30%), Equation (2-3) and Equation (2-4), respectively.

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2\text{-}3)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2\text{-}4)$$

Methanogens are very specific by their substrate. For example, species such as *Methanobacterium formicarium* uses carbon dioxide, formate, and hydrogen, while *Methanobacterium thermoantotrophicum* uses hydrogen, carbon dioxide, and carbon monoxide. *Methanococcus crisis* uses hydrogen, methanol, and methylamine. *Methanococcus mazei* uses acetate, methanol, and methylamine. *Methanosarcina bakery* uses acetate, carbon dioxide, hydrogen, methanol, and methylamine.

2.9.3. Mixed culture fermentation

Many bacterial species coexist in the anaerobic digester. Species such as *Enterobacter*, *Escherichia*, *Erwinia*, *Salmonella*, *Serratia*, and *Shigella*, and *Clostridia* are very common. They ferment carbohydrates to acids (acetate, propionate, butyrate, etc.) and alcohol when the environmental conditions are unfavorable. The mixed nature of the culture provides redundancy

so that the performance would not be affected if one species suffers from any unfavorable conditions.

2.10. Biogas Upgrading and Utilization

The term "biogas upgrading" is defined as a process that is used to remove impurities (H_2O, H_2S, CO_2, etc.) from a biogas stream to obtain a nearly pure CH_4 product. It is also called as biogas purification, biogas enrichment, or biogas cleaning. Biogas primarily comprises CH_4 in a range of 50-70% and CO_2 with concentrations ranging from 30 to 50%. The relative composition of CH_4 and CO_2 depends on the substrate nature and operating conditions such as pH. In addition to these two major components, several components are considered biogas impurities. The most common impurities in biogas are water vapor (H_2O), NH_3, H_2S, N_2, and O_2. In addition to CO_2, all these impurities are unwanted because they reduce the useful biogas heat content, which is normally expressed as Lower Calorific Value (LCV). The LCV of methane is 50.4 MJ/kg-CH_4 or 36 MJ/m^3-CH_4 at standard conditions (Angelidaki et al., 2018). Considering biogas with 60-65% CH_4 content, the LCV will be 20-25 MJ/m^3-biogas. Moreover, these unfavorable components in biogas lead to various operational problems and physical damages to the process equipment. H_2S and NH_3 are toxic and corrosive compounds, and when they go through the combustion engine, the resultant SO_2 will be harmful to the equipment. Thus, there is a necessity for treating the undesirable biogas components to make it as CH_4-pure as possible for subsequent use.

The first treatment is "biogas cleaning" which removes the harmful and toxic materials (NH_3, H_2S, volatile organic compounds (VOCs), and other toxic compounds) from the biogas. In practice, the main target is removing H_2S, and many real biogas plants have the H_2S removal units. After cleaning biogas, the second treatment aims to increase CH_4 content, by removing CO_2, which

leads to higher LCV and is called “biogas upgrading” (Angelidaki et al., 2018). The purified biogas with specifications similar to natural gas is called biomethane, in which the extent of purity of CH_4 in biogas depends on specific country’s regulations and standards. Still, generally, it should be more than 96% (Khan et al., 2017). However, only 5% of biogas was upgraded to biomethane in 2018, and about 65% of biogas was used for electricity production (International Energy Agency, 2020). Thus, the biogas upgrading involves CO_2 removal and/or its conversion to CH_4.

Several technologies have been used for biogas upgrading (Awe et al. (2017), Khan et al. (2017), Miltner et al. (2017)). Generally, these technologies can be categorized as 1) physical and chemical technologies or 2) biological technologies. The physical technologies of biogas upgrading are developed based on the physical separation phenomenon, such as pressure difference and solubility. On the other hand, the chemical methods separate CO_2/H_2S from the biogas stream due to chemical reactions. Both physical and chemical methods are placed in the same category to differentiate them from the biological methods in which live species perform the biogas upgrading. Multiple physical/chemical biogas upgrading technologies (absorption, adsorption, membrane separation, and cryogenic separation (Hosseinipour and Mehrpooya, 2019)) have been developed mainly to remove CO_2 from the biogas.

Absorption is the most common process in the industry for separating CO_2 from flue gas streams. The basic principle of the absorption process is the solubility of various gases in a liquid solvent. In biogas upgrading systems, raw biogas containing CH_4 and CO_2 will contact a solvent counter-currently within a column. Then column can tray- and/or and packing-type. As the solubility of CO_2 is higher, it will be absorbed by the solvent, and the CH_4-rich biogas will leave the column, while the CO_2-rich solvent will then be routed to the solvent regeneration process (Cozma et al., 2013). The solvents used in the absorption biogas upgrading systems could be

physical or chemical. Some examples of the physical solvents are high-pressure water (Cozma et al., 2015) or organics fluids (e.g., methanol, N-methyl pyrrolidone (NMP), and polyethylene glycol ethers (PEG)) (Khan et al., 2017). The CO_2 solubility in PEG is five times higher than its solubility in water (Tock et al., 2010). Amines are the most common chemical solvents, while some other inorganic solvents separate CO_2 from raw biogas. The most common amines are monoethanolamine (MEA), diethanolamine (DEA), and methyl-diethanolamine (MDEA) (Chen et al., 2015b). On the other hand, an aqueous solution of alkaline salts are classified as the inorganic chemical solvents, in which the salt could be potassium, sodium, ammonium, and calcium (Huang et al., 2002). One advantage of the absorption process is its capability of removing CO_2 and H_2S at the same time (Abatzoglou and Boivin, 2009); thus, there might be no need for the biogas cleaning step. However, when the concentration of H_2S is high, biogas cleaning is necessary (Sun et al., 2015b).

The adsorption process is capable of producing high CH_4 concentrations (95-99%). In the adsorption method, the target component (i.e., CO_2) will be transferred from the biogas stream to the surface of an adsorbent material due to physical or Van der Waals forces. The most common adsorption process is the Pressure Swing Adsorption (PSA) (Kim et al., 2015). The PSA process comprises two steps for adsorption and desorption (regeneration). The adsorption step involves adsorption of the desired component on the surface of the adsorbent. When the adsorbent material reaches a specific point in which more adsorption is not possible, it will be bypassed for regeneration purposes. In the PSA process, the adsorption occurs at relatively high pressures and the regeneration occurs by reducing the pressure.

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Chapter Three

EFFECT OF ORGANIC LOADING RATE ON METHANE PRODUCTION IN PSYCHROPHILIC ANAEROBIC DIGESTION OF DAIRY MANURE AND GRASS SILAGE

3.1. Abstract

This study aims to examine the early stage of the culture's adaptation during psychrophilic AD (20 ºC) of complex substrates (dairy manure and grass silage) at increasing organic loading rate (OLR; 1-5 g VS/L.d) in batch reactors. Kinetically, the first-order, Cone, and modified Gompertz models showed that the inoculum degraded the substrates and produced biogas at its early stage of adaptation. The methane yield from mono-digestion of dairy manure was higher at lower OLRs, while in co-digestion the trend was the opposite. The microbiome analysis revealed that the *Bacteroidetes* phylum was dominant, indicating the inoculum's capability to degrade and ferment the organic matter in the complex substrates. Increasing the OLR from 1 to 5 g VS/L.d increased the relative abundance of *Bacteroidetes* in dairy manure-fed samples from 30.5 to 42.9%; however, it decreased from 48.2% to 38.4% in dairy manure- and grass silage-fed samples. Generally, dairy manure increased the culture diversity compared to the inoculum (Shannon Index = 1.65 for dairy manure and 1.18 for inoculum). Shannon Index increased with the OLR in dairy manure-fed samples, but the co-digestate reversed the trend.

3.2. Introduction

This study investigated the first cycle of culture adaptation during anaerobic digestion experiments with varying organic loading rates (OLR). The OLR is defined as the amount of the volatile solids fraction of the substrate fed to the digester per day (Yao et al., 2020). It is considered a key operating parameter in the design and operation of the anaerobic digesters. The high OLR refers to a high methane yield and treatment capacity, but it also means overloading which may cause process instability (Duan et al., 2019), VFA accumulation (Ahring et al., 1995), and foam formation (Kougias et al., 2013).

Implementing AD in cold regions and under ambient conditions could be enhanced by co-digestion with a specific range of OLR. The co-digestion of cow and pig manure at low temperature (18-25 ºC) conditions can be enhanced considering the OLRs up to 4-6 kg VS/m^3.d (Garfí et al., 2011). The upper limit of OLR differs for various substrates. For example, the maximum OLR for anaerobic digestion of swine manure is 0.2 kg VS/m^3.d at operating temperatures below 20 ºC because of the high biodegradability of the swine manure (Hill et al., 2001). Also, the impact of different OLR values on the process performance varies at different temperatures (Tamkin et al., 2015). Tamkin et al. (2015) investigated three OLR values: high OLR (1.3 kg VS/m^3.d), medium OLR (0.8 kg VS/m^3.d), and low OLR (0.3 kg VS/m^3.d) at varying temperatures of the bioreactor. The results showed that by decreasing the operating temperature from 27 ºC to 10 ºC (during 72 days), the digester performance significantly reduced for all reactors leading to 77-94% reduction in biogas production. When the temperature was gradually increased from 10 ºC to 27 ºC during 72 days, the reactor with the low OLR recovered and started to produce biogas as high as before reducing the temperature (100-270 mL/d). However, the high and medium OLR reactors failed to recover and their biogas production remained less than 100 mL/d (Tamkin

et al., 2015). Numerous studies recommended low OLRs for digesters operating at temperatures below 20 ºC (Alvarez and Lidén, 2008; 2009; Kalia and Kanwar, 1998; Khoiyangbam et al., 2004; Safley Jr and Westerman, 1994). Alvarez and Lidén (2008) and Alvarez and Lidén (2009) explored the performance of the anaerobic digesters at low temperature (18 ºC) with a mixture of manures; generally they recommended the OLRs of 4-6 kg VS/m^3.d.

The objective of this study is to investigate the effect of the OLR on the performance of the low-temperature anaerobic digester. The experiments performed in this section are in the first cycle of the culture adaptation. Three kinetic models have been applied to correlate the experimental methane yield data with incubation time. Various statistical analyses have been used to clarify the relationship between the experimental results and kinetic parameters. The molecular biology technique has been implemented to explore the behavior of microbial communities. The microbial community's changes due to varying the type of substrate (glucose, manure, and a mixture of manure and silage) and OLR (1, 2, 3, 4, and 5 g VS/L.d) have been monitored. The principal component analysis (PCA) has been utilized to categorize the response of the microbial community towards the examined factors (e.g., type of substrate, OLR, and TS); this helps determine the dominant phylum/class/species of microorganisms in each reactor. The brief objectives of this chapter are provided below:

- Investigating the performance of low-temperature anaerobic digester fed with dairy manure with varying OLR
- Investigating the performance of low-temperature anaerobic digester fed with dairy manure and grass silage with varying OLR
- Kinetic modeling to explore the kinetic parameters while changing OLR in the bioreactor

- Exploring the microbial community composition under various OLR values and substrates (i.e., dairy manure and the combination of dairy manure and grass silage)

3.3. Materials and Methods

3.3.1. Inoculum

The initial inoculum was obtained from the Riverhead Wastewater Treatment Facility (RWTF), which is a conventional treatment plant in St. John's, NL, Canada. The culture structure was in the form of small black granules, which were disintegrated and converted into flocculent culture upon storage and incubation. The dilute culture's alkalinity was 1700 mg/L as $CaCO_3$ and was adjusted by adding 4.9 g of $CaCO_3$ into 7 L of dilute culture. The final measured alkalinity was 2,595 mg/L as $CaCO_3$.

3.3.2. Feedstock

Fresh manure from dairy cows (M) was collected from the Lester's Dairy Farm (LDF) on the Avalon Peninsula, Newfoundland, from the wet barn and in front of the manure scraper. The manure was transferred into a plastic drum and stored at 4 ºC before being fed to the reactors. The experiments employed chopped (< 2.5-5.0 mm) grass silage (S). The dairy manure and grass silage were subjected to complete physiochemical characterization before they were fed to the reactors (Table 3.1). The characterization results are provided in Appendix A. In addition, the experimental procedure of determining feedstock characteristics is presented in Appendix B.

Table 3.1. Physiochemical characteristics of the substrates

Parameter	Dairy Manure (M)	Grass Silage (S)
pH	6.1	4.1
Total Solids, TS (%)	8.1	33.3
Volatile Solids, VS (%)	4.5	31.3
Acid Detergent Fiber, ADF (%)	33.7	33.0
Neutral Detergent Fiber, NDF (%)	35.0	58.1
Cellulose (%)	1.2	7.2
Hemicellulose (%)	2.23	3.71
Lignin (%)	2.07	1.37
Total Nitrogen (%)	3.24	-
Fat (%)	7.2	1.33
Protein (%)	4.29	4.22
Sugars (%)	-	0.32

3.4. Batch Anaerobic Digestion Tests

Two experiments have been conducted using two sets of duplicate batch reactors have been operated at different increasing OLRs (from 1 to 5 g VS/L.d). The reactors were bench-top air-tight batch reactors. The batch reactor is usueful for conducting characterization studies and investigating the effects of many design and operational parameters. It is simple to operate with the standard protocols are available for test methodology. The system properties, such as microbial growth and degradability could be directly observed and measured (IEA Bioenergy Task 37, 2018). In addition, it is possible to perform several experiments and duplicate experiments at the same time. Batch reactor excludes the effects of some parameters that are associated with the continuous reactors. The bioreactors were 500-mL Wheaton glass bottles with a working volume of 300 mL. Mono-digestion of dairy manure and co-digestion of dairy manure and grass silage (3:1 mass ratio) have been carried out in the batch reactors (Lehtomäki et al., 2007). After adding the inoculum and substrate(s), the reactors have been purged with N_2 gas and sealed to achieve

anaerobic conditions (Figure 3.1). The reactors have been incubated in dark containers to prevent their exposure to light. The bioreactors were maintained at constant temperature (20±1 ºC). The reactors have been manually mixed daily for 1 minute.

Figure 3.1. The culture is purged with nitrogen gas to maintain anaerobic conditions. Notice the black color of the culture and its flocculent consistency

3.5. Experimental Design

Multiple sets of duplicate batch reactors were operated to investigate the effect of the OLR on the methane production rate and methane yield of dairy manure and the mixture of dairy manure and grass silage. The OLR was changed from 1 to 5 g VS/L.d and the reactors were maintained at a constant temperature of 20 ºC. The experimental is given in Table 3.2 and Figure 2.1.

3.5.1. Biogas production measurement

Produced biogas volume was measured daily using a calibrated gas pressure meter (Dwyer Instruments Inc.; model: DPGA-10). Methane (CH_4) production is reported in normalized liters

(NL CH_4), i.e., the CH_4 volume produced was corrected to standard temperature and pressure (STP) (273 K; 1 atm) using Equation (3-1):

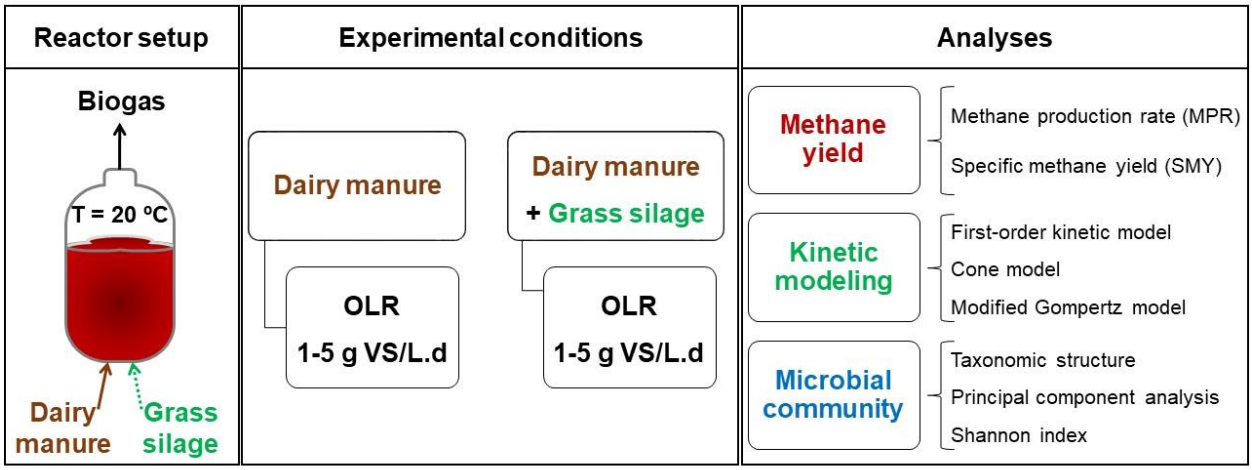

Figure 3.2. Schematic of the experimental setup, conditions, and analyses in the first cycle of culture adaptation

Table 3.2. The experimental design of first cycle of inoculum adaptation at increasing OLR

Test no.	Substrate(s)	OLR (g VS/L)	TS (%)	Label
1	Dairy Manure	1.0	2.8	M1
2	Dairy Manure + Grass Silage	1.0	2.8	MS1
3	Dairy Manure	2.0	3.5	M2
4	Dairy Manure + Grass Silage	2.0	3.5	MS2
5	Dairy Manure	3.0	4.3	M3
6	Dairy Manure + Grass Silage	3.0	4.3	MS3
7	Dairy Manure	4.0	5.0	M4
8	Dairy Manure + Grass Silage	4.0	5.0	MS4
9	Dairy Manure	5.0	5.8	M5
10	Dairy Manure + Grass Silage	5.0	5.8	MS5

$$V_{CH_{4,STP}} = \varepsilon V_m \frac{T_s P_m}{T_m P_s} \quad (3\text{-}1)$$

where, V_m stands for the measured volume of biogas; ε is the percentage of CH_4 in biogas; T_m and P_m are the actual temperature and atmospheric pressure at the time of measurement; and T_s and P_s are the standard temperature and pressure. $V_{CH_{4,STP}}$ is the volume of methane at standard temperature and atmospheric pressure.

The CH_4 volume produced is corrected to the CH_4 produced by the control bioreactors (CH_4 produced in each bioreactor has been calculated by subtracting the amount of CH_4 produced in the controls). The total specific methane yield (SMY) reported in this study is the cumulative specific CH_4 yield. Specific CH_4 yield has been calculated by dividing the CH_4 produced (NL) over the mass of the total VS fed to the bioreactor at the beginning of the experiment.

3.5.2. Biogas composition analysis

Biogas components (CH_4 and CO_2) were determined weekly using a portable biogas analyzer (model: SAZQ) and Agilent gas chromatograph (GC) model 6890. The gas analysis protocol used a column (30 m × 0.32 mm × 3.00 µm). The column and the thermal conductivity detector have been operated at 200 ºC. Calibration was performed weekly with standard gas. The inlet temperature and split ratio were 185 ºC and 30:1, respectively. 200 µL of the sample was injected for analysis. The carrier gas was Helium with a rate of 2.0 mL/min.

3.5.3. Characterization of dairy manure and grass silage

Dairy manure and grass silage have been tested for total chemical oxygen demand (TCOD), pH, alkalinity, and volatile and total solids (VS and TS, respectively) according to the standard methods (Carranzo, 2012) before they have been fed to the reactors. After incubation, mixed liquor

samples have been collected and analyzed weekly for total pH, alkalinity, and VS according to the standard method number 5560D.

3.5.4. Volatile fatty acid analysis

The concentrations of individual volatile fatty acids (VFAs), including acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids, have been measured using Agilent gas chromatograph (GC) model 6890 fitted with Flame Ionization Detector (FID) and equipped with a J&W Scientific DB-FFAP high-resolution column (25 m × 0.32 mm × 0.50 µm). Calibration has been performed using 10, 20, 50, 100, and 200 mg/L of the standard VFAs solution. The inlet temperature was set to be 260 ºC with a 20:1 split ratio of the carrier gas (Helium, 2.4 mL/min) and the sample. In each injection, 0.5 µL of the sample was used. A vial containing a standard solution of VFAs was injected after every 5 unknown samples in each analysis.

3.5.5. Fiber analysis

Fiber analysis has been conducted on dairy manure and grass silage to determine their cellulose, hemicellulose, and lignin contents. Serial extractions have been conducted for neutral detergent fiber (NDF), acid detergent fiber (ADF), and then acid detergent lignin (ADL), respectively. The NDF extraction has been conducted using a sample-containing bag in a soapy water solution. During NDF, soluble cell contents such as carbohydrates, lipids, pectin, starch, soluble proteins and non-protein nitrogen have been washed off while hemicellulose, proteins bound to the cell walls, cellulose, lignin, and recalcitrant materials remained in the bag. During ADF, hemicellulose and bound proteins have been washed off using a 1.00 normal H_2SO_4 and detergent solution while cellulose, lignin, and recalcitrant materials have been left. During ADL,

cellulose has been washed off using 72% H_2SO_4 solution while only lignin and recalcitrant materials have been left. Hemicellulose has been determined as the difference between NDF and ADF, cellulose as the difference between ADF and ADL while lignin has been considered equivalent to ADL (Bauer et al., 2009).

3.6. Kinetic Models

Mathematical models are analytical expressions of system behavior representing the actual system. They can simulate the behavior of any system under investigation under different conditions. Kinetic models reveal changes in the kinetics of microbial cultures due to adaptation. Three kinetic models, including the first-order kinetics (Equation (3-2)), Cone model (Equation (3-3)), and the modified Gompertz model (Equation (3-4)), which are commonly used to model biogas production (Zhen et al., 2016), have been applied to the experimental methane yield data in this study. Each kinetic model has its parameters obtained based on the experimental data and used as indicators for system analysis. In addition, calibrating kinetic models on CH_4 production data generates useful information on culture adaptation. The first-order kinetic and Cone models are intended to determine the cumulative methane yield and the hydrolysis rate of the organic matter. Besides cumulative methane yield and hydrolysis rate, the modified Gompertz model gives evidence on the lag-phase duration.

$$B_{(t)} = f_d \left(1 - \exp\left(-k_{hyd} t\right)\right), \quad t \geq 0 \tag{3-2}$$

$$B_{(t)} = \frac{f_d}{1 + \left(k_{hyd} t\right)^{-n}}, \quad t > 0 \tag{3-3}$$

$$B_{(t)} = f_d . \exp\left\{-\exp\left[\frac{2.71828 R_m}{f_d}(\lambda - t) + 1\right]\right\}, \quad t \geq 0 \tag{3-4}$$

where $B_{(t)}$ is the specific methane yield (NL CH_4/kg VS_{added}); f_d stands for the maximum specific methane yield (NL CH_4/kg VS_{added}); k_{hyd} refers to the hydrolysis kinetic constant (1/day); t introduces the hydraulic retention time (d); n is the shape factor; R_m is the maximum methane production rate (NL CH_4/kg VS_{added}.d), and λ shows the lag-phase duration (d).

3.7. Microbial Community Analysis

Microbial community profiling has performed on nine DNA samples extracted from the samples taken from the bioreactors: Original culture from the wastewater treatment plant (OC); inoculum from the original culture after incubation for four weeks without any feed (IN); glucose-fed culture (GL); dairy manure-fed culture at OLRs of 1, 3, and 5 g VS/L.d (M1, M3, and M5, respectively); dairy manure- and silage-fed culture at OLR 1, 3, and 5 g VS/L.d (MS1, MS3, and MS5, respectively). The extraction of DNA from culture samples has been conducted using QIAGEN RNeasy PowerSoil DNA Elution Kit as per the manufacturer's instructions.

The kit is designed to isolate DNA to recover the total nucleic acid content of the original sample of soli and sludge. The procedure of DNA isolation is given schematically in Figure 3.3.

DNA samples have been sent to the Integrated Microbiome Resource facility (Halifax, NS, Canada), where they have been processed for the determination of community 16S rDNA (regions V4-V5) sequences as described (Comeau et al., 2017) using an Illumina MiSeq. The sequence data have been then analyzed using the CACTUS pipeline (Verhoeven et al., 2018), with each sequence assigned to the appropriate phylum and class. The step-wise procedure is provided below:

- Transfer the RNA Capture Column of the RNeasy PowerSoil Total RNA Kit to a 15 ml Collection Tube

- Add 1 ml of Solution SR8 (is a salt solution that allows for the preferential release of DNA from the RNA Capture Column, leaving residual debris and inhibiting substances in the column) to the RNA Capture Column to elute the bound DNA into the 15 ml Collection Tube. Allow the solution SR8 to gravity flow into the Collection Tube

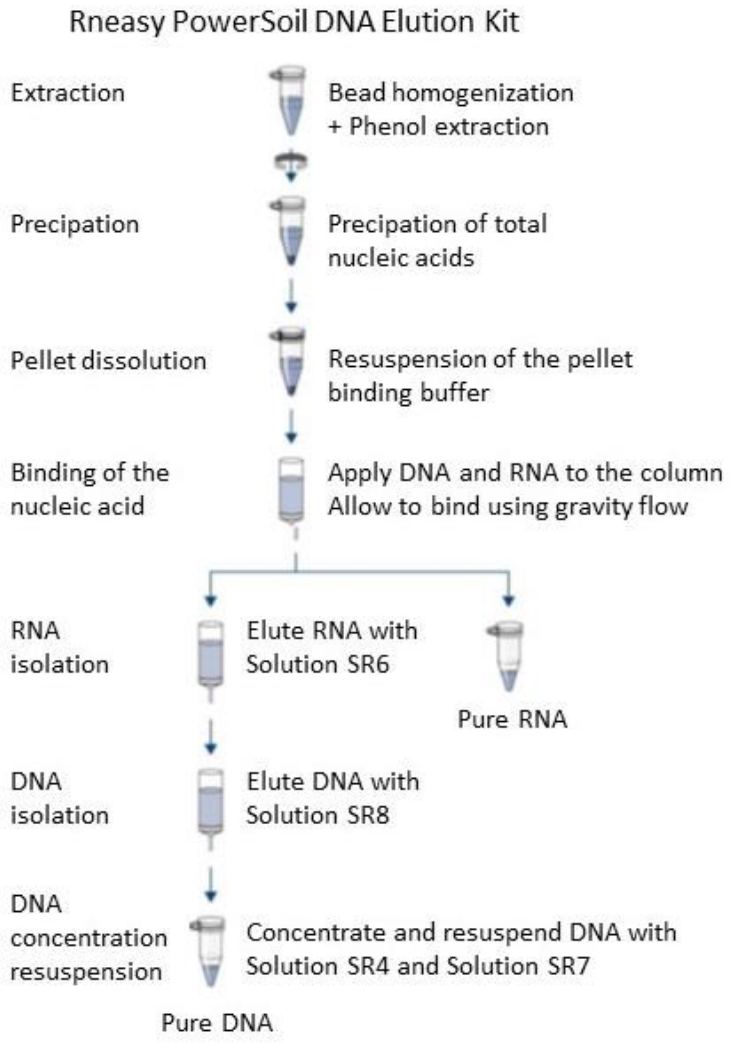

Figure 3.3. The procedure of DNA elution using the RNeasy PowerSoil Kit

- Transfer the eluted DNA to a 2.2 ml Collection Tube (provided) and add 1 ml of Solution SR4 (is 100% Isopropanol). Invert at least once to mix and incubate at –15°C to –30 °C for 10 min
- Centrifuge the 2.2 ml Collection Tube at 13,000 x g for 15 min at room temperature to pellet the DNA
- Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 min to air dry the DNA pellet
- Re-suspend the DNA pellet in 100 µl of Solution SR7 (is RNase/DNase-free water and is used to re-suspend the pelleted, it contains no EDTA)
- The eluted DNA is now ready for downstream applications

Diversity analysis (Shannon Index (Shannon, 1948)) has been performed by calculating the Shannon Index for each sample according to Equation (3-5) where RA_i represents the relative abundance of phylum/class i in the sample.

$$Shannon\ Index = -\sum RA_i \times \ln(RA_i) \quad (3\text{-}5)$$

3.8. Statistical Analysis

The kinetic models' accuracy in predicting the methane yield data with time was measured by the statistical error analysis. The coefficient of determination (R^2; Equation (3-6)) and average relative deviation (ARD; Equation (3-7)) have been calculated to show the accuracy of the kinetic models. Pearson's correlation has been implemented to correlate the experimental ultimate methane yield and the maximum specific methane yield with hydrolysis kinetic constant. The *p*-value associated with Pearson's correlation coefficient indicates the significance of the variable. It

is well accepted that $p < 0.05$ shows a significant relationship while $p < 0.01$ denotes a highly significant relationship between the two variables.

$$R^2 = \left(\frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}} \right)^2 \quad (3\text{-}6)$$

$$ARD = \frac{|B_{exp} - B_{est}|}{B_{exp}} \quad (3\text{-}7)$$

The principal component analysis (PCA) has been carried out to explore possible relationships among different phyla/classes/species in the samples. The PCA method is useful when there are many variables because it reduces the number of dimensions (Behbahani et al., 2017). It creates a new set of variables named principal components (PC), describing as much variation in the data as possible. The Eigenvalue specifies a PC parameter's significance when it is larger than or equal to 1. The PCA's product includes two figures named score plot and loading plot, which should be interpreted together. The score plot shows the score of each sample in the first and second PC domain (PC1 vs. PC2). It identifies each sample's similarity relative to other samples; the closer samples, the more similar they are (Minitab Inc., United States). The possible groups of samples (i.e., clusters) could be observed depending on how they share similar microorganisms.

On the other hand, the loading plot shows the significance of and relationship between variables. The more considerable distance from the plot origin denotes the more substantial influence of that variable. In addition, the variables placed next to each other in the loading plot are positively correlated, and the opposed variables are negatively correlated. The PCA has been conducted using Minitab® v19 software (Minitab Inc., United States).

3.9. Results and Discussion

3.9.1. Effects of the OLR on the mono-digestion of dairy manure

Methane production rate (MPR) and accumulative specific methane yield (SMY) obtained during the initial stage of adaptation from the mono-digestion of dairy manure at different OLRs are reported in Figure 3.4 and Figure 3.5, respectively. The highest MPR has been detected in the first 2 days of incubation in the reactors fed at OLR of 1 g VS/L.d (Figure 3.4). These reactors also exhibited the highest SMY (10.1 NL/kg VS_{added}.d) during the first 2 days of incubation. It has been followed by M2 (2 g VS/L.d), M3 (3 g VS/L.d), M4 (4 g VS/L.d), and M5 (5 g VS/L.d), which produced 8.6, 7.4, 6.3, and 4.8 NL CH_4/kg VS_{added}.d, respectively. After a sharp peak has been observed on day 2 in all reactors, the reactors experienced a declining MPR with some slight fluctuations. The early peak might have resulted from the conversion of readily biodegradable soluble organic matter such as VFAs in substrates. The biodegradability of dairy manure is low as it is a lignocellulosic feedstock in which the portion of easy-to-digest material has already been eliminated by the cow intestine (Zheng et al., 2015). Thus, degrading dairy manure by microorganisms that are in the early stages of acclimation to the substrate and low temperature is limited.

Generally, all reactors reached 90% of their ultimate methane yield during the first 10 days of the tests. As is seen in Figure 3.5, the reactor fed the lowest OLR produced the highest methane yield. The ultimate methane yields (NL CH_4/kg VS_{added}) of reactors M1 to M5 were 28.7, 25.9, 23.8, 20.7, and 18.3, respectively.

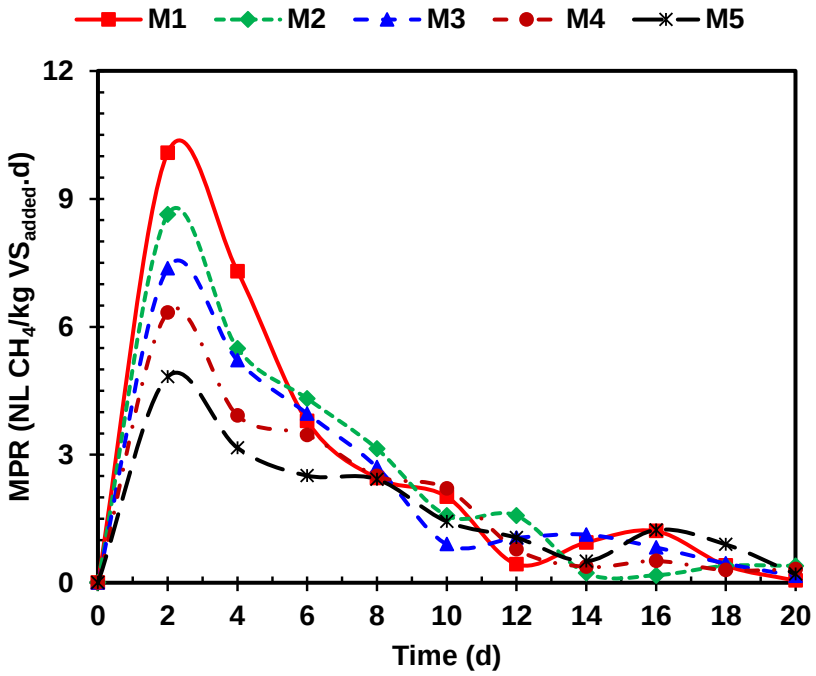

Figure 3.4. Methane production rate (MPR) during psychrophilic mono-digestion of dairy manure (M) with increasing organic loading rates of 1 to 5 g VS/L.d at 20 ºC

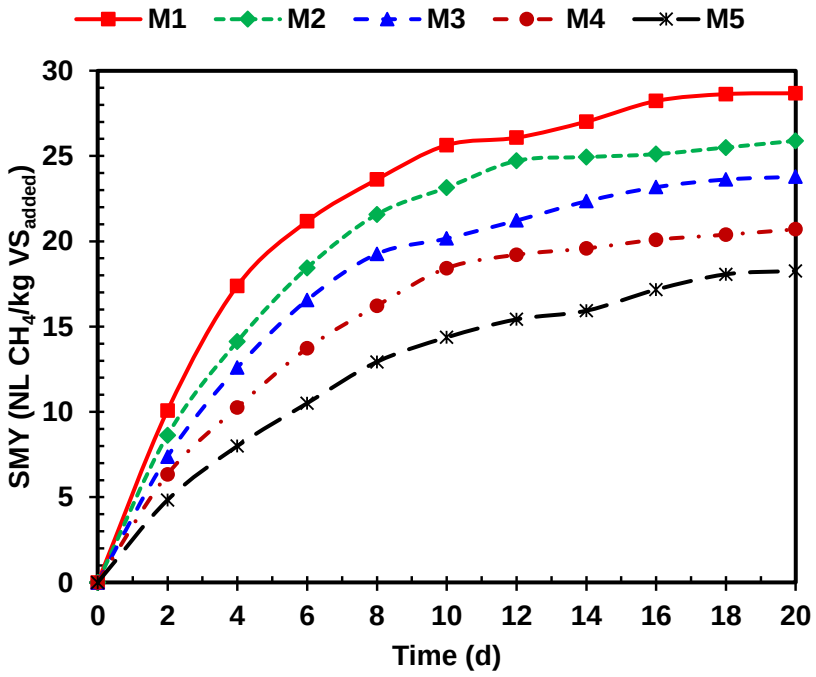

Figure 3.5. Accumulative specific methane yield (SMY) during psychrophilic mono-digestion of dairy manure (M) with increasing organic loading rates of 1 to 5 g VS/L.d at 20 ºC

A range of methane yield could be found in the literature from mono-digestion of dairy manure at psychrophilic temperatures. Saady and Massé (2013) obtained 145±12 NL CH_4/kg VS_{added} from 31 days incubation of cow feces at psychrophilic temperature using a fully substrate-acclimated inoculum. Considering the first 20 days of incubation in Saady and Massé (2013) work, the methane yield was 112 NL CH_4/kg VS_{added}, indicating an effect of the incubation period on methane yield. Martí-Herrero et al. (2015) conducted psychrophilic AD of cow manure using a tubular reactor and obtained a yield of 81 NL CH_4/kg VS_{added} during 80 days of incubation at 16.6 ºC. Alvarez et al. (2006) carried out psychrophilic AD of cow manure at 11 ºC for two hydraulic retention times (HRT) of 20 and 50 days. They fed the reactors with 1.29 kg VS/ m^3.d cow manure and obtained 12.5 NL CH_4/kg VS_{added} during 20 days and 33.6 NL CH_4/kg VS_{added} during 50 days of incubation. The methane yields obtained in the current study are higher than those reported by Martí-Herrero et al. (2015) at 20 days and Alvarez et al. (2006) at 50 days.

Table 3.3 summarizes the results from low-temperature anaerobic digestion of dairy manure from this and previous studies. The methane yield (NL CH_4/kg VS) varies widely (from 6.4 at 20 days HRT (Alvarez et al., 2006) to 237.6 at 94 days HRT (Saady and Massé, 2013)), depending on the different parameters such as temperature, HRT, TS, OLR, and ISR. However, adaptation has rarely been considered in quantitative studies. The inoculum adaptation is a key factor that should be considered in comparing the results. Saady and Massé (2013) used an adapted inoculum for the experiments, while there is no information on the inoculum adaptation stage in other studies.

3.9.2. Effects of the OLR on the co-digestion of dairy manure and grass silage

The profiles of MPR and SMY of co-digestion of dairy manure and grass silage at different OLRs (1, 2, 3, 4, and 5 g VS/L.d) are given in Figure 3.6 and Figure 3.7, respectively. Compared to the mono-digestion of dairy manure (Figure 3.6), a higher maximum MPR was observed on day 2 of the co-digestion (Figure 3.6). In contrast to the mono-digestion of dairy manure, the highest MPR on day 2 was obtained in the reactor that was fed the highest OLR (i.e., MS5). The MPR values were 20.0, 17.8, 14.3, 13.2, and 12.0 NL CH_4/kg VS_{added}.d for MS5, MS4, MS3, MS1, and MS2, respectively. A second lower peak of MPR was observed on day 6 in all reactors; this showed a jump in the microorganisms' performance. Multiple successive peaks of the MPR revealed a difference in the fermentation kinetics. The first peak was likely due to the conversion of pre-existing soluble organic matter in the substrate. Moreover, the higher MPR observed in the co-digestion experiment compared to those observed in the mono-digestion experiment indicate better biodegradability of grass silage and dairy manure than dairy manure alone. Then, the microorganisms were able to consume the easy-to-digest part of grass silage.

The same behavior was observed by Zheng et al. (2015), where multiple peaks were obtained in the co-digestion of dairy manure and switchgrass. These researchers obtained two main peaks when the dairy manure and switchgrass were fed at a 3:1 ratio; the same ratio used in this study. Interestingly, upon increasing the ratio of switchgrass in the substrate, the number of peaks and their sharpness increased (Zheng et al., 2015).

The ultimate methane yield measured in the reactors MS5, MS4, MS3, MS2, and MS1 were 62.0, 58.8, 54.5, 48.8, and 43.5 NL CH_4/kg VS_{added}, respectively (Figure 3.7). Compared to mono-digestion of dairy manure, the co-digestion of dairy manure and grass silage produced more methane at the same OLR. The ultimate methane yield increased by 51.7, 88.3, 129.2, 184.0, and

239.4% in the co-digestion experiments for reactors with OLR 1, 2, 3, 4, and 5 g VS/L.d, respectively.

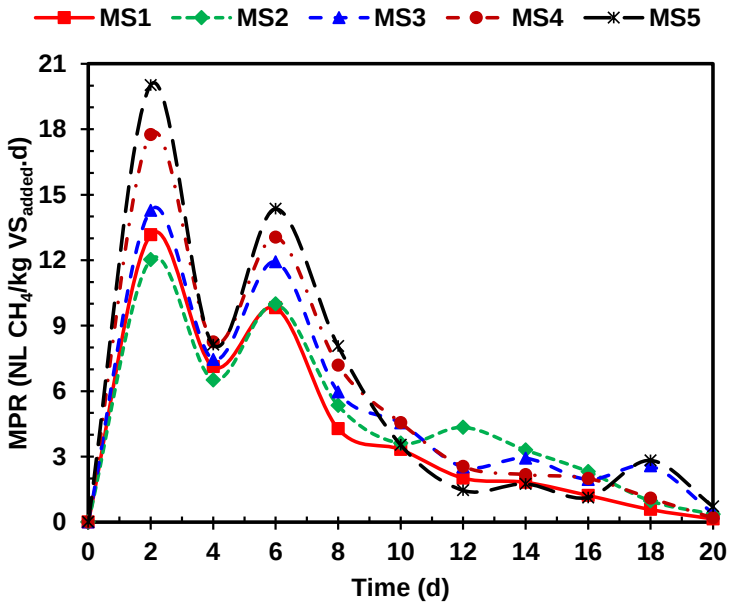

Figure 3.6. Methane production rate (MPR) during psychrophilic co-digestion of dairy manure and grass silage (MS) with increasing organic loading rates of 1 to 5 g VS/L.d at 20 ºC

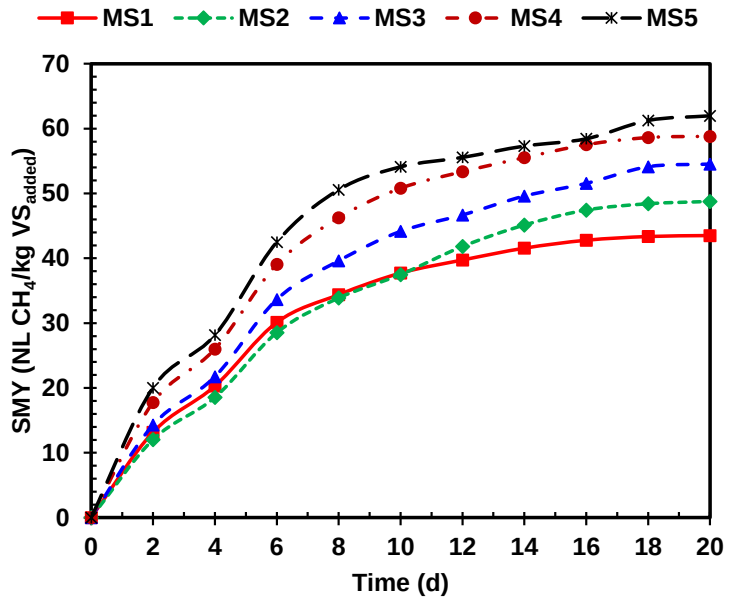

Figure 3.7. Accumulative specific methane yield (SMY) during psychrophilic co-digestion of dairy manure and grass silage (MS) with increasing organic loading rates of 1 to 5 g VS/L.d at 20 ºC

Table 3.3. Comparative performance of low-temperature anaerobic digestion of dairy manure

Substrate	**Year**	**Temperature (ºC)**	**OLR (kg VS/ m^3.d)**	**TS %**	**HRT (day)**	**Methane yield (NL CH_4/kg VS_{added})**	**Reference**
Dairy manure	2020	20	1.0	2.8	20	28.68	This study
			2.0	3.5		25.89	This study
			3.0	4.3		23.79	This study
			4.0	5.0		20.70	This study
			5.0	5.8		18.26	This study
			5.0	7.0		38.12	This study
			5.0	8.0		34.88	This study
			5.0	9.0		28.63	This study
			5.0	10.0		25.58	This study
Cow manure	2015	16.6	0.43	-	80	81	(Martí-Herrero et al., 2015)
Cow manure	2014	16.6	0.26	-	120	108	(Martí-Herrero et al., 2014)
		16.6	0.26	-	120	155.1	
Dairy manure	2013	20	3.04	-	31	145	(Saady and Massé, 2013)
					61	179	
					94	237.6	
Dairy manure	2013	22	1.26	-	6	190	(Ma et al., 2013)
Cow manure	2012	20	0.34	-	90	198	(Ferrer et al., 2011)
Cow manure	2011	25	-	-	90	185.6	(Ferrer et al., 2011)
Cow manure	2009	18	6.2	-	10	12	(Alvarez and Lidén, 2009)
					50	100	
Cow manure	2006	11	1.29	20	20	12.5	(Alvarez et al., 2006)
			3.22	50	20	6.4	
			1.29	50	50	33.6	
			0.52	20	50	24.4	
Dairy manure	1997	23	4.07	1.3	2.3	100	(Powers et al., 1997)

3.9.3. Kinetic modeling with OLR variation

Three kinetic models were used to model the experimental methane yield from anaerobic digestion of dairy manure and grass silage. Table 3.4 presents the kinetic parameters: experimental ultimate methane yield (B_{exp}), the estimated ultimate methane yield (B_{est}), and maximum methane potential (f_d) estimated by the models employed for the reactors fed different OLRs. The estimated f_d decreased with the increase of OLR in dairy manure-fed reactors. Feeding grass silage and dairy manure to the reactors, an opposite trend was observed and the f_d, estimated by first-order kinetic and modified Gompertz models increased with the increase of OLR. However, the Cone model showed a reverse trend in reactors with OLR of 4 g VS/L.d. The R_m estimated by a modified Gompertz model showed a trend of variation similar to that observed for f_d. Mono-digestion of dairy manure in reactor M5 exhibited the lowest R_m (1.79 NL CH_4/kg VS_{added}.d); this indicated difficulty in accessing the digestible portion of the lignocellulosic material in dairy manure (Zheng et al., 2015). The highest R_m was obtained by reactor MS5 (7.52 NL CH_4/kg VS_{added}.d), which showed the significance of adding grass silage to dairy manure. Moreover, no lag-phase (λ) was experienced in both mono-digestion and co-digestion experiments. It should be noted that the Cone model estimated the highest f_d among all models. In summary, the results indicated that co-digestion of dairy manure with grass silage highly enhanced dairy manure biodegradability and methane production rate (Yangin-Gomec and Ozturk, 2013). The hydrolysis reaction rate constant (k_{hyd}) indicates the biodegradability of a substrate and the efficiency of digestion. It is believed that hydrolysis is the rate-limiting step in the anaerobic digestion process most of the time, especially at low temperatures, which leads to poor substrate degradation and methane production (Rebac et al., 1999; Vavilin et al., 2008). The present study showed that high k_{hyd} led to high methane production (Figure 3.8 to Figure 3.11). This trend supported the importance of hydrolysis

in the anaerobic digestion (Ge et al., 2011). Figure 3.8 presents the changes in B_{exp}, $f_{d,}$ and k_{hyd} with the OLR in the reactors fed with dairy manure. Increasing the OLR decreased the values of B_{exp}, $f_{d,}$ and k_{hyd} (Figure 3.8). The highest k_{hyd} value (1/d), i.e., 0.220 for the first-order kinetic and 0.285 for the Cone model, corresponded to the reactor M1. The k_{hyd} of the first-order kinetic model decreased to 0.201, 0.185, 0.173, and 0.131 1/d for M2, M3, M4, and M5, respectively, upon increasing the OLR. The same trend is detected by the Cone model when the k_{hyd} decreased to 0.262, 0.233, 0.220, and 0.180 1/d for M2, M3, M4, and M5, respectively.

The variation of B_{exp} and f_d with the k_{hyd} reveals the significance of hydrolysis rate in the fermentation process. Figure 3.9 shows that by increasing the k_{hyd}, the B_{exp} and f_d also increased. For the first-order kinetic model, when k_{hyd} increased from 0.131 to 0.22 1/d, f_d increased from 19.59 to 28.79 NL CH_4/kg VS_{added}. The Cone model detected the same improvement; when k_{hyd} increased from 0.18 to 0.285 1/d, f_d also increased from 21.27 to 31.64 NL CH_4/kg VS_{added}. Although many studies reported the same trend for the effect of hydrolysis on B_{exp} and f_d (Ge et al., 2011), there are studies showing that a higher k_{hyd} does not always mean a higher methane production (Fernández-Rodríguez et al., 2014; Zhen et al., 2016). Moreover, it was reported that the temperature has a great impact on hydrolysis (Donoso-Bravo et al., 2009); higher temperatures lead to higher k_{hyd} (Ge et al., 2011).

Table 3.4. Predicted parameters of three kinetic models for mono-digestion of manure (M) and co-digestion of dairy manure and grass silage (MS) with increasing OLR

Parameter	**Manure**					**Manure and Silage**				
	M1*	**M2**	**M3**	**M4**	**M5**	**MS1****	**MS2**	**MS3**	**MS4**	**MS5**
Experiment										
$B_{exp}\ (NL/kg\ VS_{added})$	28.68	25.89	23.79	20.70	18.26	43.50	48.76	54.52	58.79	61.97
First-order Kinetic Model (Equation (3-2))										
$B_{est}\ (NL/kg\ VS_{added})$	28.44	26.02	23.72	20.88	18.17	48.29	49.85	54.86	59.71	61.69
$f_d\ (NL/kg\ VS_{added})$	28.79	26.50	24.32	21.56	19.59	54.45	55.23	58.93	62.25	63.53
$k_{hyd}\ (1/d)$	0.220	0.201	0.185	0.173	0.131	0.109	0.116	0.134	0.160	0.177
Cone Model (Equation (3-3))										
$B_{est}\ (NL/kg\ VS_{added})$	28.79	26.17	23.92	20.99	17.52	45.82	49.95	54.76	59.94	62.10
$f_d\ (NL/kg\ VS_{added})$	31.64	28.61	27.06	23.76	21.27	64.69	64.95	67.29	69.03	70.24
$k_{hyd}\ (1/d)$	0.285	0.262	0.233	0.220	0.180	0.124	0.133	0.159	0.201	0.224
n	1.33	1.43	1.32	1.37	1.20	0.98	1.23	1.27	1.36	1.36
Modified Gompertz Model (Equation (3-4))										
$B_{est}\ (NL/kg\ VS_{added})$	27.55	25.27	22.92	20.25	17.54	42.75	48.21	52.94	57.82	59.77
$f_d\ (NL/kg\ VS_{added})$	27.56	25.31	22.97	20.32	17.74	42.91	49.06	53.49	58.11	59.95
$R_m\ (NL/kg\ VS_{added}.d)$	4.21	3.50	3.02	2.49	1.79	5.18	4.56	5.49	6.73	7.52
$\lambda\ (d)$	0	0	0	0	0	0	0	0	0	0

* M1 to M5 denote the reactors fed with dairy manure changing OLR 1 to 5, respectively.

** MS1 to MS5 represent the reactors fed with dairy manure and grass silage varying OLR 1 to 5, respectively.

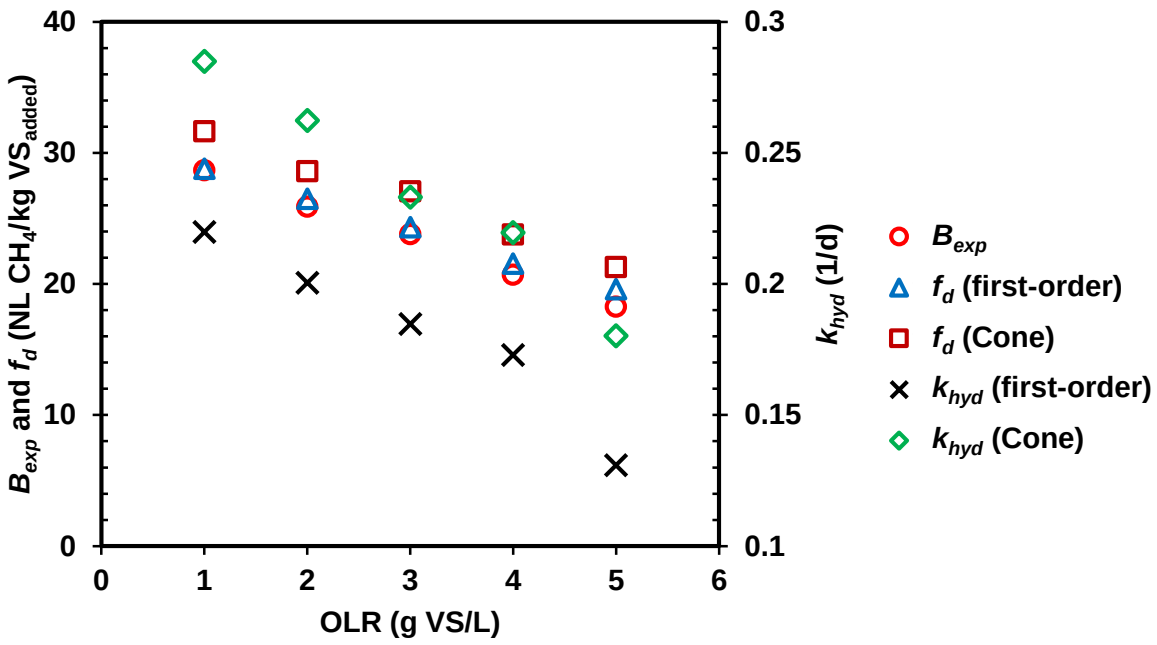

Figure 3.8. Relationship of the experimental ultimate methane yield (B_{exp}), maximum methane potential (f_d), and hydrolysis reaction rate constant (k_{hyd}) with the organic loading rate for mono-digestion of dairy manure

Pearson's correlation was conducted to relate the variations of B_{exp} and f_d with k_{hyd}. The results (Figure 3.9) show that the Pearson's correlation positively related the B_{exp} and f_d with k_{hyd}. The Pearson's correlation coefficient (R_P) values obtained for the first-order kinetic and Cone models in predicting the experimental ultimate methane yield (B_{exp}) were 0.9690 and 0.9857, respectively. The R_P values from first-order kinetic and Cone models for prediction of maximum methane potential (f_d) were 0.9633 and 0.9817, respectively. Despite having the R_P for the first-order kinetic model lower than that of the Cone model for both B_{exp} and f_d, the p-values obtained based on both the first-order and Cone models showed the significance of the relationship between the studied variables (B_{exp} and f_d with k_{hyd}). The p-values obtained for the first-order kinetic and Cone models in predicting B_{exp} are 0.0065 and 0.0021, respectively. Similarly, both

first-order kinetic and Cone models showed a significant relationship between k_{hyd} and f_d, having p-values equal to 0.0084 and 0.0030, respectively.

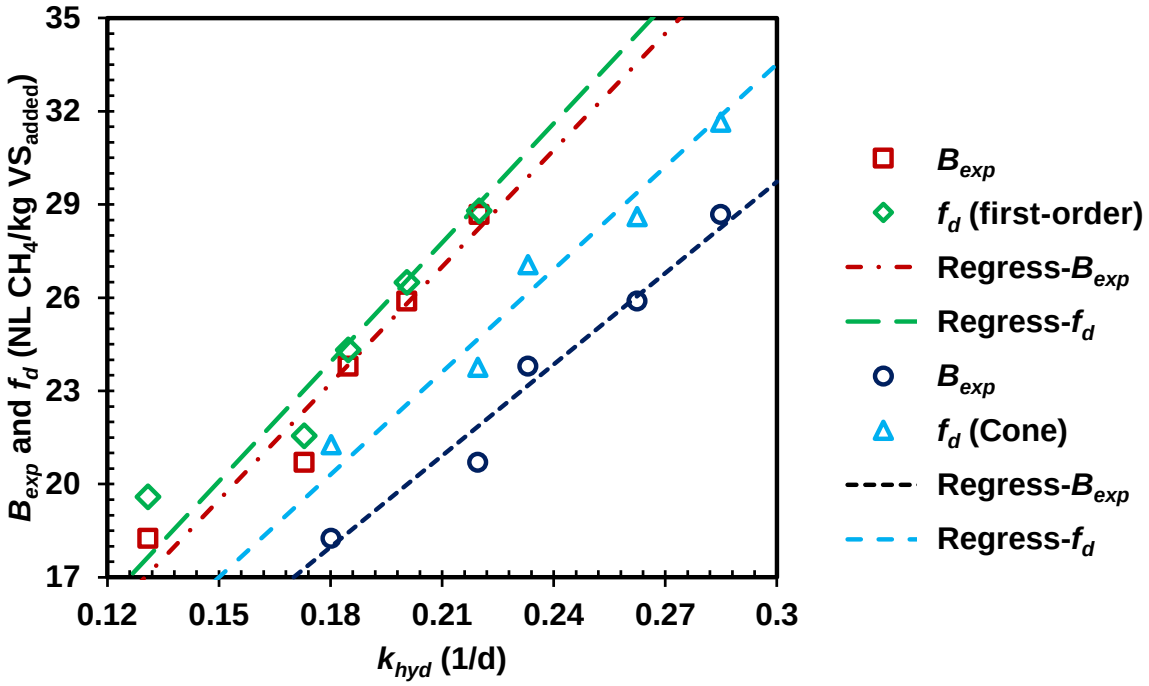

Figure 3.9. Pearson's correlations of the experimental ultimate methane yield (B_{exp}) and maximum methane potential (f_d) against hydrolysis reaction rate constant (k_{hyd}) for mono-digestion of dairy manure

Figure 3.10 presents the relationship of B_{exp}, f_d and the k_{hyd} with OLR for the co-digestion of dairy manure and grass silage. Unlike the digestion of dairy manure alone, B_{exp}, f_d and the k_{hyd} for co-digestion increased with the increase of OLR. The highest k_{hyd} (1/d) was obtained for MS5, i.e., 0.177 and 0.224 for the first-order kinetic and Cone models, respectively. However, comparing k_{hyd} in mono-digestion and co-digestion experiments at the same OLR revealed that the values of k_{hyd} for the co-digestion were lower than its values in the corresponding mono-

digestion reactors. In other words, despite that the magnitudes of B_{exp} and f_d in the co-digestion were higher than their magnitudes in the mono-digestion, the value of k_{hyd} in the co-digestion was lower than its value in the mono digestion. This observed behavior supports the conclusion that a higher k_{hyd} does not always mean a higher methane yield, i.e., in this case, the hydrolysis rate is not the limiting step (Neves et al., 2006a).

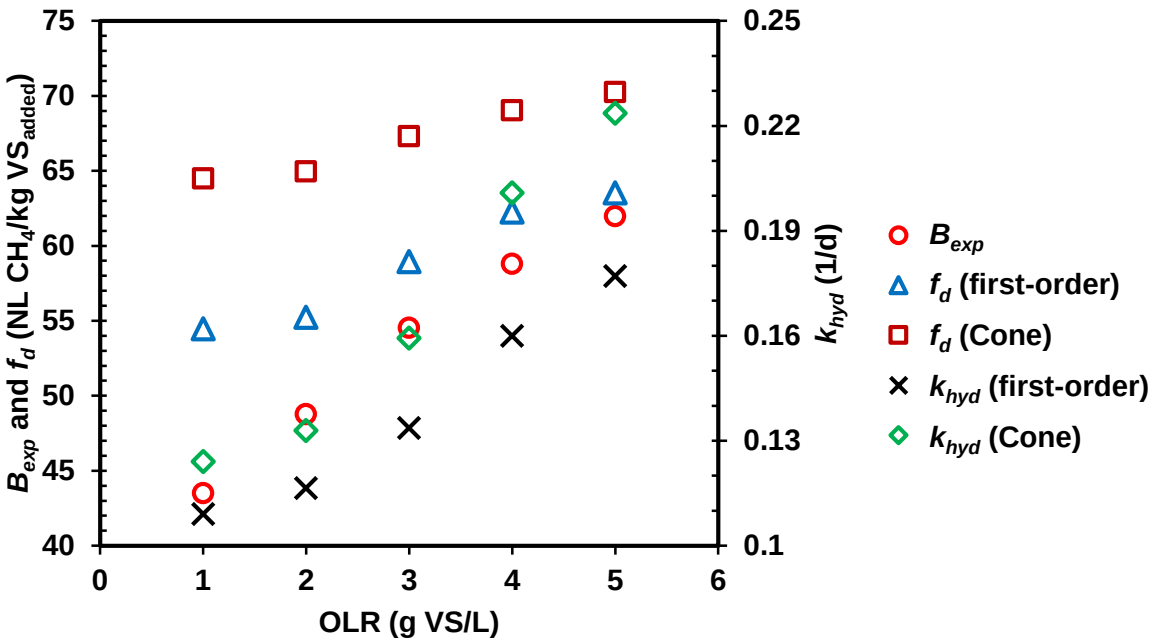

Figure 3.10. Relationship of the experimental ultimate methane yield (B_{exp}), maximum methane potential (f_d), and hydrolysis reaction rate constant (k_{hyd}) with the organic loading rate for co-digestion of dairy manure and grass silage

The variation of B_{exp} and f_d with the k_{hyd} for the co-digestion of dairy manure and grass silage is shown in Figure 3.11. Similar to the digestion of dairy manure alone, the B_{exp} and f_d of the co-digestion showed an increasing trend with the increase in k_{hyd}. Pearson's correlation detected a strong direct (positive) relationship ($R_P > 0.96$) for all pairs of variables (B_{exp} with k_{hyd}

and f_d with k_{hyd}). The R_P for first-order kinetic and Cone models in predicting B_{exp} are 0.9687 and 0.9656, respectively; and in correlating the two models with f_d are 0.9880 and 0.9906, respectively. Again, a significant correlation between variables was indicted by the *p*-values, which were 0.0066, 0.0076, 0.0016, and 0.0011 for first-order kinetic and Cone models correlations with B_{exp} and first-order kinetic and Cone models correlations with f_d, respectively.

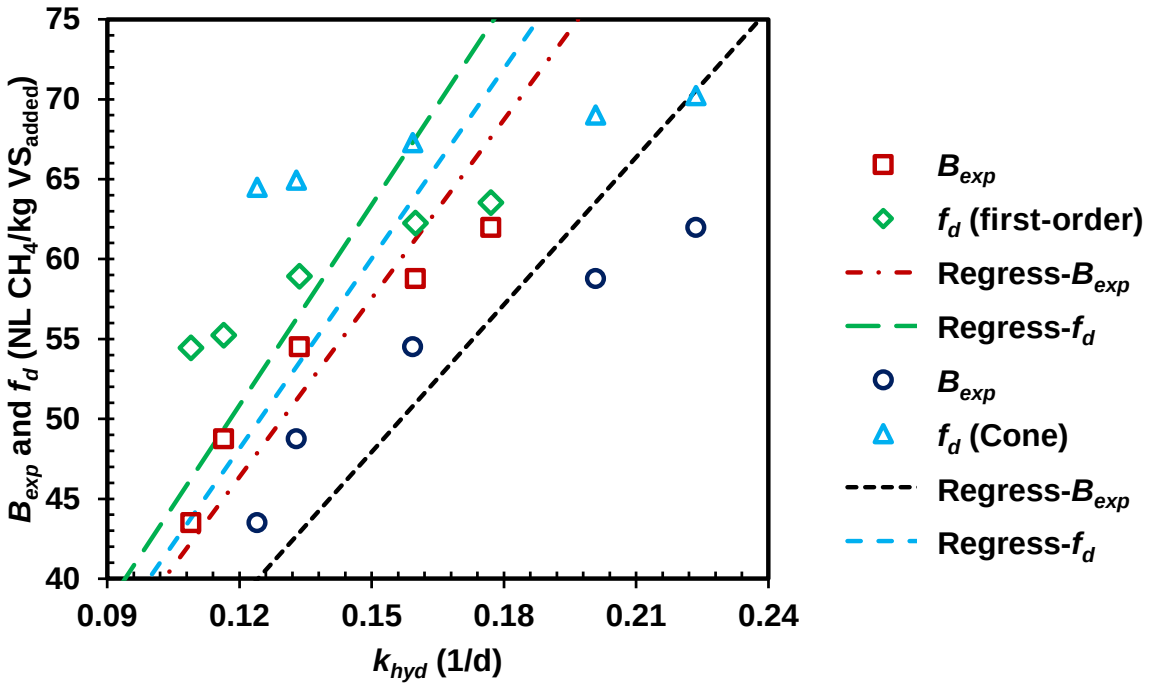

Figure 3.11. Pearson's correlations of the experimental ultimate methane yield (B_{exp}) and maximum methane potential (f_d) against hydrolysis reaction rate constant (k_{hyd}) for co-digestion of dairy manure and grass silage

A few studies investigated the kinetic parameters of dairy manure at psychrophilic conditions. Ahmed et al. (2019) validated the first-order kinetic model parameter to the cumulative biomethane production data for cow manure at 20 ºC and obtained 0.0688 1/d for the k_{hyd}. A comparison of kinetic parameters for AD of dairy manure has been reported in Table 3.5.

According to Table 3.5 a range of first-order kinetic hydrolysis constants (k_{hyd}) have been reported by different authors. Nikolaeva et al. (2009) reported a k_{hyd} equal to 0.34 1/d for anaerobic digestion of dairy manure in an up-flow anaerobic fixed-bed digester (UAFBD) at reactor temperature varying from 22 to 26 ºC during the experiments. Other studies presented lower values for the k_{hyd} of first-order kinetic model. Li et al. (2015b) showed that the k_{hyd} in first-order kinetic model decreased by increasing OLR, which was also observed in this study. The k_{hyd} of the Cone model calculated in this study showed the same behavior; it decreased with increasing OLR, which is in agreement with the results of Li et al. (2015b). In terms of the modified Gompertz model, no lag-phase was observed in this study, and Kafle and Chen (2016) reported the same result. These results confirm the accessibility of microorganisms to the digestible parts of dairy manure. However, the results of Li et al. (2015b) showed that there could be a maximum of 9 hours lag-phase duration. The maximum methane production rate (R_m) in the modified Gompertz model also followed the same trend as k_{hyd} in first-order kinetic and Cone models and experienced a reduction when the OLR increased.

Figure 3.12 compares B_{exp} and k_{hyd} (first-order kinetic model) for the mono-digestion of dairy manure and co-digestion of dairy manure and grass silage at the same OLRs. At the same OLR, the reactors fed only dairy manure had lower B_{exp} compared to the corresponding reactors fed dairy manure and grass silage. On the other hand, the values of k_{hyd} of the first-order kinetic model showed an opposite trend; values of k_{hyd} were higher for the reactors fed with dairy manure only, except for the reactor with OLR of 5 g VS/L.d.

Table 3.5. Selected kinetic studies on anaerobic digestion of dairy manure

Reactor	**T (°C)**	**OLR (kg VS/ m^3.d)**	**Equation (3-2)**	**Equation (3-3)**		**Equation (3-4)**		**Reference**
			k_{hyd} $(1/d)$	k_{hyd} $(1/d)$	n	λ (d)	R_m $(NL/kg\ VS.d)$	
Batch	20	1.0	0.220	0.285	1.33	0	4.21	This study
		2.0	0.201	0.262	1.43	0	3.50	
		3.0	0.185	0.233	1.32	0	3.02	
		4.0	0.173	0.220	1.37	0	2.49	
		5.0	0.131	0.180	1.20	0	1.79	
Batch	20	3.04	0.0688	-	-	-	-	(Ahmed et al., 2019)
Batch	36.5	3.5	0.084	-	-	0	11.9	(Kafle and Chen, 2016)
Batch	37	8	0.0690	0.10	1.36	0.36	19.08	(Li et al., 2015b)
		16	0.0788	0.11	1.22	0.15	18.86	
		32	0.0660	0.09	1.16	0.10	13.73	
		64	0.0478	0.06	1.26	0.26	8.33	
Batch	37	7 (TS%)	-	-	-	10.74	7.4	(Adiga et al., 2012)
UAFBD	22-26	4.4-24 g COD/L.d	0.34	-	-	-	-	(Nikolaeva et al., 2009)

OLR: organic loading rate; Equation (3-2): first-order kinetic model; Equation (3-3): Cone model; Equation (3-4): modified Gompertz model; k_{hyd}: hydrolysis rate constant; n: shape factor; λ: lag-phase duration; R_m: maximum methane production rate; UAFBD: up-flow anaerobic fixed bed digester.

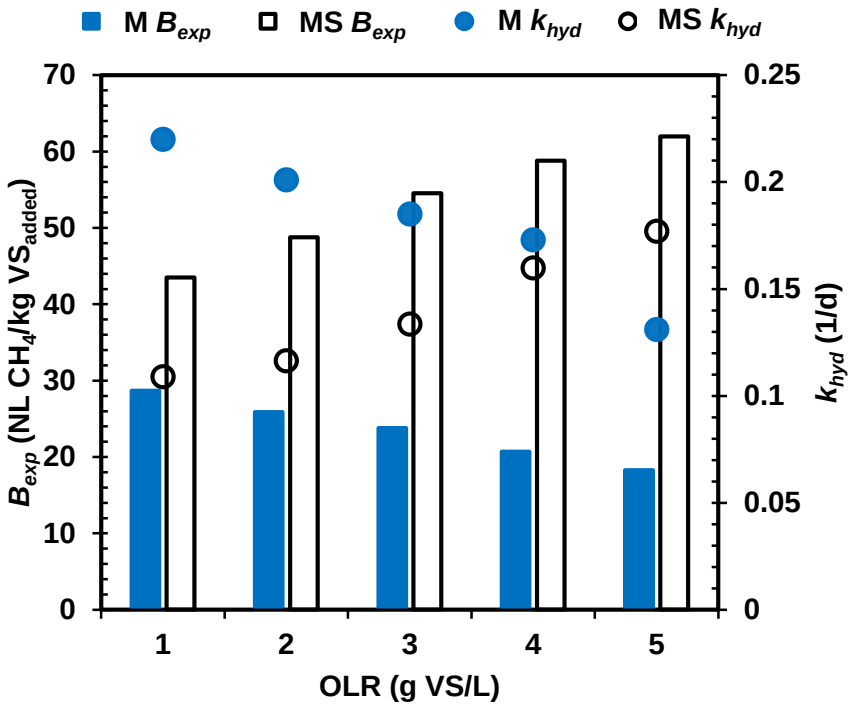

Figure 3.12. Comparison of B_{expt} and k_{hyd} of the first-order kinetic model for the mono-digestion of dairy manure and co-digestion of dairy manure and grass silage at the same organic loading rate (OLR)

The results imply that for the reactors with the same OLR, the reactor with lower k_{hyd} has a higher B_{exp} and vice versa, except for the reactor with OLR of 5 g VS/L.d. It can be concluded that the relationship between B_{exp} and k_{hyd} depends on many parameters, including the type of substrate(s), operating temperature, OLR fed to the reactor, and the nature of the inoculum (Gavala et al., 1999).

A statistical error analysis was carried out to assess the accuracy and reliability of the kinetic models. The coefficient of determination (R^2) and the average relative deviation (ARD) were used for the analysis. Generally, all three models predicted the methane yields accurately considering R^2 was > 0.97 for all models. The first-order kinetic model showed the highest R^2 in predicting the methane yield results for reactors M2 (0.9986), M4 (0.9979), M5 (0.9984), MS2 (0.9971), MS3

(0.9967), MS4 (0.9957), and MS5 (0.9927). The Cone model led in predicting the methane yields for reactors M1 and M3 with R^2 of 0.9993 and 0.9991, respectively. The reactor MS1 was the only reactor for which the modified Gompertz model showed a better R^2 (0.9906) than the first-order kinetic and Cone models. All three models were applicable to the methane yield data over time. However, first-order kinetic and Cone models presented more reliable results (lower ARD compared to modified-Gompertz model). When applying models on methane yield data from the co-digestion of dairy manure and grass silage, the accuracy of first-order kinetic and Cone models decreased. The ARD percentages (ARD%) of the first-order kinetic model in predicting methane yield from M1, M2, M3, M4, and M5 reactors were 1.35, 1.38, 1.28, 1.58, and 1.66, respectively, which were lower than the ARD% of this model in estimating the methane yield data for corresponding co-digestion experiments (7.59, 2.69, 2.66, 2.63, and 3.51 for MS1, MS2, MS3, MS4, and MS5, respectively). Table 3.6 described the discrepancy between the experimental and predicted cumulative methane yield by the three studied kinetic models over the 20-day retention time. Generally, all three models predicted the methane yields accurately ($R^2 > 0.97$ for all models). The first-order kinetic model showed the highest R^2 in predicting the methane yield results for reactors M2 (0.9986), M4 (0.9979), M5 (0.9984), MS2 (0.9971), MS3 (0.9967), MS4 (0.9957), and MS5 (0.9927). The Cone model led in predicting the methane yields for reactors M1 and M3 with R^2 of 0.9993 and 0.9991, respectively. The reactor MS1 was the only reactor for which the modified Gompertz model showed a better R^2 (0.9906) than the first-order kinetic and Cone models. All three models were applicable to the methane yield data over time. However, first-order kinetic and Cone models presented more reliable results (lower ARD compared to modified-Gompertz model). When applying models on methane yield data from the co-digestion of dairy manure and grass silage, the accuracy of first-order kinetic and Cone models decreased.

The ARD percentages (ARD%) of the first-order kinetic model in predicting methane yield from M1, M2, M3, M4, and M5 reactors were 1.35, 1.38, 1.28, 1.58, and 1.66, respectively, which were lower than the ARD% of this model in estimating the methane yield data for corresponding co-digestion experiments (7.59, 2.69, 2.66, 2.63, and 3.51 for MS1, MS2, MS3, MS4, and MS5, respectively).

The Cone model showed the same behavior in which the ARD% for M1 to M5 were 0.87, 2.22, 1.15, 2.57, and 2.84, respectively, which were less than ARD% for the corresponding MS1 to MS5 reactors (3.89, 3.27, 3.17, 3.39, and 3.95, respectively). In contrast, the modified Gompertz model predicted the methane yield data obtained from co-digestion of dairy manure and grass silage as accurately as the results of mono-digestion experiments. The ARD% of the modified Gompertz model for M1 to M5 were 5.00, 3.34, 4.48, 3.53, and 5.58, respectively, which were close to those ARD% of this model in predicting the results of co-digestion experiments (3.75, 4.16, 4.27, 3.84, and 4.63 for MS1 to MS5, respectively). It could be concluded that the first-order kinetic and Cone models were more accurate in the prediction of methane yield data from mono-digestions. In contrast, the modified Gompertz model could be accurately applied to both mono-digestion and co-digestion methane yield results.

Table 3.6. Statistical error analysis of studied kinetic models

Model	Error	Manure					Manure + Silage				
		M1*	M2	M3	M4	M5	MS1**	MS2	MS3	MS4	MS5
First-order kinetic (Equation (3-2))	R^2	0.9984	0.9986	0.9988	0.9979	0.9984	0.9769	0.9971	0.9967	0.9957	0.9927
	ARD	0.0135	0.0138	0.0128	0.0158	0.0166	0.0759	0.0269	0.0266	0.0263	0.0351
	Min	0.0014	0.0014	0.0029	0.0032	0.0004	0.0001	0.0007	0.0053	0.0013	0.0045
	Max	0.0308	0.0350	0.0255	0.0494	0.0651	0.1901	0.1094	0.1228	0.1312	0.1453
Cone (Equation (3-3))	R^2	0.9993	0.9974	0.9991	0.9966	0.9949	0.9887	0.9966	0.9965	0.9947	0.9916
	ARD	0.0087	0.0222	0.0115	0.0257	0.0284	0.0389	0.0327	0.0317	0.0339	0.0395
	Min	0.0028	0.0066	0.0004	0.0013	0.0001	0.0001	0.0001	0.0013	0.0020	0.0018
	Max	0.0153	0.0578	0.0253	0.0810	0.0728	0.0804	0.1156	0.1162	0.1316	0.1536
Modified Gompertz (Equation (3-4))	R^2	0.9831	0.9904	0.9864	0.9906	0.9838	0.9906	0.9904	0.9893	0.9902	0.9863
	ARD	0.0500	0.0334	0.0448	0.0353	0.0558	0.0375	0.0416	0.0427	0.0384	0.0463
	Min	0.0121	0.0007	0.0093	0.0008	0.0004	0.0047	0.0046	0.0058	0.0072	0.0069
	Max	0.1641	0.1867	0.1757	0.2060	0.2363	0.2041	0.2086	0.2092	0.2307	0.2423

* M1 to M5 denote the reactors fed with dairy manure changing OLR 1 to 5, respectively.

** MS1 to MS5 represent the reactors fed with dairy manure and grass silage varying OLR 1 to 5, respectively.

3.9.4. Microbial community dynamics during psychrophilic anaerobic digestion of dairy manure and grass silage

Microbial community structure is strongly affected by environmental factors (Gerardi, 2003). Temperature is one of the critical factors that change the microbial community structure and low temperatures (< 20 ºC) impose inhibitory stress on the degradation of organic matter and thus lower the methane yield (Mao et al., 2015). It is reported that the operational taxonomic units (OUT) numbers and diversity variations decrease with increasing temperature (Sun et al., 2016). Additionally, the substrate impacts the structure of the microbial community by changing the buffering capacity of the solution, which causes the accumulation/dissipation of volatile fatty acids in the mixture (Zheng et al., 2015). The taxonomic structure of microbial communities is a crucial indicator of culture concentration and activity. Figure 3.13 and Figure 3.14 present the taxonomic structure of the microbial communities at the phylum and class levels, respectively. The relative abundances (RA) of microbes in representative samples of the original culture (OC), inoculum (IN), glucose-fed culture (GL), dairy manure-fed culture (M), and dairy manure- and grass silage-fed culture (MS) are analyzed. Most sequences were classified within eight phyla of Bacteria (*Bacteroidetes*, *Chloroflexi*, *Cloacimonetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*, *Spirochaetes*, and *Synergistetes*) and one of Archaea (*Euryarchaeota*).

The *Bacteroidetes* dominated all samples (Figure 3.13) despite the variation in their relative abundance from 30.5% to 64.3% within the various reactors. The GL culture showed the highest relative abundance of *Bacteroidetes* (64.3%), followed by IN (59.4%). The dairy manure-fed sample at OLR 1 g VS/L.d (M1) showed the lowest relative abundance of *Bacteroidetes* (30.5%). The *Bacteroidetes* relative abundance increased from 30.5% to 46.9% to 42.9% with increasing OLR from 1 to 3 to 5 g VS/L.d in dairy manure-fed samples (M1, M3, and M5, respectively).

Compared to GL, the relative abundance of *Bacteroidetes* decreased in M1 (64.3% vs 30.5%); however, it increased with OLR to a higher level 46.9% in M3 and 42.9% in M5. This trend was completely the opposite in dairy manure- and grass silage-fed cultures where the relative abundance of *Bacteroidetes* decreased from 48.2% in MS1 to 36.7% in MS3 and 38.4% in MS5. It is likely that grass silage introduced a certain component or created a certain micro-environmental condition that affected the *Bacteroidetes*. Members of the *Bacteroidetes* are known to contribute to the hydrolysis of complex large molecules of organic matter. For example, they are known to help with the degradation of carbohydrates to monosaccharides and then to smaller acids, such as acetic acid and lactic acid (Yue et al., 2013). Sun et al. (2016) investigated the effect of temperature on the bacterial community during AD of dairy manure. It was found that the *Bacteroidetes* became the dominant phylum at psychrophilic (20 ºC) and mesophilic (35 ºC) temperatures, varying from 41.3 to 50.3% after 30 days of incubation (Sun et al., 2016). The *Bacteroidetes* phylum contains organisms with various physiological capabilities, including hydrolytic, acidogenic, and fermentative representatives, which play many functions during AD (Ariunbaatar et al., 2014). Also, previous research showed that the percentage of *Bacteroidetes* in the microbial community was positively correlated with the hydrolysis of biomass (Regueiro et al., 2012).

The *Chloroflexi* composition increased upon the co-digestion of dairy manure and grass silage, particularly at the higher OLR V/S. The relative abundance (RA) of *Chloroflexi* in M1, M3, and M5 was 18.7, 6, and 11.6%, respectively. In the co-digestion experiments, i.e., MS1, MS3, and MS5, the concentration of *Chloroflexi* was 4.6, 14.1, and 16%, respectively. The same experience was observed in a study by Zheng et al. (2015) in which adding switchgrass to dairy manure led to an increase in *Chloroflexi* composition. Additionally, it was reported that the

Chloroflexi have a positive impact on acetic acid production and the degradation of polysaccharides and monosaccharides (St-Pierre and Wright, 2014). Acetic acid is the major substrate to acetoclastic methanogens and it thus results in higher methane production. This might explain the results of the current study that the MS5 reactor, which contained the highest amount of *Chloroflexi* among dairy manure- and grass silage-fed reactors, produced the highest methane yield.

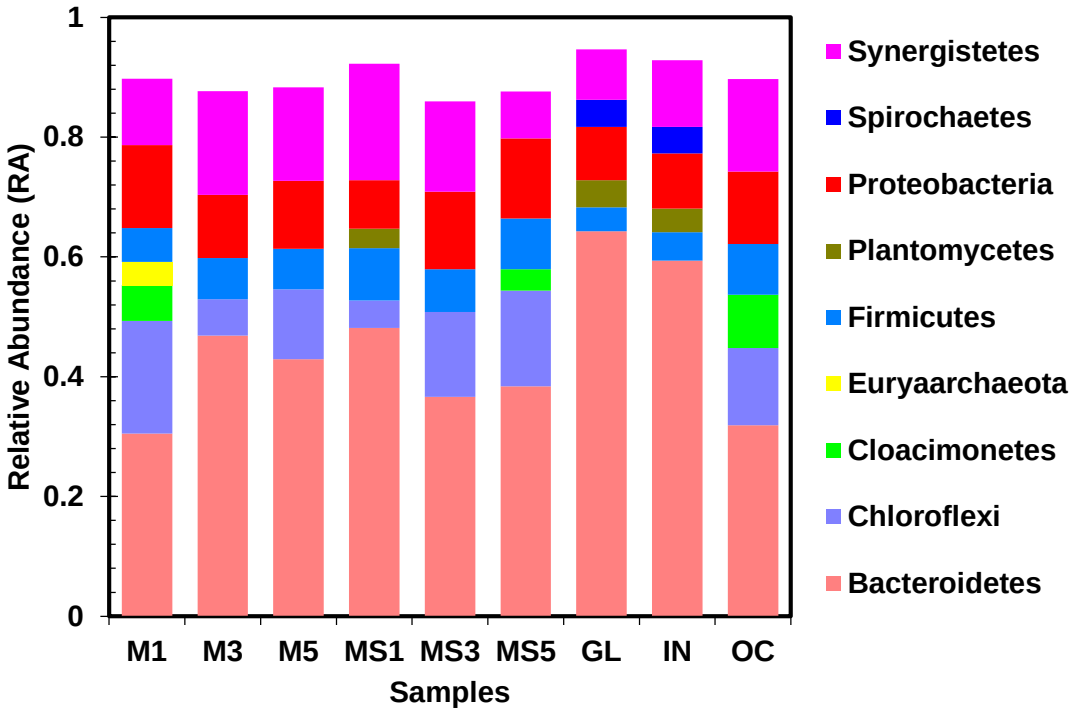

Figure 3.13. The relative abundance (RA) of bacteria and archaea at the phylum level

The relative abundance of *Synergistetes* was 15.4% in the OC and 11.1% in the IN; it decreased in GL sample to 8.4%. Its relative abundance decreased with the increase of OLR in dairy manure- and grass silage-fed cultures from 19.4% to 15.1% to 7.9% in MS1, MS3, and MS5, respectively. However, in dairy manure-fed cultures, it increased with the increase in OLR from 11.1% to 17.4% and 15.6% in M1, M3, and M5, respectively. It seems that grass silage at high

OLR enabled the *Synergistetes* to grow and maintain a high presence in the reactors. *Synergistetes* members can be found in various anaerobic environments, including soil, oil wells, and wastewater treatment plants (Vartoukian et al., 2007). The high relative abundance of *Synergistetes* in samples may imply that they significantly contribute to methane production during the AD process (Zhao et al., 2017).

The *Firmicutes* is a phylum of bacteria in which many produce endospores. The *Firmicutes* was shown to be the most abundant phylum in biogas reactors fed protein-rich substrates (Pap and Maróti, 2016). The *Firmicutes* RAs ranged from 8.4% in OC to 4.7% in IN and to 4.0% in the GL culture. Its RA was quite constant, at 8.8%, 7.2%, and 8.4% at OLR 1 (MS1), 3 (MS3), and 5 (MS5) g VS/L.d in dairy manure- and grass silage-fed cultures, respectively. Similarly, its RA was quite constant, at 5.7%, 6.9%, and 6.8% at OLR 1 (M1), 3 (M3), and 5 (M5) g VS/L.d in dairy manure-fed cultures, respectively. *Clostridia* were the prevailing class within the *Firmicutes* phylum. The *Clostridia* species formed about 6.7% in OC (Figure 3.14), but they decreased to about 3.7% in GL. Generally, feeding dairy manure alone did not affect their abundance, particularly at OLR 3 (M3) and 5 (M5) g VS/L.d. Feeding dairy manure and grass silage increased their abundance to 7.7 and 7.9% at OLR of 1 (MS1) and 5 (MS5) g VS/L.d, respectively. The class *Clostridia* represents a large group of bacteria characterized by being obligate (or strict) anaerobes and oxygen is toxic to them. Most species in the *Clostridia* are saprophytic and able to ferment plant polysaccharides (Boutard et al., 2014). They are common in the environment, particularly in the soil, and were dominant in landfills, and mesophilic and thermophilic anaerobic digesters (Burrell et al., 2004; Schnürer et al., 1996; Van Dyke and McCarthy, 2002). *Clostridia* formed about 7.03% of the operational taxonomic units (OTUs) in digesters at a wastewater treatment plant operated at 34–35 ºC (Świątczak et al., 2017). Cai et al. (2016) found *Clostridia* dominant in

a biogas-producing anaerobic digester that digests municipal sludge and that the culture was rich in *Clostridia* genes encoding functions related to fatty acid and lipid metabolism. Therefore, the finding of Cai et al. (2016) explains the decrease in the abundance of *Clostridia* in glucose-fed cultures and its increase in dairy manure-fed cultures; obviously, the dairy manure contained fatty acids, fats and lipids, and protein whereas glucose is a simple and pure carbohydrate. The results from the current study reveal that members of the *Clostridia* survived and performed well in the dairy manure-fed culture at low temperature (20 °C). However, it was reported that the contribution of *Clostridia* in dairy manure anaerobic digesters increased with temperature (from 30.4-34.9% at 37 ºC to 66.8-77.2% at 44 ºC, and 92.4-93.8% at 52 ºC) (Sun et al., 2015a). Thus, these reported findings explain the low composition of *Clostridia* (5.4-6.4% for the M1-M3 samples) that was observed in this study at low temperatures (20 ºC).

The *Spirochaeta* were only detected in two samples: the GL and IN (Figure 3.14). The relative abundance of *Spirochaeta* was 4.27% in IN, while it increased slightly upon incubation with glucose (4.38% in GL). Members of the *Spirochaeta* are common inhabitants of many aquatic environments, including both freshwater and marine, and in the sediments and mud of ponds, marshes, lakes, rivers, and oceans. They are highly motile and able to move through high viscosity environments (Leschine et al., 2006). They include saccharolytic representatives, which decompose carbohydrate polymers. Members of the *Spirochaeta* ferment carbohydrates and produce acetate, ethanol, CO_2, and H_2 (Zhang et al., 2019). One studied species, *Spirochaeta isovalerica*, ferments some amino acids in addition to carbohydrates (Paster, 2010). Interestingly, the *Spirochaeta* contains extremophilic species, such as anaerobic thermophiles isolated from hot springs, the moderately thermophilic *Spirochaeta caldaria* isolated from cyanobacterial mats of hot springs, and the extremely thermophilic *Spirochaeta thermophila* isolated from a marine

environment (Paster, 2010). Some species are alkaliphilic, such as *S. alkalica*, *S. africana*, *S. asiatica* that were isolated from an alkaline Lake and from sulfide-saturated mud sediments (Hoover et al., 2003).

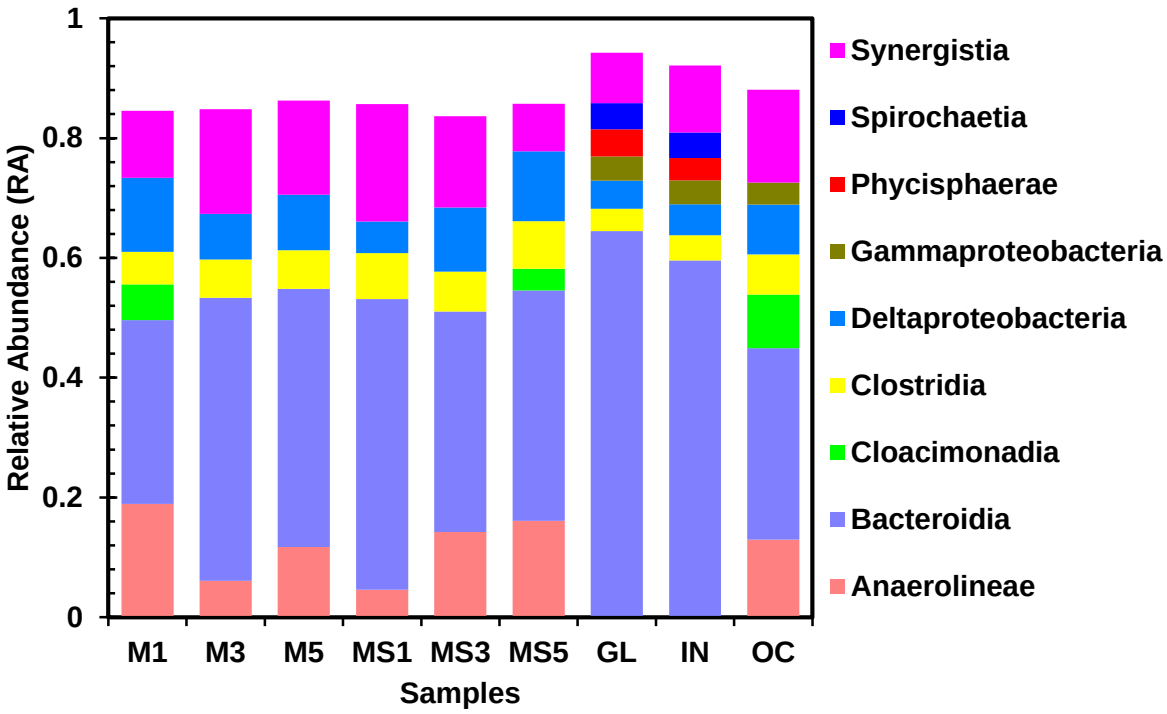

Figure 3.14. The relative abundance (RA) of bacteria and archaea at class level

Generally, the *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* phyla have been found to dominate in mesophilic anaerobic digesters fed lignocellulosic substrates (Hollister et al., 2012). It seems that the decrease in temperature from 35 ºC in the original reactor in the wastewater treatment plant to 20 ºC in the current study did not affect the abundance of *Firmicutes*. However, the slight variation in their abundance was likely due to the differences in the substrates (dairy manure, dairy manure plus grass silage versus wastewater).

The PCA of the abundance of bacteria and archaea at the phylum and class levels helps with comparing the microbial communities within the samples. Figure 3.15 and Figure 3.16 present the first and second principal components (PC1 and PC2) for the relative abundance dataset in samples of OC, IN, GL, M1, M3, M5, MS1, MS3, and MS5 at phylum and class levels, respectively. Two PCs accounted for 83.6% of the total variation in the bacterial communities. The GL and IN samples formed a cluster (Figure 3.15(a)), which shows the similarities in their communities. On the other hand, the OC was in the opposite quadrant compared to the GL and IN samples. In terms of PC1, the dairy manure-fed samples are different while based on PC2, M2 and M3 were very close to each other. Dairy manure- and grass silage-fed samples (MS1, MS2, and MS3) were almost across a straight line with a positive slope. The variables show a correlation among the presence of *Bacteroidetes*, *Spirochaetes*, and *Planctomycetes* in the samples.

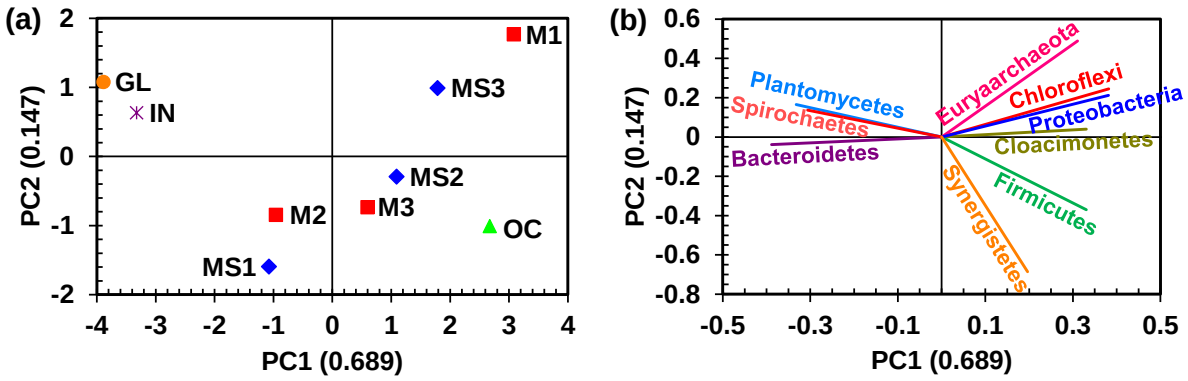

Figure 3.15. The PCA-based relationship between (a) samples and (b) variables at the phylum level

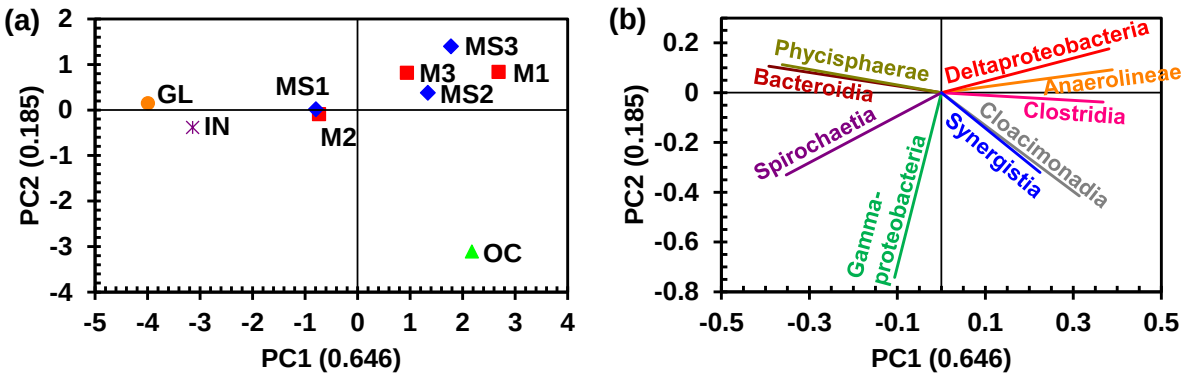

Figure 3.16. The PCA-based relationship between (a) samples and (b) variables at the class level

The Shannon Index is a metric that correlates directly with diversity within communities (Kim et al., 2017). Table 3.7 gives the number of OTUs and Shannon Index in each sample at three taxonomic levels. The least diverse samples were GL and IN with Shannon indices of 1.12 and 1.18 at the phylum level, respectively. On the contrary, the most diverse samples were the dairy manure-fed reactor at 1 g VS/L.d (M1) and the OC. Increasing the OLR of dairy manure- and grass silage-fed reactors increased the Shannon Index from 1.34 to 1.38 to 1.46 for MS1, MS2, and MS3, respectively.

Table 3.7. Number of OTUs and Shannon Indices in various samples and taxonomic level

Sample	No. of OTUs			Shannon Index		
	Phylum	Class	Species	Phylum	Class	Species
M1	32666	32426	21153	1.65	1.51	1.53
M3	14113	13990	8477	1.25	1.20	1.34
M5	14054	13969	8653	1.33	1.30	1.34
MS1	13900	13761	7716	1.34	1.16	1.26
MS3	17011	16885	10841	1.38	1.35	1.46
MS5	17444	17336	11104	1.46	1.43	1.34
GL	596	594	269	1.12	1.16	1.19
IN	11218	11161	5846	1.18	1.23	1.21
OC	13841	13777	9419	1.60	1.64	1.44

3.10. Summary

This study investigated the early stage of the culture's adaptation to psychrophilic (20 ºC) anaerobic digestion of dairy manure and co-digestion of dairy manure and grass silage at increasing organic loading rates (1-5 g VS/L.d). In mono-digestion of dairy manure, the higher organic loading rate resulted in a lower methane yield. By co-digesting grass silage and dairy manure, an opposite trend was observed and higher methane yield was obtained at the higher organic loading rate. The first-order kinetic and Cone models are more accurate in predicting the methane yield from mono-digestion of dairy manure than co-digestion of dairy manure and grass silage. However, the modified Gompertz model predicted the results of both mono-digestion and co-digestion with the same order of accuracy. The hydrolysis rate constant of first-order kinetic and Cone models as well as R_m of modified Gompertz model decreased upon an increase in the organic loading rate. *Bacteroidetes* dominated in all reactors, and its relative abundance increased with the increase of organic loading rate in dairy manure-fed reactors, while it decreased in dairy manure- and grass silage-fed reactors. Inoculum sample was the least diverse, while the incubation with dairy manure and a mixture of dairy manure and grass silage increased the diversity. In addition, the Shannon Diversity Index increased with increasing the organic loading rate in dairy manure-fed reactors, but it decreased with increasing the organic loading rate in dairy manure- and grass silage-fed reactors. The dynamics of the psychrophilic enzymes during the early stage of the culture's adaptation in anaerobic digestion needs to be investigated. Investigating the effects of other variables, such as salinity, ammonia, and pH, on the microbial community composition dynamics during the early stage is important.

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Chapter Four

EFFECT OF TOTAL SOLIDS ON METHANE PRODUCTION DURING PSYCHROPHILIC ANAEROBIC DIGESTION OF DAIRY MANURE AND GRASS SILAGE

4.1. Abstract

The total solids content of the anaerobic digester affects its performance significantly. This study investigates the early stage of the culture's adaptation during psychrophilic AD (20 ºC) of complex substrates (dairy manure and grass silage) at increasing total solids content (TS; 7-10%) in batch reactors. The first-order, Cone, and modified Gompertz kinetic models showed that the inoculum degraded the substrates and produced biogas at its early adaptation stage. The methane yield from mono-digestion of dairy manure was higher at lower TSs, while in co-digestion the trend was the opposite. In mono-digestion of dairy manure, the highest methane yield obtained for the reactor with 7% total solids is 38.1 NL CH_4/kg VS_{added}. In the co-digestion experiments, the reactor with the highest total solids (10%) showed the highest methane yield (103.9 NL CH_4/kg VS_{added}).

4.2. Introduction

The water content of the anaerobic digester plays a critical role that can affect the entire AD process (Forster-Carneiro et al., 2008; Karthikeyan and Visvanathan, 2013; Le Hyaric et al., 2012). Thus, the amount of solids fed to the reactor, called total solids, is used to define two types of anaerobic digesters: wet vs. dry. The wet anaerobic digestion is when the TS is less than 15%,

while the reactors with more than 15% TS are known as dry anaerobic digesters (Karthikeyan and Visvanathan, 2013). Wet anaerobic digestion is commonly used to treat livestock manure and agricultural wastes, especially when the TS content is less than or equal to 10% (Yi et al., 2014). Increasing the TS content of the anaerobic digester may negatively affect the methane production rate as the higher TS contents (i.e., dry AD) lower the diffusion rates of soluble intermediate products within the digester (Bollon et al., 2013; Xu et al., 2014). Abbassi-Guendouz et al. (2012) has reported that increasing the TS content from 10 to 30% causes up to 60% decrease in daily methane production due to lower hydrolysis rates. Despite the mentioned disadvantages of the dry AD, increasing the TS content leads to increased volumetric efficiency (defined as the unit of volume of methane production per unit volume of bulk sludge) (Indren et al., 2020). Also, the higher TS content decreases the capital costs of the process as dry AD needs smaller reactors and less water addition resulting in a greater economic feasibility (Li et al., 2018b).

Di Maria et al. (2017) compared the performance of the dry (solid-state) and wet anaerobic digestion of organic fraction of municipal solid waste (OFMSW) at a mesophilic temperature of 35 ºC through batch experiments. The solid-state AD was operated at OLR of 4.55 kg VS/m^3.d and TS of 37% while the wet AD experiment was conducted at OLR of 0.9 kg VS/m^3.d and TS of 4%. The results showed that the methane production in wet AD was higher than that of the dry AD, 320 and 252 NL CH_4/kg VS, respectively (Di Maria et al., 2017). In another study, a comparison was made among the wet AD, semi-dry AD, and dry AD for chicken manure at 37 ºC (Li et al., 2013). The operating TS contents of the bioreactors were 5.1-5.6% in wet AD, 10.1-11.2% in semi-dry AD, and 20.1-22.4% in dry AD. The results indicated that increasing the TS content decreased the methane yield; the wet AD showed higher methane yield than semi-dry AD and dry AD (Li et al., 2013).

This study aims to investigate the effects of the total solids (7-10%) on the adaptation of the anaerobic mixed culture in psychrophilic AD of dairy manure and grass silage. The culture used in this study has been acclimated in one cycle (20 days) under varying OLR (1-5 $gVS/m^3.d$). Three kinetic models (first-order kinetic, Cone, and modified Gompertz) have been applied to the experimental methane yield data with incubation time. Statistical analyses also have been used to explain the relationship between the experimental results and kinetic parameters.

4.3. Materials and Methods

4.3.1. Inoculum

The initial inoculum was obtained from the Riverhead Wastewater Treatment Facility (RWTF), a conventional treatment plant in St. John's, NL, Canada. The culture structure was in the form of small black granules, which were disintegrated and converted into flocculent culture upon storage and incubation. The dilute culture's alkalinity was 1700 mg/L as $CaCO_3$ and was adjusted by adding 4.9 g of $CaCO_3$ into 7 L of dilute culture. The final measured alkalinity was 2,595 mg/L as $CaCO_3$.

4.3.2. Feedstock

Fresh manure from dairy cows (M) was collected from the Lester's Dairy Farm (LDF) on the Avalon Peninsula, Newfoundland, from the wet barn and in front of the manure scraper. The manure was transferred into a plastic drum and stored at 4 ºC before being fed to the reactors. The experiments employed chopped (< 2.5-5.0 mm) grass silage (S). The dairy manure and grass silage were subjected to complete physiochemical characterization before they were fed to the reactors

(refer to Table 3.1). The characterization results are provided in Appendix A. Appendix B presents the experimental procedure of determining feedstock characteristics.

4.4. Batch Anaerobic Digestion Tests

Multiple sets of duplicate batch reactors have been operated at different increasing total solids (7-10%). The reactors were bench-top air-tight batch reactors. The batch experiments were done according to the procedure described in Chapter 3, section 3.4.

4.5. Experimental Design

Multiple sets of duplicate batch reactors were fed increasing TS content of dairy manure and grass silage. The TS was changed from 7 to 10% while maintaining the reactors at a constant temperature of 20 ºC. Figure 4.1 and Table 4.1 give the experimental design.

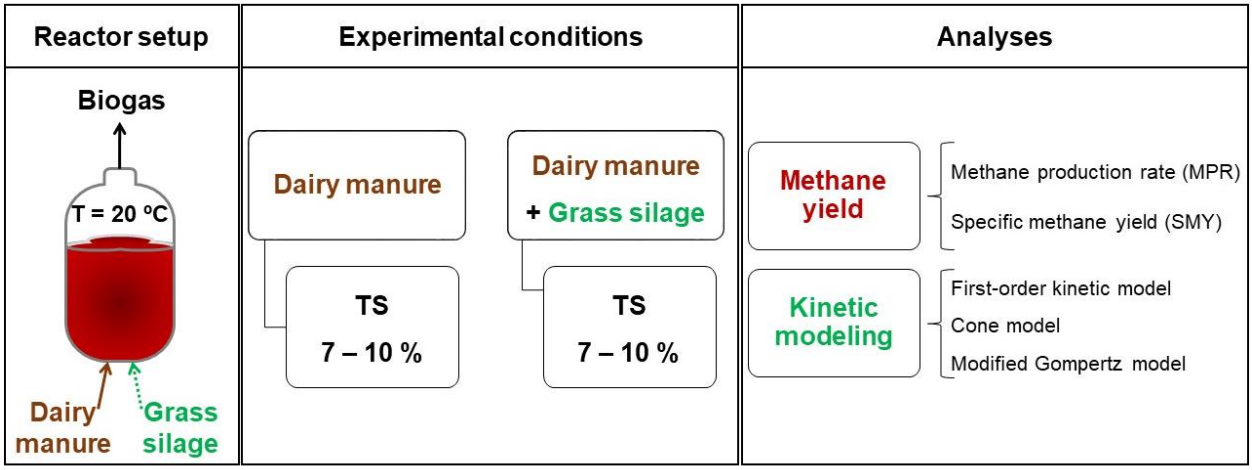

Figure 4.1. Schematic of the experimental setup, conditions, and analyses in the second cycle of culture adaptation

Table 4.1. The experimental design of the second cycle of inoculum adaptation at increasing TS

Test no.	Substrate(s)	OLR (g VS/L.d)	TS (%)	Label
1	Dairy Manure	5.0	7.0	7M
2	Dairy Manure + Grass Silage	5.0	7.0	7MS
3	Dairy Manure	5.0	8.0	8M
4	Dairy Manure + Grass Silage	5.0	8.0	8MS
5	Dairy Manure	5.0	9.0	9M
6	Dairy Manure + Grass Silage	5.0	9.0	9MS
7	Dairy Manure	5.0	10.0	10M
8	Dairy Manure + Grass Silage	5.0	10.0	10MS

The experimental and analytical protocols were carried out as described in Chapter 3, sections 3.5.1 to 3.5.5. The kinetic modeling of the biogas production process was performed according to the procedure and models expressed in Chaper 3, section 3.6. The statistical analyses were carried out based on the statistical error analyses described in Chapter 3, section 3.7.

4.6. Results and Discussion

4.6.1. Effects of the TS on the mono-digestion of dairy manure

Profiles of MPR and methane yield of mono-digestion of dairy manure at total solids of 7.0, 8.0, 9.0, and 10.0% are shown in Figure 4.2 and Figure 4.3, respectively. The maximum MPR has been observed on day 2 for all reactors (Figure 4.2). The MPR on day 2 was 16.2, 15.0, 15.3, 14.2 NL CH_4/kg VS_{added}.d for reactors 7M, 8M, 9M, and 10M, respectively. After day 2, reactors 9M and 10M experienced a dramatic reduction in MPR, while reactors 7M and 8M remained at relatively high MPR for 2 more days. MPR for reactors 9M and 10M dropped from 15.3 to 4.5 NL CH_4/kg VS_{added}.d and 14.2 to 4.1 NL CH_4/kg VS_{added}.d, respectively; this shows more than 70% reduction for both reactors. Comparatively, this reduction was observed to be 17.0% and 40.5%

for reactors 7M (from 16.3 to 13.5 NL CH_4/kg VS_{added}.d) and 8M (from 15.0 to 8.9 NL CH_4/kg VS_{added}.d), respectively. Then, all reactors continued smooth decreasing rates until the end of the experiment. The ultimate methane yield (Figure 4.3) showed an opposite trend with the increase of total solids in the reactors. The maximum cumulative methane yield (NL CH_4/kg VS_{added}) measured was 38.1 (7M) > 34.9 (8M) > 28.6 (9M) > 25.6 (10M). The reactors fed the lowest total solids (i.e., 7M) produced the highest methane yield. Zheng et al. (2015) obtained 84.3 NL CH_4/kg VS_{added} during 20 days incubation of dairy manure with 6% total solids at 35 ºC. Wei et al. (2014) digested dairy manure at 15 ºC and total solids of 20%; they obtained 47.3, 42.8, and 24.9 L CH_4/kg VS during the first 20 days of incubation at inoculum to substrate ratios (ISR) of 2, 1, and 0.5, respectively. They concluded that the higher ISR was more favorable for methane yield.

The results of the current study are in agreement with those reported by Wei et al. (2014), as the methane yields obtained from reactor 7M (ISR = 1.5) were more than those of reactor 8M (ISR = 1.1), followed by the reactors 9M (ISR = 0.8) and 10M (ISR = 0.7).

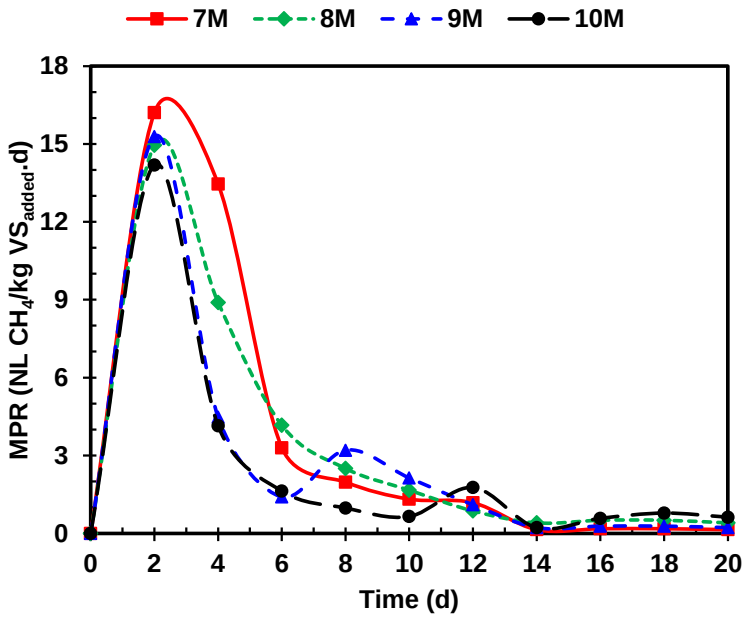

Figure 4.2. Methane production rate (MPR) during psychrophilic mono-digestion of dairy manure (M) with increasing total solids of 7 to 10% at 20 ºC

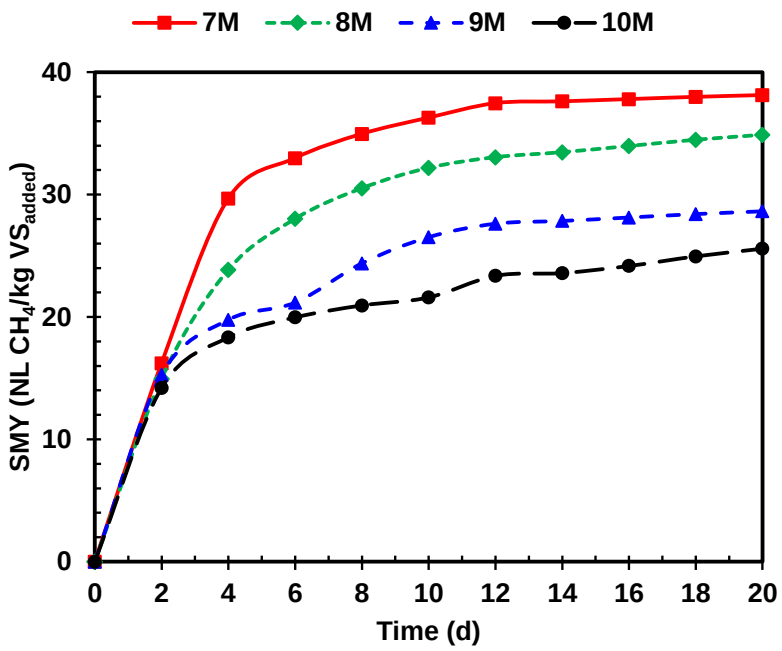

Figure 4.3. Accumulative specific methane yield (SMY) during psychrophilic mono-digestion of dairy manure (M) with increasing total solids of 7 to 10% at 20 ºC

4.6.2. Effects of the TS on the co-digestion of dairy manure and grass silage

Figure 4.4 and Figure 4.5 present the profiles of the MPR and SMY of the co-digestion of dairy manure and grass silage. Similar to the results of co-digestion at increasing OLRs, there were multiple peaks observed in the MPR profile (Figure 4.4). On day 2, the maximum MPR (NL CH_4/kg VS_{added}.d) was 36.9 (7MS) > 33.0 (8MS) > 23.6 9MS) > 18.2 (10MS). Afterward, the reactors with the highest TS contents (9MS and 10MS) maintained higher MPR whereas the other reactors (7MS and 8MS) showed a drop in the MPR.

Although reactor 8MS was leading until day 8 in terms of the cumulative methane yield, the reactors 9MS and 10MS overtook it and showed the highest cumulative methane yield at the end of the digestion period (Figure 4.5).

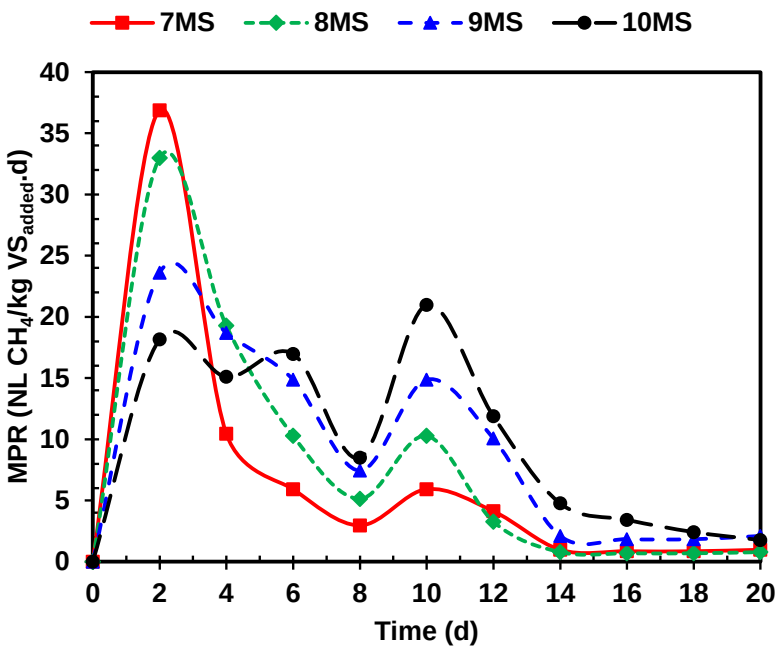

Figure 4.4. Methane production rate (MPR) during psychrophilic co-digestion of dairy manure and grass silage (MS) with increasing total solids of 7 to 10% at 20 ºC

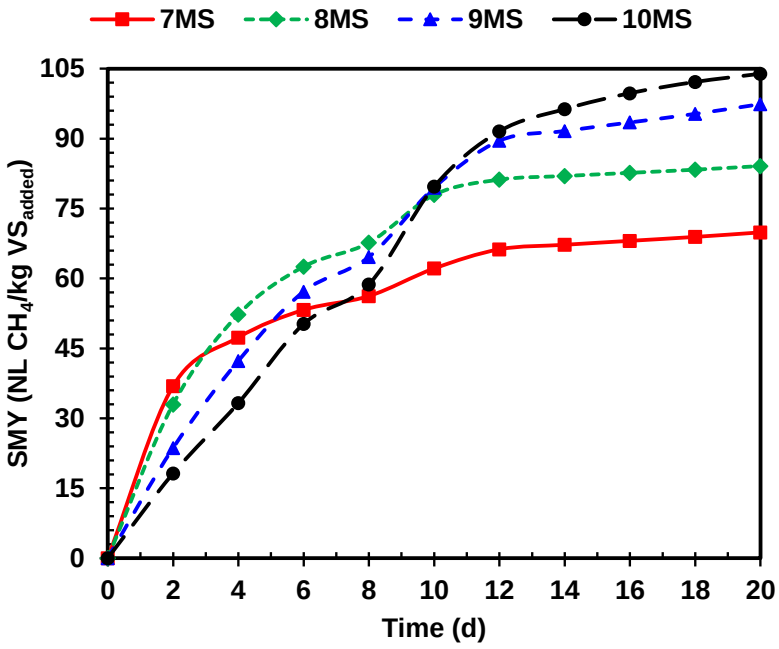

Figure 4.5. Accumulative specific methane yield (SMY) during psychrophilic co-digestion of dairy manure and grass silage (MS) with increasing total solids of 7 to 10% at 20 ºC

The reactors of the lower TS% (7MS and 8MS) showed higher methane yield rates at the beginning of the experiment while those with higher TS% (10MS and 9MS) started with a lower methane yield rate and then accelerated and achieved the highest methane yields in the experiments. The ultimate methane yield (NL CH_4/kg VS_{added}) obtained in this experiment for different reactors was 103.9 for 10MS, 97.4 for 9MS, 84.1 for 8MS, and 69.9 for 7MS.

4.6.3. Kinetic modeling with TS variation

The first-order kinetic, Cone, and modified Gompertz models were used to correlate the experimental methane yield from anaerobic digestion of dairy manure and grass silage. The experimental ultimate methane yield (B_{exp}) as well as estimated kinetic parameters, including the estimated ultimate methane yield (B_{est}) and maximum methane potential (f_d) are presented in Table 4.2 for the reactors fed different TSs. The estimated f_d by the first-order kinetic and modified Gompertz models always decreased with the increase of TS in dairy manure-fed reactors. The opposite trend was observed with feeding grass silage and dairy manure to the reactors in which the f_d estimated by first-order kinetic and modified Gompertz models increased with the increase of TS. In dairy manure-fed reactors, the f_d predicted by the Cone model first decreased by increasing the TS, but it increased in the reactor with a TS of 10%. On the other hand, the f_d estimated by the Cone model in reactors fed with dairy manure and grass silage first decreased by increasing TS from 95.23 to 94.98 NL/kg VS and then increased by increasing TS.

The maximum methane production rate (R_m) mostly decreased by increasing the TS content in the reactors fed dairy manure or dairy manure and grass silage except when the TS increased from 9% to 10% in the dairy manure-fed reactors which increased the R_m. This trend is the opposite of the first phase of the experiments, where the R_m was decreased by increasing the OLR

in dairy manure-fed reactors, but feeding the reactors with dairy manure and grass silage led to an increase in the R_m. The f_d estimated by the first-order kinetic model for dairy manure-fed reactors follows the same trend as the experimental methane yield (B_{exp}) as is seen in Figure 4.6. Increasing the TS content in the reactors led to a decrease in both B_{exp} and f_d estimated by first-order kinetic model. The f_d predicted by Cone model first decreased by increasing TS content from 7% to 9%, but it then increased in TS content of 10%.

Table 4.2. Predicted parameters of three kinetic models for mono-digestion of manure (M) and co-digestion of dairy manure and grass silage (MS) with increasing TS

Parameter	**Manure**				**Manure and Silage**			
	7M*	**8M**	**9M**	**10M**	**7MS****	**8MS**	**9MS**	**10MS**
Experiment								
$B_{exp}\ (NL/kg\ VS_{added})$	38.12	34.88	28.63	25.58	69.87	84.09	97.40	103.89
First-order Kinetic Model (Equation (3-2))								
$B_{est}\ (N\,L/kg\ VS_{added})$	38.07	34.29	27.99	23.81	67.40	84.08	99.31	108.91
$f_d\ (NL/kg\ VS_{added})$	38.12	34.40	28.06	23.83	67.57	84.92	107.81	135.79
$k_{hyd}\ (1/d)$	0.324	0.284	0.302	0.364	0.300	0.231	0.127	0.081
Cone Model (Equation (3-3))								
$B_{est}\ (N\,L/kg\ VS_{added})$	37.99	34.76	29.20	25.32	70.82	85.50	99.45	107.16
$f_d\ (NL/kg\ VS_{added})$	38.50	36.65	36.05	37.42	95.23	94.98	122.49	135.10
$k_{hyd}\ (1/d)$	0.432	0.385	0.322	0.207	0.246	0.294	0.154	0.121
n	2	1.43	0.78	0.52	0.67	1.24	1.30	1.52
Modified Gompertz Model (Equation (3-4))								
$B_{est}\ (N\,L/kg\ VS_{added})$	37.28	33.43	27.21	23.15	65.34	81.72	96.28	104.82
$f_d\ (NL/kg\ VS_{added})$	37.29	33.43	27.21	23.15	65.34	81.77	97.57	108.40
$R_m\ (NL/kg\ VS_{added}.d)$	8.01	6.39	5.65	6.07	13.76	12.79	9.55	9.00
$\lambda\ (d)$	0	0	0	0	0	0	0	0.54

* 7M to 10M denote the reactors fed with dairy manure changing TS% 7 to 10, respectively.

** 7MS to 10MS represent the reactors fed with dairy manure and grass silage varying TS% 7 to 10, respectively.

However, the k_{hyd} of the Cone model showed a steady decreasing trend by increasing the TS% in the reactors. This behavior shows an inconsistency between the predictions of kinetic models.

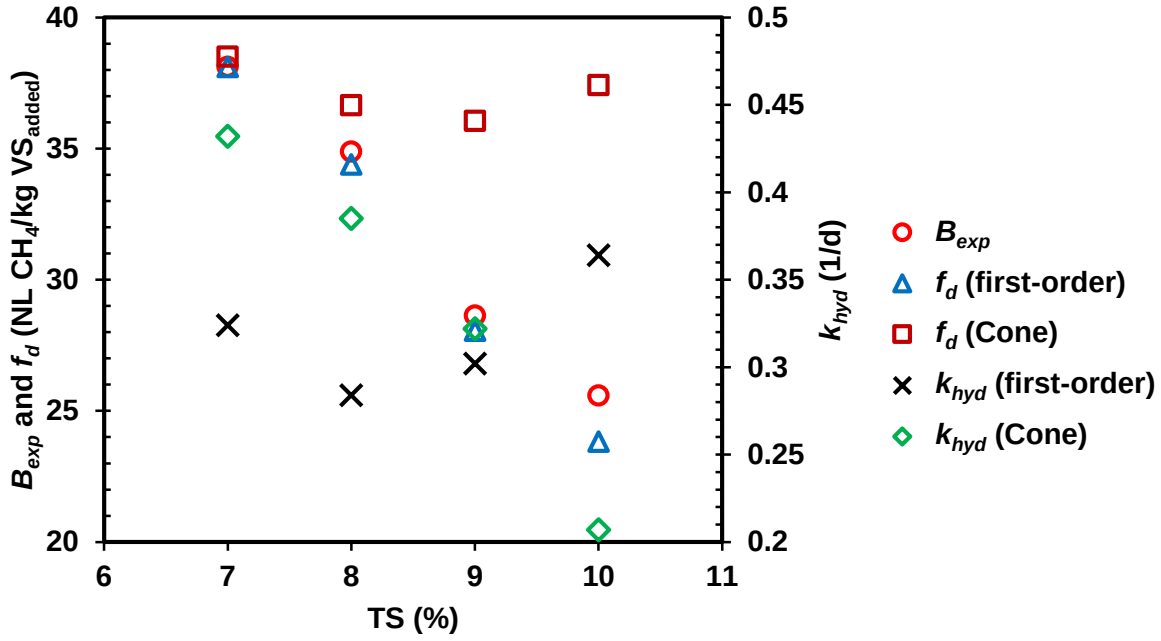

Figure 4.6. Relationship of the experimental ultimate methane yield (B_{exp}), maximum methane potential (f_d), and hydrolysis reaction rate constant (k_{hyd}) with the total solids for mono-digestion of dairy manure

Figure 4.7 presents the correlation between B_{exp} and f_d with k_{hyd} in the dairy manure-fed reactors. According to Pearson's correlation, no significant relationship was observed between B_{exp} and f_d with k_{hyd} for first-order kinetic model. However, the R_P values were negative, showing the opposite trend of B_{exp} and f_d with k_{hyd} for first-order kinetic model. Considering the Cone model, the B_{exp} correlated with k_{hyd} while no correlation was shown between the f_d and k_{hyd}. The R_P between the B_{exp} and k_{hyd} of the Cone model was 0.96. These observations show that the experimental data obtained are not consistent.

Figure 4.8 presents the relationship between the experimental methane yield data and kinetic parameters for the co-digestion of dairy manure and grass silage. As is seen, increasing the TS% in the reactor led to a constant increase in B_{exp} and f_d predicted by the first-order kinetic model.

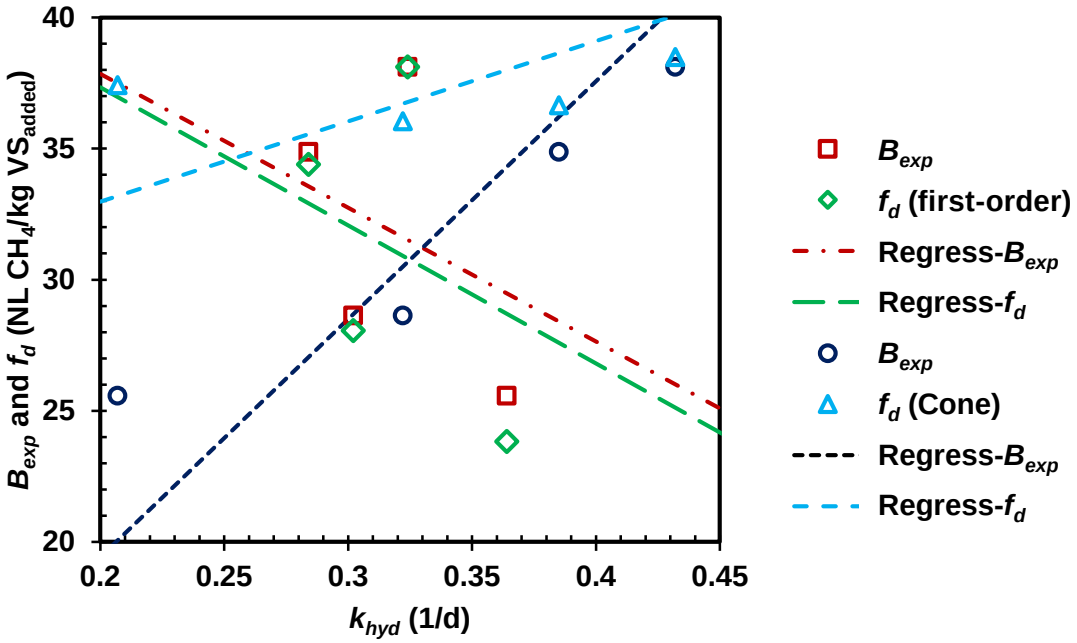

Figure 4.7. Pearson's correlations of the experimental ultimate methane yield (B_{exp}) and maximum methane potential (f_d) against hydrolysis reaction rate constant (k_{hyd}) for mono-digestion of dairy manure

The f_d estimated by the Cone model also was higher at higher TS% unless for the change in TS% from 7% to 8% that led to a slight reduction in the f_d of the Cone model. On the other hand, the k_{hyd} of the first-order kinetic model decreased by increasing the TS% in the bioreactor. However, the k_{hyd} of the Cone model first increased by increasing the TS content from 7% to 8% and then decreased by increasing the TS.

The Pearson's correlation was also applied to the co-digestion experiments while changing the TS content of the reactor (Figure 4.9). The results showed that the B_{exp} and f_d of the first-order kinetic model significantly correlated with the k_{hyd} (p-values: 0.0038 and 0.0146, respectively). The R_P obtained between B_{exp} and f_d with k_{hyd} for the first-order kinetic model were -0.9942 and -0.9783, respectively.

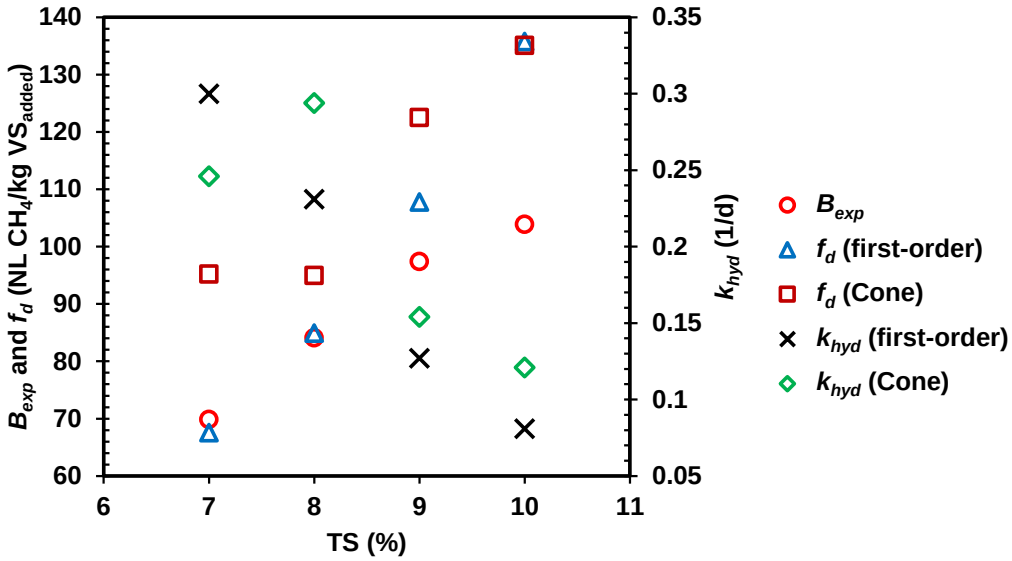

Figure 4.8. Relationship of the experimental ultimate methane yield (B_{exp}), maximum methane potential (f_d), and hydrolysis reaction rate constant (k_{hyd}) with the total solids for co-digestion of dairy manure and grass silage

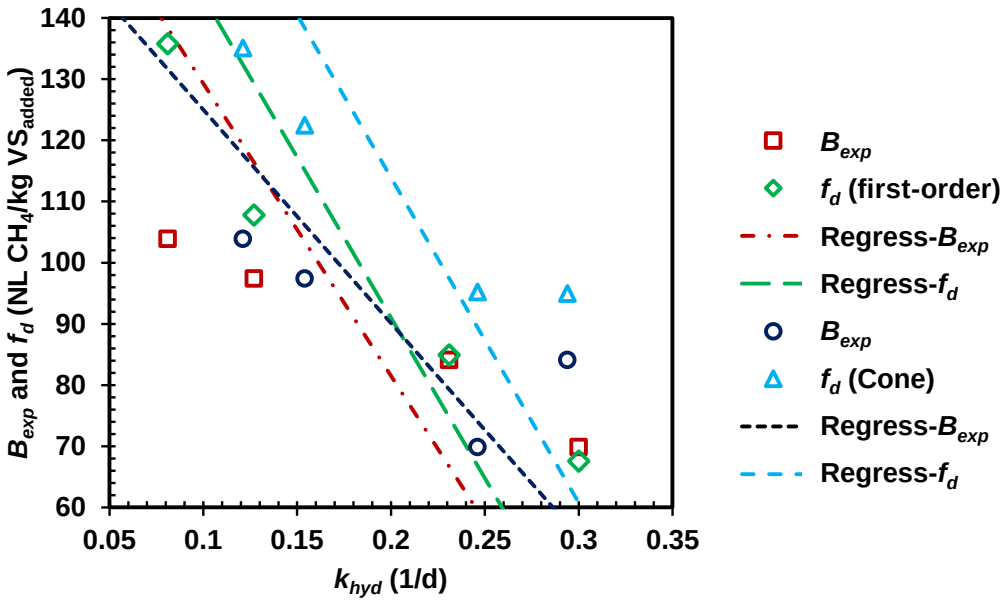

Figure 4.9. Pearson's correlations of the experimental ultimate methane yield (B_{exp}) and maximum methane potential (f_d) against hydrolysis reaction rate constant (k_{hyd}) for co-digestion of dairy manure and grass silage

The negative values of R_P indicate that the B_{exp} and f_d have an opposite trend compared to k_{hyd}. For the Cone model, no significant relationship was found between the experimental methane yield (B_{exp}) data with the k_{hyd}. However, a significant relationship was observed between the f_d and k_{hyd} of the Cone model.

4.7. Summary

This chapter studied the early stage of the culture's adaptation to psychrophilic (20 °C) anaerobic digestion of dairy manure and co-digestion of dairy manure and grass silage at increasing total solids content (7-10%). In mono-digestion of dairy manure, the higher total solid content resulted in a lower methane yield. By co-digesting grass silage and dairy manure, an opposite trend was observed and higher methane yield was obtained at the higher organic loading rate. The first-

order kinetic and Cone models are more accurate in predicting the methane yield from mono-digestion of dairy manure than co-digestion of dairy manure and grass silage. However, the modified Gompertz model predicted the results of both mono-digestion and co-digestion with the same order of accuracy. The hydrolysis rate constant of first-order kinetic and Cone models as well as R_m of modified Gompertz model decreased upon an increase in the organic loading rate.

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Chapter Five

ECONOMICS OF BIOGAS PLANT: CASE STUDY OF THE LESTER'S DAIRY FARM

5.1. Abstract

The establishment of a biogas plant requires cost analysis according to the estimated biogas production and the potential of electricity generation. This study investigated the economics of biogas plants, in which the potential of biogas production in Newfoundland and Labrador (NL) has been assessed. The data required for the cost analysis were obtained from the accessible literature, databases, and Lester's Dairy Farm, St. John's, and the conversion of biogas to electricity was estimated. The economic assessment was accomplished for NL considering various substrates, such as dairy manure, mink manure, and chicken manure. The results show that the total dairy manure in NL could produce 6,675,738 kWh electricity annually valued at 867,846 CAD. Considering other agricultural wastes, income from the biogas industry exceeds one million CAD. In Lester' dairy farm, using dairy manure only, there is an electricity generation potential of 219,300 kWh/month. This amount of electricity production covers not only their own energy demand (i.e., 25,000 kWh/month plus 65,790 kWh/month = 90,790 kWh/month), but there is a surplus that could be sold (128,510 kWh/month). The surplus generated electricity could earn 200,475 CAD annually for the farm.

5.2. Introduction

The successful completion of a biogas production project requires estimating the major costs involved in the project considering various assumptions and predictions regarding the economic,

technological, and legal aspects and using techniques from engineering economics. This chapter describes the economic analysis assesses the profit of converting NL livestock manure to Biogas. Analysis that is more detailed was applied to Lester's Dairy Farm, St. John's. The Canadian waste biomass has great potential. Canadian Biogas Association produced a map (Figure 5.1) showing the locations of biogas plants across Canada. The map shows digesters processing agricultural and food waste, sludge from wastewater treatment plants, and landfills. It also identifies the product of the facility: heat, natural gas, or electricity. Figure 5.1 shows that most of Canada's biogas plants are in Southern Ontario followed by Southern British Columbia. The other provinces also have some biogas plants. Most of the plants are agricultural and food waste digesters aiming at producing heat and electricity.

In Newfoundland and Labrador, there is only one on-farm biogas plant that processes dairy manure from the New World Dairy Farm; it is located at the island's west coast. It produces both heat and electricity. The New World Dairy started the biogas production project in 2010 with 1200 cattle (CBC Canada, 2013). Their estimation showed that they can produce about four million kilowatt-hours of electricity per year. The farms consumes a 20-25% of this value, and the rest is available for selling to the grid.

The Provincial Department of Natural Resources released Newfoundland and Labrador Farm Guide which provides relevant useful statistics on the Newfoundland and Labrador agriculture sector such as (CHFour Biogas Inc., 2012):

- Over 500 farms in NL
- 11,400 cattle, including dairy and beef, and 1,600 hogs
- 3,100 sheep
- 214,700 mink pelts from 15 farms in 2010

- 2,405 fox pelts from eight farms in 2010
- 359,000 chickens producing 110 million eggs (worth $16 million); over 60% growth since 2010
- Over 13 million kilogram poultry meat (valued at $23 million)

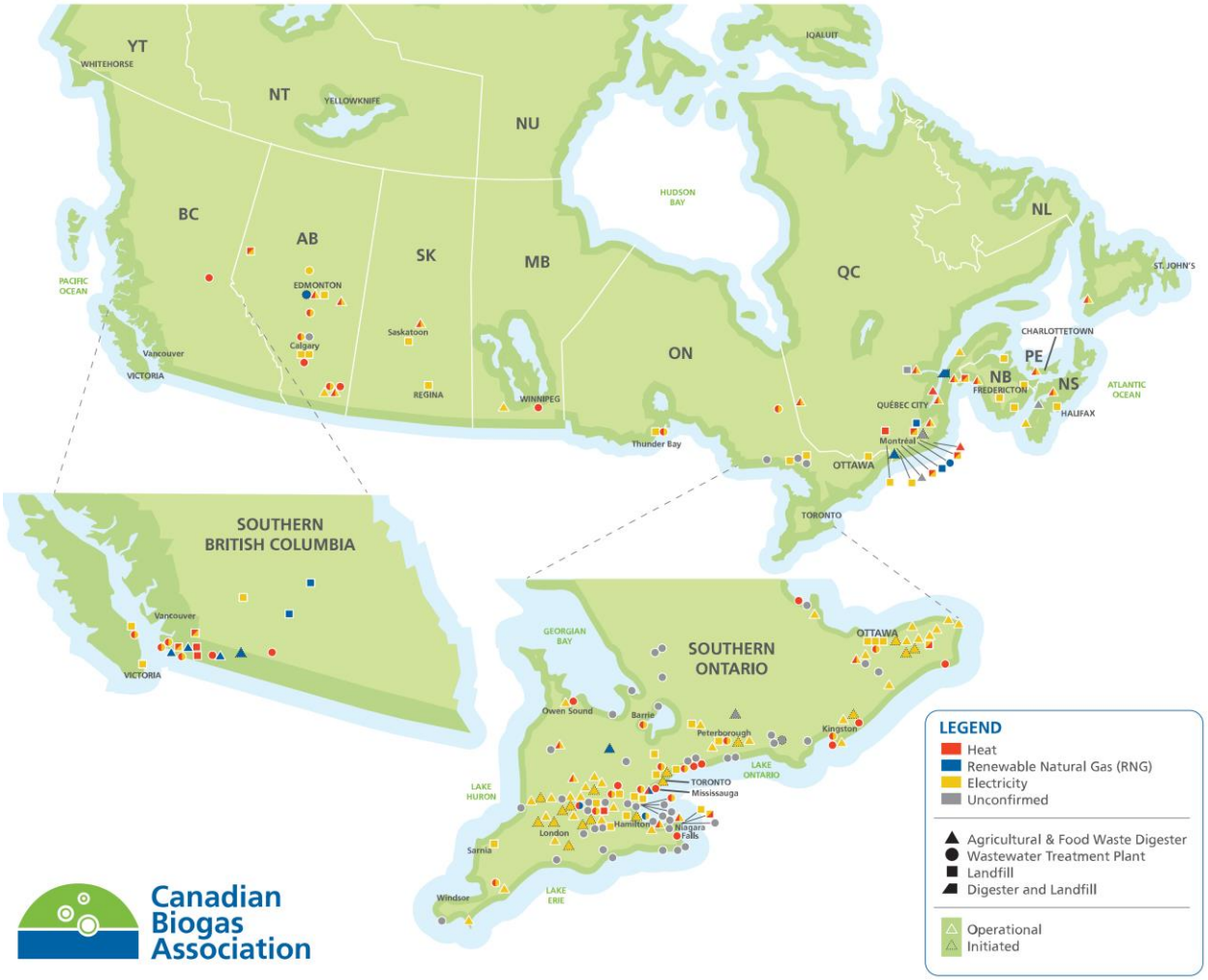

Figure 5.1. Biogas plants across Canada (2019)

There are some reports which investigated the options for organic waste processing in NL (Dillon Consulting, 2014), and the feasibility study of anaerobic digestion in NL (CHFour Biogas Inc., 2012). However, the economic assessment of on-farm biogas plants in NL with a focus on Lester's dairy farm provides a better understanding of the potential of biogas production in cold

environments. Thus, the objective of this study is to provide an estimation of biogas potential in NL. The Lester's dairy farm is also considered a case study to assess its biogas and electricity production potential.

5.3. Biogas Production Potential in NL

The agriculture industry in NL can be divided into three regions. Region 1 includes the Avalon Peninsula and Burin Peninsula. It is located at the east of NL. Region 2 consists of Bonavista Peninsula and Baie Verte Peninsula, and is located in the middle part of the NL. Region 3 comprises West Coast and Labrador. Statistics Canada reported the numbers of cattle, mink, and sheep in these regions in 2016 (Table 5.1). Based on this information, there are 9,995 cattle in the province including 5,299 dairy cows. In addition, the total numbers of mink and sheep are estimated to be 47,392 and 2,645, respectively.

A review conducted by CHFour biogas Inc. reported the amount of agricultural waste produced in different regions of the province (Table 5.2) (CHFour Biogas Inc., 2012). In total, 21,576 kg/day poultry litter along with 1,438.4 poultry mortalities were produced in the province in 2012. Dairy manure and mink manure were also estimated as 12,942 and 517.44 cubic feet per day. In addition to mink manure, there were 485,100 kg/year mink carcasses. Figure 5.2 shows the locations of farms in NL. There are many producers of different types of manure and other agriculture waste products in St. John's area with dairy farms have the largest portion of the waste produced.

Table 5.1. Distribution of livestock by agriculture division

Region	Total cattle	Dairy cows	Other cattle	Total mink	Total sheep
Region 1	3,365	1,642	1,723	*	1,756
Region 2	905	450	455	*	490
Region 3	5,725	3,207	2,518	13,771	399
Province	9,995	5,299	4,696	47,392	2,645

Note: Region 1: Avalon Pen. & Burin Pen.; Region 2: Bonavista Pen. & Baie Verte Pen.; Region 3: West Coast and Labrador
* Confidential, number included in the provincial total
Source: Statistics Canada, Census of Agriculture, 2016

Table 5.2. Agricultural waste production by feedstock and region in NL

Region	Poultry litter (kg/day)	Poultry mortalities (kg/day)	Dairy manure (ft^3/day)	Mink manure (ft^3/day)	Mink carcasses (kg/year)
Avalon	16,098	1,073.2	4,972	125.76	117,900
Clarenville (Bonavista Bay)	1,512	100.8	1,640	32.64	30,600
Gander	-	-	-	6.72	6,300
Grand Falls-Windsor	3,966	264.4	-	121.92	114,300
Corner Brook (West Coast)	-	-	6,330	230.4	216,000
Total	21,576	1,438.4	12,942	517.44	485,100

The average biogas yield for a tone dairy manure ranges between 15-30 m^3 based on the quality of the manure's organic matter (Angelidaki and Ellegaard, 2003). This biogas production per tone of chicken manure and mink manure is 75 and 75-100 m^3, respectively (CHFour Biogas Inc., 2012). On the other hand, the potential of electricity production is 2 kWh per 1 m^3 of biogas (CHFour Biogas Inc., 2012). It means that the electricity production from a ton of dairy manure, chicken manure, and mink manure is 46, 150, and 150-200 kWh, respectively.

The potential of biogas and energy production from agricultural livestock in NL was estimated in Table 5.3. The results show that there is excellent potential for energy production in NL. Dairy manure has the most potential in biogas and electricity production. The total dairy

manure in NL could produce 6,675,738 kWh electricity annually, valued at 867,846 CAD assuming 0.13 CAD/kWh.

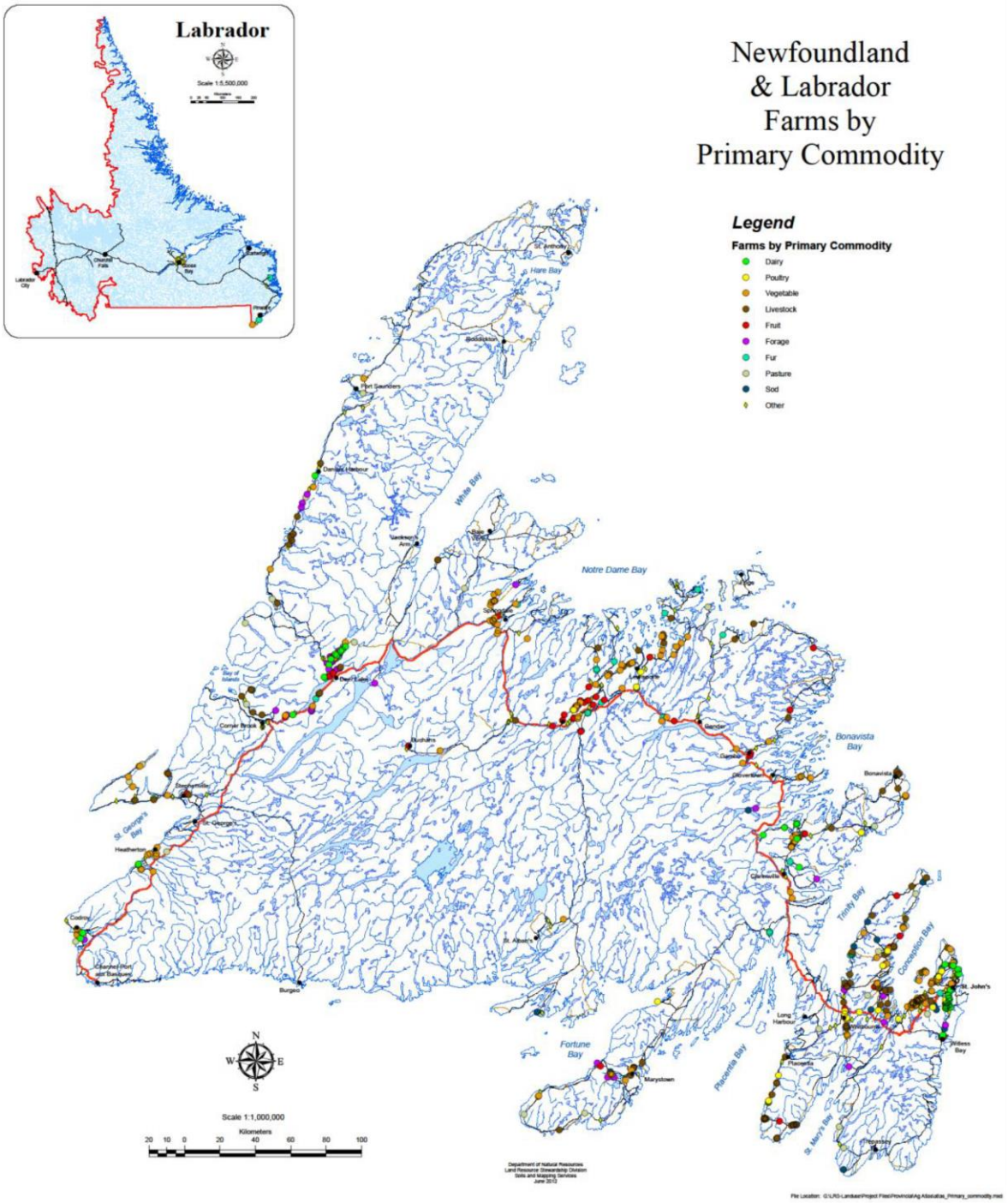

Figure 5.2. Agricultural production throughout the Newfoundland

Table 5.3. Estimation of biogas and energy potential from agricultural livestock in NL

Substrate	Amount (kg/day)	Ave. biogas production (m^3/tone of substrate)	Total biogas production (m^3/year)	Total electricity production** (kWh/year)	Total income*** (CAD)
Poultry litter	21,576	94	730,131.8	1,460,253.6	189,833
Poultry mortalities	1,438.4	75	38,836.8	77,673.6	10,098
Dairy manure*	403,124.3	23	3,337,869.2	6,675,738.4	867,846
Mink manure*	16,117.5	88	510,602.4	1,021,204.8	132,757
Mink carcasses	1,347.5	77	37,352.7	74,705.4	9,712

* The volume is converted to weight by the following factor: 1 m^3 = 1100 kg
** The conversion rate is assumed 2 kWh = 1 m^3
*** The conversion rate is assumed 0.13 CAD = 1 kWh

5.4. Biogas Production Potential in Lester's Dairy Farm

The Lester's dairy farm houses 550 cows. It means that the daily manure production (8% total solids) in Lester's Farm is 141 m^3/day (155 ton/day). Hence, the total biogas production potential in Lester's Farm is estimated to be 3,565 m^3/day. Thus, the electricity production potential in Lester's Farm is 7,130 kWh/day (297 kW capacity power plant). This means the nominal power of the biogas plant that would be installed is 300 kW. The total energy consumption in Lester's Farm ranges from 19,000 kWh/month in August to 30,000 kWh/month in April. The average electricity consumption on the farm is 25,000 kWh/month. The total electricity production potential in the farm is estimated to be 7,130 kWh/day (213,900 kWh/month). About 30% of the produced energy will be used to heat the digester and maintain its temperature at 20 ºC. This percentage increases to 50% if the digester will be operated at 35 ºC. The farm could increase its energy production by co-digesting any organic waste that is currently disposed of at the landfill. Therefore, the results show that the farm can produce 219,300 kWh/month by digesting manure only. The farm will use some of that energy to meet its energy demand (i.e., 25,000 kWh/month

plus 65,790 kWh/month = 90,790 kWh/month), and sale the surplus (around 128,510 kWh of electricity per month) (Figure 5.3).

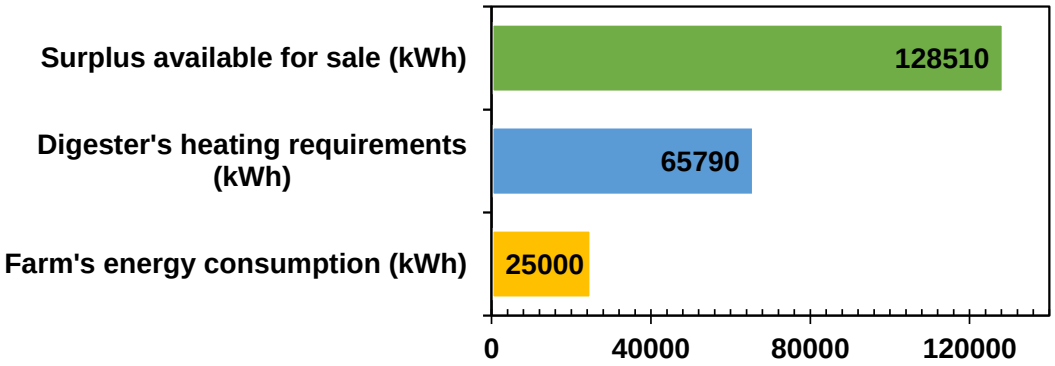

Figure 5.3. The contribution of electricity production from biogas

Of the total electricity produced in the farm, 11% will be consumed to fulfill the farm's electricity demand, and 30% to heat the digester. A 58% of the produced electricity (i.e., 128,510 kWh/month) could be sold.

Considering the total electricity production by the biogas plant in a year, it is expected to generate about 16,706.3 CAD/month (i.e., 200,475 CAD annually). Based on the closest and most relevant example of on-farm biogas plant processing dairy manure at the New World Dairy, NL, the investment was 5 million CAD for constructing and operating the biogas plant (CBC Canada, 2013). Since the New World Dairy has 1,200 cows compared to 550 at Lester's Dairy Farm and given that the actual investment was about 7 years ago, it is expected that the size of the reactor and the nominal power of the generator required for Lester's farm would be half as much. Therefore, the total investment costs required for Lester's farm biogas plant would be between 3 and 4 million CAD. Considering the 4 million dollars as the expenses, the payback period would

be 20 years. Notice that this scenario is based on processing dairy manure only. Any off-farm feedstock would increase energy production and shorten the payback period.

5.5. Benefits of Biogas Plants

However, the previous analyses considered only the biogas as the useful product of the project. There are useful products and sources of income in addition to the energy; these include the heat produced as a by-product, greenhouse gas emission credit, and the digestate (liquid and solid) as rich fertilizer concentrated with the nutrients, soil amendments. The fibers separated from the effluent stream can reduce the farm's operational costs and increase the income by production and sale of animal bedding. Table 5.4 summarizes the benefits and costs associated with the on-farm biogas plant.

Table 5.4. Comparison of advantages and costs associated with on-farm biogas plant

Benefits	**Costs**
Electricity production	Capital investment
Heat production	Operating and maintenance
GHG credit	Transportation
Fertilizer production	Culture adaptation
Job creation	
Environmental protection	

The on-farm digester will require adapted microbial culture. There are two ways to provide this culture. The first option is adaptating the culture during the start-up period. This option uses the culture from the nearby Riverhead wastewater treatment plant, St. John's (as used in this work) and adapts the culture based on the manure and other co-substrate. The culture adaptation process requires about a year to obtain a good culture. During this adaptation period, the biogas yield will start small and increase gradually. The second option is to import the adapted culture from an

operating active dairy farm biogas plant. The nearest and the only dairy farm biogas plant in NL is the New World Dairy which is located in the west of the island. The distance between the Lester's Dairy Farm, St. John's and the New World Dairy biogas plant is more than 800 km. The transportation of the culture (considered dangerous good) from the New World Dairy will impose a high cost.

The financial analysis depends on the final contracts that the farm would secure for selling the energy and other valuable products. Generally, it considers loans and interest rate, costs and savings from the product generated, purchase rate for the excess electricity.

Additional opportunities exist for income generation from environmental incentives, such as Renewable Energy Certificates (RECs) for electricity generation or Renewable Identification Number (RIN) credits under the Renewable Fuel Standard for the generation and use of biogas as vehicle fuel. There are also developing markets for carbon emission and nutrient offset credits. All these potential financial returns can benefit project stakeholders and others involved in the biogas system.

In addition to reducing methane emissions, some of the many environmental benefits of biogas systems include:

- Stabilization of nutrients for reduced water contamination risks, including substantial reduction of pathogens in manures and food wastes
- Nutrient recovery and recycling
- Reduction of odors during storage and decomposition
- Providing a natural waste treatment process
- Smaller physical footprint for organics waste processing versus composting
- Reduced volume of waste for transport and land application

- Efficient organic decomposition

As with the development of any energy project, biogas projects can benefit the local economy. Temporary jobs are created during the construction phase, and long-term jobs are created for the operation, maintenance, and logistics of transporting the off-farm feedstock, digestate, and fertilizers. Biogas energy projects involve engineers, construction firms, equipment vendors, and utilities or end-users of the power produced. Some materials for the overall project may be purchased locally, and often local firms handle construction, electrical, plumbing, and other services.

5.6. Summary

The costs associated with a biogas plant should be evaluated for the feasibility of the biogas plant. In this chapter, a cost analysis was performed, where the potential of biogas production in Newfoundland and Labrador (NL) has been assessed. The required data about the amount of manure production in NL and Lester's dairy farm was collected and used in the costs analysis. The results reveal that the total dairy manure in NL could produce 6,675,738 kWh electricity annually, valued at 867,846 CAD. Feeding other sources of agricultural wastes would increase the income from the biogas industry by over one million CAD. In Lester's dairy farm, using dairy manure only, there is an electricity generation potential of 219,300 kWh/month. This amount of electricity production covers not only their own energy demand (i.e., 25,000 kWh/month plus 65,790 kWh/month = 90,790 kWh/month), but there is a surplus that could be sold (128,510 kWh/month). The surplus generated electricity could earn 200,475 CAD annually for the farm.

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Chapter Six

ASSESSMENT OF HYDROGEN PRODUCTION FROM BIOMASS THROUGH THE INTEGRATION OF THE ANAEROBIC DIGESTION AND BIOGAS REFORMING

6.1. Abstract

Hydrogen is a clean fuel that can be used for heat and power generation and an intermediate component for chemical synthesis. This study presents the assessment of hydrogen production from biomass through integrating the psychrophilic anaerobic digestion with the dry methane reforming. For the first time, a rigorous model was developed for the low-temperature anaerobic digestion process by implementing the complex kinetics of the fermentation bioreactions. The produced biogas from the anaerobic digestion process is fed to the reforming process for hydrogen production. The kinetics of the dry methane reforming and water gas shift reactions over the Co-Ni-Al_2O_3 catalyst are employed in the model. The results of the proposed process are validated using the experimental data and show a less than 5% relative deviation. The effects of process operating variables, such as the total solids content, organic loading rate, hydraulic retention time, and digestion recirculation fraction on biogas and CH_4 yield are investigated. The optimum operating parameters in the anaerobic digestion process as well as the dry methane reforming process is obtained. The process aimed at achieving the highest CH_4 to H_2 conversion and the lowest energy consumption. The results indicate that 48.07 kg/h biogas could produce 8.11 kg/h hydrogen. The biomass to H_2 process offers an energetic efficiency of 72.85%, showing its superiority to similar processes, such as steam and auto-thermal reforming. Moreover, the results

show a high potential for CO_2 emission reduction (e.g., 398,736 tonnes/year), compared to the direct biogas combustion for electricity production.

6.2. Introduction

Civilization and population growth demand massive energy sources. Fossil fuels have always taken the primary responsibility of providing energy. Currently, fossil fuels contribute to 81% of the total energy consumption (Chen and Chen, 2020). The utilization of fossil fuels accounts for several environmental problems, such as air pollution, global warming, and soil degradation (Nicoletti et al., 2015). The increasing rate of fossil fuels consumption has diminished fossil energy resources (Cong et al., 2017). Thereby, researchers actively seek alternative energy sources to mitigate anthropogenic CO_2 emissions.

Hydrogen, the most abundant element in the universe and the only non-carbon fuel, possesses the highest energy content per unit mass. The heat of combustion of hydrogen (34 kcal/g) is significantly greater than that of petroleum (10.3-8.4 kcal/g), paraffin (10.3-9.8 kcal/g), graphite (7.8 kcal/g), and wood (4.2 kcal/g) (Jain, 2009). Hydrogen can be utilized as a fuel in the combustion process or a raw material in manufacturing chemicals (e.g., ammonia, methanol, hydrochloric acid, and hydrogen peroxide) (Abdalla et al., 2018; Rand and Dell, 2007). Having that said, hydrogen is globally accepted as an environmentally safe and secure alternative to fossil fuels.

In nature, hydrogen is not readily available as it is bounded by other elements, such as oxygen and carbon. It exists in various compounds, including water, biomass, and hydrocarbons. Multiple pathways are developed to extract hydrogen from these compounds, categorized into conventional and renewable technologies based on the raw materials used (Nikolaidis and

Poullikkas, 2017). In the former technology, fossil fuels will be used as raw material. The hydrogen will be produced through hydrocarbon pyrolysis (Schneider et al., 2020) and reforming (i.e., steam methane reforming (SMR), auto-thermal methane reforming (ATR), dry methane reforming (DMR), and partial oxidation) (Carapellucci and Giordano, 2020). The conventional hydrogen production methods are responsible for more than 95% of the industry's hydrogen requirements. According to the stats, 48% of the current hydrogen is produced from natural gas, 30% from heavy oils and naphtha, and 18% from coal (Dincer and Acar, 2015; Kothari et al., 2008). The second category utilizes renewable sources (e.g., biomass) for hydrogen production. Biohydrogen can be produced through either thermochemical or biological processes. The thermochemical methods include pyrolysis (Setiabudi et al., 2020), gasification (Salkuyeh et al., 2018), combustion, and liquefaction, while the biological processes involve dark fermentation (Hajizadeh et al., 2021), direct and in-direct bio-photolysis (Bechara et al., 2021), photo-fermentation, and sequential photo and dark fermentation (Zhang et al., 2020).

Biogas could be served as a bridge to use the advantages of the conventional technologies to produce renewable hydrogen. Biogas is similar to natural gas, which means it could be used as an alternative raw material to conventional hydrogen production technology. Another beneficiary feature of biohydrogen is its biomass origin. Biogas can be produced from various organic waste through anaerobic digestion (AD) process in a microbial environment (Saady and Massé, 2013). It is mainly composed of methane (CH_4: 40-65%), carbon dioxide (CO_2: 35-55%), and small fractions of water (H_2O), ammonia (NH_3), hydrogen sulfide (H_2S), and traces of other constituents. Generally, two types of processes are employed for producing H_2 from biogas. The first category utilizes biomethane, obtained by biogas upgrading technologies. The hydrogen is commonly produced from biomethane toward the SMR process. The most popular biogas upgrading

technologies are chemical absorption, physical absorption, pressure swing adsorption, membrane technology, and cryogenic distillation (Angelidaki et al., 2018). Note that hydrogen production using biomethane needs additional units for methane purification and compression; this leads to a 5-15% decrease in plant energy efficiency (Hashemi et al., 2019; Sun et al., 2015). The second category takes advantage of the CO_2 in the biogas stream. In this approach, the biogas stream containing CH_4 and CO_2 is sent to the reforming process (i.e., DMR process); this process has no separation/compression stage. Accordingly, any additional energy requirement is avoided. The presence of CO_2 in the feed enhances the CH_4 conversion to syngas. Thus, dry methane reforming (DMR) technology sounds the most suitable way to convert biogas to hydrogen. In the DMR, CH_4 reacts directly with CO_2 to produce hydrogen. Compared to other thermochemical methods, the DMR is an environmentally friendly process because GHGs (CH_4 and CO_2) are consumed in this process. The DMR method can be applied to the gas sources with high CO_2 content (i.e., biogas) (Kohn et al., 2014; Lunsford, 2000).

Biogas production through anaerobic digestion is a complex process involving substrate decomposition and multiple fermentation reactions. In addition, substrate complexity and presence of numerous microorganisms in the system makes the system multifaceted (Wang et al., 2017). Moreover, many parameters affect the anaerobic digestion (AD), including temperature, organic loading rate, total solids content, hydraulic retention time, carbon-to-nitrogen ratio, pH, and ammonia concentration (Panigrahi and Dubey, 2019). Thus, it is time-consuming and costly to examine the impact of all parameters through experiments.

Mathematical models allow the understanding of the system and represent the main features of a process. They could be used to formulate and validate hypotheses and to predict the behavior of the system at different conditions. The models also reduce the risk of performing experiments.

The mathematical modeling of the anaerobic digestion process was started in early 70's by developing simple models (Donoso-Bravo et al., 2011). However, upon the understanding of microbiological processes more successful and comprehensive models for AD were developed (Angelidaki et al., 1993; Angelidaki et al., 1999; Batstone et al., 2000; Vavilin et al., 1994). These models considered additional involving processes and species. In addition, more detailed kinetics were taken into account, which incorporated the inhibition of microorganisms during AD. Then, Batstone et al. (2002) developed a comprehensive model called the anaerobic digestion model no. 1 (ADM1), which describes the dynamics of 24 species with 19 bioconversion processes. ADM1 has shown many applications (Batstone and Keller, 2003; Fezzani and Cheikh, 2008; Jurado et al., 2016; Ozkan-Yucel and Gökçay, 2010) and several authors modified it to cover more species and processes (Fedorovich et al., 2003; Lübken et al., 2015; Ramirez et al., 2009). Although the ADM1 is a powerful model, the large number of parameters and variables makes ADM1 complex. Thus, simplifying the model and implementing it in a process simulation tool will be beneficial. It provides a reliable and easy-to-use platform to be further employed for anaerobic digestion modeling. In addition, process simulation tools will enable integrating upstream and downstream unit operations with the anaerobic digestion process. Process simulation/modeling is enormously endorsed by industry and researchers due to its broad application in different industries and academic fields. The process simulation studies can predict future scenarios, enhance the process operation, and reduce the costs associated with the plant design. Peris Serrano (2010) and Rajendran et al. (2014) used AD concepts from earlier studies and developed a process simulation framework. They used a two-stage process to simulate the AD process, a reactor for hydrolysis reactions and another reactor for acidogenesis, acetogenesis, and methanogenesis reactions. Lorenzo-Llanes et al. (2020) developed a simulation framework for up-flow anaerobic sludge

blanket (UASB) reactor to model the AD of vinasses in Aspen Plus. They integrated the ADM1 model and considered the sulfate reduction reactions through calculator blocks in Aspen Plus. Al-Rubaye et al. (2019) also simulated the AD of animal manure and wastewater using Aspen Plus. They considered a total of 46 reactions to represent the AD process. They studied the effects of hydrogen addition, pressure, and hydraulic retention time on methane yield. A simulation study was done by Harun et al. (2019) to investigate the AD of food waste at thermophilic temperature. They found that the produced biogas contains 52.91% CH_4, 42.52% CO_2, and 5% traces. In another study, Nguyen et al. (2014) developed a simulation model to assess the energy potential of food waste in the municipal solid waste stream. They used a simple equilibrium model to build the anaerobic reactor. The biogas composition was calculated according to the Buswell equation (Symons and Buswell, 1933). Bravo et al. (2018) developed a simulation framework to model the AD-based biogas production from volatile organic compounds (VOC). They used stoichiometric and kinetic reactors. The Monod-type expressions were used to account for the kinetics of the acidogenesis and methanogenesis steps. According to the literature, all previous simulation investigations aimed to model the AD process at mesophilic (35-45 °C) and thermophilic (55-65 °C) temperature ranges. However, the psychrophilic (< 20 °C) anaerobic digestion process could have many applications since a significant portion of the biosphere of Earth is in cold environments; yet no study is done on simulation of the psychrophilic anaerobic digestion process.

On the other hand, numerous studies have been conducted on modeling and simulating H_2 production from biogas through thermochemical methods. Marcoberardino et al. (2018) investigated two conventional methods of producing H_2 from biogas, including steam methane reforming and auto-thermal reforming, through a techno-economic analysis. They considered a reformer and two water gas shift reactors for conversion of biogas to H_2 and a pressure swing

adsorption model for H_2 purification for production of 100 kg/d H_2. In addition, they used the Gibbs free energy minimization concept to simulate the reactors. They obtained the maximum efficiency of 52% for the steam methane reforming process. Yao et al. (2017) compared three routes of H_2 production from biomass, including biomass steam gasification, biogas steam reforming, and water electrolysis. They found that water electrolysis has the highest energy efficiency (66%), followed by biogas steam reforming with an efficiency of 47%, and biomass gasification with an efficiency of 39%. They also used a Gibbs free energy minimization reactor to simulate the reformer and water gas shift reactor. Hajjaji et al. (2016) conducted a life cycle assessment of hydrogen production from biogas using steam reforming. The biogas production data were obtained from the literature. At the same time, an Aspen Plus simulation was used to attain the required data for the reforming process. The reforming and water gas shift reactors were modeled to minimize the Gibbs free energy. According to the simulation results, 11.53 kg/h H_2 was produced from 97.17 kg/h biogas (60% CH_4 and 35% CO_2). In addition, the thermal efficiency of the plant was equal to 76.8%. Chattanathan et al. (2014) explored the effect of H_2S concentration (0.5, 1, and 1.5%) on converting biogas to hydrogen through the dry reforming process. They used the Gibbs reactor in Aspen Plus to model the reforming reactions by minimizing the Gibbs free energy. Adding H_2S (0.5%) to the feed reduced the conversion of CH_4 and CO_2 by 20%, compared to the case in which only CH_4 (59%), CO_2 (39%), and N_2 (2%) were fed to the reactor. Cruz et al. (2018) evaluated the thermodynamic efficiency of the dry reforming of methane for hydrogen production from biogas (65% (v/v) CH_4 and 35% CO_2 (v/v)). They used Aspen Plus to evaluate the performance of the system using exergy analysis. The results indicated that the exergy efficiency of the dry methane reforming process for H_2 production is 55%. Minutillo et al. (2020) evaluated the green hydrogen production using steam methane reforming and auto-thermal

reforming through energy and exergy analyses. Their results indicated that the steam methane reforming has a 19% higher energy efficiency than the auto-thermal reforming process, showing a 59.8% H_2 energy efficiency.

According to the literature review, the integration of the anaerobic digestion model and dry methane reforming is rarely investigated in previous studies. Thus, the current study aimed to develop and integrate the low-temperature AD process with the DMR simulation model for the first time to produce H_2 from biomass. A detailed simulation model is developed using Aspen Plus V11, considering the kinetics of fermentation and reforming reactions. The knowledge gaps and novelties of the current study are highlighted below:

- Low-temperature AD simulation applicable for the temperature range of 10-65 °C
- Developing a temperature-dependent equation for the maximum specific growth rate of microorganisms
- Modification of the fermentation reaction rates according to the existing components in feed as well as operating conditions
- Integration of AD process with dry methane reforming process for the production of H_2 from biomass
- Simulation of reforming and water gas shift reactions using kinetic models

After the introduction, the theory and process description of the AD process and the dry methane reforming process are presented in section 6.3. The implemented methodology, including reaction kinetics and process simulation, is stated in section 6.4. Section 6.5 presents the results and discussion on biogas production and conversion to hydrogen production, sensitivity analysis,

and optimal process conditions. Finally, the major outcomes of this study are presented in section 6.6.

6.3. Theory and Process Description

This section consists of a brief review of the theoretical aspects and a description of the proposed process for producing hydrogen from biomass. Hydrogen production from biomass involves two main processes: 1) biogas production within anaerobic digester and 2) dry methane reforming for generation of H_2.

6.3.1. Anaerobic digestion

The first process deals with producing biogas from organic waste through anaerobic digestion. In this process, raw organic waste is fed to a bioreactor containing inoculum where microbial reactions occur (Holm-Nielsen et al., 2009). The microorganisms convert the organic fraction of the feed to CH_4 and CO_2 through the four steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Khan et al., 2016). Hydrolysis, the first step in the anaerobic digestion process, involves the physical conversion of large organic materials (polymers) such as carbohydrates, lipids, and proteins into monomers and small substances (Adekunle and Okolie, 2015). As a result, carbohydrates are broken down into simple sugars, proteins are degraded into amino acids, and lipids are hydrolyzed to long-chain fatty acids (LCFAs). Hydrolytic bacteria secrete extracellular enzymes to catalyze these parallel degradation processes. The bacteria directly use the soluble products. The hydrolysis step is the rate-limiting step when the substrate is complex or hard, such as lignocellulosic biomass (Tomei et al., 2009). Therefore, the hydrolysis step dramatically affects biogas production. In the hydrolysis step, enzymes play a leading role.

The enzymes such as cellulase, amylase, protease, and lipase break down large molecules into small molecules. A set of biochemical reactions can be defined to express the hydrolysis step (Equations (6-1) to (6-12)) (Rajendran et al., 2014). These reactions represent the degradation of large molecules, such as cellulose, hemicellulose, protein, triolein, etc., to smaller molecules.

$$\text{Cellulose } ((C_6H_{12}O_6)_n) + H_2O \rightarrow nC_6H_{12}O_6 \quad (6\text{-}1)$$

$$\text{Cellulose } (C_6H_{12}O_6) + H_2O \rightarrow 2C_2H_6O + 2CO_2 \quad (6\text{-}2)$$

$$\text{Hemicellulose } (C_5H_8O_4) + H_2O \rightarrow 2.5C_2H_4O_2 \quad (6\text{-}3)$$

$$\text{Hemicellulose } (C_5H_8O_4) + H_2O \rightarrow C_5H_{10}O_5 \quad (6\text{-}4)$$

$$\text{Xylose } (C_5H_{10}O_5) \rightarrow C_5H_4O_2 + 3H_2O \quad (6\text{-}5)$$

$$2 \text{ Ethanol } (C_2H_6O) + CO_2 \rightarrow 2C_2H_4O_2 + CH_4 \quad (6\text{-}6)$$

$$\text{Soluble protein } (C_{13}H_{25}O_7N_3S) + 6H_2O \rightarrow 6.5CO_2 + 6.5CH_4 + 3H_3N + H_2S \quad (6\text{-}7)$$

$$\text{Insoluble protein} + 0.3337H_2O \rightarrow 0.045C_6H_{14}N_4O_2 + 0.048C_4H_7NO_4 + 0.047C_4H_9NO_3 + 0.172C_3H_7NO_3 + 0.074C_5H_9NO_4 + 0.111C_5H_9NO_2 + 0.25C_2H_5NO_2 + 0.047C_3H_7NO_2 + 0.067C_3H_6NO_2S + 0.074C_5H_{11}NO_2 + 0.07C_6H_{13}NO_2 + 0.046C_6H_{13}NO_2 + 0.036C_9H_{11}NO_2 \quad (6\text{-}8)$$

$$\text{Triolein } (C_{57}H_{104}O_6) + 3H_2O \rightarrow C_3H_8O_3 + 3C_{18}H_{34}O_2 \quad (6\text{-}9)$$

$$\text{Tripalmate } (C_{51}H_{98}O_6) + 8.436H_2O \rightarrow 4C_3H_8O_3 + 2.43C_{16}H_{34}O \quad (6\text{-}10)$$

$$\text{Palmito-olein } (C_{37}H_{70}O_5) + 4.1H_2O \rightarrow 2.1C_3H_8O_3 + 0.9C_{16}H_{34}O + 0.9C_{18}H_{34}O_2 \quad (6\text{-}11)$$

$$\text{Palmito-linolein } (C_{37}H_{68}O_5) + 4.3H_2O \rightarrow 2.2C_3H_8O_3 + 0.9C_{16}H_{34}O + 0.9C_{18}H_{32}O_2 \quad (6\text{-}12)$$

During the hydrolysis step, some large molecules are directly converted to hydrogen and acetate, enabling the methanogens (i.e., the methane-producing microorganisms) produce methane. However, most hydrolysis products are still relatively large and require further conversion to smaller molecules. In the next step, the products of the hydrolysis step will be converted to forms usable by methanogens. This step is called acidogenesis and is carried out by acidogenic bacteria. In this step, simple sugars, amino acids, and fatty acids are degraded to

produce acetate, carbon dioxide, and hydrogen. In addition, short-chain volatile fatty acids (VFAs) and alcohols are produced. The reactions occurring in the acidogenesis step are provided in Equations (6-13) to (6-37) (Rajendran et al., 2014).

Glycine ($C_2H_5NO_2$) + H_2 → $C_2H_4O_2$ + NH_3	(6-13)
Threonine ($C_4H_9NO_3$) + H_2 → $C_2H_4O_2$ + 0.5$C_4H_8O_2$ + H_3N	(6-14)
Histidine ($C_6H_8N_3O_2$) + 4H_2O + 0.5H_2 → CH_3NO + $C_2H_4O_2$ + 0.5$C_4H_8O_2$ + 2H_3N + CO_2	(6-15)
Arginine ($C_6H_{14}N_4O$) + 3H_2O + H_2 → 0.5$C_2H_4O_2$ + 0.5$C_3H_6O_2$ + 0.5$C_5H_{10}O_2$ + 4H_3N + CO_2	(6-16)
Proline ($C_5H_9NO_2$) + H_2O +H_2 → 0.5C_2H_4O + 0.5$C_3H_6O_2$ + 0.5$C_5H_{10}O_2$ + H_3N	(6-17)
Methionine ($C_5H_{11}NO_2S$) + 2H_2O → $C_3H_6O_2$ + CO_2 + H_3N + H_2 + CH_4S	(6-18)
Serine ($C_3H_7NO_3$) + H_2O → $C_2H_4O_2$ + H_3N + CO_2 + H_2	(6-19)
Threonine ($C_4H_9NO_3$) + H_2O → $C_3H_6O_2$ + H_3N +H_2 + CO_2	(6-20)
Aspartic acid ($C_4H_7NO_4$) + 2H_2O → $C_2H_4O_2$ + H_3N + 2CO_2 + 2H_2	(6-21)
Glutamic acid ($C_5H_9NO_4$) + H_2O → $C_2H_4O_2$ + 0.5$C_4H_8O_2$ + H_3N + CO_2	(6-22)
Glutamic acid ($C_5H_9NO_4$) + 2H_2O → 2$C_2H_4O_2$ + H_3N + CO_2 + H_2	(6-23)
Histidine ($C_6H_8N_3O_2$) + 5H_2O → CH_3NO + 2$C_2H_4O_2$ + 2H_3N + CO_2 + 0.5H_2	(6-24)
Arginine ($C_6H_{14}N_4O_2$) + 6H_2O → 2$C_2H_4O_2$ + 4H_3N + 2CO_2 + 3H_2	(6-25)
Lysine ($C_6H_{14}N_2O_2$) + 2H_2O → $C_2H_4O_2$ + $C_4H_8O_2$ + 2H_3N	(6-26)
Leucine ($C_6H_{13}NO_2$) + 2H_2O → $C_5H_{10}O_2$ + H_3N + CO_2 + 2H_2	(6-27)
Iso-leucine ($C_6H_{13}NO_2$) + 2H_2O → $C_5H_{10}O_2$ + H_3N + CO_2 + 2H_2	(6-28)
Valine ($C_5H_{11}NO_2$) + 2H_2O → $C_4H_8O_2$ + H_3N + CO_2 + 2H_2	(6-29)
Phenyl alanine ($C_9H_{11}NO_2$) + 2H_2O → C_6H_6 + $C_2H_4O_2$ + H_3N + CO_2 + H_2	(6-30)
Tyrosine ($C_9H_{11}NO_3$) + 2H_2O → C_6H_6O + $C_2H_4O_2$ + H_3N + CO_2 + H_2	(6-31)
Tryptophan ($C_{11}H_{12}N_2O_2$) + 2H_2O → C_8H_7N + $C_2H_4O_2$ + H_3N + CO_2 + H_2	(6-32)
Glycine ($C_2H_5NO_2$) + 0.5H_2O → 0.75$C_2H_4O_2$ + H_3N + 0.5CO_2	(6-33)
Alanine ($C_3H_7NO_2$) + 2H_2O → $C_2H_4O_2$ + H_3N + CO_2 + 2H_2	(6-34)
Cysteine ($C_3H_6NO_2S$) + 2H_2O → $C_2H_4O_2$ + H_3N + CO_2 + 0.5H_2 + H_2S	(6-35)

$$\text{Dextrose } (C_6H_{12}O_6) + 0.1115H_3N \rightarrow 0.1115C_5H_7NO_2 + 0.744C_2H_4O_2 + 0.5C_3H_6O_2 + 0.4409C_4H_8O_2 + 0.6909CO_2 + 1.0254H_2O \quad (6\text{-}36)$$

$$\text{Glycerol } (C_3H_8O_3) + 0.4071H_3N + 0.0291CO_2 + 0.0005H_2 \rightarrow 0.04071C_5H_7NO_2 + 0.94185C_3H_6O_2 + 1.09308H_2O \quad (6\text{-}37)$$

The next step is acetogenesis in which acetogenic bacteria convert the acidic products (e.g., propionic acid, butyric acid, and valeric acid) to acetic acid, hydrogen, and carbon dioxide; substrates that methanogens consume to produce methane. Methanogenesis is the final stage of AD, where methane (CH_4) is generated either from VFAs or directly from hydrogen and carbon dioxide. Methane is an ideal waste product for AD as it is sparingly soluble in water. Once it is generated, it leaves the aqueous phase as gas and does not further affect the microbial ecology. Equations (6-38) to (6-43) represent the acetogenic reactions, and the methanogenesis reactions are expressed as in Equations (6-44) and (6-45) (Rajendran et al., 2014).

$$\text{Oleic acid } (C_{18}H_{34}O_2) + 15.2396H_2O + 0.2501CO_2 + 0.1701H_3N \rightarrow 0.1701C_5H_7NO_2 + 8.6998C_2H_4O_2 + 14.4978H_2 \quad (6\text{-}38)$$

$$\text{Propionic acid } (C_3H_6O_2) + 0.06198H_3N + 0.314336H_2O \rightarrow 0.06198C_5H_7NO_2 + 0.9345C_2H_4O_2 + 0.660412CH_4 + 0.160688CO_2 + 0.00055H_2 \quad (6\text{-}39)$$

$$\text{Iso-butyric acid } (C_4H_8O_2) + 0.0653H_3N + 0.8038H_2O + 0.0006H_2 + 0.5543CO_2 \rightarrow 0.0653C_5H_7NO_2 + 1.8909C_2H_4O_2 + 0.446CH_4 \quad (6\text{-}40)$$

$$\text{Iso-valeric acid } (C_5H_{10}O_2) + 0.0653H_3N + 0.5543CO_2 + 0.8044H_2O \rightarrow 0.0653C_5H_7NO_2 + 0.8912C_2H_4O_2 + C_3H_6O_2 + 0.4454CH_4 + 0.0006H_2 \quad (6\text{-}41)$$

$$\text{Linoleic acid } (C_{18}H_{32}O_2) + 15.356H_2O + 0.482CO_2 + 0.1701H_3N \rightarrow 0.1701C_5H_7NO_2 + 9.02C_2H_4O_2 + 10.0723H_2 \quad (6\text{-}42)$$

$$\text{Palmitic acid } (C_{16}H_{34}O) + 15.253H_2O + 0.482CO_2 + 0.1701H_3N \rightarrow 0.1701C_5H_7NO_2 + 8.4402C_2H_4O_2 + 14.9748H_2 \quad (6\text{-}43)$$

$$\text{Acetic acid } (C_2H_4O_2) + 0.022H_3N \rightarrow 0.022C_5H_7NO_2 + 0.945CH_4 + 0.066H_2O + 0.945CO_2 \quad (6\text{-}44)$$

$$14.4976H_2 + 3.8334CO_2 + 0.0836H_3N \rightarrow 0.0836C_5H_7NO_2 + 3.4154CH_4 + 7.4996H_2O \quad (6\text{-}45)$$

The performance of AD depends on parameters that should be considered in the design and operation of an anaerobic digester. The most critical parameters involving in the anaerobic digestion process are total solids (TS) content, volatile solids (VS) content (it refers to the amount of organic matter), Carbon-to-Nitrogen (C/N) ratio of the substrate, pH and alkalinity, temperature, hydraulic retention time (HRT), organic loading rate (OLR), and inoculum-to-substrate ratio (ISR) (Rocamora et al., 2020; Zamri et al., 2021). The water content of the feed should be modified (by dilution) to achieve the desired total solids content within the bioreactor. The produced biogas mainly contains CH_4 and CO_2. Also, the undigested fraction of the organic waste could be gathered and disposed of or partially recirculated to the inlet to achieve higher biogas production (Algapani et al., 2019).

6.3.2. Dry methane reforming (DMR)

The main step in the dry methane reforming (DMR) is syngas production from CH_4 and CO_2, according to Equation (6-46). Since this reaction is highly endothermic (Lavoie, 2014), it is promoted at higher temperatures (Gao et al., 2018). In addition, side reactions take place in the reformer; the most common side reaction is the reverse water gas shift (RWGS) reaction (Equation (6-47)). Although the RWGS reaction is an unwanted, it helps adjust the H_2/CO ratio for the production of fuels with higher hydrocarbons (Abdullah et al., 2017) and improves the reformer's performance in terms of CH_4 and CO_2 conversion (Kathiraser et al., 2015).

$$CH_4 + CO_2 \rightarrow 2H_2 + 2CO \qquad \Delta H = 247.3\ kJ/mol \qquad (6\text{-}46)$$

$$CO_2 + H_2 \leftrightarrow CO + H_2O \qquad \Delta H = 41\ kJ/mol \qquad (6\text{-}47)$$

The DMR is a heterogeneous catalytic reaction and is carried out in fixed bed reactors. The catalysts used in the DMR are usually based on nickel or noble metals, such as rhodium (Rh), ruthenium (Ru), palladium (Pd), platinum (Pt), and iron (Fe), on supports such as Al_2O_3, SiO_2, La_2O_3, ZrO_2 (Aramouni et al., 2018; Pakhare and Spivey, 2014; Requies et al., 2009). The nickel-based catalysts are the most feasible catalysts to use in industry because of their availability and lower costs (Abdullah et al., 2017).

In addition to the high temperature required for the DMR reaction, the catalyst deactivation is a problem associated with this process. The catalyst deactivation happens because of the coke deposition over the catalyst particles and the resulting growth of carbon when it is dissolves in nickel (Ginsburg et al., 2005; Snoeck et al., 1997). This problem can cause the catalyst loss and even blockage in the reactor. The noble metals have shown a high resistance over coking and are reported as a solution to prevent the coke deposition, but they are expensive. Therefore, bimetallic Ni-Co is commonly hired in large-scale industry as it implements the advantages of both metals in controlling coke and catalyst deactivation (Luisetto et al., 2012; Zhang et al., 2007a).

The syngas produced in the reformer contains H_2, CO, and unreacted CH_4 and CO_2. The water gas shift (WGS) reaction (Equation (6-48)) is used to enrich H_2 in the product. In WGS reaction, the CO produced in the reformer reacts with steam to produce H_2 and CO_2 (Chein et al., 2013). Thus, the WGS reaction is important in producing H_2 because it is also used for converting CO to CO_2, which helps to capture CO_2.

$$CO + H_2O \leftrightarrow H_2 + CO_2 \qquad \Delta H = -41\ kJ/mol \tag{6-48}$$

6.4. Methodology

Biogas mainly contains CH_4 and CO_2, which can be used as feed to produce various products. The interest is increasing in producing H_2 from biomass because it is a clean fuel. H_2 can be produced from biogas through the reforming process, which uses biogas to produce syngas (i.e., CO and H_2). Then, the produced syngas is fed to a water gas shift (WGS) reactor to convert carbon monoxide to hydrogen.

The process of H_2 production from biomass is modeled using Aspen Plus V11 since it provides a flexible environment for considering various aspects of the process. The first step is selecting/defining the chemical components. The major components in the overall process are H_2, CH_4, CO_2, H_2O, CO, O_2, N_2, NH_3, H_2S, as well as the biomass components such as cellulose, hemicellulose, dextrose, proteins, etc. (refer to Equations (6-1) to (6-45) for the all components). The components are provided based on the different feedstocks, intermediate and final products of the anaerobic digestion process. Aspen Plus databanks provide the pure component specifications for most of the components involved in the process. However, some components, such as proteins, need to be defined by user. The next step is to define a proper thermodynamic model. Since the vapor phase in the anaerobic digestion process mainly contains CH_4 and CO_2, and the pressure is low, the vapor phase is treated as an ideal gas. However, the liquid phase is not ideal as many compounds and interactions occur in the liquid phase. Based on this fact, the Non-Random Two Liquids (NRTL) thermodynamic model is a suitable model to predict the physical/chemical equilibrium of the system. The NRTL model in Aspen Plus takes the vapor phase as an ideal gas

and considers the non-ideality of the liquid phase by calculating the activity coefficient of components in the liquid phase (Aspen Technology, 2020).

Anaerobic Digestion Reactor. The anaerobic digestion model is developed according to the model introduced by Angelidaki et al. (1999). This model was successfully used by Rajendran et al. (2014) where they developed the process simulation model (PSM). The current study modifies the PSM to be applicable to low temperature anaerobic digestion. As is said, the anaerobic digestion process comprises hydrolysis, acidogenesis, acetogenesis, and methanogenesis steps. Biomass hydrolysis is a complex process, and the reaction kinetics are not completely available (Balaji et al., 2020). However, the stoichiometry of the hydrolysis reactions are available. Thus, the biomass hydrolysis is modeled in this study using a stoichiometric reactor (i.e., RStoic) in Aspen Plus. The RStoic model is used when the reaction kinetics are not available, but the stoichiometry of the reactions is known. The RStoic model requires the data that shows the extent of the reaction or conversion (Aspen Technology, 2020). In this regard, the hydrolysis reactions (Equations (6-1) to (6-12)) for the components that existed in the feed are adapted from Rajendran et al. (2014). This set of reactions provides a comprehensive framework to model various substrates. Each substrate has its specific components, which could be specified in the feed specifications. The hydrolysis products are routed to a continuous stirred tank reactor (CSTR) in which the fermentation reactions (acidogenesis, acetogenesis, and methanogenesis) will occur. The CSTR reactor in Aspen Plus (called RCSTR) is capable of modeling reactions with known stoichiometry and reaction kinetics (Aspen Technology, 2020). This study considers 33 reactions to take place in the RCSTR simultaneously (Equations (6-13) to (6-45)). These reactions are 25 (acidogenic), 6 (acetogenic), and 2 (methanogenic). The rate of the fermentation reactions (μ) is

presented as a first-order kinetic equation, shown in Equation (6-49). The reaction rate is a function of the maximum specific growth rate of microorganisms (μ_{max}) and inhibition terms. The performance of microorganisms might be inhibited by several factors. For instance, the rate of the VFA acetogenic step could be represented as Equation (49) (Angelidaki et al., 1999).

$$\mu = \mu_{max}(T)\left(\frac{1}{1+\frac{K_s(T)}{[A]}}\right)\left(\frac{1}{1+\frac{K_{s,NH_3}}{[T-NH_3]}}\right)\left(\frac{1}{1+\frac{[HAc]}{K_{i,HAc}}}\right)\left(\frac{1}{1+\frac{[LCFA]}{K_{i,LCFA}}}\right)\left(\frac{1}{1+\frac{[H_2]}{K_{i,H_2(T)}}}\right)F(pH) \quad (6\text{-}49)$$

In Equation (6-49), μ is the reaction rate; S stands for the substrate for insoluble carbohydrates or for the insoluble proteins; $\mu_{max}(T)$ is identical for the temperature-dependent maximum specific growth rate of microorganisms; K_s presents the half-saturation constant; K_{s,NH_3} shows the half-saturation constant for total ammonia; [T-NH_3] indicates the total ammonia concentration; K_i denotes inhibition constants; $F(pH)$ is the pH growth-modulating function.

$\mu_{max}(T)$, the temperature-dependent maximum specific growth rate of microorganisms is expressed in Equation (6-50) to consider the effect of temperature change on the maximum specific growth rate.

$$\mu_{max}(T) = \mu_{max}e^{-\frac{E_A}{R}(\frac{1}{T}-\frac{1}{T_0})} \quad (6\text{-}50)$$

where μ_{max} indicates the maximum specific growth rate constant; E_A represents the activation energy; R is the gas universal; T stands for the absolute temperature; and T_0 denotes the absolute reference temperature.

The study of Rajendran et al. (2014) used a temperature function to calculate the maximum specific growth rate, which is suitable for the mesophilic and thermophilic temperature range. However, to use the model for the psychrophilic temperatures, the maximum specific growth rate equation should be modified. In this regard, the constant parameters in the maximum specific growth rate equation are calculated for a wider temperature range (10 to 65 ºC). In addition, the maximum specific growth rate has a positive relationship with temperature; when the temperature increases, the maximum specific growth rate increases. It was observed that in the model developed by Rajendran et al. (2014) the equation predicts lower maximum specific growth rate at higher temperatures (for example, 20 d^{-1} at 55 ºC and 30.8 d^{-1} at 35 ºC for propionic acid degradation). This lead to non-reliable results when the temperature changes.

The basic parameters reported for the specific growth rate are at temperatures of 35 ºC and 55 ºC in ADM1 (Batstone et al., 2002). Donoso-Bravo et al. (2009) reported the relative changes of maximum specific growth rate with temperature for sugars, amino acids, and fatty acids uptake in the temperature range of 10 ºC to 35 ºC. Also, Rebac et al. (1995) provided the maximum specific growth rate for butyrate and propionate uptake, and acetoclastic methanogens and hydrogenotrophic methanogens in the same temperature range. Thus, using the reported relative changes of maximum specific growth rate with temperature, the new constants are calculated and implemented in this study.

The biochemical reaction rate could be inhibited by ammonia, short-chain fatty acids, long-chain fatty acids, and H_2. Thus, the inhibition factors should be considered in the modeling of reactions in Aspen Plus. Since Aspen Plus does not provide a built-in reaction rate format for the biochemical reactions, several calculator blocks are developed in Aspen Plus to model the fermentation reactions accurately. In other words, the current study does not use a fixed reaction

rate for the fermentation reactions. The calculator blocks use the ForTran programming language to calculate the reaction rate of each reaction according to the operating conditions and the concentration of each component. For example, altering the NH_3 concentration in the feed changes the reaction rate accordingly. The calculator blocks improve the accuracy of the model by calculating and updating the reactions rate. However, the use of calculator blocks increases the computation time and makes the simulation difficult to converge. This is due to accounting a large number of reactions (i.e., 33 reactions) with their respective calculations in each iteration when solving the RCSTR. A list of full reaction rates, constant parameters, and all required inputs for the calculator blocks can be found in the literature (Angelidaki et al., 1993; Angelidaki et al., 1999; Batstone et al., 2002). In this study, cow manure is used as feed to produce biogas. The feed characteristics are presented in Table 6.1. based on Kaparaju et al. (2009) study. Also, the composition of cow manure is obtained from Budiyono et al. (2011).

Table 6.1. The feed characteristics based on the study of Kaparaju et al. (2009)

Characteristics	Value
Substrate	Cow manure
Temperature (ºC)	23
Pressure (kPa)	101.33
Loading rate (L/d)	0.333
TS%	6
VS% (of TS)	80

Dry Reforming Process. The biogas produced in the AD process is fed to the dry methane reforming (DMR) process. The reforming reaction (Equation (6-46)) proceeds using a catalyst. In this study, 5Co-15Ni/80Al_2O_3 (wt.%) catalyst is used in the reforming reactor (Balaji et al., 2020). The Langmuir-Hinshelwood-Hougen-Watson (LHHW) in Aspen Plus is selected to model the reaction. The reforming reaction is adapted from Foo et al. (2011) (Equation (6-51)).

$$-r_{DMR} = \frac{k_1\sqrt{P_{CH_4}}\sqrt{P_{CO_2}}}{1 + k_2\sqrt{P_{CH_4}} + k_3\sqrt{P_{CO_2}} + k_4\sqrt{P_{CH_4}}\sqrt{P_{CO_2}}} \quad (6\text{-}51)$$

The rate of the water-gas shift (WGS) reaction (Equation (6-48)) was explained by Hou and Hughes (2001) (Equation (6-52)). The kinetic parameters are obtained by regressing experimental data from Hou and Hughes (2001).

$$-r_{WGS} = \frac{k_5\left(k_6\frac{P_{CO_2}\sqrt{P_{H_2}}}{\sqrt{P_{H_2O}}} - \frac{P_{CO}\sqrt{P_{H_2O}}}{\sqrt{P_{H_2}}}\right)}{\left(1 + k_7P_{CO} + k_8\sqrt{P_{H_2}} + k_9\frac{\sqrt{P_{H_2O}}}{\sqrt{P_{H_2}}}\right)^2} \quad (6\text{-}52)$$

The k_i in Equations (6-51) and (6-52) follows the simple Arrhenius equation as shown below:

$$k_i = k_{0i}e^{-\frac{E_i}{RT}} \quad (6\text{-}53)$$

The kinetic parameters of the dry methane reforming and the water-gas shift reactions are presented in Table 6.2 according to Equation (6-53).

Table 6.2. The kinetic parameters of the dry methane reforming and the water-gas shift reactions

Kinetic Parameter (k_i)	**Pre-exponential factor (k_{0i})**	**Activation energy, E_i ($\frac{J}{mol}$)**	**Kinetic Parameter (k_i)**	**Pre-exponential factor (k_{0i})**	**Activation energy, E_i ($\frac{J}{mol}$)**
k_1	1.232×10-5 $\frac{kmol}{kg_{cat.}.s.Pa}$	56400	k_5	5.169×10-4 $\frac{kmol}{kg_{cat.}.s.Pa}$	15542.115
k_2	1.037×10-6 $\frac{1}{kPa^{0.5}}$	49155	k_6	5.659×101 $\frac{1}{kPa}$	-36583.64
k_3	3.716×10-3 $\frac{1}{kPa^{0.5}}$	-30960	k_7	5.248×10-13 $\frac{1}{kPa^{0.5}}$	-139800
k_4	3.854×10-9 $\frac{1}{kPa}$	18195	k_8	5.636×10-10 $\frac{1}{kPa^{0.5}}$	-93400
			k_9	6.549	11096.798

The steps that are taken in account to conduct the current study are summarized in Figure 6.1. After finalizing the simulation model, the energetic performance of the proposed process is evaluated using Equation (6-54). The thermal energetic efficiency (η_{th}) is defined as the output energy divided by the input energy (Simpson and Lutz, 2007).

$$\eta_{th} = \frac{\dot{m}_{H_2} \times LHV_{H_2}}{\dot{m}_{BG} \times LHV_{BG} + \frac{W_c + W_P}{\eta_E} + \sum \dot{Q}} \quad (6\text{-}54)$$

where, $\dot{m}_{H_2}$ stands for the mass flow rate of produced H_2 in kg/h; LHV represents the lower heating value in MJ/kg (which is 119.9 for H_2 and 20.2 for biogas); $\dot{m}_{BG}$ is the mass flow rate of biogas in kg/h; W_C denotes the compressor power; W_P indicates the pump power; $\dot{Q}$ is the heat supplied to the system; and η_E stands for the efficiency of the heat energy supplied and is set to 45%.

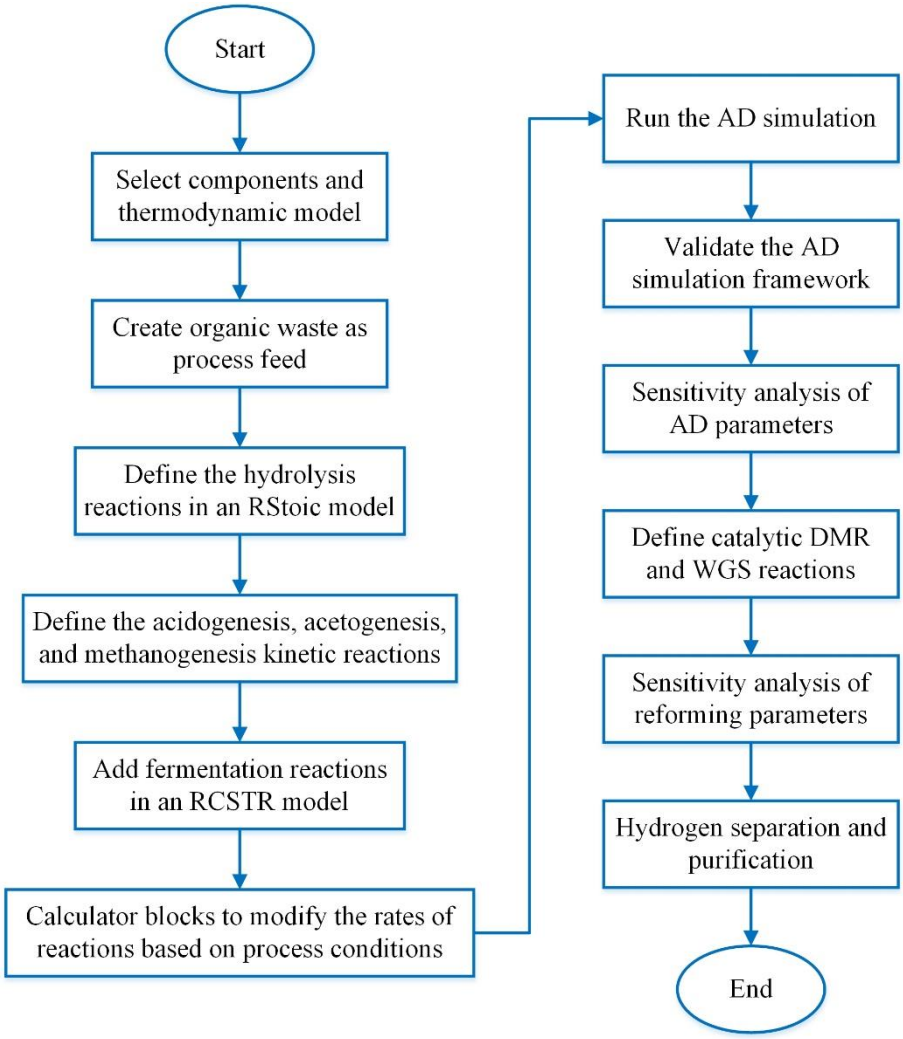

Figure 6.1. The flowchart of constructing the biomass-to-H_2 production process simulation

Process Simulation. The process flowsheet is shown in Figure 6.2. Before entering the raw biomass to the process, its properties (e.g., water content) should be manipulated. The water (or the total solids) content of the substrate is a parameter that could be modified according to the process conditions. After preparing the suitable substrate for the process, it could be fed to the anaerobic digester. The anaerobic reactor in the simulation consists of two parts. The RStoic accounts for the hydrolysis reactions. Then, the hydrolyzed biomass (stream 2) goes to the RCSTR for fermentation reactions. The RCSTR produces two streams (biogas and digestate). The digestate

could be partially recycled to the digester inlet. The biogas (the red line in the flowsheet) is directed to the hydrogen production plant. In order to manipulate the CH_4/CO_2 ratio in reforming feed, stream 18 that contains CO_2, is mixed with the biogas in the MX2. Then, stream 3 is routed to a compressor (C1) to increase the pressure of the reforming feed. The pressurized reforming feed (stream 4) is heated to the desired temperature of the reforming reactions in HE1 and HE2 before entering the DMR, which is a plug flow reactor. The dry methane reforming reaction occurs in the DMR, and the produced syngas leaves the reactor. Since the produced syngas is at a high temperature and the operating temperature of the downstream processes is lower, the syngas is sent to HE3 as hot fluid to preheat the reforming feed (stream 4). The hot outlet stream from HE1 (stream 7) still contains high energy content and is used to preheat water (stream 10). The cooled syngas (stream 8) is sent to a valve (V1) for pressure adjustment before feeding to the WGS reactor. In addition, two more heat transfer equipment, HE4 and HE5, are employed to manipulate the inlet temperature of the WGS reactor. The cooled syngas (stream 9) contains H_2, CO, and unreacted CH_4 and CO_2. The WGS reaction requires the presence of CO_2 and H_2O to go forward. Thus, CO_2 is provided from the separated CO_2 from the H_2/CO_2 separation unit (stream 19). Water is heated in HE3, HE4, and HE5 to reach the WGS temperature. Then, the CO_2 and steam (streams 19 and 13, respectively) are mixed with syngas in MX3. Stream 14 is fed to the WGS reactor, where the WGS reaction occurs, and the mixture of H_2 and CO_2 is produced (stream 15). The H_2 and CO_2 mixture is routed to the separator (SEP) to achieve a pure H_2 product. The separated CO_2 is routed to a splitter (SP2) where the CO_2 required in the process is collected and recycled to the plant (streams 18 and 19), and the rest is captured (stream 20). The produced H_2 (stream 16) is sent to HE4 to cool down before sending for storage.

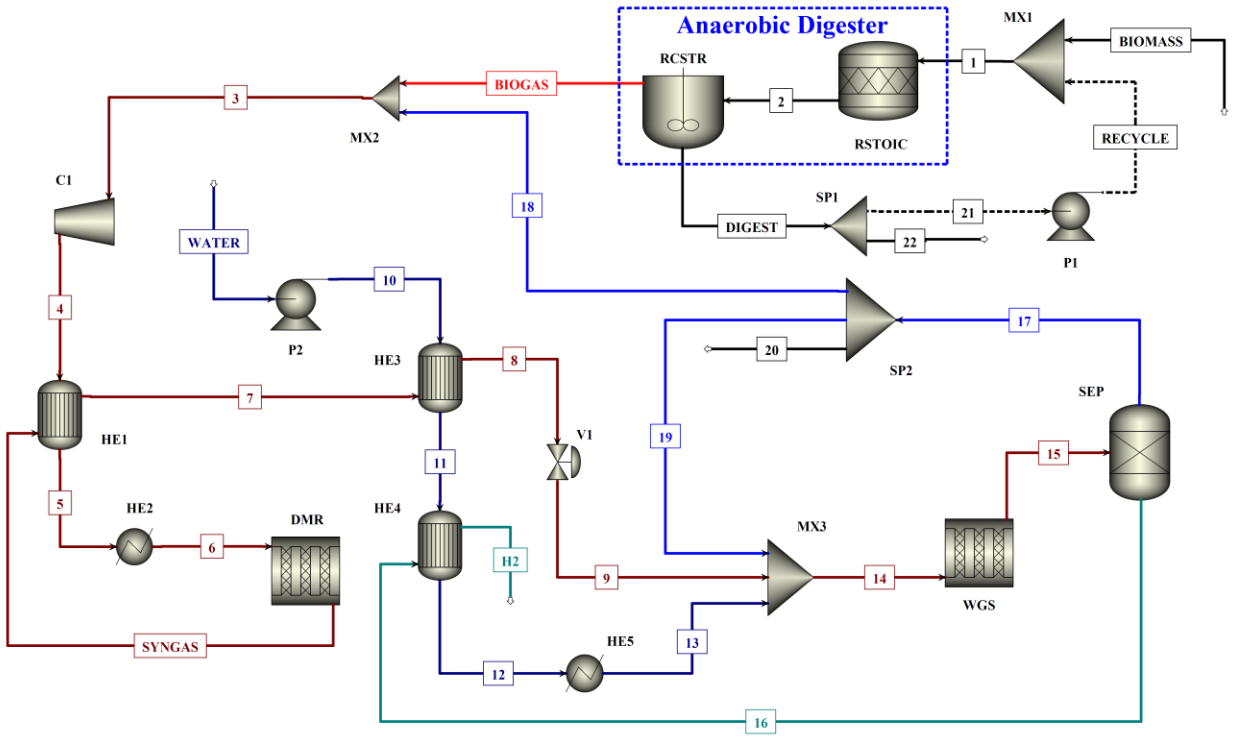

Figure 6.2. The process flowsheet developed for hydrogen production from biomass

Assumptions, Challenges, and Limitations. The current study comprises modeling and simulation of multiple unit operations. The following hypotheses are considered in order to simulate the process:

- Continuous operation of the plant with fixed feed rate and composition
- Steady state condition
- No solid component in the feed (aqueous solution feed)
- Constant conversion factors for hydrolysis reactions
- Constant inhibition parameters in the kinetics of the fermentation reactions
- Constant and the same temperature over the RStoic and RCSTR
- Perfect mixing in the anaerobic digester
- Negligible H_2S and NH_3 composition in the produced biogas

- Perfect mixing in the reforming reactors
- Uniform shape and size of the catalyst particles in the reforming reactors
- Constant porosity throughout the reforming reactors
- Constant separation efficiency in separation of H_2 from CO_2

In addition to the assumptions, several challenges and limitations could be reported for the current model:

- Disintegrating step is not considered in the model. Thus, user should provide the substrate composition in order to use the model
- The energy consumption due to the agitation in the CSTR is not considered
- The catalyst deactivation is not considered in the reforming reactors
- The carbon formation is not considered in the reforming reactors

6.5. Results and Discussion

The anaerobic digestion process is modeled with Aspen Plus V11. The AD process simulation contains an RStoic model for simulating hydrolysis reactions and an RCSTR to model the acidogenesis, acetogenesis, and methanogenesis. Kinetic equations and parameters are gathered from previous studies (Angelidaki et al., 1993; Angelidaki et al., 1999; Batstone et al., 2002) to account for degradation of intermediate products as well as biogas production, except for the parameters of the maximum specific growth rate which are calculated in the current study. The kinetics of the reforming reactions are also calculated according to the LHHW model. The results are presented in three parts: 1- the main results obtained from the simulation model along with a validation of the model with experimental data obtained from the accessible literature; 2- a

sensitivity analysis performed to investigate the effect of various parameters on the process performance; and 3- exploring the optimal process conditions.

6.5.1. Process simulation and validation

The kinetic equations are used to model the anaerobic digestion process. The kinetic constants are modified and extended to the low-temperature anaerobic digestion. Before using the constructed simulation framework (Figure 6.2), it is necessary to ensure the validity of the simulation model. To this end, two experimental case studies are selected and simulated. The first case study is experimental biogas production from cow manure reported by Kaparaju et al. (2009). The experiment was conducted at 55 ºC, with a feed rate of 0.333 L/d, 6% total solids, and 80% (TS%) volatile solids content. The second case study is also biogas production from cow manure reported by Ahring et al. (2001) at 55 ºC using cow manure feed at 0.1 kg/d, 6.07% total solids, and 4.54% volatile solids. Despite that, both cases are at thermophilic temperatures, they are chosen, as enough information are available for these case studies. In addition, it is possible to compare the results of the current study with other simulation studies. Moreover, the constant parameters of the temperature-dependent maximum specific growth rate of microorganisms calculated and implemented in this study are valid for a wide range of temperature (10-65 ºC). Thus, the constants will be the same for low, moderate, and high temperature AD. The simulation results from the current study are compared to the experimental results of two case studies in Table 6.3. In simulating case study 1, the simulation model predicted 361.5 L/kg VS_{added} biogas which is close to the 353.5 L/kg VS_{added} of biogas experimentally obtained by Kaparaju et al. (2009). The relative deviation percent was calculated to evaluate the accuracy of the simulation, which was 2.3%. Rajendran et al. (2014) also simulated the first case study and obtained 365.8 L/kg VS_{added}

biogas showing a 3.4% deviation from the experimental biogas yield. According to the second case study, the current simulation model predicted the methane yield equal to 213.9 L CH_4/kg VS_{added}, which shows a 5.9% deviation from the experimental methane yield of 202 L CH_4/kg VS_{added} obtained by Ahring et al. (2001).

Table 6.3. The validation of the anaerobic digester model in Aspen Plus

Case	Literature experimental Results	Simulation Results	Relative Deviation (RD%)	Reference
Case 1	353.5 L/kg VS_{added} (Kaparaju et al., 2009)	361.5 L/kg VS_{added}	2.3	This study
		365.8 L/kg VS_{added}	3.4	(Rajendran et al., 2014)
Case 2	202 L CH_4/kg VS_{added} (Ahring et al., 2001)	213.9 L CH_4/kg VS_{added}	5.9	This study

The biogas produced from the anaerobic digester contains 50.66% CH_4, 31.87% CO_2, 15.43% H_2O, and 2.04% other constituents (see Table 6.4). In a dry basis analysis, the biogas contains 59.9% CH_4 and 37.7% CO_2, similar to the typical biogas composition reported in the literature for dairy manure (Harikishan and Sung, 2003; Kalia and Singh, 2001).

Table 6.4. The composition of biogas obtained from the simulation

Component	Composition (Mole %)	Dry basis composition (Mole %)
CH_4	50.66	59.90
CO_2	31.87	37.68
H_2O	15.43	-
H_2	0.17	0.20
NH_3	0.29	0.34
H_2S	0.15	0.18
Others	1.43	1.69

In addition, the simulation results revealed that the digestate still contains organic matter and can produce more biogas. Table 6.5 provides the composition of digestate. The analysis of digestate shows that its total and volatile solids content is 4.73% and 3.25% (68.7% VS/TS). Möller and Müller (2012) analyzed the digestate of anaerobic digestion of various sources and reported that the total solids content is in the range of 1.5-13.2% containing 63.8-75% VS/TS ratio. Thus, the TS and VS of the digestate produced in the current simulation agree with the results previously reported in the literature.

Table 6.5. Composition analysis of the digestate effluent

Component	**Composition (Mass%)**
CH_4	0.09
CO_2	0.59
H_2O	95.27
H_2	Trace
NH_3	0.09
H_2S	0.01
HAc (acetic acid)	0.04
HPr (propionic acid)	0.42
HBu (butyric acid)	0.34
Cellulose	0.45
Xylose	0.58
Ethanol	0.50
Protein	0.14
Inert	1.48

6.5.2. Sensitivity analysis

The sensitivity analysis is a vital step in investigating various aspects of the process and evaluating the performance of the simulation model. It is also used to further understand the system behavior with changing the operating parameters. This section first explores the impact of various parameters on the performance of the anaerobic digestion process in terms of biogas and CH_4

yield. Then, the variation of the reforming process operating parameters is investigated in terms of CH_4 conversion, H_2 production, and energy consumption.

Anaerobic digestion. As is mentioned previously, several parameters are important in the anaerobic digestion process. This section explores the effects of the feed rate, hydraulic retention time, total solids content of the feed, and the digestate recirculation fraction in an anaerobic digester on the performance of biogas production. Figure 6.3 shows the simulation results obtained from sensitivity analysis. Figure 6.3(a) shows the effect of feed rate on the biogas yield in the simulation framework. Increasing the feed rate to the anaerobic digester decreases the biogas yield. At the lowest feed rate of 0.1 L/d, the biogas yield is 400.97 L/kg VS_{added}. However, by increasing the feed rate to 1 L/d, the biogas yield drops to 343.01 L/kg VS_{added}. This decreasing trend is expected as the feed rate indicates the organic loading rate fed to the reactor. Thus, biogas yield is decreased with increasing organic loading rate, which is similarly observed in the experimental studies (Babæe and Shayegan, 2011). This phenomenon could be justified based on the inoculum-to-substrate (IS) ratio. In anaerobic digestion systems, the ratio of inoculum to the amount of substrate fed to the bioreactor is a key parameter. At higher IS ratios, the substrate is easily accessible to the microorganisms, and more biogas yield is expected (Latifi et al., 2019) but the effective space of the reactor will be reduced (Wu et al., 2015). When the IS ratio is low, microorganisms do not have access to all available substrate, and thus, the ultimate digestion of the substrate is not happening (Lü et al., 2012). Therefore, the optimum IS ratio should be specified to produce the highest CH_4 yield. The effects of increasing the feed rate on the CH_4 content are presented in Figure 6.3(a); it is decreased from 51.8% to 50% upon increasing the feed rate from 0.1 to 1 L/kg VS_{added}. Similar behavior was observed by Liu et al. (2017) in mesophilic anaerobic

digestion of food waste operated at different increasing OLRs. They showed that the higher OLR, the higher the drop in the CH_4 content, meaning that the declining line was sharper for the reactor with higher OLR than the reactor with lower OLR.

Hydraulic retention time is also an important parameter in anaerobic digestion that impacts the process performance. The effect of hydraulic retention time on biogas yield and CH_4 content is investigated in Figure 6.3(b). The results show that increasing the hydraulic retention time leads to an increase in both biogas yield and CH_4 content in biogas. The highest increase in biogas yield is observed in the first week of incubation. After that, the biogas yield increases gradually and became a flat line in the last week. This is an important outcome as the industrial anaerobic digesters typically operate for one week hydraulic retention time as it is not economically feasible to operate them for a longer time. The simulation results indicate that it is possible to achieve more than 87% of the biogas yield in the first week of incubation compared to 30 days of retention time. It agrees with the experimental results obtained by Aramrueang et al. (2016), in which 90% of the total biogas yield was obtained in the first week of incubation.

The importance of the first week of incubation could also be observed in the results obtained for the CH_4 content. The CH_4 content is too low at the beginning of the process (i.e., the first day) but it increases with time and reaches to 50% at day 7. The highest CH_4 content is obtained at day 30 (CH_4 content equal to 51.4%), showing that more incubation time results in more CH_4 content. The higher hydraulic retention time also results in a higher CH_4 content in biogas. However, it is acceptable to operate the reactor for one week incubation time (batch fermentation) before feeding it with fresh feed since the CH_4 content at the end of the first week is satisfactory. The same trend was observed by Bi et al. (2020) in co-digestion of cattle manure and food waste. They showed that the CH_4 content was increased from 56% at day 5 to 62% at day 7 and then to 67% at day 25.

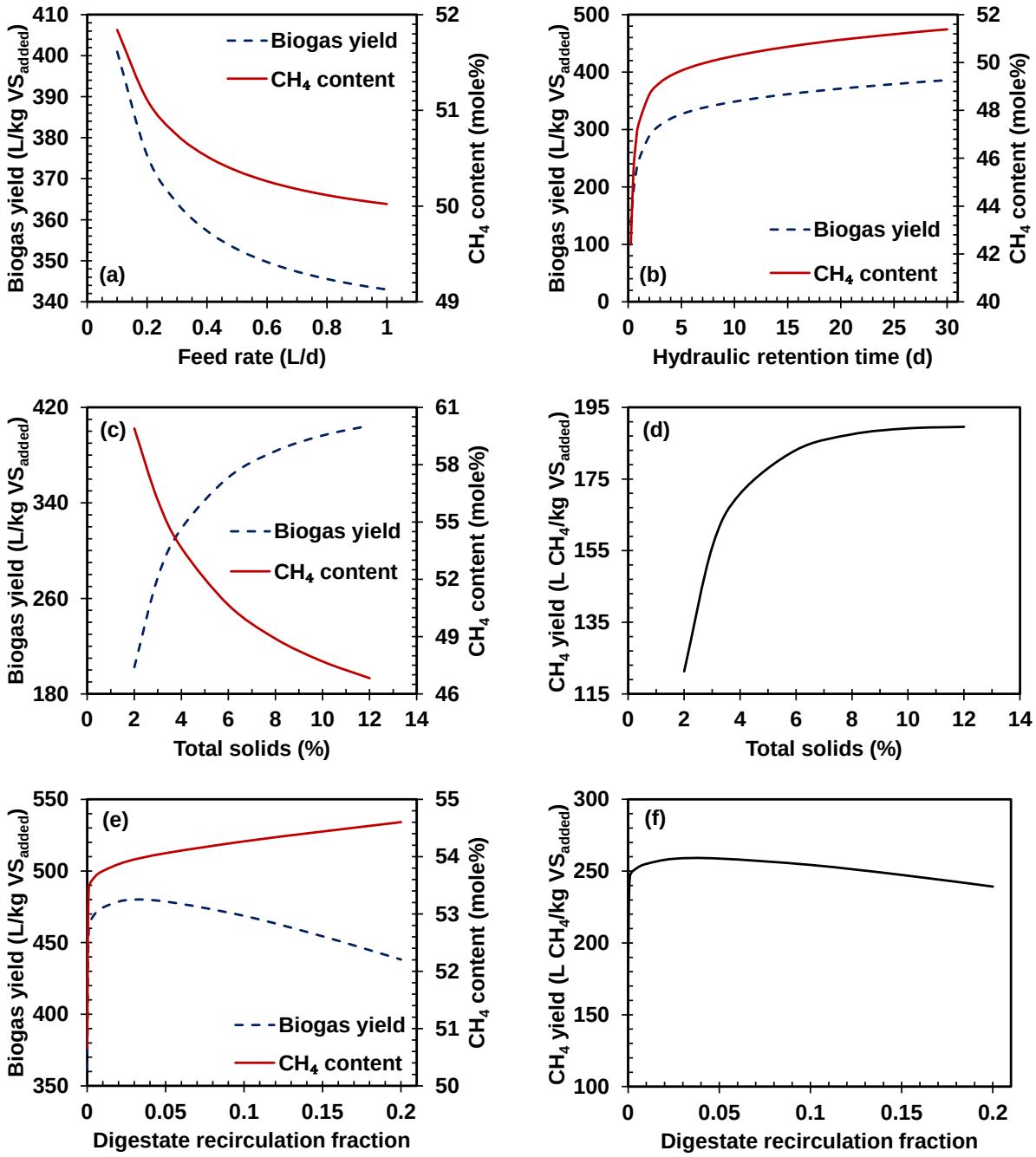

Figure 6.3. The simulation of the effect of various parameters on biogas yield, CH_4 content, and CH_4 yield

The total solid is a critical parameter in the operation of anaerobic digestion. The simulation model is used to explore the effect of the total solids content on the process performance in terms of biogas yield (Figure 6.3(c)). Increasing the total solids content leads to an increase in biogas

yield since more organic matter is available to be utilized by microorganisms. A significant increase in biogas yield is observed when increasing the total solids from 2% to 4%. By increasing the total solids content beyond 6%, the biogas yield increases, but its rate of increasing is lower than the rate of biogas yield increase when the total solids changed from 2% to 6%.

Despite having the biogas yield increased by increasing the total solids content, the CH_4 content in biogas shows a decreasing trend (Figure 6.3(c)). The highest CH_4 content (59.9%) is obtained at the lowest total solids content (i.e., 2%). By increasing the total solids content, the CH_4 content dramatically decreases and reaches to 46.8% at total solids equal to 12%. This is an important finding as the upgrading of low-quality biogas (low content of CH_4) is costly. According to the Intergovernmental Panel on Climate Change (IPCC) report, the costs of CO_2 capture using amine solutions in several plants are estimated to be between 29 to 51 USD/tonnes CO_2 (Metz et al., 2005). Thus, having a biogas stream with lower CH_4 content (higher content of CO_2) leads to higher costs of biogas upgrading. Hence, the anaerobic digester should be operated at a total solids at which the biogas CH_4 content is 50% or more.

Figure 6.3(d) presents the CH_4 yield variation with total solids content. As is discussed earlier, increasing the total solids content increases the biogas yield but decreases the CH_4 content in biogas. A similar trend was observed by Latifi et al. (2019) where the CH_4 content was decreased from 70% to 50% upon increasing the total solids from 5% to 7%. Thus, exploring the change of CH_4 yield with the total solids content seems necessary. According to the results, the CH_4 yield follows the same trend as biogas yield; it increases with increasing the total solids content. The lowest CH_4 yield is obtained at total solids equal to 2%. Having a feed with 4% total solids leads to a 40.9% increase in CH_4 yield (from 121.3 to 170.9 L CH_4/kg VS_{added}). Increasing the total solids content of the feed to 6% increases the CH_4 yield to 183.1 L CH_4/kg VS_{added}, showing a

7.1% rise. The same behavior was observed by Maamri and Amrani (2014) where the biogas yield increased by increasing the TS. By increasing the TS beyond 6%, the CH_4 yield almost remains constant and its increase is negligible considering the associated costs with handling a high total solids feed.

As is shown in Table 6.5, the digestate leaving the reactor contains organic materials that are not converted to the final products (CH_4 and CO_2). Thus, it is useful to use the organic matter of the digestate in a way that enhances the process in terms of biogas and CH_4 yield. One of the practical solutions is recirculating a fraction of the digestate to the reactor inlet. Recycling is a common practice in the chemical industry to obtain products with a higher rate and quality. This study explores the effect of recirculation of the digestate to the reactor inlet on the biogas yield (Figure 6.3(e)). Recycling a small amount of the digestate to the reactor inlet significantly increases the biogas yield. The biogas yield in the main case without digestate recirculation is 361.5 L/kg VS_{added} while recycling 0.5% of the digestate dramatically increases the biogas yield to 470.1 L/kg VS_{added}. Recirculating more of the digestate enhances the biogas yield until 2.5% of the digestate is recirculated. After that, recirculating more digestate decreases the biogas yield. The positive impact of digestate recirculation was also observed by Chen et al. (2020). They found that 60% digestate recirculation improves the cumulative CH_4 production by 65.8% (115.9 L), compared to the CH_4 production when 50% of the digestate was recirculated (69.9 L).

The digestate recirculation also changes the CH_4 content in produced biogas (Figure 6.3(e)). The results indicate that increasing the digestate recirculation rate increases the CH_4 content. Even at a low fraction of digestate recirculation (e.g., 0.5%) the CH_4 content increases by 3%. The constant increasing trend of the CH_4 content in biogas by increasing the digestate recirculation fraction encourages higher digestate recirculation rates. However, it should be noted that the higher

digestate recirculation rate means higher costs for transferring the digestate to the reactor inlet. In addition, the reactor volume and available inoculum are two critical factors that should be capable of handling the fresh feed and the recycle stream.

The CH_4 yield variation with digestate recirculation fraction is shown in Figure 6.3(f). The results indicate two trends for the CH_4 content in response to digestate recirculation: an increasing trend when the digestate recirculation fraction is 0 to 5% and a decreasing trend when it is > 5%. Thus, the highest CH_4 yield is obtained at the digestate recirculation fraction of 5% (258.8 L CH_4/kg VS_{added}), which is 41.3% higher than the original case (no recirculation). Since the CH_4 yield is the critical factor of AD performance, the 5% digestate recirculation is the optimal value.

Low-temperature anaerobic digestion. Simulation of low-temperature anaerobic digestion requires some parameters to be modified to make the simulation capable of predicting various variables affected by temperature change. The current study provides the maximum specific growth rate parameters (Table 6.6) according to Equation (6-50) for the temperature range of 10 to 65 ºC.

Table 6.6. The maximum specific growth rate parameters calculated in this study

Group	$\mu_{max}\ (d^{-1})$	$E_A\ (J.mol^{-1})$	$T_0\ (K)$
Glucose-consuming acidogens	2.6670	44400	273.15
LCFA-degraders	0.7149	36200	273.15
Amino-acid degraders	6.7180	32450	273.15
Propionate degraders	2.4630	28880	273.15
Butyrate degraders	4.4400	26360	273.15
Valerate degraders	0.7149	36200	273.15
Methanogen	0.9436	38610	273.15
Hydrogen utilizing step	0.9436	38610	273.15

Figure 6.4 presents the results of the simulation of anaerobic digestion performance at varying temperatures. The temperature has a significant impact on the biogas yield (Figure 6.4(a)). As the temperature increases, the biogas yield increases as well. The biogas yield at low temperatures (10-15 ºC) is very low, while increasing the temperature to 25 ºC causes a significant rise in biogas yield. The biogas yield at 25 ºC is twice that obtained at 10 ºC. By increasing the temperature from 25 ºC to 40 ºC, the biogas yield smoothly increases. Then, having a temperature of more than 40 ºC, the biogas yield dramatically increases.

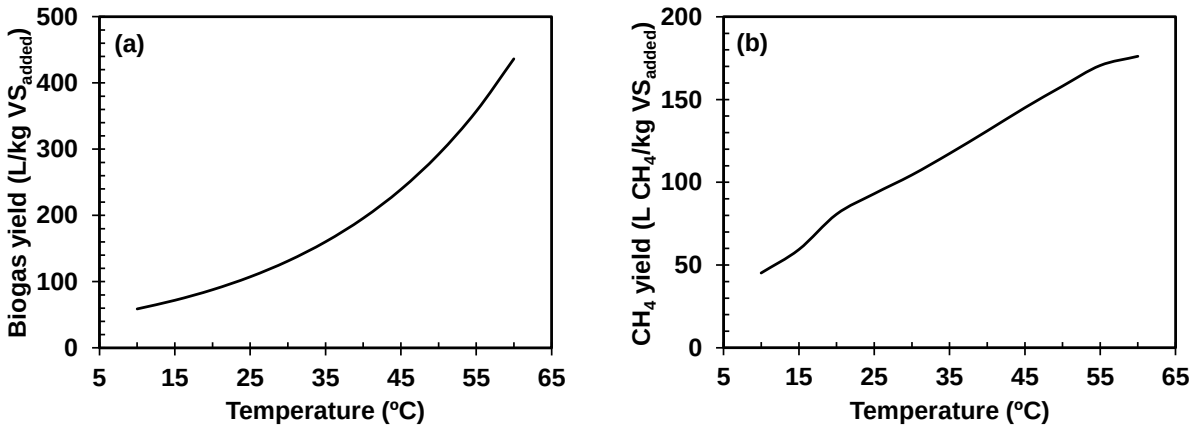

Figure 6.4. The simulation of the effect of temperature on the (a) biogas yield; and (b) CH_4 yield

The effect of temperature on the CH_4 yield is shown in Figure 6.4(b). In contrast with the biogas yield, the CH_4 yield diagram shows an almost linear increasing trend with temperature. The CH_4 yield obtained at 20 ºC is 80.6 L CH_4/kg VS_{added} which is 69% and 47% of the CH_4 yields obtained at 35 and 55 ºC, respectively. The results indicate the importance of high temperature on the CH_4 yield and the efficiency of the anaerobic digestion plant. A few studies compared the results of low-temperature anaerobic digestion with high temperature. Arikan et al. (2015) measured the CH_4 yield from dairy manure at temperatures of 22 ºC, 28 ºC, and 35 ºC. They found that the CH_4 yields at 22 ºC and 28 ºC are 70% and 87% of the CH_4 yield at 35 ºC, respectively.

The simulation model developed in the current study shows that the CH_4 yields at 22 ºC and 28 ºC are 70% and 83% of the CH_4 yield at 35 ºC, respectively, which agree with the results of Arikan et al. (2015). Bouallagui et al. (2004) investigated the effect of temperature (20, 35, and 55 ºC) on CH_4 yield from fruit and vegetable wastes in a tubular reactor. They showed that the CH_4 yield at 55 ºC is 31% and 164% higher than that obtained at 35 ºC and 20 ºC, respectively. Considering the results obtained using the simulation model developed in the current study, the CH_4 yield at 55 ºC is 170.54 L CH_4/kg VS_{added}, which is 45% higher than that of 35 ºC and 111% higher compared to CH_4 yield at 20 ºC. In addition, the nearly linear trend of CH_4 yields with temperature is in agreement with the studies of Arikan et al. (2015) and Bouallagui et al. (2004). It is mainly due to a higher concentration of CH_4 in biogas at lower temperatures.

Dry methane reforming. After validating and analyzing the simulation model in the previous sections, this section presents the results of the simulation of hydrogen production using dry methane reforming. In order to define a basis for the DMR simulation, a biogas production plant with cow manure feed (6% total solids and 80% volatile solids) of 150 m^3/day and operating at 293.15 K with 7 days hydraulic retention time is considered. The results show that 553.5 m^3/day biogas (65% CH_4 and 35% CO_2) was produced.

The CO_2 composition in reformer feed influences the conversion of CH_4 to syngas. According to Equation (6-46), one mole of CH_4 will react to one mole of CO_2 to produce an equimolar mixture of H_2 and CO. Figure 6.5 shows the effect of CO_2 composition in the DMR feed on CH_4 conversion and H_2 composition in the produced syngas. For a biogas feed (65% CH_4 and 35% CO_2), the CH_4 conversion is very low (53.8%). Thus, it is necessary to add extra CO_2 to the reformer feed to achieve a higher CH_4 conversion. Increasing CO_2 in the reformer feed

increases the CH_4 conversion; considering the 1:1 ratio of CH_4:CO_2 in the reformer feed, the CH_4 conversion is more than 99%. Therefore, a higher CO_2 content in the biogas results in a higher rate of syngas production. It should be noted that the CO_2 proportion should be kept at an optimum level because too-high inlet CO_2 decreases the H_2 proportion in the syngas. Increasing the CO_2 proportion in the reformer feed from 35% to 50%, increases the H_2 proportion in the syngas. Then, feeding more CO_2 to the reactor decreases the H_2 composition in the produced syngas. The lowest H_2 content of syngas is 41.2% when no CO_2 is added to the feed. The H_2 proportion in syngas product reaches its highest amount when equal moles of CH_4 and CO_2 are present in the reformer feed. Next, adding more CO_2 reduces the H_2 content of syngas because the unreacted CO_2 in the feed directly goes to the syngas. Thus, an external CO_2 stream is added to the biogas to achieve an equimolar CH_4/CO_2 feed for the reformer.

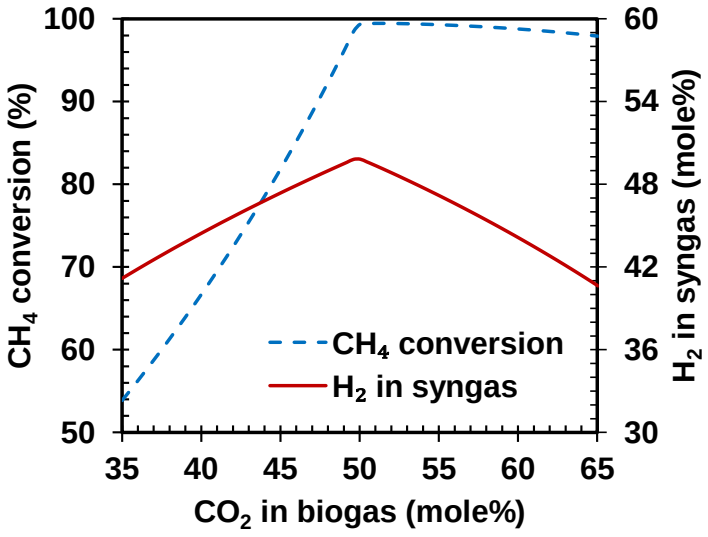

Figure 6.5. The CH_4 conversion in the reformer (left axis) and the H_2 composition in syngas (right axis) by changing the feed CO_2 composition

The length of the reactor is a critical parameter since it directly affects the costs of the plant. The effect of DMR length on CH_4 conversion is investigated in Figure 6.6(a). The CH_4 conversion is calculated at changing pressures of 1 to 4 bar. It is observed that at constant pressure, the CH_4 conversion is increased by increasing the DMR length. The CH_4 conversion reached more than 99% at 0.5 m when the pressure is 1 bar. By increasing the pressure, the required length of the reactor needed for the 99% CH_4 conversion is decreased; this shows the positive impact of pressure. The 99% CH_4 conversion was obtained at 0.36, 0.29, and 0.25 m length of DMR at pressures of 2, 3, and 4 bar, respectively. Increasing the pressure from 1 bar to 4 bar could be done in a single-stage compressor, while going beyond 4 bar requires a second stage of compression. Note that the typical compression ratio of compressors is between 3-5 (Hajizadeh et al., 2018). In addition, changing the pressure to more than 4 bar is becoming less impactful on the reactor length.

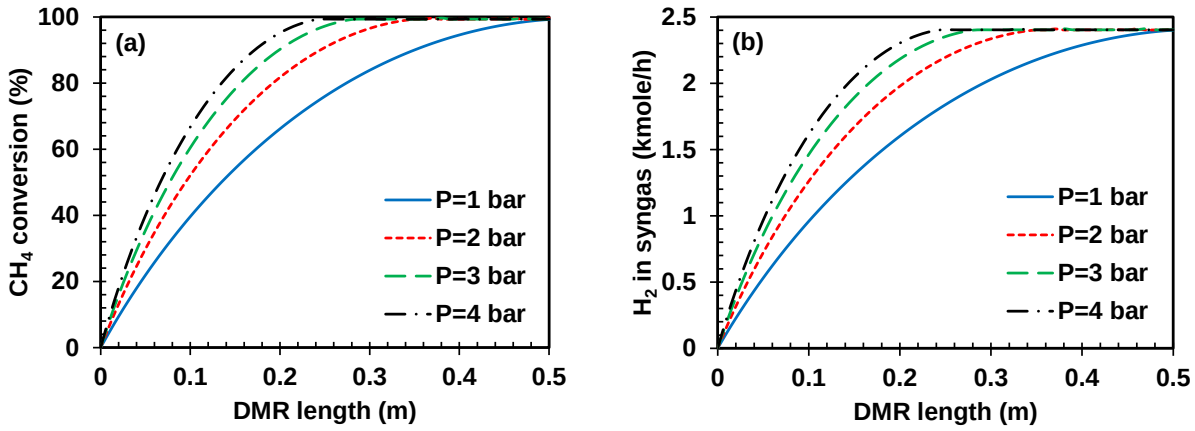

Figure 6.6. The effect of DMR length on (a) CH_4 conversion; and (b) H_2 rate in syngas

The reformer temperature is the most critical parameter that affects the reactor's CH_4 conversion and energy efficiency. The reformer temperature is varied from 350 ºC to 650 ºC at several pressures to examine its effect on CH_4 conversion (Figure 6.7(a)). Increasing the reformer

temperature increases the CH_4 conversion with the highest effect observed between 450 ºC and 580 ºC, which increased the CH_4 conversion sharply (19.8% to 94.9%). Then, increasing the temperature beyond 580 ºC, the CH_4 conversion gradually increases to achieve its highest value at 600 ºC (99.6%). Again, the impact of pressure on the temperature of the reactor is significant. As is seen, increasing the pressure from 1 bar to 4 bar reduces the highest temperature of the reactor required to achieve the highest CH_4 conversion. The highest CH_4 conversion is obtained at temperatures of 600, 570, 560, and 550 ºC at pressures of 1, 2, 3, and 4 bar, respectively. The required energies to maintain the reactor temperature at the mentioned values play a vital role in having an efficient plant. The outlet stream from compressor will have a higher temperature because of compression. Thus, compressor already provides a part of the energy needed for increasing the temperature.

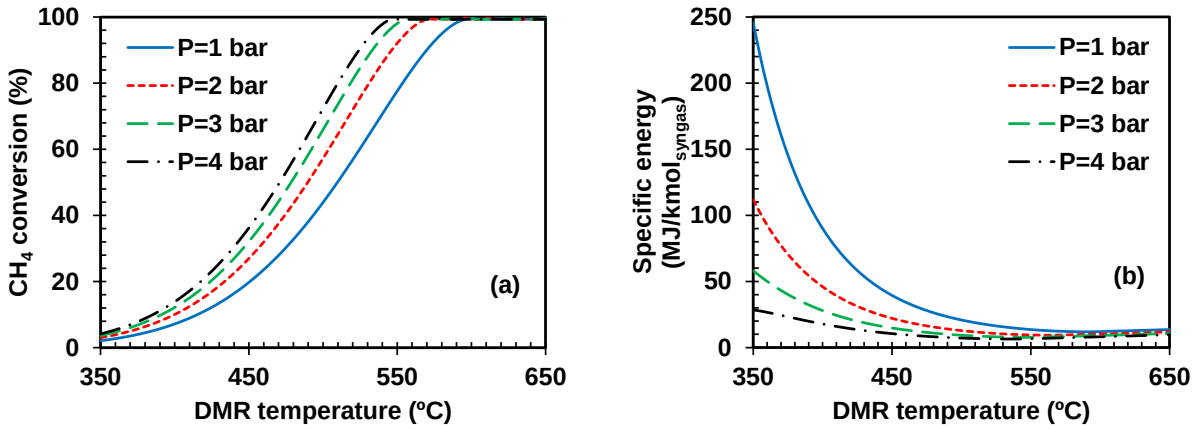

Figure 6.7. The effect of DMR temperature on (a) CH_4 conversion; and (b) specific energy consumption

As the reforming reactions occur at a high temperature, there is a need to find the optimum temperature to achieve the highest conversion and lowest energy consumption. Figure 6.7(b) presents the specific energy of the reformer in terms of reformer temperature. The specific energy

is the energy supplied to the system per unit of mole/mass of the desired product (syngas (H_2 + CO)). Thus, the specific energy (MJ/kmol$_{syngas}$) is the heat supplied to the reformer per unit mole of syngas. Although heat supplied is low at low reformer's temperatures, the specific energy is too high (245.89 MJ/kmol$_{syngas}$) because the syngas production is minimal (at atmospheric pressure). By increasing the temperature, syngas production and the energy supplied grow, but the specific energy declines, showing a higher production rate of syngas compared to the heat supplied to the system. The lowest specific energy (11.99 MJ/kmol$_{syngas}$) is achieved at 590 ºC. Then, the specific energy gradually increases by rising the reformer temperature, as the syngas production rate remains relatively constant. The specific energy decreased at higher pressures of biogas. When the biogas pressure is 2 bar, the specific energy is 9.35 MJ/kmol$_{syngas}$ at temperature of 565 ºC, which is 5 ºC lower than the optimum temperature of the DMR at pressure of 2 bar. The same trend is observed with biogas pressures of 3 and 4 bar, where the minimum specific energy consumptions are 7.69 and 6.45 MJ/kmol$_{syngas}$, respectively. According to the results shown in Figure 6.7, it could be concluded that the optimum temperature for the reformer is between 590 ºC and 600 ºC at atmospheric pressure. At these temperatures, the highest CH_4 conversion and the lowest specific energy consumption is achieved.

The produced syngas, which contains a high energy content, should be routed to the WGS reactor for CO to H_2 and CO_2 conversion. In order to find the optimum operating conditions of the WGS reactor, several factors are examined. The WGS reaction rate (Equation (6-52)) shows that CO_2 is required in the WGS feed to make the reaction go forward. Therefore, along with the syngas, CO_2 and H_2O are also added to the inlet of the WGS reactor. The flow rates of the CO_2 and H_2O streams affect the performance of the WGS. Figure 6.8(a) presents the effect of CO_2/H_2O supply rate on the WGS and its composition on CO conversion. The purpose of the WGS reactor

is to convert CO to H_2 and CO_2 according to Equation (6-48). Thus, the highest CO conversion is desired. Increasing the flow rate of the CO_2/H_2O supply increases the CO conversion (Figure 6.8(a)). However, when the supply contains 33.3% CO_2 and 66.6% H_2O (CO_2/H_2O = 0.5), the CO conversion is higher than in other cases in which more CO_2 exists in the supply. The CO conversion reaches more than 97% when 1.1655 kmol/h CO_2 and 2.3345 kmol/h H_2O are supplied (a total of 3.5 kmol/h of mixture of CO_2 and H_2O with a ratio kept at CO_2/H_2O = 0.5). For CO_2/H_2O = 1 (equimolar CO_2/H_2O mixture), achieving more than 97% CO conversion requires a higher flow rate. The higher flow rate of the CO_2/H_2O supply stream means more costs associated with heating it to the temperature of the WGS reactor. Also, for the case of CO_2/H_2O = 2 (66.67% CO_2 and 33.33% H_2O in their mixture), the required mole flow of the stream is higher than the other two cases to achieve the 97% CO conversion.

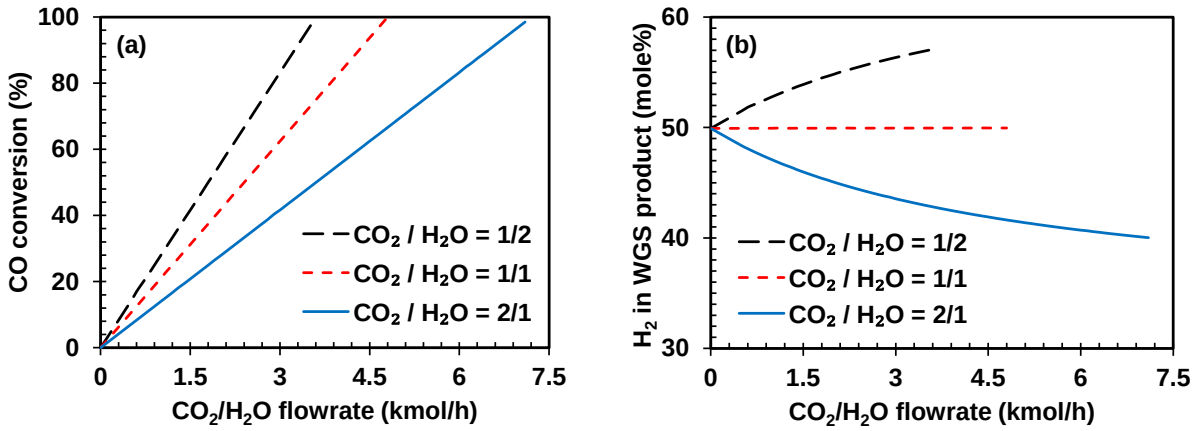

Figure 6.8. The effect of CO_2/H_2O flow rate and its composition on (a) CO conversion and (b) H_2 composition in WGS product

The change in CO_2/H_2O flow rate and composition also influences the H_2 composition (mole%) in the WGS product (Figure 6.8(b)). Generally, CO_2/H_2O needs to be supplied because

it helps with CO conversion, as observed in Figure 6.8(a); however, the CO_2/H_2O ratio is a key factor in H_2 composition in the product as well. Increasing the flow rate of the supply stream (33.3% CO_2 and 66.6% H_2O) increased the H_2 composition. The lowest H_2 composition (51.5%) is obtained when 0.5 kmol/h of CO_2/H_2O with a ratio of 0.5 is fed to the WGS reactor. By increasing the flow rate to 3.5 kmol/h, the H_2 composition increases to 57%. This result is important because the increase in the H_2 composition reduces the energy required in the next step to separate H_2 from CO_2. When feeding the CO_2/H_2O at a ratio of 1, the H_2 composition remains almost constant, but decreased upon increasing the CO_2/H_2O ratio to 2.

Figure 6.9 represents the change in CO_2 composition in the WGS product with CO conversion in the WGS reactor. As the objective of this process is to convert CO to H_2 and CO_2, its performance will be optimum when it achieves the highest CO conversion and the lowest CO_2 composition in the product. The results indicate that the CO_2 composition in the WGS product increases with CO conversion for all cases. However, considering the CO conversion of more than 97%, the CO_2 composition in the product will be lower when feeding the CO_2/H_2O at a lower ratio.

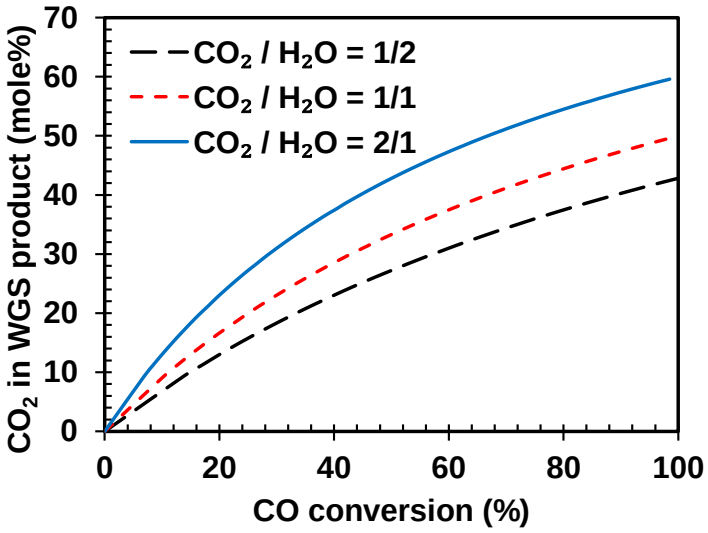

Figure 6.9. The variation of CO_2 mole% in WGS product with CO conversion at various CO_2/H_2O ratios

The length and temperature of the WGS reactor affects the CO conversion as well as the energy efficiency of the system. Figure 6.10 shows the effect of the length and temperature of the WGS on the CO conversion at several pressures. As is expected, the CO conversion increases with increase in the WGS length. However, the pressure negatively affects the WGS reactor. As is seen in Figure 6.10(a), less length of the WGS reactor is required to achieve the highest CO conversion at lower pressures. Figure 6.10(b) shows the effect of WGS temperature on CO conversion. It shows that the CO conversion is negligible at low temperatures (i.e., 0.67% at 255 ºC) but increasing the temperature enhances the CO conversion. When the temperature is greater than 275 ºC, the CO conversion sharply increases and reaches more than 97% at 288 ºC at atmospheric pressure. Therefore, the minimum temperature of the WGS at which the highest CO conversion is achieved is 288 ºC when the WGS pressure is 1 bar. Increasing the pressure of syngas, from 1 to 4 bar shifts the temperature to higher values to obtain the highest CO conversion. The more than 97% of CO conversion is obtained at temperatures of 296, 300, and 302 ºC at pressures of 2, 3, and 4 bar, respectively.

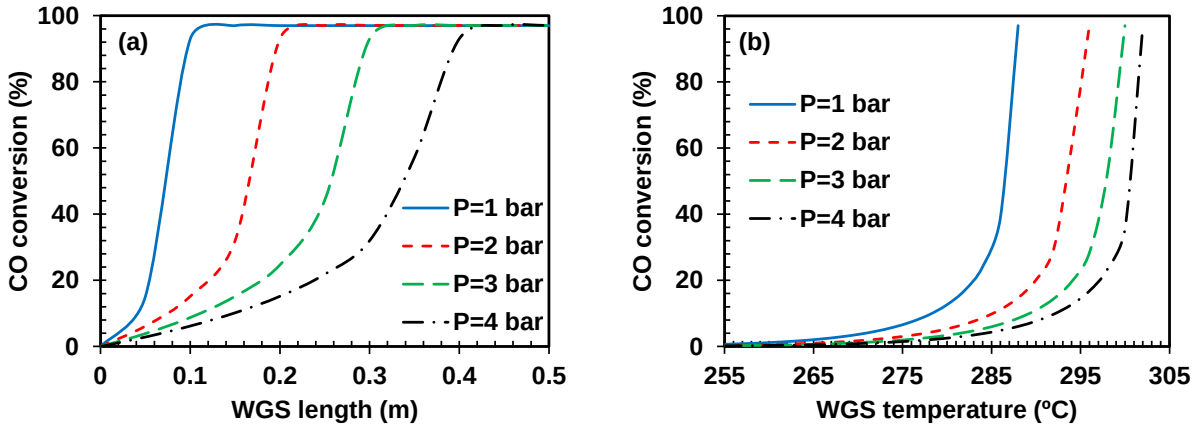

Figure 6.10. The effect of WGS reactor (a) length and (b) temperature on the CO conversion

The specific energy consumption in the WGS is defined as the heat supplied to the reactor per unit mole/mass of H_2 produced. Accordingly, the energy efficiency of the WGS reactor is determined. Figure 6.11 presents the change in the specific energy in $MJ/kmol_{H_2}$ versus the CO conversion in the WGS reactor and various pressures. At 1 bar, the specific energy first increased with increasing the CO conversion from 0 to 1.8% (Figure 6.11); after that, it decreased and reached a minimum of 29.71 $MJ/kmol_{H_2}$ when the CO conversion is as high as 97%. This point is equivalent to the WGS temperature of 288 ºC, which shows the highest CO conversion and the lowest specific energy.

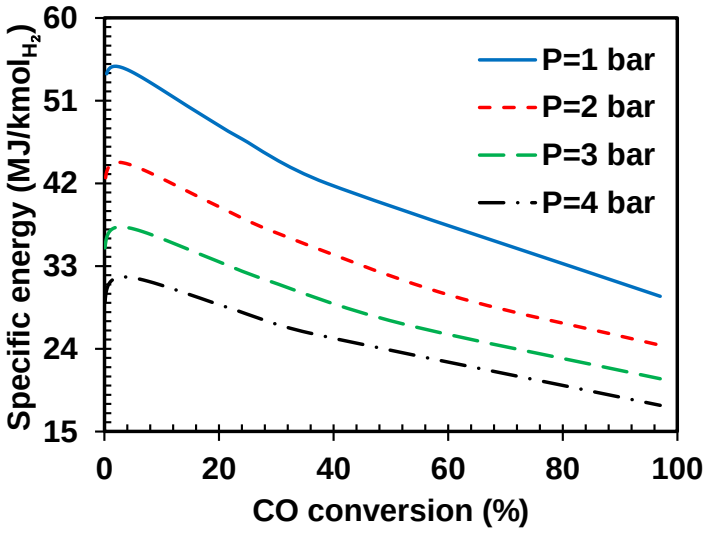

Figure 6.11. The specific energy consumption of the WGS reactor with the CO conversion

The WGS product contains 56.94% H_2, 42.1% CO_2, and traces of CH_4 and CO. The next step is separating H_2 from CO_2 to achieve H_2 as a final product. The H_2/CO_2 separation in this study is simulated using an ideal separator with 85% efficiency, as proposed by Cruz et al. (2018). Integrating the H_2/CO_2 separation unit with the dry reforming process resulted in the production of 4.02 kmol/h (8.11 kg/h) H_2 as the final product. Moreover, the separated CO_2 stream, which

contains 3.5 kmol/h CO_2 (154.04 kg/h), is used as a CO_2 source for CO_2 supply in the reformer feed and CO_2 supply for the WGS reactor.

6.5.3. Optimal process conditions

Integration of the psychrophilic anaerobic digestion with the dry methane reforming process is promising method of H_2 production from biomass. This section provides the optimum process parameters according to the results obtained in the previous section. The thermal energetic efficiency of the plant at its optimum condition is presented. In addition, the environmental impact of the proposed process is compared with the direct use of biogas for heat and power generation.

According to the sensitivity analyses, the most critical parameters in the anaerobic digestion process are the total solids content and the digestate recirculation fraction. It is assumed that the feed rate and composition are fixed and the anaerobic digester operates for one week. In the reforming process, the CO_2 supply rate to the reformer, reformer temperature and pressure, CO_2 and H_2O supply to WGS, and the WGS temperature and pressure, are the crucial parameters. The optimum values of the mentioned parameters are reported in Table 6.7.

Table 6.7. The optimum process parameters obtained in this study

Plant section	Parameter	Unit	Value
Anaerobic digestion	Temperature	°C	20
	Total solids content	%	6
	Digestate recirculation fraction	%	5
Reforming (DMR)	CO_2 supply rate to DMR	kg/h	24.21
	DMR pressure	bar	4
	DMR temperature	°C	595
Reforming (WGS)	CO_2 supply rate to WGS	kg/h	51.45
	H_2O supply rate to WGS	kg/h	41.99
	WGS pressure	bar	1
	WGS temperature	°C	288

The mass balance on the reforming section shows that potential of 8.11 kg/h H_2 production from 48.07 kg/h of biogas. The total mass balance is presented in Table 6.8. The net amount of each component (Table 6.8) indicates that CH_4 and H_2O are consumed while CO_2, H_2, and CO are produced during the process.

Table 6.8. The mass balance of components in the hydrogen production plant from biogas

Component	**Inlet streams (kg/h)**		**Outlet streams (kg/h)**		**Net (kg/h)**
	Biogas	**Steam**	**H_2 product**	**CO_2 captured**	
CH_4	-19.4045	0	0	0.1321	-19.2724
CO_2	-28.6634	0	0	78.3831	49.7197
H_2	0	0	8.1111	1.4314	9.5425
CO	0	0	0	2.0054	2.0054
H_2O	0	-41.9936	0	0	-41.9936
Total	-48.0679	-41.9936	8.1111	81.952	0

The proposed process shows a thermal energetic efficiency of 72.85%, which is in the range of the previous studies (Figure 6.12). Among the previous studies, studies 2, 4, 6, 7, 8, and 9 used steam reforming, while studies 3, 5, and 10 employed the auto-thermal reforming to convert biogas to H_2.

The hydrogen production from biogas is a promising method of greenhouse gas emission reduction. Based on the results obtained from the overall mass balance in the process, the biogas contains 19.4045 kg/h CH_4 and 28.6634 kg/h CO_2. Utilizing the biogas to produce H_2 through the proposed process also captures CO_2. The sources of CO_2 emission in the current process are the supplied heat in heaters, reactors, pumps, and compressor. According to the results, the CO_2 emission from the proposed process is 35.73 kg/h CO_2-equivalent. Considering the direct combustion of the biogas for electricity production, 81.88 kg/h CO_2 will be released to the

atmosphere. The net difference between the CO_2 emission from the direct combustion of biogas and H_2 production plant is 46.15 kg/h, equivalent to 398,736 tonnes/year CO_2 emission reduction.

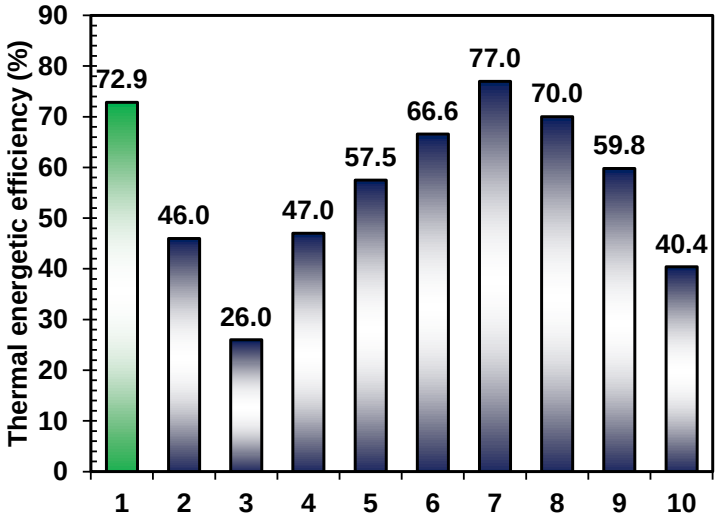

Figure 6.12. Comparison of the thermal energetic efficiency of the current study with literature. 1: This study; 2, 3: Marcoberardino et al. (2018); 4: Yao et al. (2017); 5: Di Marcoberardino et al. (2018); 6: Simpson and Lutz (2007); 7: Bargigli et al. (2004); 8: Hajjaji et al. (2013); 9, 10: Minutillo et al. (2020)

H_2 is the fuel of the future, and it is expected to be the main component of energy consumption. Hence, various methods of H_2 production are applied to different hydrogen sources. The usage of biomass for H_2 production is an effective step in sustainable energy production. Several benefits accompany the anaerobic digestion process for biomass conversion to biogas and hydrogen production, using the dry methane reforming process. The results of this study are useful in both agricultural and energy industries. A rigorous simulation of the AD process is helping the agriculture industry to forecast the amount of recoverable energy and nutrients from waste biomass. The developed model is applicable in a wide range of temperatures and resolves one of the major challenges in modeling and simulation of the AD process (i.e., low-temperature AD).

Considering the potential of applying AD technology in cold regions, the current simulation model will greatly help wastewater treatment facilities and agricultural sectors in modeling the potential of energy production from municipal agricultural wastes. In addition, the application of the dry methane reforming method for conversion of natural gas with high CO_2 content is increasing. Since the biogas contains a large portion of CO_2, the DMR method is promising to convert CH_4 and CO_2 to syngas.

6.6. Summary

This study aimed at producing hydrogen from biomass through integration of psychrophilic anaerobic digestion with dry methane reforming process. The advantage of the current simulation model is its ability to model the low-temperature anaerobic digestion process. The kinetic models are implemented within the simulator to calculate the kinetic rate of reactions. The simulation model was validated with the experimental data, and a sensitivity analysis was performed to show its performance with changing variables. The major outcomes of the current study are listed below:

- A mathematical expression is developed for the first time for calculating the temperature-dependent maximum specific growth rate of microorganisms, which is valid in a temperature range of 10 ºC to 65 ºC.
- The simulation model shows a good agreement with experimental studies (less than 6% relative deviation in predicting CH_4 yield).
- The simulation model well predicts the behavior of the system. Increasing the feed rate decreases the biogas yield and CH_4 content.

- More than 87% of the biogas yield is produced in the first week of incubation, which indicates the importance of short hydraulic retention time for industrial digesters.
- The total solids content in the digester feed impacts the rate and quality of biogas. Increasing the total solids content results in increasing the biogas yield, while the CH_4 content decreases. The CH_4 yield significantly increases with total solids content increment from 2% to 6%.
- Recirculation of a fraction of digestate improves the quality of biogas and increases the CH_4 yield; the highest CH_4 yield is obtained when 5% of the digestate is recirculated which is 41.3% higher than the original case (no recirculation).
- The simulation of the low-temperature anaerobic digestion process also shows consistent and sensible results compared to experimental data. The CH_4 composition of biogas at lower temperatures is higher, and thus, the CH_4 yield follows a quite linear trend with temperature.
- 150 m^3/day of cow manure (6% total solids and 80% volatile solids) fed to the anaerobic digester operating at 20 °C could produce 553.5 m^3/day biogas.
- Temperature and pressure are the most critical parameters in dry methane reforming reaction. The optimum temperature and pressure for the current study is 595 °C and 4 bar, respectively, which gives more than 97% CH_4 conversion to syngas.
- The water-gas shift reaction achieves more than 99% CO conversion at temperature of 288 °C and atmospheric pressure.
- 8.11 kg/h H_2 could be produced via dry reforming of biogas obtained from anaerobic digestion of 150 m^3/day cow manure.

- The proposed process captures CO_2 while producing H_2. A total of 78.38 kg/h CO_2 is eliminated to be released to the atmosphere.
- The thermal energetic efficiency of the dry methane reforming process offered in this study is 72.85%, which shows its advantage compared to steam methane reforming and auto-thermal reforming.
- The CO_2 emission from the current process is 35.73 kg/h CO_2-equivalent, which is less than a half of CO_2 emission from the direct combustion of biogas for electricity and heat generation (81.88 kg/h CO_2 emission). This shows a total of 398,736 tonnes/year CO_2 emission reduction.

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Chapter Seven

CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

This study investigated the early stage of the culture's adaptation to psychrophilic (20 ºC) anaerobic digestion of dairy manure and co-digestion of dairy manure and grass silage at increasing organic loading rates (1-5 g VS/L.d) and the total solids content (7-10%). Also, for the first time, the psychrophilic anaerobic digestion was simulated in Aspen Plus V11. The biogas utilization for H_2 production through the methane reforming process was integrated with the anaerobic digestion process. The major results obtained in this study are listed below:

- The CH_4 yield obtained from digesting manure alone (first cycle) ranged from 18.3 NL CH_4/kg VS_{added} at OLR of 5 g VS/L.d to 28.7 NL CH_4/kg VS_{added} at OLR of 1 g VS/L.d. However, the CH_4 yield obtained from digesting manure and silage (first cycle) ranged from 43.5 NL CH_4/kg VS_{added} at OLR of 1 g VS/L.d to 62 NL CH_4/kg VS_{added} at OLR of 5 g VS/L.d
- The CH_4 yield obtained from digesting manure alone (second cycle) ranged from 25.6 NL CH_4/kg VS_{added} at TS of 10% to 38.1 NL CH_4/kg VS_{added} at TS of 7%. However, the CH_4 yield obtained from digesting manure and silage (second cycle) ranged from 69.9 NL CH_4/kg VS_{added} at TS of 7% to 103.9 NL CH_4/kg VS_{added} at TS of 10%
- Adding silage increased the methane yield significantly even in the seed microbial culture, which is not adapted to the new complex substrates and operation temperature.

- Modeling the kinetic of the CH_4 yield data in each phase determined the hydrolysis reaction rate constants. In the first cycle (culture adaptation at different organic loading rates), the reaction rate constant in the first-order kinetic model for digesting manure alone was 0.131 to 0.220 1/d for OLRs 5 to 1 g VS/L.d, respectively, whereas for digesting dairy manure and grass silage, it was 0.109 to 0.177 1/d for OLRs 1 to 5 g VS/L.d
- Adding grass silage to the system decreased the hydrolysis reactions rate constant at lower OLRs, while increased the hydrolysis reaction rate constant as the OLR increased
- In the second cycle (culture adaptation at increasing total solids in the bioreactor), the reaction rate constant for digesting dairy manure alone was 0.284-0.364 1/d, whereas for digesting dairy manure and grass silage it was 0.081-0.3 1/d. Obviously, the effect of the short term of adaptation is apparent in the value of the reaction rate constant, which increased on the upper end measured in the first cycle
- The ecology of the psychrophilic anaerobic digestion of dairy manure and grass silage using molecular biology methods was studied to identify the dominant species of microorganisms and the change in their abundance upon adaptation. The analysis was applied to the original culture (from the wastewater treatment plant facility), the inoculum, and samples from the experiments of the culture which was fed cow manure and cow manure and grass silage at organic loading rates of 1, 3, and 5 g VS/L.d. Generally, bacterial species belonging to the phyla of *Bacteroidetes*, *Synergistetes*, *Colsridia*, *Spirochaeta*, *Syntrophobacterales*, and *Firmicutes* dominated in the culture; most of them are capable of fermenting sugars, fats, and

proteins. Their dynamic reflected the effect of the substrate type and nature rather than the organic loading rate. The diversity is promising for the performance of biogas production

- The economic assessment was conducted on the total biogas production potential using dairy manure in Newfoundland and Labrador; about 3,330,000 m^3 of biogas could be produced annually. This is equivalent to about 6,675,000 kWh electricity per year and worth about CAD 860,000
- The economic analysis for Lester's Farm showed that there is potential for production of 219,300 kWh electricity per month, which is equivalent to a biogas power plant of 300 kW nominal capacity. From this production, about 11% would be consumed by the farm to meet its energy demand, 30% would be used to heat the reactor to maintain its temperature at 20 ºC reactor, and 59% would be the surplus that could be sold as electricity. The on-farm biogas production could earn more than 200,000 CAD annually. The payback period for the biogas plant construction is estimated to be 20 years assuming the biogas plant would cost 4 million CAD for the construction and commissioning
- Adaptation of the culture is a challenging step that needs about one year; otherwise, the culture should be sourced or imported from an actively operating dairy manure biogas plant
- A simulation study was performed to model the low-temperature anaerobic digestion process, for the first time, using commercial simulation software. The simulation results agreed with the experimental results obtained in the current study and were validated with the experimental results from the literature.

- The integration of hydrogen production plant with anaerobic digestion process was investigated. The results showed that there is a potential of producing hydrogen from biogas, which is a valuable method in producing a green source of energy and reducing greenhouse gas emissions.
- Temperature and pressure are the most critical parameters in dry methane reforming reaction. The optimum temperature and pressure for the current study is 595 °C and 4 bar, respectively, which gives more than 97% CH_4 conversion to syngas.
- The water-gas shift reaction achieves more than 99% CO conversion at temperature of 288 °C and atmospheric pressure.
- 8.11 kg/h H_2 could be produced via dry reforming of biogas obtained from anaerobic digestion of 150 m^3/day cow manure.
- The proposed process captures CO_2 while producing H_2. A total of 78.38 kg/h CO_2 is eliminated to be released to the atmosphere.
- The thermal energetic efficiency of the dry methane reforming process offered in this study is 72.85%, which shows its advantage compared to steam methane reforming and auto-thermal reforming.
- Biogas to H_2 production plant using is better than the biogas-to-electricity by direct combustion in terms of CO_2 emissions. The results revealed that using H_2 production plant could reduce the CO_2 emissions by 398,736 tonnes/year.

7.2. Recommendation for Future Studies

The current study investigated the biogas production potential of dairy manure and grass silage at low temperatures. The experimental results are obtained at increasing organic loading rate

and increasing total solids content, two important operating parameters of the anaerobic digestion process. The simulation framework was developed to model the anaerobic digestion process and assess the hydrogen production potential from biomass. The following suggestions could be carried out to understand better the various aspects of the low-temperature anaerobic digestion process as well as the potential of biofuel production from biogas:

- Given that this study was impacted by COVID-19 outbreak and the following restrictions, it is recommended to conduct the experiments in multiple cycles to completely acclimate the culture to the new conditions. Generally, it could take several months to a year to fully acclimate the culture, in which the performance of microorganisms in the production of methane will enhance accordingly. The acclimation phase is a vital step as it represents the operation of the biogas plant at its start-up phase.
- Investigating the microbial community during the experiments of multiple cycles of acclimation to explore the dynamics of the microbiome. The composition of microorganisms determines the extent of acclimation. Having enough information about the dynamics of microorganisms assists with better scenarios for culture adaptation, such as feeding rate, feeding ratio (dairy manure to grass silage), and hydraulic retention time.
- Co-digesting other agricultural or municipal wastes to the bioreactor to enhances the methane yield of the process. Investigating the effect of various available wastes, such as municipal waste, food processing waste, and agriculture residues on the performance of the anaerobic digester would provide useful results.

- Increasing the total solids content during the experiments to greater than 10% to explore the process at solid-state anaerobic digestion.
- Performing the experiments at lower temperatures (10-15 ^{o}C) to explore the effect of temperature change in the psychrophilic region on the performance of the microorganisms. The temperature of 20 ^{o}C is the upper level of the psychrophilic region and the ambient temperature in NL is lower. Considering lower temperatures for biogas plant, less heat is required in the anaerobic digester. It can help with increasing plant efficiency.
- Mathematical modeling of the anaerobic digestion bioreactor. A rigorous and verified mathematical model is always helpful in designing the best scenarios to achieve optimum plant efficiency. Up to this date, a few studies have developed rigorous kinetic and mathematical models for the psychrophilic anaerobic systems. The Anaerobic Digestion Model No. 1 (ADM1) is a great model to be applied to psychrophilic temperatures. This model comprises several parameters that are temperature-dependent. Identifying these parameters and the specific feed parameters will help with better understanding, design, and operation of the process.
- Exploring the optimum operating conditions, such as temperature, organic loading rate, total solids content, and hydraulic retention time of anaerobic digestion process using the simulation and mathematical models.
- Investigating the economics of producing various biofuels, such as hydrogen, methanol, ethanol, etc., from biogas using the simulation model.

Appendix A:

CHARACTERIZATION LABORATORY REPORT

The Appendix A presents the characterization of raw dairy manure and grass silage samples.

Newfoundland Labrador

DEPT. OF FISHERIES & LAND RESOURCES
PRODUCTION & MARKETING DIVISION
SOIL, PLANT & FEED LABORATORY
308 BROOKFIELD RD., P O BOX 8700
ST. JOHN'S, NL, A1B 4J6

FEED TESTING RESULTS

CLIENT: Dr Noori Saady (MUN)
DATE RECEIVED: 19-11-12
DATE REPORTED: 19-11-29

Analysis Parameter	Lab # 114 Sample ID:		Lab # Sample ID:		Lab # Sample ID	
	Dry basis	As rec'd	Dry basis	As rec'd	Dry basis	As rec'd
Dry Matter, %	25.8					
Silage pH	-	4.1	-		-	
Crude Protein, %	16.4	4.2				
ADF, %	33.0	-		-		-
NDF, %	58.1	-		-		-
Est. TDN, %	62.0	16.0				
Dig. Energy, Mcal/kg	2.73	-		-		-
Net Energy, Mcal/kg	1.38	0.36				
Calcium, %	0.56	0.14				
Magnesium, %	0.32	0.08				
Potassium, %	2.91	0.75				
Phosphorous, %	0.49	0.13				
Sodium, %	0.051	0.013				
Sulphur, %	-					
Iron, ppm	452	117				
Copper, ppm	14	3.6				
Manganese, ppm	86	22				
Zinc, ppm	53	14				
Crude Fat, %						
Ash, %						

NATIONAL FORAGE TESTING ASSOCIATION 2019 Certified Chemistry by NFTA Excellence in Forage Testing DM CP ADF NDF

Soil, Plant and Feed Laboratory
Department of Fisheries and Land Resources
308 Brookfield Road, Provincial Agriculture Building
P O Box 8700, St John's NL, A1B 4J6
Telephone (709) 729-6738, Fax (709) 729-6734

MANURE ANALYTICAL REPORT

Submitted by: Name: Dr Noori Saady
Address: MUN
Tel: 864-6087
Email: 17saady@mun.ca

Date Received: 19-11-12 Date Reported: 19-11-29

Analysis	Lab # MC 135 ID: dairy	Lab # ID:	Lab # ID:
Moisture Content (as rec'd) (%)	89.4		
pH	6.1		
Total Nitrogen (%)	3.24		
ADF (%)	33.7		
NDF (%)	35.0		
Total Phosphorous (%)	0.45		
Total Potassium (%)	2.40		
Total Calcium (%)	1.28		
Total Magnesium (%)	0.49		
Total Iron (ppm)	561		
Total Copper (ppm)	36		
Total Manganese (ppm)	145		
Total Zinc (ppm)	99		
Total Boron (ppm)	14		
Total Sodium (ppm)	6670		
Fat (%)	7.2		

(Results reported on a Dry Basis)

Tom Fagner
Soil, Plant and Feed Laboratory

Copy to: Dr X. Guo

AGRI-ANALYSE ENR.
1730 WELLINGTON SOUTH
SHERBROOKE, QC, J1M 1K9
TEL: (819) 821-2152 FAX: (819) 348-1888
TOLL FREE: 1-800 567- 6045

FEED ANALYSIS REPORT

Sample Number : 191128042
Description : MANURE
Identification :
Date Printed : 12/22/2019
Client Name : MEMORIAL UNIVERSITY
240 PRINCE PHILIP DR
ST-JOHN'S NL A1B 3X5

ID :
Telephone:
Fax :
Rep : NOORI SAADY
E-mail:

	As Received	Dry Basis
Dry matter %	11.44	100
Moisture %	88.56	0
Protein % (Nx6.25)	2.02	17.63
Acid Detergent Fibre %	3.27	28.6
Neutral Detergent Fibre %	5.5	48.1
LIG % NDF	4.29	37.54
Lignin %	2.07	18.06

Mitar Mojovic, Chemist

AGRI-ANALYSE
1730 WELLINGTON S, SHERBROOKE, QC J1M 1K9
TEL: (819) 821-2152
INTERURBAINS SANS FRAIS
TOLL FREE: 1-800-567-6045

AGRI

NEAR INFRARED ANALYSIS REPORT

SAMPLE NUMBER: 191128041
DESCRIPTION: Legume Grass Haylage, NIR F SILAGE MUN
IDENTIFICATION: C/O NOORI SAADY
DATE PRINTED: 11/28/19

CLIENT NAME: MEMORIAL UNIVERSITY
ADDRESS: 240 PRINCE PHILIP DR.
ST-JOHN'S NF
A1B 3X5

	As Received Basis	100% Dry Matter Basis		As Received Basis	100% Dry Matter Basis
MOISTURE %	75.37	0.00	**Energy**		
DRY MATTER %	24.63	100.00	TDN % [ADF]	14.58	59.22
Protein			NEL, MCAL/KG [ADF]	0.33	1.33
CRUDE PROTEIN %	4.22	17.14	NEM, MCAL/KG [ADF]	0.32	1.28
ADF-N %	0.40	1.62	NEG, MCAL/KG [ADF]	0.18	0.71
ADF-N (%CP) %	9.46	9.46			
AVAILABLE PROTEIN %	4.22	17.14	TDN % [WEISS]	17.33	70.37
SOLUBLE PROTEIN (% CP)	61.60	61.60	NEL, MCAL/Kg [WEISS]	0.40	1.61
DEGRADABLE PROTEIN (% CP)	80.80	80.80	NEM, MCAL/KG [WEISS]	0.37	1.48
NDICP %	0.64	2.60	NEG, MCAL/KG [WEISS]	0.19	0.76
NDICP (%CP) %	15.14	15.14	**VFA**		
Fiber			pH %	3.74	3.74
ACID DET. FIBER %	8.57	34.81	LACTIC %	2.02	8.21
NEUTRAL DET. FIBER %	12.28	49.87	ACETIC %	0.22	0.89
NDFD30 (%NDF) %	60.76	60.76	BUTYRIC %	0.18	0.73
NDFD48 (%NDF) %	65.86	65.86	AMMONIA %	0.36	1.46
LIGNIN %	1.37	5.56			
LIGNIN (%NDF) %	11.16	11.16	**Milk Per Day From Forage (Kg)**	8.55	34.72
Calculations			**Milk Per Metric Ton From Forage (Kg/MT)**	465.67	1890.68
NFC %	5.19	21.08			
NSC %	0.49	1.97	DCAD, mEq/Kg	71.03	288.40
RELATIVE FEED VALUE (RFV)	114.91	114.91			
RELATIVE FORAGE QUALITY (RFQ)	170.74	170.74			
DM INTAKE (% BODY WT.)	2.98	2.98			
Predicted Variables					
FAT %	1.33	5.42			
STARCH %	0.17	0.68			
SUGARS %	0.32	1.29			
ASH %	2.24	9.09			
Minerals					
CALCIUM (Ca) %	0.15	0.61			
PHOSPHORUS (P) %	0.11	0.43			
POTASSIUM (K) %	0.62	2.53			
MAGNESIUM (Mg) %	0.08	0.31			
SULFUR (S) %	0.05	0.22			
CHLORIDE (CL) %	0.21	0.87			
SODIUM (NA) %	0.01	0.05			

Terry Winslow, Président

CHIMISTE Mitar Mojovic 2009-104 QUÉBEC

Mitar Mojovic, Chimiste

Appendix B:

EXPERIMENTAL PROCEDURE

B.1. Introduction

Appendix B provides a detailed procedure of the experimental analyses for measurement of the total solids (TS), volatile solids (VS), alkalinity, chemical oxygen demand (COD), and pH of the samples of manure, grass silage, and the bioreactor content.

B.2. Total Solids (TS)

Total solids refer to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature (105±2 ^{o}C).

Fixed solids are also considered the residue of the total, suspended, or dissolved solids after combusting for a specified time at a specified temperature (550 ^{o}C). The required apparatus are analytical balance, drying oven, desiccator, evaporating dishes, muffle furnace, filtration apparatus, volumetric pipets, aluminum pans, porcelain crucibles, and tweezers.

The step-by-step procedure of the total solids (TS) measurement is provided below:

1. Weight the evaporating dish (note as: B).

2. Pour 5 ml well-mixed sample into evaporating dish.

3. Dry in the oven at 105 ℃ for 1 hour.

4. Cool in the desiccator.

5. Weight the evaporating dish.

6. Repeat drying, cooling, and weighing process until the weight difference < 4% (note as A).

Figure B.1. The total solids determination

B.3. Volatile Solids (VS)

The weight loss on ignition is called volatile solids (VS), which is used as a measure of organic matter in the sample. The procedure for determination of the volatile solids (VS) are as follows:

1. Weigh the dried dish of the total solid test (note as B).
2. Put in the furnace for 90 min at 550 ℃ for ignition.
3. Let it cool in a desiccator and weigh the dish (note as C)



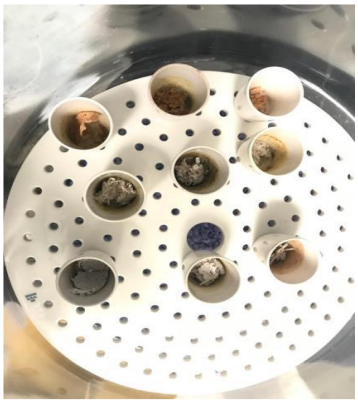

Figure B.2. Samples before and after heating to determine volatile solids

B.4. Alkalinity

Alkalinity can be defined as the acid-absorbing property of a liquid. The major acid absorbing constituents that we typically deal with are hydroxide (OH^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. The apparatus needed for determining the alkalinity are magnetic stirrer and stir bars, burette (25 mL), volumetric pipets, and miscellaneous glassware.

The procedure is of determining the alkalinity based on the colorimetric titration is given below:

1. Pipet a 50 mL aliquot of the sample into a 150 ml beaker.
2. Add a stir bar, place the beaker on magnetic stirrer and stir gently.
3. Add phenolphthalein indicator (5 drops) and note the color.
4. Titrate the sample with 0.02 N H_2SO_4. Add the acid in increments of 0.5 to 1.0 ml.
5. Record the Volume (V_p) at which the color changes from pink to colorless.
6. Add the methyl red–bromocresol green indicator (5 drops).
7. Continue titration. Record the Volume V_{mo} at which the solution changes color again.

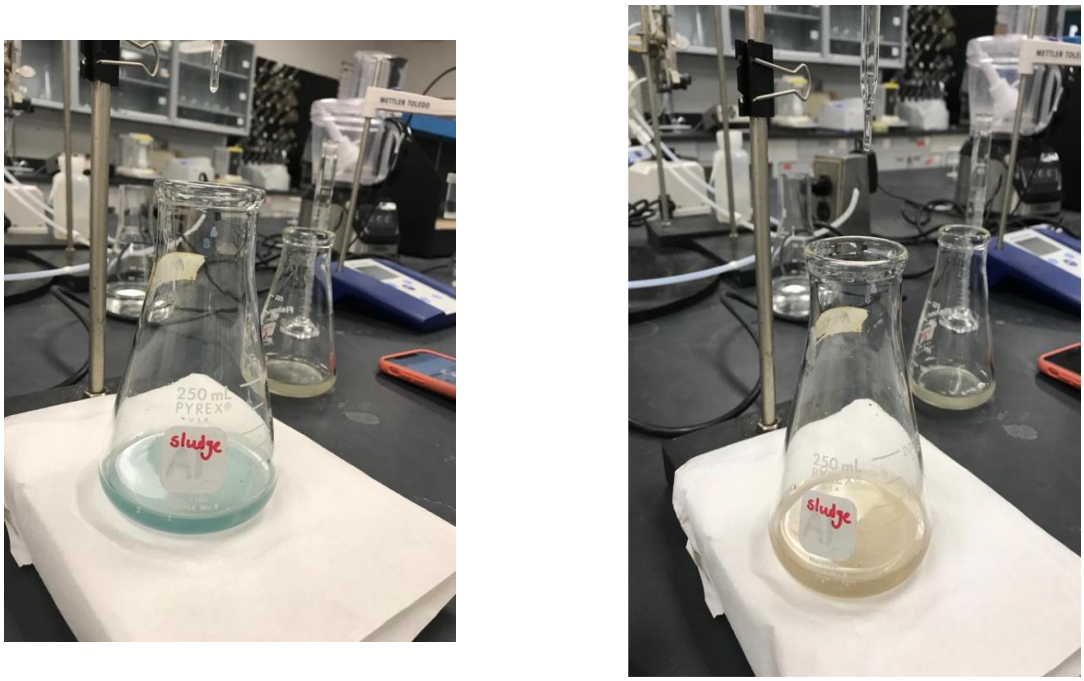

Figure B.3. Cow manure samples before and after titration

B.5. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand is a measure of the amount of oxygen required to oxidize the organic matter in the sample using a strong chemical oxidizing agent. It provides a direct measure of all organic oxidizable matter in the sample. The required apparatus are: analytical balance, volumetric flasks and pipettes, drying oven capable of maintaining 150 °C, muffle furnace capable of maintaining 500 °C, digestion station, and UV spectrophotometer.

The procedure is provided below:

1. Write labels on three tubes mentioning the sample name and concentration of standard and group number.

2. Add 2.5 mL of sample, blank, and standard to tubes accordingly.

3. In the digestion station, add 1.5 mL digester (dichromate iron) and 3 mL of H_2SO_4 + silver sulphate to each tube with the help of TA

4. Place the tubes in the oven, record the time, and let it stay there for one hour.

5. Take the tubes out of the oven after one hour let them remain out for 10 minutes to cool down to room temperature

6. Using the UV spectrophotometer, read the A600 data for blank, water sample, and standard 200 mg/L concentration.

7. Write down the data for standard 200 concentration on the board to complete required data for drawing the chart.

Considering that a strong digester such as H_2SO_4 can almost completely oxidize any carbon based or organic compounds, this experiment is also often used to determine the effectiveness of wastewater treatment facilities.

Figure B.4. Sample preparations before spectrophotometric analyses

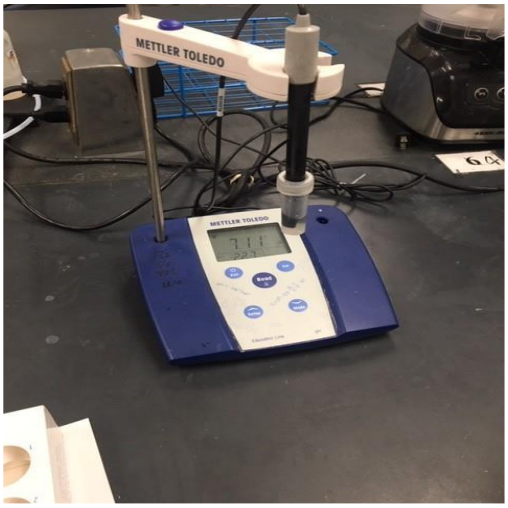

Figure B.5. pH meter indicating the pH value of a sample

B.6. pH

The pH is a measure of the acidity or alkalinity of manure. This can be quite variable, dependent upon the feed and bedding practices associated with the animal production system. The pH was measured using the pH meter shown in Figure B.5.