Acidification of In-Storage Psychrophilic Anaerobic Digestion (ISPAD) Content to Reduce Ammonia Volatilization

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ABSTRACT

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In-storage-psychrophilic-anaerobic-digestion (ISPAD) is a treatment system applicable to wastewaters stored for a period of over 100 days, such as livestock wastes and municipal sludge. The ISPAD system consists of a storage tank covered by an airtight geo-membrane, filled as a sequentially batch fed system and emptied when full except for a 0.3 m depth to inoculate the next batch. Thus, ISPAD operates at a temperature fluctuating with ambient, and its microbial community is acclimated to low temperatures. Taking advantage of existing structures and requiring little technical supervision because of its low operating temperatures, ISPAD is an anaerobic digestion (AD) system feasibly accessible to operations producing limited amount of organic wastewaters. Since ISPAD operates under conditions which are totally different as compared to conventional AD reactors, optimal management practices need to be developed through microbial kinetic estimation and process modelling.

The first objective was to evaluate the microbial behaviour of the ISPAD system by estimating its kinetic coefficients. The second and third objectives were to develop and validate a mathematical model to simulate the ISPAD process, such as the volatile fatty acid (VFA) concentration and pH of its content and its CH_4 production, based on its specific microbial kinetics. The final project objective was to predict and experimentally test conditions which lead to ISPAD content acidification to limit ammonia (NH_3) volatilization from the digestate.

The first part of the project consisted of determining microbial kinetic values by fitting the Monod model to results obtained from laboratory substrate activity test (SAT) using ISPAD inoculum and for temperatures of 8, 18 and 35 ºC. The fitting process consisted of: applying the decomposition principle to prioritize the determination of kinetic parameters, and then using the statistical least square error procedure to minimize the sum of squared errors between the measured ISPAD experimental data and the Monod model results. The results produce microbial

kinetic values specific to the ISPAD system and associated with two groups of microbial population, one acclimated to cold and another to the mesophilic conditions.

The second part of the project consisted of developing a mathematical model based on that of Keshtkar et al. (2001). Simulation of ISPAD was achieved using the Simulink/Matlab software to predict glucose, VFAs degradation, pH and CH_4 production. To predict the pH of the ISPAD content, a function was introduced based on optimized dissociation constants (K_a) for the major ions found in organic wastewaters under AD. Finally, a temperature function was added for most kinetic values to simulate the ISPAD process for temperatures ranging from 4 to 35 ºC. For this purpose, the Arrhenius and Square-Root Equations were compared. For the maximum microbial growth rate (μ_{max}), the Square Root Equation better represented acidogens and propionate degrading acetogens, while the Arrhenius Equation better represented the methanogens and butyrate degrading acetogens. The model was calibrated using experimental data, where ISPAD content and glucose was used as inoculum and substrate, respectively. The proposed model showed good agreement with the experimental data in predicting biogas generation, substrate consumption and pH at a temperature range of 4 to 35 ºC. Although microbial activity at 4 °C was much less than that at 18 and 35 ºC, it showed acclimation to lower temperature.

The third part of the project validated the ISPAD model using condition, which differed from those used for calibration, such as a different concentration of substrate. The model accuracy was checked mathematically by determining the coefficient of determination. The ISPAD model was able to predict glucose degradation, VFAs, pH, and methane. However, the model weakly predicts CO_2 production for the first 2 days likely because of its water solubility.

The final portion of the method dealt with ISPAD content acidification, to reduce NH_3 volatilization from the digestate upon removal from the system. Acidification was based on quickly increasing the sequential organic load (OL), under specific temperatures to favour acidogen growth and VFA accumulation. A mathematical equation was applied to the ISPAD model to optimize different OL strategies, where glucose was fed to simulate the hydrolysis of sugar rich wastes. By comparing with laboratory experiments, ISPAD model prediction was found adequate in selecting optimal acidification OL strategies, but did not predict accumulation of intermediate substrates, which produced a lag in pH drop, and generates a pH drop below 6.0, because of set values for its pH inhibition function. Nevertheless, sequentially feeding 13 kg

glucose/m^3 of ISPAD content over 4 days was found to drop the pH of the ISPAD content to 6.0 by day 7. Such OL competes favorably against present acidification techniques such as that using concentrated sulfuric acid.

The contribution to knowledge of this work was introducing the decomposition method to determine AD microbial kinetics; for ISPAD, establishing microbial kinetics, ion dissociation constants for pH prediction, and parameter variation with temperature producing an accurate model to predict and optimize the ISPAD process, and determination of sequential OL strategies to feasibly acidify the ISPAD content to lower NH_3 volatilization from its digestate upon system removal.

DEDICATION

To my dear husband, Mohammad, who has been a constant source of practical and emotional support during the challenges of graduate school and life. I am truly thankful for having you in my life.

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Nomenclature, Subscript and Expressions

Subscript	Description
MA	Acetoclastic methanogens
AB	Butyrate degrading acetogenic
AP	Propionate degrading acetogenic
Hom	Homoacetogens
MH	Hydrogenotrophic methanogens
A	Acidogens
Glu	Glucose
Pr	Propionate
But	Butyrate
Ac	Acetate
Am	Ammonia

Symbol	Description
AD	Anaerobic Digestion
ISPAD	In-Storage-Psychrophilic Anaerobic Digestion
VFA	Volatile Fatty Acid
NH_3	Ammonia
TAN	Total Ammonia Nitrogen
OLR	Organic Loading Rate
OL	Organic Load
HRT	Hydraulic Retention Time
GRG	General Reduced Gradient
μ_{max}	Maximum microbial growth rate
K_s	Half saturation constant
Y	Yield coefficient
K_d	Decay rate constant
BMP	Biochemical Methane Production
SAT	Specific substrate Activity Test
SSE	Sum of Square Error
WSSE	Weighted Sum of Square Error
t_i^{Obs}	Observed data
t_i^{Pred}	Model predicted output
N	Number of data
IWA	International Water Association

R^*	Universal gas constant
μ	Microbial growth rate
ΔH	Enthalpy of the reaction
R	Correlation coefficient
K_i	Inhibition constant
V	Volume
A^-	Anions
C^+	Cations
E_a	Activation energy
T_{min}	Apparent minimum temperature
FB	Fractional Bias
RSME	Root Mean Error
NMSE	Normalized Mean Squared Error
MAPE	Mean Absolute Percentage Error
R^2	Coefficient of determination
K_{a1}	First dissociation constant for carbonic acid
K_{a2}	Second dissociation constant for carbonic acid
K_{a3}	Dissociation constant for acetic acid
K_{a4}	Dissociation constant for propionic acid
K_{a5}	Dissociation constant for butyric acid
K_{a6}	Dissociation constant for ammonia
K_w	Dissociation constant for water
b	Regression coefficient
F(pH)	pH inhibition
pk_1	Lower pH drop-off value, where the growth rates are approximately 50% of the uninhibited rate
pk_h	Upper pH drop-off value, where the growth rates are approximately 50% of the uninhibited rate
CV	Coefficient of Variation
COD	Chemical Oxygen Demand
TS	Total Solid
TSS	Total Suspended Solid
VS	Volatile Solid

VSS	Volatile Suspended Solid
VDS	Volatile Dissolved Solid
FS	Fixed Solid
S_{ij}	Substrate for each group of the AD consortium (j) at any time step (i)
X_{ij}	Microbial population for each group of the AD consortium (j) at any time step (i)
V_i	Holding volume in ISPAD tank
t_r	Regular interval of time
T	Temperature

Chapter 1. Introduction

1.1.Problem Statement

Anaerobic digestion (AD) is a wastewater treatment process which generates an energy rich biogas while controlling methane (CH_4) emissions and thus lowering greenhouse gas emissions (Abbasi et al., 2012). Once scrubbed of its CO_2 and other minor gases including H_2S, this biogas can replace natural gas, because of its high CH_4 content in the range of 65% (Kapdi et al., 2005). This biogas is also a renewable energy source reducing dependence on petro fuels deposits with many deposits projected to be depleted by 2050, at the present rate of consumption. Furthermore, AD produces an odorless digestate which is rich in minerals and offers an interesting soil fertilization value (Monnet, 2003). Anaerobic digestion is therefore an interesting alternative to aerobic treatments, which consume considerable energy and generally volatilize at least 50% of the nitrogen content of waste (Mata-Alvarez et al., 2000).

Despite its potential to generate CH_4 and its lower operating cost as compared to aerobic degradation, AD is not a widely used system to treat wastewaters unless subsidized. In the provinces of Ontario and Saskatchewan, Canada, several AD systems were built in the early 1980's to be abandoned before the end of the decade because of a lack of profitability. Indeed, the conversion of the biogas to electricity is expensive at $0.25/kW-h, because of its inefficient conversion with 60 to 70% of the energy lost as heat (Biogas, 2011). The CH_4 is relatively incompressible and when used as natural gas, is difficult to transport (Kapdi et al., 2005). The CH_4 generated by AD system must be located close to a plant requiring energy to be economically transported and used as a heat source. As for feeding into pipelines, there were issues, such as the introduction of contaminants affecting the quality of the natural gas. Although less costly than aerobic processes, conventional reactor AD systems still require a relatively high level of investment, regular technical supervision and digestate disposal (Friman, 1984; Scruton et al., 2004). These costs are not compensated by the CH_4 rich biogas, which is relatively expensive to transport and use as energy.

Ever since the establishment of specialized livestock farms, AD has been considered an interesting solution to control odor, ammonia (NH_3) and greenhouse gas emissions from manure

storages. According to Statistics Canada (2012) and for the swine industry alone, 12.9 million pigs are produced in Canada annually with Quebec sharing 32% of this production. Based on 0.7 to 1.0 m^3 of excretions per finished hog, this production generates annually 9 to 13 million tons of manure, which is an excellent source of crop nutrients when properly land applied. Between land applications, the manure is stored in open tanks and generates atmospheric pollutant such as CH_4, nitrous oxide (N_2O) and NH_3 (Burton & Turner, 2003). According to Park et al. (2006), up to 23% of the potential CH_4 in the manure may be lost during storage under Canada's climatic conditions. In addition, about 20% of the total ammoniacal nitrogen (TAN or NH_4^+ and NH_3) can be lost through volatilization from storage facilities (Muck & Steenhuis, 1982). The volatilized NH_3 causes soil acidification and eutrophication when transported and deposited by wind and rain. Anaerobic digestion is a manure treatment which can minimize these atmospheric emissions.

To provide a more feasible AD systems for wastewaters stored over at least 100 days, the concept of In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) was introduced. Although AD performs best under mesophilic conditions, namely 30 to 40 °C, it can also occur under thermophilic and psychrophilic conditions corresponding generally to 55-65 °C and less than 20 °C, respectively. Psychrophilic conditions are suited to the temperate climate of Canada (Kashyap et al., 2003) because the lower temperature require less biogas to heat the system during the winter. Psychrophilic AD systems were successfully operated in temperate regions by Alvarez and Lidén (2009), Massé et al. (1997) , and Safley Jr and Westerman (1990).

The ISPAD concept consists of using a circular existing storage tank for the wastewater measuring 30m in diameter by 3.66m in depth and covering it with an airtight geomembrane. The system is started with only 0.3 to 0.6m of content, as inoculum and filled by sequential feeding of manure over the entire storage period. Once full, the system is emptied except for a bottom 0.3 to 0.6m of content to serve once more as inoculum (King, 2011). Operating at fluctuating ambient temperatures and low feeding organic loading rates, the system is extremely stable requiring little if any technical supervision. The low reaction time created by the storage facility compensates for the low reaction rate of AD at psychrophilic temperatures. Furthermore, the short hydraulic retention time (HRT) of conventional AD system generally stops before reaching the full CH_4 potential of the waste, while the long ISPAD retention time can recover part of this biogas. The low infrastructure cost and technical requirements make the ISPAD

system extremely feasible especially for small operations handling less than 3.5 tons of volatile solids/day.

The ISPAD system was investigated for microbial acclimation, protein degradation (King et al., 2012; King et al., 2011), and biogas generation (Giard et al., 2013; Nohra et al., 2003). Using the content of a field ISPAD built in 2004, in Drummondville, some 100 km North East of Montreal, Canada, King (2011) demonstrated that the system was able to effectively reduce the volatile solids (VS) content of the treated swine manure while releasing up to 63% of the CH_4 potential (King, 2011). In addition to producing biogas, ISPAD produces a nutrient rich digestate which can be used as fertilizer and offers a lower level of pathogens (Côté et al., 2006). The microbial community actively breaks down manure amino acids in the ISPAD tank with minimum loss of NH_3 through the biogas increasing the proportion of plant-available total ammoniacal nitrogen (TAN) (King et al., 2012). Accordingly, ISPAD digestate is rich in TAN and highly susceptible to NH_3 volatilization during land application, despite the fact that during a land spreading simulation, King et al. (2012) demonstrated that ISPAD treated manure lost 53 % less TAN as compared to conventionally stored manure.

The control of NH_3 volatilization from ISPAD system is an interesting option since volatilized NH_3 contributes substantially to the problem of environmental acidification. The deposition of the nitrogenous nutrient NH_3 also causes the eutrophication of natural ecosystems (Hooda et al., 2000), disturbs the nutrient balance and causes nutrient deficiencies. Other than environmental problems, NH_3 volatilization can result in a net loss of TAN, affecting the fertilizer value of ISPAD treated manure. In AD reactors, pH and buffering capacity are the two most important factors controlling NH_3 volatilization after temperature. In AD reactors where the pH ranges from 6.6 to 7.4 (Lahav & Morgan, 2004), NH_3 concentration can double with a pH changes from 7.7 to 8 (Sommer, 1997; Sommer & Husted, 1995; Sommer & Hutchings, 2001).

Several techniques were developed to lower the manure NH_3 volatilization, such as incorporation during land application and sulphuric acid addition before storage. At a rate of 5 kg of 18 M/m^3 of manure, sulphuric acid drops the manure pH to 5.5 and minimizes NH_3 volatilization. This technique changes the manure composition and can acidify the soil over a long-term basis. Instead of sulphuric acid and as compared to conventional AD systems, ISPAD is well designed to use AD to lower the manure pH. In AD, increasing the organic loading rate

(OLR) can cause a high concentration of volatile fatty acids (VFAs) as a result of a higher acidogen growth compared to methanogens (Ahring et al., 1995). The accumulation of VFAs can drop the digester pH to 6 while still maintaining its methanogen population, thus reducing NH_3 volatilization.

This study therefore focused on developing a management practice for ISPAD, to acidify its content through AD where a fast increase in organic concentration would favour the growth of acidogens over that of methanogens, and result in the accumulation of VFAs. Thus, the content of the ISPAD would offer a low pH such as 6 when removed from the system and, to be less exposed to NH_3 volatilization during land spreading. The development of such a management practice for ISPAD, can be better defined and understood through the modeling of the process once the microbial kinetics have been characterized.

1.2.Objectives

The main objective of this research is to develop operational procedures, which lead to the acidification of the ISPAD content just before land spreading to minimize the volatilization of the NH_3 accumulated during the ISPAD treatment. To achieve this main objective, the project must investigate and determine the microbial kinetics for ISPAD treating pig manure, and develop a model capable of predicting ISPAD microbial behavior, pH and VFAs accumulation.

More specifically, this research focused on the following specific scientific objectives:

I. Obtain reliable microbial kinetics for the ISPAD system while developing and demonstrating the advantages of the decomposition approach, a management optimization process; compare these kinetic values to those found in the literature;

II. Develop and calibrate a model predicting the behavior of the ISPAD microbial populations using kinetic values obtained in objective (1); as opposed to presently developed model, the ISPAD model must be able to predict pH evolution, include a function for 2 types of methanogens, namely acetoclastic and hydrogenophilic, include the conversion of CO_2 into acetate, and predict temperature effect on maximum growth rate (μ_{max}) and dissociation constant (K_a);

III. Validate the ISPAD model using experimental data;

IV. Establish optimal management strategies to acidify ISPAD systems; and validate the optimized acidification strategies using laboratory experiments.

1.3.Hypotheses

The project is designed to test the following hypotheses:

I. ISPAD community kinetic coefficients are different from those of mesophilic AD systems.

II. A mathematical model considering the ISPAD operating condition can be developed to accurately simulate the process.

III. The developed model could reasonably predict the ISPAD system.

IV. Increasing the OL for ISPAD can acidify the system; the necessary OL depends on temperature and the model developed can predict ISPAD acidification.

1.4.Scope of the Project

The project is limited to the ISPAD system and the ISPAD inoculum used in the present experiments is obtained from a field system built in 2004 in the Drummondville area of the Province of Quebec, Canada. The model developed simulates the AD process after hydrolysis, as the hydrolysis process requires another set of extensive research projects to model the degradation of complex molecules such as those found in wastewaters. The model was calibrated and validated based on data obtained from batch laboratory tests obtained using 250 mL flasks. Acidification conditions were obtained from model prediction and from laboratory tests also conducted in 2L flasks.

1.5.Organization of Thesis

This thesis consists of seven chapters which include the introduction, general literature review, four scientific articles, conclusions, and bibliography. Following the introduction in Chapter 1, Chapter 2 briefly reviews the history of AD system and its microbial degradation process. Following this, the methods to determine the kinetic parameters and their characteristics are discussed. The evolution of AD models from simple models to the complicated ones is also

reviewed. Finally, the NH_3 volatilization from anaerobically digested manure and acidification of manure through AD as one of the promising technique to reduce NH_3 volatilization are reviewed. The chapter concludes by summarizing the state of knowledge pertaining to aspects of ISPAD indicating the areas to which a contribution was needed.

Chapter 3 describes the materials and methods used to determine the kinetic parameters for ISPAD system. To determine the kinetic parameters, two methods of optimization are compared. Finally, the effect of temperature on ISPAD microbial kinetic value was also evaluated.

In Chapter 4, an AD model was developed through the kinetic parameters obtained in chapter 3. To develop a model, one of AD model was modified based on ISPAD operating condition. The model was then calibrated through experimental data.

In Chapter 5, the calibrated ISPAD model was validated where the model tested for another condition that was used for calibration.

In Chapter 6, the validated ISPAD model was used to find a suitable parameter that could be used to acidify the ISPAD content. Gradually increasing the OL was found the best practice to acidify the ISPAD content. Three different scenarios of increasing OLs were modeled and optimized. Finally, one scenario was selected as the most suitable option to acidify the ISPAD content and was tested using experimental data. The predictive ability of acidification model was evaluated through statistical analysis. Finally, the model was modified to optimize the cost of the process.

Chapter 7 presents a summary and the overall conclusions from the complete research project, including the contributions to knowledge and suggestions for further research. The complete list of references cited in the thesis is presented at the end.

Chapter 2. Literature Review

2.1. Anaerobic Digestion Background

By collecting marsh gases from lakes and performing combustion experiments, Alessandro Volta was the first scientist to investigate the anaerobic digestion (AD) process in 1770. The investigation conducted by Alessandro Volta lead to the construction of the first biogas plant in Bombay, India in 1859. In 1886, Beauchamp and Tappeiner proved that biogas is the product of an anaerobic process completed by microorganisms called methanogens (Gunnerson et al., 1986).

Further research investigated the conditions under which methanogens were able to grow and reproduce. Up to that time, it was understood that only methanogens were required to produce biogas in AD systems. However in the 1960's and from bacterial cultures isolated from anaerobic digesters, it was found that several groups of microorganisms were required to produce biogas under AD. Thus, AD was found to be a complex multi-step process requiring several parallel pathways of microbial activity.

In the early 19th century, manure and agricultural waste started to be used for biogas production. However, AD attracted poor interest because of the low fuel value of the biogas and digester problems (Arsova, 2010).

In the 20th century, Europe and the United States further push the development and utilization of AD. For cost-effectiveness, this lead to the development of digesters operating at ambient temperature and at a high rate (Van Lier et al., 2001). In 2003 and in the U.S., landfill biogas generated 43 billion kW-h (147 trillion BTU) of energy representing 0.6% of the total U.S. natural gas consumption. Furthermore, this use of landfill biogas reduced by 99 million metric tons, the U.S. greenhouse gas emissions, representing 4% of the U.S. greenhouse gas production. Since then, AD attracted interest as a potential source of energy.

2.2.Microbiology of Anaerobic Digestion

Anaerobic digestion (AD) requires four main microbial steps to degrade organic matter, each carried out by a different group of microorganisms in the absence of O_2 (Husain, 1998). These steps are series of interlinked and parallel reactions, hence they influence one another.

Hydrolysis is the first process step where complex organic polymers such as polysaccharides, proteins, and lipids are converted by extracellular enzymes into soluble products like simple sugars, amino acids and fatty acids. Hydrolysis reactions include two phases, the first being colonization over the surface of the particle, and the second being the release of enzymes to produce monomers to degrade the particle (Vavilin & Lokshina, 1996). The overall hydrolysis rate is complex to predict as it depends on: the size of the organic particles, their shape and surface area, biomass concentration, enzyme production, and adsorption rate (Grady Jr et al., 2011; Parawira et al., 2005). Hydrolysis requires a high level of energy and can be considered a limiting stage in the AD of complex polymers (Gallert & Winter, 1997).

The second step is acidogenesis where the monomers produced from hydrolysis are degraded into compounds such as volatile fatty acids (VFAs), CO_2, H_2 and acetate. The degradation end product for glucose for example, varies from VFAs, lactate, and ethanol, depending on the degradation factors such as substrate concentration, pH and dissolved H_2. When the organic load (OL) is high, lactic acid is mainly produced as opposed to VFAs when the pH exceeds 5. However, at a pH under 5, more ethanol is produced and at an even lower pH under 4, the processes may stop (Batstone et al., 2002). The H_2 partial pressure has a dominant influence on the fermentation process. When the H_2 partial pressure is low, glucose conversion to acetate and hydrogen is favoured over that of ethanol or VFAs. Therefore, when hydrogen-utilising organisms such as hydrogenotrophic methanogens maintain a low H_2 partial pressure, the fermentation pathway to acetate and hydrogen will be the main carbon flow between the carbohydrates and CH_4 end point. Yu and Fang (2003) studied the effect of temperature on acidogenesis and their products including VFAs and alcohols, to show no effect on product distribution. However, Dinopoulou et al. (1988) showed that temperature had effect on VFAs distribution, where acetate production increased with temperature, while propionate slightly decreased with increasing the temperature. The fermentation pathway to produce acetate and

hydrogen is favoured rather than that of ethanol or butyrate formation, when the partial pressure of hydrogen is low. These products cannot be utilized directly by the methanogens and must be degraded further in a subsequent process that is referred to as acetogenesis (Björnsson, 2000).

Acetogenesis is the third step conducted by acetogenic bacteria, whereby low molecular weight VFAs are converted into acetate, CO_2, and H_2. Acetate can be produced from three pathways: a) the fermentation of soluble organic compounds such as glucose; b) VFAs degradation, and; c) H_2 and CO_2 reduction to acetate. The temperature has an effect on the production pathway for acetate through the acetogenesis step. For example, homoacetogenesis which converts CO_2 and H_2 to acetate, will be dominant at low temperatures, (Batstone et al., 2002; Kotsyurbenko, 2005) and be negligible under mesophilic conditions. Acetogens are obligate hydrogen producers and they maintain a syntrophic or mutually beneficial relationship with hydrogen-consuming methanogenic archaea. This interspecies hydrogen transfer where the methanogens serve as a hydrogen sink allows the fermentation reactions to proceed. Acetogens are sensitive to environmental changes, and needs long periods to adjust themselves to new environmental conditions (Björnsson, 2000).

The fourth and final step producing CH_4 is carried out by acetoclastic methanogens decarboxylating acetate and hydrogenotrophic methanogens using H_2 to reduce CO_2 (Vavilin et al., 1998). From stoichiometry, about 70% of the CH_4 produced in AD originates from the acetate pathway. The hydrogen pathway is more energy yielding than the acetate pathway, and hydrogenotrophic methanogenesis are less sensitive to environmental changes than acetoclastic methanogens. Therefore, methanogenesis from acetate tends to be rate limiting in the anaerobic treatment of easily hydrolysable substrates (Björnsson, 2000). The operating conditions for hydrogenophilic and acetoclastic methanogenic archaea are different. At high H_2 partial pressure, hydrogenotrophic methanogens are more active, while acetoclastic methanogens are not affected by H_2 partial pressure. At thermophilic temperatures of 65 ºC, hydrogenotrophic methanogens were found to be more active than the acetoclastic methanogens (Ahring et al., 2001), while at psychrophilic temperature under 15 ºC, hydrogenotrophic methanogens showed very low activity (Kotsyurbenko et al., 2001). The hydrogenotrophic methanogens are among the fastest growing organisms in the AD process with a doubling time of six hours compared to the slow-growing acetoclastic methanogens with a doubling time of 2.6 days (Björnsson, 2000).

2.3.Microbial Kinetics Determination

Kinetic coefficients are useful parameters to evaluate the behaviour of microbial populations and the rate of substrate degradation. The most important kinetic coefficients are the maximum microbial growth rate (μ_{max}), microbial decay rate (K_d), microbial yield (Y), and half saturation constant (K_s). The maximum microbial growth rate occurs when nutrients are not limiting and the decay rate represents the death rate of cells. The microbial yield is the ratio of microorganism biomass production to the substrate consumption. The half saturation constant is the concentration of substrate required to achieve half of the μ_{max} and is thus, an indication of the affinity of microorganism to consume the substrate. These kinetic values vary as a function of substrate, microbe and temperature (Donoso-Bravo et al., 2011).

One method used to obtain kinetic values is the fitting of experimental data to a suitable model. For AD, literature has tested three different feeding methods: batch (Donoso-Bravo et al., 2009; Flotats et al., 2003), continuous (Batstone et al., 2009; Bernard et al., 2001) and fed-batch (Redzwan & Banks, 2004; Rodrigues et al., 2003). Batch tests are commonly used in AD for the determination of kinetic parameters because of their simplicity and the short experimental time required. Two batch laboratory procedures are frequently used to estimate AD kinetic coefficients: biochemical methane production (BMP) and specific substrate activity test (SAT). The biochemical methane production assay is operated in a long-term batch incubation of cultures with periodic biogas sampling to provide a temporal profile of CH_4 production from a known initial concentration of active biomass and organic substrate. It generates data to compute the rates of substrate consumption and CH_4 production (Chynoweth et al., 1993; Shelton & Tiedje, 1984). However, the SAT is operated for a shorter period of time to measure the rate of consumption of individual substrates used by the main AD microbial group. For AD, Donoso-Bravo et al. (2009) used starch, glucose and acetic acid as the main substrates in SAT to obtain kinetic parameters of hydrolysis, acidogenesis and methanogenesis, respectively. Flotats et al. (2003) also estimated kinetic parameters for the AD of gelatin using SAT.

The data obtained experimentally is used to estimate kinetic coefficients by changing their value till the model predicts a response corresponding to that obtained experimentally. The selection of an appropriate set of equations is important in designing the model and this is discussed in section 2.4. For AD, the kinetic values of μ_{max}, K_s, Y, and X are interdependent,

which makes their estimation difficult and tedious. The "decomposition approach" is introduced in this work as an innovative method to estimate interdependent kinetic coefficient through data fitting (Bahn et al., 1996; Harjunkoski & Grossmann, 2001). The advantages of this approach are: (1) to reduce the possibilities of dropping into local minima in the process of optimization; (2) to reduce the computation time; and (3) to increase the fitting accuracy compared to other approaches (Jiang & Cheng, 2005). The decomposition approach requires decomposition of the designed algorithm into sub-problems and the solving of individual parameters by data fitting, in order of importance.

Using the decomposition approach, each sub-problem can be solved through an objective or cost functions, recognized as the most common tools to fit the experimental data to a selected model. The most useful cost function is the Sum of Square Error (SSE) (Batstone et al., 2002; Donoso-Bravo et al., 2010; López & Borzacconi, 2010; Noykova & Gyllenberg, 2000) which assumes that the standard deviation of the measurement errors is constant:

$$SSE = \sum_{i=1}^{n} \left(t_i^{\text{obs}} - t_i^{\text{pred}} \right)^2 \qquad (2\text{-}1)$$

The observed data, the model-predicted outputs and the number of data are shown as t_i^{obs}, t_i^{pred}, and n, respectively.

When errors do not have a constant standard deviation, the weighting factors (W_i) can be introduced along with Eq. (2-2), leading to a Weighted Sum of Square Error (WSSE) (Flotats et al., 2003; Lokshina et al., 2001):

$$WSSE = \sum_{i=1}^{n} \left[W_i \left(t_i^{\text{obs}} - t_i^{\text{pred}} \right) \right]^2 \qquad (2\text{-}2)$$

To optimize the cost function, several algorithms have been developed, which can be categorized into two groups, namely exact and heuristic. An exact method is a mathematical procedure that generates a sequence of solutions improving order for a class of problems. This method uses convexity assumptions for the cost function to obtain the optimized values. The

main drawback of an exact method is the risk of getting trapped in local minima. The common approach to solve this trap problem is to start the search from several randomly selected initial parameters, which is called a multi-start strategy. The heuristic technique can solve the problem more quickly than the classic exact method because it is better at finding an approximate solution when classic methods fail. This is achieved by trading optimality, completeness, accuracy, and/or precision for speed.

Both exact and heuristic methods were used in the AD kinetic values determination. Application examples for the exact methods are Levenberge Marquardt (García-Ochoa et al., 1999; Lokshina et al., 2001), sequential quadratic programming (Aceves-Lara et al., 2005; Sales-Cruz & Gani, 2004), multiple shooting (López & Borzacconi, 2010; Müller et al., 2002), and the direct search method (simplex algorithme) (Haag et al., 2003; Mösche & Jördening, 1999; Simeonov, 1999). Application examples for the heuristic method are Simulated Annealing (Haag et al., 2003), Genetic Algorithms (Wichern et al., 2009), and particle Swarm Optimization (Wolf et al., 2008). The heuristic methods require a certain level of knowledge and experience, being more costly. In addition, the heuristic method is more sensitive to initial values and stopping criteria. Consequently, exact solutions may be more efficient.

2.4.Anaerobic Digestion Models

Considering that AD is such a complex process, mathematical models can provide a better understanding of the system; predict its outcome under various conditions and thus reduced operation costs and risks. The following sections discuss the evolution of AD models (Table 2.1).

Developed in the early 1970's, the first AD models assumed a simple step process based on the Monod (1950) equation computing microbial growth rate and product generation, from substrate concentration and μ_{max}. This single step process was based on the assumption during the 1930's, that one single group of microbes was responsible for AD (Buswell & Hatfield, 1936). The Monod model became the basis for most microbial models, including those pertaining to AD (Angelidaki et al., 1993; Batstone et al., 2002; Chen & Hashimoto, 1980; Contois, 1959; Hill & Barth, 1977). Based on Contois (1959) relating specific growth rate to population density, Chen and Hashimoto (1978) added a relationship determining μ_{max} as a

function of temperature. However, the first models excluded the effects of inhibition, despite the findings by Haldane (1930) and the concepts of in-competitive and non-competitive inhibition developed by Andrews (1969) and Ierusalimsky (1967), respectively.

Table 2-1: Summary of model characteristics

	Type of model	Characteristics
Simple models	First order model	• One equation for substrate uptake rate • No consideration for inhibition • No distinction between different microbial groups
	Monod (1950) model	• No consideration for inhibition • No distinction between different microbial groups
	Contois (1959) model	• Dependency between bacterial growth rate and population • No consideration for inhibition • No distinction between different microbial groups
	Chen and Hashimoto (1978) and Chen and Hashimoto (1980) model	• Based on the Contois (1959) model • Dependency between initial substrate concentration and reactor substrate concentration • Consideration of temperature effect on μ_{max} • No consideration for inhibition • No distinction between different microbial groups
Complicated models	Hill (1983) model	• Based on Monod model • Distinct acidogenesis and methanogenesis steps • Immediate availability of substrate • Consideration of the same μ_{max} for two microbial groups • VFAs inhibition for two steps and NH_3 inhibition of Methanogenesis
	Thomas and Nordstedt (1993) model	• Based on Monod model, extended by the Hill (1983) model • Distinction between acid forming bacteria and methanogens • Considers two easily and slowly biodegradable substrate • Considers VFAs inhibition for two steps
	Angelidaki et al. (1993) model	• Based on Monod model • Distinction between acid forming bacteria, acetogens and methanogens • Considers two easily and slowly biodegradable substrates • The hydrogenotrophic step is grouped with other steps • Considers inhibitions of VFAs, NH_3 and pH • Temperature dependency of μ_{max} • Considers physico-chemical reactions • Developed for CSTR reactor at thermophilic condition
	Keshtkar et al. (2001) model	• Extended Angelidaki et al. (1993) model • Developed for batch system at mesophilic condition
	Batstone et al. (2002), ADM1 model	• Based on Monod model • Distinction between acid forming bacteria, acetogens and methanogens • In addition, carbohydrate, protein and lipid substrates are described individually • Considers two easily and slowly biodegradable substrate • Considers inhibition of NH_3, hydrogen and pH • Considers physico-chemical reactions

Along with inhibition effects, the finding that AD relied on more than a single group of microorganisms provided the incentive for more complicated models. Hill and Barth (1977) proposed a model for manure based on two microbial populations, acidogens and methanogens, and the inhibition effect of NH_3. Furthermore, VFAs received increased attention as an intermediate but potential inhibitory substance in AD. Hill and Barth (1977) incorporated the inhibitory effect of NH_3 and VFA to model the growth kinetics of methanogens and acidogens. Thomas and Nordstedt (1993) extended the Hill and Barth (1977) model, considering two kinds of substrate, the readily and slowly degradable fraction of the biodegradable solids. A combination of three inhibition factors, NH_3, pH and VFA, resulted in a more comprehensive model produced by Angelidaki et al. (1993) for the thermophilic AD of manure in a continuously stirred tank reactor. This model included four microbial groups, the acid forming bacteria, the propionate degrading acetogens, the butyrate degrading acetogens and the acetoclastic methanogens. Keshtkar et al. (2001) extended this model to batch mesophilic AD, using the stoichiometry and kinetics developed by Angelidaki et al. (1993).

Developed by the International Water Association (IWA) task group for the mathematical modeling of AD, the ADM1 model expanded the modelling of AD degradation from carbohydrate to proteins and lipids (Batstone et al., 2002). The ADM1 model applied a multiple step process including disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis, considering the inhibition function of pH on all microbial groups, and free NH_3 on acetoclastic methanogens. The model also considered inhibition of H_2 on the acetogenic group. However, the ADM1 model is complex with a large number of parameters, considering 24 species and 19 bioconversion processes. All ADM1 model parameters cannot be identified due to inherent difficulty of separating the biomass concentration from the maximum specific uptake rates. In addition, the ADM1 model omits some processes and species, which are applicable in some conditions (Kleerebezem & Van Loosdrecht, 2006).

2.5.Temperature Functions

Temperature has a significant effect on the rate of reactions in AD system. In AD systems, two equations including the Arrhenius and the Square Root were applied to describe the

relationship between temperature and reaction rate. According to the Arrhenius equation (Eq. 2-3), the reaction rate roughly doubles for a temperature increase of 10 °C:

$$K = Ae^{\frac{-E_a}{R^*T}} \qquad (2\text{-}3)$$

where K is the reaction rate, A is a constant, R* is the universal gas constant (0.008314 kJ mol^{-1} K^{-1}), T is temperature (K) and E_a is the activation energy (kJ mol^{-1}).

In most AD models, the Arrhenius equation has been applied to various parameters, such as to the growth rate, μ, (Siegrist et al., 2002; Sinechal et al., 1979; Srisertpol et al., 2010), the maximum growth rate, μ_{max}, (Angelidaki & Ahring, 1993; Hashimoto, 1983), the saturation constant, K_s, (Dague et al., 1998; Siegrist et al., 2002), the hydrolysis rate, K, the death rate, K_d, (Donoso-Bravo et al., 2009; McKinney, 1963; Siegrist et al., 2002; Veeken & Hamelers, 1999), the inhibition constants, K_i, (Siegrist et al., 2002), and the yield coefficient for substrate to biomass, Y, (McKinney, 1963). However and for some studies, the Square Root Equation showed better correlation for the temperature effect than the Arrhenius Equation.

Ratkowsky et al. (1982) proposed the Square Root model, a simpler and purely descriptive model of the evolution of microbial growth rate with temperature. This model indicates a Square Root relationship below optimum temperature adequately describes bacterial growth in pure culture as follow:

$$\sqrt{K} = b(T - T_{min}) \qquad (2\text{-}4)$$

where K is the reaction rate (or growth rate in the case of bacteria) at temperature T (°C), T_{min} is the apparent minimum temperature for growth, and b is regression coefficient.

In a study where granular sludge was adapted to 10 °C during 235 days and where the temperature dependence of acetate conversion (methanogenic activity) was well described by the Arrhenius Equation, several VFAs activities, including propionate and butyrate, were better described by a Square Root Equation (Rebac et al., 1995). However, in another study, the

Arrhenius Equation was a poor predictor of the temperature dependence for the methanogenic activity of a biomass adapted to lower temperatures of 5 to 29 °C (Kettunen & Rintala, 1997).

2.6.Ammonia Volatilization from Anaerobically Digested Manure

Environmentally, the high total ammoniacal nitrogen (TAN or NH_4^+ and NH_3) of AD digestate is an issue as it is susceptible to NH_3 volatilization. Such volatilization contributes to atmospheric loading and deposition of NH_3 causes the eutrophication of natural ecosystems (Hooda et al., 2000), disturbs the nutrient balance of soils and causes nutrient deficiencies. In addition to environmental problems, manure NH_3 volatilization can result in a net loss of TAN, affecting the fertilizer value of AD digestate.

Besides TAN concentration, the two main factors controlling NH_3 volatilization are pH and temperature, simply because they determine the ratio of NH_3 to TAN. The effect of pH on the dissociation of NH_4^+ into NH_3 is described by Eq. (2-5). When the concentration of OH^- exceeds that of H^+ in solution, shift the reaction to the right and releases NH_3 free to be volatized.

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad (2\text{-}5)$$

The ratio of NH_3 to TAN is also affected by temperature as described by Eq. (2-6) and (2-7) (Loehr, 1984; Olofsson, 1975):

$$\frac{NH_3}{TAN} = \frac{1}{(1 + 10^{(pK_a - pH)})} \qquad (2\text{-}6)$$

$$pK_a = 0.09018 + (\frac{2729.92}{T}) \qquad (2\text{-}7)$$

where pK_a is the negative log of the dissociation constant for NH_3 and T is temperature (K).

If the digestate is to be land applied as a soil amendment, other factors may be involved in NH_3 volatilization. For swine manure, Fig. 2-1 produces a chart listing all factors involved (Sommer & Hutchings, 2001).

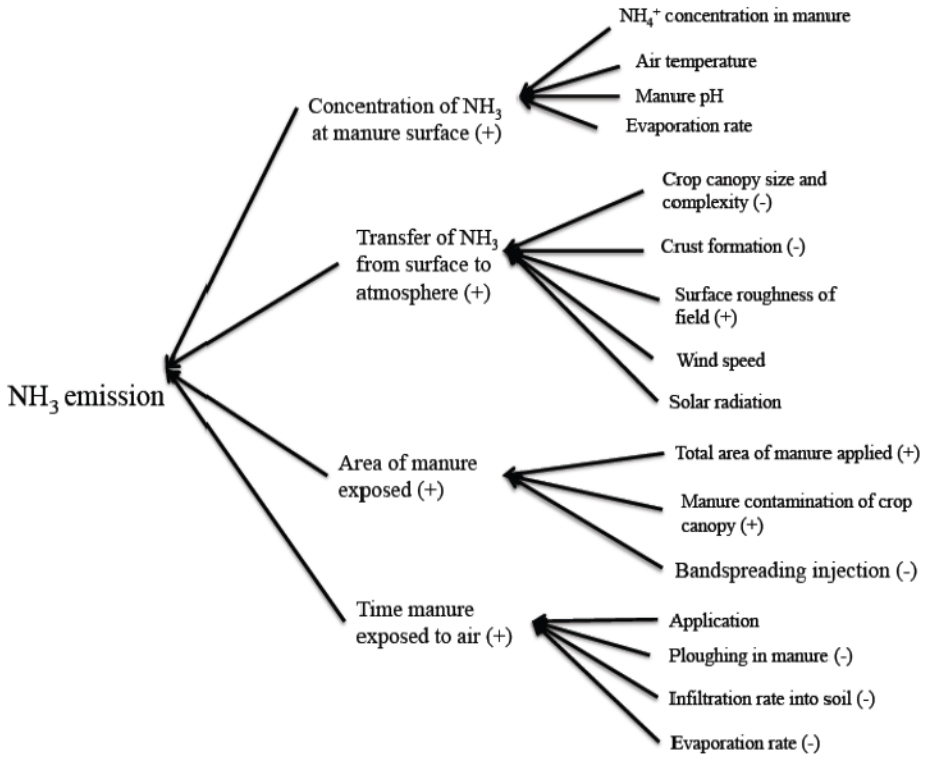

Fig. 2-1: Factors affecting NH_3 volatilization from field-applied manures adapted from Sommer and Hutchings (2001)

The manure TAN has a direct effect on NH_3 volatilization. Reducing protein content of livestock rations lowers manure TAN leading to less NH_3 volatilization during land application (Velthof et al., 2005). There is also a direct relationship between NH_3 volatilization and temperature and solar radiation (Brunke et al., 1988; Moal et al., 1995). Rainfall dilutes TAN and improves soil infiltration, thus reducing the NH_3 volatilization (Klarenbeek & Bruins, 1991).

King et al. (2012) demonstrated that ISPAD digestate can offer a TAN content higher than mesophilic digesters. During the ISPAD process, organic nitrogen is actively mineralized into TAN with very little NH_3 lost through the release of the biogas, as compared to mesophilic digesters.

There are conflicting reports pertaining to the impact of AD on digestate NH_3 volatilization when used as soil amendment. When both ISPAD and conventionally stored swine manure were applied to soils in wind tunnels, the ISPAD manure lost 53 % less TAN than conventionally stored (King et al., 2012). Accordingly, AD may change the manure TS and its particle size, thus improving soil infiltration to reduce NH_3 volatilization or even, offer a different buffering capacity affecting the ratio of NH_3 to TAN (King et al., 2012). This dry matter reduction was also observed by Gutser et al. (2005) along with a more soluble nitrogen fraction, resulting in a higher infiltration of the manure TAN with the liquid fraction into the soil and a reduced NH_3 volatilisation, resulting in higher levels of plant-available TAN in soils amended with digested manures (Gutser et al., 2005). Nevertheless, other research projects demonstrated that AD increases the manure pH, thus increasing risk of NH_3 volatilization (Sommer, 1997; Sommer & Hutchings, 2001).

Techniques are available to reduce NH_3 volatilization during digestate land application such as: dropping close to the ground with disk incorporation (Bless et al., 1991), injection into the ground, and applying during cool or rainy days. Although these methods are relatively economical, they do not control NH_3 volatilization during handling operations (Huijsmans & De Mol, 1999), which can reach 50%. Acidification is another technique to reduce NH_3 volatilization, but this treatment requires 5 kg of 18 M sulphuric acid m^{-3} of swine manure to drop its pH below 6 (Stevens et al., 1992). While NH_3 volatilization is mitigated through this expensive technique, manure composition is changed, risks of odours are introduced, soil pH can be affected and crops can show higher concentrations of sulphur.

2.7.Acidification of Wastewaters during Anaerobic Digestion

The main objective of this research is to use AD acidification to decrease the pH of the ISPAD content one week before emptying, to reduce NH_3 volatilization of its digestate. The acidification of AD systems has been observed and demonstrated to occur especially when microbial growth is disturbed. Since acidogens are faster growing organisms, as compared to methanogens, changes in factors affecting microbial growth rate can lead to acid accumulation. Thus, acidogens activity can surpass that of the methanogens under rapid changes in temperature, organic loading rate (OLR) and hydraulic retention time (HRT) (Demirel &

Yenigün, 2002). Hydraulic retention time can remove microbial populations faster than they can reproduce, thus affecting their growth. Because ISPAD is sequentially fed-batch and its temperature is allowed to fluctuate with that of ambient, only rapid changes in organic loading (OL) can be used for its acidification through imbalances in microbial growth.

Organic load, OL, is defined as the amount of organic matter treated per unit volume of digester. Organic overloading can lead to a high concentration of VFAs as a result of the unbalanced growth between acidogens and methanogens (Ahring et al., 1995). For ISPAD, the acidogen μ_{max} was found to range between 2.9 and 3.7 day^{-1} at 18 °C, as compared to 0.2 to 0.23 day^{-1} for acetoclastic methanogens (Madani-Hosseini et al., 2014c).

There are optimum values in terms of OLR to achieve acidification. In the AD of sugar-beet and beet pulp processing wastewaters, Alkaya and Demirer (2011) reached the highest VFA concentration by increasing OLR from 1.35 to 5.4 g COD l^{-1} d^{-1}, leading to an acidification degree of 46.9%. With maize grains treated in a two-stage AD, namely hydrolysis and acidification, maximum VFA concentrations of 11 400 mg l^{-1} was reached at an OLR of 6.3 kg VSS m^{-3} d^{-1}, dropping the pH from 5.9 to 3.3 after 7 days (Hutňan et al., 2010). However, Ho (2010) showed that increasing the OLR from 2.2 to 6.4 g COD l^{-1} d^{-1} had no effect on lowering pig manure pH due to its high natural buffering capacity. For pig manure under AD, increasing the OLR merely released more TAN and bicarbonate from urea and proteinaceous organics degradation. These products not only increase the pH from 7.3 to 8.1, but also increased the buffering capacity of the manure. However, reducing the pH of high-strength pig manure to pH 5.5 with concentrated hydrochloric acid proved to be effective in increasing VFA concentrations in reactor effluents (Ho, 2010). Kim et al. (2004) accelerated the conversion of synthetic wastewater containing long-chain fatty acids and glucose to VFAs, when the OLR was increased from 1 to 5 g COD l^{-1} d^{-1}. No pH drop resulted from the VFA accumulation because of sufficient initial alkalinity. The acidification of synthetic dairy wastewater dropped from 57.1% to 28.8% when OLR increased from 2 to 30 g COD l^{-1} d^{-1} for both the thermophilic and mesophilic acidogenic reactors, while the optimum OLR for VFA production was 12 g COD l^{-1} d^{-1}. However, the production of ammonium from protein degradation balanced the pH effect of VFA production with the final pH remaining between 6.1 and 6.4 (Yu & Fang, 2002; Yu & Fang, 2001).

The acidification preferences of different components of wastewater are different. Results of batch experiments showed that carbohydrates are preferentially acidified as compared to protein and lipid. For dairy manure, 92–99% of carbohydrates, 59–85% of protein and 12–42% of lipid were acidified (Yu & Fang, 2002; Yu & Fang, 2001). The distribution and concentrations of the four main VFAs (acetic, propionic, n-butyric and n-valeric acids) changed with the OLR. For dairy manure, Ince (1998) showed that acetic and propionic acids were detected up to an OLR of 4 g COD l^{-1} d^{-1}, but n-valeric acid was predominant as OLR increased to 23 g COD l^{-1} d^{-1}, followed by n-butyric, acetic and propionic acids.

Temperature impacts AD acidification. Under mesophilic condition of 37 to 45 °C, more stable acidification products were produced than for thermophilic conditions of 55 to 60 °C. For example, Kozuchowska and Evison (1995) showed that the highest concentration of VFAs for acidification of coffee waste occurred at a temperature of 45 °C. A temperature of 40 °C was found by Dinopoulou et al. (1988) to optimized acidification of beef extract. Maharaj and Elefsiniotis (2001) found that VFA production is even feasible at a low temperature of 8 °C, when diluted primary sludge is enriched with starch rich industrial wastewaters.

Optimum pH for hydrolysis and acidification of complex wastewater organics differs for carbohydrate, protein and lipids. Hydrolysis of carbohydrate generally proceeds favourably at a slightly acidic pH while hydrolysis of protein requires a neutral or weakly alkaline pH (McInerney, 1988). For complex substrates containing a mixture of organic compounds (carbohydrates, proteins, lipid), maximum acidification of synthetic and real complex wastewaters occurred at an optimum pH of 5.5 and 6.3, for mesophilic temperature of 35-37 °C (Kasapgil et al., 1996; Yu & Fang, 2002).

There are controversial results in controlling pH to optimize the acidification process. For example, Yilmaz and Demirer (2008) showed that pH control was not effective in governing acidification for dairy manure. A pH-controlled reactor, set at 5.0–5.5, had a lower acidification degree than the uncontrolled. However, Ho (2010) showed that setting the pH of piggery wastewater to 5.5 effectively increased VFA concentration. Zoetemeyer et al. (1982) studied the effect of pH on acidogenesis of glucose, over the range of 4.5 to 7.9. A pH range of 5.7 to 6 for the acidogenesis improved substrate availability for CH_4 production. However, Elefsiniotis and

Oldham (1994) showed that the rate of VFA production was not affected by pH between 4.3 and 5.2, but at higher pH values of 5.9 to 6.2, VFAs production dropped by 25 to 30%.

2.8.Research Direction

The ultimate objective of the present research project is to develop a management technique for ISPAD, to use its microbial AD communities to acidify its content, one week before emptying, to produce a digestate with a pH of about 6.0, offering a low level of NH_3 volatilization. Maintaining a pH around 6.0 will save the methanogen populations to re-inoculate the next batch treated by the ISPAD system. The literature review presented in this chapter suggests that a robust knowledge of ISPAD processes is required to achieve such an objective. This is justified by the challenges to be faced in acidifying the ISPAD content, where all experimental materials are obtained from a field system treating swine manure. Swine manures in AD are known to offer a strong buffering capacity resulting from the high bicarbonate/carbon dioxide and TAN concentrations. Furthermore, ISPAD systems are fed a low OL especially towards the end of the process, in the range of 0.5 kg VS m^{-3}, thus supporting a low microbial population, which may take some time to grow to levels required to acidify the entire ISPAD content. The time of year preferable for acidification is another issue. Since based on literature, higher and more stable amounts of VFAs are produced at mesophilic temperature; acidification should be conducted when the ISPAD content is operating under a high psychrophilic temperature coinciding with the emptying seasons. This operation is generally carried out before and after the cropping season, namely in May and late October or early November.

To have a robust knowledge of ISPAD processes, the system must be modelled using appropriate kinetic values. Accordingly, the present research will first concentrate on obtaining ISPAD kinetic values and not only comparing such values with that in the existing literature but also investigating changes which may occur if the experimental field ISPAD system is still leading to microbial acclimation. The microbial kinetic of ISPAD differs from conventional mesophilic system because of the following aspects:

I. The ISPAD process operates at temperatures varying with that of ambient and thus, the value of its microbial kinetics must be predicted based on operating temperature;

II. The ISPAD process operates at an OL which decreases over time in terms of volume under AD, for a relatively low OL as of the middle of the batch, as compared to a constant OL for conventional systems;

III. In the ISPAD, the volume under AD increases over time as compared to a fixed volume for conventional systems;

IV. The ISPAD content is not mixed while conventional AD systems are completely mixed.

Because the ISPAD system requires the determination of microbial kinetics based on a wider range of factors as compared to that of conventional AD processes, the model fitting of experimental data requires a more sophisticated technique. Thus, the present work will test the decomposition method which is a technique used in optimization to define the value of interactive coefficients such as AD kinetic coefficients.

The second step is the development of a model predicting ISPAD behaviour. To save time, an existing model will be modified based on ISPAD operating conditions. This model will also be developed to investigate ISPAD content acidification through AD. Therefore, the ISPAD model should consider the followings:

I. Under psychrophilic conditions, homoacetogenesis is important as it converts H_2 and CO_2 to acetate and links the two methanogenic pathways of acetate conversion to CH_4 by acetoclastic methanogens, and CO_2 conversion to CH_4 by hydrogenophilic methanogens;

II. The two main groups of methanogens, acetoclastic and hydrogenotrophic, cannot be lumped as for ISPAD simulation, they respond differently to environmental conditions.

III. The ISPAD process operates at temperatures varying with that of ambient and thus, the temperature function should be introduced in the model to predict the ISPAD system.

IV. For further research on ISPAD acidification, the model should be able to predict some parameters such as pH and VFAs accumulation.

Finally, the third and last step is the determination of conditions which will lead to the acidification of ISPAD, despite the low microbial populations of the system and the high buffering capacity of the wastewaters as a result of their high carbonate and TAN concentration. The identification of effective ISPAD acidification strategies require:

I. the modelling of OL to predict their effect on pH regime over time;
II. the optimization of OL to reach acidiciation;
III. the laboratory validation of such predictions.

Chapter 3. Microbial kinetic for In-Storage-Psychrophilic Anaerobic Digestion (ISPAD)

Connecting Statement

Chapter 2 provided a discussion on different anaerobic digestion models and their detailed specifications. However, In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD), which is the main focus of this thesis, operates under quite different conditions as compared to conventional anaerobic digestions. Because ISPAD is a unique system and its microbial kinetic are still to be discovered, the first step in developing the ISPAD model is to obtain the appropriate kinetic values, which is the focus of this chapter. Specifically, this chapter is designed to produce ISPAD microbial kinetic by fitting the Monod model to results obtained from laboratory substrate activity test (SAT) using ISPAD inoculum and under temperatures of 8, 18 and 35 ºC. Kinetic value fitting was improved by introducing and testing the innovative decomposition method, a technique used in optimization to define the value of interactive coefficients. The prediction of the conventional fitting approach was compared to that of the decomposition approach. Furthermore, the ISPAD model needs to predict microbial activity within a temperatures range. Both the Arrhenius and Square Root Equations were tested for their accuracy in predicting maximum microbial growth rate based on operating temperature.

This chapter is the result of an article published in the Journal of Environmental Management (Madani-Hosseini et al., 2014c). The first contributing author, Mahsa Madani-Hosseini, produced the model, analyzed the data and wrote the article. The second and third contributing authors, Dr. Catherine Mulligan and Dr. Suzelle Barrington, supervised, advised on methods of analysis, and revised the content of the article.

Abstract

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is a wastewater storage tank converted into an anaerobic digestion (AD) system by means of an airtight floating geo-membrane. For process optimization, ISPAD requires modelling with well-established microbial

kinetic coefficients. The present objectives were to: obtain kinetic coefficients for the modelling of ISPAD; compare the prediction of the conventional and decomposition fitting approach, an innovative fitting technique used in other fields of science, and; obtain equations to predict the maximum growth rate (μ_{max}) of microbial communities as a function of temperature. The method consisted in conducting specific Substrate Activity Tests (SAT) using ISPAD inoculum to monitor the rate of degradation of specific substrates at 8, 18 and 35 °C. Microbial kinetic coefficients were obtained by fitting the Monod equations to SAT. The statistical procedure of Least Square Error analysis was used to minimize the Sum of Squared Errors (SSE) between the measured ISPAD experimental data and the Monod equation values. Comparing both fitting methods, the decomposition approach gave higher correlation coefficient (R) for most kinetic values, as compared to the conventional approach. Tested to predict $\mu_{max}$with temperature, the Square Root equation better predicted temperature dependency of both acidogens and propionate degrading acetogens, while the Arrhenius equation better predicted that of methanogens and butyrate degrading acetogens. Increasing temperature from 18 to 35 °C did not affect butyrate degrading acetogens, likely because of their dominance, as demonstrated by microbial population estimation. The estimated ISPAD kinetic coefficients suggest a robust psychrophilic and mesophilic coexisting microbial community demonstrating acclimation to ambient temperature.

Keywords: Psychrophilic anaerobic digestion; Swine manure; Kinetic coefficients

3.1.Introduction

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is the conversion of a standard exterior storage tank into an anaerobic digester by means of an airtight geo-membrane cover collecting biogas (King, 2011). With ISPAD, the tank is gradually filled over an extended period of at least 100 days, thus compensating for its psychrophilic temperature fluctuating with ambient climatic conditions. For ISPAD, microbial acclimation, protein degradation (King et al., 2012; King et al., 2011) and biogas generation were investigated (Giard et al., 2013; Nohra et al., 2003). Nevertheless, process optimization is still required through modelling with well-established microbial kinetic coefficient values, such as maximum microbial growth rate (μ_{max})

and fluctuation with temperature, microbial yield (Y), and substrate half saturation constant (K_s). Microbial kinetic coefficients are useful modelling parameters to evaluate the behaviour of microbial population, the rate of substrate degradation in AD systems, and biogas production (Jiménez et al., 2006). Based on the determination of kinetic coefficients, Nwabanne et al. (2009) concluded that the rate of digestion of a municipal solid waste digester could be corrected through inoculation.

A variety of methods have been used in kinetic parameter identification, because the AD process is characterized by its high complexity and non-linearity. This causes variability in values reported for the kinetic parameters, even when the same operational and environmental conditions have been evaluated (Donoso-Bravo et al., 2011). The mode of operation (e.g., batch vs. continuous), environmental and operational conditions (e.g., pH, temperature, organic load) are other factors which cause kinetic parameters variability (Pavlostathis & Giraldo-Gomez, 1991). The ISPAD system operates at variable temperature with seasons and loading rates, while they are constant in conventional AD systems (Table 3-1). Therefore, the ISPAD requires microbial kinetic values that are not found in the literature.

Table 3-1: Features of ISPAD compared to conventional anaerobic digestion

	Conventional anaerobic systems	**ISPAD**
Temperature	Constant (20, 35 or 55 °C)	Variable with seasons (0 to 25 °C)
Pressure	Ambient or higher	Ambient
Volume	Constant	Continuously increasing
Mixing	Completely mixed or plug flow	No mixing applied
Loading rate	Constant	Decreasing over time
Retention time	5 to 40 days	200 to 365 days

The objectives of this study were to: 1) statistically fit laboratory specific substrate test (SAT) results to that of the Monod equation applied in series to the various AD microbial groups to generate ISPAD kinetic coefficients and microbial densities; 2) compare the prediction

accuracy of the conventional fitting method to that of the decomposition approach, and; 3) compare the prediction accuracy of both the Arrhenius and Square Root equations to describe the relationship between temperature and μ_{max}. The laboratory data used in this project consisted of specific substrate activity tests (SATs) obtained using ISPAD inoculum acclimated to swine manure and individual substrates namely, glucose, propionate, butyrate and acetate, at 8, 18 and 35 ºC. Kinetic parameters were limited to known value ranges. The curve fitting process also yielded microbial population densities, X, for all 4 main degradation groups including acidogen, propionate degrading acetogen, butyrate degrading acetogen, and acetoclastic methaonogen.

3.2.Kinetic Coefficient Determination

A variety of laboratory AD methods are used to obtain data for the computation of microbial kinetic coefficients through statistical fitting. Three different feeding methods are used, namely batch (Donoso-Bravo et al., 2009; Flotats et al., 2003), continuous (Batstone et al., 2009; Bernard et al., 2001) and fed-batch (Redzwan & Banks, 2004; Rodrigues et al., 2003). Two batch tests are commonly used in the determination of AD kinetic coefficients because of their simplicity and short experimental duration: biochemical methane production (BMP) and specific substrate activity test (SAT). The BMP assay is a long-term batch incubation of cultures with periodic biogas and reactor content sampling to provide a temporal profile of substrate consumption and methane production from a known initial concentration of active biomass and substrate (Chynoweth et al., 1993; Shelton & Tiedje, 1984). However, the SAT is a shorter assay measuring the rate of consumption of individual substrates used by one of the main AD microbial groups. Such an assay provides more specific data for the fitting of individual AD process. Donoso-Bravo et al. (2009) used starch, glucose and acetic acid as the main substrates in SAT to obtain kinetic coefficients for hydrolysis, acidogenesis and methanogenesis, respectively. Using SAT, Flotats et al. (2003) also estimated kinetic coefficients for the AD of valerate.

The experimental data is used to estimate kinetic coefficients by changing their value till the model predicts a response corresponding to that obtained experimentally. The selection of an appropriate set of modelling equations is important. In kinetic coefficient estimation, the simple Monod equation is accurate and simple when a limited number of parameters are unknown. For AD, the kinetic values of μ_{max},K_s, Y, and X are interdependent, which makes their estimation

difficult, challenging and tedious. Used in other scientific fields, the decomposition approach is applied in this work as an innovative method of estimating interdependent parameters through data fitting (Bahn et al., 1996; Harjunkoski & Grossmann, 2001). This approach requires the decomposition of the designed algorithms into sub-problems and the solving of individual parameters by data fitting, in order of importance. The advantages of this approach are to: 1) reduce the possibilities of dropping into local minima in the process of optimization; 2) reduce the computation time; and 3) increase the fitting accuracy compared to other approaches (Jiang & Cheng, 2005).

Using the decomposition approach, each sub-problem can be solved through an objective or cost functions, recognized as the most common fitting tool. The most useful cost function is the Sum of Square Error (SSE) (Batstone et al., 2009; Donoso-Bravo et al., 2010; López & Borzacconi, 2010; Noykova & Gyllenberg, 2000) which assumes that the standard deviation of the measurement errors is constant:

$$SSE = \sum_{i=1}^{n} \left(t_i^{obs} - t_i^{pred} \right)^2 \qquad (3\text{-}1)$$

where the observed data, the model-predicted outputs, and the number of data are shown as t_i^{obs}, t_i^{pred}, and n, respectively.

To optimize the cost function, several algorithms were developed, namely grouped under the exact and heuristic methods. An exact method is a mathematical procedure generating a sequence of solutions improving the order of a class of problems. This method uses convexity assumptions for the cost function to obtain the optimized values. The main drawback is the risk of getting trapped in local minima, specifically when the cost function is non-linear. The common approach to solve this trap is to start the search using several randomly selected initial parameters, which is called a multi-start strategy. Solving the problem more quickly, the heuristic methods are multi-start by nature, but are highly sensitive to the initial parameters. Also, the heuristic methods are better at finding an approximate solution when classic methods fail. This is achieved by trading optimality, completeness, accuracy, and/or precision for speed.

Both the exact and heuristic methods were used in AD kinetic determination. Examples for the exact methods are: Levenberge Marquardt (García-Ochoa et al., 1999; Lokshina et al., 2001), Sequential Quadratic Programming (Aceves-Lara et al., 2005; Sales-Cruz & Gani, 2004); Multiple Shooting (López & Borzacconi, 2010; Müller et al., 2002), and Direct Search also called Simplex Algorithm (Haag et al., 2003; Mösche & Jördening, 1999; Simeonov, 1999). Applications of the heuristic methods are: Simulated Annealing (Haag et al., 2003), Genetic Algorithms (Wichern et al., 2009), and Particle Swarm Optimization (Wolf et al., 2008).

The heuristic methods require a certain level of knowledge and experience, making them difficult and expensive to use, besides their sensitivity to initial values and stopping criteria. Consequently, heuristic methods may be more inefficient solutions than the exact methods with their local optima issues.

Because of limitations offered by heuristic methods, this study used an exact method, namely the multi-start strategy, to obtain ISPAD kinetic coefficients. Since the risk of getting trapped in local minima is sensitive to the initialization parameters, the kinetic coefficient obtained for the Keshtkar et al. (2001) model were used. The Keshtkar et al. (2001) model was designed for manure substrates, includes inhibition factors for VFA, pH and free NH_3, and describes a cyclic batch reactor, similar to the ISPAD. This model considers five steps: 1) the hydrolysis of particulate substrate by extracellular enzymes; 2) the consumption of soluble substrates by acid forming bacteria; 3) the consumption of VFA; 4) the formation of acetate by propionate and butyrate degrading acetogens, and finally; 5) the consumption of acetate to generate methane by methanogens.

3.3.Temperature Functions

In AD systems, two equations are generally used to predict temperature dependence, namely the Arrhenius and the Square Root equations. According to the Arrhenius equation (Eq. 3-2), the reaction rate roughly doubles for a temperature increase of 10 °C:

$$K = Ae^{\frac{-Ea}{R^*T}} \qquad (3\text{-}2)$$

where K is the reaction rate, A is a constant, R^* is the universal gas constant (0.008314 $kJ/mol\ K$), T is temperature (K) and E_a is the activation energy (kJ/mol).

The Square Root equation is simpler and purely descriptive of the evolution of microbial growth rate with temperature (Ratkowsky et al., 1982). The Square Root equation describes a less than optimal temperature adaptation of microbial growth in pure culture:

$$\sqrt{K} = b(T - T_{min}) \qquad (3\text{-}3)$$

where K is the reaction rate (or growth rate in the case of microbes) at temperature T (K), T_{min} is the apparent minimum temperature for growth (K), and b is the regression coefficient.

In most AD models, the Arrhenius equation was applied to the growth rate, μ, (Siegrist et al., 2002; Sinechal et al., 1979; Srisertpol et al., 2010), the maximum growth rate, μ_{max}, (Angelidaki & Ahring, 1993; Hashimoto, 1983), the saturation constant, K_s, (Dague et al., 1998; Siegrist et al., 2002), the hydrolysis rate, K, the death rate, K_d, (Donoso-Bravo et al., 2009; McKinney, 1963; Siegrist et al., 2002; Veeken & Hamelers, 1999), the inhibition constants, K_i, (Siegrist et al., 2002), and the yield coefficient from substrate to biomass, Y, (McKinney, 1963). However, the Square Root equation provided a better temperature prediction than the Arrhenius. With granular sludge adapted to 10 °C over 235 days, the temperature dependence for the methanogenic conversion of acetate was well described by the Arrhenius equation, but the Square Root equation better predicted the propionate, butyrate and mixed VFA activities (Rebac et al., 1995). In another study, the Arrhenius equation was a poor predictor of temperature dependence for the methanogenic activity of a biomass adapted to lower temperatures of 5 to 29 °C (Kettunen & Rintala, 1997). Accordingly, both the Arrhenius and Square Root equations will be tested to predict temperature dependence of the maximum growth rate for the AD microbial communities.

3.4.Material and Methods

3.4.1. Inoculum characterization and analytical procedure

In 2004, a full-scale swine manure ISPAD facility was established at Saint-Francois-Xavier in the central region of the Province of Quebec, Canada. This facility consisted of a circular concrete tank measuring 30 m in diameter by 3.66 m in depth, covered with an airtight membrane (GTI, Fredericton, NB, Canada). The tank received manure from the swine facility on a weekly basis and was emptied twice yearly. Manure samples from this ISPAD installation were brought to the laboratory for analysis using standard methods (Eaton & Franson, 2005) and SATs.

Samples were analyzed for solids (total solids, TS; volatile solids, VS; total suspended solids, TSS, and volatile suspended solids, VSS), COD (total and soluble chemical oxygen demand), VFA, anions and cations. Total solids were determined by drying at 103 ºC overnight (VWR, Sheldon Manufacturing, model 1327F, OR, USA). Volatile solids were determined by incineration at 500 °C for two hours (*Barnstead Thermodyne* model 48000, IA, USA). Suspended solids were separated from the supernatant by centrifuging at 1000 rpm for 10 minutes at 4 °C. Chemical oxygen demand was measured using the potassium perchromate method and a spectrophotometer (*Hach* model DR 2800, CO, USA). The pH of all samples was measured using a pH meter (Corning model 450, NY, USA).

Volatile fatty acids (VFAs) (acetic, propionic and butyric acids) were analyzed on a gas chromatograph equipped with a flame ionization detector. Anions (NO_2^-, NO_3^-, PO_4^{3-}, Cl^-) were analyzed using a polymer-based chromatography column, 250 mm × 41 mm OD, (model PRP-X100, Hamilton, NV, USA), on a high-performance liquid chromatograph (model P4000 & AS3000, TSP). Conductivity data were obtained by using a Waters Millipore detector model 432. The parameters were: mobile phase 4.0 mM p-hydroxybenzoic acid, pH 8.5 with 2.5% methanol, 100 µL injection, 1.8 cm^3/min flow rate at 40 °C. Cations (Na^+, NH_4^+, K^+) were similarly analyzed on a cation resin-based chromatography column, 250 mm × 41 mm OD, (model PRP-X200, Hamilton) with: mobile phase 4.0 mM nitric acid with 30% methanol, 20 µL injection, and 1.8 cm^3 /min flow rate at 40 °C.

3.4.2. Computer simulation and statistical fitting method

The experimental data to compute the kinetic parameters was obtained from SATs conducted by King (2011). The SATs were conducted at 8, 18 and 35 °C using ISPAD manure as inoculum. Four individual liquid substrate assays were conducted, where, excluding hydrolysis, each assay applied the substrate used by one of the main AD microbial group: glucose, acetate, propionate and butyrate. Describing the substrate uptake behaviour of the ISPAD microbial communities, this data provided parameters for curve fitting and kinetic coefficient determination (Donoso-Bravo et al., 2009).

Each experimental uptake data set can be fitted mathematically to an AD equation simulating the specific substrate uptake rate such as the Monod equation (Donoso-Bravo et al., 2009; Goudar et al., 1999; Robinson & Tiedje, 1983). For this, the Monod equation (Eq. 3-4 to 3-6) was programmed using Excel (Microsoft 2010), where μ_i, $\frac{dX_i}{dt}$ and $\frac{dS}{dt}$ were computed in steps of short time increment:

$$\mu_i = \mu_{maxi}\frac{S_j}{S_j + K_{si}} \quad \text{i} = \text{A, AP, AB, M} \qquad (3\text{-}4)$$

$$\frac{dX_i}{dt} = (\mu_i - K_{di})X_i \qquad (3\text{-}5)$$

$$\frac{dS_j}{dt} = -\frac{1}{Y_i}\frac{dX_i}{dt} \qquad \text{j} = \text{glu, pr, but, and ac} \qquad (3\text{-}6)$$

where S is the concentration of substrate in mg/L, X is the concentration of active microbial biomass in mg/L, Y is the yield of microbial biomass from the substrate in mg biomass/mg substrate, K_s is the half-saturation constant in mg substrate/L, K_d is the bacterial decay constant which is considered 5% of μ_{max}, j represents the substrates of glucose (glu), propionate (pr), butyrate (but), and acetate (ac), and A, AP, AB, and M represent acidogens, propionate degrading acetogens, butyrate degrading acetogens, and acetoclastic methanogens, respectively.

As the kinetic values of μ_{max}, K_s, Y, and X are interdependent, the decomposition approach was used and kinetic coefficients were determined in order of importance. Since the exact method used can produce local minima traps, the fitting process was initiated by computing microbial population X values using kinetic coefficients obtain by the Keshtkar et al. (2001) model at 35 °C (Table 3-2).

Table 3-2: Kinetic coefficient used as initial values (Keshtkar et al., 2001)

Process	Parameter	Unit	Value
1. Acidogenesis	μ_{maxA}	$1/d$	5.0
	K_{sglu}	mg/L	500
	Y_A	mg/mg	0.077
2. Propionate degrading acetogenic	μ_{maxAP}	$1/d$	0.54
	K_{spr}	mg/L	259
	Y_{AP}	mg/mg	0.094
3. Butyrate degrading acetogenic	μ_{maxAB}	$1/d$	0.68
	K_{Sbut}	mg/L	176
	Y_{AB}	mg/mg	0.083
4. Methanogenesis	μ_{maxM}	$1/d$	0.60
	K_{sac}	mg/L	120
	Y_M	mg/mg	0.04

Microbial population densities, X, are critical in determining ISPAD kinetic coefficients (Goudar et al., 1999; Rebac et al., 1999), especially considering their lack of reference in the literature. The fitted X value pertained to acidogens, propionate degrading acetogens, butyrate degrading acetogens, and acetoclastic methanogens. Once the X values were established, the kinetic coefficients were fitted for 35 °C, using the Monod equation results and boundary values for μ_{max}, K_s, and Y based on ADM1 (Batstone et al., 2002) (Table 3-3). Also, the 35 °C values for Y were presumed to apply to 18 and 8 °C conditions because of weak sensitivity to temperature. The 35 °C values for X and Y were used to fit the 18 and 8 °C data. The Microsoft solver (Microsoft 2010) was used to minimizing the SSE between calculated and experimental values. To assess the effectiveness of the decomposition approach, the fitting process was also performed using the conventional approach of fitting all kinetic parameters of μ_{max}, K_s, Y, and X simultaneously at 35 °C, and then using the optimized X and Y values for the fitting of μ_{max} and K_s at 8 and 18 °C.

Since the difference between the experimental substrate uptake data and the modelled values produced smooth nonlinear graphs, the Generalized Reduced Gradient algorithms (GRG) of the Excel Microsoft solver were used to reduce the likelihood of local minimum traps. An example of such a trap is the convergence defining the algorithm stopping criterion which is the amount of relative change specified in the last five iterations. To further reduce the likelihood of local minimum traps, the following features in GRG were enabled: 1) "multistart" for repeated run starting with specific decision variable values; 2) "random seeds" where GRG generates candidate starting points, and; 3) forward and central "derivatives" to find the optimal trajectory for further iterations.

Table 3-3: ISPAD kinetic coefficients at 8, 18 and 35 °C

Process	Parameter	Unit	Range of reported data (Batstone et al., 2002)	Value 8 °C	18 °C	35 °C
1. Acidogenesis	μ_{maxA}	$1/d$	0.4-21.12	0.64	2.90	6.40
	K_{sglu}	mg/L	22-1280	219.20	167.64	140.20
	Y_A	mg/mg	0.01-0.17	0.123	0.123	0.123
	X_A	mg/L	ND[1]	7.54	7.54	7.54
2. Propionate degrading acetogenesis	μ_{maxAP}	$1/d$	0-1.64	0.011	0.063	0.12
	K_{spr}	mg/L	20-1146	392.00	163.70	100.50
	Y_{AP}	mg/mg	0.019-0.089	0.053	0.053	0.053
	X_{AP}	mg/L	ND	18.32	18.32	18.32
3. Butyrate degrading acetogenesis	μ_{maxAB}	$1/d$	0.021-2.64	0.023	0.22	0.23
	K_{sbut}	mg/L	12-450	411.38	450	450
	Y_{AB}	mg/mg	0.026-0.079	0.034	0.034	0.034
	X_{AB}	mg/L	ND	85.96	85.96	85.96
4. Methanogenesis	μ_{maxM}	$1/d$	0.0192-1.2	0.045	0.20	0.40
	K_{sac}	mg/L	11-930	533.77	213.30	193.33
	Y_M	mg/mg	0.014-0.076	0.019	0.019	0.019
	X_M	mg/L	ND	23.59	23.59	23.59

Note: No Data

The trade-off between the accuracy of the solution, the optimal values of kinetic coefficients, and the time and difficulty level of the algorithm is an important issue. Specifically, central derivatives were used because their approach is more accurate when changing rapidly at the current point, but this operation requires more recalculations. The algorithm was allowed to

use the multistart method set at 10^{-3} for the convergence feature. Finally, population size was set at 20 and 3 random seeds.

To compare the temperature prediction accuracy of the Arrhenius and the Square-Root equations for μ_{max}, theses models were fitted with the obtained μ_{max} values (Table 3-3). The initially used coefficients of E_a and T_{min} were based on the literature (El-Mashad et al., 2005; Finster, 2008). Parameters of both models were then optimized using GRG algorithm, minimizing SSE between the calculated and the experimental values. The prediction accuracy of two equations was then compared by the Mean Absolute Percentage Error (MAPE), the Fractional Bias (FB), the Root Mean Square Error (RMSE), the Normalized Mean Square Error (NMSE), and the Coefficient of Determination (R^2) (Kusiak & Wei, 2012).

3.5.Results and Discussion

3.5.1. Characteristics of ISPAD manure

The analytical results of analyses performed on the ISPAD manure are presented in Table 3-4.

Table 3-4: Characteristics of the experimental manure (King, 2011)

Characteristic	Unit	Fresh manure	ISPAD manure
Solids			
TS	g/L	48.01	38.71
VS	g/L	34.34	25.40
FS	g/L	13.63	13.31
VSS	g/L	27.38	24.01
VDS	g/L	6.96	1.38
pH	-	6.90	7.46
COD			
Total	g/gVS	2.43	1.99
Soluble	g/gVS	0.88	0.08
VFA			
Acetic	mg/gVS	142.04	0.33
Propionic	mg/gVS	60.31	0.00
Butyric	mg/gVS	40.69	0.00
Anions			
Cl^-	mg/gVS	33.81	21.33
NO_2^-	mg/gVS	2.93	0.04
NO_3^-	mg/gVS	0.00	0.00
PO_4^{3-}	mg/gVS	15.61	6.94

SO_4^{2-}	mg/gVS	0.00	17.38
Cations			
Na^+	mg/gVS	19.19	13.43
NH_4^+	mg/gVS	108.88	79.16
K^+	mg/gVS	70.23	34.51
ATP			
ATP	mg/gVS	12	16.7
Active	%VSS	0.4-1.2	0.5-1.6

3.5.2. The experimental substrate consumption rate

For temperatures of 8, 18, and 35 ^{0}C, the substrate consumption rate data obtained from SAT assays can be classified into three types: exponential, linear and linear-exponential curves. The glucose activity curves at temperatures 8, 18 and 35 ^{0}C, showed a smooth exponential trend with an early low uptake rate followed by a higher rate associated with microbial growth. The curve shape is stretched out in time as temperature decreases, showing that acidogens consumed glucose faster at higher temperatures. The propionate consumption rate at 8 ^{0}C was linear compared to exponential at 18 and 35 ^{0}C (Fig 3-1). This linear 8 ^{0}C curve resulted from either a slow growth at low temperatures (Arbeli et al., 2006; McHugh et al., 2004; Öztürk, 1993) or inhibition by acetate and butyrate (Vavilin & Lokshina, 1996). Methane production from propionate is known to be slower than that from butyrate and acetate, because of its thermodynamically unfavourable AD process (Gijzen et al., 1988). Propionate consumption doubled by increasing the temperature from 18 to 35 ^{0}C. As opposed to other substrates in Fig. 3-1, butyrate consuming acetogens demonstrated a linear trend for 8, 18 and 35 ^{0}C, with the curve slope increasing especially between 8 and 18 ^{0}C. It was demonstrated that low temperatures of 3-9 ^{0}C favour the degradation of butyrate over propionate (Nozhevnikova et al., 2000). There was no important increase in butyrate consumption rate between 18 and 35 ^{0}C, indicating a lack of microbial temperature sensitiveness. Finally, the strong slope of the 8 ^{0}C linear curve, as opposed to propionate, suggests a robust butyrate-consuming acetogen population with good growth. Accordingly, butyrate did not accumulate at low temperatures as opposed to propionate, as also found by Langenhoff and Stuckey (2000). Acetate consumption curves were linear-exponential at 8, 18 and 35 ^{0}C but of a slope similar to that of butyrate. Furthermore, the curve slope

increased with temperature confirming the change in biomass activity, despite the fact that their linear-exponential trend reflected limited growth.

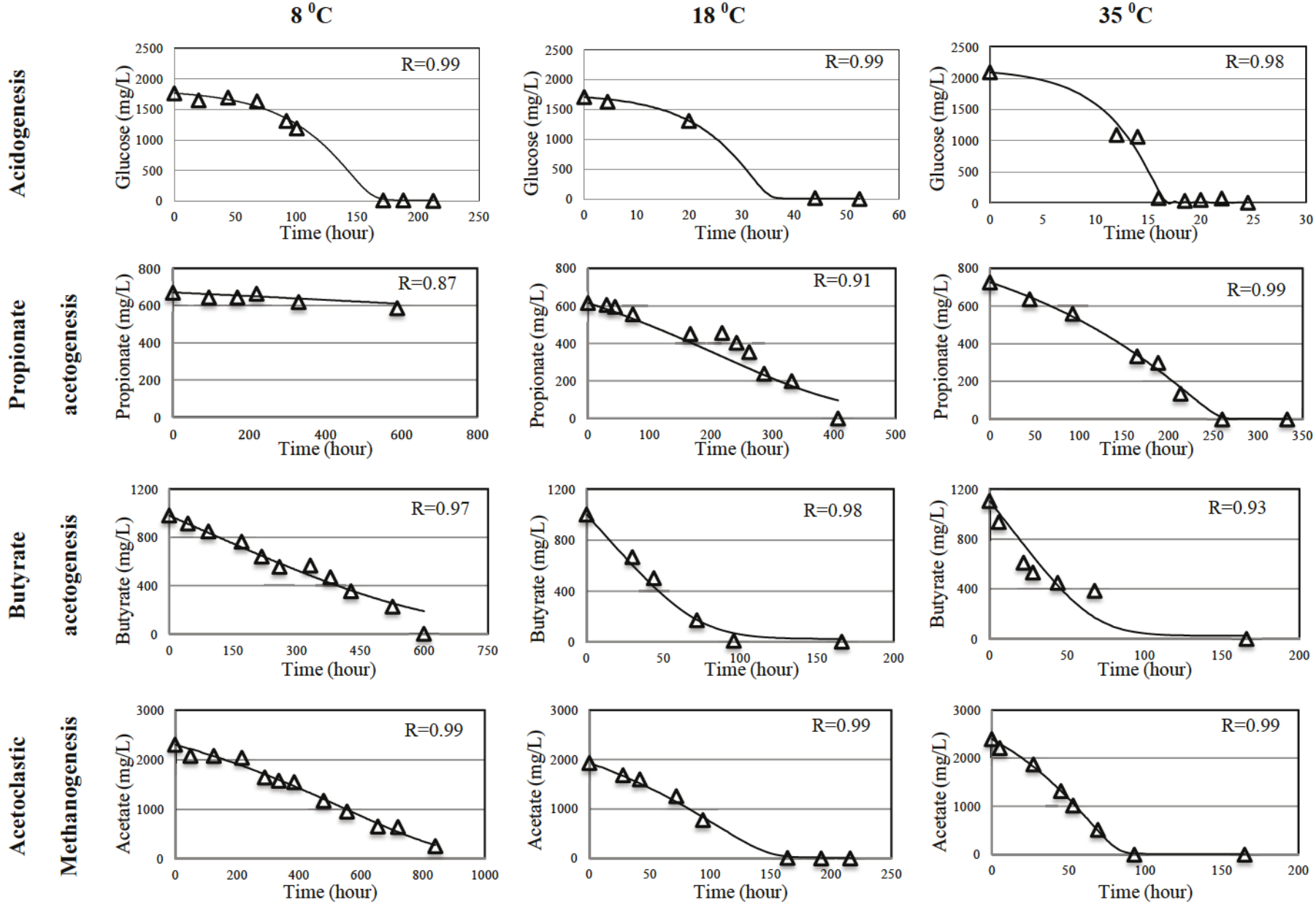

Fig. 3-1: Glucose, propionate, butyrate and acetate uptake rate by ISPAD biomass using decomposition approach: experimental data (Δ), and model prediction (-).

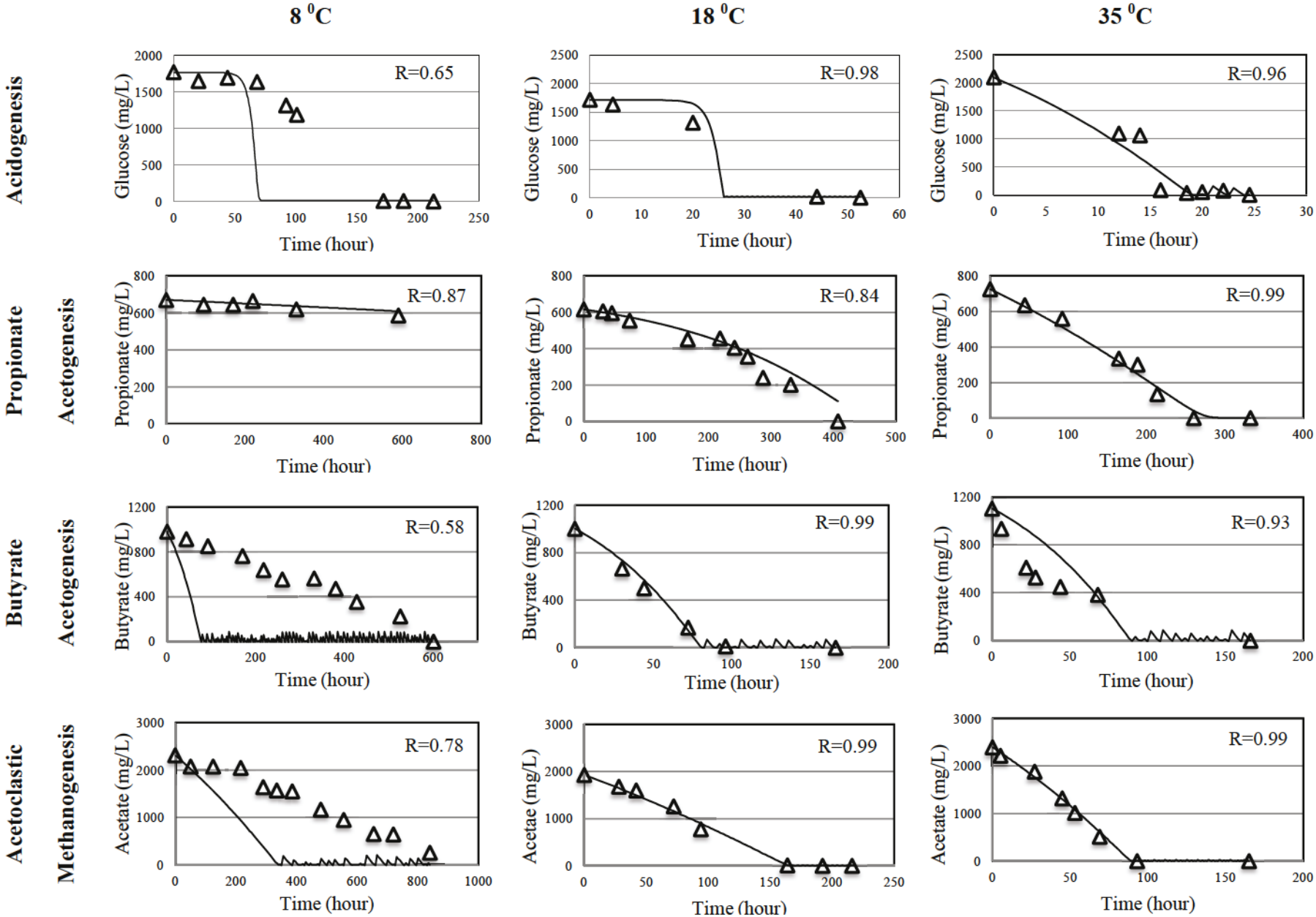

Fig. 3-2: Glucose, propionate, butyrate and acetate uptake rate by ISPAD biomass using non-decomposition approach: experimental data (Δ), and moc prediction (-).

3.5.3. The decomposition versus the conventional fitting approach

Using the decomposition approach, the curve fitting method produced reliable kinetic coefficients, with a goodness of fit represented by a Correlation Coefficients (R) for non-linear curves (Ting & Shiqiang, 2011), ranging from 0.87 to 0.99 (Fig. 3-1). However, the conventional approach produced R values ranging from 0.58 to 0.99 (Fig. 3-2). Both approaches produced similar R values for the uptake rate curves such as for propionate, butyrate, and acetate curves at 35 ^{0}C, but, for other curves, the R value for the conventional approach was lower. For example, the R value for glucose, butyrate, and acetate curves at 8 ^{0}C are respectively 52, 67, and 26% higher if the fitting process is performed using the decomposition approach. The goodness of fit was also compared using the Mean Squared Error (MSE), and the Mean Absolute Error (MAE) besides the R value for all steps at all 3 temperatures (Table 3-5).

Table 3-1: Comparison of conventional and decomposition approaches

Process	Approach	R			MAE			MSE		
		8 °C	**18 °C**	**35 °C**	**8 °C**	**18 °C**	**35 °C**	**8 °C**	**18 °C**	**35 °C**
1. Acidogenesis	Decomposition	0.99	0.99	0.98	38	13	84	2488	496	14301
	Conventional	0.65	0.98	0.96	448	88	125	542071	24072	37076
2. Propionate degrading acetogenesis	Decomposition	0.87	0.91	0.99	14	67	58	297	7493	6443
	Conventional	0.87	0.84	0.99	14	63	26	284	12659	1097
3. Butyrate degrading acetogenesis	Decomposition	0.97	0.98	0.93	41	62	188	4192	6886	63914
	Conventional	0.58	0.99	0.93	409	30	348	233745	1839	178186
4. Methanogenesis	Decomposition	0.99	0.99	0.99	62	47	28	6958	4553	1608
	Conventional	0.78	0.99	0.99	795	55	46	974922	5572	3829

For the decomposition approach, both MSE and MAE were lower as compared to the conventional approach, especially at 8 ^{0}C, compared to the conventional approach. The conventional approach was slightly more accurate at predicting propionate degrading acetogenesis at both 18 and 35 ^{0}C, and butyrate degrading acetogenesis at 18 ^{0}C. In general, the

conventional approach may result in a drop of the local minima in finding different kinetic parameters. Rather, the decomposition approach offers advantages in terms of improving data fitting.

3.5.4. Maximum growth rate coefficient and temperature effect

Acidogens, propionate degrading acetogens, butyrate degrading acetogens and methanogens demonstrated a μ_{max} decreasing by a factor of 10, 10.9, 10 and 8.8 from 35 to 8 ^{0}C, respectively (Table 3-3). For all microbial groups, temperature and μ_{max} were positively correlated due to activation of enzymatic reactions (Kayranli & Ugurlu, 2011).

Growing faster than the other AD microbial groups (Kashyap et al., 2003), the acidogens (glucose degrading bacteria) exhibited the fastest μ_{max} at all temperatures, followed by the methanogens and the butyrate degrading acetogens, and then, with the slowest growth rate, the propionate degrading acetogens. At 35 ^{0}C, the acidogens demonstrated a μ_{max} of 6.40 1/d as compared to the propionate and butyrate acetogens with a μ_{max} of 0.12 and 0.23 1/d, respectively. Methanogens demonstrated a μ_{max} of 0.40 1/d at 35 ^{0}C. When temperature increased from 18 to 35 ^{0}C, there was no increase in μ_{max} for butyrate consuming bacteria, but by increasing the temperature from 8 to 18 ^{0}C, μ_{max} increased by a factor of 10. To predict the impact of temperature on μ_{max} for each stage of AD, both the Arrhenius and the Square Root equations were tested (Table 3-6) for accuracy through their R^2 values and the statistical parameters MAPE, RMSE, NMSE and FB.

Table 1-2: Prediction accuracy of Arrhenius and Square Root equations for μ_{max}

Process	Model	R^2	MAPE	RMSE	NMSE	FB	E_a (kJ/mol)	T_{min} (K)
1. Acidogenesis	Arrhenius	0.95	0.39	0.019	0.014	0.041	43.42	-
	Square root	0.97	0.26	0.014	0.007	0.021	-	262.94
2. Propionate degrading acetogenesis	Arrhenius	0.92	0.59	0.0005	0.026	0.061	39.62	-
	Square root	0.94	0.44	0.0004	0.016	0.032	-	260.54
3. Butyrate degrading acetogenesis	Arrhenius	0.97	0.34	0.0005	0.009	0.13	42.38	-
	Square root	0.91	0.59	0.001	0.031	0.24	-	251.90
4. Methanogenesis	Arrhenius	0.95	0.46	0.001	0.016	0.11	38.07	-
	Square root	0.92	0.54	0.001	0.024	0.18	-	250.67

The FB is a nonlinear operator, which is used to represent the relative difference between model and experimental data. It varies between +2 for extreme under prediction, to -2 for extreme over prediction. The other statistical estimators of MAPE, RMSE, and NMSE represent the overall deviation between experimental data and model results.

For the Arrhenius equation, the optimized E_a values for each step of the AD process were in the range of 38.07-43.42 kJ/mol, as reported in the literature (Ngozi-Olehi et al., 2010). For the Square Root equation, the optimized T_{min} was in the range of 250-262 K (Table 3-6) for psychrophilic organisms (Bowman, 2001). For acidogens, despite close R^2 values of 0.95 and 0.97, the Square-Root equation provided a better prediction, as compared to that of Arrhenius, because of lower FB, MAPE and NMSE. King (2011) showed that ISPAD acidogen growth rate versus temperature did not obey the Arrhenius equation, because of coexisting psychrophilic and acclimated mesophilic population. Similarly for acetogens consuming propionate (Table 3-6) and although both the Square Root and Arrhenius equations gave a close R^2 of 0.94 and 0.92 respectively, the former shows a better prediction, because of a lower FB. The butyrate degrading acetogens showed a different behaviour, with a μ_{max} better estimated by the Arrhenius equation, because of its higher R^2 and lower FB values (Table 3-6). Finally for the methanogens consuming acetate, R^2 for both the Arrhenius and Square-Root equations were close, but the Arrhenius equation provided a better prediction with a lower FB value.

3.5.5. Apparent half-saturation coefficient and temperature effect

The apparent half-saturation constant, K_s, indicates the concentration at which the microbial group is able to process the substrate at half of its maximum growth rate. It also represents the affinity of the microbial population for the substrate. A relationship was observed between temperature and K_s for all substrate consumption, except for butyrate degrading acetogenesis (Table 3-3), confirming that K_s increases at low temperatures (Lawrence & McCarty, 1969; Lin et al., 1987; Nedwell, 1999). Taken up by a form of temperature sensitive active transport mechanism, the substrate is likely to become increasingly less available at lower temperatures. Also the ability of microbes to sequester the substrate declines as temperature dropped (Nedwell, 1999). Also as temperature dropped, viscosity within the cell membrane

increases reducing the effectiveness of substrate transport during metabolism and a minimum is reached at temperatures solidifying the membrane lipids.

The K_s for the acidogens remained within a range of 140-219 mg/L, but increased slightly with lower temperatures.

Temperature had a variable impact on K_s for propionate and butyrate degrading acetogenesis. Specifically, propionate degrading acetogens showed a K_s substantially larger of 392 mg/L at 8 ^{0}C as compared to that of 100 mg/L at 35 ^{0}C. However, K_s increased slightly from 8 to 18 ^{0}C and did not change from 18 to 35 ^{0}C for butyrate degrading acetogens. For butyrate consuming acetogens, K_s increased with temperature from 8 to 18 ^{0}C, as opposed to acidogens, propionate degrading acetogens, and methanogens. For methanogens, K_s decreased by a factor of 2.7 when the temperature dropped from 35 to 8 ^{0}C. Westermann et al. (1989) also observed a drop of substrate affinity for methanogens when temperature decreased. Methanogens are clearly less temperature dependent when substrate concentration is reduced to sub-saturating levels.

The propionate degrading acetogens showed the lowest $K_s$value of 100.2 mg/L at 35 ^{0}C indicating their highest affinity to substrate (Table 3-3). However, methanogens showed the lowest desire to consume substrate with K_s of 533.8 mg/L at 8 ^{0}C. A high K_s for methanogens indicated that methanogenic growth might not be sensitive to low concentrations of acetate (Chen, 2010).

3.5.6. Biomass density and temperature effect on yield coefficient

The anaerobic process generally exhibits a yield coefficient Y varying from 0.01 to 0.17 mg of biomass produced per mg of substrate consumed (Table 3-3), compared to the values determined in this work of 0.019 to 0.123. As for Jia et al. (1996), acidogens had the highest Y of 0.123 mg of biomass produced per mg of substrate consumed, indicating their higher microbial biomass production for each gram of substrate consumed and their lower sensitivity to the effect of pH and substrate concentration (Beccari et al., 1996; Lin & Chen, 1999; Shin et al., 1995). The propionate and butyrate degrading acetogens exhibited a respective Y of 0.053 and 0.034 while the methanogens exhibited a yield of 0.019 mg of biomass produced per mg of substrate consumed.

The biomass density of each AD groups lacks documentation because of difficulties in measuring individual populations. In this project, the decomposition effect was used to determine the concentration of each microbial population X by minimizing SSE between the experimental data and the selected model (Haag et al., 2003). The relative density among AD microbial population as found in the literature (Kalyuzhnyi, 1997; Torre & Stephanppoulos, 1986) indicates that 50% of the total biomass is generally associated with the acidogens (Torre & Stephanppoulos, 1986). However for the present ISPAD inoculum, acidogens and propionate degrading bacteria represented only 5.4 and 13.5 % of total population while the methanogens and the butyrate degrading bacteria made up 17.4 and 63.5 % of the AD biomass. The different ISPAD biomass distribution, as compared to the literature, resulted from the fact that the ISPAD inoculum had not been fed for over 60 days, when exposed to the SAT. Accordingly, the AD groups demonstrating the highest populations were those associated with the substrate remaining to be degraded, such as butyrate.

3.6.Conclusions

The concept of In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is a fed-batch anaerobic system with undefined kinetic coefficients. For temperatures of 8, 18 and 35 °C covering the range of ISPAD operating conditions, the objective of this study was therefore to obtain kinetic coefficients namely μ_{max}, K_s and Y, to test the innovative decomposition approach to obtain a more accurate fit, and to establish a temperature function for the maximum growth coefficient corresponding to each AD microbial consortium. Microbial population densities were also calculated to determine methane production potentials under various ambient temperatures. The kinetic coefficients were obtained by fitting laboratory Substrate Activity Test (SAT) results to the Monod model.

The kinetic coefficients obtained indicated that, at a low temperature of 8 °C:

1) except for the acidogens demonstrating an initial lag phase, the organisms consuming VFAs exhibited limited growth compared to temperatures of 18 and 35 °C;
2) the propionate-consuming organisms at 8 °C had not acclimated as much as the other organisms within the AD community and showed almost no degradation resulting in the accumulation of propionate;

3) the butyrate degrading acidogens did demonstrate acclimation, with a linear consumption rate even at 8 °C.

Also, among organisms and temperatures, the kinetic coefficients indicated that:

1) acidogens were acclimated even at 8 °C, degrading glucose at an exponential rate increasing with temperature;
2) propionate degrading acetogens exhibited activity at 18 and 35 °C, but very little at 8 °C;
3) butyrate degrading acetogens exhibited activity at 8 °C, increasing with temperatures;
4) methanogens exhibited a linear consumption rate for acetate at 8 °C, and an exponential rate at 35 °C, suggesting the existence of two groups of methanogens, one acclimated to cold conditions and another remaining mesophilic.

To predict the impact of temperature on μ_{max} for each ISPAD microorganisms group, the Square Root equation performed better for both acidogens and propionate degrading acetogens, while the Arrhenius equation performed better for methanogens and butyrate degrading acetogens.

3.7.Acknowledgement

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Chapter 4. In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) Process. Part I: Model Development and Calibration

Connecting Statement

Based on the microbial kinetics established in Chapter 3, this chapter develops a mathematical model to predict ISPAD microbial activity, CH_4 production and content pH, under the specificity of the ISPAD system. The ISPAD system has a microbial population acclimated to low psychrophilic temperatures, exposed to variable digestion temperatures and sequentially fed-batch on a regular basis. Developed for the mesophilic treatment of manure under batch systems, the Keshtkar et al. (2001) model was used as basis for the development of the ISPAD model. Specifically, the Simulink/Matlab (MathWorks, 2012a) software was used to operate the ISPAD model and calculate the sensitivity of the various kinetic parameters in terms of CH_4 production. To calibrate the model, laboratory data was obtained from batch tests where ISPAD inoculum was fed glucose, and the glucose and VFAs concentrations, and pH changes were monitored along with CH_4 production to assess the accuracy of the proposed model.

This chapter has produced a manuscript which is currently under review by the Journal of Environmental Management and Sustainable Development. The first contributing author, Mahsa Madani-Hosseini, designed the experiments, conducted the laboratory work, analyzed the data and wrote the article. The second and third contributing authors, Dr. Catherine Mulligan and Dr. Suzelle Barrington, supervised, advised on the experimental design and methods of analysis, and revised the content of the article.

Abstract

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is a treatment system applicable to wastewaters stored for over 100 days, such as livestock wastes and municipal sludge. The ISPAD system differs from conventional reactors by being a sequentially fed-batch operating at a temperature fluctuating with ambient. Operated for more than 10 years in the Drummondville area of Eastern Canada, a field ISPAD system was found to host a microbial community acclimated to low temperatures. The objective of this study was to develop a mathematical

model to simulate the ISPAD process based on that of Keshtkar et al. (2001), verify the value of its microbial kinetics, and to simulate the pH changes of its content along with its methane (CH_4) production. Furthermore, the values of the ISPAD microbial kinetics were compared to that of previous years to check for further acclimation to psychrophilic conditions. Simulation of ISPAD was achieved using the Simulink/Matlab software. The model was calibrated using laboratory data obtained from batch experiments using 7-year-old ISPAD inoculum, and glucose as substrate, and where glucose, VFAs and pH changes were monitored along with biogas production. The ISPAD model showed good agreement with the experimental data representing the system behaviour between 4 and 35 ºC. Although microbial activity at 4 °C was much slower than that at 18 and 35 ºC, it showed acclimation to low temperatures. Furthermore, comparison of microbial kineti values over 3 years of field ISPAD monitoring demonstrated population acclimation, especially for the methanogens.

Keywords: Anaerobic process; Biogas; Modelling; Kinetic parameters; Psychrophilic

4.1.Introduction

Anaerobic digestion (AD) is considered a sustainable treatment for all organic wastes because it produced an energy rich biogas while capturing emissions of methane (CH_4), a greenhouse gases. Nevertheless, AD is generally practiced when subsidized because of the many issues associated with its process. Generally operated under mesophilic conditions, heating is required under cold climatic conditions for a negative energy balance. For many applications, the biogas must be scrubbed to remove corrosive agents and concentrate CH_4. CH_4 is difficult to transport unless compressed into a liquid at high pressures. Electrical conversion of the biogas is inefficient at 35 to 40%, increasing its energy cost. Investments associated with AD are significant enough to require a large and regular input of organic waste.

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) was developed to eliminate some of the issues associated with AD. Consisting of an airtight cover installed over a storage facility, the ISPAD concept makes use of existing structures to reduce the cost of the reactor. Operating at psychrophilic temperatures fluctuating with that of ambient, ISPAD is a slower process compared to conventional mesophilic systems, which is compensated by the long storage period of over 100 days. Thus, ISPAD is managed as a sequentially fed batch system, where organic

waste is regularly added over the storage period, until the system is filled. At that time, the treated waste is removed except for a limited amount left as inoculant for the subsequent batch. The system is operated at ambient temperatures, thus requiring no heating under cold climatic conditions. Furthermore, its low operating temperature and feeding rate make it extremely stable, thus requiring little technical supervision. Because it uses existing storage facilities, ISPAD is a feasible treatment for operations producing small quantities of organic waste. Finally, ISPAD limits ammonia (NH_3) volatilization and odour emissions of the organic waste while in storage.

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) was successfully used to treat swine manures in the Eastern Canada. For a system built in 2004 in the Drummondville area of Eastern Canada, its monitoring demonstrated the effective reduction of swine manure volatile solids and the released 63% of its total CH_4 potential (King et al., 2011). Its microbial population was found to be acclimated to low psychrophilic conditions (King et al., 2011), and to be capable of generating biogas even under cold winter conditions (Giard, 2011; Nohra et al., 2003). Operated at temperatures under 20 °C, ISPAD biogas was found to contain negligible amounts of NH_3, as compared to mesophilic systems (King et al., 2012).

To further develop the ISPAD system and use its full potential, modelling and simulation are required. For example, the acidification of ISPAD content to a pH of 6.0 just before emptying for land application would reduce NH_3 volatilization and odour emissions from its digestate, while still maintaining a methanogen population capable of inoculating the next batch. Anaerobic digestion (AD) is such a complex process that only mathematical models can predict the outcomes under specific conditions.

Therefore, the main objectives of this project were to: develop a comprehensive model to predict ISPAD biogas production, substrate consumption, and pH evolution under its operating temperature ranging from 4 to 35 °C, and monitor the microbial acclimation of a field ISPAD system established in 2004 and sampled in 2009 and 2012 to inoculate laboratory batch experiments. Several already developed AD models can serve as a base to produce the ISPAD model to concentrate the present research work on including the specificity of the ISPAD operating conditons. The base model must nevertheless be designed to reflect the needs of ISPAD management practices, such as pH regime and effect of temperature change on microbial

kinetics. The present ISPAD model excluded the hydrolysis step as it represents a process as complex as that of AD from the glucose stage.

4.2.Selecting the Most Appropriate Base for the ISPAD Model

In selecting a base to develop the ISPAD model, several existing models where examined, such as the ADM1 (Batstone et al., 2002), the Hill model (Hill, 1982) and the Keshtkar et al. (2001) model. The IWA Task Group developed a complex model (ADM1) describing the dynamics of 24 species and 19 bioconversion processes (Batstone et al., 2002). Parameter values are provided for common AD conditons, which simplifies the determination of values for all species and processes. Nevertheless, when applying the ADM1 model to a non standard application, extensive laboratory work must be conducted to establish the kinetic values of all 24 species and the process values of all 19 bioconversions. Considering ISPAD conditions, the ADM1 model neglects some processes and species having a significant impact at low temperatures (Donoso-Bravo et al., 2011) such as the homoacetogenesis step. Homoacetogenesis converts hydrogen and carbon dioxide to acetate and links the two methanogenic pathways of acetate conversion to CH_4 by acetoclastic methanogens and carbon dioxide conversion to CH_4 by hydrogenophilic methanogens (Kotsyurbenko, 2005). The ADM1 model was therefore considered too complex to serve as model base.

Working especially with dairy manures, Hill (1982) developed an AD model which included the homoacetogensis step and operated under mesophilic and thermophilic temperatures. Tested at low temperatures for the treatment of swine manure, the model could not reasonably predict the degradation process, because it failed for higher organic loads for swine manure with high degradability and production of large amounts of acids and NH_3, compounds generated in large quantities by the swine manure as compared to dairy manures (Hill et al., 2001).

The psychrophilic AD model developed by Massé and Droste (2000) applies to a sequencing batch reactor, whereas ISPAD is a sequentially fed batch system. Furthermore, homoacetogenesis is not considered. In modelling low temperature AD system,Vavilin et al. (1998) demonstrated that the homoacetogenesis step was important. Also, Vavilin et al. (1998) considered only pH inhibition while NH_3 and VFA inhibition is also important (Angelidaki et al., 1993).

Developed for the batch treatment of livestock manures, the Keshtkar et al. (2001) model considers a limited number of parameters to be defined experimentally, while still considering the main AD inhibitors such as pH, NH_3, and acetate. The model is also capable of predicting the pH regime, an element essential to the modelling of the ISPAD process. Because of these capabilities, the Keshtkar et al. (2001) model was used as a base for the development of the ISPAD model.

Nevertheless, the Keshtkar et al. (2001) model must be modified to properly predict the ISPAD process. First of all, it lumps the two main groups of methanogens, acetoclastic and hydrogenotrophic, whereas for ISPAD simulation, these two main groups must be differentiated. They respond differently to environmental conditions and differ in terms of substrate consumption, namely acetate and carbon dioxide respectively. The Keshtkar et al. (2001) model also predicts CH_4 production at mesophilic temperature without considering the homoacetogenesis step, which is the conversion of carbon dioxide to acetate. This step is dominant at low temperatures and must be included in the ISPAD model to correctly predict CH_4 production. Whereas the Keshtkar et al. (2001) model only considers one operating temperature, the ISPAD model must include a temperature function for the maximum microbial growth rate, μ_{max} , and the acid/base dissociation constant (K_a). Such temperature effect can be defined by the Arrhenius and Square Root equations (Madani-Hosseini et al., 2014c).

Finally, the ISPAD model must be tested using its specific kinetic values obtained experimentally. The ISPAD system depends on microbial acclimation to low temperatures and low organic loads diluted over time as the system fills up. The developed ISPAD model will be initialized using kinetic values estimated for each AD group from 2009 ISPAD samples tested in the laboratory using substrate activity tests, and then fitted by Madani-Hosseini et al. (2014c) to the Monod equation. Such initial kinetic values facilitate the fitting process using experimental data with less chance of being trapped in local minima. Furthermore, the 2009 kinetic values were also compared with those obtained in this study from 2012 ISPAD samples to evaluate the evolution of the ISPAD microbial acclimation over 3 years of operation.

4.3.Materials and Methods

4.3.1. Model description

In this study, 3 AD steps were considered: acidogenesis, acetogenesis and methanogenesis (Fig 4-1). The hydrolysis step was not considered, because the simulation of its degradation process can be quite elaborate for complex organic molecules as found in wastewaters. Thus, the ISPAD model was run assuming that the hydrolysis process had degraded carbohydrates and amino acids into glucose and ammoniacal nitrogen. The production of ammoniacal nitrogen from the fed wastewaters was modeled through Eq. 4-12 presented later on.

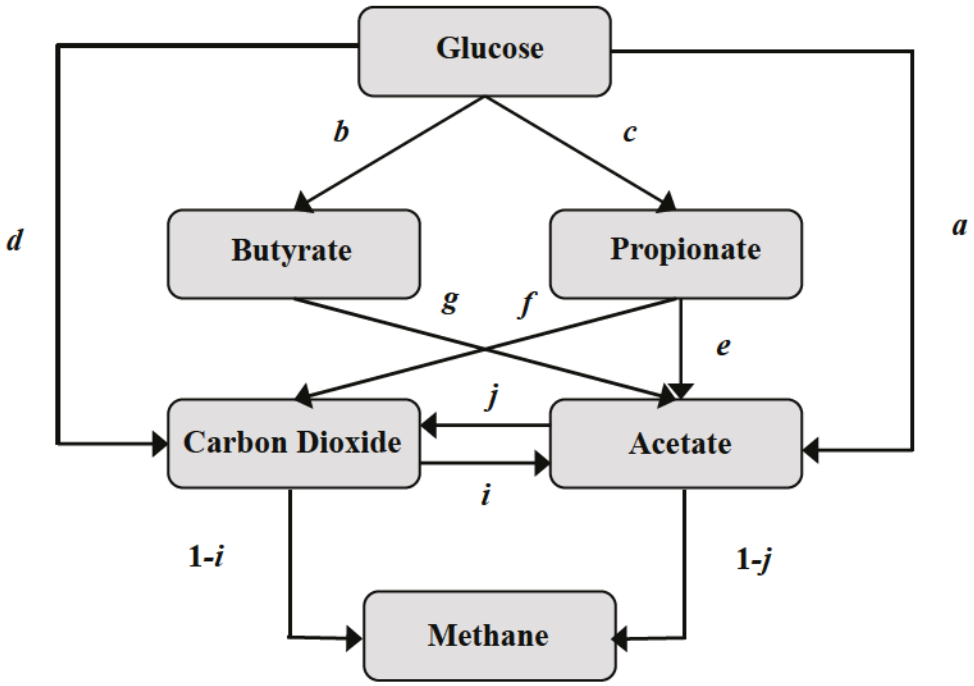

Fig. 4-1: Scheme of carbon substrate conversion in anaerobic digestion, neglecting the biomass production, Letters indicate conversion factors used in the model, shown in Table 4-5.

In the first step called acidogenesis, glucose is degraded into simple compounds such as VFAs and CO_2. The most important VFAs, in terms of biogas production, are propionic, butyric and acetic acids. The consumption of glucose and the growth of acidogens are assumed to obey Monod-type kinetics considering pH inhibition of acidogens ($F_A(pH)$) (Table 4-1).

Table 4-1: Kinetic equations used in the model

Process	Microbial growth rate	Microbial mass balance
Acidogenesis	$\mu_A = \mu_{maxA}\frac{S_{glu}}{K_{sglu}+S_{glu}}\times F_A(pH)$	$\frac{dX_A}{dt} = (\mu_A - k_{dA})X_A$
Butyrate degrading acetogenesis	$\mu_{AB} = \mu_{maxAB}\frac{S_{but}}{K_{sbut}+S_{but}} \times\frac{K_{ibut}}{K_{ibut}+S_{ac}}\times F_{AB}(pH)$	$\frac{dX_{AB}}{dt} = (\mu_{AB} - k_{dAB})X_{AB}$
Propionate degrading acetogenesis	$\mu_{AP} = \mu_{maxAP}\frac{S_{pr}}{K_{spr}+S_{pr}} \times\frac{K_{ipr}}{K_{ipr}+S_{ac}}\times F_{AP}(pH)$	$\frac{dX_{Ap}}{dt} = (\mu_{Ap} - k_{dAp})X_{Ap}$
Homoacetogenesis	$\mu_{Hom} = \mu_{maxHom}\frac{S_{CO_2}}{K_{sCO_2-Hom}+S_{CO_2}} \times F_{Hom}(pH)$	$\frac{dX_{Hom}}{dt} = (\mu_{Hom} - k_{dHom})X_{Hom}$
Acetoclastics Methanogenesis	$\mu_M = \mu_{maxM}\frac{S_{ac}}{K_{sac}+S_{ac}} \times\frac{K_{iam}}{K_{iam}+S_{am}}\times F_M(pH)$	$\frac{dX_M}{dt} = (\mu_M - k_{dM})X_M$
Hydrogenotrophic Methanogenesis	$\mu_{MH} = \mu_{maxMH}\frac{S_{CO_2}}{K_{sCO_2-MH}+S_{CO_2}} \times F_{MH}(pH)$	$\frac{dX_{MH}}{dt} = (\mu_{MH} - k_{dMH})X_{MH}$

The effect of pH inhibition ($F(pH)$) on the growth rate was described by a Michaelis pH function, normalized to give a value of 1.0 as the center value (Angelidaki et al., 1993):

$$F(pH) = \frac{1 + 2\times10^{0.5(pk_1 - pk_h)}}{1 + 10^{(pH - pk_h)} + 10^{(pk_1 - pH)}} \qquad (4\text{-}1)$$

where the coefficient pK_1 and pK_h are the lower and upper pH drop-off value with the microbial growth rate equal to 50% of the uninhibited rate. Below pK_1 and above pK_h, the growth rate is almost zero. Inhibition by pH was considered for other degradation steps including

acetogenesis and methanogens. The coefficients pK_1 and pK_h differ depending on the microbial group (Table 4-2).

Table 4-2: pK_1 and pK_h values used in the model (Keshtkar et al., 2001)

Parameter	Type of microorganism	Value
pK_{1A}	Acidogen	5
pK_{hA}	Acidogen	8
pK_{1AP}	Propionate degrading acetogen	6
pK_{hAP}	Propionate degrading acetogen	8.5
pK_{1AB}	Butyrate degrading acetogen	6
pK_{hAB}	Butyrate degrading acetogen	8.5
pK_{1MA}	Acetoclastic methanogens	6
pK_{hMA}	Acetoclastic methanogens	8.5
pK_{1MH}	Hydrogenotrophic methanogens	6
pK_{hMH}	Hydrogenotrophic methanogens	8.5
pK_{1Hom}	Homoacetogen	6
pK_{hHom}	Homoacetogen	8.5

Acetogenesis is the second step conducted by acetogenic bacteria, whereby low molecular weight VFAs are converted into acetate and CO_2. In addition, CO_2 used by other acetogenic bacteria, homoacetogens, are converted into acetate. Besides using Monod-type kinetics to simulate the consumption of propionate and butyrate, non-competitive inhibition functions were introduced in the model for acetate inhibition (Table 4-1).

The third and final step producing CH_4, is carried out by acetoclastic methanogens decarboxylating acetate, and hydrogenophilic methanogens using H_2 to reduce CO_2 (Fig. 4-1). Ammonia inhibition was considered for acetoclastic methanogens (Table 4-1), as NH_3 is expected to play an important role in AD inhibition (Batstone et al., 2002; Hill & Barth, 1977).

4.3.2. Mass balance and model equation

The ISPAD model is based on a mass balance analysis of substrate, carbon and the biomass for each microbial group of the AD consortium. The ISPAD system is an airtight tank receiving a specific volume, V, of wastewater at a regular interval of time, t_r. Once full, the ISPAD tank is emptied except for a residual volume used as inoculum for the refilling process, which is initiated immediately thereafter.

Thus, at any time t_i, being a multiple of t_r, the ISPAD tank holds a volume V_i with: a microbial population for each group of the AD consortium of X_{ij}, where i refers to the time step and j refers to the microbial group; for each microbial group of the AD consortium also, the substrate concentration is S_{ij}. If at this time t_i, a known volume of fresh wastewater is added, the microbial population of each group becomes diluted by the fresh wastewater with very little addition of AD microbes:

$$X_{(i+1)j=} \frac{V_i \times X_{ij}}{(V + V_i)} \tag{4-2}$$

This volume of wastewater also increases the substrate concentration for each microbial group of the AD consortium, as each microbial group breaks down its substrate, to produce substrate for the next AD group within the chain of reactions:

$$S_{(i+1)j=} \frac{V_i \times S_{ij} + V \times S_0}{(V + V_i)} \tag{4-3}$$

where S_0 is the substrate concentration of the volume of fresh wastewater added to the ISPAD system.

Once the ISPAD microbial populations and substrate concentrations are readjusted according to the volume of fresh wastewater received, then the ISPAD model can compute for each time sequence, changes in parameters, such as microbial population growth, substrate consumption, VFA production, pH of the digestate and finally CH_4 production over time t_r. The Monod equation is used for this purpose, assuming a mass balance in terms of microbial population,

substrate and carbon. Since there is neither inflow nor any outflow, then, the following simple equations apply:

$$\frac{dX}{dt} = (\mu - K_d)X \tag{4-4}$$

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} \tag{4-5}$$

Because the ISPAD model considers the inhibition effect of pH, acetate and NH_3, the growth rate, μ, is described in Table 4-1.

The ISPAD model therefore simulates batch processes occurring sequentially: each sequential step is initiated by the addition of a known volume V at every time interval t_r. The digestion period for each step is also t_r.

Accordingly, Eq. (4-5) can be translated for each substrate based on carbon balance:

$$\frac{ds_{glu-C}}{dt} = \frac{1}{Y_A}\frac{dX_A}{dt} \tag{4-6}$$

$$\frac{ds_{but-C}}{dt} = b\left(\frac{1}{Y_A}\frac{dX_A}{dt}\right) - \left(\frac{1}{Y_{AB}}\frac{dX_{AB}}{dt}\right) \tag{4-7}$$

$$\frac{ds_{pr-C}}{dt} = C\left(\frac{1}{Y_A}\frac{dX_A}{dt}\right) - \left(\frac{1}{Y_{AP}}\frac{dX_{AP}}{dt}\right) \tag{4-8}$$

$$\frac{ds_{CO_2-C}}{dt} = d\left(\frac{1}{Y_A}\frac{dX_A}{dt}\right) + f\left(\frac{1}{Y_{AP}}\frac{dX_{AP}}{dt}\right) + j\left(\frac{1}{Y_M}\frac{dX_M}{dt}\right) - \left[i\left(\frac{1}{Y_{Hom}}\frac{dX_{Hom}}{dt}\right) + 1 - i\left(\frac{1}{Y_{MH}}\frac{dX_{MH}}{dt}\right)\right] \tag{4-9}$$

$$\frac{ds_{ac-C}}{dt} = a\left(\frac{1}{Y_A}\frac{dX_A}{dt}\right) + e\left(\frac{1}{Y_{AP}}\frac{dX_{AP}}{dt}\right) + g\left(\frac{1}{Y_{AB}}\frac{dX_{AB}}{dt}\right) + i\left(\frac{1}{Y_{Hom}}\frac{dX_{Hom}}{dt}\right) - \left[j\left(\frac{1}{Y_M}\frac{dX_M}{dt}\right) + 1 - j\left(\frac{1}{Y_M}\frac{dX_M}{dt}\right)\right] \tag{4-10}$$

$$\frac{ds_{CH_4-C}}{dt} = 1 - i\left(\frac{1}{Y_{MH}}\frac{dX_{MH}}{dt}\right) + 1 - j\left(\frac{1}{Y_M}\frac{dX_M}{dt}\right) \quad (4\text{-}11)$$

$$\frac{dS_{H_3}}{dt} = KS_O - (\mu_A X_A + \mu_{AP} X_{AP} + \mu_{AB} X_{AB} + \mu_M X_M + \mu_{MH} X_{MH} + \mu_{Hom} X_{Hom}) \quad (4\text{-}12)$$

Eq. 4-12 was added to the ISPAD model to simulate the ammoniacal nitrogen generated from the hydrolysis of the wastewaters. Thus, Eq. 4-12 predicts the release of ammonia from organic nitrogen (S_O) at a rate constant K, because such ammoniacal nitrogen can inhibit AD when produced in excess of what the microbial groups can consume.

4.3.3. pH prediction

In AD, pH prediction is important because it affects microbial growth and total NH_3 concentration (Chen et al., 2008). Furthermore, pH is one of the most important parameters affecting NH_3 volatilization besides temperature. Therefore, the ISPAD model needs to predict the pH of its content for further investigation on controlling of NH_3 volatilization. In AD systems, pH is mainly controlled by the interaction of the carbon dioxide/bicarbonate buffer system with bases such as NH_3, and acids such as VFAs mainly propionate, butyrate and acetate. The ionic balance between the following elements is generally used to compute the pH of a system: CO_2, NH_3, VFAs (acetate, ac^-, propionate, pr^-, and butyrate, but^-), and cations (C^+) and anions (A^-). The following equation is used for that purpose:

$$[H^+] + [NH_4^+] + [C^+] = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] + [ac^-] + [pr^-] + [but^-] + [A^-] \quad (4\text{-}13)$$

To simulate pH variation with time, the ionic charge balance (Eq. 4-13) needs to be iteratively solved. Only the sum of the concentration of anions (A^-) and cations (C^+) is assumed to be independent of time and their initial values were used (Table 4-3).

The concentrations of ionic compounds can be obtained according to Eq. (4-14) to (4-20).

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \qquad K_{a1} = \frac{[HCO_3^-][H^+]}{[CO_2]} \qquad (4\text{-}14)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \qquad K_{a2} = \frac{[CO_3^{2-}][H^+]}{[HCO_3^-]} \qquad (4\text{-}15)$$

$$HAC \leftrightarrow AC^- + H^+ \qquad K_{a3} = \frac{[AC^-][H^+]}{[HAC]} \qquad (4\text{-}16)$$

$$HPr \leftrightarrow Pr^- + H^+ \qquad K_{a4} = \frac{[Pr^-][H^+]}{[HPr]} \qquad (4\text{-}17)$$

$$HBut \leftrightarrow But^- + H^+ \qquad K_{a5} = \frac{[But^-][H^+]}{[HBut]} \qquad (4\text{-}18)$$

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad K_{a6} = \frac{[NH_3][H^+]}{[NH_4^+]} \qquad (4\text{-}19)$$

$$H_2O \leftrightarrow OH^- + H^+ \qquad K_w = [OH^-][H^+] \qquad (4\text{-}20)$$

The dissociation constants (K_a) presented in the third column of Table 4-6 are theoretical values found for pure solutions. In wastewaters, K_a can change because of the interaction of especially dissolved carbon with other active species (Liu et al., 2013). Accordingly, $K_a$values where found by a process of optimization using pH data obtained from ISPAD inoculum fed glucose at 35 °C. As ISPAD operates at ambient temperatures, and temperature affects K_a, a temperature function was included for K_a determination. Therefore, the K_a values were optimized for 35 °C conditions and then computed for 18 and 4 °C based on the Van't Hoff Equation:

$$\ln\left(\frac{K_{a2}}{K_{a1}}\right) = \frac{-\Delta H}{R^*}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad (4\text{-}21)$$

where K_{a1} is the dissociation constants at T_1 (K), K_{a2} is dissociation constant at T_2 (K), ΔH is enthalpy of the reaction (J /mole), and R^* is universal gas constant (8.314 J /mole K).

4.3.4. Temperature effects on μ_{max}

The temperature effect on μ_{max}, is commonly described by the Arrhenius and the Square Root Equations. According to the Arrhenius equation (Eq. 4-22), the reaction rate roughly doubles for a temperature increase of 10°C:

$$\mu_{max} = Ae^{\frac{-Ea}{R^*T}} \quad (4\text{-}22)$$

where A is a constant, R^* is the universal gas constant (0.008314 $kJ/mol\,K$), T is temperature (K) and E_a is the activation energy (kJ/mol).

The Square Root equation describes a less than optimum temperature adaptation of bacterial growth in pure cultures:

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (4\text{-}23)$$

where T_{min} is the apparent minimum temperature for growth (K), and b is the regression coefficient.

Madani-Hosseini et al. (2014c) optimized the values of E_a and T_{min} for ISPAD population groups to compare the prediction accuracy of both the Arrhenius and the Square Root equations, describing the relationship between temperature and μ_{max}. The results showed that the Square Root equation predicted temperature dependency for both acidogens and propionate degrading acetogens, while the Arrhenius equation better predicted temperature effect for methanogens and butyrate degrading acetogens. The ISPAD model therefore incorporates both of these equations accordingly.

4.3.5. Model assumptions

Anaerobic digestion is a complex process which can be simplified through assumptions. Thus, the AD process of the ISPAD system was simplified using the following assumptions:

- The ISPAD model only focuses on carbohydrate (glucose) degradation.
- H_2 production was not considered in the model, since the ISPAD model is based on carbon balance.
- To calculate the pH, all acid/base pairs were considered in equilibrium.
- For each step, the reactor is operated at constant volume.
- Since the most important VFAs, in terms of biogas production, are propionic, butyric and acetic acids, the other VFAs such as valerate were not considered.
- Biogas contains CH_4 and CO_2, where CH_4 has low solubility in liquid phase.
- The reactor behaves like a perfectly mixed tank, and that the biomass and substrate are uniformly distributed within the reactor.

4.3.6. Model calibration and kinetic parameter estimation

For the calibration of the model, batch experiments were conducted in the laboratory using inoculum obtained from a 7-year-old field ISPAD in the spring of 2012. These batch experiments produced curves for glucose degradation and VFAs and CH_4 production over time at temperatures of 4, 18, and 35 °C. All samples were duplicated and the result averaged. The inoculum consisting of 100 ml of ISPAD content (7.2 gVS/L) was placed in 250 ml bottles containing 50 ml glucose at a concentration of 1000 mg/L. The total volume of liquid in each 250 ml bottle was therefore 150 ml with a VS concentration of 4.8 g/L and a glucose concentration of 333 mg/L. Bottles were capped, sealed and flushed with N_2 gas to establish anaerobic conditions, before starting the AD process and monitoring glucose and VFA concentrations, and CH_4 production. The mixture was shaken by hand once a day. The system was run at 3 controlled temperatures of 4, 18, and 35 °C: for 18 and 35 °C, the bottles were incubated, while for 4 °C, the bottles were refrigerated. For each temperature, duplicate control bottles were prepared with ISPAD inoculum and water instead of glucose. Samples of 2 ml were regularly withdrawn from the bottle headspace for gas production analysis using a gas

chromatograph while gas production was monitored until gas production ceased, using a water displacement apparatus. Also, 2 ml liquid samples were removed from each bottle at specified time intervals to monitor pH, glucose and VFA concentrations.

A fitting process was used to obtain kinetic coefficient values from the experimental data to calibrate the ISPAD model (Fig. 4-2). The experimental data was corrected by subtracting the control value, for CO_2, CH_4, VFAs, and glucose.

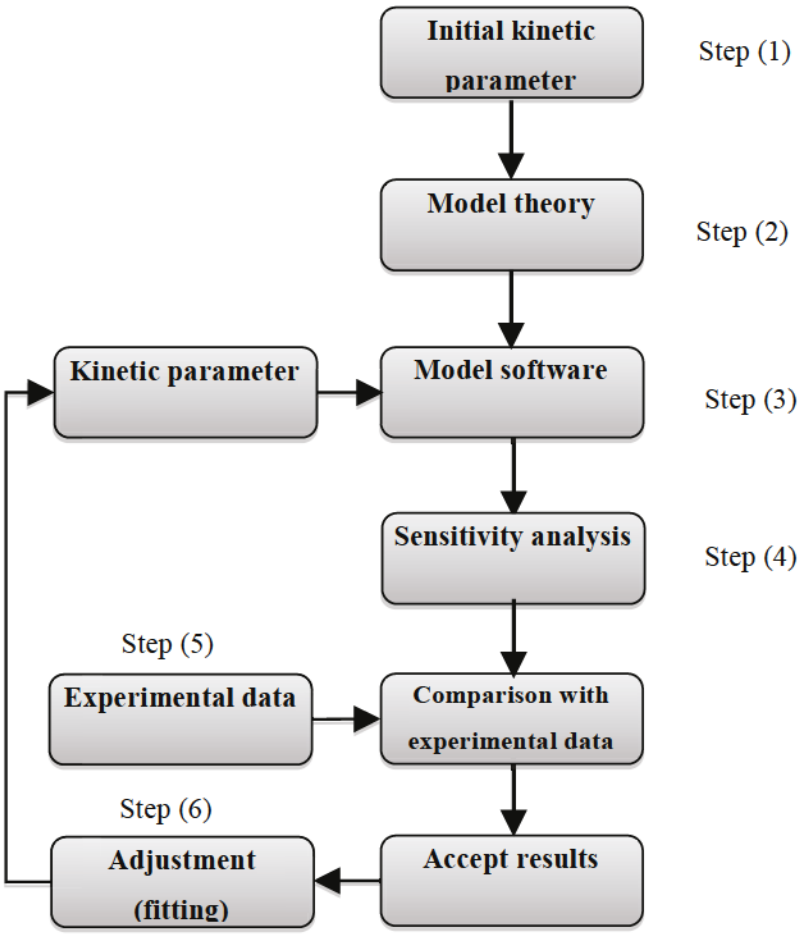

Fig. 4-2: Steps in modeling process

The fitting process was initiated at 35 °C using the values of μ_{max}, K_s, Y, and bacterial density (X) determined by Madani-Hosseini et al. (2014c) and inhibition kinetic values based on Keshtkar et al. (2001) (step 1, Fig. 4-2). The fitting process consisted of integrating the theoretical equations of the ISPAD model (step 2, Fig. 4-2) into the software Matlab/Simulink (MathWorks, 2012a) (step 3, Fig. 4-2). Furthermore, a batch system was reproduced in the

Matlab/Simulink software to match the experimental batch tests. Then, an iterative loop consisting of steps 3, 4 and 6 (Fig. 4-2) was started using the ISPAD model to obtain the best fitting kinetic parameters.

Once the kinetic coefficient and microbial population values were obtained for 35 °C conditions, curve fitting was extended to the 18 and 8 °C data. Microbial population values for 35 °C were presumed to remain the same for the 18 and 4 °C curve fitting operation.

To find the coefficient of variation (C.V) for the kinetic coefficients, the data from each individual quadruplet runs at 18 °C was fitted using the ISPAD model (Table 4-4). The quadruplet runs were obtained from the duplicate fed glucose and the control fed no glucose.

4.3.7. Analytical procedure

The experimental inoculum was obtained in 2012 from a 7-year-old field ISPAD system treating swine manure in the Drummondville area of Eastern Canada. The ISPAD samples were analyzed according to standard methods (Eaton & Franson, 2005) to establish: solids (TS, VS, TSS, VSS, FS and VDS) and pH (Table 3). To measure COD, commercial COD test kits for ultra-high rate COD were used (DR/4000, HACH Corp). Anions including Cl^-, NO_3^{2-}, NO_2^-, SO_4^{2-} and PO_4^{3-} and the cation of NH_4^+ were determined by HACH kit/high rate.

To monitor gas production from the batch tests, the biogas composition (CH_4, and CO_2) was measured by injecting 2 ml samples into a gas chromatograph (Varian, model 3800) equipped with TCD detector and CARBOXEN 1010 PLOT (capillary column) from SUPELCO, 30mm× 0.53mm column. The carrier gas was helium/argon. The column temperature was held at 50-100 °C for 5 °C/min. The injection flow was 5 ml/min.

The liquid samples were analyzed for VFAs by HPLC (Beckman Coulter Inc, Gold system) where the HPLC spectra were analyzed using the Beckman Coulter Inc. software (32 Karat Software, Beckman Coulter Inc.). Before injection, samples were filtered using a syringe filter, PTFE (polytetrafluoroethylene), 25 mm diameter, 0.45 µm to remove solids. The samples were injected into a polystyrene resin chromatography column (30cm× 7.8mm ID, SUPLECOGEL model C-610H, USA). The parameters were: mobile phase of 0.1% phosphoric acid, 100 µL injection, UV detection wavelength of 210 nm, and 0.5 ml/min flow rate at 30 ^{0}C. The glucose concentration was measured by the colorimetric method (Lever, 1972).

4.4.Results and Discussion

4.4.1. Inoculum characterization

The ISPAD inoculum is characterized in Table 4-3 along with fresh swine manure assumed to be diluted to give the same Fixed Solid (FS) level, because this component is not affected by AD.

Table 4-3: Characteristics of experimental ISPAD inoculum and fresh swine manure

Characteristic	ISPAD inoculum	Fresh swine manure[2]
Solids (g/L)		
- Total	14.03 (0.50)[1]	30.0 (1.44)
- Volatile	7.22 (0.09)	23.2 (0.15)
- Fixed	6.81 (0.50)	6.8
- Dissolved	13.8 (0.50)	-
- Suspended	0.23 (0.07)	-
pH	8.17 (0.09)	7.5
Nitrogen (g/L)		
- TKN	1.89 (0.25)	1.40 (0.56)
- NH_4-N	1.71 (0.085)	0.78 (0.27)
- NO_3-N	0.013 (0.001)	-
Phosphorous (g/L)		
- Total	-	0.50 (0.27)
- PO_4^{-3} - P	0.53 (0.011)	0.33
Mineral (g/L)		
- Ca	-	0.90 (0.49)
- K	0.85 (0.24)	0.80 (0.44)
- Mg	-	0.18 (0.09)
- Na	0.34 (0.29)	0.18 (0.14)
VFAs (g/L)		
- Acetic	0.03 (0.00)	-
- Propionic	0.00 (0.00)	-
- Butyric	0.00 (0.00)	-
COD (g/L)	5.95 (0.45)	-

[1]Standard deviation in brackets
[2] (ASABE, 2000)

The ISPAD inoculum offers much lower TS and VS levels because of the loss of carbon during AD degradation (King, 2011). As for NH_4^+-N and PO_4^{-3}, their values are higher for the ISPAD inoculum because of AD degrading organic components releasing such elements.

Mineral concentrations are similar, because these are soluble elements not affected by AD, but rather by diet. The low concentrations of VFAs in the ISPAD inoculum, and the resulting increase in pH, confirm that the process is well acclimated to operating conditions (Kotsyurbenko, 2005). Therefore, the 7-year old ISPAD content could be used as inoculum as it was considered to offer a fully functional microbial population (Wilkie, 2005).

4.4.2. Estimated kinetic parameters and conversion factors

Table 4-4 compared the kinetic coefficients obtained in this study using 2012 ISPAD inoculum, to that of a previous study (Madani-Hosseini et al., 2014c) using 2009 ISPAD inoculum and that of another research conducted at 6°C but for cattle manure. Model fitting of the AD experimental data was used to establish the 2012 ISPAD kinetic values.

For the ISPAD system, comparing the 2012 to the 2009 kinetic values provides an indicator of acclimation for the microbial communities. Kinetic values obtained at 35 °C depend on the freshness of the inoculum because the experimental ISPAD system was regularly fed with fresh manure containing mesophilic populations which become active for a certain amount of time, during the initial stages of AD (King, 2011). The significant differences will therefore concentrate on results obtained at 4 and 18 °C:

1) At 4 °C, higher maximum growth rates for the acidogens, butyrate degrading acetogens, and acetoclastic methanogens, but a drop for the propionate degrading acetogens; this observation confirms the fact that lower temperatures favour butyrate rather than propionate degradation;
2) At 18 °C, higher maximum growth rates for the acidogens and butyrate degraders, but no change for the propionate degrading acetogens and the acetoclastic methanogens;
3) At 4 °C, a higher substrate affinity for the propionate and butyrate degrading acetogens, and the acetoclastic methanogens, but a lower affinity for the acidogens;
4) At 18 °C, a higher substrate affinity for the buryrate degrading acetogens, but no change for all other microbial groups;
5) a higher microbial growth rate for the acidogens and acetoclastic methanogens.

Accordingly, the acclimation of the ISPAD microbial population has been evolving over time, from 2009 to 2012.

Table 4-4: Estimated ISPAD kinetic values at 2009 and 2012

Process	Parameter	Units	Value						
			8 ^{0}C	4 ^{0}C	18 ^{0}C		35 ^{0}C		6 ^{0}C[3]
			2009[1]	2012	2009	2012	2009	2012	
1. Acidogenesis	μ_{maxA}	$1/day$	0.64	1.10	2.9	3.70 (0.066)[2]	6.4	7.90	2
	K_{sglu}	mg/L	219	321	167	140 (0.11)	140	35	113.4
	Y_A	mg/mg	0.123	0.110	0.123	0.110 (0.16)	0.123	0.030	0.2
	X_A	mg/L	7.54	2.2	7.54	22 (0.05)	7.54	2.2	-
2. Propionate degrading acetogenesis	μ_{maxAP}	$1/day$	0.11	0.01	0.06	0.05 (0.12)	0.12	0.03	0.07-0.08
	K_{spr}	mg/L	392	189	163	111 (0.08)	100	21	19.98
	Y_{AP}	mg/mg	0.053	0.01	0.053	0.03 (0.01)	0.053	0.090	0.05
	X_{AP}	mg/L	18.32	59.0	18.32	59.0 (0.00)	18.32	59.0	-
	K_{ipr}	mg/L	-	960	-	960 (0.00)	-	960	-
3. Butyrate degrading acetogenesis	μ_{maxAB}	$1/day$	0.023	0.14	0.22	0.9 (0.00)	0.23	0.08	0.09-0.13
	K_{sbut}	mg/L	411	213	450	124 (0.00)	450	150	20.24
	Y_{AB}	mg/mg	0.034	0.020	0.034	0.008 (0.00)	0.034	0.026	0.08-0.1
	X_{AB}	mg/L	85.96	15.50	85.96	15.50 (0.00)	85.96	15.50	-
	K_{ibut}	mg/L	-	720	-	720 (0.00)	-	720	-
4. Homoacetogenesis	μ_{maxHom}	$1/day$	-	0.44	-	0.73 (0.02)	-	-	1
	K_{sCO_2}	mg/L	-	300	-	160 (0.04)	-	-	10.56
	Y_{Hom}	mg/mg	-	0.042	-	0.058 (0.1)	-	-	0.05
	X_{Hom}	mg/L	-	65.0	-	65 (0.05)	-	-	-
5. Acetoclastic Methanogenesis	μ_{maxMA}	$1/day$	0.045	0.19	0.2	0.23 (0.11)	0.4	0.60	0.008-0.022
	K_{sac}	mg/L	533	210	213	351 (0.02)	193	113	348
	Y_{MA}	mg/mg	0.019	0.010	0.019	0.038 (0.06)	0.019	0.060	0.01
	X_{MA}	mg/L	23.59	11.50	23.59	11.50 (0.08)	23.59	11.50	-
	K_{iam}	mg/L	-	260	-	260 (0.00)	-	260	
6. Hydrogenotrophic Methanogenesis	μ_{maxMH}	$1/day$	-	0.1	-	0.23 (0.20)	-	0.32	0.8
	K_{sCO_2}	mg/L	-	170.00	-	32.5 (0.1)	-	29.00	10.56
	Y_{MH}	mg/mg	-	0.015	-	0.005 (0.28)	-	0.03	0.04
	X_{MH}	mg/L	-	45.0	-	45.0 (0.08)	-	45.0	-

[1]Madani-Hosseini et al. (2014c)

[2]Coefficient of variance from four sets of data (fraction)

[3] Vavilin et al. (1998)

Comparing the 2012 ISPAD kinetics to that of Vavilin et al. (1998):

1) the maximum growth rate of the ISPAD acidogens is increasing towards that of the Vavilin value, but the substrate affinity is becoming less sensitive with time as compared to that of Vavilin;

2) the maximum growth rate of the ISPAD propionate and butyrate degrading acetogens is within the range of that of Vavilin, but offers a much lower affinity for the substrate;
3) the maximum growth rate and substrate affinity for the ISPAD homoacetogens is much lower than that of Vavilin.

A comparison of the Vavilin kinetics indicate that the ISPAD microbial populations can further acclimate to psychrophilic conditions.

The coefficients of variation (C.V.) were calculated for the batch test conducted at 18 °C (Table 4-4), to check the level of result variability. The maximum growth rate, μ_{max}, of the acidogens and hydrogenotrophic methanogens showed the highest C.V. of 6 to 20%, while all other kinetic parameters showed a C.V. of under 5%. As for other kinetic parameters, variation of K_s values with temperature was larger than the C.V., indicating that temperature had a significant effect.

Table 4-5 presents the optimized conversion factors at 4, 18, and 35 °C. The main carbon flow in the acidogenesis step happened between glucose and acetate at a higher temperature, in agreement with Husain (1998). However, at 4 °C, the carbon flow in acidogenesis occurred between glucose and CO_2. The Vavilin et al. (1998) model calibration indicated that most of the glucose was converted to CO_2 under psychrophilic conditions. At higher temperatures, higher levels of propionate rather than butyrate resulted from glucose degradation. When temperature dropped from 35 to 4 °C, glucose conversion to butyrate was almost 2.5 times higher that of propionate. Temperature also affects propionate conversion, where most of the propionate converted to acetate at a low temperature of 4 °C and CO_2 at higher temperatures 18 and 35 °C. For methanogenesis at higher temperatures, most of the CH_4 production came from hydrogenotrophic methanogenesis. However, at lower temperatures, CH_4 was mostly produced by the acetoclastic methanogens. Kotsyurbenko et al. (2001) showed that under psychrophilic conditions (<15 ^{0}C), the hydrogenotrophic methanogen activity was very low. Homoacetogenesis is mainly responsible for H_2 consumption and under such conditions; CH_4 formation through acetoclastic methanogens becomes dominant. Homoacetogenesis under psychrophilic temperatures can be responsible for 95% of the total CH_4 production (Kotsyurbenko, 2005).

Table 4-5: Conversion factors used in the model to achieve a carbon mass balance, where Fig 4-1 illustrates the process associated with each factor.

Symbol	Conversion factor (%) 4 °C	18 °C	35 °C
a	20	47	30
b	25	23	13
c	10	10	27
d	40	20	30
e	67	5	20
f	33	95	80
g	100	100	100
i	67	67	5
j	5	10	50

4.4.3. Sensitivity analysis

To reduce the number of parameters requiring calibration, a sensitivity analysis was conducted to determine which independent variable impacts a specific dependent variable under a given set of assumptions. Local and global sensitivity analysis (Bernard et al., 2001; Noykova & Gyllenberg, 2000; Tartakovsky et al., 2008; Vavilin et al., 2003) evaluate respectively: linear perturbations for the output for a specific set of parameters, and the sensitivity for a broader spectrum of input parameters in nonlinear models. Because AD models represent a complex usually nonlinear system, the global sensitivity method is preferred.

Sensitivity was quantified in terms of variation in measurable process under the perturbation of model parameters in their neighbourhood domain. The variance-based method was chosen for the global sensitivity analysis, correlating the variance of inputs and outputs for steady state simulations. The variance in the parameters will lead to a variance in output, and an important parameter will have a bigger impact on the output variance than a parameter for which the model is less sensitive.

Simulations using Keshtkar et al. (2001) inhibition kinetic values showed less than 50% discrepancies between the experimental results and the model predictions. Thus, the parameters

in the Keshtkar et al. (2001) model were kept constant. However, all other kinetic parameters of μ_{max}, K_s, Y, and X were optimized because of their high sensitivity range.

4.4.4. Model prediction

To analyse the ability of the model to simulate the ISPAD system, the correlation coefficients (R) were calculated. The results showed that the R values were in the range of 0.6 to 0.98, indicating a reasonable prediction. A correlation greater than 0.8 is generally described as strong, whereas a correlation under 0.5 is generally described as weak. The model prediction for CO_2, CH_4, glucose, VFAs, and pH are described in detail as follows.

4.4.4.1. Simulation of CH_4 and CO_2

Fig. 4-3 shows measured and simulated results for CH_4 and CO_2 production per glucose consumption, at 4, 18 and 35 °C after model calibration. Overall, model prediction at 18 and 4 °C was better than at 35 °C, which increased with time.

The duration of the lag phase increased as temperature dropped, with 4 °C showing the longest duration. At 4 °C, CH_4 conversion rate, about 0.6 CH_4 (mg-C)/glucose (mg-C), was less than that at 18 and 35 °C at 0.8 CH_4 (mg-C)/Glucose (mg-C) after 6 and 10 days of incubation, respectively. Production of CH_4 at 4 °C started to slowly increase after 10 days of incubation and reached 0.6 CH_4 (mg-C)/glucose (mg-C) after 22 days. Production of CH_4 as per glucose consumption at a 6 day incubation for 35 °C was 1.7 and 32.4 times higher than at 18, and 4 °C, respectively.

The model predicted a CO_2 production at 35 °C sharply increasing at the beginning of the experiment, compared to a smooth increase at 4 °C. The model CO_2 prediction ability at 4 °C was better than those of 35 and 18 °C. The biogas CO_2 fraction increased when temperature decreased to 4 °C. This behaviour could be due to the methanogens lag phase at lower temperatures.

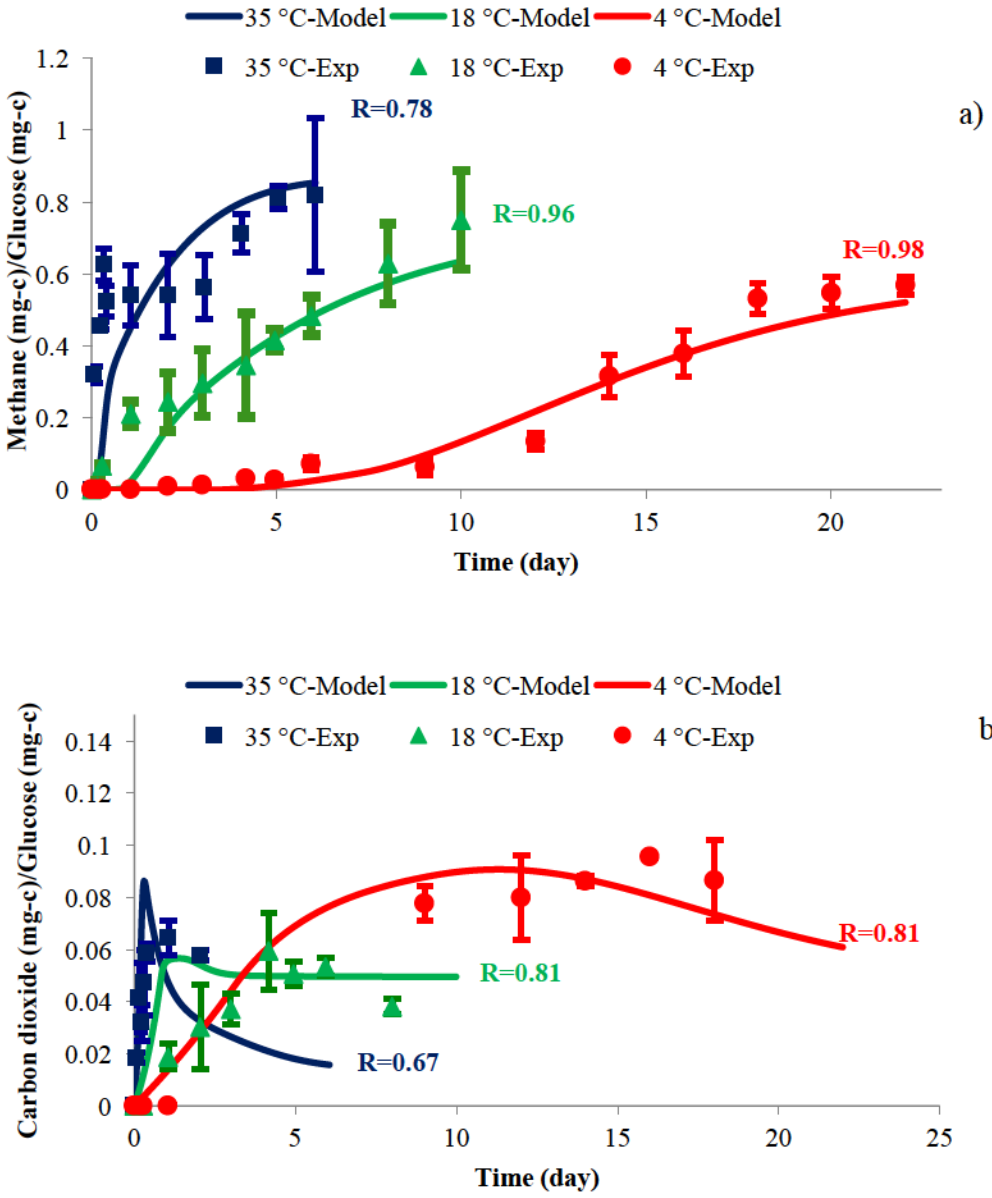

Fig. 4-3: Simulation of biogas production, a) for CH_4 and b) for CO_2, from glucose degradation at 35, 18, and 4 °C inoculated with ISPAD manure. Experimental data, point; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

4.4.4.2. Simulation of glucose and VFAs

Glucose degradation by the ISPAD inoculum at 35, 18 and 4 °C is illustrated by Fig. 4-4. The model accurately predicted glucose consumption at all temperatures with the curve stretched in time as temperature drops, showing that acidogens consumed glucose faster at higher

temperatures. Complete glucose consumption at 4 °C required over 22 days as compared to 1 and 3 days at 18 and 35 °C.

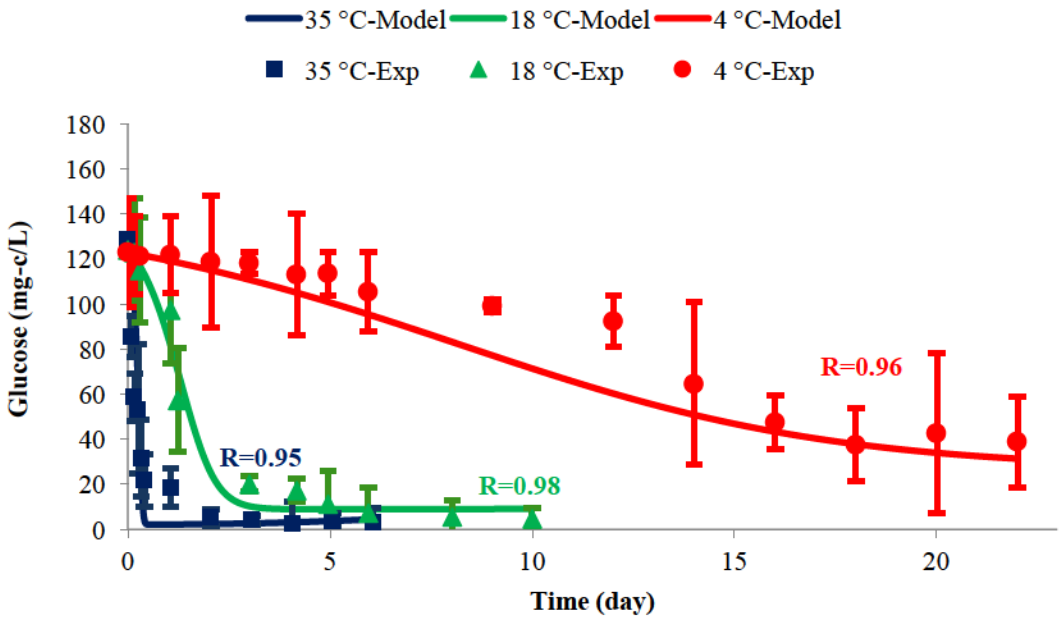

Fig. 4-4: Simulation of glucose degradation at 35, 18, and 4 °C, with the 2012 ISPAD inoculum. Experimental data, point; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

At all temperatures, acetate was the major VFA produced (Fig. 4-5) (Wang et al., 1999). At 35 and 18 °C, acetate production showed the steepest slope at the beginning of the experiment, as compared to a slow increase at 4 °C, as a result of a lag phase. While the temperature affected the acetate and propionate concentration, it had no significant effect on butyrate concentration. While butyrate was consumed by methanogens, propionate persisted in the reactor, specifically at 4 °C. Low temperatures of 3 to 9 °C are known to favour the degradation of butyrate over propionate (Nozhevnikova et al., 2000). Furthermore, CH_4 production from propionate is slower than that from butyrate and acetate, because of its thermodynamically unfavourable AD process (Gijzen et al., 1988).

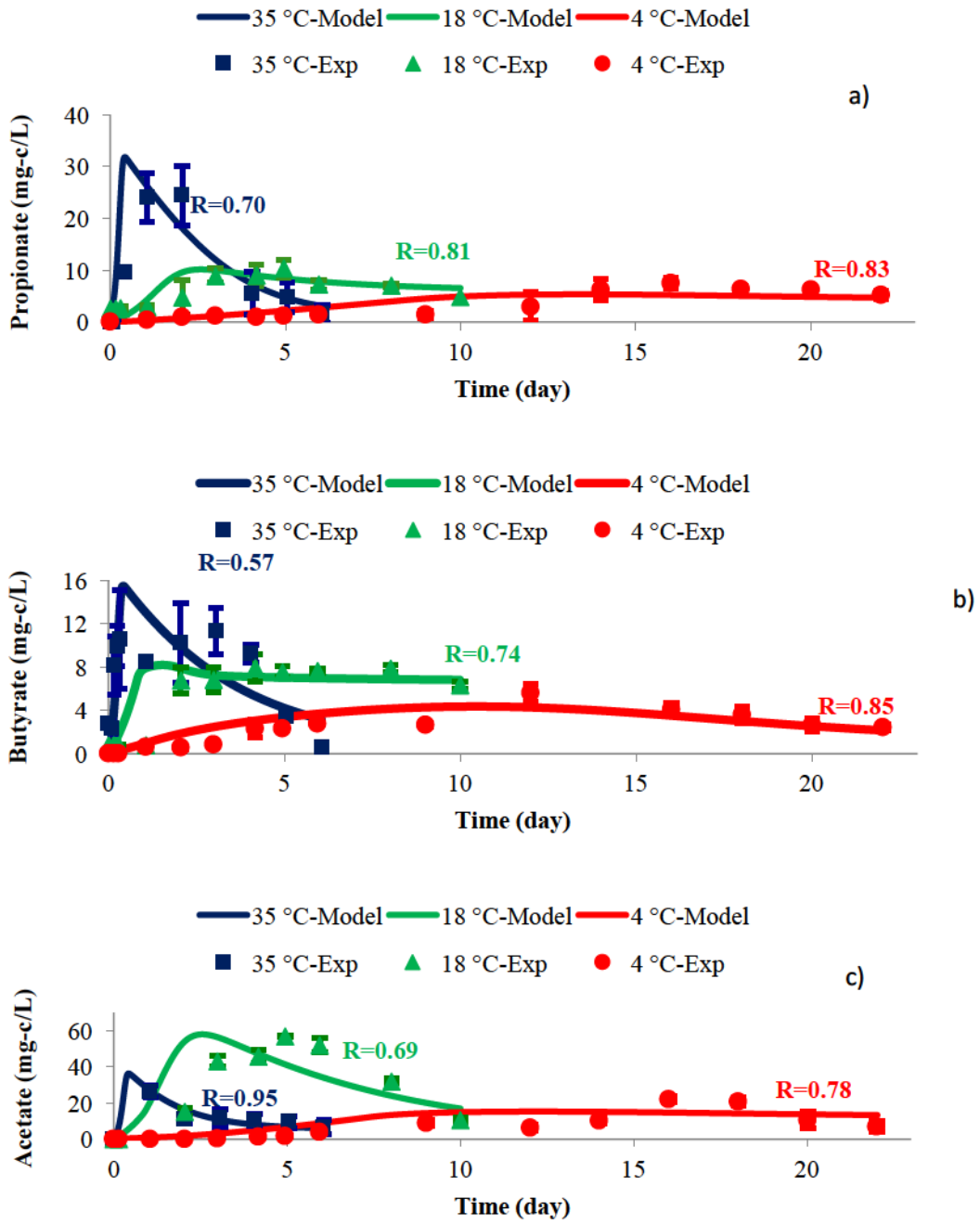

Fig. 4-5: Simulation of VFAs production from glucose degradation at 35, 18, and 4 °C, with the 2012 ISPAD inoculum. Experimental data, point; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

4.4.4.3. Simulation of pH

The optimized dissociation constants for the main active acids and bases, K_a, for ISPAD content at all 3 temperatures are presented in Table 4-6. At 35 °C, the K_a fitting process produced a major change in the second dissociation constant for carbonic acid, and a slight change in the dissociation constant for propionic and butyric acid. Values at 35 °C were adjusted to 18 and 4 °C using Eq. 4-21.

Table 4-6: Dissociation coefficients at 35, 18, and 4 °C

Parameter	**Units**	**Reported value**[1]	**Fitted value 35 ^{0}C**	**Temperature corrected fitted value 18 ^{0}C**	**Temperature corrected fitted value 4 ^{0}C**
K_{a1}	$mmol/L$	4.909×10^{-4}	4.909×10^{-4}	3.69×10^{-11}	1.59×10^{-62}
K_{a2}	$mmol/L$	5.623×10^{-8}	$3.82\times10^{-}$	1.95×10^{-14}	5.08×10^{-6}
K_{a3}	$mmol/L$	1.73×10^{-2}	1.73×10^{-2}	2.03×10^{-9}	2.12×10^{-59}
K_{a4}	$mmol/L$	1.445×10^{-2}	1.5×10^{-3}	2.69×10^{-11}	6.01×10^{-59}
K_{a5}	$mmol/L$	1.445×10^{-2}	1.5×10^{-3}	2.69×10^{-11}	6.01×10^{-59}
K_{a6}	$mmol/L$	1.567×10^{-6}	1.567×10^{-6}	1.527×10^{-14}	2.55×10^{-2}
K_w	$mmol/L$	2.065×10^{-11}	2.065×10^{-10}	1.039×10^{-11}	5.5×10^{-12}

Dissociation constants at 35 0C (Dean, 1992) K_{a1} K_{a2} K_{a3}, K_{a4} K_{a5}, K_{a6}, and K_w are first dissociation constant for carbonic acid, second dissociation constant for carbonic acid, dissociation constant for acetic acid, propionic acid, butyric acid, ammonia, and water, respectively.

Fig. 4-6 shows the fitting of the experimental data and model prediction for pH at all three temperatures. The 35 °C experimental results showed a pH dropping from 8.1 to 7.5 during the first day, to start climbing thereafter. At 18 °C, the pH dropped from 8.0 to 7.7 on day 2, to remain constant thereafter, whereas at 4 °C, the pH remained at 8.0 during the full experimental period.

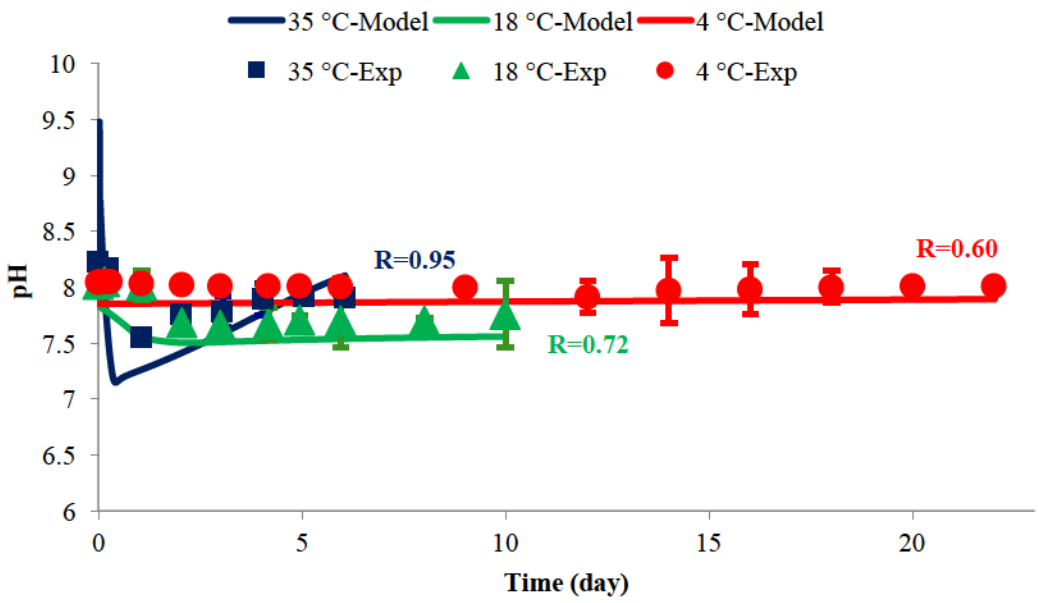

Fig. 4-6: Simulation of pH from glucose degradation at 35, 18, and 4 °C, with the 2012 ISPAD inoculum. Experimental data, point; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

The model was able to predict the pH regime with an R value of 0.95, 0.72 and 0.6 at 35, 18 and 4 °C, respectively. Nevertheless, at 18 °C, the model predicted a pH of 7.5 compared to 7.7 for the experimental data, and at 4 °C, the model predicted a pH of 7.9 compared to 8.0 for the experimental data. Such close estimation by the model indicates its capability in reproducing the pH regime of the ISPAD system.

4.5.Conclusions

An alternative to the conventional anaerobic digestion reactor, In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is a system offering an affordable process to units producing a limited amount of organic wastewaters which must be stored for at least 100 days. Nevertheless, ISPAD is a sequentially fed batch process relying on acclimated microbial groups which have not been fully characterized. The objective of the research was to develop the knowledge and a model capable of optimizing the operation of ISPAD by predicting its behaviour under operating conditions. To do so, the research evaluated ISPAD kinetic values and developed an anaerobic

digestion (AD) model capable of predicting the process. Also, by comparing 2009 and 2012 fitted kinetic values, the research work was able to verify if the microbial communities were still acclimating to the psychrophilic conditions imposed by the ISPAD system. The mathematical ISPAD model developed is able to predict substrate consumption, VFA levels, biogas generation and pH evolution. The model did not include hydrolysis, as this step is quite complex and requires research as extensive as the present predicting methane production and pH regime from glucose degradation.

The model was developed from that of Keshtkar et al. (2001), by adding functions specific to low temperature AD such as determining the activity of the two main groups of methanogens, acetoclastic and hydrogenotrophic, and including a temperature function for the maximum microbial growth rate, μ_{max} , and the acid/base dissociation constant (K_a). The fitted kinetic values obtained from the developed model showed that the microbial communities were still acclimating to the low operating temperatures. Furthermore, the ISPAD model was able to predict glucose concentration with an R value of 0.95 to 0.98, methane production with an R value of 0.78 to 0.98, pH regime with an R value of 0.60 to 0.95 and acetate with an R value of 0.69 to 0.95. Full testing of the ISPAD model still requires validation.

4.6.Acknowledgments

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Chapter 5. In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) Process. Part II: Model Validation

Connecting Statement

Using the ISPAD model successfully calibrated using experimental data in Chapter 4, Chapter 5 proceeds with its calibration under different conditions. The prediction accuracy of the model using experimental data was validated mathematically by determining its coefficients of determination.

This chapter resulted in a manuscript currently under review at the Journal of Journal of Environmental Management and Sustainable Development. The first contributing author, Mahsa Madani-Hosseini, designed the experiments, conducted the laboratory work, analyzed the data and wrote the article. The second and third contributing authors, Dr. Catherine Mulligan and Dr. Suzelle Barrington, supervised, advised on the experimental design and methods of analysis, and revised the content of the article.

Abstract

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is a sequentially fed batch treatment system operating at a temperature fluctuating with that of ambient conditions. Because of its specific operation modes and the acclimation of its microbial groups, a mathematical ISPAD model was built, on principles of microbial kinetics, to optimize its management. The objective of this study is therefore to validate this ISPAD model using laboratory data obtained from batch tests. For this purpose, glucose at 630 mg/L, was fed to 8-year-old ISPAD inoculum and digested at 18 °C. Changes in glucose, VFAs and pH were monitored along with biogas production. The cross-validated coefficient of determination (Q^2) was used to determine the fit between the model prediction and the experimental values. The ISPAD model was able to strongly predict glucose degradation, VFAs, pH, and methane. However, the model weakly predict the early CO_2 changes over time, likely because of its water solubility.

Keywords: Anaerobic process; Biogas; Modelling; Kinetic parameters; Psychrophilic; Validation

5.1.Introduction

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) consists of a wastewater storage tank converted into an anaerobic digester by means of an airtight floating geo-membrane cover. This anaerobic digestion (AD) system was developed for Canadian climatic conditions to improve system feasibility, reduce odours, conserve nitrogen and produce biogas. The ISPAD system is a long term sequentially fed batch operation functioning under ambient temperature, where wastewater is added sequentially over the treatment period of at least 100 days. A mathematical model predicting the behaviour of the ISPAD system under its operating conditions was developed (Madani-Hosseini et al., 2014b; Madani-Hosseini et al., 2014c). The proposed model was calibrated with experimental data obtained with ISPAD inoculum fed glucose as the substrate. However, this ISPAD model requires validation to check its predictive capacity. Two types of validation methods are found in the literature, direct and cross validation. Both validation methods can be used if sufficient data is available to produce two subsets, one for parameter identification and direct validation, and the other for cross validation.

In direct validation, the model is tested with data used for parameter identification. A good test in direct validation is based on residual analysis such as the correlation coefficient (R). The correlation coefficient (R) has been used alone to evaluate model fit in several studies (Flotats et al., 2006; Palatsi et al., 2010; Redzwan & Banks, 2004). Madani-Hosseini et al. (2014b) used the R value to check the predictive quality of the developed ISPAD model. The results showed that the R values for biogas generation, glucose, VFAs, and pH ranged from 0.57 to 0.98, indicating reasonable prediction. Although the model may provide a reasonable fit with respect to the calibration data, it may perform poorly when asked to predict different conditions. As AD is a complicated multi-stage dynamic process, the model should be validated to truly represent the kinetic of the system through obtained kinetic constants. Therefore, cross validation is needed to test the model using different operating conditions. Cross validation was applied to check the ADM1 model validity in several studies (Boubaker & Ridha, 2008; Fezzani & Cheikh, 2009; Ozkan Yucel & Gökçay, 2010; Souza et al., 2013). For example, Boubaker and Ridha (2008) used the ADM1 model to check its applicability for mesophilic anaerobic co-digestion of olive mill wastewater with olive mill solid wastes. Experimental results of the mesophilic anaerobic co-digestion of olive mill wastewater with influent total COD of 56 g/L were used for model

calibration. The calibrated model was cross-validated with the experimental results using an influent total COD of 24 and 80 g/L to check the model predictability. Souza et al. (2013) determined the feasibility of using biochemical methane production (BMP) tests as a data source for ADM1 model calibration. The calibrated model was then cross-validated with continuous digester data sets.

The principal objective of this study was to validate the ISPAD model. Accordingly, laboratory experiments were conducted using inoculum from an 8-year-old ISPAD system and glucose was used as substrate. The inoculum characteristics were similar to that used to calibrate the ISPAD model using the Simulink/Matlab software. As opposed to calibration conditions, the validation laboratory tests used a double glucose concentration of 630 mg/L, but the same temperature of 18 °C. During the laboratory experiments, glucose, VFAs and pH changes were monitored along with biogas production.

5.2. Material and Methods

5.2.1. Experimental data

For the model validation, batch experiments were conducted at 18 °C in the laboratory using an inoculum obtained from 8-year-old field ISPAD. The field ISPAD is used to treat the manure produced by a swine farrowing unit, in the central part of the Province of Quebec, Canada, near Sherbrooke. The inoculum was collected during the tank emptying operation and, because the mixing of the tank content is difficult, the inoculum solids content varies between sampling.

The batch experiments produced using the ISPAD inoculum generated curves for glucose degradation and VFAs and CH_4 production over time at temperature 18 °C. All samples were duplicated and the results were averaged. The 250 ml test bottles were filled with 150 ml of ISPAD inoculum (5.3 gVS/L) and 15 ml of 7000 mg/L of glucose solution. The total volume of liquid in each 250 ml bottle was therefore 165 ml with a VS and glucose concentration of 4.8 g/l and 630 mg/L, respectively. Duplicate control bottles were prepared with ISPAD inoculum and water instead of glucose. Bottles were capped, sealed and flushed with N_2 gas to establish anaerobic conditions, before starting the AD process and monitoring glucose and VFAs concentrations, pH changes and CH_4 production. All bottles were placed in an incubator maintained at 18 °C. The mixture was shaken by hand once a day.

Samples of 2 ml were regularly withdrawn from the bottle headspace for gas production analysis using a gas chromatograph while gas production was monitored until gas production ceased, using a water displacement apparatus. Also, 2 ml liquid samples were removed from each bottle at specified time intervals to monitor pH, and glucose and VFA concentrations.

The ISPAD inoculum was analyzed according to standard methods (Eaton & Franson, 2005) to establish: Solids (TS, VS, TSS, and VSS) and pH. To measure COD, the commercial COD test kit for ultra-high rate COD were used (DR/4000, HACH Corp). Anions including NO_3^{2-}, NO_2^-, and PO_4^{3-} and cation of NH_4^+ and total VFAs were determined by HACH kit/high rate.

To monitor gas production from the batch tests, the biogas composition (CH_4, and CO_2) was measured by injecting the 2 ml samples into a gas chromatograph (Varian, model 3800) equipped with TCD detector and CARBOXEN 1010 PLOT (capillary column) from SUPELCO, 30mm× 0.53mm column. The carrier gas was helium/argon. The column temperature was held at 50-100 °C for 5 °C/min. The injection flow was 5 ml/min.

The liquid samples were analyzed for glucose concentration, total VFAs, and pH. The glucose concentration was measured by the colorimetric method (Lever, 1972).

5.2.2. Statistical procedure

To validate the model, the ISPAD model was run using an operating temperature of 18 °C, and a glucose concentration of 630 mg/L. The model was run using the Simulink/Matlab software and the kinetic parameters obtained through model calibration (Madani-Hosseini et al., 2014b). The model prediction was then compared with experimental data using the cross-validated coefficient of determination (Q^2) as statistical parameters (Eq. 5-1). The cross-validated coefficient of determination (Q^2) quantifies the quality of the fit between the model prediction and the experimental values and the ability to correctly predict new data, respectively.

$$^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \qquad ^2 \leq 1 \qquad (5\text{-}1)$$

where n, y_i, $\hat{y}_i$, and $\bar{y}$ represent the number of data, experimental data, model predicted data, and mean of data, respectively. A Q^2 value approaching 1 shows a good prediction for the model.

The Q^2 values testing the validation were compared to the R^2 values used to test the calibration of the ISPAD model (Madani-Hosseini et al., 2014b). The Q^2 and R^2 are the same parameters with different names to distinguish between validation and calibration.

5.3. Results and Discussion

5.3.1. Inoculum characterization

The analytical results of analyses performed on the 8-year old ISPAD inoculum are presented in Table 5-1. The 8-year old ISPAD inoculum had fewer solids than 7-year-old field ISPAD inoculum used for calibration. For example, the VS of 8-year old ISPAD inoculum was half of that of 7-year-old field ISPAD inoculum (Madani-Hosseini et al., 2014b), because the sampling was conducted without reaching the bottom layer of settled solids. The other components such as pH, COD, anions, and cations did not change significantly over a year.

Table 5-1: Characteristics of the experimental inoculum

Characteristic		**Unit**	**Value**	**STD**[1]
Solids	TS	g/L	8.74	0.27
	VS	g/L	5.29	0.35
	FS	g/L	3.45	0.13
	VSS	g/L	4.01	0.09
	VDS	g/L	1.28	0.27
	TSS	g/L	4.17	0.26
pH		-	8.22	0.10
Total VFAs		gHOAC$/L$	1.19	0.25
COD		g/L	6.10	0.21
Anions	NO_2^-	g/L	0.01	0.00
	NO_3^-	g/L	0.08	0.001
	PO_4^{3-}	g/L	0.47	0.047
	SO_4^{2-}	g/L	0.83	0.035
Cation	NH_4^+	g/L	1.04	0.045

5.3.2. Model prediction and validation

Figs. 5-1 to 5-4 present the results of the laboratory experiment monitoring the glucose and VFAs concentrations, the biogas generation and the changes in pH, along with the model

prediction. The performance of the model in predicting the results was determined using the statistical parameters of Q^2 . The results showed that Q^2 values ranged from 0.49 to 0.98. Methane prediction had the highest Q^2 value of 0.98. However, CO_2 prediction had the lowest Q^2 value of 0.49. The calibration results showed that the coefficients of determination (R^2) were in the range of 0.47 to 0.96 (Table 5-2). The Table 5-2 shows that the model prediction ability is increased through validation of pH, CH_4, and VFAs.

Table 5-2: Values of R^2 from calibration and ^{2} from cross validation

Component		
Glucose	0.96	0.76
VFA	0.65, 0.54, 0.47*	0.85
CH_4	0.92	0.98
CO_2	0.65	0.49
pH	0.51	0.78

Note: *For propionate, butyrate, and acetate, respectively.

The ISPAD model predicted the glucose experimental data with a Q^2 value of 0.76. However, the model calibration showed the better R^2 value of 0.96. For the glucose concentration, the model prediction ability decreases over time, while at the beginning the model strongly predicted the glucose consumption. This is likely the result of low glucose concentrations after 3 days of experimentation, and error in the analytical results (Fig. 5-1).

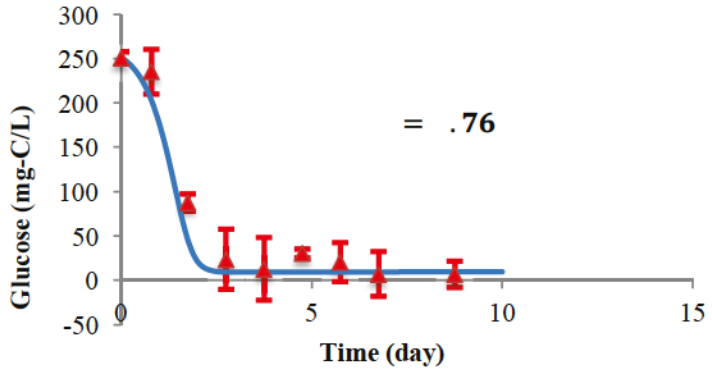

Fig. 5-1: Simulation of glucose degradation at 18 ^{0}C inoculated with ISPAD inoculum. Experimental data, triangle; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

Fig. 5-2 shows the model prediction ability for total VFAs. The model predicted the ISPAD system with the Q^2 value of 0.85, which is higher than the R^2 value for calibration of individual VFAs, propionate, butyrate, and acetate with the R^2 values of 0.65, 0.54, and 0.47, respectively. There are some occasional discrepancies between model prediction and the experimental data. The first discrepancy resulted from the one day lag phase, where the model indicated a high VFA production from the start, whereas the experimental data indicated that VFA production started after day 1. Also after day 5, VFA levels were low leading to some analytical error in the experimental data. This resulted in a general ISPAD model over predicting total VFAs.

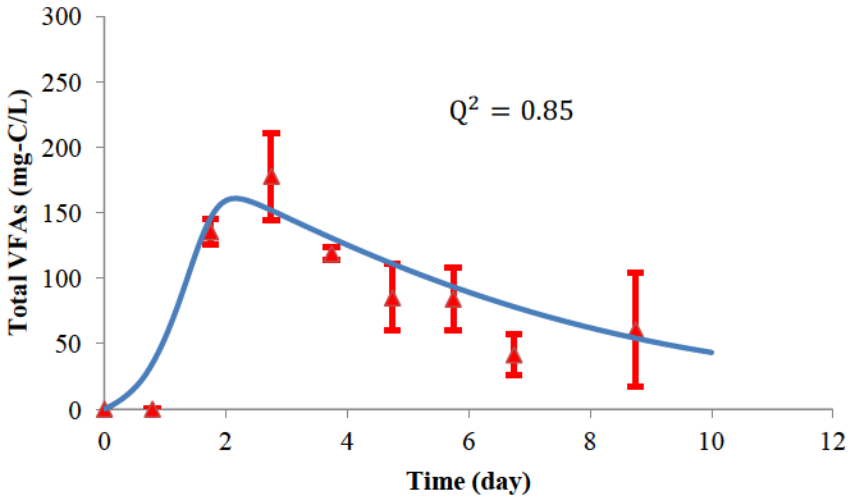

Fig. 5-2: Simulation of VFAs production of glucose degradation at 18 ^{0}C inoculated with ISPAD inoculum. Experimental data, triangle; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation

The ISPAD model was capable of accurately predicting CH_4 production, with a Q^2 value of 0.98 (Fig. 5-3), very close to the R^2 value for calibration of 0.92. The ISPAD prediction ability for CH_4 increased over time, as the model tended to under estimate values in the beginning, as also observed with the model calibration. This early over-prediction for both calibration and validation curve resulted from a one day lag phase in CH_4 production, as the inoculum had been stored at 4 °C for several months before being used.

The ISPAD model was not so accurate in predicting CO_2 especially at the beginning of the assay because of its absorption by the wastewaters, being highly soluble as compared to CH_4 (Fig. 5-3). After 3 days, the calibration curve provided a better prediction of the experimental data better than validation curve.

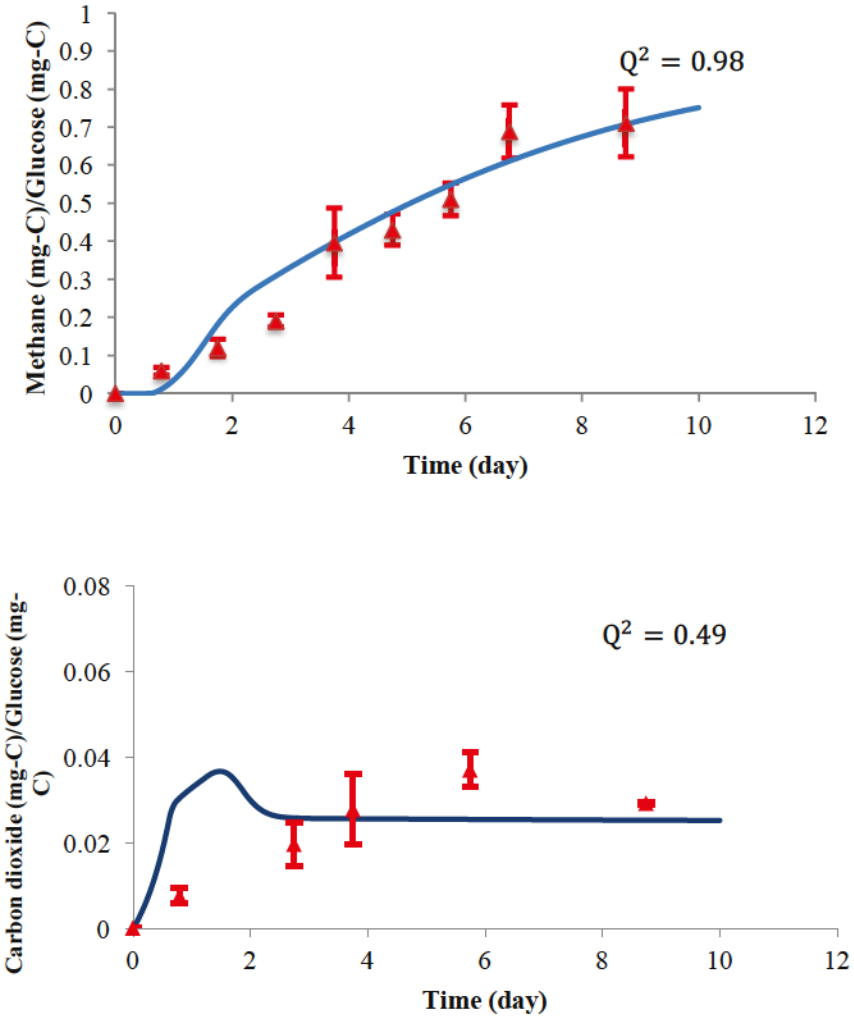

Fig. 5-3: Simulation of biogas production of glucose degradation at 18 ^{0}C inoculated with ISPAD inoculum. Experimental data, triangle; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

The model predicted the pH of ISPAD system with a $Q^2$of 0.78. However, some significant discrepancies between model prediction and the experimental data were observed specifically at

the beginning of the essay (Fig. 5-4). The model predicted a fast drop in pH right from the start, while the experimental data demonstrated a slower drop, likely as a result of the one day lag phase observed in VFA production. The validation curve was better able to predict pH changes, with a Q^2 value of 0.78 as compared to the calibration ^{2} value of 0.51.

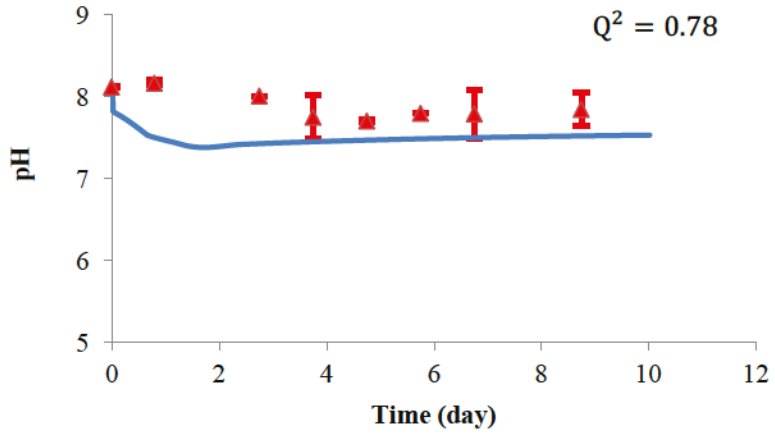

Fig. 5-4: Simulation of glucose degradation and pH at 18 °C, with the ISPAD inoculum. Experimental data, triangle; model prediction, line. Note: Data points represent the average of two replicates and error bars represent +/- one standard deviation.

The ISPAD model was able to properly predict the behavior of the ISPAD system during the validation process. However, the several assumptions made while building the ISPAD model introduce some prediction limitations:

- all parameters are calibrated with inoculum from the same ISPAD system fed swine manure;
- only glucose was tested as substrate, and a change in substrate may not be modeled as well;
- The ISPAD model was designed to predict the performance of batch fed systems;
- The ISPAD model was calibrated by laboratory scale data, and needs to be validated using prototype and field scale experimental data.

5.4.Conclusions

The capacity of the model to predict ISPAD behaviour was validated using laboratory data using a substrate concentration twice that used for its calibration at a temperature of 18 °C. In general, the cross-validation procedure produced a Q^2 value over 0.65, indicating few discrepancies with both over- and under-prediction. Therefore, the kinetic parameters obtained by Madani-Hosseini et al. (2014b) and the ISPAD model built for batch-fed systems produced a tool capable of predicting the behaviour of ISPAD inoculum when glucose was used as substrate under temperatures ranging from 4 to 35 °C. Future research will concentrate on aspects of hydrolysis to adapt the ISPAD model to various substrates.

5.5.Acknowledgments

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Chapter 6. Acidification of In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) Process to Reduce Ammonia Volatilization: Model Development and Validation

Connecting Statement

In chapter 5, the model validation using experimental data showed that it could predict the ISPAD behaviour with reasonable accuracy. Therefore, in this chapter 6, the ISPAD model was used to address the main objective of this thesis; defining management practices leading to the acidification of the ISPAD content using excessive organic loading (OL). Such acidification reduces ammonia volatilization from the digestate once removed from the system. Specifically, a mathematical equation was developed to optimize 3 different OL strategies. The 3 strategies were applied to the ISPAD model and their acidification capacity was assessed. Finally, the best strategy was selected for ISPAD model validation using laboratory tests. The appropriate acidification strategy was tested by experimental data using glucose as substrate and 8-year old ISPAD inoculum.

This chapter resulted in a manuscript which is currently under review by Journal of Waste Management. The first contributing author, Mahsa Madani-Hosseini, designed the experiments, conducted the laboratory work, analyzed the data and wrote the article. The second and third contributing authors, Dr. Catherine Mulligan and Dr. Suzelle Barrington, supervised, advised on the experimental design and methods of analysis, and revised the content of the article.

Abstract

In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) is an ambient temperature treatment system for wastewaters stored for over 100 days under temperate climates, which produces a nitrogen rich digestate susceptible to ammonia (NH_3) volatilization. Present acidification techniques reducing NH_3 volatilization are not only expensive and with secondary environmental effects, but do not apply to ISPAD relying on batch-to-batch inoculation. This study aimed at identifying and validating sequential organic loading (OL) strategies producing imbalances in acidogen and methanogen growth, acidifying ISPAD content one week before emptying to a pH

of 6, thus also preserving the inoculation potential. This acidification process is challenging as wastewaters often offer a high buffering capacity and ISPAD operational practices foster low microbial populations. A model simulating ISPAD pH regime was used to optimize 3 different sequential OLs and obtain ISPAD content acidification to a pH of 6.0. All 3 strategies were compared in terms of biogas production, VFAs concentration, microbial activity, glucose consumption, and pH drop. Laboratory validation of the model outputs confirmed that a sequential OL of 13 kg glucose/m^3 of ISPAD content over 4 days could indeed drop the pH to 6.0. Such OL competes feasibly with present acidification techniques. Nevertheless, more research is required to explain the 3-day lag between the model results and the experimental data, resulting possibly from alcohol formation.

Keywords: Anaerobic digestion, ammonia, acidification, organic load

6.1.Introduction

Operating between 0 and 20 °C, In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) accommodates temperate climatic conditions such as that of Canada to treat wastewater stored for more than 100 days. The ISPAD system is sequentially batch fed without being emptied except at the end of the storage period. A minimum depth of wastewater is kept in the ISPAD when emptied to inoculate the next batch. Tested with swine manure, ISPAD released 64% of the methane potential, because of an acclimated microbial population (King et al., 2011). Operated under psychrophilic temperatures, ISPAD biogas had a low ammonia (NH_3) content as compared to mesophilic systems (King, 2011), thus conserving nitrogen to produce a digestate rich in Total Ammoniacal Nitrogen (TAN representing NH_4^+ and NH_3) (King et al., 2012). Highly susceptible to NH_3 volatilization, such digestate can contribute to the acidification of natural ecosystems and the eutrophication of surface water bodies (Hooda et al., 2000), besides losing TAN affecting its fertilizer value.

Besides TAN concentration, the two main factors controlling NH_3 volatilization are pH and temperature, simply because they determine the ration of NH_3 to TAN. The effect of pH on the dissociation of NH_4^+ into NH_3 is described by Eq. (6-1). When the concentration of OH^- exceeds that of H^+ in solution, shift the reaction to the right and releases NH_3 free to be volatized.

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad (6\text{-}1)$$

The ratio of NH_3 to TAN is also affected by temperature as described by Eq. (6-2) and (6-3) (Loehr, 1984; Olofsson, 1975):

$$\frac{NH_3}{TAN} = \frac{1}{(1 + 10^{(pK_a - pH)})} \quad (6\text{-}2)$$

$$pK_a = 0.09018 + (\frac{2729.92}{T}) \quad (6\text{-}3)$$

where pK_a is the negative log of the dissociation constant for NH_3 and T is temperature (K). Therefore, acidification of the digestate before its removal from ISPAD could greatly reduce NH_3 volatilization.

Techniques presently used to acidify wastewaters are not only expensive but also lead to secondary environmental effects. For example, swine manure can be acidified to a pH of 5.5, using 5kg of 18M sulfuric acid/m^3 of manure. This technique increases the manure sulfur content which can produce odors and acidifies the soil receiving digestate over a long-term basis. Furthermore, it cannot be applied to ISPAD relying on batch-to-batch inoculation. An alternative solution consists in using the microbial anaerobic process to acidify the ISPAD digestate one week before emptying. Acidification of anaerobic digestion (AD) systems often results from a fast change in temperature and/or organic loading rate, as VFAs producing acidogen can adapt and grow more quickly than VFAs consuming methanogens. For example, ISPAD acidogens produced an estimated maximum microbial growth rate (μ_{max}) of 1.10 to 7.9/day between 4 and 35 °C as compared to 0.19 to 0.60/day for methanogens (Madani-Hosseini et al., 2014b). Considering the operation of ISPAD, organic loading (OL) is the main acidification strategy as temperature is governed by ambient conditions.

For ISPAD to inoculate itself from batch to batch, pH drop should be limited to 6.0 to maintain an active methanogen population (Lahav & Morgan, 2004) while still substantially lowering NH_3 volatilization. For wastewaters with a TAN concentration of 3000 mg/L, a drop in pH from 7.0 to 6.0 resulted in a drop in NH_3 from 0.5 mg/L to 0.015 mg/L (Bussink et al., 1994;

Frost et al., 1990; Loehr, 1984; Stevens et al., 1992).

The acidification effect of OL was investigated for two stage AD systems, with an accompanying drop in pH of 1.5 to 2.6 unit after 7 days of operation (Alkaya & Demirer, 2011; Hutňan et al., 2010). Stamatelatou et al. (2003a) simulated the effects of sequentially overloading an anaerobic baffled reactor, using glucose dosages increased over time. Stamatelatou et al. (2003b) were able to accumulate sufficient VFAs to drop the pH to 4.0. Although increasing the OL can drop the pH of AD systems, the buffering capacity of the wastewater can be a challenge. Ho (2010) showed that increasing the organic loading rate had no effect on lowering pig manure pH due to its high natural buffering capacity.

The buffering capacity of an AD reactor depends on the alkalinity of its content namely, the concentration of bicarbonate ion (HCO_3^-), ammonium (NH_4^+), and total dissolved solids. Governed by its dissolved CO_2, HCO_3^- is the main source of buffering capacity while NH_4^+ concentration is governed by the TAN content of the wastewater (Procházka et al., 2012). There is a direct positive relationship between total dissolve solids and alkalinity (Rtins & Probst, 1991). For example, municipal wastewaters have a Total Solids (TS) and alkalinity of 0.0350-1.2% and 50-200 mg $CaCO_3$/L, respectively, as compared to swine manure with 0.4 to 4.0% TS and a buffering capacity of 1750 to 7900 mg $CaCO_3$/L, respectively.

The acidification of ISPAD can be challenging. In this project, inoculum was obtained from a field ISPAD system treating swine manure. Thus, a high buffering capacity was expected along with a low microbial population, resulting from the limited OL of ISPAD especially towards the end of the batch. At such time, the OL can be as low as 0.4 kg of VS/m^3 of ISPAD content, whereas conventional systems receive in the range of 1.5 to 4.0 kg VS/m^3 of reactor.

The main objective of this study was therefore to identify OL strategies capable of acidifying ISPAD content within one week before emptying. To achieve such objective, the project aimed at: i) identifying optimal OL strategies capable of acidifying the ISPAD content to pH of 6; ii) verifying if ISPAD acidification can be achieved using the optimized OL despite of ISPAD high buffering capacity and low microbial population; iii) validating the model by laboratory experiments, using one of the optimized acidification strategy, and; iv) further refining acidification strategies to optimize the process cost.

Acidification of ISPAD was simulated using the model developed by Madani-Hosseini et al. (2014b) which is capable of simulating pH regime, glucose and VFA concentrations and methane generation. The ISPAD model simulates all AD processes except for hydrolysis, explaining the use of glucose as OL to represent hydrolyzed sugar rich wastes. Three linearly increasing OL strategies were optimized using the ISPAD model and simulated for their acidification potential. The optimal OL strategy was determined by investigating: i) the feeding frequency, namely the optimal time interval, Δt, between two successive additions of glucose, and; ii) the OL or amount of glucose to be fed at each time interval. The acidification modeling was presumed to occur at 22 °C, a normal and achievable temperature for the ISPAD content in late spring and early summer, corresponding to the land spreading season. The simulation extended over 8 days to produce sufficient data to observe differences among strategies and to experimentally validate the results. Finally, the appropriate acidification strategy was selected and validated by experimentation where the inoculum was collected at the end of the storage period of an 8-year-old field ISPAD system treating swine manure.

6.2.Materials and Methods

6.2.1. The ISPAD model

The ISPAD model developed by Madani-Hosseini et al. (2014b) was used to predict optimize the acidification strategy using a linearly increasing OL fed at a fixed time interval. The ISPAD model was specifically designed to:

i) simulate a sequentially fed batch reactor totally emptied at intervals of at least 100 days, except for a volume remaining to inoculate the new batch;

ii) adjust microbial kinetics to a range of temperatures between 4 and 35 °C, covering psychrophilic and mesophilic conditions (Madani-Hosseini et al., 2014c);

iii) compute methane production from two types of methanogens, the acetoclastic and the hydrogenotrophic, consuming different substrates and requiring different environmental growth conditions, and;

iv) include a homoacetogenesis step, a dominant process at low temperatures, which converts carbon dioxide to acetate.

The kinetic values used for the ISPAD model (Table 6-1) were obtained using inoculum from an 8-year-old field ISPAD system treating swine manures in the Drummondville area, in the center of the province of Quebec, Canada (Madani-Hosseini et al., 2014b).

Table 6-1: Estimated kinetic coefficients (Madani-Hosseini et al., 2014b)

Process	Parameter	Unit	Value		
			4 ^{0}C	18 ^{0}C	35 ^{0}C
1. Acidogenesis	μ_{max}	$1/day$	1.10	3.70	7.90
	K_s	mg/L	321	140	35
	Y	mg/mg	0.110	0.110	0.030
2.Propionate degrading acetogenesis	μ_{max}	$1/day$	0.01	0.05	0.03
	K_s	mg/L	189	111	21
	Y	mg/mg	0.010	0.030	0.090
	K_i	mg/L	960	960	960
3. Butyrate degrading acetogenesis	μ_{max}	$1/day$	0.14	0.90	0.08
	K_s	mg/L	213	124	150
	Y	mg/mg	0.020	0.008	0.026
	K_i	mg/L	720	720	720
4. Homoacetogenesis	μ_{max}	$1/day$	0.44	0.73	-
	K_s	mg/L	300	160	-
	Y	mg/mg	0.042	0.058	-
	X	mg/L	65.0	65.0	-
5.Acetoclastic Methanogenesis	μ_{max}	$1/day$	0.19	0.23	0.60
	K_s	mg/L	210	351	113
	Y	mg/mg	0.010	0.038	0.060
	K_i	mg/L	260	260	260
6.Hydrogenotrophic Methanogenesis	μ_{max}	$1/day$	0.1	0.23	0.32
	K_s	mg/L	170.00	32.5	29.00
	Y	mg/mg	0.015	0.005	0.03

Note: μ_{max}: Maximum microbial growth rate, K_s: half saturation constant, Y: microbial yield, X: microbial biomass, and K_i: inhibition kinetic

The ISPAD model was calibrated and validated for all steps of AD, except hydrolysis.

Accordingly, the model was operated assuming that the OL, consisting of a sugar rich organic waste, has been degraded into glucose. The ISPAD model was found to predict glucose degradation, system pH regime, VFAs concentrations and methane production with an accuracy represented by a coefficient of determination (R^2) of 0.76, 0.78, 0.85, and 0.98, respectively (Madani-Hosseini et al., 2014a). The experimental swine manure ISPAD inoculum offered a relatively high buffering capacity because of its alkalinity of 4500 mg carbonate/L and its TAN of 1040 mg/L.

6.2.2. Feeding scenarios and parameter variation for acidification

The nature of the ISPAD system is such that OL is the main manageable parameter. For ISPAD, acidification can be carried out one week before emptying, at a temperature corresponding to before and after the cropping season, namely May and October. At this time of year, the temperature of ISPAD content ranges from 20 to 25 °C (Giard et al., 2013). To represent such conditions, a temperature of 22 °C was selected to run the model and laboratory experiment.

In this study, the acidification strategies were developed based on sequentially applying an OL, represented by glucose, at specific time intervals and increasing linearly over time, to avoid microbial inhibition. Thus, OL can be sequentially fed and increased over time in parallel with acidogen growth. Therefore, OL optimization requires the determination of frequency and load evolution.

The glucose fed at each sequence or OL can thus be defined and fixed as $\{A(t) = (A_f)\}$, or if variable as $\{A(t) = (A_v(t)\}$, or even if mixed as $\{A(t) = (A_f + A_v(t))\}$, where $A(t)$ is the OL fed at time t. By definition, $A(t) = 0$ when t is not a integer multiple of Δt. A simple strategy is a fixed OL such that $A(t = 0) = A(t = \Delta t) = A(t = 2\Delta t) = \quad = A_f$. A variable OL strategy can be defined from $A_v(t)$ representing the variable component at time t. The third approach is to consider a mixed OL, $(A_f + A_v(t))$, where both A_f and $A_v(t)$ are optimized.

Accordingly, the OL optimization can be defined as:

$$A(t) = \begin{cases} A_f + A_v\,(t) & \text{i t i an integer multiple o } \Delta t \\ 0 & \text{otherwi e} \end{cases} \quad (6\text{-}4)$$

where

Δt: Time interval between two successive additions of glucose to the digester;

$A(t)$: The amount of glucose to be added at time t;

A_f: Fixed amount of glucose to be added to the digester at time t;

A_v: Variable amount of glucose to be added to the digester at time t.

Note that Eq. (6-4) gives a general formula for the loading model. Specifically, glucose is added only if t is an integer multiple of Δt. Also, $A_v(t)$ can be further defined by letting $K_t = \frac{t}{\Delta t}$. Clearly, from Eq. (6-4), glucose will be added at time t, if K_t is an integer number. Therefore:

$$A(t) = \begin{cases} A_f + K_t A_v & \text{i } K_t \text{ i an integer number} \\ 0 & \text{otherwi e} \end{cases} \quad (6\text{-}5)$$

The OL and its variation over time was defined by applying Eq. (6-5) to the ISPAD model of Madani-Hosseini et al. (2014b), where OL was simulated as glucose produced from the hydrolysis of a sugar rich waste. Whereas the fixed strategy was simulate using a constant feeding regime, the mixed and variable strategies were designed to increase the OL in parallel with the growth of acidogen population.

The values for A_f, A_v, and Δt were optimized initially for all 3 strategies using both the optimization tool Matlab/Simulink and the Solver in Excel. For all 3 strategies, the pH drop was limited to 6.0. Using the fixed strategy as an example, a series of A_f and Δt were used in the ISPAD model, each generating a pH drop regime down to 6.0 over 8 days. The optimization tools were given the obtained data to find the optimized values for A_f and Δt.

6.2.3. Experimental set up and operation

To validate the acidification model, laboratory tests were conducted using a 2L anaerobic digester (Fig. 6-1) which included: the inlet to feed the substrate; the reactor; the liquid sampling port, and; the gas outlet with its measurement system. Using a mixer rotating at 50 rpm, the

ISPAD reactor was regularly mixed to provide sufficient contact between the substrate and the microbial population. Although field ISPAD systems are not mixed, the laboratory set-up was mixed to obtain more consistent results and to shorten the reaction period. An L/S Precision variable-speed drive pump (Master Flex, Canada) was used to feed the substrate.

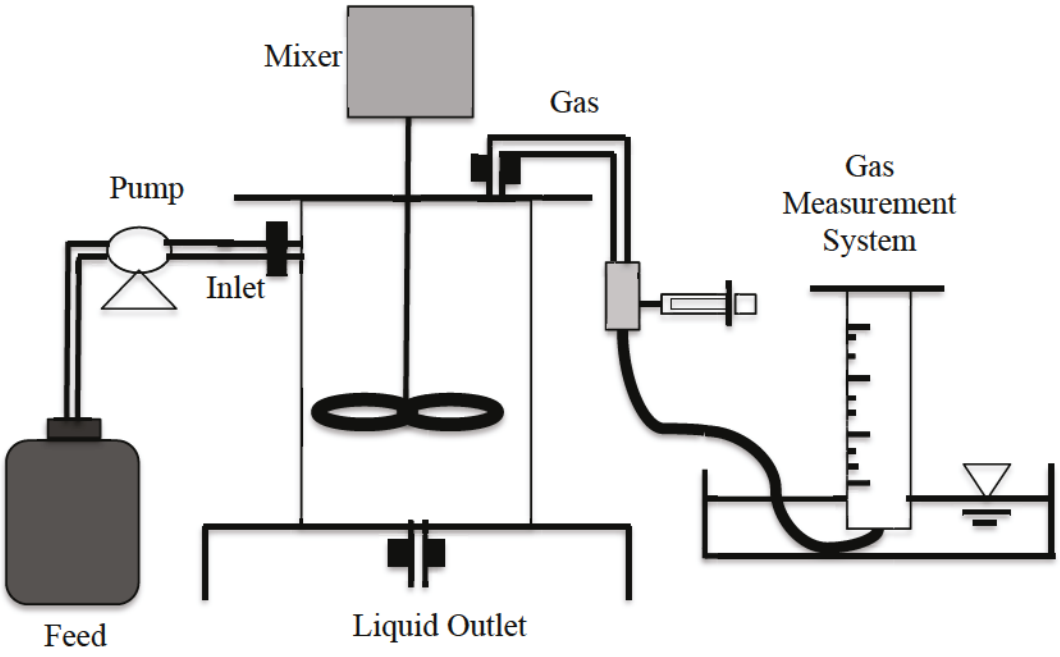


Fig. 6-1: Experimental set up.

The experimental procedure used inoculum collected from a 8-year-old field ISPAD treating swine manure in St-Francois-Xavier of the Drummondville region, Quebec, Canada. After receiving 500 ml of ISPAD inoculum, the reactor was capped, sealed and flushed with N_2 gas to establish anaerobic conditions. The glucose solution was added at the optimized OL interval (Table 6-2). Once in operation, gas production was measured using a water displacement system. The volume of water displaced is equal to the volume of gas produced, as measured by a graduated cylinder.

For 7 days, samples were regularly taken from the reactor to follow the glucose concentration,

VFAs alkalinity, and TAN concentration, and pH. Biogas production was monitored using a water displacement system and sampled for CH_4 quantification. Duplicate samples were collected, analysed and the results were averaged.

Table 6-2: Amount of glucose solution addition

Time of addition (day)	Glucose (mg/L)[1]	Glucose solution (ml) with concentration of 20000 mg/L	Total volume of digester[2] (ml)
0	312.5	7.94	507.94
1	1585	43.72	551.66
2	2197.5	68.10	619.75
3	2492.5	88.23	707.98
4	3042.5	127.03	835.01
5	3592.5	182.83	1017.84
6	4142.5	265.89	1283.73

Note: [1]The glucose concentration reached after the feeding operation. The glucose solution had a concentration of 20000 mg/L. [2]Includes inoculum and glucose solution.

Results obtained experimentally were compared with those predicted by the ISPAD model through the correlation coefficient (R) calculation (Eq. 6-6). The correlation coefficients were computed for glucose, VFAs, methane, and pH prediction for 7 days of operation.

$$R = \sqrt{1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}} \qquad (6\text{-}6)$$

where n, $\boldsymbol{y_i}$, $\boldsymbol{\hat{y}_i}$, and $\boldsymbol{\bar{y}}$ represent the number of data, experimental data, model predicted data, and mean of data, respectively.

6.2.4. Analytical analysis

The ISPAD inoculum was analyzed for solids, COD, total VFAs, NH_4^+, anions, and pH. The solids were analyzed according to standard methods (Eaton & Franson, 2005) to establish TS, VS, VSS, FS,VDS, VSS/FS, and VDS/FS. The COD, total VFAs, anions including NO_2^-, NO_3^-, PO_4^{3-}, Cl^- and cations of NH_4^+ were analyzed by commercial Hach kits.

The samples from reactor were taken on a regular basis and analyzed for glucose concentration, pH, total VFAs, alkalinity, TAN, and methane. The liquid samples were analyzed for total VFAs and TAN by commercial Hach kit. The alkalinity was monitored by titration according to the method No. 2320B (Clesceri et al., 1998). The pH of all samples was measured using pH meter. Gaseous samples were analyzed immediately by gas chromatograph (Varian, model 3800) equipped with TCD detector and CARBOXEN 1010 PLOT (capillary column) from SUPELCO, 30mm× 0.53mm column. The carrier gas was helium/argon. The column temperature was held at 50-100 °C for 5 °C/min. The injection flow was 5 ml/min. The glucose concentration will be measured by the colorimetric method (Lever, 1972).

6.3.Results and Discussion

Results are categorized into 3 parts, namely the model prediction (sections 6.3.1 and 6.3.2), the model validation using experimental data (sections 6.3.3 and 6.3.4), and acidification optimization (section 6.3.5).

6.3.1. Optimized acidification parameters

The acidification model parameters were optimized to drop the pH to 6.0, for an active methanogen population to inoculate the next ISPAD batch. Table 6-3 shows the optimized values of Δt, A_f, and A_v for the fixed, variable, and mixed strategies. The time interval between glucose substrate addition, Δt, for the fixed strategy was found to be 12 h, while that of the variable and mixed strategies was 24 h**.** For the fixed strategy, the optimized A_f value was 550 mg-C/L as compared to that of the mixed and variable strategies at 330 mg-C/L, respectively. For the variable and mixed strategies, the A_v values were 300 and 220 mg-C/L, respectively.

Table 6-3: Optimized acidification parameters for all three organic loading (OL) strategies.

Parameters	Strategy		
	Fixed	**Variable**	**Mixed**
Δt (day)	0.5	1	1
A_f (mg-C/L)	550	0	330
A_v (mg-C/L)	0	300	220

The optimized parameters produced cumulative amounts of fed glucose differing over time but totaling similar loads on day 7. The fixed OL produced the highest initial cumulative OL followed by the mixed and then the variable OL (Fig. 6-2).

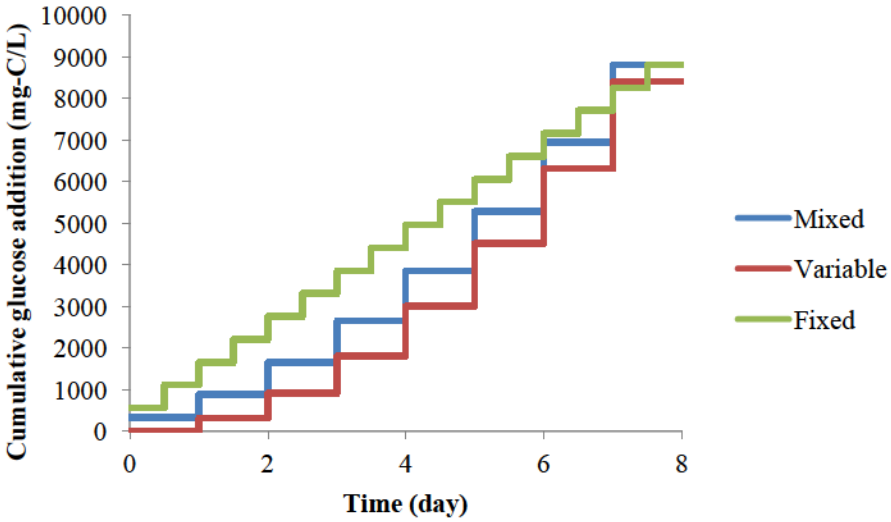
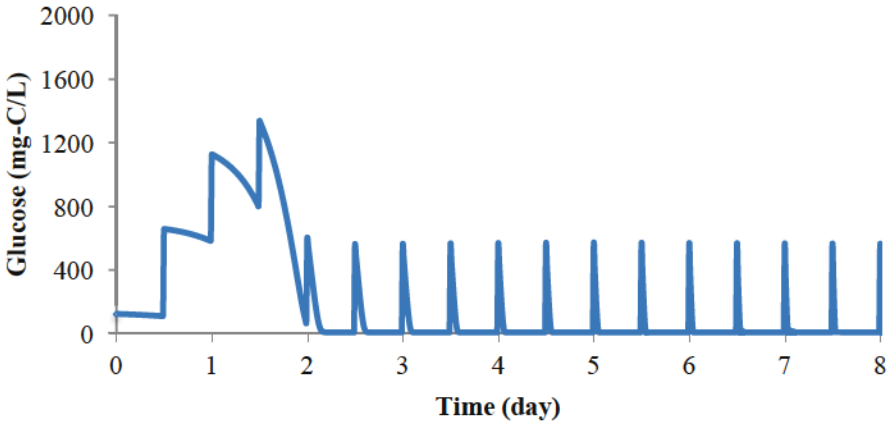


Fig. 6-2: Cumulative mass of glucose fed for each one of the three organic loading (OL) strategies.

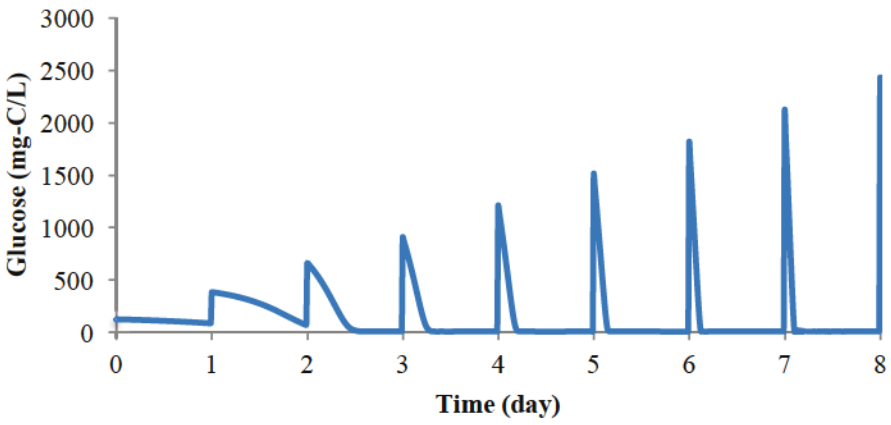
6.3.2. Acidification prediction by ISPAD model

For all 3 strategies, predicted glucose concentration demonstrated a residual effect only for

the first 2 days after which glucose levels would drop to practically zero before the next sequential feeding (Fig. 6-3a, 6-3b and 6-3c). The fixed OL produced glucose peaks of 557 mg-C/L after each feeding, which quickly dropped to 7 mg-C/L after 12 h. The variable and mixed OL produced increasing peaks in glucose concentration after each feeding as prescribed by their strategy, also falling back to almost zero before the next sequential feeding. Thus, the OL strategies were able within a few days, to build an ISPAD microbial population capable of degrading high levels of glucose.



(a)



(b)

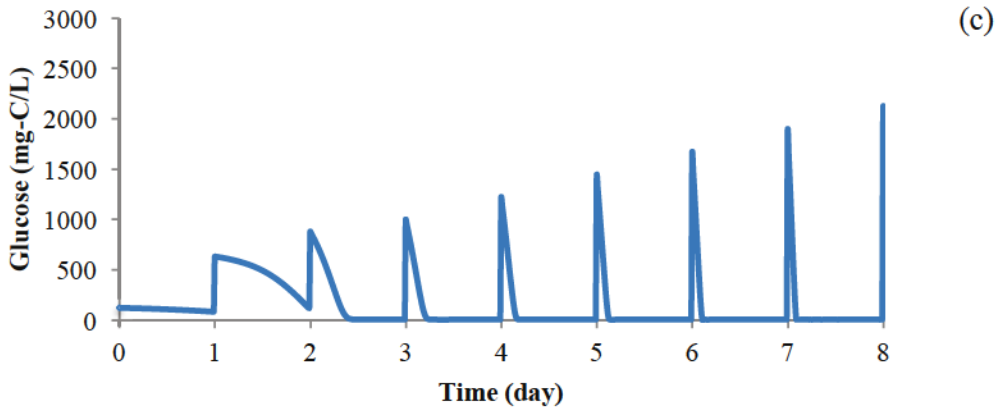
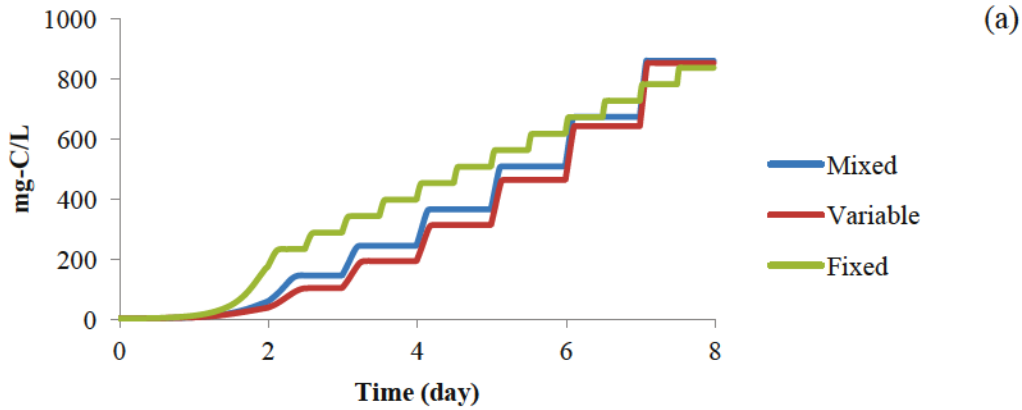


Fig. 6-3: Glucose levels prediction for the fixed (a), variable (b) and mixed (c) organic loading (OL) strategies.

Indeed, Fig. 6-4a illustrates an acidogen population which follows the stepwise cumulative glucose OL for all 3 strategies. As compared to the other 2 strategies, the fixed OL resulted in a faster climb in acidogen population because of the larger mass of glucose fed initially. On day 7, the 2 other OL strategies produced acidogen populations matching that of the fixed strategy, because of a similar cumulative glucose OL. As for the methanogens, their population growth followed a smooth curve, still at a density matching the glucose OL of each strategy (Fig. 6-4b). Among all 3 strategies, the fixed OL resulted in a slighlty higher methanogen population as of day 2, whereas that of the variable and mixed remained quite similar. The higher methanogen population associated with the fixed strategy resulted from the stimulation of higher VFAs concentrations with a pH above 6.0.



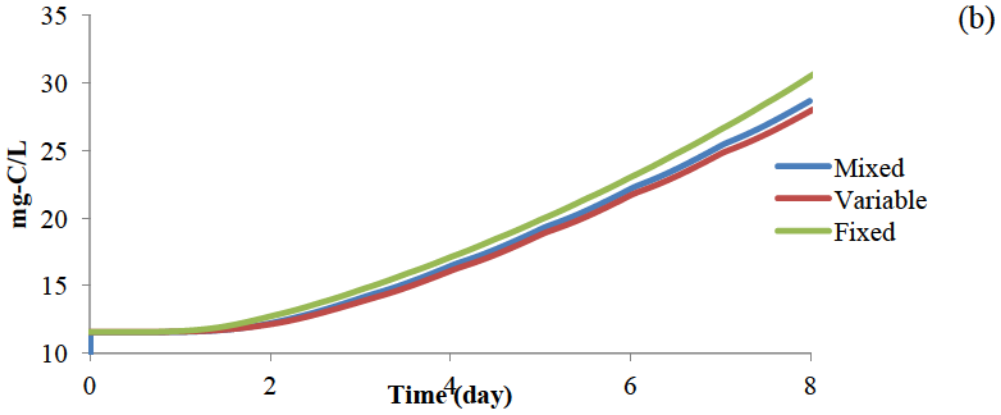


Fig. 6-4: Population size of acidogens (a) and acetoclastic methanogens (b) for the three organic loading (OL) strategies.

From day 2, the fixed strategy produced higher VFA accumulations compared to the other 2 strategies with the variable strategy producing slightly less VFAs levels (Fig. 6-5), in parallel with the cumulative feeding of glucose. By day 7, the mixed and variable strategies had produced VFA accumulations similar to that of the fixed strategy.

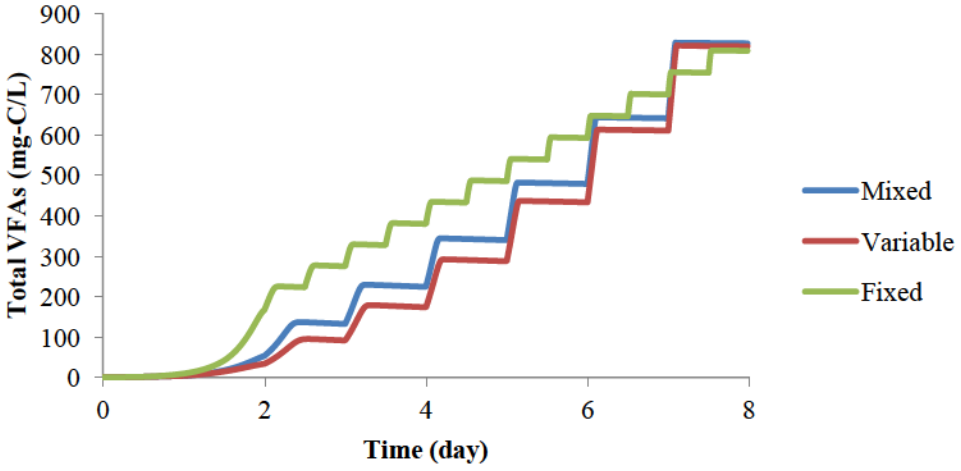


Fig. 6-5: Predicted VFAs concentration for all three organic loading (OL) strategies.

Initially at 8.0, the predicted pH regime also followed a trend representative of the cumulative glucose OL (Fig. 6-6). The 3 strategies produced similar pH levels during the first day, after which, the fixed strategy quickly dropped the pH to 6.3 on day 2, whereas the pH of the other 2 strategies dropped to 6.5 and 6.6. Nevertheless, the fixed strategy suffered a very slow pH drop after day 3 to finish at 6.2 on day 8, whereas the variable and mixed strategies produced faster pH drops reaching 6.0 and 5.8 respectively. The simulation predicted that the ISPAD microbial populations were able to drop the pH to 6.2 or lower, within one week.

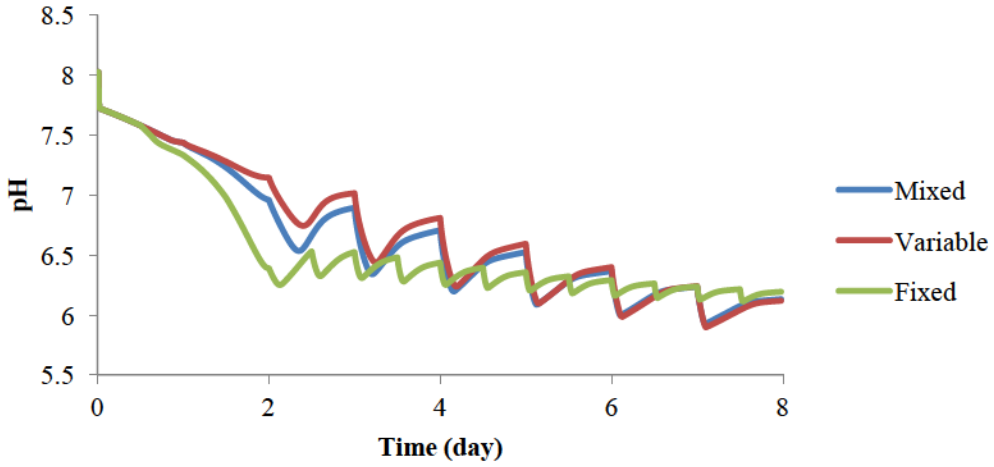


Fig. 6-6: The predicted pH regime for all three organic loading (OL) strategies.

All strategies produced CH_4 during the test period (Fig. 6-7). The fixed strategy produced more methane from day 2 to 7, because of its higher cumulative glucose feeding. On day 8, the cumulative CH_4 production of the variable and mixed strategy had reached that of the fixed strategy.

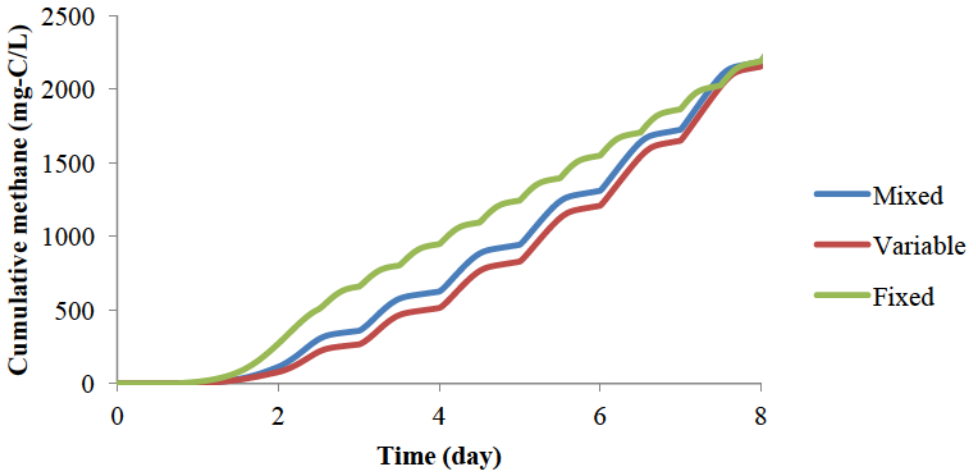


Fig. 6-7: Cumulative methane production for all three organic loading (OL) strategies.

After 7 days of feeding, the fixed, variable and mixed strategy required 51.0, 46.7, and 47.0 kg of glucose/m^3 of ISPAD content, respectively, using swine manure inoculum from a 8-year-old field ISPAD. With industrial grade glucose selling for \$200/ton, these 3 strategies cost \$10.20, \$9.30, and \$9.40/m^3 of ISPAD content, respectively. A lower cost can be achieved with sugar rich wastes valued at \$0.2/ton, with a 50% sugar content. For example, the mixed strategy would require 94 kg of sugar rich waste/m^3 of reactor volume representing a cost of \$1.88/m^3. Such an acidification method compares with the use of 5kg of 18M sulfuric acid/m^3 at a cost of \$2.00/m^3. Furthermore, AD acidification is more sustainable environmentally, considering the impact of not only manufacturing but also using sulfuric acid to acidify wastewaters.

To validate the acidification model in the laboratory, the mixed strategy was selected because of its lower cost and slightly greater acidification potential while still allowing for an active methanogens growth in parallel with that of the acidogens. The fixed strategy used more glucose than the mixed and variable strategies, indicating that a gradual increase in OL is preferred if the biomass is to adjust to new conditions (Stamatelatou et al., 2003b).

6.3.3. Inoculum characteristics

The experimental ISPAD inoculum is characterized in Table 6-4. As compared to fresh swine manure, the ISPAD inoculum offered lower VSS/FS and VDS/FS ratios indicating the loss of a major portion of its organic fraction through AD. The ISPAD inoculum also offered more solids, anions, cations, and alkalinity compared to typical municipal wastewater (Table 6-4), indicating a greater challenge in AD acidification. Based on its low VFA concentration and high pH, the ISPAD inoculum was acclimated to its operational conditions (Kotsyurbenko, 2005) and offered a fully functional microbial population (Wilkie, 2005).

Table 6-4: Characteristics of the ISPAD inoculum compared to fresh swine manure and municipal wastewater.

Characteristic		Unit	Fresh manure[1]	Municipal Wastewater[2]	ISPAD inoculum
Solids	TS	g/L	48.01	0.35-1.2	8.74
	VS	g/L	34.34	0.105-0.325	5.29
	VSS	g/L	27.38	0.08-0.275	4.01
	VDS	g/L	6.96	0.025-0.05	1.28
	FS	g/L	13.67	0.02-0.07	3.45
	VSS/FS		2.01	3.92-4	1.16
	VDS/FS		0.51	0.71-1.25	0.37
pH		-	6.90	-	8.22
COD		g/L	83.4	0.25-1	5.95
Total VFAs		$g\ acetate/L$	8.34	<0.1- >0.4	1.19
Anions	Cl^-	g/L	1.16	0.03-0.10	0.02
	NO_2^-	g/L	0.10	0	0.01
	NO_3^-	g/L	0.00	0	0.08
	PO_4^{3-}	g/L	0.53	0.004-0.0015	0.47
	SO_4^{2-}	g/L	0.00	0.002-0.005	0.83
Cation	NH_4^+	g/L	3.73	0.012-0.050	1.04
Alkalinity		$g\ carbonate/L$	-	0.05-0.20	4.4

[1] King (2011)

[2] Tehobanoglous and Burton (1991)

6.3.4. Acidification model validation

The mixed OL strategy was selected to validate acidification prediction using the ISPAD model with laboratory data sequentially feeding over 6 days, 31 g glucose/L of initial ISPAD content. Glucose degradation and VFA accumulation were both predicted with a strong R of 0.96 (Figs.6- 8 and 6-9).

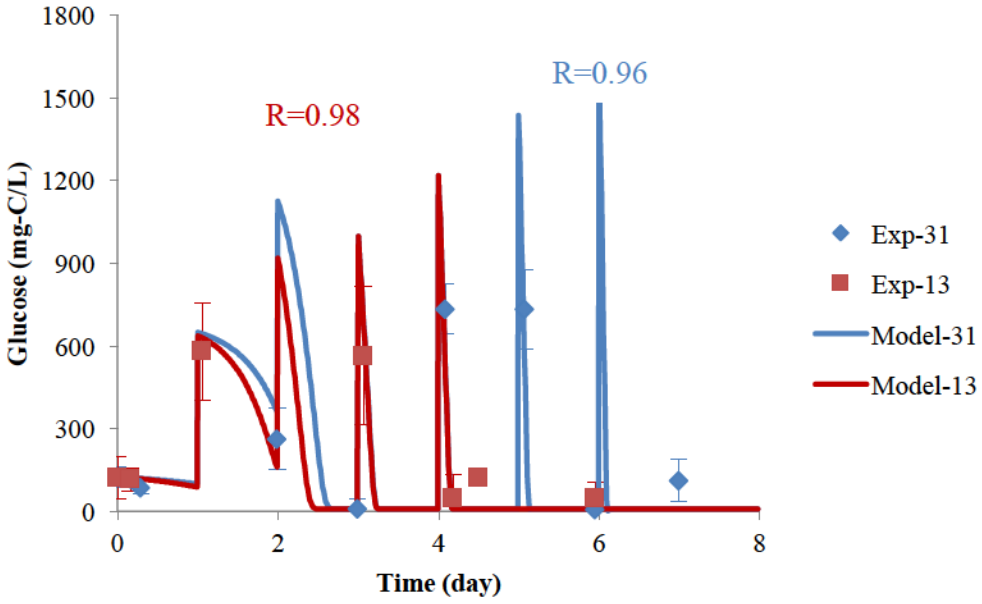


Fig. 6-8: Simulation of glucose degradation with the ISPAD inoculum for a total organic load (OL) of 31 and 13 g glucose/L of initial ISPAD content. Experimental data, point; model prediction, line. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

For VFAs, the model showed a 2-day lag phase followed by a stepwise increase reaching 7500 mg-acetate/L of digester after 7 days of glucose feeding. As compared to the experimental data, the ISPAD model under predicted VFA concentration during this 2-day lag phase, and then over predicted VFA concentration from day 2 to 7 day. This over prediction likely resulted from the production of an intermediate product, such as alcohols observed for AD in an mixed-acid fermentation environment when pH decreases (Stamatelatou et al., 2003a). This kind of shift, following a drop in pH, results from microorganisms trying to maintain their intracellular pH (Lowe et al., 1993). A carbon balance calculation estimates that the accumulation of intermediate

products, such as alcohol, represented 45% of total glucose fed, when averaged over the 7-day test period.

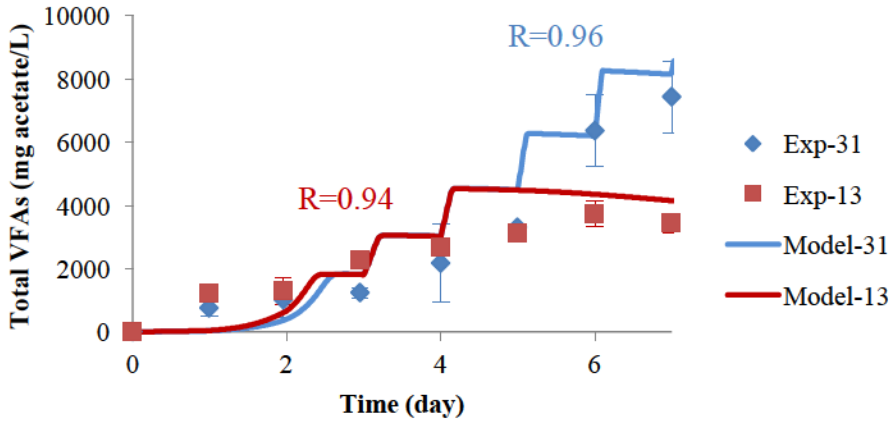


Fig. 6-9: Simulation of VFAs concentration with the ISPAD inoculum for a total organic load (OL) of 31 and 13 g glucose/L of initial ISPAD content. Experimental data, point; model prediction, line. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

Although respecting an R of 0.94 (Fig. 6-10), the model over predicted CH_4 production in parallel with the over predicted VFAs.

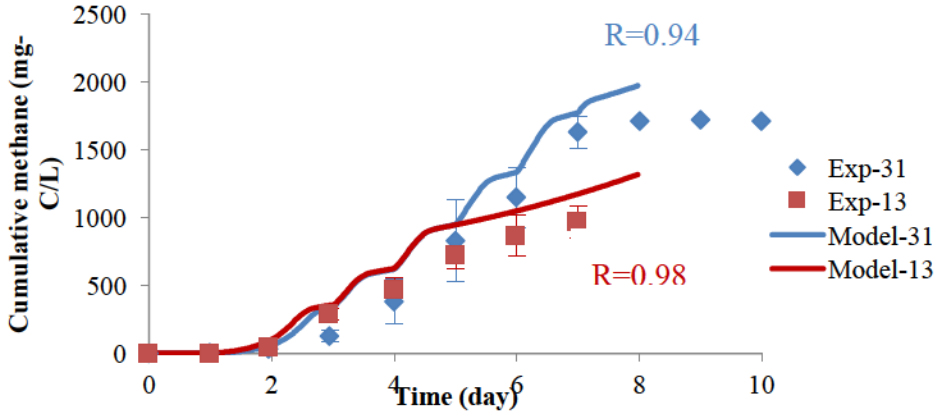


Fig. 6 10: Simulation of cumulative methane production with the ISPAD inoculum for a total organic load (OL) of 31 and 13 g glucose/L of initial ISPAD content. Experimental data, point; model prediction, line. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

After 7 days, the cumulative CH_4 production reached 1630 mg-C/L, and 3 days after stopping the glucose feeding, namely on day 10, CH_4 production ceased because of a pH drop to 4.5, inhibiting methanogen activity. The calculated CH_4 potential at 22 °C was 0.21 L CH_4/g VS added, for a 50% conversion of glucose into CH_4, because of an important amount of accumulated as VFAs.

Despite being able to follow the trend with a corresponding R of 0.92, the model predicted a lower pH during the first 3 days of essay (Fig. 6-11) and a higher pH up to day 7. Interestingly enough, the experimental data continued to drop after stopping glucose feeding on day 7, and reached 4.5 on day 9 to remain at that value on day 10. The ISPAD model stopped dropping the pH below 6.0 because of its pH inhibition function increasing exponentially at this value.

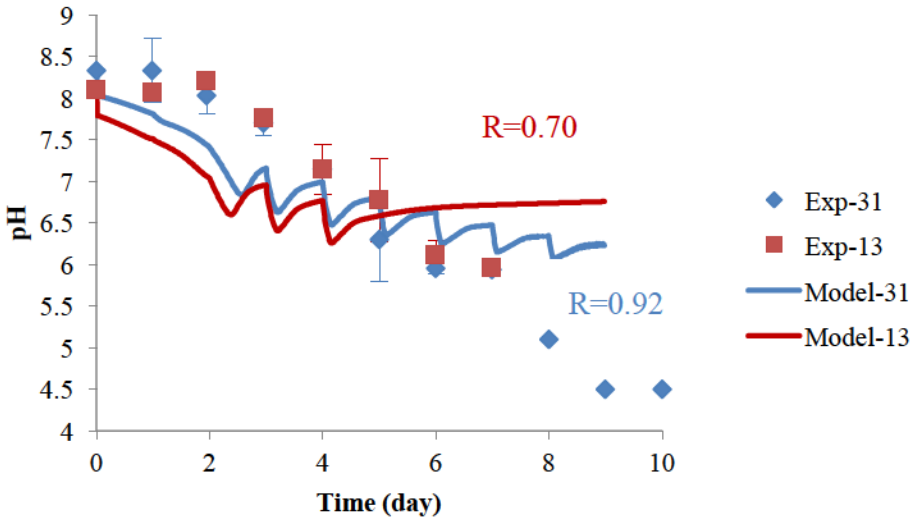


Fig. 6-10: Simulation of pH regime with the ISPAD inoculum for a total feeding of 31 and 13 g glucose/L of initial ISPAD content. Experimental data, point; model prediction, line. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

Such experimental results suggest that a shorter and thus lower glucose OL can be applied for the ISPAD content to reach a pH of 6.0. Accordingly, a second experiment was conducted with

the same OL as that of 31 g glucose/L of initial ISPAD content fed over 7 days, but stopped on day 4 for the cumulative feeding of only 13 g glucose/L.

6.3.5. Testing of an alternative organic loading strategy

Validation of the ISPAD model indicated that there is a lag in acidification prediction because of the accumulation of intermediate products such as alcohols, and whereas the model stops at a pH drop to 6.0, the experimental data indicates a drop which continues beyond that point while still maintaining CH_4 production. To lower even further the OL below 31 g glucose/L of ISPAD content over 7 days, a shorter but just as intensive OL strategy was tested in the laboratory experiments and its outcome was predicted using the ISPAD model.

Figures 6-8 to 6-11 compare the results obtained experimentally and by modeling the 13 and 31 g of glucose/L of ISPAD content, fed respectively over 4 and 7 days. Figure 6-8 illustrated similar glucose concentrations for both OL and a good model prediction for an R value of 0.98 and 0.96, for the low and high OL respectively. With 13 g/L strategy, the experimental VFA data peaked on day 6, at 3500 mg/l, as compared to that predicted peaking on day 4, at 4500 mg/l. In parallel and while still generating CH_4 (Fig. 6-10), the 13 mg/L OL produced a pH of 6.0 on day 6, as compared to that predicted which dropped to 6.2 on day 4, and quickly bounced back to 6.5 on day 5 (Fig. 6-11). Thus, the experimental data indicated that: i) an OL of 13 g of glucose/L of ISPAD content was able to drop the pH to 6.0 within 6 days, and; ii) the ISPAD model needs to be further improved to account for the accumulation of intermediate product such as alcohol, under conditions where the pH is dropping quickly. According to Eq. 6-2 and Eq. 6-3, when the ISPAD digestate pH dropped from 8 to 6 with TAN of 1100 mg/L at 22 ^{0}C, the NH_3 volatilization will be decreased by 100 times.

For the lower OL strategy of 13 g glucose/L, cumulative methane production reached 0.22 LCH_4/g VS added, which corresponds to that of the higher OL strategy at 0.21 LCH_4/g VS added of 31 g glucose/L.

While testing the low OL feeding strategy, the buffering capacity of the system was evaluated by measuring its TAN and alkalinity (Figs. 6-12 and 6-13). The monitoring of TAN showed a slight increase only for the higher OL strategy after 6 days, likely because of the higher energy

level required to break down organic N.

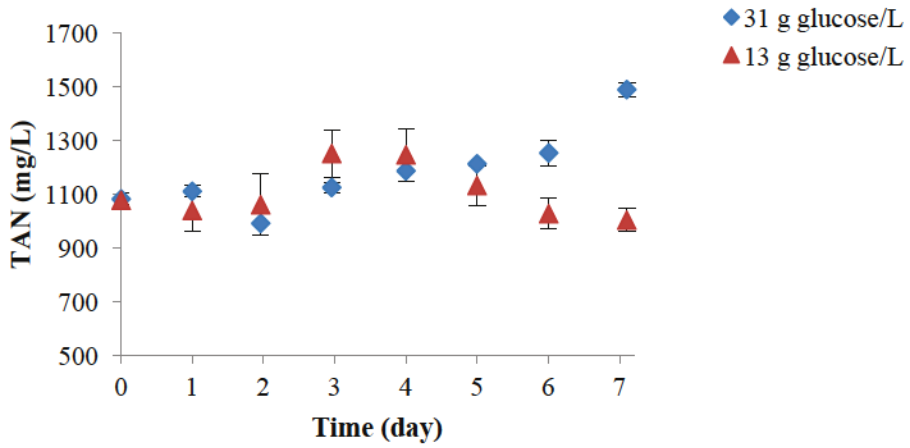


Fig. 6-11: Total ammoniacal nitrogen (TAN) concentration for the 2 organic loading (OL) strategies. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

The OL of 13 g glucose/L dropped the alkalinity from 4400 to 3300 mg carbonate/L over 7 days, because of VFA accumulation (Fig. 6-13). Such high alkalinity value confirms that AD can acidify the ISPAD content when the OL strategy is optimized, and despite its low initial microbial populations.

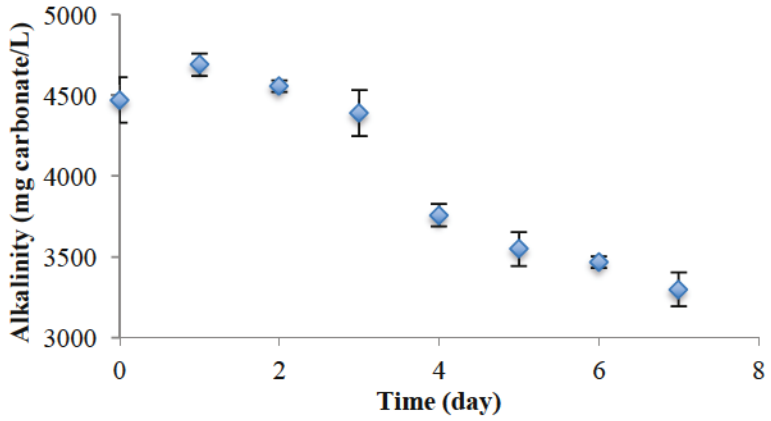


Fig. 6-12: Alkalinity for a total feeding of 13 g glucose/L of initial ISPAD content. Data points represent the average of two replicates and error bars represent +/- one standard deviation.

6.4.Conclusions

The objective of this study was to define management practices to use microbial populations to acidify the content of In-Storage-Psychrophilic-Anaerobic-Digestion (ISPAD) systems, one week before emptying, to lower its digestate NH_3 volatilization. Acidification was induced by sequentially feeding a high organic load (OL), under early summer temperatures, to favor acidogen growth, VFAs accumulation and thus pH drop. A pH of 6.0 was considered sufficient in controlling NH_3 volatilization while still maintain an active methanogen population to inoculate the new ISPAD batch treatment. The acidification of ISPAD required optimization because its wastewaters under treatment generally offer a high alkalinity and Total Ammoniacal Nitrogen (TAN). Thus, a method was developed to use the ISPAD model to predict optimal OL strategies. The results were further validated with experimental data. Because the ISPAD model does not have a hydrolysis simulation compartment, the experiment was conducted assuming that glucose represents hydrolyzed sugar rich wastes.

Both the simulation and experimental tests demonstrated that an optimized OL could acidify the ISPAD content, despite its high alkalinity, TAN and low microbial populations. Nevertheless, the experimental data indicated that the ISPAD model needs further development to predict an accumulation of intermediate products, under conditions of fast pH drop. Such accumulation not only creates a lag but also a greater pH drop, as compared to that predicted.

The experimental tests demonstrated that the sequential feeding of 13 g of glucose/L of ISPAD content, over 4 days, could drop the pH to 6.0 on day 6. If achieved using sugar rich wastes, this OL strategy could acidify the ISPAD content at a low cost \$0.80/m^3, with relatively limited secondary environmental impacts. Such a technique compared quite favourably against present methods such as using 18M sulfuric acid at \$2.00/m^3.

6.5.Acknowledgements

The authors acknowledge the financial contribution of Geomembrane Technology Inc. (Fredericton, New Brunswick, Canada) and the Natural Science and Engineering Research Council of Canada (NSERC) and Concordia University.

Chapter 7. Summary and Conclusions

7.1.Summary

This research was designed to address 4 critical issues affecting the potential for success of In-Storage Psychrophilic Anaerobic Digestion (ISPAD): (i) the determination of microbial kinetics for the ISPAD system; (ii) the development of best management practices for the ISPAD system by developing a model predicting the system behaviour; (iii) the validation of the model to determine the accuracy of the model in predicting ISPAD behaviour; (iv) the optimization of organic loading strategies and their laboratory validation, to acidify the ISPAD content and reduce ammonia (NH_3) volatilization from its digestate once removed. These issues were investigated sequentially by performing a variety of laboratory analyses, incubations and simulations on samples of inoculum obtained from a full-scale ISPAD installation treating swine manure.

Stage 1: Kinetic Parameters Determination

For process optimization, ISPAD requires modelling with well-established microbial kinetic coefficients. The reported kinetic coefficients in the literature could not be applied to the ISPAD system, because it differs from conventional systems and their operating condition. Therefore, ISPAD kinetic coefficients were obtained through 2 fitting approaches: conventional and decomposition. The method consisted in conducting specific Substrate Activity Tests (SAT) using ISPAD inoculum to monitor the rate of degradation of specific substrates at 8, 18 and 35 ºC. Microbial kinetic coefficients were obtained by fitting the Monod equations to SAT. The statistical procedure of Least Square Error analysis was used to minimize the Sum of Squared Errors (SSE) between the measured ISPAD experimental data and the Monod equation values. Comparing both fitting methods, the decomposition approach gave higher correlation coefficient (R) for most kinetic values, as compared to the conventional approach.

To obtain equations to predict the maximum growth rate (μ_{max}) of microbial communities as a function of temperature, two equations, namely the Square Root and Arrhenius were tested. The Square Root Equation better predicted temperature dependency of both acidogens and propionate degrading acetogens, while the Arrhenius Equation better predicted that of methanogens and butyrate degrading acetogens.

Stage 2: ISPAD Model development and calibration

A model predicting the ISPAD system was developed from a base model found in the literature to be most appropriate considering ISPAD operation. The developed ISPAD model was calibrated and validated using laboratory data obtained with 2009 field ISPAD inoculum. The microbial kinetic values obtained in the first part of this thesis validated the ISPAD model for pH, VFAs and methane (CH_4) production prediction. In addition, 2 temperature functions namely the Square Root and Arrhenius Equations, were calibrated to simulate changes in maximum microbial growth rate at different operating temperatures. Simulation of ISPAD was achieved using the Simulink/Matlab software. To calibrate the model, laboratory data was obtained from batch experiments using 2012 ISPAD inoculum, and glucose as substrate, and where glucose, VFAs and pH changes were monitored along with CH_4 production. The proposed model showed good agreement with the experimental data to predict CH_4 production, substrate consumption and pH at a temperature range of 4 to 35 ºC. Furthermore, comparison of microbial kinetic values over 3 years of field ISPAD monitoring demonstrated population acclimation, especially for the methanogens.

Stage 3: ISPAD model validation

Calibrated in stage 2 and found to provide a reasonable fit, the ISPAD model was validated with new laboratory data conducted using inoculum from the field ISPAD system now 8-year-old and fed glucose as substrate. The glucose concentration was as high as that used for model calibration. Changes in glucose, VFAs and pH were monitored along with biogas production. The cross-validated coefficient of determination (Q^2) was used to determine the fit between the model prediction and the experimental values. The ISPAD model was able to strongly predict glucose degradation, VFAs, pH, and methane. However, the model weakly predicted the early CO_2 changes over time, likely because of its water solubility.

Stage 4: Acidification of ISPAD content

Since in the third stage of research, the developed ISPAD model provided reasonable predictions, the fourth stage consisted in determining best practices to acidify ISPAD digestate to minimize its NH_3 volatilization when removed. The acidification of ISPAD one week before emptying is challenging considering the high buffering capacity of wastewaters and the low microbial densities at such time. The ISPAD system only allows for the control of temperature

(time of the year) and organic loading (OL). Therefore, OL strategies were optimized to promote the growth of acidogens producing VFAs at a faster rate than their degradation by slower growing methanogens. The acidification process was simulated over an 8 day period, where glucose represented a sugar rich waste, considering that the ISPAD model does not have a hydrolysis component. The acidification modeling was presumed to occur at 22 °C, a normal and achievable temperature for the ISPAD content in late spring and early summer, corresponding to the land spreading season. Feeding 31 g glucose/L of ISPAD content over 8 days under a different regime, the 3 optimized OL strategies were all found to acidify the ISPAD content. There validation in the laboratory, using a 2 L digester, and 8-year-old ISPAD inoculum indicated reasonable prediction of pH drop, except for an observed lag in VFA production resulting under such circumstances, from the accumulation of intermediate products, such as alcohol. Also, the model was designed to predict a sharp drop in methanogen population when the pH dropped below 6.0, which was not observed with the laboratory results. The model and laboratory tests were thus repeated with 13 g glucose/L of ISPAD content. Although this lower glucose OL was found to drop the pH to 6.0, the ISPAD model needs to be further improved to account for the accumulation of intermediate products later transformed into VFA.

7.2.Conclusions

The most important conclusion of this research project is that gradually feeding an increasing organic load (OL) can feasibly acidify the ISPAD content despite its high buffering capacity and low microbial populations. Three major conclusions to simulate the ISPAD system and also to achieve its acidification may be drawn from the research summarized in the preceding sections:

I. The microbial kinetics obtained for the ISPAD system was different from those reported in the literature, as ISPAD operates under conditions which differ from the classic anaerobic system are operated.

II. The developed model reasonably predicted the behavior of the ISPAD system in terms of the glucose degradation, VFAs concentration, CH_4 production, and pH. Furthermore, comparison of microbial kinetics over 3 years of monitoring showed the ISPAD microbial acclimation.

III. A gradually increasing OL, simulated as glucose, can successful acidify ISPAD digestate one week before land spreading to minimize the NH_3 volatilization while maintaining a viable methanogen population to inoculate the next batch.

7.3.Contribution to Knowledge

The primary scientific objective of this study was to identify the management conditions leading to the acidification of ISPAD systems through its AD process, thus reducing NH_3 volatilization during land spreading.

The contributions to knowledge made in this study are summarized as follows:

I. For fed-batch psychrophilic AD systems such as ISPAD, introduce for the first time in microbial modelling, the decomposition approach to produce interdependent kinetic values for μ_{max}, K_s, Y, and X as a function of temperature. Compared to traditional optimization process, the decomposition approach produced kinetic values improving model prediction of ISPAD systems. The relationship between μ_{max} and temperature for different microbial groups was also obtained using the decomposition approach. This relationship developed the prediction ability of the ISPAD model for a temperature range of 4 to 35 °C.

II. Based on the Keshtkar et al. (2001) model, the ISPAD model was developed to predict its operation under temperatures of 4 to 35 °C. The Keshtkar et al. (2001) model lumps two types of methanogens, acetoclastic and hydrogenotrophic, which differ in terms of substrate consumption and environmental growth conditions. Thus, the ISPAD model distinguished between these 2 types of methanogens. The Keshtkar et al. (2001) model also predicts CH_4 production at mesophilic temperature without considering the homoacetogenesis step dominant at low temperatures. Thus, the ISPAD model included this step to predict CH_4 production. Whereas the Keshtkar et al. (2001) model only considers one operating temperature, the ISPAD model included the effect of temperature on both the maximum growth factor μ_{max} and the dissociation constant (K_a) values.

III. The concept of favouring the growth of acidogens over that of methanogens was introduced to acidify the ISPAD content before land spreading, to reduce NH_3

volatilization from its nitrogen rich digestate. For this purpose, the ISPAD model was used to predict optimized management conditions required to drop the manure pH to 6.0. Considering the field operation of ISPAD, OL and temperature (time of the year) are the two main management parameters which can be used for acidification.

IV. The management practices required to feasibly acidify ISPAD within one week were determined, despite the high buffering capacity and low microbial population of the system. For this purpose, the ISPAD model was further calibrated and validated. Dissociation constants (K_a) for the major ions found in manure under AD were optimized to better predict pH variations for ISPAD.

7.4.Directions for Further Research

From the conclusions listed above, further research on ISPAD is recommended:

I. The ISPAD model requires a hydrolysis component, adapted to complex organic wastes. Hydrolysis is an important component of anaerobic digestion as it is generally the limiting step. The hydrolysis step for the ISPAD model can further help to determine best management practices for the acidification technique using as substrate, a sugar or starch rich organic waste.

II. To improve the acidification and pH prediction, the ISPAD model needs to be expended to consider alcohol production and conversion to VFAs.

III. The validation of the acidification process was limited to laboratory scale experiments; it therefore needs to be validated using a prototype and field scale experiment.

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