HYPERTHERMOPHILIC ANAEROBIC DIGESTION

OF FOOD WASTE

Luis Ortega Charleston

May, 2008

Department of Bioresource Engineering

McGill University, Montreal

A thesis submitted to McGill University in partial fulfillment of the requirements of the Ph. D. degree

SUGGESTED SHORT TITLE:

Hyperthermophilic digestion of food waste

CONTENTS

CONTENTS (Continuation)

CONTENTS (Continuation)

CONTENTS (Continuation)

CONTENTS (Continuation)

CONTENTS (Continuation)

LIST OF FIGURES

LIST OF FIGURES (Continuation)

LIST OF FIGURES (Continuation)

LIST OF TABLES

LIST OF TABLES (Continuation)

LIST OF TABLES (Continuation)

ABBREVIATIONS AND NOTATIONS

AD	Anaerobic digestion
ATP	Adenosine triphosphate
Bo	Ultimate biodegradation potential ($L_{STP}CH_4/gVS_{fed}$)
C0, C1, C2, C3, C4	Batch stages
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand (g/L)
CSTR	Continuously stirred tank reactor
16S-rDNA	Genomic deoxyribonucleic acid
DBP	Dibutylphthalate
DEHP	Phthalic acid ester di-(2-ethylhexyl)-phthalate
DGGE	Denaturing Gradient Gel Electrophoresis
F/M	Food to microorganism ratio (gVS/gVSS)
Go	Ultimate gas potential (L_{STP}/gVS_{fed})
HRT	Hydraulic retention time (day)
L_{-STP}	Litre of gas corrected to standard pressure (103.25 kPa) and temperature (273.15 K)
MA	Specific methanogenic activity (mmol CH_4/gVSS/day)
MSW	Municipal solid waste
OFMSW	The organic fraction of municipal solid waste
OLR	Organic loading rate (gVS/L/day)
PCR	Polymerase chain reaction
PDA	Photodiode array

R1, R2, & C	Reactors 1, 2, and Control
SA	Specific substrate activity (mmol Substrate/gVSS/day)
SC-OFMSW	Selectively collected organic fraction of municipal solid waste
SS	Suspended solids (g/L)
T1	Thermal transition 1
T2	Thermal transition 2
TKN	Total Kjeldahl nitrogen (% of gVS)
TS	Total solids (g/L)
TU	Temperature upgrading procedure (2.5 °C *per* week)
UASB	Up flow anaerobic sludge blanket reactors
VFA	Volatile fatty acids (mg/L)
VS	Volatile solids (g/L)
VSS	Volatile suspended solids (g/L)

ACKNOWLEDGEMENTS AND DEDICATORY

The author is very grateful to Céline Husser and Catrin Barleben for conducting batch tests and operating reactors, and to Alain Corriveau and Stephan Deschamps for their technical support, and to Susan King for English proof-reading some of the scientific papers. Acknowledgements to given to the National Research Council of Canada (NRCC), the Mexican Council of Science and Technology (CONACYT) and the Natural Science and Engineering Research of Canada for their financial support.

Many, many thanks to Dr. Serge Guiot for his exceptional academics support and for his welcome to one of the most skilled, hard-working team I have ever joined; to Professor Suzelle Barrington, for the great advices, guidance, and encouragement; to Professor Robert Kok, from whom I learned the value of communication in a foreign language, and to McGill University for its financial support and all the helpful attitude of its employees, especially those of the Department of Bioresources Engineering at the Macdonald Campus and the people of the Scholarships and Student Aid Office.

Many, many thanks to my thesis defense examiners; Professor Benjamin Simpson, Dr. Valerie Orsat, Dr. Dominic Frigon, Dr. Clark Grant, and Dr. Darwin Lyew for the pain-taking time taken to read this work and for their valuable remarks, observations, and advice for improving and enhancing this document.

I'm very grateful to the NRCC and its personnel at BRI in Montreal. Among them, I would especially like to thank Mrs. Marie J. Lorraine, Mr. Alain Corriveau, Mr. Stephan Deschamps, Dr. Boris Tratakovsky, Mr. Jèrôme Breton, Mr. J.C. Frigon, Mrs. Marie J. Levesque, Mrs. Ruxandra Cimpoia, and Mrs. Michelle Manuel France.

Many thanks to Catrin Barleben, Benoit Persin, Viktoria Lytvyn, Patrick Benoist, Zully León, my *compadre* Stephan Brey (Stephx, the German Santos), Stephan Grosse, Alberto Mazza, Darwin Lyew, Pauline Gourland, Céline Husser, Angela Angusti, Aurelia Chanteperdrix, and last but not least Olivia Wojciechowicz; for all the fun, support, friendship, and above all, for making of this a great, living experience.

Thanks to all the soccer teams we did beat and to the soccer teams that did beat us. To my soccer and hockey friends; many thanks…

Thank you to all the people close to me from *Mexicalpán de las tunas*: My *compallín* Marchelo *marqués de Chiconautla*, *la* Nathalie Cabirol, *la* "Praticia", Lucy, Anabel, *la* Rosa, Xavier Reivax R. Tolentino, *la flota de* Biotecnología Ambiental, my *compadre* Ariel, and to my *entenados* Uriel y Alejandro.

Finally, I want to dedicate this thesis to the people that made it worth while: To Julianna and Sebastián, to my father -Andrés Ortega in loving memory-, to my mom -Anastasia R. Charleston-, to Denisse, Andrea, Lluvia, Nuve, Paquito, Dinorihta, Alex, Ademir, Paco, Erick, Cesar, Sandra, and Judith, and to my broccolis –Dinorah, Denisse, Andrés, Gaby, and Yuri-, to all of you with all my love and gratitude.

ABSTRACT

The anaerobic digestion (AD) of the organic fraction (OFMSW) of municipal solid waste (MSW) is a sustainable and environmentally interesting solution as compared to landfilling. Anaerobic digestion operated under mesophilic temperatures degrades only a fraction of the complex compounds found in OFMSW, whereas under higher temperatures, such as thermophilic and hyperthermophilic, the degradation could be more extensive. The objective of this study was therefore to evaluate the effect of a thermal transition from 35°C (mesophilic) to 70°C (hyperthermophilic), on an anaerobic inoculum fed OFMSW. The research methodology was divided into the following stages: 1) a mesophilic, industrial wastewater treating granular sludge was upgraded to thermophilic conditions and adapted for the treatment of OFMSW (Chapter Three); 2) during the thermal transition from mesophilic to thermophilic conditions, a microbial phylogenetic characterization (16sDNA PCR followed by DGGE) of the sludge was carried out (Chapter Four); 3) to evaluate substrate (protein and vegetable oil) limiting degradation rates for the production of methane, a serum bottle batch test (MP) was developed (Chapter Five); and 4) the thermophilically adapted and OFMSW treating sludge was upgraded to hyperthermophilic conditions and both reactor performance and sludge methane and substrate activities were evaluated (Chapter Six).

The one-step temperature upgrading from 35 to 55 °C and the feeding of OFMSW resulted in a fully adapted inoculum without an initial time-consuming temperature acclimatization stage. The substrate loading rate program was a key factor in increase the population of thermophilic methanogens. Genomic deoxyribonucleic acid (16S rDNA) tools were used to characterize the newly adapted thermophilic biomass. As opposed to

Archaea, only the bacterial domain tolerated the acidic conditions which occurred after loading the reactor with an F/M of 4.43 gVS/gVSS.

The rate of hydrolysis and methanization *via* acetogenesis of specific substrates was evaluated using both the initial mesophilic and the newly adapted thermophilic inoculums. Using a batch test, methane production (MP) was monitored to follow the degradation of complex compounds such as albumin and amino acids, and oils such as olive oil, glycerol and oleic acid. These rates were compared to the non limiting substrate, acetic acid. Using mesophilic sludge, the methanization of albumin and olive oil was limited to 22 and 47%, respectively, and was almost completely eliminated under thermophilic conditions. The newly adapted thermophilic sludge had a good proteolytic activity, but was unable to methanize olive oil, because of the inhibitory effect of the release oleic acid.

The newly adapted thermophilic sludge was exposed to a step wise temperature increase of 2.5 °C/week up to 70 °C (hyperthermophilic condition). The acetoclastic methanogens were unable to tolerate temperatures above 62.5 °C while hydrogenophilic methanogens resisted the thermal transition and managed to sustain low methane production rates of 0.09 and 0.07 $L_{STP}CH_4$/L/day. The hyperthermophilic temperature displaced hydrogen-related methane production towards a low, fermentative hydrogen production rate of 0.011 $L_{STP}H_2$/L/d, which was maintained for 45 days.

RESUMÉ

Le procédé de digestion anaérobie, visant à traiter la fraction organique des déchets solides, a l'avantage d'être plus intéressant que le procédé d'enfouissement des déchets en termes d'impact sur l'environnement. Le procédé de digestion anaérobie opère à des températures mésophiles et permet la dégradation partielle des composés complexes. A des températures plus élevées, telles thermophiles et hyper thermophiles, la dégradation de ces composés est plus efficace. Par conséquent, l'objectif fut d'évaluer l'effet d'une transition de température de 35°C (mésophile) à 70°C (hyper-thermophile) sur les microorganismes anaérobiques et leur dégradation des déchets alimentaire. La méthodologie a été divisée en plusieurs étapes, soit: 1) une boue de traitement d'effluents industriels du type granulaire mésophile a été soumise à des température thermophiles et à un traitement visant à l'adapter au traitement des déchets alimentaires (Chapitre 3); 2) les populations microbiennes présentes pendant la phase de transition de température mésophile à thermophile ont été caractérisées par un outil moléculaire (PCR et DGGE-ADNr16S) (Chapitre 4); 3) un test en batch a été développé afin d'étudier les vitesses limitantes de dégradation des substrats (protéines et huile végétale) (Chapitre 5); 4) la boue préalablement adaptée aux températures thermophiles et au traitement des déchets a été soumise à des températures hyper-thermophiles. La performance du réacteur et les activités de production de méthane et de dégradation du substrat ont alors été évaluées (Chapitre 6).

L'étape d'adaptation a consisté à augmenter la température d'un seul coup, de 35 à 55°C, afin d'adapter les microorganismes au traitement des déchets. Cette méthode a permis d'obtenir un inoculum acclimaté en un seul coup et évite l'étape d'acclimatation à

des températures thermophiles. La méthode rapide employée avantage l'enrichissement de la population en microorganismes thermophiles méthanogènes. L'analyse moléculaire à l'ADN ribosomal 16S pour caractériser la nouvelle biomasse adaptée aux conditions thermophiles a démontré que contrairement aux Archaea, seul les bactéries ont été capables de tolérer les conditions acides présentes après avoir alimenter le réacteur avec un ratio charge/bactérie (F/M) de 4.43 g VS/gVSS.

Le taux d'hydrolyse et de méthanisation fut évalué via l'acétogénèse de substrats spécifiques par la biomasse mésophile et la nouvelle biomasse thermophile. Un test en batch basé sur la production de méthane fut développé pour suivre la dégradation de composés complexes tels l'albumine et acide aminés, et les huiles telles l'huile d'olive, le glycérol et l'acide oléique. Ces taux furent mesurés à celui obtenu avec la dégradation sans inhibition de l'acide acétique. La boue mésophile n'a dégradé que 22 et 47 % de l'albumine et de l'huile d'olive. La nouvelle boue thermophile a démontré une forte activité protéolytique.

La nouvellement biomasse thermophiles fut exposée à un augmentation graduelle de température de 2.5°C/semaine jusqu'à 70°C. Les organismes méthanogènes acétoclastiques n'ont pas pu tolérer plus de 62.5°C tandis que les méthanogènes hydrogénophiliques ont résisté à la transition de température et ont produit de faible taux de production du méthane de 0.09 et 0.07 $L_{STP}CH_4$/L/jour. La température thermophile a favorisé la production d'hydrogène par voie fermentative plutôt que la production de méthane à partir d'hydrogène au taux de 0.011 $L_{STP}H_2$/L/jour, et un tel taux de production fut conservé pendant 45 jours.

CHAPTER ONE

1 INTRODUCTION

1.1 PROBLEM STATEMENT

By definition, a waste is considered to be any material which offers little or no value to mankind. All human activities produce waste so eventually, clothing, food, equipment and buildings become wastes. Furthermore, a control approach produces a solid or semisolid waste stream which attracts environmental and health afflicting agents.

According to Rhyner *et al.* (1995), US municipal activities account for up to 9% of the total mass of municipal solid waste (MSW) generated and designating unwanted or discarded material with an insufficient moisture content below 85 – 90% to be free flowing. Such MSW consists of durable and non-durable goods, containers and packaging materials, food waste, yard waste and trimmings, and miscellaneous organic matter arising from residential, commercial, institutional and some industrial sources (US E.P.A. 2001). Mining and demolition waste are not considered as part of MSW.

With a *per capita* waste generation of 864 kg/year, the US is the biggest MSW producer with a total production of 229 x 10^6 metric ton/ year which represents 60% of the total amount of MSW generated within the Top-ten World economies (US E.P.A. 2001). Without reliable data on annual MSW production rates, highly populated developing countries produce MSW in amounts that could be as high as those in developed countries. For China, Hongalo and Yongfeng (2001) reported an annual garbage production per capita of 380 kg which, once compounded with China's population, represents twice the annual MSW production in the US.

Sustainable practices now recycle and reuse 30% of wasted materials such as paper, glass, plastic, compost, textile fibers, and ferrous and non-ferrous metals. The remaining waste, usually referred to as the organic fraction of municipal solid waste (OFMSW), is generally landfilled or incinerated. This is the fraction producing greenhouse gas (GHG) emissions and leachate.

Recycling, reuse, landfilling, and incineration contribute to the overall risk reduction associated with waste accumulation. Nevertheless, improving and optimizing strategies of MSW reduction is certainly a challenge. With respect to AD, both conventional and non-conventional practices can be modified to specifically improve the treatment of OFMSW. Such an approach is by far the most promising and reliable technology capable of overcoming health and environmental problems associated with MSW accumulation and landfilling.

Basically, AD is the biodegradation of organic matter in the absence of oxygen and is performed in a series of successive steps:

– Hydrolysis of complex macromolecules such as proteins, carbohydrates and lipids;
– Fermentation of single molecules to produce organic acids such as volatile fatty acids (VFA) and other intermediate metabolites;
– Production of acetate, hydrogen, and carbon dioxide, and;
– Production of methane and carbon dioxide.

The layout of a conventional AD process includes a reception tank, an anaerobic digester where the organic matter is transformed into methane and carbon dioxide, and a digested paste dewatering system. Normally, the biogas produced generates heat and electricity. Water from the dewatering system is recycled to adjust the concentration of

solids of the incoming stream and finally the digested paste is composted or can be used as fuel for waste-to-energy incinerators.

Some of the important features of anaerobic digestion are:

1st Oxygen-independent process, eliminating the costly oxygen supply infrastructure;

2nd One of the earliest biological interactions, where its inefficiency was explained in terms of cell production; such an inefficiency reduces the cost of sludge treatment and disposal;

3rd With 10% of the waste COD transformed into cells, 55% is transformed into methane which can produce electricity or heat;

4th Recently, AD was found to produce hydrogen, a fuel with a simple and clean conversion to heat and power, since energy derived from hydrogen only produces H_2O.

Despite its advantages, AD is not intensively used. In Europe, the treatment capacity of conventional and non-conventional AD systems increased by 750% from 1990 to 2000, or from 122 kton to 1037 kton of OFMSW/year. This 2000 capacity is less than 10% of the total MSW produciton in Europe. The rest of MSW is landfilled or composted (De Baere, 2000). Neither the US E.P.A. nor Environment Canada is promoting the use of conventional or non-conventional AD for the treatment of MSW. As a result, AD is practically not used for organic waste treatment in North America and most of the 229×10^6 and 20×10^6 tons/yr of MSW generated in the USA and Canada respectively are being disposed in landfills.

Between Europe and North America, the discrepancy observed in the use of AD technology is culturally motivated (Lalonde, 2003), but also linked to economic factors. The City of Toronto, Canada, has closed the operation of its landfill at Keele Valley and all MSW produced (3,500 ton per day) is currently sent for final disposal at the Carleton Farms landfill south-west of Detroit, Michigan in the US, an 800-km round trip from Toronto (Robson, 2003). Besides the implications associated with bilateral collaboration, such a practice represents significant business potential for those in the transportation industry. If it is true that the large-scale implementation of an anaerobic digestion system for the treatment of Toronto's MSW is a riskier strategy than what is currently being used (Allen Kain and Enviros RIS, 2001), arguably, a constant flow of hazardous material is perhaps more risky on a long term basis and, as a consequence, more problematic for decision makers. Meanwhile, MSW in North America continues to be mainly disposed in landfills.

Because of the lack of landfill mixing and heating, waste biodegradation is incomplete and the total methane production is significantly lower than that generated by conventional AD systems. As a result, the gas energy potential from a landfill is at least 22 times lower (7 kWh per ton, US E.P.A. 2003) than that of conventional or non-conventional AD systems of 150 kW-h or 165 to 245 kW-h / ton treated (Baldasano and Soriano, 2000 and De Baere, 2000), respectively.

Landfilling cannot totally be substituted by conventional AD. According to Baldasano and Soriano (2000), the combined use of anaerobic digestion, sludge incineration and ash landfilling could reduce greenhouse gas emission from OFMSW by almost 40%. Thus, the use of conventional anaerobic digestion may feasibly reduce the

problem associated with MSW accumulation by allowing a better reuse of the waste, producing a renewable source of energy and reducing the production of GHG emission.

1.2 PROPOSED STUDY

Recycling not only allows for the recuperation of valuable materials such as paper, glass, plastic and metals, but also increases both the concentration of organic matter contained in MSW as well as its biodegradability, further contributing to more efficient landfilling and composting operations. The application of AD to MSW may further reduce the MSW stream by reusing the organic fraction for biogas production. Anaerobic digestion was shown to be a sound technology for the treatment of high-strength polluted water and sewage sludge. During the last decade, and because of its capacity to stabilize MSW, AD has been tested world-wide, consequently increasing its application.

The nature of the OFMSW has created some challenges such as the high solids content of over 25%, which made it difficult to be handled by a conventional AD process. The utilization of more robust equipment not only allowed MSW to be treated by conventional AD systems, but also reduced the complexity of the facilities. Consequently, during the 1980's, concepts such as solid fermentation became quite common in terms of research and large-scale applications.

As part of the effort to improve the biodegradability of OFMSW, source sorting (SS-OFMSW) and separate collection (SC-OFMSW) were promoted, to improved waste biodegradability by 30% and also methane production. Nevertheless, wastes with a low biodegradability such as food waste, paper, paper board, cardboard, yard waste, and

highly shielded compounds such as lignin, lignocellulose, cellulose, and hemi-cellulose, has a significant impact on the overall gas production.

During the last decade, the implementation of AD under thermophilic temperatures of 55°C proved to be effective in increasing the loading potential, and reducing the hydraulic retention time (HRT), pathogen counts and sludge production. Thermophilic temperatures improved the degradation of large-complex bio-molecules by a better microbial performance beyond the rapidly biodegradable substrate. Other practices conducted at the pilot-scale, included pre-treatments such as high-pressure steam which physically breakdown lignin and lignocellulose. Such treatments known as thermal hydrolysis (Schieder *et al.,* 2000) or steam pressure disruption (Liu *et al.,* 2001), enhanced methane yield by up to 40% as compared to conventional thermophilic AD system. However, applications to a larger scale are still uncertain and more research is necessary, especially in terms of technical and economic benefits.

In this context, if AD is indeed potentially the best and most energy-efficient method to treat OFMSW, several concerns need to be addressed, and thus warrant further investigation. Greater emphasis is needed to investigate energy efficient options hyper-thermophilic fermentation to improve biomass degradability and simultaneously, stabilization particularly for slowly biodegradable matter.

1.3 OBJECTIVES

The objective of this research was to evaluate the effect of increasing AD temperature from thermophilic or 55°C, to hyper-thermophilic or 70°C, for the treatment of OFMSW.

The specific objectives of the project were:

1. To develop a thermophilic anaerobic inoculum for the treatment of OFMSW, by instantaneously increasing to 55°C, the operating temperature of a reactor inoculated with mesophilic anaerobic sludge. The inoculum adaptation was conducted using a bench scale reactor seeded with a mesophilic granular sludge obtained from two upflow anaerobic sludge blanket digester, treating respectively apple and milk wastewaters. Substrate activity tests were conducted to characterize the changes in biomass during this transition. Using a bench scale reactor, the new thermophilic sludge was adapted by allowing its stabilization for 7 days, and then supplying it with an increasing load of OFMSW at a frequency of 21 days.
2. To evaluate the composition of microbial community after adaptation to thermophilic conditions and OFMSW. This characterization was performed using substrate Genomic 16S-rDNA phylogenic analysis.
3. To develop a simple methane potential test (MP) to measure the degradation of albumin and olive oil in serum bottles under mesophilic and thermophilic conditions, and to demonstrate the application of the technique. The project first evaluated the methane and carbon dioxide production curves with time of a non limiting substrate, namely acetic acid. Then, the MP test was repeated with albumin and olive oil and their monomer-like compounds, namely a mixture of the amino acids, alanine and glycine for albumin, and oleic acid and glycerol for olive oil.
4. To evaluate the impact of a step wise temperature upgrading (TU) of 2.5°C/week, from 55°C-thermophilic to 70°C-hyperthermophilic conditions. The performance of the hyperthermophilic upgraded reactor was compared to that of a reactor maintained under thermophilic conditions. Both anaerobic reactors were continuously stirred

1.4 SCOPE

The present study was limited to the use of OFMSW generated by a cafeteria. The AD experiments were carried out in completely mixed and temperature controlled, laboratory-scale batch fed digesters during the mesophilic to thermophilic transition and continuously fed during the thermophilic to hyperthermophilic transition. During the first thermal transition, the microbial community structure was studied by phylogenic analysis of amplified and denatured deoxyribonucleic acid (DNA) extracted from samples of the anaerobic biomass. During the second thermal transition, microbial kinetic studies were carried out by using specific activity tests (SA and MP) as measured by specific substrate consumption and methane production rates. These tests also evaluated the presence and activity of trophic groups associated with the overall treatment process.

ORGANIZATION OF THE THESIS

The following chapter is a general literature review which includes the subjects in which this research is framed: 1) the justification of why AD is an energy-efficient way to treat OFMSW; 2) a general review of MSW, with some statistics and characteristics; 3) elements of modern MSW management systems and how these change our perspective towards organic wastes, and; 4) the generalities of the AD process, where its characteristics, microbiology and biochemistry are discussed as well as the different kinds of technologies currently available for the treatment of the OFMSW.

The literature review is followed by four experiments, Chapter 3 to 6, each written as a separate scientific paper. Chapter 3 characterizes the food waste selected to feed the sludge used in all experiments and adapts mesophilic sludge to thermophilic conditions while following its methane production and microbial substrate activity. The sludge produced by with the thermal transition experiment described in Chapter 3, was subjected to a microbial phylogenetic characterization (16sDNA PCR amplification followed by DGGE). Chapter 5 presents a serum bottle batch test (MP) developed to evaluate substrate (protein and vegetable oil) limiting degradation rates for the production of methane. Finally in Chapter 6, the thermophilically adapted food waste treating sludge was upgraded to hyperthermophilic conditions and the performance of two reactors and their sludge methane and substrate activities were evaluated.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 MUNICIPAL SOLID WASTE DEFINITION AND CHARACTERISTICS

Nearly all-human activities produce waste, defined as material perceived to have little or no value by society's producers or consumers. Solid waste is further defined as unwanted or discarded material with insufficient moisture content of less than 85 % to be free flowing and which is generated during the acquisition of raw material, refining and manufacturing processes and customer products usage. Wastes are hazardous and require special handling or treatment to prevent serious health impact to humans or environmental impact to ecosystems. In addition to solid waste, human activities generate liquid and gaseous by-products which often exceed the assimilative capacity of the natural environment.

A unified approach which includes a study of all forms of waste –solid, liquid and gaseous- is theoretically appealing, but a practical justification for giving priority to solid waste management and wastewater treatment is that many other pollution problems tend to reduced one or both of these domains (Figure 2.1.1).

Accurate projections of waste quantities and composition are essential for the planning of efficient and economical waste transport, processing, and disposal systems. The US Environmental Protection Agency (US EPA, 2001) estimated that 226.3 million tons of MSW were generated in 1999, an increase of 6.8 million tons from 1998. Of this, 163.4 million tons were disposed in landfills or incinerators and the remainder of 62.9 millions tons was recovered. Of the total MSW recovered, 78% was recycled and 22% was recovered for composting.

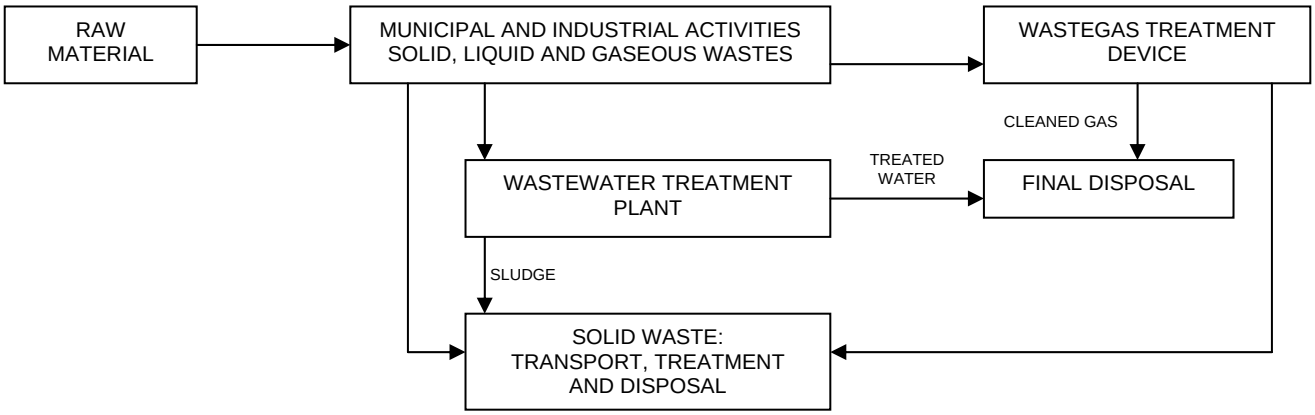


Figure 2.1.1 Solid waste generation.

Industrial wastes, wastewater sludge, construction and demolition waste, junked automobiles, agricultural wastes, and mining wastes are not included in the definition of MSW. The estimated annual generation rates for these wastes in the United States are shown in table 2.1.1.

Municipal solid waste (MSW) consists of those durable and non-durable goods, containers and packaging material, food waste, yard trimmings, and miscellaneous organic wastes arising from residential, commercial, institutional and industrial sources. Other types of waste typically excluded from MSW are industrial waste produced by manufacturing and processing operations, construction and demolition waste, agricultural waste, oil and gas waste, and mining waste resulting from the extracting and processing of minerals (Rhyner *et al*., 1995). Examples of the types of MSW are listed in table 2.1.2.

Table 2.1.1 Estimated annual solid waste generated in the US (Rhyner *et al.*, 1995).

Type of work	Annual quantities (million of tonne)
Agricultural	-
Construction/demolition	30.5
Household (hazardous)	0.3
Industrial (non-hazardous, dry basis)	423
Industrial hazardous	193.1
Mining	1377.5
Municipal solid waste	192.6
Municipal sludge (dry basis)	8.4
Municipal combustion ash	2.3

Notes: - not known

Table 2.1.2. Sources of municipal solid waste (MSW).

Source	Types of solid waste
Residential	Appliances, newspapers, clothing, disposable tableware, food, packaging, cans, bottles, food scraps, yard trimmings
Commercial	Corrugated boxes, food wastes, office papers, disposable tableware, yard trimmings
Institutional	Office papers, cafeteria and restrooms wastes, classroom wastes, yard trimmings
Industrial	Corrugated boxes, lunchroom wastes, office papers, wood pallets

Rhyner *et al.* (1995)

Commercial waste results from retail, wholesale, and community service activities. Institutional waste is produced by schools, hospitals, and public or private services facilities. The industrial waste included in MSW is primarily from offices and support operations, excluding waste from processing and manufacturing (table 2.1.3). In contrast with Rhyner *et al.* (1995), Mata-Alvarez *et al.* (2000) included dewatered municipal sewage in the MSW stream.

In 1990, the US EPA estimated the MSW generation rate to be 2.0 kg/person/day and projected a generation increases by 1.3% from 1990 to 2000. Residential waste accounts for 55 to 65% of the total MSW generation, while commercial waste ranges between 35 and 45% (Rhyner *et al*., 1995). Local variations result from factors such as type of dwelling, household size, type of housing, urban versus rural households, socioeconomic factors, and season. Rural households, for example, tend to generate about 25% less waste/capita than their urban counterparts. Household size and lifestyle account mainly for the higher per capita generation rates in apartments and multifamily dwellings (Rhyner *et al*. 1995).

Commercial waste quantities are harder to estimate than for residential because of a wider range in types and magnitudes of activities such as for restaurants, hospitals, schools, and others private and public service facilities. Detailed estimates of the waste generation by commercial activities may be based on measures such as the number of employees in a building, the floor area of a store, the number of meals served at restaurant, and the number of beds in a hospital (Table 2.1.4). The implicit assumption is a linear relationship between the parameter indicating the magnitude of the activity and the quantity of the waste produced (table 2.1.5).

Table 2.1.3 Description of MSW components.

Category	Description
Paper and paperboard	
High grade paper	Office paper and computer paper
Mixed paper	Mixed colored paper, magazines, glossy paper, and other paper not fitting the categories of high grade, newsprint, and corrugated
Newsprint	Newspaper
Corrugated	Corrugated boxes, corrugated and brown (Kraft) paper
Yard waste	Branches, twigs, leaves, grass, and other plant material
Food waste	All food waste excluding bones
Glass	Clear and colored glass
Plastics	All types of plastics
Ferrous metals	Iron, steel, tin cans, and bi-metal cans
Non-ferrous metals	Primarily aluminum, aluminum cans, copper, brass, and lead
Wood	Lumber, wood products, pallets, and furniture
Rubber	Tires, footwear, wire cords, gaskets
Textiles	Clothing, furniture, footwear
Leather	Clothing, furniture, footwear
Miscellaneous	Other organic and inorganic materials, including rock, sand, dirt, ceramics, plaster, bones, ashes, etc.

Ref.: Rhyner *et al.* (1995).

Table 2.1.4 Typical waste multipliers for hospital, schools, hotels and restaurants.

Category	Waste generation rate
Hospitals	4.5 to 8.0 kg per occupied bed per day
Schools	0.2 to 0.5 kg per student per day
Hotels	0.5 kg per occupied room per day (non-checkout day)
	1.0 kg per suite or for checkout days
Restaurants	90 kg per thousand dollars sales

Ref: Rhyner *et al.* (1995).

2.1.1 OFMSW CHARACTERISTICS

Collecting MSW is the first treatment. The different choices made by municipalities to separate the different MSW fractions before any technological treatment are strictly linked to material characteristic. In Europe, the daily organic fraction of MSW (OFMSW) production is estimated at 400,000 tons (Mata-Alvarez *et al*. 2000) and represents that produced by households, industries and institutions (Mata-Alvarez, 2002).

Two important practices influence OFMSW characteristics: a) unsorted collection (UC-OFMSW), and; b) source separated collection. The last one can be divided in: 1) separated collection (SC-OFMSW) representing the organic fraction collected from markets, canteens and restaurants, and; 2) the source sorted (SS-OFMSW) representing the organic fraction sorted within homes. Source separation generally produces a high grade of OFMSW but depends on the participation of the producers and the information and education received (Mata-Alvarez, 2002).

Table 2.1.5 Representative composition of waste from various commercial sources expressed as percentages by weight (Rhyner *et al.* 1995).

Waste component	Retail trade	Restaurant	Office	School	Government
Paper	41.5	36.6	64.2	47.8	53.8
Newspaper	2.9	2.5	3.6	3.3	6.7
Corrugated	22.0	15.6	11.5	11.6	8.4
High grade white	1.4	0.0	10.6	6.3	7.2
Mixed recyclable	10.3	4.4	29.0	21.6	25.0
No recyclable	4.9	14.1	9.5	5.0	6.5
Plastics	12.0	13.7	4.3	5.1	3.5
PET (1)	0.1	0.0	0.1	0.1	0.1
HDPE (2)	0.0	0.1	0.0	0.0	0.0
Other	11.9	13.6	4.2	5.0	3.4
Glass	2.5	5.9	3.9	3.2	2.7
Container	2.3	5.8	2.9	1.0	2.4
No recyclable glass	0.2	0.1	1.0	2.2	0.3
Metals	20.5	4.9	2.9	5.8	9.8
Aluminum cans	0.2	0.5	0.5	0.8	0.5
Tin/steel cans	0.2	3.8	0.2	0.2	0.4
Other ferrous	19.5	0.4	2.2	3.7	8.6
Other non-ferrous	0.6	0.2	0.0	1.1	0.3
Organic	18.8	36.6	10.8	35.0	23.2
Food waste	8.1	36.0	3.0	14.0	3.2
Yard debris & wood	10.7	0.6	7.8	21.0	20.0
Others	4.7	2.3	13.9	3.1	7.0

2.1.1.1 UC-OFMSW characteristics

Unsorted collection represents all activities related to MSW collection and transport from the source, such as from institutions, dwelling, and commercial and industrial facilities. According to the equipment complexity and process technology, UC-OFMSW can be classified into three main groups: a) simplified, b) medium complex, and c) complex (Mata-Alvarez, 2002).

Simplified plants consist of a primary shredder, a trommel screen (hole size 50-100 mm) and a magnetic belt from which three streams are obtained: the over –sieve, also called gray waste such as paper, plastics and small amounts of putrescible matter, which can be incinerated, the organic fraction which can be biologically treated and the iron material fraction which is recycled.

Medium complex plants consist of at least one size reduction step, iron separation, and more than one screen operation. All oversize material is sent for incineration or landfilling, while the "pure" OFMSW coming from the screen operations is sent for biological treatment, and the iron material fraction is recycled.

Complex plants are designed with a more complete sorting line: size reduction, iron separation, screenings, dry matter shredder and pellet formation for refuse-derived fuel (RDF) production. The products are more "pure", and the OFMSW obtained is a substrate more suitable for biological processes. The UC-OFMSW is a substrate studied for both its composition and chemical-physical parameters (table 2.1.6).

Table 2.1.6 Chemical-physical characteristics of UC-OFMSW sorted in "complex" plants.

Parameter	Average
Total solids (TS), g/kg	763.0
Total volatile solids (TVS), %TS	43.9
Total chemical oxygen demand (TCOD), %TS	59.6
Total Kjeldahl nitrogen (TKN), %TS	2.2
Total phosphorus (P), %TS	0.11

Mata-Alvarez (2002).

Table 2.1.7 Composition of UC-OFMSW sorted in complex plants in terms of %TS and TVS of each fraction (Mata-Alvarez, 2002).

Fraction	%TS	%TVS
Putrescible	59.0	78.0
Paper	4.6	7.1
Wood	1.1	2.2
Plastic	1.8	3.4
Inert	33.5	9.3

The UC-OFMSW generally offers a high total solid content (TS) because of the inert fraction of the unsorted waste which is incompletely separated with this sorting approach. Five fractions are considered: putrescible matter, paper, wood, plastic and inert materials. Such waste also has a total volatile solid content (TVS) under 50% (table 2.1.7). Accordingly, over 30% of the substrate is actually unusable as feed for AD. Furthermore, these inert materials will be present in the digested sludge, making any

agronomic recovery more difficult in terms of nitrogen and phosphorus (Mata-Alvarez, 2002).

2.1.1.2 Separate collected (SC-OFMSW) and source sorted (SS-OFMSW) characteristics

The separate collection of the OFMSW (SC-OFMSW) is defined as the disposal in a separate system, to lower the quantity of foreighn materials and promote the efficient use of materials and energy. Through educational programs, the public recycles rather than reduces the quantity of waste produced, which changes the characteristics of the MSW stream.

The typical range of total solids (TS) content in SC-OFMSW is 15 - 25%, and of total volatile solids is 70 – 90% TS. Nitrogen and phosphorous ranges are between 2.5 – 3.5% TS and 0.5 – 1.0% TS, respectively, which is similar to UC-OFMSW (Mata-Alvarez, 2002). Table 2.1.8 characterizes SC-OFMSW collected from canteens, which is rich in fried foods and bread increasing the TS of the waste.

Table 2.1.8 Characteristics of SC-OFMSW collected in canteens Mata-Alvarez (2002).

Parameter	Range
Total solids (TS), %	21.4 – 27.4
Total volatile solids (TVS), %TS	91.3 – 99.7
Total chemical oxygen demand (TCOD), gO_2/gTS	1.2 – 1.3
Total Kjeldahl nitrogen (TKN), %TS	2.6 – 3.7
Total phosphorus (P), %TS	0.13 – 0.28

Table 2.1.9 Characteristics of SC-OFMSW collected in fruits and vegetables markets.

Parameter	Average
Total solids (TS), g/kg	81.8
Total volatile solids (TVS), %TS	81.9
Total chemical oxygen demand (TCOD), gO_2/gTS	1.0
Total Kjeldahl nitrogen (TKN), %TS	2.1
Total phosphorus (P), %TS	2.8

Pavan *et al*., (2000b)

The organic fraction from fruits and vegetables markets has a high water content often exceeding 90% (table 2.1.9) according to Pavan *et al*. (2000). The SS-OFMSW exhibits 10 % TS for fruit and vegetables wastes, and 20-25% TS for kitchen waste mixed with garden waste. The TVS ranges from 85-90% for both residues, while the nitrogen and phosphorous content ranges between 2-3% TS and 0.2-0.5% TS, respectively (Mata-Alvarez, 2002).

The ultimate biodegradation potential, B_0, is the most appropriate parameter for evaluating the biological characteristics of OFMSW (Mata-Alvarez, 2002) and represents the maximum amount of methane obtained from the AD of a substrate at a specific temperature. Table 2.1.10 shows the range of methane and biogas productions (assuming 55% methane content) from UC-, SC-, and SS-OFMSW.

Table 2.1.10 Ultimate methane and biogas production for OFMSW.

Substrate	UC-OFMSW	SC-OFMSW	SS-OFMSW
B_0, m^3CH_4/kgTVS	0.16 – 0.37	0.45 – 0.49	0.37 – 0.40
G_0*, m^3/kgTVS	0.29 – 0.66	0.81 – 0.89	0.67 – 0.72

* G_0 stands for the ultimate biogas production (Mata-Alvarez, 2002)

The substrates with the least and most biodegradable potential are UC-OFMSW and SC-OMSW and both cannot therefore be treated with the same AD system. Garden wastes are presumed to explain the difference in biodegradability between SC-OFMSW and SS-OFMSW (Mata-Alvarez, 2002).

2.1.2 WASTE MANAGEMENT

Integral waste management must consider the Life Cycle Analysis (LCA) to assure the best possible use of the MSW. There are four possible alternatives for the treatment of the organic wastes: a) biological treatment, b) incineration with energy recovery, c) incineration without energy recovery, and; d) landfilling or permanent storage.

The treatment selected for the OFMSW depends highly on their biodegradability. Biological treatments treating OFMSW are composting, AD and fermentation, where composting and anaerobic digestion are most developed. Others organic waste amenable to biological treatment include sewage sludge, some industrial wastes, and agricultural and food processing residues. Both composting and AD can biologically treat OFMSW and transform them into soil amendments. The high temperature achieved with composting has a stabilization effect on pathogens, while the same is obtained with AD when operated at thermophilic temperatures.

Fermentation is a treatment process particularly well adapted to agricultural residues and food processing waste. Fermentation can produce alcohol from fruits and grains but requires a costly pre-treatment when applied to the organic portion of MSW which contains paper with a high level of cellulosic material (Rhyner *et al*. 1995).

Incineration is the controlled burning of wastes at high temperatures in a facility designed for efficient and complete combustion. By definition, complete combustion involves the conversion of all carbon compounds into carbon dioxide (CO_2), hydrogen to water (H_2O), and sulfur to sulfur dioxide (SO_2). Incineration generates ash but also gas and heat energy. Wastes are incinerated for one or more of the following reasons: volume reduction, destruction of certain chemicals or alteration of chemical characteristics, destruction of pathogens, and/or energy recovery. The lack of available land for landfilling and the high cost of energy are the two major incentives for including incineration as part of a solid waste management system. Incineration reduces, but does not eliminate, the need for landfilling space. Typically, only 50% of MSW are combustible and incineration reduces the volume and mass of this combustible portion by 90 and 70%, respectively (Rhyner *et al*. 1995).

Most modern incinerators are designed to recover energy. In Europe, the stream and hot water produced by central incinerators are commonly used for the district heating of houses and businesses. Approximately 75% of US incinerators are waste-to-energy plants where the energy is used to produce steam or generate electricity, generating revenues to offset the high capital and operating costs.

An alternative to directly burning wastes for energy is to produce refuse-derived fuels (RDF) from MSW defined as a homogeneous shredded mixture of the combustible

organic fraction of MSW. Existing industrial or utility boilers use RDF as supplementary fuel otherwise RDF are burned in specifically designed incinerators (Rhyner *et al*. 1995).

Landfilling wastes is a modern variation of the long-used practice of discharging wastes in a dumpsite at the outskirts of a community, to isolate the community from health and nuisance issues associated with decomposing wastes. Marginal agricultural land is used when available. A modern sanitary landfill is an engineered site, selected, designed, and operated to minimize environmental impacts such as rodents, flies, leachate losses and odours. Municipal wastes are dumped in a confined area, compacted in thin layers, and covered with earth at the end of each day. Certain types of industrial and non-putrescible wastes may not require daily covering. Site design, construction, and operation are subject to state and federal regulations and standards (Rhyner *et al*. 1995).

Landfilling is a necessary component of any MSW management system. Waste reduction efforts, recycling, incineration and biological treatment can reduce the quantity of materials sent to landfills, but anyone experienced in waste management acknowledges the presence of residual materials requiring landfilling. The number of landfills in the US is decreasing for three reasons: a) the closure of many old landfills not meeting current design and operation standards; b) the difficulty in finding sites for new landfills, and; c) the use of larger landfills to reduce the costs of site design, construction, operation, leachate and gas monitoring, collection and treatment, administration, and engineering (Rhyner *et al*. 1995).

Public opposition is likely the most important factor preventing municipalities from identifying and developing new landfill sites, rather than the lack of suitably located land with the required soil and hydro geological characteristics. People object to landfills

because of the aesthetics, nuisance issues such as dust, noise, traffic, odours, and environmental concerns such as groundwater pollution, landfill gases and loss of agricultural land.

2.2 ANAEROBIC DIGESTION

The overall AD microbiology of OFMSW is similar to that observed during the anaerobic treatment of either industrial wastewater or sewage sludge. The difference lies in the proportion of trophic groups such as hydrolytic, fermentative and acidogenic bacteria, and acetoclastic or hydrogenophilic methanogens. In this sense, AD is a metabolic process where organic matter is degraded in the absence of oxygen. The following sections will review the basic biochemistry and microbiology of the process.

2.2.1 BIOCHEMISTRY AND MICROBIOLOGY

The overall process includes a series and/or parallel biochemical reactions and compounds serving as electron acceptors, other than oxygen, and namely nitrates, sulfates, and/or carbon dioxide. Overall anaerobic reactions can be described as follows:

Denitrification (anoxic):

$$C_6H_{12}O_6 + 4.8\ NO_3^- + 4.8\ H^+ \rightarrow 6\ CO_2 + 2.4\ N_2 + 8.4\ H_2O$$

Sulfate- reduction:

$$C_6H_{12}O_6 + 3\ SO_4^- + 3\ H^+ \rightarrow 6\ CO_2 + 3\ HS^- + 6\ H_2O$$

Methanogenesis:

$$C_6H_{12}O_6 \rightarrow 3\ CH_4 + 3\ CO_2$$

In AD via methanogenesis, 90% of the energy contained within organic matter, expressed as chemical oxygen demand (COD), is converted into biogas and only 10% is transformed into cellular growth. Such low and inefficient cell production limits the size of infrastructures and resources associated with the disposal of sludge.

The overall anaerobic process involves the coordinated and combined metabolic activity of various groups of micro-organisms. Substrates for certain micro-organisms are the metabolic consequence of others. Such a coordinated action represents a typical multisubstrate/multiorganism system in which reactions occur sequentially.

Gujer and Zehnder (1983), proposed a simultaneous, six-step mechanism for the conversion of particulate organic matter into methane (figure 2.2.1): 1) Hydrolysis of protein, carbohydrates, and lipids; 2) fermentation of sugar and amino-acids; 3) β–oxidation of long chain fatty acids and alcohol fermentation; 4) anaerobic oxidation of intermediate organic acids, except acetate; 5) transformation of acetate into methane by acetoclastic methanogenesis, and; 6) transformation of molecular hydrogen and carbon dioxide into methane (hydrogenophilic methanogenesis).

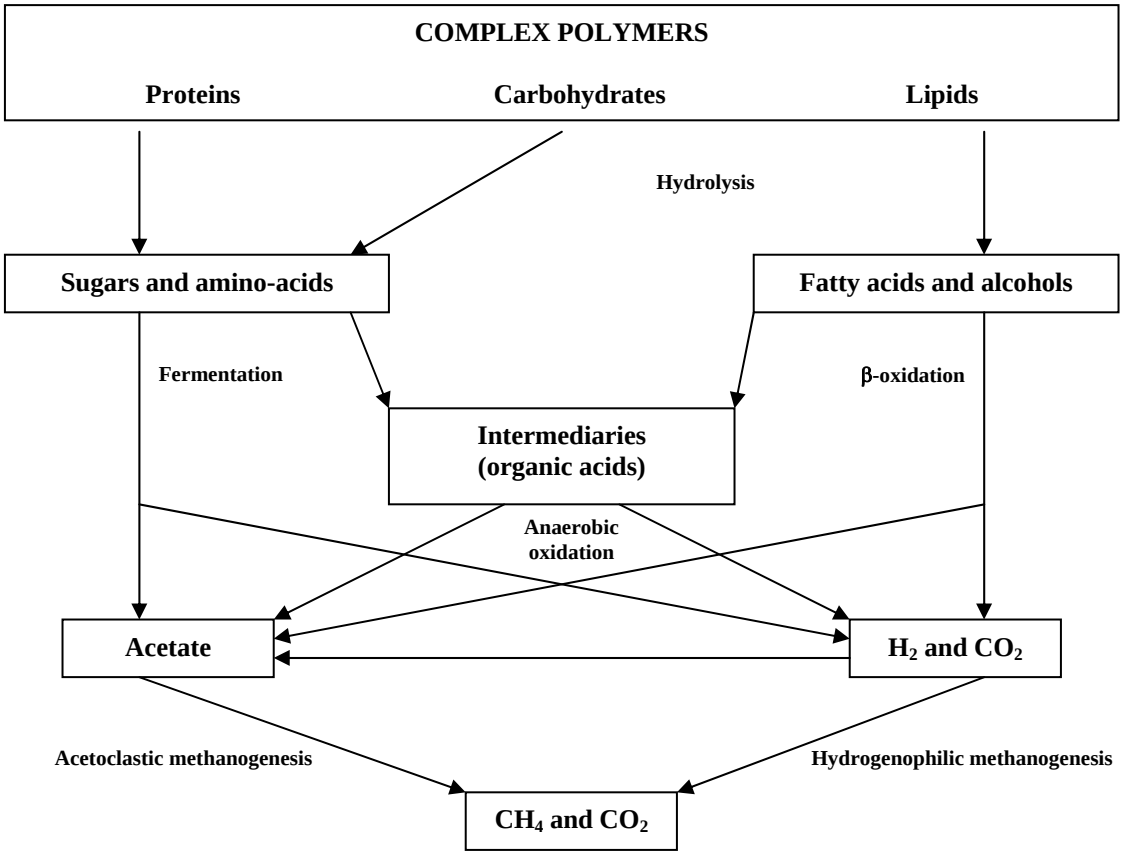


Figure 2.2.1 Metabolic pathway of anaerobic digestion (Gujer and Zehnder, 1983).

Four different groups of microorganisms are responsible for the AD biochemical process: a) primary fermenter bacteria hydrolyzing polymers and fermenting products of hydrolysis, producing volatile fatty acid (VFA), carbon dioxide (CO_2), and hydrogen (H_2); b) acetate, CO_2 and H_2-producing micro-organisms known as obligate hydrogen producing acidogens (OHPA), which use VFA, propionate, butyrate, and some aromatic compounds as substrate; c) CO_2 and H_2-consuming methanogens known as hydrogenophilic methanogens and acetate-consuming methanogens known as

acetoclastic methanogens, and; d) sulfate-reducing bacteria which produces H_2S if SO_4^{-2} is available in the media.

In the AD process, the proportion of each bacterial group depends on the amount of specific substrate. Bacteria or microbial groups actually act as bio-catalyst for each metabolic reaction. The reaction velocity is limited by the substrate or nutrient concentration rather than amount of bio-catalyst and not all substrates are susceptible to anaerobic degradation. According to Field (2002), recalcitrant compounds composed of high molecular weight polymers are not degraded by the anaerobic microbial consortia, such as: 1) plastic, lignin and humus; 2) plastics built out of hydrocarbons (e.g. polyethylene or polystyrenes); 3) natural random aromatic polymers, lignin and humus; 4) peat, coal, and mineral oils, and; 5) lignin and humus. During the AD of pulping wastewater, the high molecular weight fractions remain inert, while the lowest molecular fractions, such as monomers and oligomers, are metabolized.

The principal gases produced during AD are methane and carbon dioxide, and generally, the flow of intermediate or specific substrates can be expressed as chemical oxygen demand (COD). If carbon dioxide is the principal electron acceptor, instead of nitrates or sulfates, methane results from the net reduction of COD. The bicarbonate produced during AD makes it harder to quantify the volume of carbon dioxide formed. If the substrate is completely mineralized, the relative proportion of the different biogas components will be defined by the mean state of oxidation of organic matter contained within the substrate.

The heterogenic flora contained in an anaerobic reactor is not only responsible for the supply of substrates for subsequent process steps, but also for the prevalence of

anaerobic conditions. If accidentally oxygen reaches the anaerobic system, one fraction of the micro-organisms can reduce the concentration of a compound, stabilizing also the red-ox potential to more convenient values of –300 mV for methanogenic micro-organisms.

2.2.1.1 Hydrolysis

Because bacteria only consume soluble substrates, the microbial assimilation of particulate polymers requires a break down or hydrolysis step. Extra-cellular enzymes usually carry out this process and the reaction rate depends on several factors such as the pH of the media, cellular retention time and waste composition. Particulate material includes both substrate and recently formed biomass. There are several limitations describing the hydrolysis phenomena. However, microbial reactions within a reactor generally exhibit rates increasing exponentially. Thus, non-biodegradable polymers such as wax or lignin can reduce the hydrolysis rate of associated particulate organic matter.

The hydrolysis of dissolved macromolecules (> 1,000 Dalton) is carried out by extra-cellular enzymes attached to the cell wall. In biofilm cultures fed macromolecules substrates, for example, no more than 8% of the total hydrolytic activity (leucine, amino peptidase and α-glycosidase) was found to be located in the cell-free bulk solution. Thus, the generalized mechanism for macromolecules degradation by biofilms features cell-associated hydrolysis, followed by the release of hydrolytic fragments in the bulk solution (Confer and Logan, 1998).

The process of hydrolysis can be exemplified by describing the biodegradation of cellulose which is a linear polymer formed by cellobiose units joined by β-1,4-glycoside bonds. Two glucose linked units form what is known as one cellobiose unit (figure 2.2.2).

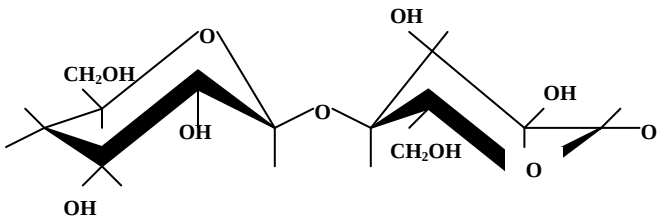


Figure 2.2.2 Cellobiose's molecular structure.

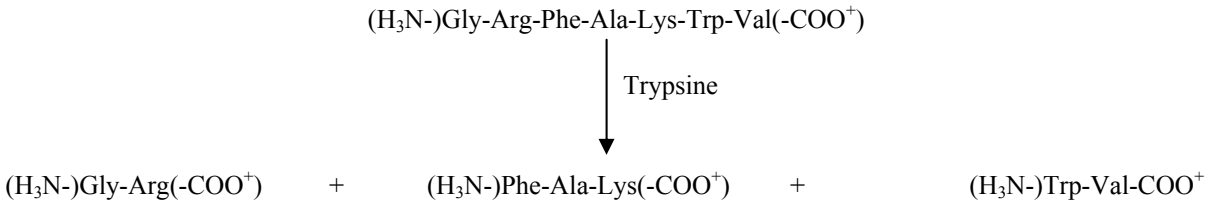
The degree of polymerization (PD) for cellulose represents 50 000 or more D-glucose units. Cellulodextrines with 1 to 6 PD units are water soluble, but the solubility diminishes with increasing PD. While increasing the length of the cellulose chain, the hydrogen bonds and van der Waals forces strongly join with adjacent cellulose chains, thus forming zones of highly packed cellulose from which water is excluded.

Hydrolysis of cellulose is carried out by the substitution of water into the β-1,4 bond of cellobiose. Solubilization of cellulose through extra cellular enzymes starts with the slow reduction of cellulose PD and the conversion of substrate into soluble sugar. Generally, the enzymatic reaction rate depends on the available surface area of cellulose. The complete hydrolysis of cellulose requires at least the action of three enzymes known as C_1, C_x, and β-glycosidase. Generally, C_1 is an extra-cellular enzyme which produces cellobiose units from cellulose. Then, C_x is assumed to randomly transform the cellobiose β-1,4 glycosidic bond and finally glucose is produced from the β-glycosidase catalysis of cellobiose compounds (Brock and Madigan, 1991).

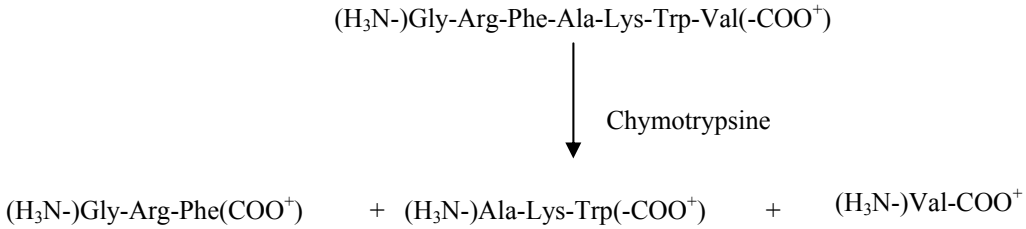
Since the degradative mechanism is not clearly known, the micro flora responsible for the break down of fibrous polysaccharides is currently a subject of a controversy. Eleven types of cellulolytic bacteria were identified and all of them are strict anaerobes, Gram positive micro-organisms. An anaerobic effluent could account for up to $4x10^5$ micro-organisms/mL and only some of them are capable of producing endo-glucanase or β-glycosidase. Consequently, these micro-organisms are capable of hydrolyzing only soluble cellulose derivates such as carboxymethylcellulose. For example, *Cl. thermocellum* produces C_1 and C_x enzymes. Additionally, hemi cellulose-degrading micro-organisms such as *Bacteroides ruminicola* and some Gram-negative micro-organisms have been isolated from anaerobic reactors treating swine waste (Brock and Madigan, 1991).

The attack of the α-1,4- and/or α-1,6-glycosidic bond by amylases releases starch, glycogen and some related polysaccharides. Examples are *Cl. butyricum*, Bacteriodes sp., Lactobacillus sp., *Bacillus subtillis*, *B. cereus*, and *B. licheniformis*. In anaerobic effluents, these micro-organisms can reach counts of $4x10^4$ /mL.

Besides the hydrolysis of fibrous or high molecular weight carbohydrates, protein hydrolysis is carried out by extra-cellular enzymes or proteases, producing polypeptides and amino-acids. Depending on the original protein characteristics, these compounds are subsequently metabolized into VFA, CO_2, H_2, NH_4^+, and S_2^- (figure 2.2.3).



a)



b)

Figure 2.2.3 Protease mechanism: a) Trypsin, and; b) Chymotrypsin (Rawn, 1990).

Proteolytic bacteria play an important role in stabilizing solid or semi-solid waste. Clostridia and Cocci are the most numerous proteolytic micro-organisms found in anaerobic digesters with counts of 10^4 to 10^6/mL. Examples are *Clostridium bifermentans, Cl. butyricum, Cl. perfringens, Cl. mangenotti, Cl. litusburense*, *Peptococcus anaerobius* and *Staphilococcus aureus*, besides Sarcina, Bacteroides and Propionibacterium. The majority of proteolytic organisms isolated are also able to hydrolyze carbohydrates producing CO_2 and organic acids (Brock and Madigan, 1991).

Lipases are the extra-cellular enzymes responsible for lipids conversion into long-chain VFA. In mixed cultures, proteases inhibit some lipases. The majority of lipases attack the 1 and 3 positions of glycerol, while other can attack all three positions producing VFA and glycerol is produced as end products (figure 2.2.4).

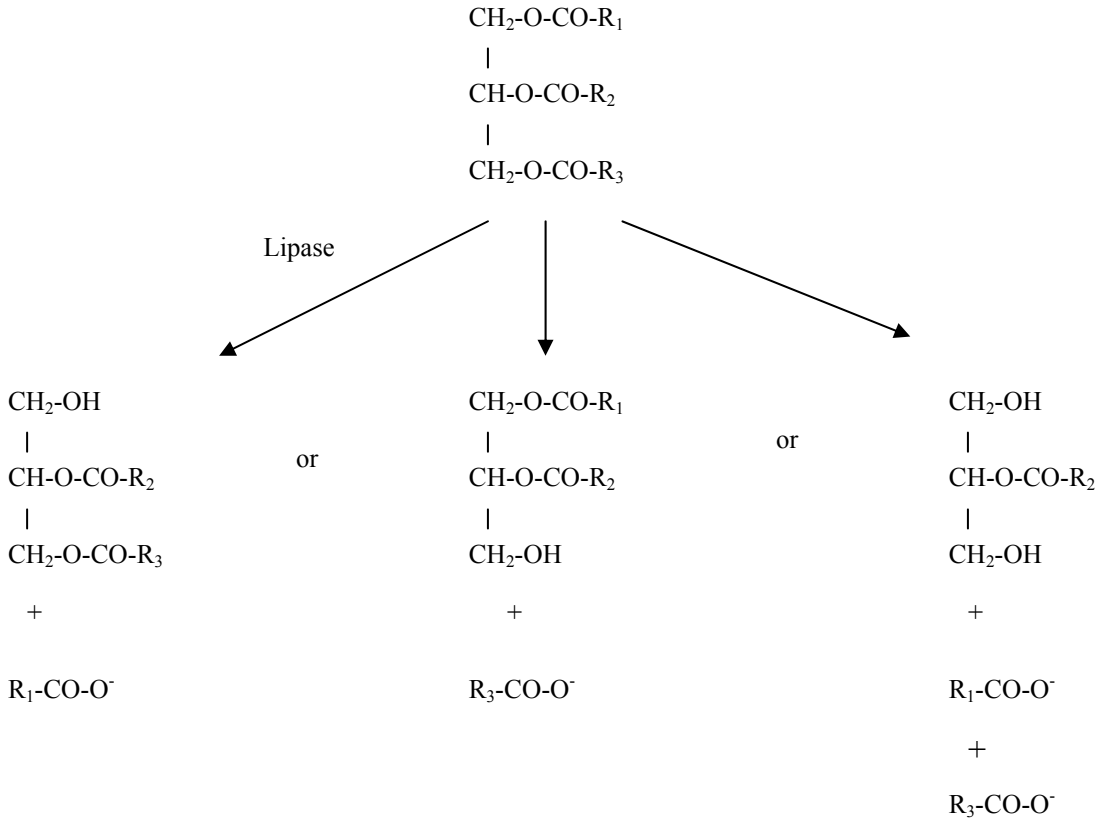


Figure 2.2.4 Lipase activity inside the human intestine (Rawn, 1990).

The concentration of lipolytic micro-organisms in wastewater can reach 10^4 to 10^5 counts/mL. In anaerobic environments, Clostridia and Micrococci seem to be responsible for the production of the majority of extra-cellular lipases (Brock and Madigan, 1991).

2.2.1.2 Fermentation and β-oxidation

Fermentation and β-oxidation are the steps following the hydrolysis of complex polymers whether particulate or soluble. Acetogenic bacteria are the principal micro-flora and acetate is one of the main products of those micro-organisms, besides higher molecular weight VFAs such as propionate, butyrate, iso-butyrate, valerate and iso-valerate (figure 2.2.5).

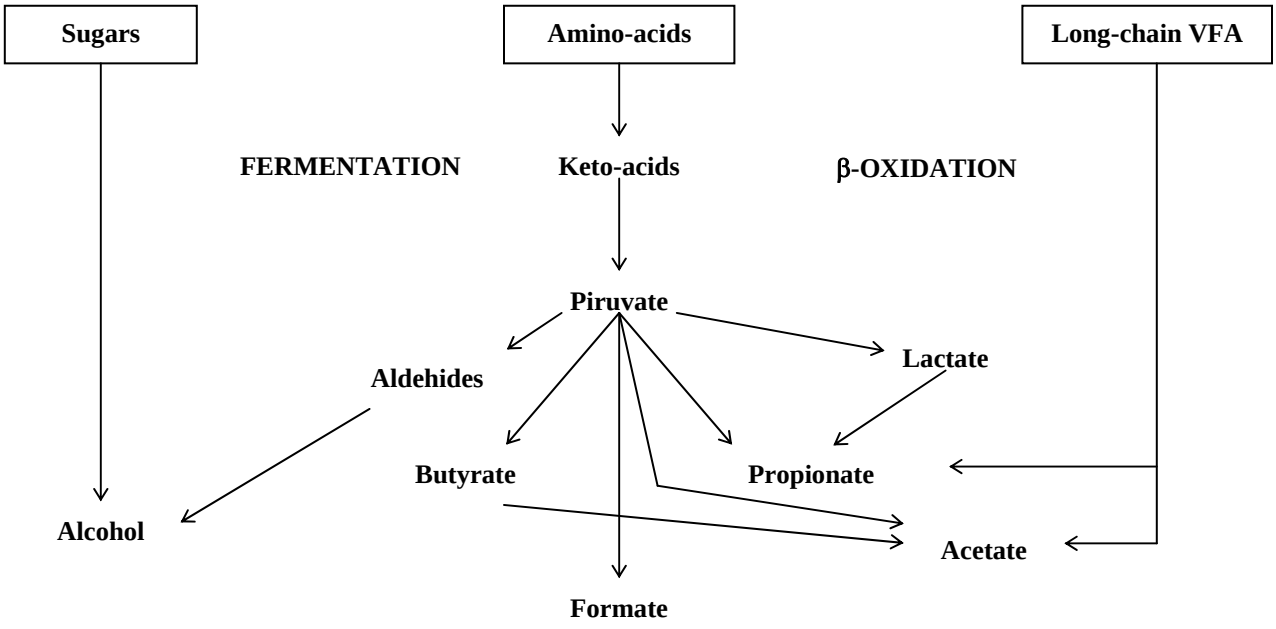


Figure 2.2.5 Acetate formation during fermentative and β-oxidative activities.

2.2.1.3 Methanogenesis

Nearly 70% of methane produced during AD is generated from acetate through a decarboxylation reaction:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$$

After culture enrichment in the absence of Methanosarcina, Methanosaeta was identified as mainly responsible for this reaction. Since the reaction of acetyl-CoA is closely related to methane production from methyl compounds and acetate, the growth of acetoclastic methanogens including energy production is also related to this metabolic pathway.

Acetate is presumed to activate acetyl-CoA in the presence of the carbon monoxide dehydrogenase which transfers the acetate methylated group onto a B_{12}-vitamin molecule to produce CH_3-B_{12}. Then, the methylated group is transformed into tetrahydromethanopterine and then Co-M to form CH_3-CoM. This last metabolite is reduced by electrons generated from the oxidation of CO into CO_2 by the enzyme CO-dehydrogenase. Since no method is known to couple phosphorylation at the substrate level during acetoclastic methanogenesis, the synthesis of ATP is carried out through the pathway of electro-carrying phosphorylation (figure 2.2.6).

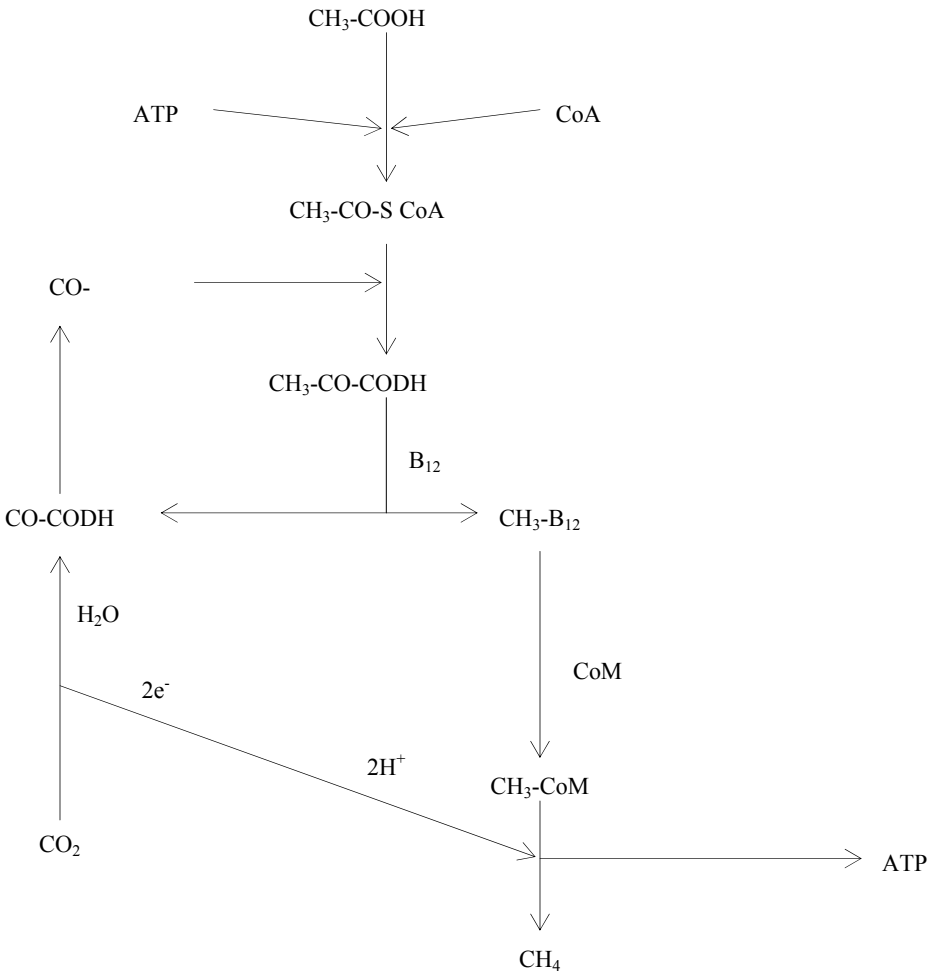


Figure 2.2.6 Acetoclastic methanogenesis.

Besides acetoclastic methanogenesis, methane is produced by another metabolic pathway, namely reductive methanogenesis, where CO_2 is reduced by H_2. Methanogens able to perform the reductive methanogenesis are highly affected by medium pH:

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

This reaction is governed by the enzyme CoM exclusively found in methanogens and because of its methyl group, is required by methyl-Co-M-reductase and is active during the final stage of CO_2 reduction.

Reeve *et al*. (1997) found that *Methanobacterium thermoautotrophicum*[1], common to anaerobic reactors, regulates the synthesis of enzymes promoting cell duplication or increases methane yield/cell in response to substrate availability. In H_2-limiting conditions, the cell switches off the enzyme codification from the transcription of the mrtBDGA operon responsible for the synthesis of the enzyme methyl coenzyme M reductase II, at the last step of CO_2 reduction to methane. Rather, the cell uses the transcription of the mcrBDCGA operon responsible of the synthesis of the enzyme methyl coenzyme M reductase I in the same last step of CO_2 reduction (figure 2.2.7). This change in enzyme synthesis is accompanied by a drop in methane production without a drop in cell growth, a process consistent with natural environments where micro-organisms can maximize either growth rate or growth yield.

[1] *Methanobacterium thermoautotrophicum*: strictly $H_2 + CO_2$ consuming methanogen. Growth temperature 55°C. Substrate condition H_2/CO_2 gas mixture 89%/11%.

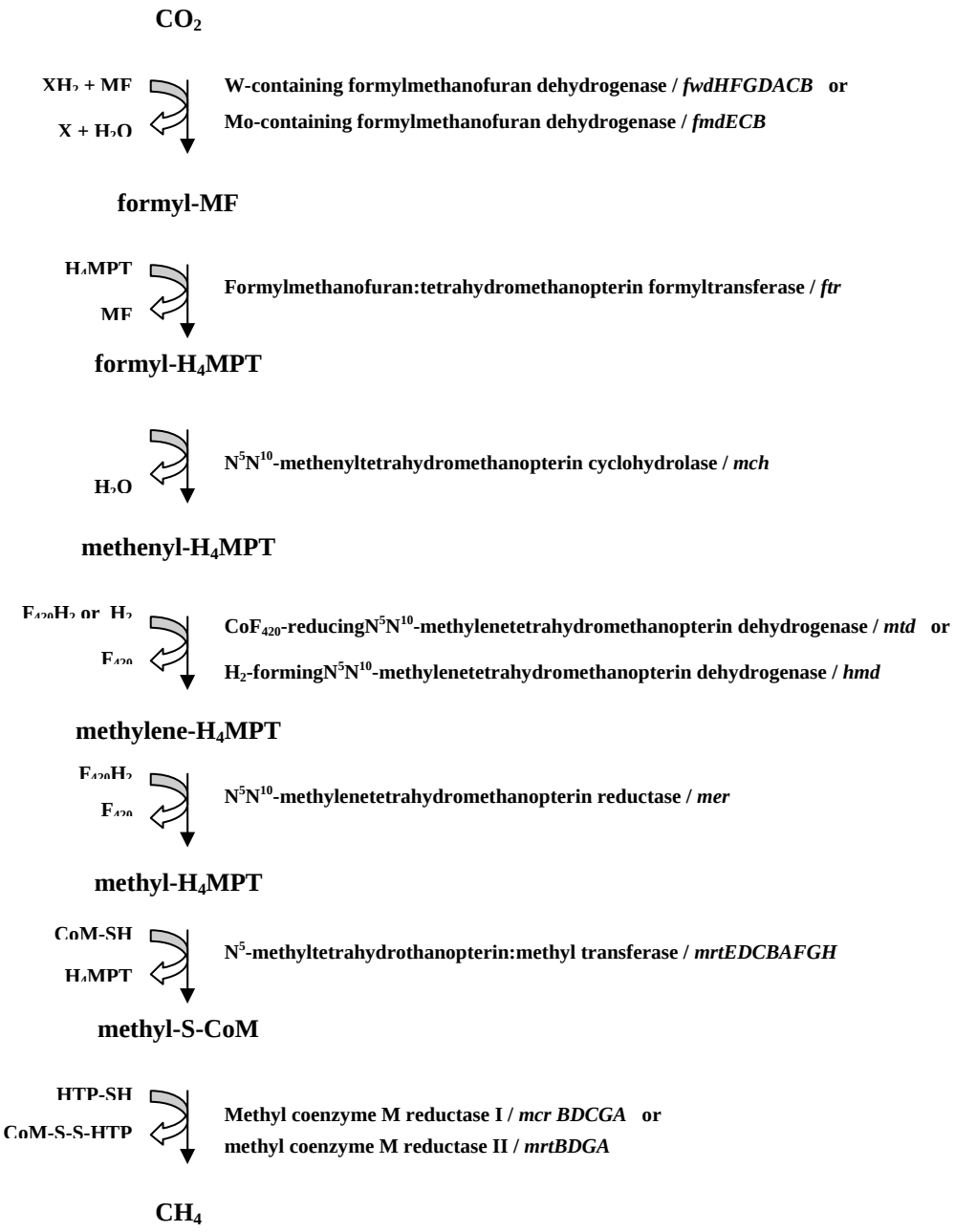


Figure 2.2.7 The H_2-dependent pathway for CO_2 reduction to CH_4 in *M. thermoautotrophicum* (Reeve *et al*. 1997).

2.3 ANAEROBIC TECHNOLOGY FOR THE TREATMENT OF MSW

Food waste causes landfilling issues, such as malodours and slow stabilization (Kim *et al*., 2000). In landfills, the degree and rate of organic matter degradation are often assumed to be higher than they actually are, when after two decades in the landfill, 33 % of non-plastic organic waste are still recognizable. Besides odours, OFMSW produces greenhouse gases, terrain settlement and leachate (Rhyner *et al*. 1995).

Biological treatment can maximize recycling and recovery of its components. Among biological treatments, anaerobic digestion is often cost-effective, owing to the high energy recovery and the limited environmental impact. Indeed and when considered in the context of Life Cycle Analysis (LCA), AD offers a number of interesting features. Biogas is an important fuel for heating in Third World countries. Moreover, aerobic treatment inevitably gives rise to extensive emission of undesirable volatile compounds such as ketones, aldehydes, ammonia and even methane. In AD, all gases are contained and can be eliminated by flaming the biogas.

The AD of MSW is not the most popular process when stabilizing wastes, but over the last decade, it has become an interesting alternative. One of the main factors promoting the AD of MSW is the practice of source-separated collection, since the waste becomes “cleaner” and this facilitates the process of energy and materials recovery.

De Baere (2000) investigated an full size plant treating where at least 10% of the treated organic waste came from markets or municipalities (excluding sewage sludge and manure) and clearly, AD was a reliable technology. In Europe, the total treatment

capacity for solid waste evolved from 122,000 tons/year in 1990 to 1,037,000 tons/year and 53 plants, in 2000, for 750 % increase.

Lissens *et al.* (2001) observed the trend in the AD of OFMSW sorted mechanically in central plants or organic wastes separated at the source (biowaste; mainly paper, vegetables, fruits, food left-over and garden waste). Batch reactors for wet and dry systems were the most common types of reliable anaerobic digesters. The process configuration varied according to needs and included at times, one or two phase digestion systems.

Almost all treatment plants include both pre and post-treatments, depending on several factors: characteristics of the substrate, the type of anaerobic technology, and the final use of the biosolid produced. Pre-treatment steps may include magnetic separation, comminuting in a rotating drum or shredder, screening, pulping, gravity separation (dry separation), or pasteurization. In the post-treatment steps, the typical sequence involves mechanical dewatering, aerobic maturation and water and/or gas treatment (figure 2.3.1).

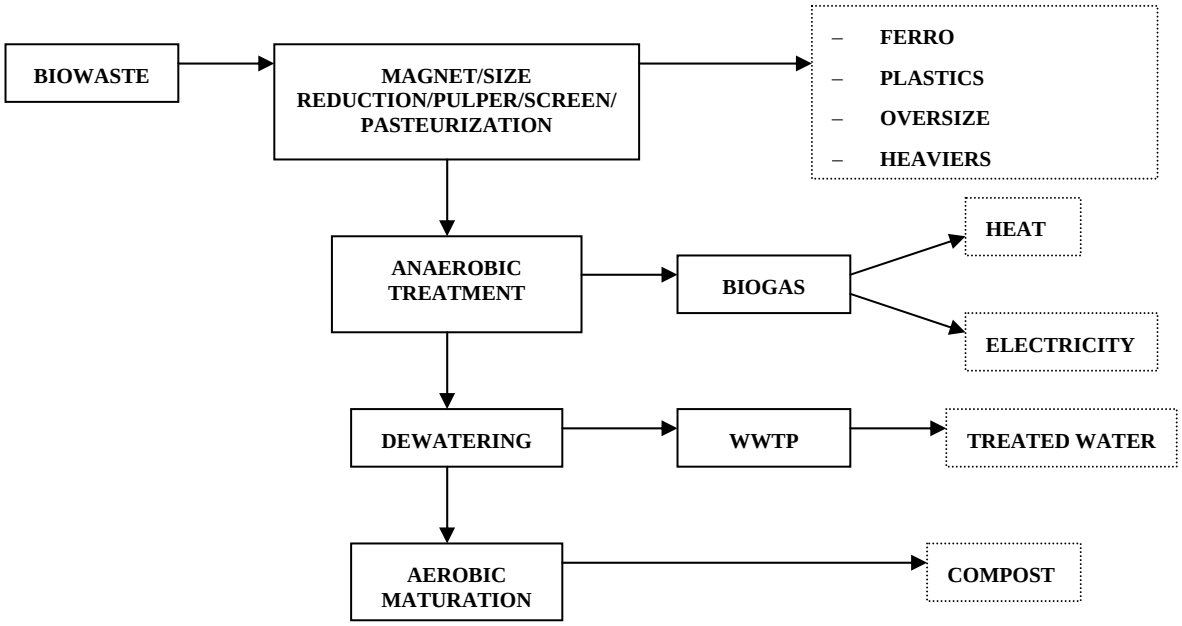


Figure 2.3.1 Overview of pre and post-treatment technologies in OFMSW digestion.

Because AD requires a series of biochemical transformations roughly separated into a first step where hydrolysis, acidification and liquefaction take place and a second step where acetate, hydrogen and carbon dioxide are transformed into methane, commonly used technology relate to one-stage, two-stage and batch reactor. In one-stage and batch systems, all these reactions take place simultaneously in a single reactor, while in two- or multiple-stage systems, the reactions take place sequentially in at least two reactors.

A useful tool in evaluating the biological performance of AD is the maximum sustainable reaction rate expressed as both a rate of substrate utilization or maximum organic loading rate (OLR) expressed as $kgVS/m^3 \cdot d$, or as the rate of product formation such as methane production rate expressed as $m^3CH_4/m^3 \cdot d$, under standard pressure and temperature. Hydraulic retention time (HRT) is another useful tool evaluating reactor performance.

2.3.1 ONE-STAGE SYSTEMS

About 90% of the current European full-scale plants for AD of OFMSW and biowaste rely on one-stage systems, approximately evenly split between wet and dry operating conditions (De Baere, 2000).

2.3.1.1 One-stage wet systems

The term “solid waste” generally means organic biodegradable waste with over 15% TS. In wet, complete mixed one-stage systems, the organic solid waste is diluted with water

via pulping and slurrying to less than 15 % TS. One of the first full-scale plants for the treatment of biowaste, built in the city of Waasa, Finland, in 1989, is based on this principle. Usually a pulper with vertical auger mixers is used to shred, homogenize and dilute the waste in sequential batches. To this end, both fresh and recycled process waters are added to attain 10 – 15 % TS. The obtained slurry is then digested in large complete mix reactors where the solids are kept in suspension by vertical impellers (figure 2.3.2).

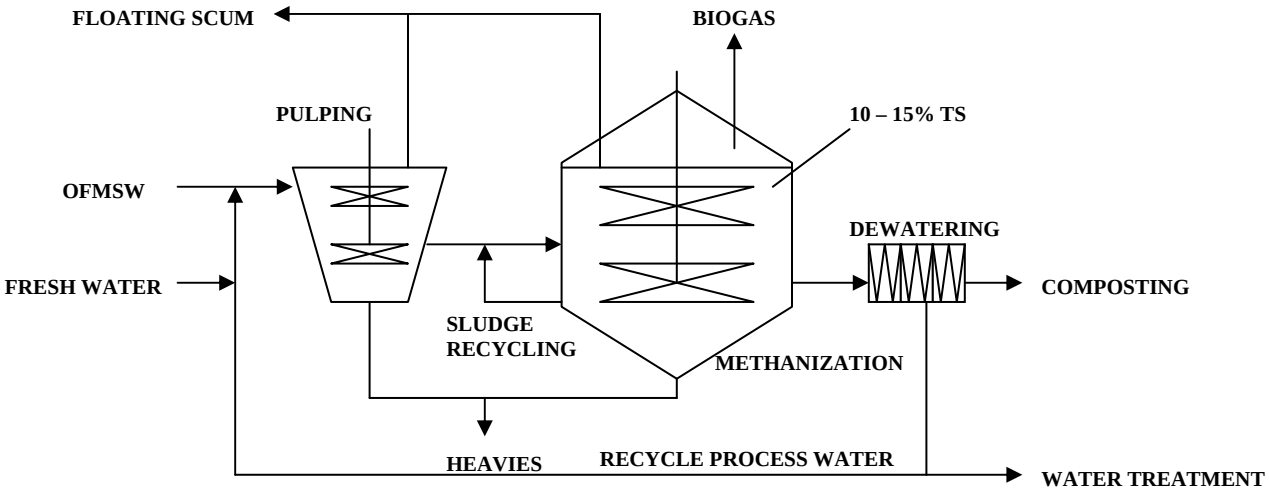


Figure 2.3.2 The typical design of a one-stage wet system (Waasa, Finland).

In complete mixed one-stage systems, slurry-wastes do not always keep a homogeneous consistency because the heavier fraction and contaminants may sink and a floating scum layer forms because of substances present in plant materials. The heaviest particles accumulating at the bottom of the reactor may damage the propellers while several meters of floating scum can accumulate to hamper mixing. Both of these bottom and top fractions must be extracted. Heavy particles damage pumps and often are removed from the waste before entering the reactor (Lissens *et al*. 2001).

A technical drawback of the complete mixed one-stage reactor is the occurrence of short-circuiting, such as the passage of a feed fraction through the reactor with a shorter HRT than that of the average bulk stream. This generally results in a decreased biogas production and lower kill-off of microbial pathogens. The removal of the floating scum layer and the heavy fraction leads to the incomplete biogas recovery. Water consumption is generally high because the waste needs to be diluted at a ration of $1m^3$ tap water/ton solid wastes. Typical OLR values for one-stage wet systems treating OFMSW range from 5 to 10 kg SV/m^3·d, but the variation depends on the origin and composition of the substrate (Lissens *et al*. 2001).

2.3.1.2 One-stage dry systems

Research during the 1980's demonstrated that biogas yield and production rate were at least as high in systems where the waste was kept at its original TS. The challenge with high TS values is the handling, pumping and mixing of the waste stream.

In dry systems, the fermenting mass within the reactor is kept at a TS of 20 to 40 % and only very dry substrates with more than 60 % TS need to be diluted with process water. Transport and handling of these wastes is carried out with conveyor belts, screws and powerful pumps especially designed for highly viscous streams. This type of equipment is more expensive than centrifugal pumps used in wet systems. However, this equipment is much more robust and flexible since wastes with TS of 20 to 50 % can be readily handled including impurities such as stones, glass or wood. The only pre-treatment required is the removal of the coarse impurities larger than 30 mm, and this is far simpler with dry as compared to wet systems (Lissens *et al*. 2001).

Due to their high viscosity, the fermenting wastes move via principles of plug flow inside the reactors, which is technically simple, as no mechanical devices need to be installed within the reactor. At least three industrial designs are effective for the adequate mixing of solid wastes with dry anerobic systems (figure 2.3.3).

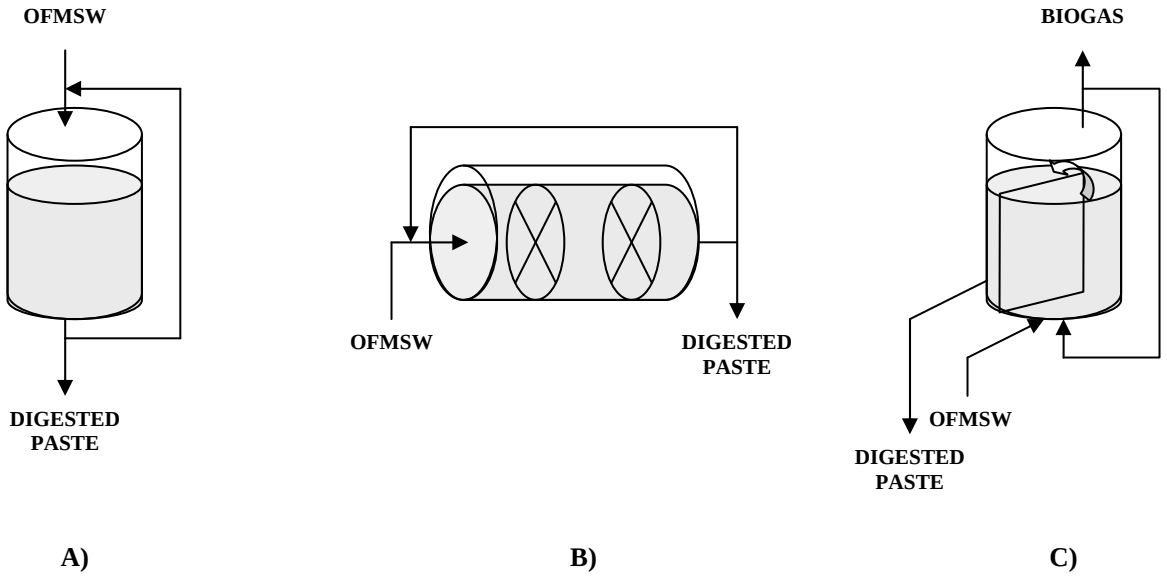


Figure 2.3.3 Typical designs for one-stage dry systems: A) Dranco; B) Kompogas and BRV, and; C) Valorga.

In the Dranco process, one part of fresh waste is mixed with six parts of digested waste extracted from the bottom of the mixer. This simple design is effective for the treatment of wastes ranging from 20 to 50 % TS (Lissens *et al*. 2001).

The Kompogas process works similarly to the Dranco design, except that the plug flow takes place horizontally in cylindrical reactors and mixing occurs by slowly rotating impellers inside the reactor, which also degas and resuspend heavy particles. This system requires careful adjustment of TS around 23 % because at lower values, heavy particles

such as sand and glass tend to sink and accumulate inside the reactor while higher TS cause excessive flow resistance (Lissens *et al*. 2001).

The Valorga system is quite different in that the horizontal plug flow is circular in a cylindrical reactor and mixing occurs via biogas injection at high pressure at the bottom of the reactor. This biogas injection takes place every 15 minutes through a network of injectors, but these require regular unclogging and maintenance (Lissens *et al*. 2001).

The dry and wet reactors remove the same amount of VS, ranging between 50 to 70 %, resulting in a biogas yields of 90 m^3/ton fresh food waste or 210 to 300 m^3CH_4/ton VS destroyed (De Baere, 2000). The OLR of dry systems is generally higher than that of wet systems. The Valorga dry system at Tilburg, Netherlands, treats 5 kg VS/m^3·d, a value comparable to that of wet systems. The optimized dry systems of the Dranco plant in Brecht, Belgium, treats 15 kg VS/m^3·d on a yearly average (De Baere, 2000).

Inhibitors, such as ammonia, can often limit the OLR of reactors treating OFMSW. One-stage wet reactors generally suffer from their fully homogenized content, where spatial niches are elminated aqnd bacteria are fully exposed to high concentration of inhibitors. For solid wastes with a C/N ratio above 20, water dilution generally reduces ammonia inhibition (Lissens *et al*. 2001).

Small economical differences separate wet and dry systems, both in terms of investment and operational costs. The higher costs for the sturdy waste handling devices, such as pumps, screws and valves required for dry systems, are compensated by cheaper pre-treatment systems and reactor design (Lissens *et al*. 2001). As compared to one-stage wet systems, dry systems acheive higher OLRs in both bench-scale and full-scale applications. Moreover, slightly higher biogas yields are expected in dry systems

compared to wet systems, since neither heavy inert nor scum layer needs to be removed before or during digestion (Lissens *et al*. 2001).

2.3.2 TWO-STAGE SYSTEMS

For the conversion of OFMSW into biogas, two- and multi-stage systems are justified by a sequence of biochemical reactions which do not necessarily share the same optimal environmental conditions. Typically, two-stage systems consist of: a first for the liquefaction-acidification limited in rqate by the biodegradability of the substrates, and; the second stage for the acetogenic and methanogenic process with a rate limited by the slow microbial growth of the methanogenic bacteria. The main advantage of a two-stage system is not its higher biogas yield but its increased biological stability for wastes which cause unstable performance in one-stage systems. Examples of such wastes are cellulose-poor wastes with C/N ratios lower than 10 or rapidly degradable wastes such as the market wastes rich in fruits and vegetables (Pavan *et al*. 2000b). Figure 2.3.4 illustrates a two-stage system, excluding the pre-treatment equipment.

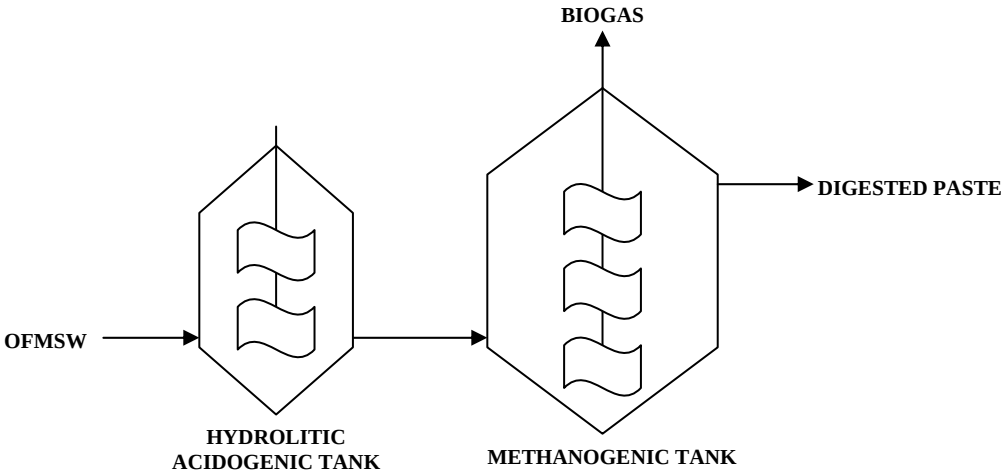


Figure 2.3.4 The typical design of two-stage systems ("dry-dry" or "wet-wet")

As with dry one-stage systems, the two-stage dry system requires less pre-treatment for the removal of dense materials and scum. A possible drawback of two-stage systems is the occurrence of methanogenesis in the first reactor when hydrolysis becomes rate-limiting (Lissens *et al*. 2001). Typical HRT in the first acidogenic tank range from 2 to 4 days, while that of the mesophilic and thermophilic methanogenic tank range from 15 to 25 and 7 to 15 days, respectivity (Pavan *et al*. 2000b; Ghosh *et al*. 2000). For the different agro-industrial residues with mainly vegetable residues, 50 to 70 % of the organic matter can be degraded within a HRT of 10 to 20 days including both phases (Lissens *et al*. 2001).

In terms of OLR under thermophilic temperatures for source sorted OFMSW, Pavan *et al*. (2000b) and Scherer *et al*. (2000) fed the first and second reactors with 31 and 7 kg SV/m^3·d, and 29 and 10 kg SV/m^3·d, respectively, resulting in a specific gas production of 0.5 m^3/kg TVS_{added} and 0.7 m^3/kg TVS_{added}. Two stage systems can

effectively treat OFMSW with a C/N-ratio below 10 because methanogens can be protected from high ammonium levels, while for C/N ratios above 15, the one-stage process is preferred.

The first tank of the two-phase anaerobic treatment of OFMSW improves the rheological characteristics of the waste. Battistoni *et al*. (2000) found that the acidogenic phase reduces over 10% of both rigidity and yield stress coefficients of both source collected OFMSW and separately collected OFMSW. Despite the increase of both coefficients during the second thermophilic methanogenic phase, its net sludge flow capacity is not affected because of the drop in VST. The energy required to mix the sludge in the second tank ranges from 25 to 50 W/m^3.

2.3.3 BATCH SYSTEMS

In batch systems, digesters are filled once with fresh waste, with or without addition of seed material, and allowed to react sequentially either in the dry mode at 30 to 40 % TS, or the wet mode with less than 15% TS. Though batch systems appear as an in-box landfill, they achieve 50 to 100-fold higher biogas production rates because: the leachate is continuously recirculated, which allows for the dispersion of inoculant, nutrients and acids, and; AD occurs at higher temperatures.

In the first Dutch full-scale batch mesophilic anaerobic digester of OFMSW with a capacity of 1,000 ton/week, ten Brummeler (2000) found that the inactivation of *Salmonella typhimurium* (from 105 to < 1 cfu/mL) was associated with high concentrations of acetate, propionate and butyrate of 3,500, 3,000 and 1,100 mg/L, respectively. The HRT of the batch process plant was 21 days and the highest VFA

concentrations were reached in the first five days after closing the digester. The plant was designed for an average OLR of 3.6 kg VS/m^3·d and s summer OLR of 5.1 kg VS/m^3·d. With a process temperature of 35 to 40°C, the Biocel plant produced weekly 70 kg of biogas, 500 kg of compost products, 230 kg of wastewater sent to the sewage system, 30 kg of aerobic post treatment-requiring products, 120 kg of vaporized water, and 50 kg of non-recyclable products. The heat and electricity produced from the biogas were reused in the plant facilities and offices; excess electricity was directed to the municipal system.

Because batch systems are technically simple, the investment costs are significantly lower than those continuously fed. The land area required by batch systems is, however, considerably larger than that for continuously fed dry systems, since the height of batch reactors is five-fold less and the OLR two-fold less, resulting in a footprint enlarge ten times/ton of treated waste. Operational costs, on the other hand, are comparable (ten Brummeler, 2000).

2.4 PERSPECTIVES

A remarkable evolution has occurred in the attitude towards the in-reactor digestion of solid waste. Skepticism with respect to its feasibility has changed towards a general acceptance that various digester types can function reliably at full scale. The choice of AD technologies depends on substrate characteristics, whether one or two-stages, and mesophilic or thermophilic. According to De Baere's (2000), anaerobic plants were initially operated at mesophilic temperature, but since the introduction of thermophilic systems in 1992 and 1993, AD became more efficient and the cumulative capacity has increased from 56,000 in 1997 to 280,000 tons/year in 2000. Other benefits of

thermophilic AD are the higher pathogen kill-off and the added amount of heat and electricity recovered.

2.5 THERMOPHILIC ANAEROBIC TREATMENT OF MSW

The effect of temperature whether mesophilic, thermophilic and even psycrophilic, on the treatment of low and high strength wastewater, excess sludge and OFMSW was extensively studied. Thermophilic AD is an attractive technology due to its advantages, such as high loading potential, shorter HRT, pathogen control and low sludge production.

Anaerobic digestion can function over a large range of temperatures, from psycrophilic temperatures at 10°C to some extreme thermophilic temperatures over 70°C, namely hyperthermophilic conditions. Temperature influences both the kinetics and the thermodynamics of AD, because microbial degradation rates and yields increase with temperature. The average temperature over a long period fixes the bacterial population and the kinetic coefficients of two majors groups of micro-organisms (figure 2.5.1), namely the thermophilic (45 – 60°C) and psycro-/mesophilic (10-40°C) organisms. However, it is more common to classify them as thermophilic (45 – 65°C) and mesophilic (25 – 40°C).

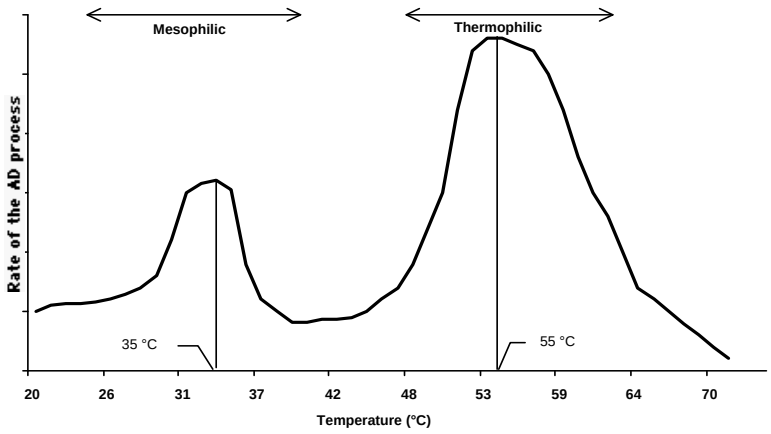


Figure 2.5.1 Temperatures range for anaerobic digestion.

Nozhevnikova *et al*. (1999) introduced the concept of moderate and extreme thermophilic conditions, corresponding to 50 - 55 °C and 55 - 82 °C, respectively. A rapid change in temperature from one range to another introduces a population shift because the groups are incompatible (Mata-Alvarez, 2002). Nozhevnikova *et al*. (1999) assume that thermophilic and mesophilic methanogenic communities differ with respect to the microbial species. Nevertheless, some evidence demonstrated that is not the case and micro-organisms have mechanisms to evolve from one specific state to another depending on the transition conditions. According to Nielsen and Petersen (2000), the start of full-scale thermophilic anaerobic digesters in Denmark was achieved with and without transition conditions and all the strategies resulted in a quick and satisfactory start-up. For all the plants started at thermophilic temperature, a few days were required to transform high levels of volatile fatty acids and CO_2 concentrations into biogas. This period requires carefull monitoring and a lower loading rate may be necessary. This period can be avoided when the digester is started directly at the thermophilic

temperature. Mata-Alvarez (2002) concluded that for the mechanically sorted organic fraction of MSW (MS-OFMSW) and dry or wet AD, a 10-day transition from mesophilic to thermophilic conditions (35 to 55°C) lead to significant variations in the organic loading rate, from 15 to 40 % in 48 hours, but the system recovers its original performance.

2.5.1 OPERATING CONDITIONS

Thermophilic anaerobic digestion was tested using different substrates, from excess wastewater sludge to agricultural waste and even refuse-derived fuel (RDF). Komatsu *et al*. (2002) describe the first dry thermophilic anaerobic plant for the treatment of the organic fraction of solid wastes. The substrate components were commercial waste (garbage and Kyoto's hotel leftovers), yard waste (branches trimmed from street trees), and used papers. The substrates had the following average TS and VS, respectively on a wet basis: 28.0 and 26.6 % for commercial waste, 93 and 89 % for used paper and; 59.3 and 48.0 % for yard waste. The generated biogas supplied both the required electric and heat energy to operate the plant. Because of the low biodegradability and high humidity of the biosolids produced, reuse alternatives were not obvious.

A semi-dry thermophilic anaerobic digester treating OFMSW was reported by Pavan *et al*. (2000a). Increasing the amount of SS-OFMSW from 0 to 100 % increased the specific gas production from 0.32 to 0.78 m^3/kg TVS_f, and TVS biodegradability from 35 to 80 %. Source separation practices for OFMSW improve reactor performance, reduce xcess sludge and increase biogas production.

Pavan *et al*. (2000b) also tested the effect of HRT, OLR and temperature (mesophilic and thermophilic) on the performance of a two-phase anaerobic digester using SS-OFMSW as substrate. The market collected SS-OFMSW, from the city of Treviso (Italy), are described in the table 2.5.1. The best performance for phase separation, gas production and VS removal was achieved with mesophilic and thermophilic conditions, a HRT of 3 to 5 days and 7 to 10 days for the hydrolysis phase and methanogenic reactors, respectively. Under such conditions, the acidogenic reactor reached concentrations exceeding 20 g TVFA/L and the methanogenic phase reached a gas production of 5.1 $m^3/m^3 \cdot d$, or 0.5 $m^3/kgTVS_f$. The OLRs were 31.2 and 6.9 kg $TVS/m^3 \cdot d$ for the acidogenic and methanogenic reactors, respectively, and the overall %VS removal reached 77 %.

Table 2.5.1 Market SS-OFMSW, Treviso, Italy (Pavan *et al*. 2000b).

	TS	**VS**	**TCOD**	**TKN**	**P**
Parameter	**g/kg**	**%TS**	**gO_2/gTS**	**%TS**	**%TS**
Average value	81.8	81.9	1.0	2.1	2.8

Scherer *et al*. (2000) tested thermophilic anaerobic digestion using substrates with a very lower biodegradability such as the gray OFMSW defined as the waste remaining after source separated collection of biowaste, but still containing a significant amount of organic material, mainly food and non-recyclable paper (De Baere, 2000). The two-step thermophilic anaerobic digestion had HRT of 4.3 and 14.2 days for the hydrolysis and methanogenic reactors, respectively, and the feed TS and VS ranged between 6.6 and 18% and less than 10% of the TS, respectively. The respective OLR of the thermophilic digesters ranged between 10.6 to 29 g and 2 to 10 g SV/L·d for the hydrolysis and

methanogenic reactors, depending on the source of the raw material. The VS removal efficiencies of the hydrolysis and methanogenic reactors varied from 13 and 68% and 31 to 68% respectively, depending on the type of material used. Under these conditions, 737 and 156 L/kgVS_{fed} of gas were produced.

According to Kim *et al*. (2000), substrate sodium ion concentration and particle size influence the thermophilic AD of food waste. For a fundamental batch glucose-enriched kinetic test with 5 g/L of sodium salt, an OLR under 2 g COD/L caused no inhibition and the substrate utilization rate coefficient, k, and the half-saturation coefficient, Ks, were 0.24 hr^{-1} and 700 mg/L, respectively. The substrate inhibition coefficient, Ki, was 1000 mg/L for the inhibiting OLR of 2 to 4 mg COD/L. Above 5 g/L of sodium ion, methane gas production dropped even under an OLR under 2 g COD/L. As the average particle size increased from 1.02 to 2.14 mm, the maximum substrate utilization rate coefficient, k_H, dropped from 0.0033/h to 0.0015/h.

Ghosh *et al*. (2000) monitored thermophilic anaerobic digestion using a blend of 80 % refuse-derived fuel (RDF) and 20 % excess activated sludge. The main constituents of RDF were 42.4 % paper products, 15.3 % food waste, 13.8 % garden waste, 10.1 % glass and ceramics, 7.2 % rock and ash, 6.7 % metal, 1.8 % plastic, rubber and leather,1.6 % textiles and 1.1 % wood. The one-phase digestion was conducted with an OLR of 4.8 kg VS/m^3·d and a HRT of 7 days. At 55°C, the specific methane yield was 0.156 m^3_{SPT}/kgVS_{add}.

2.5.2 LOADING CAPACITY

Under steady state conditions and using a full-scale thermophilic digester, Zábranská *et al*. (2002) fed sludge up to 4.1 kg VS/m^3d, as compared to the maximum loading rate of 3.1 kg VS/m^3d used for a similar mesophilic reactor. The production of more gas demonstrated that thermophilic condition increases methanogenic activity.

Thermophilic anaerobic digestion for OFMSW was evaluated by Li *et al*. (2002) with a 5-L laboratory scale reactor. Under thermophilic conditions and a 7.5 day HRT, no VFA accumulation resulted from an OLR of 33 gCOD/L·d which is twice the load accommodated under mesophilic conditions with a 15 day HRT. Thus, increasing the organic load from 10 to 33 g COD/L·d resulted in the same average VS removal of 75 to 80% for thermophilic conditions while for mesophilic conditions, VS removal dropped from 75 to 65 % with an organic load increasing from 13 g COD/l·d to 22 g COD/l·d.

2.5.3 BIOGAS AND CH_4 PRODUCTION

Thermophilic conditions are known to increase the metabolism of methanogenic bacteria and, for several substrates, improve methane production. In the laboratory with a substrate containing 9.1 % chicken slurry, 25.5 % poultry manure, 41.4 % biological municipal waste, 4 % kitchen refuse, 8 % grease, and 3 % food refuse, thermophilic conditions produced 450 L mehtane/$gVSS_{added}$, or twice the amount obtained under the same 4 day HRT but with mesophilic conditions. Longer HRT did not substantially increase methane production for both conditions as a HRT of 15 to 20 days produced 500 L of methane/$gVSS_{added..}$ With a 4 days HRT, thermophilic and mesophilic AD reduced VS by 38 and 22%, respectively (Maibaum and Kuehn, 1999).

With eigth full-scale thermophilic anaerobic reactors in Denmark, Nielsen and Petersen (2000) found that thermophilic and mesophilic conditions performed similarly when fed chemical, primary and excess sludge but thermophilic temperatures reduced HRT from 20 to 10-12 days, and increased biogas production by 160 L/kg of fed VS.

Under a batch test exposed to a stepwise temperature increment, Zábranská *et al*. (2000) found that thermophilic conditions promoted the methanogenic activity of sludge fed glucose, acetic and propionic. The sludge methane yield was increased to 0.36, 0.37 and 0.30 L/gCOD, from 0.32, 0.18 and 0.2 L/gCOD, as compared to mesophilic temperatures and the same respective substrates. Nevertheless, primary and raw sludge reduced thermophilic methane production to 0.26 and 0.20 L/g COD, as compared to 0.27 and 0.17 L/g COD under mesophilic conditions, respectively.

In a pilot scale reactor treating 3 tons/day of organic waste, Komatsu *et al*. (2002) observed that thermophilic AD had a variable performance depending on the type of waste. An average decomposition of 84 % was achieved with commercial waste, 66 % with used paper and only 20 % with yard waste. The associated biogas production was 808, 827 and 825 m^3_N/ton fed VS, respectively, with 58 % methane.

With full scale facilities, Zábranská *et al*. (2002) obtained *ca.* 50% more the biogas under thermophilic as compare to mesophilic conditions (0.68 and 0.48 m^3_{STP} / kg of VS, respectively). Nevertheless, similar methane percentages of 66.1 to 66.5 % were observed indicating higher water evaporation at higher temperature.

Using a 5 L thermophilic anaerobic digester, Li *et al*. (2002) obtained 8 to 11 m^3 methane/m^3·d, with an average OLR of 33 kg COD/m^3·d and a HRT of 7.5 days or twice that under mesophilic conditions with an OLR of 15 kgCOD/m^3·d and a 15 day HRT.

Thermophilic AD does not always produce a higher biogas yield as compared to mesophilic conditions. With a 2-L reactor treating proteinaceous wastewater, Fang and Wai-Chung (1999) obtained better results with a mesophilic biomass. With an organic load of 8 to 16 g COD/L·d, 79 to 98 % of the COD was removed with the mesophilic biomass as compared to 68 to 80 % with the thermophilic biomass. When the loading rate was further increased to 42 g COD/L·d, only the mesophilic reactor experienced a severe sludge washout, but the thermophilic reactor could only remove 54 % of the COD. The lower capacity of the thermophilic reactors was attributable to the short 9 h HRT and to the high propionate concentration of the sludge bed blanket of 540 to 1680 mg/L.

Watanabe *et al*. (1997) obtained a high pathogen kill-off with sludge treated by thermophilic AD, despite the low gas production rate. For continuously fed 6-L laboratory reactors with a 20 day HRT operated under mesophilic (35°C) and thermophilic (55°C) temperatures, gas production and VS digestion ratios were similar, at 0.5 L/gVS and 55 %, respectively.

2.5.4 PATHOGEN REDUCTION

Thermophilic AD of solid or semi-solid waste can produce biosolids with low pathogen and parasite counts because of the pasteurization effect of its temperature and gas pressure. Watanabe *et al*. (1997) found that in steady state thermophilic AD of sludge, Fecal Coliforms dropped from $10^2 – 10^4$ NMP/g to 10^0 NMP/g, with a 10 to 20 day HRT. Under the same conditions, Enterococci and Salmonella dropped from $10^3 – 10^4$ NMP/g to 10^0 NMP/g and from 1.8 - 30 NMP/4g to less than 1.8 NMP/4g, respectively.

With a pilot scale completely mixed reactor, Huyard *et al*. (2000) found that 60°C and 2±1 days of HRT reduced VS by 61 % and Fecal Coliforms by 5.5 log, Polio Virus by 4 log and *Ascari ova*bu by 2.6 log. Such results meet the EPA/PEC requirements for a Process Further Reduce Pathogen (PFPR) equivalency (table 2.5.2). The initial pathogen counts of the sludge were for Fecal Coliforms 5.59 to 7.47 log NMP/g TS, for Ascaris ova 100 eggs /gTS and for Poliomyelitis Virus 1,000 PFU /gTS.

In 2000, new Danish regulations encouraged the thermophilic AD of sludge in eight municipal wastewater treatment plants (Nielsen and Petersen, 2000). Despite the reduction in pathogen counts, the Horsens plant added an additional pasteurization step to produce "controlled pasteurized" sludge for land spreading, according to Danish regulations. This additional treatment requires a guaranteed HRT based on the temperature, such as 2.5 hours at 60°C. Furthermore, the sludge must respect maximum Salmonella and Faecal streptococcus counts (table 2.5.3). At the Holbæk wastewater treatment plant, the sludge pathogen count was measured before and after the change-over to thermophilic anaerobic digestion (table 2.5.3).

Table 2.5.2 Comparison of USA and French regulations.

	EPA 40 CFR 503 (USA)	Decree of 8/1/98 (France)
Volatile solid reduction	38%	-
Faecal coliforms	< 1000 cfu/gTS	-
Salmonella	< 3 NMP/4gTS	< 8 NMP/10gTS
Enterovirus	< 1 NMP/4gTS	< 3 NMP/10gTS
Viable helminth eggs	< 1 ova/4gTS	< 3 ova/10gTS

Huyard A., Ferran B., and Audic J-M.. (2000).

Table 2.5.3 Sludge pathogen count at the Holbæk wastewater treatment plant.

	Activated sludge before digestion	After mesophilic digestion	After thermophilic digestion	Danish requirements
Salmonella (/100mL)	100 – 2,000	100 – 250	< 2	Must not be found
Faecal streptococcus (/mL)	3,000-30,000	250 - 400	< 10	< 100

With a two step 9,600 m^3 themophilic reactor, Zábranská *et al*. (2002) reduced the count of thermotolerant Coliforms to 10^3-10^4 cfu/g TS, which is still higher than that required to meet the class A biosolids standard established by the USEPA. This was attributed to both the short HRT of 5.1 days and to the lack of proper mixing inside the reactor. Tracer studies revealed that only 75 and 40% of the first and second reactor volumes, respectively, were utilized.

United Utilities and Montgomery Watson Hazra of the UK (Mayhew *et al*., 2002), developed a treatment system with enzymic hydrolyzer that achieved at least 3.5 log and sometimes up to 6.8 log reduction in *E. coli* when treating combined municipal and industrial sludge to meet giosolids regulations:

- Treated sludge must achieve a minimum 2 log reduction in *E. coli* and a final product standard of 1 x 10^5 cfu *E. coli* /g of TS;
- Enhanced treated sludge must achieve a minimum of 6 log reduction in *E. coli*, a final product standard of 1x10^3 cfu *E. coli* /g of TS and contain no Salmonella.

The enzymatic hydrolyzer described by Mayhew *et al*. (2002) was a two-step, mesophilic anaerobic digestion. The first AD step increased the sludge temperature from

20 to 40 °C over four days while the second AD step maintained 40 °C-mesophilic conditions for three to four days.

2.5.5 ENERGY RECOVERY

Anaerobic digestion, even under mesophilic and thermophilic conditions, produces sufficient methane to justify its conversion into electricity and heat. This biogas is a renewable energy because of its potential used in the treatment facilities, thus reducing and even avoiding consumption from the municipal supply.

The first Japanese demonstration plant thermophilically treated 3 tons/day of OFMSW under dry fermentation and produced 820 m^3_N/ton-VS (50% CH_4) of gas supplying all the heat and part of the electric energy required by the plant (Komatsu *et al*. 2002). The biogas produced by the eight Danish full-scale thermophilic anaerobic sludge plants is used in boilers to heat the sludge at an efficiency 50 – 70 % (Nielsen and Petersen 2000). The thermophilic anaerobic digester at Holbæk recovers 70 % of the biogas heat. Even with cold sludge at 5 °C, the energy cost was reduced from 200 kW to 70 kW. Thus, natural gas was supplemented only during cold periods.

With thermophilic anaerobic digestion, Thierbach and Hanssen (2002) stabilized 106,000 tons/year of sewage sludge and municipal solid waste at 42 % TS, and used the biogas to produce biosolids. Some 63,000 MW-h/y of net energy was recovered to supply 60 % of the electricity requirementsby the wastewater treatment facility.

2.5.6 OTHER ADVANTAGES

At the Holbæk plant in Denmark, Nielsen and Petersen (2000) fed a thermophilic reactor with high TS sludge dewatered using a centrifuge separator, a belt filter press, and a chamber filter press. The produced thermophilic digested sludge increased by 5% more the TS and despite the use of more conditioning polymer, the smaller mass of sludge solids resulted in no net increase in cost of treatment.

2.6 OFMSW PRE-TREATMENT AND HYPERTHERMOPHILIC ANAEROBIC DIGESTION

Because 35 to 50 % of the OFMSW mass is composed of fibrous matter such as paper, wood, and yard waste, improving the degradation of cellulose- and ligno-cellulose-related materials is a key issue in improving their VS reduction through AD. Lipid- or protein-rich wastes also offer a low biodegradability. As pre-treatments, mechanical reduction of the particle size, high pressure and temperature exposition, and hyperthermophilic digestion were tested.

To meet German regulations on the disposal of carcasses, Schieder *et al*. (2000), used Thermal Hydrolysis (TDH) followed by AD to treat wet organic waste and bio-solids produced by canteens and restaurants, including food scraps, biological waste and carcasses. The TDH pre-treatment was tested in a 150 L prototype tank heated at 220°C and pressurized to 40 atmospheres. The TDH pre-treatment solubilized 70 % of the TS, before feeding the hydrolyzed effluent into an up-flow fixed bed anaerobic reactor and the separated solids were dehydrated and composted. After only five days at 35°C, the TDH and AD system produced gas at a constant rate of 500 L/kg of COD, which is 4.5

times the amount of gas produced after 20 days with a 35°C-mesophilic AD without pre-treatment.

By pre-treating with steam pressure disruption, Liu *et al.* (2002) improve the thermophilic (55°C) AD of a lignin and cellulose rich waste consisting of paper, wood and yard waste, with a 25 % TS. Despite its exposure to AD for 35 days, the waste was still undigested. The rapid depressurization of 5 kg of waste, after steam heating at 240°C for 5 min and 55 atmospheres, resulted in a visible disruption of the fiber and the released of some of their soluble organic components. The disrupted material, after re-inoculation, provided a lag-free rapid burst in methane production at rates twice as high as those observed with the initial digestion, and produced 40% more methane and VS reduction. The secondary digestion product was enriched in lignin but had lost some of its cellulose and hemi-cellulose as compared to the waste from the primary digestion.

Nevertheless, thermal disruption was found to be costly and the separation at the course of cellulose, lignin or ligno-cellulose before AD can be more interesting. The separated fraction can be treated by composting. Wood and yard waste could even serve as compost bulking material.

Although not extensively tested, hyperthermophilic temperatures of 60 to 80°C have the potential to further increase the methane yield of OFMSW. There is enough evidence that bio-transformation of organic matter under anaerobic conditions can be conducted at such extreme temperatures. Nozhevnikova *et al*. (1997) studied the effect of temperature on anaerobic communities of deep northern lake sediments. Under hyperthermophilic temperature of 70°C, with a substrate mixture of H_2/CO_2 (1/4) and after incubating for 25 days, the sediments produced 17 mmol CH_4/L, which was

equivalent to that produced during 55 days at 30°C. With the same substrate, sediments incubated at 50°C for 15 days produced 20 mmolCH_4 /L. At 70°C, a *Methanobacterium thermoautotrophicum* like culture was obtained, corresponding to a methanogen strictly consuming H_2 (Reeve *et al*. 1997). Compared to 50°C, the low methane production is related to the substrate being slowly available. This was confirmed by Reeve *et al*. (1997) using a gas mixture of 89% H_2 and 11% CO_2 to feed a ***M. thermoautotrophicum*** culture at 55°C. The substrate availability was reduced by mixing the medium at 280 rather than 600 rpm, and biogas production dropped from 500 μmoles CH_4/min to less than 100 μmoles CH_4/min. When mixing at 600 rpm, methane production peaked after 10 h, at 1000 μmolesCH_4/min.

After a short pre-incubation of digested sludge as seed at 55 °C, Nozhevnikova *et al*. (1999) fed cattle and pig manure and measured a methane gas content of 4 and 48 %, respectively, while under hyperthermophilic conditions of 73 °C, the methane content dropped to 6 and 3.2%. At hyperthermophilic temperature of 82 °C, methane production stopped mainly because of the proliferation of acidogenic communities with acetate, propionate and butyrate levels reaching 31 to 39 mM.

In the laboratory, Scherer *et al*. (2000) evaluated the anaerobic digestion of gray-OFMSW using a two phase hyperthermophilic anaerobic reactor separating hydrolysis and methanogenic activities. Respective HRT of 4.3 and 14 day and temperatures of 70 and 60°C were used. The initial gray-OFMSW fed VS, with 6 to 7% TS, was reduced by 6 and 79% by the hydrolytic and methanogenic reactors for a respective gas production of 27 and 540 $L_N/kgSV_{fed}$. The methanogenic effluent contained 0.25 to 3.0 g/L VFA. The high hydrogen-utilizer count of 10^8 – 10^9 /g TS suggested that syntrophic acetate

oxidation followed by autotrophic methanogenesis were the predominant last step in the microbial food chain.

Accordingly, methanogenesis occurs at 60 to 73°C since all relevant groups still function. More research is needed to establish the different groups of microorganisms involved, their characteristics, their tolerance to changes in temperature, their capability to accommodate substrates with high concentration of organic matter such as for OFMSW, and their kinetic behavior.

CONNECTING STATEMENT (First scientific article)

Chapter 3 characterizes the food waste selected to feed the sludge in Chapters 3, 4 and 6, and adapts mesophilic sludge to thermophilic conditions while following its methane production and microbial substrate activity. This scientific paper was submitted and accepted for publication at the *Journal of Environmental Management*, a peer reviewed Journal published by Elsevier on behalf of the International Water Association (IWA).

Authors and affiliation:

Luis Ortega[1,2] (i); Suzelle Barrington[1] (ii); Serge Guiot[2] (iii)

(1) McGill University, Bioresource Eng. Department. 21111 Lakeshore Road, Ste-Anne-de-Bellevue (Quebec), H9X 3V9 Canada. E-mail: luis.ortega@nrc.ca

(2) National Research Council of Canada, Biotechnology Research Institute. 6100 Royalmount Av. Montreal (Quebec), H4P 2R2 Canada.

The candidate (i) conducted the experimental work including that of the AD of OFMSW, along with laboratory analyses, statistical analysis, and the writing of manuscripts. Author (ii) further enhanced the analysis of data, edited the manuscripts and contributed to the content of the article. Author (iii) lead the research project, supervised the laboratory and analytical work and further enhanced the analysis of data and edited the manuscripts.

CHAPTER THREE

Thermophilic adaptation of a mesophilic anaerobic sludge for food waste treatment

3.0 ABSTRACT

As opposed to mesophilic, the thermophilic anaerobic digestion of food waste can increase the biogas output of reactors. To facilitate the transition of anaerobic digesters, this paper investigated the impact of adapting mesophilic sludge to thermophilic conditions. A 5 L bench scale reactor was seeded with mesophilic granular sludge obtained from an up-flow anaerobic sludge blanket digester. After 13 days of operation at 35 °C, the reactor temperature was instantaneously increased to 55 °C and operated at this temperature until day 21. The biomass was then fed food waste on days 21, 42 and 63, each time with an F/M (Food / Microorganism) ratio increasing from 0.12 to 4.43 gVS/gVSS. Sludge samples were collected on days 0, 21, 42 and 63 to conduct substrate activity tests, and reactor biogas production was monitored during the full experimental period. The sludge collected on day 21 demonstrated that the abrupt temperature change has no pasteurization effect, but rather lead to a biomass with a fermentative activity of 3.58 g Glucose/gVSS/d and a methanogenic activity of 0.47 and 0.26 g Substrate/gVSS/d, related respectively, to acetoclastic and hydrogenophilic microorganisms. At 55 °C, an ultimate gas production (Go) and a biodegradation potential (Bo) of 0.2 to 1.4 $L_{\text{-STP}}/gVS_{fed}$ and of 0.1 to 0.84 $L_{\text{-STP}}$ CH_4/gVS_{fed} were obtained, respectively. For the treatment of food waste, a fully adapted inoculum was developed. The feeding stage was initiated within 20 days, but to increase the population

of thermophilic methanogenic microorganisms, a substrate supply program must be carefully observed.

Keywords: anaerobic digestion, biogas production, food waste, mesophilic conditions, thermophilic adaptation.

3.1 INTRODUCTION

In North America and Europe, up to 35% of the total mass of generated municipal solid waste can be made up of organic components (Rhyner *et al*., 1995). To divert the organic fraction of municipal solid wastes (OFMSW) from landfills while beneficially reusing such waste, more energy-efficient practices are required. For OFMSW, the conventional mesophilic-anaerobic/composting coupled system offers an energy potential of 150 kWh/t_{MSW} which is 21 times higher than that reported for landfill municipal solid waste of 6.9 kW-h/t_{MSW} recovered from collected biogas (Baldasano and Soriano, 2000). Despite this higher energy yield, there are only a few conventional anaerobic digesters (AD) in North America for the transformation of OFMSW into green-energy carriers (U.S. EPA 2003). In comparison, Europeans have long accepted to treat the OFMSW using AD, but also know that the choice of technologies depends on the substrate characteristics.

Thermophilic conditions to operate one or two-stage AD systems can further improve the energy efficiency of the technology. According to De Baere's (2000) and as opposed to mesophilic plants, the start-up of the first thermophilic plant in 1992 has increased the cumulative capacity (t/year) of treating OFMSW from 56 000 to 280 000 t/year, and this from 1997 through 2000. Under thermophilic conditions, a high level of

pathogen kill-off is obtained and more heat and electricity are recovered. Nevertheless, the optimal transition of AD systems from mesophilic to thermophilic conditions is not clearly defined. The transition depends on the microbial adaptation to new operational conditions or the presence of different groups of microorganisms capable of performing their biological functions at specific temperatures.

With a rapid temperature change from mesophilic to thermophilic, there may not be a population shift if the groups are not compatible (Mata-Alvarez, 2002). Nozhevnikova *et al*. (1999) have assumed that thermophilic and mesophilic methanogenic communities differ in terms of microbial species. This assumption may not be completely true as some mechanisms may permit the microorganisms to evolve from one specific state to another depending on the transition conditions. Nielsen and Petersen (2000) mentioned that the quick and satisfactory start up of full-scale thermophilic anaerobic digesters in Denmark has been achieved with and without a transitional period. The first two days of starting the plant at thermophilic temperatures were associated with a high content of volatile fatty acids and a high biogas CO_2 concentration, requiring the careful monitoring of the loading rate. This period was avoided when the digester was immediately started using thermophilic temperatures. For food waste mechanically sorted from MSW and treated under either a dry or wet anaerobic process, Mata-Alvarez (2002) observed a transition from mesophilic to thermophilic conditions (35 to 55°C in 10 d) requiring significant variations in organic loading (from 15 to 40% over 2 d) without permanent effect on the process performance.

The objective of this experiment was therefore to develop a thermophilic anaerobic inoculum for the treatment of food waste, by adapting anaerobic mesophilic

sludge. The adaptation was conducted using a bench scale reactor seeded with a mesophilic granular sludge obtained from two Upflow Anaerobic Sludge Blanket digester (UASB), one treating apple wastewater while the other treating milk wastewater. Substrate activity tests were conducted to characterize the changes in biomass, and the new thermophilic inoculum was fed selectively collected OFMSW (SC-OFMSW) to test its treatment capacity.

3.2 METHODOLOGY

The project consisted in achieving the adaptation of:

1) a granular UASB biomass from mesophilic to thermophilic conditions, and;

2) the resulting thermophilic biomass to a new type of substrate (food waste) at different loading rates, under completely mixed and thermophilically controlled anaerobic conditions using a bench scale reactor.

3.2.1 SUBSTRATE

The SC-OFMSW used in this study was the food waste collected from the solid waste produced by a cafeteria. Immediately after collecting the cafeteria solid waste, the organic fraction was manually sorted and the plastic, glass, metal and wood portions were removed. Despite manual sorting, the substrate was mostly made up of food scraps (meat, egg, fruit and vegetables, bread, pastries), but also contained some paper napkins, paper and plastic packaging, wood sticks and plastic table-settings.

The resulting substrate was shredded using a 50 mm stainless-steel blade and then chopped into a paste using an 87/Polytron/PTA 36/2 blade connected to a CH-6010

Kiennz-Lu electric rotor (*Kinematica*, Switzerland). All particles larger than 4.75 mm were separated with a No. 4 U.S.A. standard testing sieve (*W.S. Tyler*, USA). During the first shredding operation, tap water was added to drop the solid content to 21.8%. The meat and egg fractions were visibly high explaining the high Total Kjeldahl Nitrogen (TKN) of 17% and carbon/nitrogen (C/N) ratio of 3:1 associated with the substrate (Table 3.2.1).

3.2.2 SEED

There could be several options for the choice of sludge for this study, but for availability reasons, anaerobic granular sludge was the best of those options. The seed then consisted of a 50/50 % mixture of granular and non-granular sludge taken from two separate-UASB reactors treating dairy process wastewater and apple-juice process wastewater, respectively. The total and volatile solids were typical for anaerobic granular sludge (Table 3.1).

Table 3.1 Experimental substrate (SC-OFMSW) and seed characteristics

Parameter	SC-OFMSW	Seed mixture
Density (kg/L)	1.22	1.042
Solid (%)	21.8	4.2
TS (g/L)	218.2	42.1
VS (% TS)	95.1	67.0
SS (g/L)	161.8	38.1
VSS (% SS)	96.5	70.0
Total COD (g/gVSS)	1.16	0.97
Soluble COD (% TCOD)	n.e.	12.12
TKN (% TS)	17	n.e.
C/N	3.1	n.e.

Notes: n.e.: not evaluated; The seed mixture consisted of 50% UASB sludge treating apple wastewater and 50% UASB sludge treating milk wastewater.

3.2.3 REACTOR SET-UP

The experimental 5 L glass reactor is illustrated in Figure 3.1. The reactor's working volume was 4 L. The system included a 6-600 peristaltic pump (*Cole-Parmer,* Chicago, IL) and a Type-RZR50 stirrer (*Caframo,* Wiarton, ON). The temperature was automatically controlled using a heating system surrounding the digester and encompassing a 305mm -LT-type probe (*Cole-Parmer,* Vernon Hill, IL), a *Digi-Sense* standard model temperature controller (*Cole-Parmer,* Vernon Hill, IL) and a *Brisk Heat* silicone extruded, flexible electric heating tape (*Thermolyne*, Dubuque, IO). The pH monitoring system consisted of an epoxy-pH probe (*Cole-Parmer,* Niles, IL) and an *Accumet* pH-meter model 825 MP (*Fisher*, USA). After recovering the condensate, the biogas generated was sampled through the biogas sampling port and measured by means of a Wet Tip-like water displacement system.

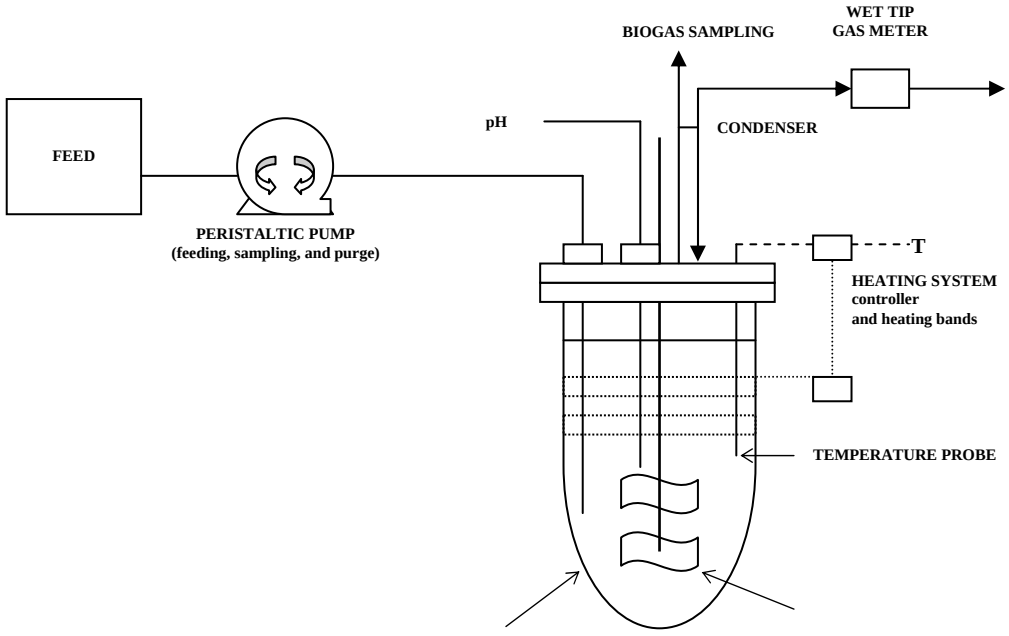


Fig. 3.1 Schematic diagram of the batch reactor system.

3.2.4 EXPERIMENTAL STAGES

The first stage of the experiment took 20 days (stage C0) and consisted of adapting the seed to thermophilic conditions. At the beginning of stage C0, the reactor was seeded with 4 L of the seed mixture (Table 3.1) and operated at 35 °C to generate inoculum, without exogenous substrate supply. On day 13, the temperature of the process was instantaneously increased from 35 to 55 °C, and maintained at that temperature until day 20.

This C0 stage was followed from days 21 to 84, by the thermophilic batch stages C1, C2, and C3 each lasting 21 d, to assess the treatment capability of the new biomass at 55 °C and at various SC-OFMSW loading rates. At the start of each stage C1, C2 and C3, the SC-OFMSW was fed with a food to microorganism ratio (F/M) increasing sequentially with stage from 0.12 to 4.43 gVS/gVSS. Because of medium acidification at the beginning of C3, a fourth 21-days stage (C4) was carried out to restore methanogenic conditions. At the beginning of stage C4 (day 84), the reactor biomass received a mixture of 1.6 L of sludge produced at the end of stage C2, 0.3 L of the original seed blend and 0.25 L of a 0.2M $NaHCO_3$ solution at pH of 8, to restore the reactor's pH to 7.0. Biomass activity was monitored during stage C4 by measuring gas production and subjecting the final (21 d) sludge to substrate activity (SA) tests.

During the complete 104d experimental period, gas production was continuously measured and samples were taken at regular 24h intervals. A sludge sample was obtained from the reactor on days 0 and 20 of stage C0, and used for substrate activity tests (SA). Both samples were incubated at 35 and 55 °C during such SA tests to check for the presence and character of the remaining mesophilic and thermophilic microorganisms.

Sludge samples were also collected from the reactor at the end of stages C1, C2, C3 and C4, for additional SA testing under an incubation temperature of 55° C.

3.2.5 ANALYTICAL METHODS

To calculate the F/M ratio supplied during stages C1 to C3, the substrate, the seed sludge and the reactor's biomass were analyzed for chemical oxygen demand (COD), total and suspended solids (TS and SS), and total and suspended volatile solids (VS and VSS), according to standard methods (APHA *et al.,* 1995).

Acetic, propionic and butyric acids were measured using an *Agilent* 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector of 0.2 microL. The samples were mixed at a ratio of 1:1 (V/V) using an internal standard of iso-butyric acid dissolved in 6% formic acid and directly injected into a glass column of 1 m x 2 mm *Carbopack C* (60 to 80 mesh) coated with 0.3% *Carbowax* 20M and 0.1 % H_3PO_4. The column was held at 130 °C for 4 minutes and helium as carrier gas was injected at a rate of 20 mL/minute. The injector and the detector were both maintained at 200 °C.

For the SA tests, glucose was measured using an HPLC (*Waters Chromatography Division*, Milford MA, USA) equipped with an injector (model 717+), a photodiode array (PDA) detector (model 2996), a pump (model 600) and a 2414 refractive index detector. The separation unit consisted of an ICsep ICE-ION-300 column (*Transgenomic*, San Jose, CA) of 300 mm x 7.8 mm id and an ion guard GC-801 column (*Transgenomics*). The mobile phase consisted of 0.035N of sulfuric acid at a pH of 4, flowing at a rate of 0.4 mL/min. The measurements were conducted using a wavelength of 210 nm.

The biogas composition (hydrogen, nitrogen, oxygen, methane, and carbon dioxide) was measured by means of an HP gas chromatograph (68900 Series, *Hewlett Packard,* Wilmington, DE) equipped with a thermal conductivity detector (TCD) and a 900mm x 3 mm (36" x 1/8") 60/80 mesh Chromosorb 102 column (*Supelco*, Bellefonte, PA). Daily determinations of gas production rate (GPR: L_{-STP} /L/d), ultimate gas production potential (Go in L_{-STP} /gVS_{fed}), and ultimate biodegradation potential (Bo in L_{-STP} -CH_4/gVS_{fed}) were calculated as specified by Mata-Alvarez (2002).

Mesophilic and/or thermophilic microbial diversity of the biomass produced during stages C0 to C4 was evaluated through substrate activity tests (SA) carried out in 60 mL serum bottles according to Guiot *et al*. (1995). Triplicate bottles were inoculated with biomass diluted with a phosphate buffer to obtain 2000 mgVSS/L for hydrogenophilic activity and 5 000 mg VSS/L for the bottles used for measuring activity of glucose, acetic and propionic acid. The bottles were flushed with N_2/CO_2 (80 % / 20 %) to remove all oxygen and incubated in a rotary shaker (*New Brunswick Scientific Co*., Edison, NJ). While being incubated at either 35 or 55 °C, the serum bottles and their content were shaken at 100 rpm for the soluble substrate tests and at 400 rpm for the hydrogen substrate test.

3.2.6 STATISTICAL ANALYSIS

For the C0 stage, the statistical significance for the biomass activity (SA) before and after the temperature shift and incubated at temperatures of 35 and 55 °C, was determined using a *t-test* with paired data (Wardlaw, 1985). The SA results obtained were tested for significant differences using:

$$t = \frac{h(N)^{1/2}}{Sh} \qquad (1)$$

where:

t = Value of student *t*-found

N = the test number of pairs

h = mean of differences of SA-values of each bottle-pair (mesophilic and thermophilic)

Sh = standard deviation of h

The *t-found* value was compared to that of a Student *t-Statistic* table for a degree of freedom of 2 (2 d.f.).

Likewise, the statistical significance of the SA measured under incubation at 55°C for samples of biomass taken at the end of C1, C2 and C3 was determined by a one-way analysis of variance or *F-test* (Wardlaw, 1985). The values obtained from the SA of these sludge samples were tested for significant difference using the following equation:

$$F = \frac{Vb}{Vw} \qquad (2)$$

where:

F = Variance ratio

Vb= Between groups variance (stages C1, C2 and C3)

Vw= Within groups variance (between triplicates)

The F value was compared at a critical *F-Value* table for 2 d.f. between groups and 6 d.f. within groups. A multiple range test for comparing means in an analysis of variance was evaluated for significance within groups. The Multiple range test was used to identify the treatment(s) demonstrating a significant difference.

3.3 RESULTS AND DISCUSSION

3.3.1 MESOPHILIC TO THERMOPHILIC TRANSITION (STAGE C0)

The biogas production rate for the first stage, C0, is illustrated in Figure 3.2. The biogas production peaked at 1.9 L_{STP}/day after 24 hrs, but dropped thereafter to reach a low of 0.32 L_{STP}/day on day 13, indicating that all available substrate had been consumed by the mesophilic microorganisms and that the sludge was stabilized. On day 13, the system's temperature was instantaneously increased from 35 to 55 °C and the reactor's content reached 55 °C within ten minutes. Thermophilic condition was maintained thereafter.

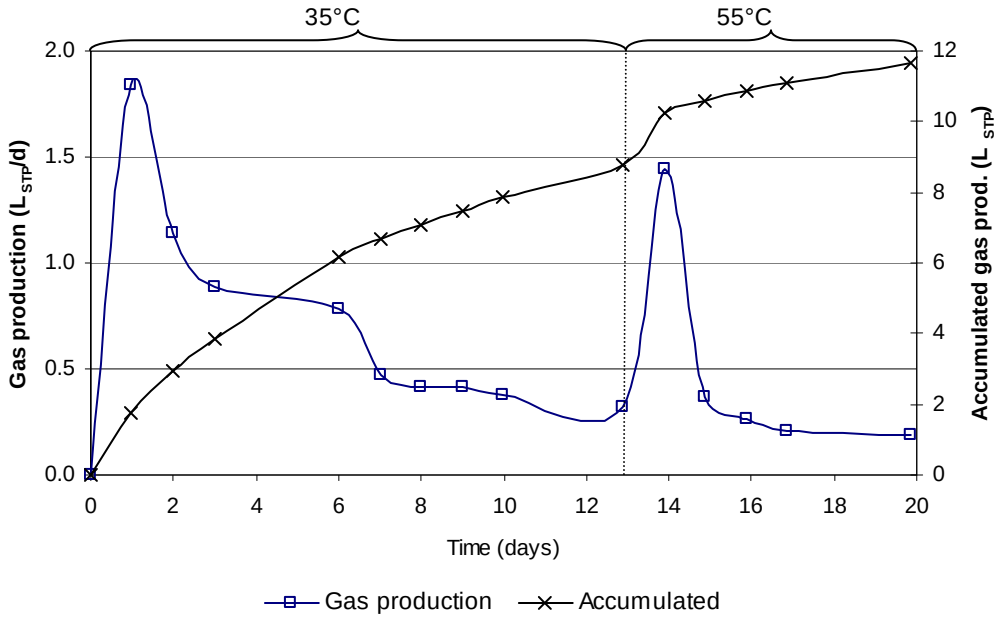


Fig. 3.2 Gas production profile during the C0 stage.

After 22 h of thermophilic operation at 55 °C, 1.4 L_{STP} of gas was produced, only 50% of which was produced by the microbial activity. The other 50% biogas volume resulted from the increase in temperature of the reactor and the lower CO_2 water solubility. Accordingly, this temperature change also affected the water solubility of carbon dioxide and the methane fraction of the biogas composition which dropped after day 1 from 65 to 38% and came up slightly to reach 41% on day 20. Because methane was still being produced, the methanogenic microorganisms had survived the temperature transition.

During the mesophilic phase (from day 0 to day 12), the volumetric production of methane varied between 0.05 and 0.27 L_{STP}/L/d, while during the thermophilic phase (day 13 to day 20), the volumetric production of methane varied between 0.02 and 0.14 L_{STP}/L/d. Therefore, the temperature transition was successful in extracting a slight but additional volume of gas from the food waste.

The shift of microbial population within the sludge collected at the beginning and at the end of the seeding stage (C0) was evaluated by means of SA tests. The SA values obtained at both 35 and 55 °C demonstrated that increasing the temperature did not produce a pasteurization effect on either bacterial or methanogenic populations. Except for hydrogenophilic methanogens, thermophilic population showed no significant difference (P>0.10) between the SA values for the 0 and 20 day sludge, but the abrupt change in reactor temperature, from 35 to 55°C on day 13, resulted in a positive selection of thermophilic fermentative bacteria (Table 3.2). Thermophilic glucose consuming bacteria were observed to almost double their activity from 1.85 to 3.58 g Glucose/gVSS/d, from day 0 to 20.

Table 3.2 Substrate activity for the C0 initial and adapted sludge measured at 35 and 55 °C.

Substrate	***Incubation T (°C)***	Activity (g substrate/gVSS /d)		Probability
		Initial Sludge (Day 0)	Adapted Sludge (Day 20)	
Glucose	35	5.09 ± 1.82	2.12 ± 1.09	> 0.02
	55	1.85 ± 0.49	3.58 ± 2.06	> 0.10
Propionate	35	0.09 ± 0.02	0.06 ± 0.006	> 0.10
	55	0.03 ± 0.02	0.02 ± 0.01	> 0.10
Acetate	35	0.62 ± 0.4	1.18 ± 0.39	> 0.10
	55	0.6 ± 0.11	0.47 ± 0.51	> 0.10
Hydrogen	35	1.04 ± 0.39	0.01 ± 0.001	> 0.02
	55	2.52 ± 1.12	0.26 ± 0.02	> 0.05

Notes: Initial sludge = mesophilic sludge; Adapted sludge = sludge adapted after 7 days of operation at 55°C.

For the mesophilic glucose consuming bacteria, their activity was significantly reduced ($0.05>P>0.02$) by 58% on day 20 after being exposed to 55 °C, and as compared to day 0. Although not significantly different ($P>0.10$), mesophilic or thermophilic propionic acid consuming bacteria reduced their activity from 0.09 to 0.06 and from 0.03 to 0.02 g propionate/gVSS/d, respectively, after increasing the reactor temperature to 55 °C.

Similarly, the activity of the hydrogen consuming mesophilic methanogens was significantly reduced ($0.05>P>0.02$) during stage C0, because by the end of day 20, their SA dropped 100 fold to a final value of 0.01 g Hydrogen/gVSS/d. Although not statistically different ($0.10>P>0.05$), thermophilic hydrogen-consuming methanogens also reduced their SA from 2.52 to 0.26 g Hydrogen/gVSS/d. Interestingly, mesophilic acetoclastic methanogens were not affected by the abrupt change of temperature, because they doubled their SA from 0.62 to 1.18 g Acetate/gVSS/d, likely as a result of

starvation. Without being significantly different, thermophilic acetoclastic methanogens slightly reduced their activity from 0.6 to 0.47 g Acetate/gVSS/d.

In summary, the mesophilic seed used for this experiment already had a thermophilic potential which was expressed by the sudden change of temperature carried out on day 13. Nevertheless, the trophic groups which were significantly affected by the temperature change were the mesophilic fermentative bacteria and mesophilic hydrogen-consuming methanogens.

3.3.2 EFFECT OF F/M ON THERMOPHILIC FERMENTATION (STAGES C1 TO C3)

Following the C0 stage, the SC-OFMSW-feeding stage was initiated through three batch cycles (C1 to C3) during which the substrate was supplied every 21 days using a F/M ratios of 0.12, 1.15 and 4.43 gVS/gVSS. The average process temperature were controlled at 55.2 ±0.1, 55.3 ±0.1 and 56.5 ±2.5 °C, respectively (Table 3.3).

Table 3.3 Operation conditions and reactor performance from C1, C2 and C3.

Stage	F/M gVS/gVSS	Average pH	Go L_{STP}/gVS_{fed}	Bo $L_{STP}CH_4/gVS_{fed}$
C1	0.12	-	1.40	0.84
C2	1.15	7.3	0.89	0.61
C3	4.43	5.17	0.2	0.01

Notes: Go: Ultimate gas production.
Bo: Ultimate methane production.

The reactor biomass accommodated the low and intermediate F/M ratios (C1 and C2). If the amount of VS in the initial (C1) F/M ratio of 0.12 gVS/gVSS would have been applied proportionally during the first 21 days, it would be equivalent to an organic

loading rate (OLR) of 0.12 gVS/L/d. This OLR is low compared to the typical organic load of 3.6 gVS/L/d suggested by ten-Brummeler (2000) for continuous systems. The intermediate (C2) F/M ratio was increased ten fold to 1.15 gVS/gVSS (OLR of 1.09 gVS/L/d) and still did not negatively affect the reactor biomass, since the production of gas increased substantially from 11 L_{STP} during stage C1 to 56 L_{STP} during stage C2. The F/M ratio of 4.43 gVS/gVSS applied during stage C3 produced two short burst of gas production: the first of 106.3 L_{STP} /d occurred 6 h after feeding, and; the second of 15.95 L_{STP} /d occurred two days after feeding, (Figure 3.3).

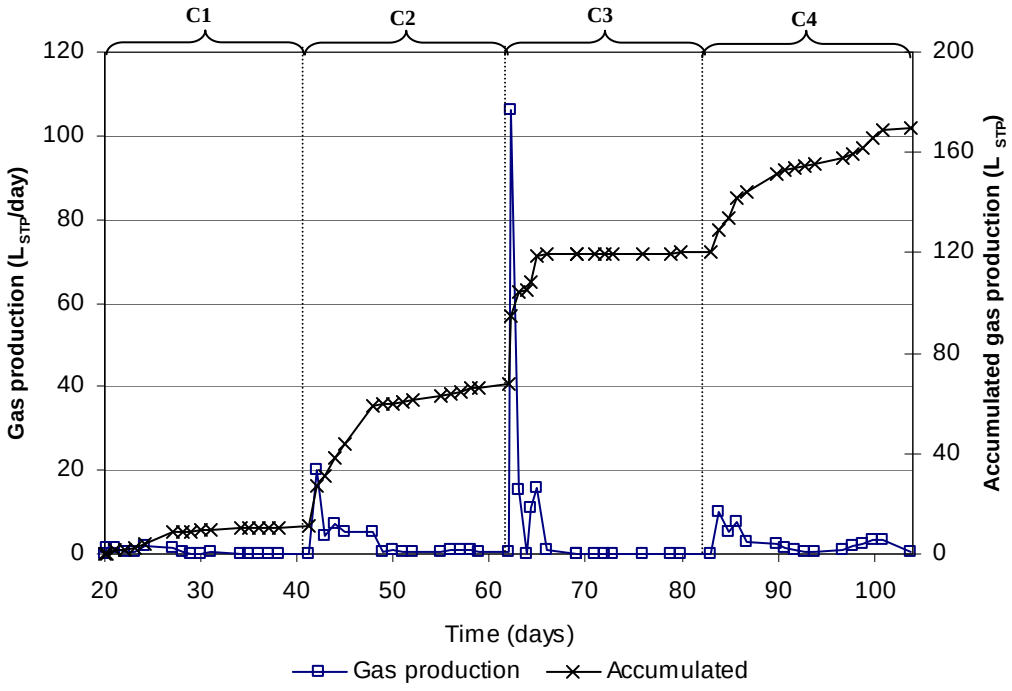


Fig. 3.3 Daily and accumulated gas production profile from stages C1 to C4.

Within two days, the F/M ratio of stage C3 acidified the reactor medium and its pH fell from 7.75 to 5.45 (Figure 3.4), as a result of accumulating volatile fatty acids (VFA). Within the first day of C3, the concentrations of acetate, propionate and butyrate reached levels of 4 580, 65 and 7 000 mg/L, respectively. By the end of C3, only 45 and 40 % of the acetate and butyrate, respectively, were consumed, while the propionate concentration remained unchanged.

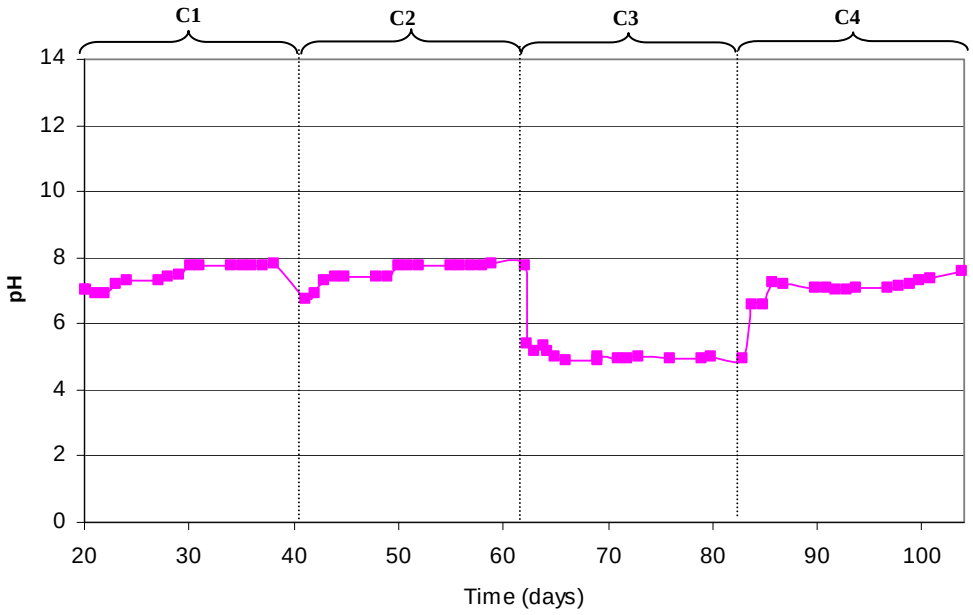


Fig. 3.4 Daily pH profile from stages C1 to C4.

Stages C1 and C2 resulted in ultimate gas (Go) and biodegradable potential (Bo) values of 0.85 L/gVS_{fed} and 0.46 $L\text{-}CH_4/gVS_{fed}$, respectively, exceeding those for SC-OFMSW degradation presented by Mata-Alvarez (2002). Nevertheless, the acidification of the reactor during stage C3, following a high F/M application, reduced the biogas-production capacity and both values for Go and Bo dropped from 0.89 to 0.2 L_{STP}/gVS_{fed} and from 0.61 to 0.01 $L_{STP}CH_4/gVS_{fed}$, respectively.

Figure 3.5 shows the profile of the gas composition throughout C1 to C4. The concentration of methane increased from 60 % during stage C1 to 64 % during stage C2. During stage C3, methanogenesis was displaced towards hydrogen production because on day 64, the biogas contained 30 % hydrogen suggesting strong fermentative activity. Notably, a decrease in total gas production rate from 106.3 to 15.95 L_{STP} /d was observed during this period (Figure 3.3). To check for hydrogen production on day 65, the reactor's headspace was replaced with a mixture of inert gas (80 % N_2 / 20 % CO_2) and biogas hydrogen content was once more monitored. Hydrogen was immediately observed again reaching 55 % by the end of day 69.

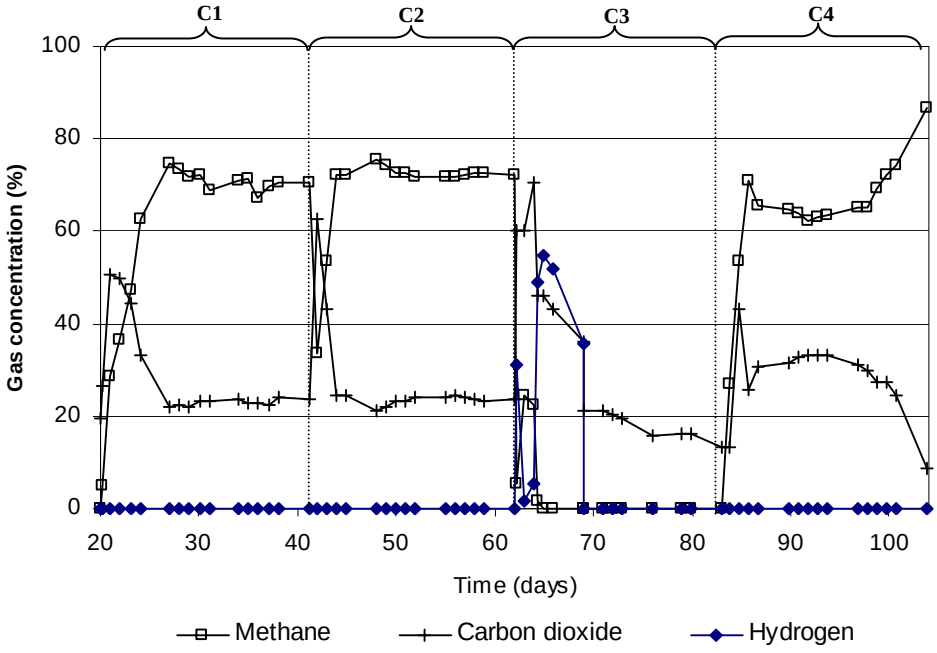


Fig. 3.5 Daily gas composition from stages C1 to C4.

Methanogenic activity and anaerobic digestion in general is tightly regulated by the production of hydrogen. In this sense, for all the organisms involved to conserve energy, the anaerobic media must sustain a hydrogen partial pressure within the window of 6 x

10^{-5} to 10^{-2} atm (Ferry, 1993). For that reason it is not easy to determine in this case whether the production of hydrogen during C3 is related to the consumption of rapidly available carbohydrates by fermentative bacteria or the degradation of butyric acid by syntrophic bacteria; more likely the first burst of hydrogen (accompanied by a small production of methane) was related to the action of hydrogen-producing, carbohydrate-fermenting bacteria. After the stripping of hydrogen from the media and the headspace of the reactor on day 65 (since fermentative bacteria could be inhibited) the second stage of hydrogen production could be largely related to syntrophic activity. It is well known for example that while degrading butyric acid, *Syntrophomonas wolfei* produces propionate, hydrogen and carbon dioxide (McCarty and Mosey, 1991). Since during the second burst of hydrogen after day 65 there was no uptake of hydrogen and carbon dioxide for the production of methane, the build up of hydrogen could have increased again the hydrogen partial pressure in the media thus inhibiting synthrophic activity. Notably, after day 71 and until day 84, gas production ceased completely (Figure 3.3) and no more hydrogen was observed in the headspace of the reactor (Figure 3.5).

3.3.3 SUBSTRATE ACTIVITY TEST FOR THERMOPHILIC FERMENTATION (STAGES C1 TO C3)

The SA tests conducted on the biomass collected at the end of stages C1, C2 and C3 indicated that the F/M ratio affected the capability of the biomass to adapt to different substrates (Table 3.4). Among all three stages, C2 improved the fermentative biomass activity while C3 reduced it by more than 50 %. Hydrogen production rather than uptake was observed with the C3 biomass indicating that the hydrogen producing fermentative

bacteria outpaced the hydrogenophilic capacity of the biomass to convert H_2 into methane. Similarly, the uptake of acetate for the production of methane was affected by the C3 biomass because the activity of acetoclastic methanogens dropped from 0.88 during C2 to -0.005g Acetate/gVSS/d.

Table 3.4 Substrate activity evolution of thermophilic sludge during the adaptation stage.

	Substrate Activity, g/gVSS /d			
Sludge	Glucose	Propionate	Acetate	Hydrogen
C1	0.21 ± 0.27^{b}	0.011 ± 0.006^{c}	0.08 ± 0.01^{d}	0.23 ± 0.06^{f}
C2	2.27 ± 0.98^{a}	0.005 ± 0.017^{c}	0.88 ± 0.12^{d}	0.36 ± 0.04^{g}
C3	1.93 ± 0.86^{a}	0.007 ± 0.019^{c}	-0.005 ± 0.09^{e}	-0.17 ± 0.07^{f}

Notes: Values with the same letter are not significantly different within groups at the level of $P \leq 0.05$.

The analysis of variance conducted on the SA values for stages C1, C2, and C3 suggested that the selected F/M program significantly affected the fermentative (glucose degrading) bacteria (Table 3.4), where stage C1 produced significantly lower values than stages C2 and C3 ($P< 0.05$). As well, both acetoclastic and hydrogenophilic methanogenesis were specially affected by the F/M supplied ($P< 0.01$), where stage C2 produced significantly higher values than stages C1 and C3. Finally, there was no significant difference in syntrophic-propionic degrading activity ($P>0.05$), as all stages had activity results under 0.015 g of propionic acid/ g VSS/d.

3.3.4 Restoration of thermophilic sludge (stage C4)

After stage C3 and on day 84, a new non-substrate cycle (C4) was started to restore the pH of the medium and the methanogenic activity. For this purpose, 2.15 L of the reactor's acidified medium were replaced with a mixture of 1.6 L of sludge produced at the end of stage C2, 0.3 L of the original seed blend, and 0.25 L of a 0.2M $NaHCO_3$ solution at pH 8. At the end of this 21-day stage (C4), the biomass was producing gas (Figure 3.3) and the pH was restored to 7.0 (Figure 3.4).

With C4, both fermentative (glucose consumption) and acetoclastic methanogenesis activities were recovered, giving SA values of 0.6 and 0.26 g substrate/gVSS/d. The propionate activity was recovered and measured 0.09 g Propionic/gVSS/d while the hydrogenophilic methanogenesis activity was at a low value of 0.02 g H_2/gVSS/d.

3.4 CONCLUSIONS

The present experiment demonstrated that a mesophilic wastewater-treating anaerobic sludge can be adapted for the treatment of the organic fraction of municipal solid waste (OFMSW) under thermophilic conditions. Nevertheless, the organic load program must be carefully selected and monitored, since the methanogenic activity can easily be displaced towards a fermentative activity.

The instantaneous upgrading procedure from mesophilic to thermophilic conditions positively selected thermophilic fermentative bacteria. The upgrading procedure also reduced thermophilic acetoclastic methanogens and significantly reduced thermophilic hydrogenophilic methanogens.

Despite the loss of thermophilic methanogenic biomass, under batch conditions, the reactor could later sustain a moderate F/M ratio of 1.5 gVS/gVSS while a high F/M ratio of 4.43 gVS/gVSS displaced methanogenesis towards hydrogen production. Although hydrogen production was not the goal of this process development, its observation might be of interest as an alternative energy source. More research is needed with that respect.

3.5 Acknowledgments

The technical assistance of A. Corriveau and S. Deschamps is gratefully acknowledged. This research was supported by the National Research Council of Canada and the Natural Science and Engineering Research Council of Canada. Additional support was granted by the Mexican Council of Science and Technology (CONACYT in Spanish), towards a graduate scholarship for Mr. L. Ortega.

3.6 References

Baldasano, J.M., Soriano, C., 2000. Emission of greenhouse gases from anaerobic digestion processes: comparison with other municipal solid waste treatments. Water Science and Technology 41 (3), 275.

De Baere, L., 2000. Anaerobic digestion of solid waste: state-of-the-art. Water Science and Technology 41 (3), 283.

Ferry James, G., 1993. Methanogenesis: Ecology, Physiology, Biochemistry & Genetics. Chapman & Hall, USA.

Guiot, S.R., Safi, B., Frigon, J.-C., Mercier, P., Mulligan, C., Tremblay, R., Samson, R., 1995. Performances of a full-scale novel multiplate anaerobic reactor treating cheese whey effluent. Biotechnology and Bioengineering 45, 398–405.

Mata-Alvarez, J., 2002. Biomethanization of the Organic Fraction Municipal Solid Wastes. IWA Publishing, UK.

McCarty, P.L., Mosey, F.E., 1991. Modeling of anaerobic digestion processes (a discussion of concepts). Water Science and Technology 24 (8), 17–33.

Nielsen, B., Petersen, G., 2000. Thermophilic anaerobic digestion and pasteurization. Practical experience from Danish wastewater treatment. Water Science and Technology 42 (9), 65.

Nozhevnikova, A.N., Kostsyurbenko, O.R., Parshina, S.N., 1999. Anaerobic manure treatment under extreme temperature conditions. Water Science and Technology 40 (1), 215.

Rhynder, C.R., Schwartz, L.J., Wenger, R.B., Kohrell, M.G., 1995. Waste Management and Resource Recovery, first ed. Lewis Publishers (CRC Press), USA.

Standard methods for the examination of water and wastewater, 1995. 19th ed. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington, DC, USA.

ten Brummeler, E., 2000. Full scale experience with the Biocel process. Water Science and Technology 41 (3), 299.

US Environmental Protection Agency (USEPA), 2003. Landfill Methane Outreach Program. Available from the web via the Internet /www.epa.gov/Imop/about.htm#ifgteS.

Wardlaw, A.C., 1985. Practical Statistics for Experimental Biologists. Wiley, Great Britain.

CONNECTING STATEMENT (Second scientific article)

Chapter 4 deals with the description of the microbial community of a mesophilic granular sludge before and after adaptation to thermophilic conditions as well as its evolution after being fed different food/microorganism ratios (F/M) under a batch regime and thermophilic conditions. This scientific paper will be submitted for publication in a peer reviewed journal. The election of the journal is currently under evaluation.

Authors and affiliation:

Luis Ortega[a,b] (i); Marie-Josée Lévesque (ii),[b] Suzelle Barrington[a] (iii); Serge Guiot[b] (iv)

(a) McGill University, Bioresource Eng. Department. 21111 Lakeshore Road, Ste-Anne-de-Bellevue (Quebec), H9X 3V9 Canada. E-mail: luis.ortega@nrc.ca

(b) National Research Council of Canada, Biotechnology Research Institute. 6100 Royalmount Av. Montreal (Quebec), H4P 2R2 Canada.

The candidate (i) carried out the experimental work on the AD of OFMSW, along with all laboratory analyses and the writing of the manuscript. The candidate (i) also conducted part of the molecular biology analyses (PCR and DGGE) which were performed by author (ii). Author (iii) further enhanced the analysis of data, edited the manuscripts and contributed to the content of the article. Author (iv) lead the research project, supervised the laboratory and analytical work carried out by the candidate, further enhanced the analysis of data and edited the manuscript.

CHAPTER FOUR

PHYLOGENETIC DESCRIPTION OF A THERMOPHILICALLY ADAPTED MICROBIAL ANAEROBIC MESOPHILIC SLUDGE

4.0 ABSTRACT

As compared to mesophilic, thermophilic anaerobic digestion (TAD) can further stabilize pathogens and deliver more biogas with a shorter hydraulic retention time (HRT), as long as the mesophilic inoculum can adapt to thermophilic conditions. The objective of this work was to observe the microbial genetic changes occurring under such transition. A bench scale reactor was seeded with mesophilic granular sludge and, after 13 days of operation at 35 °C, was instantaneously increased to 55 °C. Once adapted to 55 °C and on days 21, 42 and 63, the biomass was fed food waste, each time with a Food/Microorganism ratio (F/M) increasing from 0.12 to 4.43 gVS/gVSS. Molecular biology tools were used for the phylogenic analysis of the anaerobic sludge to characterize transitions in biomass composition. From the results, the thermal shock had practically no effect on the biomass which continued to produce methane at low and intermediate F/M. For the high F/M ratio of 4.43 gVS/gVSS, acid conditions developed and, as opposed to *Archaea*, only the bacterial domain survived. A fully adapted inoculum can be developed by eliminating the initial time-consuming acclimatization stage from mesophilic to thermophilic conditions, but the feeding stage must be delayed for 20 days, to increase the thermophilic methanogenic population and the substrate supply program must be carefully observed.

Keywords: Anaerobic digestion, thermophilic adaptation, food waste, *Methanothermobacter wolfeii, Methanothermobacter thermautotrophicus, Methanosaeta concilii, Clostridium thermopalmarium.*

4.1 INTRODUCTION

In Europe and especially North America, the development of more energy efficient technologies can help divert from landfills, a larger proportion of the organic fraction of municipal solid waste (OFMSW). As compared to mesophilic and to operate one or two-stage anaerobic digestion (AD) systems, thermophilic conditions can further improve the energy efficiency of the technology. Furthermore, thermophilic digestion can result in a higher kill-off of pathogens and can process OFMSW faster, thus increasing the capacity of anaerobic digesters (De Baere, 2000). For example, the introduction of thermophilic plants in Europe has increased the cumulative capacity (t/year) to treat OFMSW from 56 000 to 280 000 t/year, from 1997 to 2000.

Nevertheless, the successful transition of AD systems from mesophilic to thermophilic depends on the adaptation of the microbial population and the presence of specific groups of micro-organisms capable of performing their biological functions under thermophilic conditions. After a rapid change of temperature, no population shift was assumed by Mata-Alvarez (2002) because of group incompatibility. In this sense, Nozhevnikova *et al*. (1999) assumed that thermophilic and mesophilic methanogenic communities differ. Nevertheless, there is evidence that this assumption is not completely true and that there are mechanisms that permit the micro-organisms to evolve from one specific state to another depending on the transition conditions. According to Nielsen and

Petersen (2000), full-scale thermophilic anaerobic digesters in Denmark were started with and without a transition stage and all strategies used have resulted in a quick and satisfactory start-up. For the anaerobic treatment of mechanically sorted OFMSW, using a dry or wet process, Mata-Alvarez (2002) concluded that the transition from mesophilic to thermophilic conditions (from 35 to 55 °C in 10 days), using significant variations in organic loading (from 15 to 40 % in 48 h), did not have any permanent effect on the process performance.

Due to its widespread distribution, from hot springs to thermophilic anaerobic digesters worldwide, *Methanobacterium thermoautotrophicum* (the first thermophilic methanogen isolated) is perhaps one of the most important methanogens ever described. After its first description by Zeikus and Wolfe in 1972, a considerable number of thermophilic methanogens have been identified, and among the most important in terms of waste treatment systems are: *Methanothermobacter thermoautotrophicus*, *Methanothermobacter wolfeii*, *Methanobacterium thermophilum*, and *Methanosarcina thermophila* (Ferry, 1993).

The objective of this experiment was to observe the change in biomass character and genetic type when mesopholic sludge was adapted to thermophilic conditions and supplied with various loads of food waste. This characterization was performed using substrate Genomic 16S-rDNA phylogenic analyses. The adaptation was conducted by instantaneously exposing a mesophilic granular anaerobic sludge to thermophilic conditions using a bench scale reactor, and after letting it stabilize for 7 days, supplying it with an increasing load of food waste at a frequency of 21 days.

4.2 METHODOLOGY

4.2.1 EXPERIMENTAL MATERIALS

The 22% total solids substrate used in this study was food waste separated from the solid waste produced by a cafeteria and then chopped into a paste and diluted with distilled water (Table 4.1). The mesophilic seed consisted of a 50/50 % mixture of granular and non-granular sludge taken from two separate up-flow anaerobic sludge blanket reactors (UASB) treating apple-juice and dairy process wastewaters respectively, at 35 °C (Table 4.1).

Table 4.1 Experimental food waste substrate and seed characteristics.

Parameter	Food waste	Seed mixture
Density (kg/L)	1.22	1.042
Solid (%)	21.8	4.2
TS (g/L)	218.2	42.1
VS (% TS)	95.1	67.0
SS (g/L)	161.8	38.1
VSS (% SS)	96.5	70.0
Total COD (g/gVSS)	1.16	0.97
Soluble COD (% TCOD)	n.e.	12.12
TKN (% TS)	17	n.e.
C/N	3.1	n.e.

Notes: n.e.: not evaluated.
The seed mixture consisted of 50% UASB sludge treating apple wastewater and 50% UASB sludge treating milk wastewater.

The experimental 5 L glass reactor used to adapt the mesophilic sludge had a 4 L working volume and was continuously stirred and controlled for temperature and pH (Ortega *et al.*, 2007 – chapter 3).

4.2.2. THERMOPHILIC ADAPTATION OF THE MESOPHILIC SLUDGE

The mesophilic sludge was first adapted to thermophilic conditions during an initial 20 day stage (C0). On day 0, the reactor was seeded with 4 L of the mesophilic sludge mixture and operated at 35 °C without extraneous substrate supply to generate an inoculum. On day 13, the process temperature was instantaneously increased from 35 to 55 °C, and maintained at that temperature until day 20, still without extraneous substrate supply.

Following stage C0, the reactor biomass received some food waste to further observe its thermophilic adaptation. At the beginning of each 21 day stages namely C1, C2, and C3, the reactor was loaded according to a food to biomass ratio (F/M) of 0.12, 1.15 and 4.43 gVS/gVSS, respectively. Because the medium was acidified following the loading operation during stage C3, a fourth 21-days stage (C4) was conducted to restore methanogenic conditions. Starting on day 84 and to restore the thermophilic inoculum, stage C4 was initiated by replacing 2.15 L of the reactor's biomass with 1.6 L of sludge produced at the end of stage C2, 0.3 L of the original seed blend and 0.25 L of a 0.2M $NaHCO_3$ solution at pH of 8. Following this replacement, the reactor was operated for an additional 21 days.

To observe the biomass activity during the complete experimental period of 105 days, gas production was continuously measured and samples were taken at regular 24h intervals (weekdays) to determined its composition. Sludge samples (1.5 mL) were collected at the beginning of each stage C0, C1, C2, C3 and C4. Along with a sample of the seed (inoculum on day 0 of stage C0) these samples were kept frozen at –70 °C until analyzed for microbial diversity through genomic DNA identification.

4.2.3. ANALYTICAL METHODS

The food waste substrate, the original mesophilic seed and the reactor's biomass were analyzed to compute the F/M to load the reactor during stages C1, C2 and C3. These were analyzed for chemical oxygen demand (COD), total and suspended solids (TS and SS), and total and suspended volatile solids (VS and VSS). These analyses were carried according to standard methods (APHA *et al.,* 1995). Acetic, propionic and butyric acids were analyzed using an *Agilent* 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector. The composition of the biogas produced was determined using an HP gas chromatograph (68900 Series, *Hewlett Packard,* Wilmington, DE) equipped with a 900mm x 3 mm (36" x 1/8") 60/80 mesh Chromosorb 102 column (*Supelco*, Bellefonte, PA).

4.2.4. MICROBIAL DIVERSITY

Microbial diversity analyses were conducted using the DNA-profiling techniques. From sludge samples of *ca*. 1.5 mL, the total DNA was extracted as described by Tresse *et al*. (2002). From -70 °C storage, the samples were first glass-bead cell disrupted through hi-speed shaking (13 000 rpm). Then, the nucleic acids were separated and extracted by several stages of 20%-sodium-dodecyl-sulfate protein segregation and phenol/chloroform extraction. Finally, the extracted DNA was precipitated with a mixture of 3 M sodium acetate (1/10) and 100%-ethanol. Once re-suspended in distilled water, the optical density of the DNA/Protein ratio was measured at 260/280 (nm/nm) by means of a DU-640 *Beckman* spectrophotometer (*Beckman Coulter*, USA).

Afterwards, the 16S rRNA genes were amplified using the polymerase chain reaction (PCR). The Domains *Bacteria* and *Archaea* were amplified separately using the primers described in Table 4.2.

Table 4.2 PCR primers used in this study.

Primer	rDNA Target	Primer sequence (5'-3')	Reference
341f	*Bacteria*	CCTACGGGAGGCAGCAG	Muyzer *et al.* (1993)
758r	Universal	CTACCAGGGTATCTAATCC	Muyzer *et al*. (1996)
931f	*Archaea*	AGG AAT TGG CGG GGG AGC A	Amann *et al.* (1995)
1392r	Universal	ACG GGC GGT GTG T(G/A)C	Lane (1991)

Notes: f: forward, r: reverse.
All forward primers were attached with a GC-clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3') according to Muyzer *et al.* (1993) for DGGE analysis.

The PCR amplification conditions for *Bacteria* were: denaturation at 95°C for 5 minutes followed by 30 cycles under annealing temperatures consisting of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and finally 72 °C for 7 minutes. The PCR amplification conditions used for *Archaea* were: denaturation at 94°C for 5 minutes, followed by 20 cycles under annealing temperatures consisting of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and finally 72 °C for 7 minutes.

Denaturing Gradient Gel Electrophoresis (DGGE) of amplified fragments were performed as previously described by Tresse *et al*. (2002). PCR products were separated in 8% acryl amide gels with a 40 to 60% denaturing gradient. The gels were run in a 1X-Tris-EDTA-Acetate buffer solution for 16 h at 60 °C and 80V. Stained bands were

excised from the gel, purified and re-amplified with the same primers. The sequencing was realized in both directions for each DNA fragments using the *BIG DYE Terminator reaction kit* (*Perkin Elmer Applied Biosystems Division*, Foster City, CA). For comparison, the sequences were submitted to the Genbank databases using the National Center for Biotechnology Information BLASTN program (Altschul *et al*., 1997).

4.3 RESULTS

4.3.1 ADAPTATION FROM MESOPHILIC TO THERMOPHILIC CONDITIONS AND F/M LOADING

The 5 L bench scale reactor was seeded with mesophilic granular sludge obtained from up-flow anaerobic sludge blanket digesters. After 13 days of operation at 35 °C, the reactor temperature was instantaneously increased to 55 °C and operated at this temperature until day 21. The sludge collected on day 21 demonstrated that the abrupt temperature change had no pasteurization effect, but rather lead to a biomass with a fermentative activity of 3.58 g Glucose/gVSS/d and a methanogenic activity of 0.47 and 0.26 g Substrate/gVSS/d, related respectively, to acetoclastic and hydrogenophilic microorganisms (Ortega *et al.* 2007 – chapter 3).

The biomass was then fed food waste at the beginning of stages C1, C2 and C3, corresponding to days 21, 42 and 63, each time with an F/M (Food / Microorganism) ratio increasing from 0.12 to 1.15 and then 4.43 gVS/gVSS, respectively. Sludge samples were collected on days 0, 21, 42 and 63 to conduct substrate activity tests, and reactor biogas production was monitored during the full experimental period. The reactor's biomass was able to accommodate the low and intermediate F/M ratios (stages C1 and

C2) by producing biogas, but the high F/M of stage C3 resulted in the production of hydrogen instead of methane, the acidification of the medium, and the accumulation of volatile fatty acids (4 580 mg acetic acid/L, 65 mg propionic acid/L and 7 000 mg butyric acid/L). For stages C1 and C2, the ultimate gas production (Go) and the ultimate biodegradation potential (Bo) decreased with loading rate from 1.4 to 0.89 L/gVS_{fed} and 0.84 to 0.61 $L\text{-}CH_4/gVS_{fed}$, respectively. (Ortega *et al.* 2007 – chapter 3).

As a result of the reactor's acidification during stage C3, the reactor reduced its biogas-production capacity and both Go and Bo dropped to 0.2 L_{STP}/gVS_{fed} and 0.01 $L_{STP}CH_4/gVS_{fed}$, respectively. A non-substrate stage C4 followed the loading stage C3, as of day 84 to restore the pH and methanogenic activity. The replacement of 2.15 L of the reactor's acidified medium produced a pH of 7.0 and resulted in the additional production of 29.5 L of biogas by day 105 (Ortega *et al.* 2007 – chapter 3).

4.3.2. Sludge microbial evolution

The phylogenic analysis of DNA extracted from the biomass samples at the end of every stage (C0 to C4) and from the initial inoculum (day 0 for stage C0) indicated the presence of two types of bacteria and several species of methanogens (Tables 4.3 and 4.4).

These DNA analyses first indicated that hydrolytic and fermentative activities and volatile fatty acid oxidation were carried out by Clostridium-like and syntroph-like microorganisms, respectively. Secondly, the application a high F/M favored the activity of bacteria rather than that of methanogens.

For the DNA-sequencing and *BLAST* comparison of the domain *Bacteria*, several bands were related to the class of Clostridium sp. (Table 4.3 and Figure 4.1). Thus, band

10 was identified as *Clostridium thermopalmarium* and its presence was relevant during stages C3 and C4. As well, during the food loading stages C2 and C4, bands 22 and 23 were related to *Coprothermobacter proteolyticus* and *C. platensis*, respectively, both classified as Clostridium-like microorganisms, belonging to the phylum *Fermicutes*.

Table 4.3 Results of the DNA-profiling analysis and the BLAST comparison for the domain Bacteria.

Band	Microorganism	BLAST reference	Compared nucleotides	Identities (%)	Stage
10	*Clostridium thermopalmarium*	AF286862.1	386	100	C3; C4
15	Uncultured bacteria (fatty acid oxidizing syntrophs in granular sludge)	AF482435.1	412	96	C0; C1; C2
19	Uncultured bacterium UASB_TL13 (Genus Cytophaga in environmental samples)	AF254391.1	407	99	All
26	Uncultured bacterium clone R2b21 (fatty acid oxidizing syntrophs in granular sludge)	AF482436.1	383	98	Seed
22	*Coprothermobacter proteolyticus* (Phylum: Fermicutes; Class: Clostridium)	X69335.1	388	99	C2; C4
23	*Coprothermobacter platensis* gene 16SrRNA (Phylum: Fermicutes; Class: Clostridium)	Y08935.1	389	96	C2; C4

Three syntroph-like microorganisms were also identified (Table 4.3). Band 15 was related to a syntroph uncultured bacteria present during stages C0, C1, and C2. For band 19 (uncultured bacterium UASB-TL13), the presence of a Cytophaga, syntroph-like microorganism was detected in all samples analyzed (C0 to C4). Finally, a syntroph-like

microorganism related to an uncultured bacterium clone R2b21, a fatty acid oxidizing syntroph in granular sludge, was detected only in the anaerobic seed used at the beginning of the experiment (day 0 of C0).

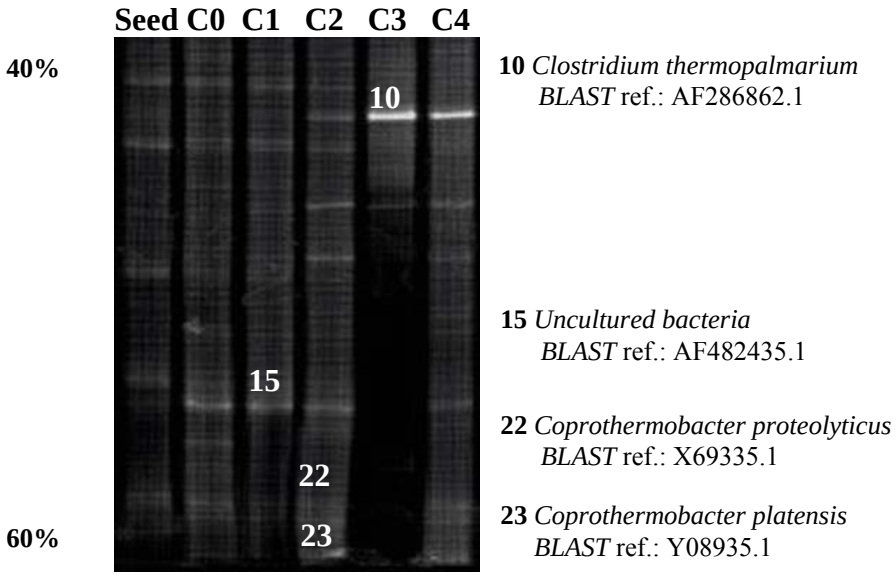


Fig. 4. 1 DGGE-*Bacteria* gel (negative-photograph) for the adaptation stage C0 with no substrate addition; C1 with a loading of 0.12 gVS/gVSS; C2 with a loading of 1.15 gVS/gVSS; C3 with a loading of 4.43 gVS/gVSS, and; C4 with no substrate addition.

For the DNA-sequencing and *BLAST* comparison of the domain *Archaea* (Table 4.4), all bands analyzed were related to the phylum *Euryarchaeota* of which 56 % was related to the class methanobacteria, 30 % to the class methanomicrobia, and 14 % to non-cultured methanogens.

Table 4.4 Results of the DNA-profiling analysis and the BLAST comparison for the domain Archaea.

Band	Microorganism	BLAST reference	Compared nucleotides	Identities (%)	Stage
3	*Methabacterium beijingense*	ay552778	381	98	All but C3
4	*Methanoculleus palmeoli*	y16382	427	98	"
9	*Methanothermobacter wolfeii*	ab104858	244	98	"
19	Methanobacterium sp.	ay350742	447	98	"
21	*Methanosaeta concilii*	x51423	325	99	"
25	*Methanothermobacter thermautotrophicus*	x68717	243	97	C2; C4
31	Uncultured methanosarcinales	ab077214	431	99	All but C3

Although the biomass had resisted the abrupt change of temperature during stage C0, no DNA methanogen-related was recuperated from the biomass sample at the end of the loading stage C3. Since methanogenic conditions were re-established by replacing the acidified biomass with a mixture of the original seed and the sludge produced during stage C2, all methanogens were completely re-seeded during stage C4 (Figure 4.2).

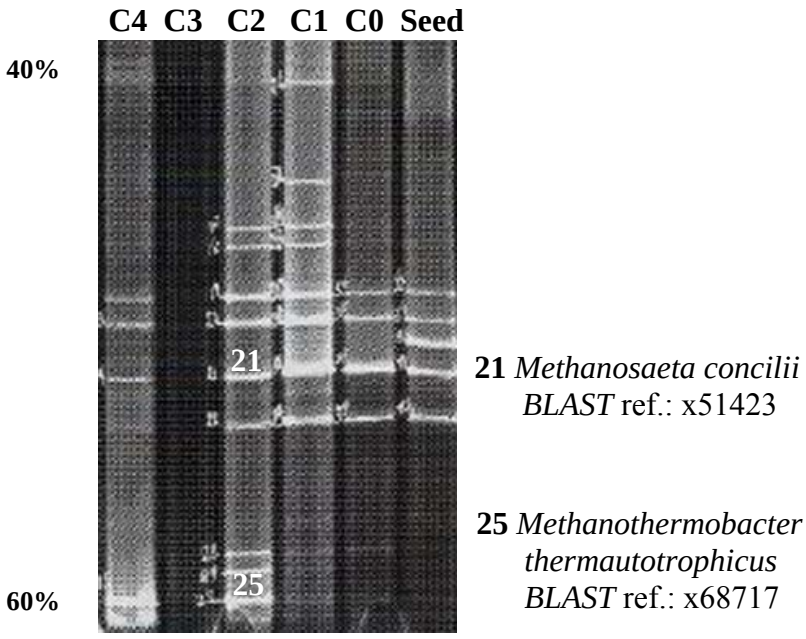


Fig. 4.2 DGGE-*Archaea* gel (negative-photograph) for the adaptation stage C0 with no substrate addition; C1 with a loading of 0.12 gVS/gVSS; C2 with a loading of 1.15 gVS/gVSS; C3 with a loading of 4.43 gVS/gVSS, and; C4 with no substrate addition.

4.4 DISCUSSION

These results indicate that the abrupt change in temperature during stage C0 did not negatively affect the methanogenic capability of the reactor's biomass because it was able to produce biogas afterwards while being loaded with a low and intermediate F/M. Thus and after the immediate thermophilic transition, the phylogenic analysis together with the methanogenic capability of the reactor indicated that both mechanisms (adaptation and microbial shift) were present during the transient temperature conditions. This fact is reinforced by the detection of both *Bacteria* and *Archaea* on day 0 (original seed) and day 20 of the adaptation stage C0.

Nevertheless, the supply of increasing amounts of food waste substrate proved to be an effective selection force for microorganisms. Although no shift was noticed while applying low and intermediate F/M, the application of 4.43 gVS/gVSS and its resulting acidic conditions negatively affected both the acetoclastic and the hydrogenophilic methanogenic microorganisms. Only bacterial 16S-rDNA was extracted after the acidification of the reactor at the end of stage C3.

The concentration of ionized-volatile fatty acids (VFA) was relatively low at 4 580 mg acetic acid/L, 65 mg propionic acid/L and 7 000 mg butyric acid/L. Nevertheless, the concentration of un-ionized acetic acid was more likely to exert a negative effect on the methanogenic microorganisms as a result of the pH drop from 7.8 to 5.2 early during stage C3. According to the Henderson-Hasselbach dissociation equation (Guiot 1991), such a pH drop displaced the chemical equilibrium for un-ionized acetic acid and increased its concentrations from 2 to 506 mg/L, which in turn, can inhibit the methanogen biomass. These observations were consistent with those of Guiot (1991) who observed that a concentration of 200 mg/L of un-ionized acetic acid in a hybrid anaerobic process lead to the reduction of its methane production.

The phylogenic analysis showed that hydrolytic activity in general was related to Clostridium-like microorganisms. Meanwhile, fermentative activity responsible for the production of H_2 could be related to both Clostridium-like and syntroph-like microorganisms. Thus, microbial interaction between fermentative bacteria and methanogens are energetically favored compared to the axenic growth of the bacteria. For example, *Clostridium thermocellum*, growing together with *Methanobacterium thermoautotrophicum*, consume glucose producing acetate, CO_2 and H_2. This improves

energy conservation for fermentative organisms, since the acetyl-CoA intermediates in this biochemical pathway can be conserved to adenosine triphosphate (ATP) (Weimer and Zeikus, 1977; Ferry, 1993). For stage C3, this condition existed only during the first two days of operation. As a result, syntrophy between Clostridium sp. and methanogens took place and methanogen activity was suppressed as a result of acidic conditions. This interaction was present during the precedent stages (C0 to C2). Members of the obligate interspecies of the H_2-transfer chain (McCarty and Mosey, 1991), syntrophic organisms participated as well in the production of H_2 between day 65 and 67 of stage C3. These organisms are known to partially consumed butyrate to produce even more acetate, CO_2 and H_2.

The presence of such microorganism within the samples analyzed suggest that during the earliest phase of stage C3, sugars of low molecular weight such as glucose and even cellobiose were consumed by Clostridium-like organisms producing H_2 and acetate. Thus, the important accumulation of acetate during stage C3 was related to carbohydrate degradation by Clostridium-like microorganisms, a product of the partial oxidation of butyrate by syntroph-like microorganisms as well. Besides the high H_2-partial pressure in the media, the accumulation of acetate and butyrate was accompanied by a drop in pH from 7 to 5. Consequently, the microbial activity of both bacteria and methanogens almost ceased along with the production of biogas.

4.5 CONCLUSIONS

Mesophilic wastewater-treating anaerobic sludge was found to easily and quickly adapt to thermophilic conditions for the treatment of food waste representing the organic

fraction of municipal solid waste. Nevertheless, the organic load program must be carefully selected and monitored, since the methanogenic activity can easily be displaced towards a fermentative activity.

The instantaneous upgrading procedure from mesophilic to thermophilic conditions did not have a pasteurization effect on anaerobic microorganisms, since no shift of microbial populations was observed. Instead, low and intermediate F/M ratios positively selected the mesophilic acetoclastic methanogens and mesophilic fermentative bacteria.

As opposed to temperature effect, low pH conditions provoked a shift of the microbial content. Under batch operation, a low pH environment related to a high F/M ratio of 4.43 gVS/gVSS displaced methanogens by favoring the growth of hydrogen producing-bacteria. Although hydrogen production was not the goal of this process development, its observation might be of interest as an alternative energy source. More research is needed with that respect.

CONNECTING STATEMENT (Third scientific article)

Chapter 5 deals with the development of a serum bottle laboratory batch test for measuring the limiting step (initial degradation rate and biochemical methane potential) during the degradation of food waste. The methodology is able to use complex substrates such as proteins and vegetable oil (bovine albumin and olive oil degradations are described in this chapter). This scientific paper will be submitted for publication in a peer reviewed journal. The election of the journal is currently under evaluation.

Authors and affiliation:

Luis Ortega[1,2] (i); Suzelle Barrington[1] (ii); Serge Guiot[2] (iii)

(1) McGill University, Bioresource Eng. Department. 21111 Lakeshore Road, Ste-Anne-de-Bellevue (Quebec), H9X 3V9 Canada. E-mail: luis.ortega@nrc.ca

(2) National Research Council of Canada, Biotechnology Research Institute. 6100 Royalmount Av. Montreal (Quebec), H4P 2R2 Canada.

The candidate (i) carried out the experiments, statistical analysis, and the writing of manuscripts. Author (ii) further enhanced the analysis of data, edited the manuscript and contributed to the content of the article. Author (iii) lead the research project, supervised the laboratory and analytical work carried out by the candidate, further enhanced the analysis of data, edited the manuscript and contributed to the content of the article.

CHAPTER FIVE

METHANE PRODUCTION TEST: A TOOL FOR EVALUATING ANAEROBIC LIMITING-STEP DEGRADATION OF FOOD WASTE

5.0 ABSTRACT

The anaerobic degradation of organic mater is an interactive process requiring a coordinated interaction of several groups of microorganisms, and hydrolysis is often the limiting step when treating complex substrates such as food waste. To evaluate and compare the rate of hydrolysis and methanization *via* acetogenesis for a specific substrate and inoculum, this project developed a batch test based on methane production (MP). This batch test examined the degradation of complex compounds, namely proteins (albumin and amino acids) and oils (olive oil, glycerol and oleic acid) , and compared them to that of acetic acid, under mesophilic and thermophilic conditions. The acetoclastic and hydrogenophilic methanogenesis were not found to be the limiting steps for albumin or olive oil, and their respective monomer-like molecules namely amino acids, and glycerol and oleic acid. As compared to acetic acid and for the same COD load, their methanization rate was reduced by 22 and 47% respectively under mesophilic conditions, and under thermophilic conditions, was almost nonexistent. Additional tests showed that the same sludge, but thermophilically adapted during 10 months while treating food waste, had a good proteolytic activity, but was unable to methanize olive oil. The low degradability of olive oil was not related to the breakdown of its triglyceride molecules (lipolysis), but rather to the accumulation of oleic acid which exerted an

inhibitory effect on thermophilic acetoclastic methanogens. This inhibitory effect of oleic acid slowly disappeared with its degradation by other microorganisms in the system.

Keywords: Albumin; anaerobic digestion; food waste; oleic acid; olive oil; thermophilic conditions

5.1 INTRODUCTION

The anaerobic degradation of organic matter is a complex process requiring a coordinated interaction of several groups of microorganisms: hydrolytic and acidogenic fermentative bacteria, syntrophic acetogenic bacteria, and acetoclastic and hydrogenophilic methanogenic archaea. Normally, the anaerobic degradation rate of highly degradable substrates is limited by the well-known slow growing action of acetoclastic methanogens: their growing rate is several times slower than that of the general bacterial populations (Ferry, 1993). In food waste containing degradable but complex macromolecules like proteins, carbohydrates, and lipids, the degradation-limiting step is displaced towards the hydrolysis of such compounds instead of the degradation of acetic acid. Thus, both the methanization rate and its potential are governed by the presence of complex organic matter and the microorganisms capable of breaking down such compounds.

Since during a methanogenic anaerobic process, 70% of the organic matter is transformed into methane *via* acetoclastic methanogenesis (Gujer and Zehnder, 1983), a methane producing-based monitoring methodology (MP) can be developped. This MP methodology consists in observing the degradation of substrates involved in specific steps of the methanization process *via* acetogenesis. The objective of this study was therefore to use MP to investigate the degradation of albumin and olive oil in serum

bottles under mesophilic and thermophilic conditions, and to demonstrate the application of the technique. The project first evaluated the MP test under non-limiting conditions using acetic acid as substrate. Then, the MP test was used to measure the degradation of albumin and olive oil and their transformation into methane and carbon dioxide. To compare the rate of hydrolysis of these two compounds, methanization rates and potentials of their monomer-like compounds (a mixture of alanine and glycine for albumin and oleic acid and glycerol for olive oil) were evaluated as well.

5.2 METHODOLOGY

5.2.1 SLUDGE

The first series of tests, conducted under mesophilic (35 °C) and thermophilic (55 °C) temperatures were carried out using a mixture of mesophilic granular sludge taken from two industrial up-flow anaerobic sludge blanket reactor, the first treating wastewater from a dairy plant and the second treating wastewater from an apple-juice plant. A second series of tests conducted under thermophilic temperatures (55 °C) were carried out with this same mesophilic sludge acclimated to 55 °C during 7 months in a mixed anaerobic reactor fed with a source sorted food waste. This food waste was collected from the solid waste produced by a cafeteria which contained 218.2 g l^{-1} of totals solids (TS), 95 % volatile solids (VS on a dry basis), 1.16 g COD g^{-1}VSS and 17 % nitrogen (on a dry basis) measured as total Kjeldahl nitrogen (Ortega *et al*., 2007 –Chapter 3-).

5.2.2 Substrates

The substrates selected to run the MP tests corresponded to specific degradation steps in the methanization process *via* acetogenesis: 1) *Acetic acid (CH_3COOH)* which is transformed into methane by acetoclastic methanogens; 2) *Albumin* which is hydrolyzed into peptides, then amino acids, and then acetic acid (Ramsay and Pullammanappallil, 2001), 3) *Alanine ($C_3H_7O_2N$)* and *Glycine($C_2H_5O_2N$)* amino acids which are metabolized into acetic acid (Ramsay and Pullammanappallil, 2001), 4) *Olive oil* which contains triglycerides transformed by the hydrolytic and fermentative bacteria into long chain volatile fatty acids (VFA) and glycerol (Rawn, 1990); 5) *Oleic acid ($C_{18}H_{34}O_2$)* which can be transformed into acetate throughout β-oxidation (Rawn, 1990) and which determines the production of methane resulting from the degradation of unsaturated long chain VFA; olive oil consists of 85% oleic acid by mass (Michigan State University - Department of Chemistry, 2007); and finally 6) *Glycerol ($C_3H_8O_3$)* which, following the hydrolysis of olive oil, can serve as methanogenic substrate via its transformation into acetic acid.

Table 5.1 shows the equivalent stoichiometric substrate chemical oxygen demand (g $COD_{Substrate}$ g^{-1} Substrate) for acetic acid, alanine, glycine, glycerol and oleic acid and the measured $COD_{Substrate}$ (g $COD_{Substrate}$ g^{-1} Substrate) for albumin and olive oil. These values were used to calculate the initial concentration of substrate to use in the serum bottles which, depending on the MP tests, were loaded with 4.5, 9, or 12 g $COD_{Substrate}$ l^{-1} as described below.

Table 5.1. Stoichiometric $COD_{Substrate}$ equivalence of experimental substrates.

Substrate (Stoichiometry of oxidation)	COD equivalent (g COD g^{-1} Substrate)
Acetic acid ($CH_3CO_2H + ½ O_2 \rightarrow 2 CO_2 + 2 H_2O$)	1.06
Albumin*	1.56 ±0.23
Alanine ($C_3H_7O_2N + 5¼ O_2 \rightarrow 3 CO_2 + NO_3 + 3½ H_2O$)	1.88
Glycine ($C_2H_5O_2N + 3¾ O_2 \rightarrow 2 CO_2 + NO_3 + 2½ H_2O$)	1.59
Olive oil*	2.39 ±0.15
Glycerol ($C_3H_8O_3 + 3½ O_2 \rightarrow 3 CO_2 + 4 H_2O$)	1.21
Oleic acid ($C_{18}H_{34}O_2 + 25½ O_2 \rightarrow 18 CO_2 + 17 H_2O$)	2.87

Notes: * Measured in the laboratory, otherwise calculated.

5.2.3 Methane production (MP) tests

The MP test measures the ultimate limiting step in the process of anaerobic digestion assuming that the sludge contains all the trophic groups leading to the production of acetate as the last substrate for the production of methane. The degradation results are expressed in mmol of CH_4 *per* gVSS of sludge *per* day. The tests were carried out in 120 ml serum bottles as described previously (Owen *et al*., 1979; Shelton and Tiedje, 1984; and Cornacchio *et al*., 1986).

Acetic acid was first used as substrate to determine its rate of methane production as well as its methanization potential (amount of methane produced per COD equivalent of acetic acid used in the bottle). Then, albumin was tested along with two amino acids, alanine and glycine to compare their rate of methanization and methanization potential to that of acetic acid; being intermediate substrates found in the degradation process of proteins, the amino acids served as indicators of the rate of hydrolysis of proteins. Then, olive oil along with glycerol and oleic acid were tested, where again glycerol and oleic acid are intermediate substrates found in the degradation process of olive oil.

Additional control bottles were tested without substrate to monitor the methanogenic activity resulting from the degradation of the organic matter remaining in the sludge after sampling and the production of biogas related to the endogenous respiration of methanogenic cells.

For each treatment, four bottles were inoculated with sludge diluted with a phosphate buffer to obtain an inoculum concentration of 10 to 15 gVSS l^{-1}. The bottles were supplied with 9 g $COD_{Substrate}$ l^{-1} of each substrate and then flushed with N_2:CO_2 (80 %: 20 %) to remove all oxygen. Three of those bottles were used to monitor and calculate the sludge's methanogenic production rate and potential. The fourth bottle was used to monitor over time the degradation of acetic acid and, intermediate substrates, depending on the test. No gas production was monitored in this fourth bottle. For the acetic acid substrate test, and to eliminate acetoclastic methanogenesis as limiting step, evaluations were conducted using bottles containing 0.06, 0.15 and 0.21 M of acetic acid.

While being incubated at either 35 or 55 °C, the serum bottles and their content were shaken at 100 rpm. The volume of biogas from each bottle was periodically

measured by displacing water in an attached inverted filled-burette. During the first week of the test, gas was sampled and analyzed daily and every other day afterwards. The specific methane production rate ($mmolCH_4\ g^{-1}VSS\ d^{-1}$) was calculated using the maximum slope of the curve representing the accumulation of methane over time. The final MP result was obtained by subtracting the activity of the substrateless control bottles. The yield of methane ($mmolCH_4\ mmol^{-1}$acetic acid) was calculated using the final molecular production of methane from each bottle divided by the molar mass of acetic acid consumed in the bottle. Using the ideal gas law, the molar mass of methane was expressed based on standard conditions (1 atm, 273.15 K and R of 0.0821 L atm $mol^{-1}K^{-1}$).

The substrate biodegradation efficiency (%) was calculated as the ratio of methane produced and the mass of substrate added, both expressed in COD equivalent (g COD-CH_4 and $gCOD_{Substrate}$, respectively). In theory, a value of 3.975 g COD g^{-1} CH_4 equivalent should be obtained from the following stoichiometric equation:

$$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O \qquad (1)$$

Mesophilic and thermophilic hydrogenophilic methanogenesis was evaluated through substrate activity tests (SA) carried out in 60 ml serum bottles. Triplicate bottles were inoculated with biomass diluted with a phosphate buffer to obtain 2 gVSS l^{-1} and then flushed with H_2:CO_2 (80:20 at *ca*. 138 kPa) as substrate (Guiot *et al*. 1995). The bottles were then incubated in a rotary shaker (*New Brunswick Scientific Co*., Edison, NJ). The bottles were incubated at either 35 or 55 °C and at 400 rpm. The initial H_2 activity (mmol

H_2 g^{-1}VSS d^{-1}) was calculated from the highest slope of the H_2-degradation *vs.* time curve.

5.2.4 ANALYTICAL METHODS

The amount of substrate supplied to the bottles was based on chemical oxygen demand (COD in g l^{-1}), while the amount of inoculating sludge added to each bottle was based on volatile suspended solids (VSS in g l^{-1}). These analyses were carried out according to standard procedures (APHA *et al*., 1995). Chemical oxygen demand was measured using the potassium perchromate method while volatile solids (VS) were measured by incinerating dry solids at 500 °C.

Acetic acid was measured using an *Agilent* 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector of 0.2 μl. The samples were fortified at a ratio of 1:1 (V:V) using an internal standard of iso-butyric acid dissolved in 6% formic acid and directly injected into a glass column of 1 m x 2 mm *Carbopack C* (60 to 80 mesh) coated with 0.3% *Carbowax* 20M and 0.1 % H_3PO_4. The column was held at 130 °C for 4 minutes and helium as carrier gas was injected at a rate of 20 ml min^{-1}. The injector and the detector were both maintained at 200 °C.

The biogas composition (hydrogen, nitrogen plus oxygen, methane, and carbon dioxide) was measured using an HP gas chromatograph (68900 Series, *Hewlett Packard,* Wilmington, DE) equipped with a 900 mm x 3 mm (36” x 1/8”) 60/80 mesh.

5.2.5 STATISTICAL ANALYSIS

The one-way analysis of variance (ANOVA) or *F-test* were used to determine the statistical significance of the CH_4 yield for samples of biomass tested using the MP test for 0.06, 0.15 and 0.21 M of acetic acid (Wardlaw, 1985). The values obtained from these sludge samples were tested for significant difference using the following equation:

$$F = \frac{Vb}{Vw} \qquad (2)$$

where:

F = Variance ratio

Vb= Between groups variance (0.06, 0.15 and 0.21 M of acetic acid)

Vw= Within groups variance (between triplicates)

The F value was compared in a critical *F-Value* table for 2 d.f. between groups and 6 d.f. within groups. A multiple range test (MRT) for comparing means in an analysis of variance was evaluated for significance within groups. The MRT was used to identify the treatment(s) demonstrating a significant difference.

5.3 RESULTS AND DISCUSSION

5.3.1 ACETOCLASTIC METHANOGENESIS UNDER MESOPHILIC CONDITIONS

The first experiment was conducted under mesophilic temperatures, with the mesophilic sludge fed acetic acid. It represents the last and most limiting step in the anaerobic degradation of organic matter into methane. The objective of this first experiment was to measure the rate of methane production from various concentrations of acetate.

The bottles containing 0.06, 0.15 and 0.21M of acetic acid, corresponded to low, average and high acetic acid concentrations of 3.4, 8.9 and 12.8 g l^{-1}. These concentrations verified any possible negative limiting (low concentration) or inhibiting (high concentration) of the substrate on the biomass.

Acetic acid degradation with time is illustrated in Figure 5.1a. With 0.06 and 0.15 M of acetic acid, a one day lag phase was observed and it took 3.8 and 8.4 days, respectively, to consume all the substrate available. With 0.21 M of acetic acid, no lag phase was observed and all the acetic acid was consumed in 10.4 days. The production of methane from the consumed acetic acid (Figure 5.1b) took place with a small lag phase of *ca*. 2 days. The concentration of 0.06M of acetic acid was first to reach a plateau after almost 4.0 days, while those with 0.15 and 0.21M of acetic acid reached this plateau after 9 and 13 days, respectively.

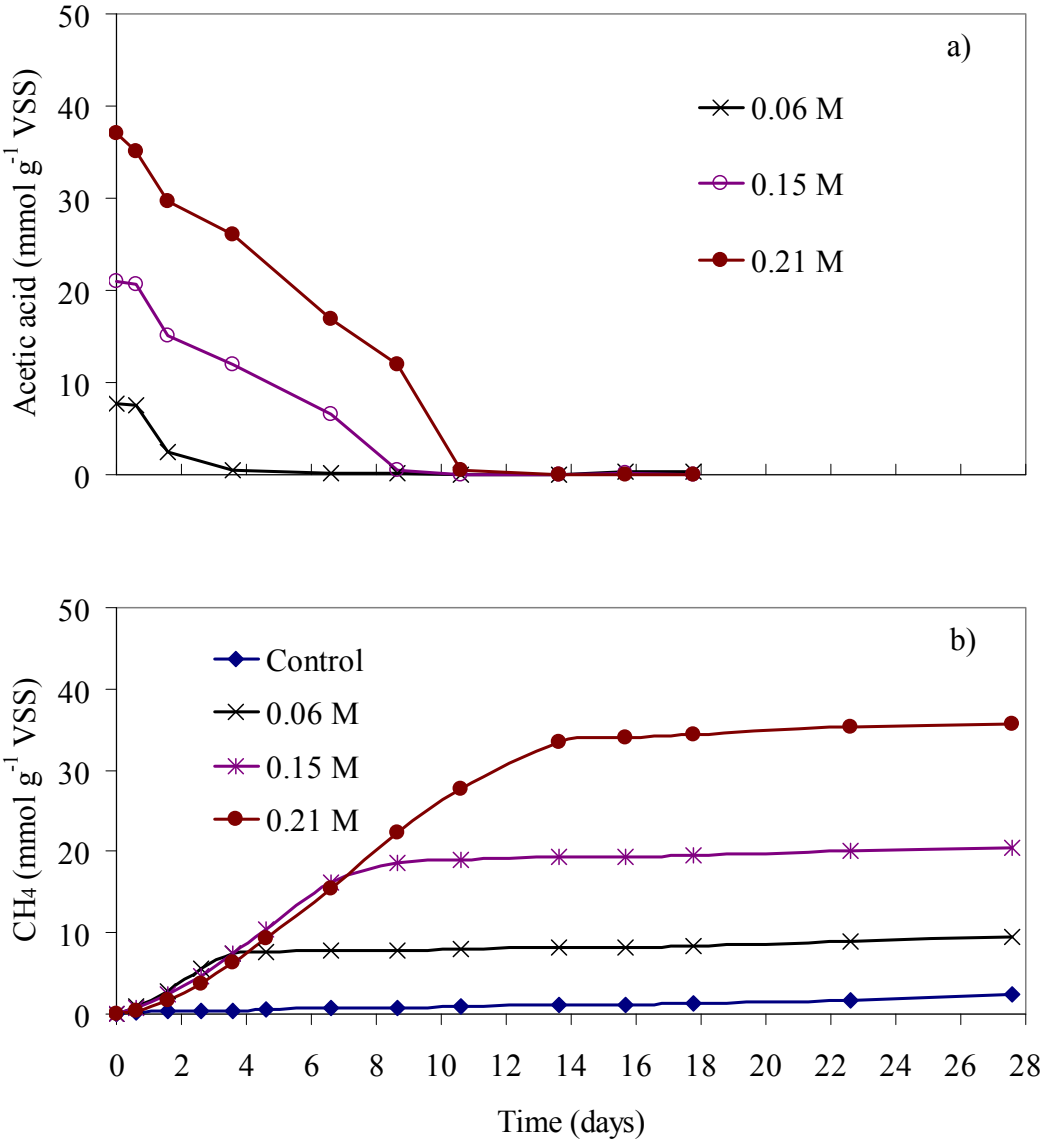


Figure 5.1. Acetate depletion (a) and MP activity (b) with 0.06, 0.15 and 0.21 M of acetic acid, using the mesophilic sludge under mesophilic temperatures.

Table 5.2 reports the initial production rate of CH_4 by the sludge, its acetate consumption rate and methane yield, for mesophilic conditions. The low and high concentrations of

0.06 and 0.21 M of acetic acid showed no limiting and inhibiting effect, respectively. Because all three concentrations of acetic acid produced the same methane yield, the average concentration of 9 g $COD_{Substrate}$ l^{-1} (0.15 M acetic acid) was used to conduct the following methanization tests with albumin, olive oil, glycerol, and oleic acid. The specific rate of acetic acid depletion (2.2, 2.3, and 3.2 mmol acetic acid g^{-1}VSS d^{-1}, respectively) followed the methane production trend (2.3, 2.6, and 3 mmol CH_4 g^{-1}VSS d^{-1}, respectively). This suggests a stochiometrically proportional transformation of acetic acid into methane. Those results correspond to the methane yield obtained in all cases (0.94, 0.87, and 0.92 mmolCH_4 $mmol^{-1}$ acetic acid, respectively).

Table 5.2. MP activity with 0.06, 0.15 and 0.21 M Acetic acid, under mesophilic temperatures and using the mesophilic sludge.

Acetic acid concentration (M)	**0.06**	**0.15**	**0.21**
Initial rate (mmolCH_4 g^{-1} VSS d^{-1})	2.3 ±0.9[a]	2.6 ±0.6[a]	3 ±0.05[b]
Acetate depletion (mmol acetic acid g^{-1} VSS d^{-1})	2.2	2.3	3.2
Yield (mmolCH_4 $mmol^{-1}$ acetic acid)	0.95 ±0.03[c]	0.87 ±0.01[c]	0.93 ±0.05[c]

Notes: Values with the same letter are not significantly different within groups at the level of $P \leq 0.05$.

The H_2-SA tests evaluated hydrogenophilic methanogenesis (results not shown). These tests showed no hydrogen and carbon dioxide limitations since initial SA rates of 518 ±197 and 120 ±13 mmol H_2 g^{-1} VSS d^{-1} were observed, respectively, with bottles incubated at mesophilic temperatures with the mesophilic sludge.

Under conditions of no acetic acid limitations, a methane production rate of 2.2 mmol of CH_4 g^{-1} VSS d^{-1} was obtained for a range of acetic acid concentrations of 0.06 to 0.21 M.

5.3.2 MESOPHILIC AND THERMOPHILIC MP TESTS FOR ALBUMIN AND AMINO ACIDS

For the MP tests conducted under mesophilic and thermophilic temperatures, using the mesophilic sludge, the rate of methane production from acetic acid (0.15 M equivalent to 9.0 g COD l^{-1}) and albumin (9 g COD l^{-1}) is illustrated in Figure 5.2a, as compared to the control test without substrate. Under mesophilic conditions, albumin produced a methanization rate (2.0 ±0.02 mmol CH_4 g^{-1} VSS d^{-1}) similar to that of 0.15 M of acetic acid (2.6 ±0.6 mmol CH_4 g^{-1} VSS d^{-1}). After 27 days, albumin had produced 16.76 mmol CH_4 g^{-1} VSS (0.41 g COD-CH_4), equivalent to a COD-protein degradation of 78%.

Under thermophilic temperatures and still with the mesophilic sludge, albumin produced an initial methanization rate (0.7 ±0.06 mmol CH_4 g^{-1} VSS d^{-1}) similar to its own and 0.15 M acetic acid under mesophilic condition (0.8 ±0.04 mmol CH_4 g^{-1} VSS d^{-1}). After only 5 days, methane production reached a plateau and stopped, indicating that the soluble substrate contained in the inoculum, rather than from albumin was the source of COD. The total cumulative methane production (4.6 mmol CH_4 g^{-1} VSS) was lower than that of the control bottles at 10.2 mmol CH_4 g^{-1} VSS, suggesting that the acetoclastic methanogens of the mesophilic sludge were inhibited by the change in temperature from 35 to 55 °C. In the control bottles exposed to 55 °C but still with the mesophilic sludge, the methanogenic microorganisms showed a 5 days lag phase before starting to produce

methane. Thus, the thermophilic temperatures affected the proteolytic microorganisms of the mesophilic sludge which were unable to degrade the albumin into methane.

A second series of MP test were conducted with the thermophilically adapted sludge to determine if temperature or the fermentative microorganisms were responsible for this low protein hydrolysis into amino acids or for the conversion of amino acids into acetic acid and then methane (Fig. 5.2b). This test was conducted with 0.06 M acetic acid, 4.5 g COD l^{-1} of albumin and an equimolar mixture of alanine and glycine (amino acids) as substrates (4.5 g $COD_{substrate}$ l^{-1}). This combination of amino acids was selected to produce acetic acids through the *Sitckland* reaction and to prevent the accumulation of hydrogen, since the degradation of alanine into acetic acid produces hydrogen while that of glycine requires hydrogen (Ramsay and Pullammanappallil, 2001).

With the thermophilically adapted sludge under thermophilic temperatures, the process was not limited by the thermophilic methanogenesis of acetic acid (Figure 5.2b) since the initial thermophilic MP rate was 13 times higher (9.5 ±0.3 mmol CH_4 g^{-1} VSS d^{-1}) than that of 0.7 ±0.06 mmol CH_4 g^{-1} VSS d^{-1}, obtained with mesophilic sludge exposed to thermophilic temperatures and fed 0.15 M acetic acid (Figure 5.2a). The SA test results of 120 ±13 mmolH_2 g^{-1} VSS d^{-1} also demonstrated that hydrogenophilic methanogenesis did not limit the degradation of substrates (results not shown). Albumin degradation with the thermophilically adapted sludge produced 1.5 ±0.2 mmol CH_4 g^{-1} VSS d^{-1}, a rate slightly lower than that obtained with the mesophilic sludge (Figure 5.2a) under mesophilic conditions (2 ±0.02 mmol CH_4 g^{-1} VSS d^{-1}). Methane production with albumin (0.19 gCOD-CH4) corresponded to a protein degradation of only 37%.

The thermophilic MP test for the mixture of amino acids (Figure 5.2b) produced an activity of 5.2 ±0.1 mmol CH_4 g^{-1} VSS d^{-1} which was almost equivalent to that of acetic acid (4.4 mmol CH_4 g^{-1} VSS), indicating a stochiometric transformation of the amino acids into acetic acid and then methane. The average methane production (0.23 g COD-CH_4) corresponded to an amino acid degradation of 51 %. Thus, the thermophilic sludge offered the proper populations but albumin (protein) degradation was limited by the proteolysis into peptides and not by the ultimate hydrolysis of amino acids into acetic acid, and then methane. Fang and Chung (1999) also observed a higher degradation (83-85%) of a proteinaceous wastewater under mesophilic conditions as compared to that under thermophilic conditions (62-82%). The limiting step in the reaction under thermophilic conditions was presumed to be protein hydrolysis.

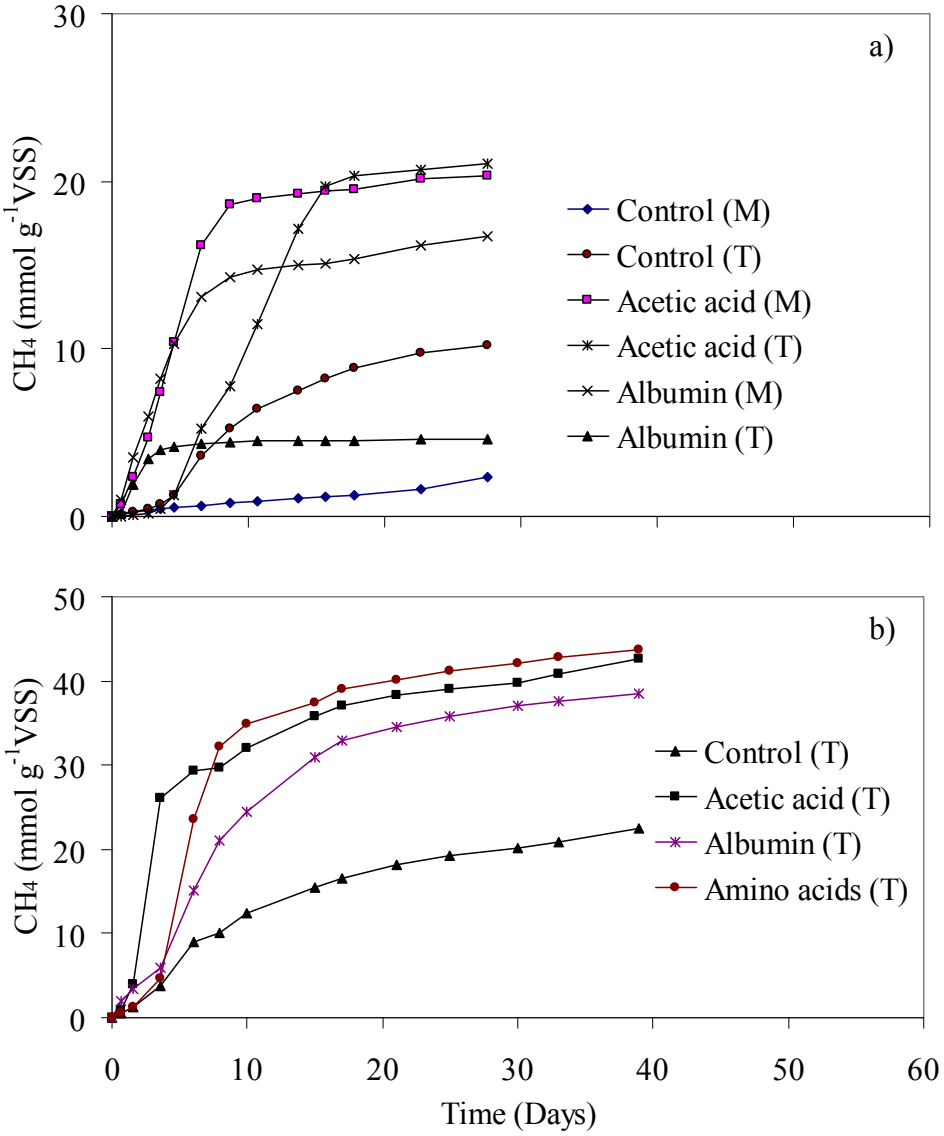


Figure 5.2. Mesophilic and thermophilic MP test: a) acetic acid at 0.15 M and albumin at 9 g COD l^{-1}, incubated with the mesophilic sludge under mesophilic (35 °C) and thermophilic (55 °C) temperatures; b) acetic acid at 0.06 M and albumin at 4.5 g COD l^{-1} and amino acids at 4.5 g COD l^{-1} incubated with the thermophilic sludge under thermophilic (55 °C) temperatures.

5.3.3 Mesophilic and thermophilic MP tests for olive oil, glycerol, and oleic acid

The specific production of methane from olive oil, glycerol and oleic acid was conducted under mesophilic and thermophilic temperatures using the mesophilic sludge (Figures 5.3a and 5.3b).

For mesophilic temperatures (Figure 5.3a), olive oil was degraded into methane almost without a lag phase. Although its initial methane production rate was slightly lower than that for acetic acid, its cumulative methane production reached that of acetic acid after 22 days. Glycerol demonstrated an initial methane production rate equivalent to that of olive oil, but reached a plateau on day 8, with only a small additional amount produced until day 27. Oleic acid was not degraded by the sludge because its activity was only slightly higher than that of the substrate-less control, and by day 16, its cumulative methane production was equal to that of the control.

Under thermophilic temperatures using the mesophilic sludge (Figure 5.3b), glycerol initially produced methane at the same rate as under mesophilic temperatures, but after 5 days, showed a much higher rate surpassing that of acetic acid. Olive oil demonstrated only a limited initial methane production rate which stopped after 5 days and a methane production of 3 mmol CH_4 g^{-1} VSS. Oleic acid produced an insignificant amount of methane as compared to the substrate-less control which suffered a 3 day lag phase but produced 10 mmol CH_4 g^{-1} VSS. The results obtained with olive oil and oleic acid showed that the acetoclastic methanogens of the mesophilic sludge suffered an inhibitory effect under thermophilic temperatures.

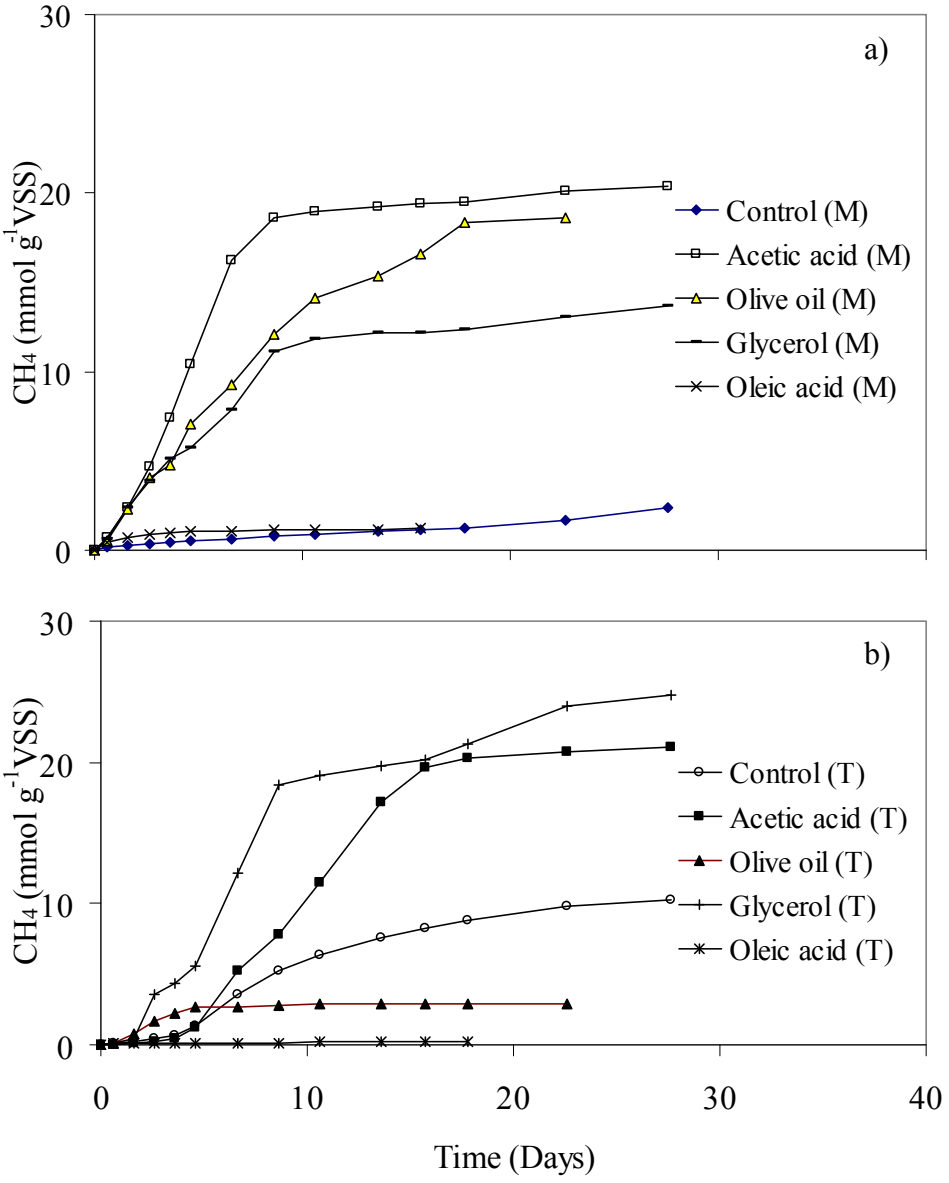


Figure 5.3 a) Mesophilic (M); b) thermophilic (T) MP test for acetic acid, olive oil, glycerol and oleic acid (all at 9 $gCOD_{Substrate}$ l^{-1}) conducted with mesophilic inoculum.

Under mesophilic temperatures and using the mesophilic sludge, (Table 5.3), glycerol and olive oil showed almost the same initial MP methane activity (1.3 ±0.06 mmol CH_4 g^{-1} VSS d^{-1} and 1.2 ±0.02 mmol CH_4 g^{-1} VSS d^{-1}, respectively) suggesting that oil hydrolysis was not the limiting factor. Although both compounds showed a similar methanization rate, only 53% of the olive oil (including its hydrolysis products, glycerol, and oleic acids among other long chain volatile fatty acids) was degraded as compared to 65% of the glycerol. This suggests that the degradation of olive oil released long chain volatile acids such as oleic acid which, as sole substrate, showed a low initial mesophilic methanization rate (0.3 ±0.02 mmolCH_4 g^{-1} VSS d^{-1}). Accordingly, the β-oxidation of oleic acid and other fatty acid was slow as compared to the hydrolysis of olive oil into glycerol and the fermentation of glycerol into acetic acid. Oleic acid accounts for 85% of the triglyceride mass of olive oil, and was degraded at a much lower rate. Under thermophilic temperatures, 42% of the glycerol was degraded, which was slightly better (1.6 ±0.02 mmolCH_4 g^{-1} VSS d^{-1}) than under mesophilic temperatures. Therefore, both olive oil and oleic acid had a strong inhibition effect on the acetoclastic methanogens of the mesophilic sludge exposed to thermophilic temperatures, since initial rates and biodegradation percentage decreased significantly ($P < 0.05$), even showing negative values. When inoculated with mesophilic sludge, olive oil degradation was not controlled by hydrolysis, but by the release of long chain volatile fatty acids (oleic acid included). It is not clear whether the limiting step was related to the process of β-oxidation or to the observed inhibitory effect.

Table 5.3. MP activity and biodegradation for olive oil, glycerol, and oleic acid, under mesophilic and thermophilic temperatures, using the mesophilic sludge.

	Substrate (9 gCOD l^{-1})		
Parameter	**Olive oil**	**Glycerol**	**Oleic acid**
Initial rate (mmolCH_4 g^{-1} VSS d^{-1})^M	1.2 ±0.2	1.3 ±0.06	0.3 ±0.02
Initial rate (mmolCH_4 g^{-1} VSS d^{-1})^T	0.4 ±0.3	1.6 ±0.02	-8.2 ±0.04
Biodegradation (%)^M	53	65	-6
Biodegradation (%)^T	-28	42	-41

Notes: *M*: Mesophilic; *T*: Thermophilic.

$$\% = \frac{(gCOD \cdot CH_4 Substrate - gCOD \cdot CH_4 Control) * 100}{(gCODSubstrate_{Added})}$$

Olive oil and oleic acid degradations were further tested with the 7 month acclimated thermophilic sludge under thermophilic temperatures (Figure 5.4a). Glycerol was not used since it had proved to be non-limiting in the degradation of olive oil. Olive oil showed a very low MP value (-0.04 ±0.1 mmolCH_4 g^{-1} VSS day^{-1}). Since substrate limitation was earlier not related to the solubility of the oil nor to hydrolysis, the low degradability of olive oil was related to the release of long chain volatile fatty acids, mostly oleic acid. Oleic acid as a substrate had an inhibitory effect on thermophilic methanogenic microorganisms especially at the beginning of the test. Such inhibitory effect slowly disappeared because the sludge improved its activity after 33 days and produced -1.1 ±0.3 mmol CH_4 g^{-1} VSS day^{-1}. The acetic acid profiles obtained with olive oil (Figure 5.4b) indicated that the production of acetic acid never exceeded that of the

control, and that olive oil was practically not degraded although no inhibitory effect could be assumed *per se*.

The gradual drop in inhibition effect is related to the degradation of the long chain volatile acids, especially oleic acid. This was confirmed by the acetic acid profile observed in the fourth MP bottle using oleic acid as substrate (results not shown). Although oleic acid was completely transformed into acetic acid (30 mmol acetic acid g^{-1} VSS), the degradation process took 49 days. Since the monitoring was stopped after 50 days, it is anticipated that this acetic acid would eventually be transformed into methane. Thus, the limiting step in the degradation of olive oil (vegetable oil) is not related to the hydrolysis of the triglyceride molecule, but to the inhibitory effect of long chain volatile fatty acids on acetoclastic methanogens in the first place. It also takes some time for the syntrophic microorganisms to multiply and to develop the special metabolic pathway degrading oleic acid, and for that matter any unsaturated volatile fatty acid. Accordingly, the β–oxidation process requires three additional enzymes, *cis-Δ^3 Enoyl-CoA isomerase*, *Enoyl-CoA hydratase*, and *S-3-hyroxyacyl-CoA epimerase* respectively promoting: first, the transformation of a cis double bond into a trans-δ^2 bond; then, stereo-specifically promoting the hydration of the trans-δ^2 bond, and; finally producing S3-hydroxyacyl CoA which is the regular substrate for the *S-3-hyroxyacyl CoA deshydrogenase* (a β-oxidation regular enzyme) (Rawn, 1990). Nevertheless, more research is needed to evaluate the nature of those acids since oleic acid only accounts for 85% of the mass of triglycerides contained in olive oil and such a proportion could vary depending of the source of the oil.

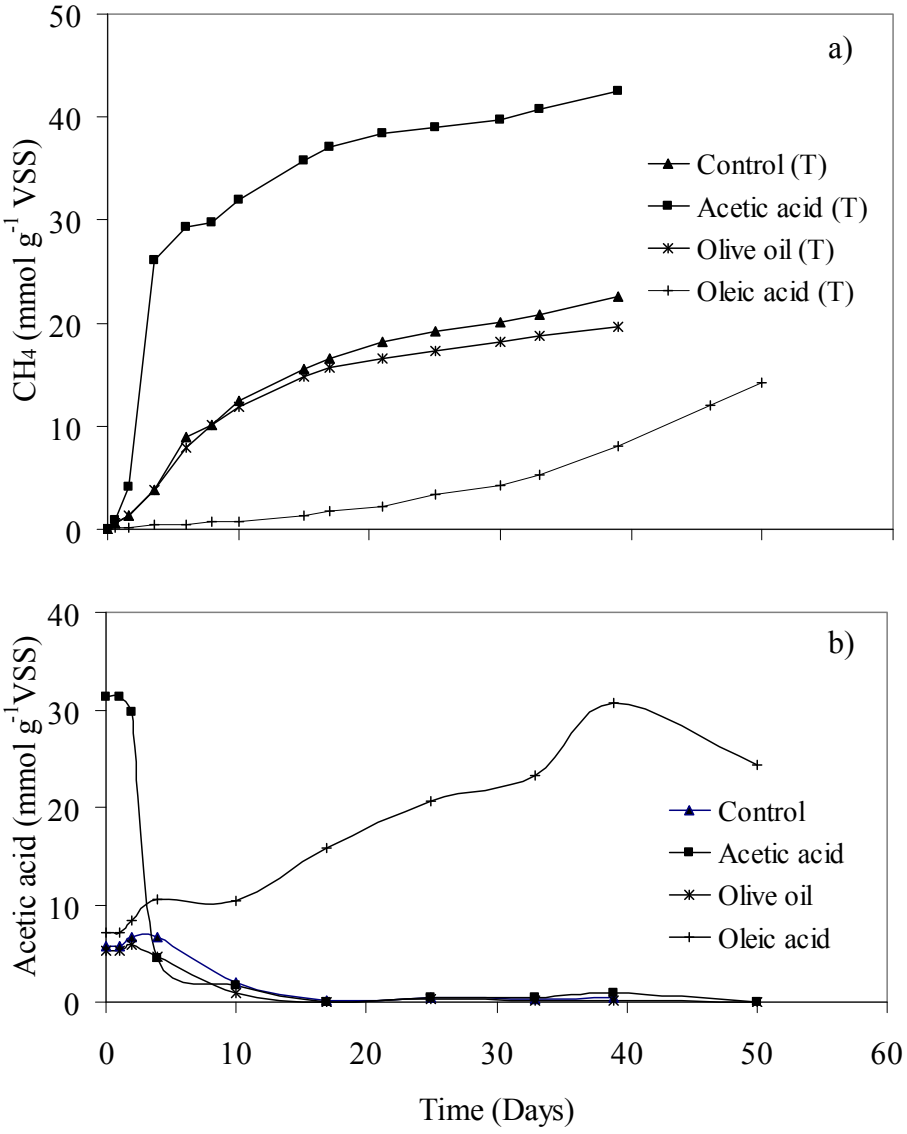


Figure 5.4. MP test using the thermophilic sludge exposed to thermophilic temperatures and acetic acid, olive oil, and oleic acid (4.5 g COD l-1): a) methane production rate; b) acetic acid profile.

5.4 CONCLUSIONS

The developed MP methodology proved to be useful in determining the relative presence of the different trophic groups involved in the process of anaerobic digestion. As well, it was useful in determining the limiting step in the degradation of compounds such as albumin and olive oil.

For MP tests using a mesophilic sludge and temperature, both albumin and olive oil were partially transformed into methane, although the degradation of albumin was faster during the initial phase. The limiting step for the degradation of albumin was related to the hydrolysis of the protein into peptides, since the mixture of amino acids showed a quick and efficient transformation into acetic acid first and then into methane.

The MP tests also demonstrated that olive oil hydrolysis is not the limiting factor in its degradation, which rather comes from the inhibitory effect of long chain volatile acids released from the hydrolysis of triglycerides. Oleic acid proved to have a slowly disappearing inhibitory effect over acetoclastic methanogens, under thermophilic conditions.

CONNECTING STATEMENT (Fourth scientific article)

Chapter 6 deals with the description of the thermal transition of a food waste treating anaerobic thermophilic sludge from 55 to 70 °C. Reactor performance as well as methane and substrate activities of the sludge were evaluated throughout the upgrading procedure. This scientific paper will be submitted for publication in a peer reviewed journal. The election of the journal is currently under evaluation.

Authors and affiliation:

Luis Ortega[1,2] (i); Suzelle Barrington[1] (ii); Serge Guiot[2] (iii)

(1) McGill University, Bioresource Eng. Department. 21111 Lakeshore Road, Ste-Anne-de-Bellevue (Quebec), H9X 3V9 Canada. E-mail: luis.ortega@nrc.ca

(2) National Research Council of Canada, Biotechnology Research Institute. 6100 Royalmount Av. Montreal (Quebec), H4P 2R2 Canada.

The candidate (i) carried out the experiments along with data analyses, statistical analysis, and the writing of the manuscript. Author (ii) further enhanced the analysis of data, edited the manuscript and contributed to the content of the article. Author (iii) lead the research project, supervised the laboratory and analytical work carried out by the candidate, further enhanced the analysis of data, edited the manuscript and contributed to the content of the article.

CHAPTER SIX

ANAEROBIC TREATMENT OF FOOD WASTE: IMPACT OF A STEP-WISE TEMPERATURE INCREASE FROM THERMOPHILIC TO HYPERTHERMOPHILIC CONDITIONS

6.0 ABSTRACT

If thermophilic anaerobic digestion can improve biogas yield as compared to mesophilic anaerobic conditions, then hyperthermophilic digestion may improve gas yield even more so, as long as the transitional feeding regimes acclimatize the microbial populations. The present objective was to gradually expose a thermophilic anaerobic biomass to hyperthermophilic conditions and monitor microbial activity during and after the thermal transition. Two bench-scale food-waste treating tank reactors (R1 and R2) operating under thermophilic anaerobic conditions were upgraded from 55 to 70°C at a rate of 2.5°C/week, while a third one remained under thermophilic conditions as control (C). Conducting a first run of 85 days (T1) using R2, the average organic loading rate (OLR) and the hydraulic retention time (HRT) were 2g VS/L/day and 35 days, respectively and the pH was not controlled. During T1, the pH dropped to 5.9 and the accumulation volatile fatty acids (VFA) lead to the digester breakdown. A second 85 day run (T2) was then conducted using R1 where the pH was artificially controlled at 8.0 and the OLR was stepwise reduced from 3 to 0.3g VS/L/d along with TU and maintained at this low level for 44 days of operation at 70 °C. The TU negatively affected the performance of both R2 and R1 since methane production dropped from 0.8 to 0.2 $L_{STP}CH_4$/L/day during T1 and from 1.0 to 0.04 $L_{STP}CH_4$/L/day during T2. Methanogens produced and sustained a low

methane production for 14 days after TU (average 0.09 and 0.07 $L_{STP}CH_4$/L/day, for T1 and T2, respectively). In both cases, the hyperthermophilic production of methane was related to hydrogenophilic methanogen activity since production of methane from acetic acid ceased when R2 and R1 reached 62.5 and 65 °C, respectively. After 14 days of hyperthermophilic operation in R1, H_2 replaced CH_4 production and a low volumetric production rate (0.011 $L_{STP}H_2$/L/d) was maintained during the last 45 days of T2. Further research is needed to improve either H_2 production and/or hydrolysis of macromolecules at hyperthermophilic conditions.

Keywords: Anaerobic digestion, food waste, thermophilic conditions, hyperthermophilic adaptation, hydrogen production.

6.1 INTRODUCTION

The organic fraction of municipal solid wastes (OFMSW) is of low biodegradability because of its rich content (35 to 50 %) in fibrous materials such as paper, wood and yard waste. With a high cellulose and ligno-cellulose content, this low biodegradability directly impacts the profitability of industrial anaerobic facilities. Pretreatment practices such as mechanical reduction and exposure to high temperatures or pressures, can improve the biodegradability of such wastes as well as those rich in lipids and proteins (Schieder *et al*., 2000; Liu *et al*., 2002).

Anaerobic digestion of OFMSW under hyperthermophilic conditions (60 to 80°C) could represent an alternative to better degrade organic matter especially complex macromolecules such as cellulose and lingo-cellulose among others. While studying anaerobic communities from lake sediment at 2 and 70 °C, Nozevnikova *et al*. (1997)

found that hyperthermophilic temperature (70°C) for 25 days allowed for a methane yield, from a substrate mixture of H_2/CO_2 (1/4), corresponding almost to that (17 mmolCH_4/L) obtained during a 55 day incubation at 30 °C; the culture was similar to *Methanobacterium thermoautotrophicum*.

Using a seed of thermophilically (55°C) pre-incubated digested sludge, Nozhevnikova *et al*. (1999) observed that the digestion of cattle and pig manure produced a biogas with 4 and 48% methane, respectively. When increasing the digester temperature to 73°C (hyperthermophilic conditions), the digestion of cattle manure increased the proportion of methane by up to 6% while in the case of pig manure, the proportion of methane decreased down to 3.2%. Nevertheless, digestion of the same seeds at 82°C did not produced any biogas due to the displacement of the methanogen activity resulting from the proliferation of acidogenic communities and the accumulation of 31 to 39 mM of volatile fatty acids such as acetate, propionate, and butyrate.

Ahring *et al.*, (2001) investigated the effect on microbial populations of increasing the temperature of a cattle-manure treating reactor from 55 to 65°C. The reactor still produced methane, but at a lower rate, and volatile fatty acids accumulated while hydrolysis was not improved. Extreme thermophiles, mainly *Archaea* communities, were observed to conduct the hydrolysis since their ribosomal ribonucleic acid increased significantly above 65°C.

Hartmann and Ahring (2003) investigated the degradation of xenobiotic compounds, namely phthalic acid ester di-(2-ethylhexyl)-phthalate (DEHP) and dibutylphthalate (DBP) contained in OFMSW under thermophilic (55°C) and hyperthermophilic (68°C) conditions. At 55°C and using a hydraulic retention time

(HRT) of 15 days, 38 to 70% of DBP was removed, but no consistent removal of DEHP was observed. However, after treating the thermophilic effluent at 68°C with an HRT of 5 days, 34 to 53 % of DEHP was removed, while DBP removal was further increased to 62-74 %. Removal rates for DEHP and DBP of 0.11-0.32 d^{-1} and 0.41-0.79 d^{-1} were higher than previously observed with lower anaerobic temperatures. This was attributed to a higher level of substrate degradation, resulting in a higher concentration and therefore higher bioavailability of DEHP and DBP. The sequential thermophilic and hyperthermophilic reactor configuration improved the degradation of organic matter and biogas yields, while also reducing levels of phthalic acid esters.

The experiments described above suggest that between 60 and 73°C, both fermentation and methanogenesis occurred under hyperthermophilic conditions. Although temperature upgrading (TU) is likely an important facet of developing hyperthermophilic conditions, this process has received limited attention.

The objective of this research was to evaluate the impact of a step wise TU from 55°C-thermophilic to 70°C-hyperthermophilic conditions. The performance of the hyperthermophilic upgraded reactor was compared to that maintained under thermophilic conditions. Both anaerobic reactors were continuously stirred tanks (CSTR) treating food waste. The accumulation of VFA, especially in the form of ionized and un-ionized acetic acid (acetate and acetic acid), was closely monitored while specific substrate activity and methanogenic activity were also evaluated.

6.2 METHODOLOGY

6.2.1. REACTOR SET-UP AND SUBSTRATE

Three 5-L CSTRs were similarly equipped (R1, R2 and C). The systems included a 4 L working volume glass vessel, a 6-600 peristaltic pump (*Cole-Parmer,* Chicago, IL) and a *Type-RZR50* stirrer (*Caframo,* Wiarton, ON). Daily, a timer (*ChonTrol* timer model *CD, Lindburg Enterprises*, San Diego, CA) activated the pump which fed the substrate to all three reactors. Using a heating system surrounding the digester, the temperature was automatically controlled by a 305mm -*LT-type* temperature probe (*Cole-Parmer,* Vernon Hill, IL), a *Digi-Sense* standard model temperature controller (*Cole-Parmer,* Vernon Hill, IL) and a *Brisk Heat* silicone extruded, flexible electric heating tape (*Thermolyne*, Dubuque, IO). After recovering the condensate, the biogas generated was sampled with a *1750-Gastight Hamilton* syringe (*Hamilton Co*., Reno, Nevada) through the biogas sampling port and measured by means of a Wet Tip-like water displacement system (Figure 6.1).

The substrate was a source sorted food waste collected from the solid waste produced by a cafeteria and it contained 218.2 g l^{-1} of totals solids (TS), 95 % volatile solids (VS on a dry basis), 1.16 g COD g^{-1}VSS and 17 % nitrogen (on a dry basis) measured as total Kjeldahl nitrogen (Ortega *et al.*, 2007 –Chapter 3-). All reactors were seeded with a sludge that was adapted to thermophilic conditions (Ortega *et al.*, 2007b – Chapter 5-) and were operated under thermophilic conditions (55°C) for seven months of acclimatization before initiating the present study. During the seven month acclimatization stage, the average temperature of the reactors was 54.7°C, the average pH value was 7.8, and the OLR supplied was 1.3 gVS L^{-1} d^{-1} (data not shown).

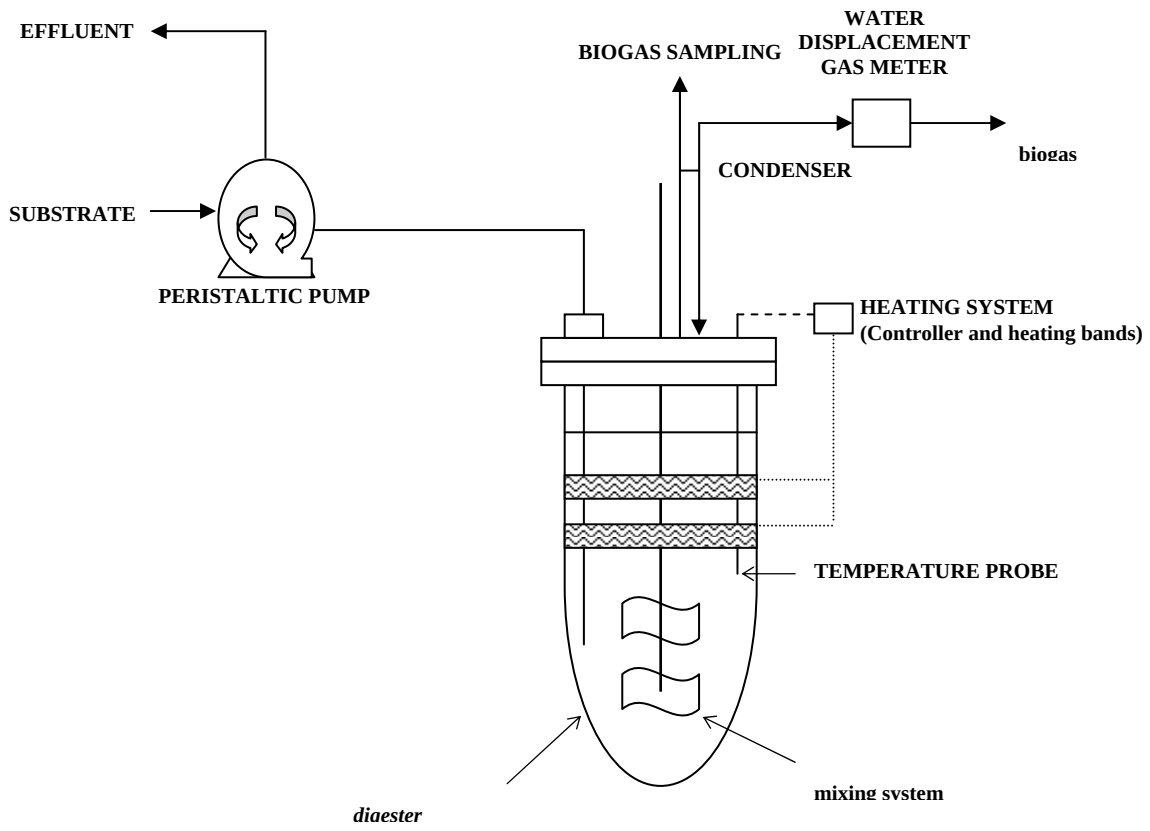


Figure 6.1. Schematic diagram of reactors R1, R2, and Control.

6.2.2. EXPERIMENTAL STAGES

The present experiment involved slowly upgrading the temperature of two reactors, one at a time, while keeping the third one as control, under thermophilic temperatures. Each experimental reactor was upgraded to hyperthermophilic conditions (70°C) over 42 days using a TU of 2.5 °C/week.

The first run, T1, involved upgrading reactor R2 which failed after 43 d of operation at 70°C, because of VFA accumulation causing the pH to drop from 7.0 to 6.1. During T1, all three reactors (R1, R2 and C) were fed daily, and operated at an OLR varying between 1.7 and 3.0 g VS/L/day and a HRT of 35 days.

Run T2 was then initiated with reactor R1, while artificially maintaining its pH at 8 using a saturated bicarbonate solution of Na and K (pH of 10). To separately evaluate the effect of temperature, pH and the accumulation of VFA (specifically the effect of acetic acid), the OLR was stepwise reduced from 3.0 to 0.3g VS/L/day, along with a TU of 2.5°C/week. After upgrading the temperature of R1 as carried out for R2, both reactors (R2 and C) were fed daily, using a hydraulic retention time of 44 days.

During both experiments and five times weekly, the reactors were monitored for temperature, pH, VFA, volumetric gas production and volumetric H_2 and CH_4 content. As well, the reactor biomass (R1 or R2 and C) was analyzed for specific substrate activity (SA) and specific methanogenic activity (MA) before and after T1 and T2. These SA and MA tests were carried out at both 55 and 70 °C, to evaluate the presence of thermophilic and hyperthermophilic organisms.

6.2.3 THE SUBSTRATE AND METHANOGENIC ACTIVITY TESTS

The SA tests measures the proportion of the trophic biomass involved in the anaerobic digestion process, in terms of mmol of substrate consumed daily *per* gVSS of sludge (mmol/ gVSS/d). The tests were carried out according to Guiot *et al*. (1995), in triplicate 60 mL serum bottles inoculated with biomass diluted with a phosphate buffer to 2 000 mg VSS/L for the hydrogenophilic activity test, and to 5 000 mg VSS/L for the glucose, acetic, butyric and propionic acids activity test. The bottles were flushed with N_2/CO_2 (80 % / 20 %) to remove all oxygen and incubated in a rotary shaker (*New Brunswick Scientific Co*., Edison, NJ). While being incubated at either 55 or 70°C, triplicate serum bottles and their content were shaken at 100 rpm for the soluble substrate tests and at 400

rpm for the bottles containing H_2 as substrate. Acetic acid and H_2 consumption indicated the activity of the acetoclastic and hydrogenophilic methanogenesis, while propionic and butyric acids consumption measured the syntrophic activity and glucose measured the fermentation activity.

The MA tests measured methane production from acetic acid, which is the ultimate limiting step in anaerobic digestion and assumes the presence of trophic groups producing acetate as last substrate before methane production. The methodology for this test was based on the recommendation and practices described previously and used for the biochemical methane potential test (Owen *et al*., 1979; Shelton and Tiedje, 1984; and Cornacchio *et al*., 1986). The results of this test are expressed in terms of mmol of methane produced / g VSS of sludge /d, where sludge is the viable biomass. The tests were conducted in 120 mL serum bottles where acetic acid, albumin, a mixture of alanine and glycine (two amino acids), glycerol, olive oil and oleic acid were used as substrates (Ortega *et al*., 2007b –Chapter 5-). For each MA tests, triplicate bottles were inoculated with biomass diluted with a phosphate buffer to obtain from 10 to 15 g VSS/L. The bottles were supplied with 4.5 g COD/L of each substrate and then flushed with N_2/CO_2 (80 % / 20 %) to remove all oxygen. Additional control bottles were tested without substrate to monitor the methanogenic activity related to endogenous respiration and to the biodegradable organic matter remaining in the sludge after sampling. While being incubated at either 55 or 70°C, the serum bottles and their content were shaken at 100 rpm.

The volume of biogas produced by each bottle was periodically measured by water displacement using an inverted burette filled with water and attached to the bottle.

Once the pressure in the bottle was equilibrated with that of the column of water in the burette, the bottle was removed from the burette and the gas was sampled and analyzed. The specific methanogenic activity was calculated by measuring the rate of methane production; the maximum slope of the curve expressed the rate of accumulation of methane over time, and this rate was calculated in terms of VSS supplied. The final activity results were obtained by subtracting the activity of the control bottles from that of the bottle with substrate.

6.2.4 ANALYTICAL METHODS

To calculate the OLR supplied (gVS/L/day), the substrate was analyzed for volatile solids (VS). For the SA and MA tests, volatile suspended solids (VSS) and chemical oxygen demand (COD) were quantified using standard methods (*APHA et al.*, 1995). Volatile solids were measured by drying at 103 °C and then incinerating at 550°C. Chemical oxygen demand (COD) was determined colorimetrically after reacting with potassium perchromate at 150 °C for 2.0 h.

Acetic, propionic, and butyric acids were measured using an *Agilent* 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector of 0.2 microL. The samples were enriched to a ratio of 1:1 (V/V) using an internal standard of iso-butyric acid dissolved in 6% formic acid and directly injected into a glass column of 1 m x 2 mm *Carbopack C* (60 to 80 mesh) coated with 0.3% *Carbowax* 20M and 0.1 % H_3PO_4. The column was held at 130°C for 4 minutes and helium as carrier gas was injected at a rate of 20 mL/minute. The injector and the detector were both maintained at 200 °C.

Since the measurement of VFA was conducted at a pH of 1 (due to the formic and iso-butyric acids added), the concentration of un-ionized acetic acid at the pH of the reactors was estimated based on the Henderson-Hasselbach dissociation equation (Guiot, 1991). The pH of the reactors was monitored off-line by means of an epoxy-pH probe (*Cole-Parmer,* Niles, IL) and an *Accumet* pH-meter model 825 MP (*Fisher*, USA). The biogas composition (hydrogen, nitrogen, oxygen, methane, and carbon dioxide) was measured by means of an HP gas chromatograph (68900 Series, *Hewlett Packard,* Wilmington, DE) equipped with a 900mm x 3 mm (36" x 1/8") 60/80 mesh.

Glucose was measured using an HPLC chromatograph (*Waters Chromatography Division*, Milford MA, USA) equipped with a model 717+ injector, a photodiode array (PDA) detector (model 2996), a model 600 pump and a 2414 refractive index detector. The separation unit consisted of an ICsep ICE-ION-300 column (*Transgenomic*, San Jose, CA) of 300 mm x 7.8 mm inside diameter, and an ion guard GC-801 column *Transgenomics,* San Jose, CA). The mobile phase consisted of 0.035N of sulfuric acid at a pH of 4, flowing at a rate of 0.4 mL/min. The measurements were conducted using a wavelength of 210 nm.

The biogas composition (hydrogen and methane) was measured using an HP gas chromatograph (68900 Series, *Hewlett Packard,* Wilmington, DE) equipped with a 900mm x 3 mm (36" x 1/8") 60/80 mesh Chromosorb 102 column (*Supelco*, Bellefonte, PA). All daily gas production rates were calculated based on standard temperature and atmospheric pressure (L_{-STP} /L/d).

6.2.5 STATISTICAL ANALYSIS

Statistical significance for the thermophilic and hyperthermophilic biomass activity (SA tests) compared to the control reactor C, at the end of the experiments (day 427), was determined using a t-test with paired data (Wardlaw, 1985). Significance was evaluated using a 95% confidence level using:

$$t = \frac{h(N)^{1/2}}{Sh}$$

where:

t = Value of student *t*-found

N = the test number of pairs

h = mean of differences of SA-values of each bottle-pair (mesophilic and thermophilic)

Sh = standard deviation of h

The *t-found* value was compared to that of a Student *t-Statistic* table for a degree of freedom of 2 (2 d.f.).

6.3 RESULTS AND DISCUSSION

6.3.1 FIRST TEMPERATURE UPGRADING EXPERIMENT (T1)

The average operating conditions for reactors R1, R2 and C, 48 days before initiating T1, are illustrated in Table 6.1. All reactors were operating under similar conditions, except for the slightly lower average operating temperature of reactor R2 (54.0 ±4.4°C versus 55.0 ±0.2°C and 54.9 ±0.1°C) resulting from two previous power outages. Because of their similar methane production and their stable condition, all three reactors were considered replicates.

Table 6.1. Average operating conditions and performance of the experimental reactors, 48 days before starting to upgrade the temperature (T1).

Parameter	R1	R2	Control
Temperature (°C)	55 ±0.2	54 ±4.4	54.9 ±0.1
HRT (days)	57.26 ±6.8	57.3 ±6.6	57.13 ±6.7
OLR (gVS/L/day)	1.37 ±0.17	1.37 ±0.17	1.37 ±0.16
PH	7.7 ±0.2	7.8 ±0.25	7.9 ±0.24
Vol. gas prod. (L_{STP}/L/day)	0.7 ±0.25	0.7 ±0.3	0.8 ±0.3
Vol. CH_4 prod. ($L_{STP}CH_4$/L/day)	0.4 ±0.14	0.37 ±0.17	0.43 ±0.16
Go (L_{STP}/gVS_{fed})	0.54 ±0.21	0.50 ±0.22	0.59 ±0.22
Bo ($L_{STP}CH_4/gVS_{fed}$)	0.31 ±0.13	0.29 ±0.13	0.32 ±0.13
Acetic acid (mg/L)	1293 ±701	1702 ±1066	1417 ±1098
Propionic acid (mg/L)	2416 ±204	2420 ±178	2315 ±215
Butyric acid (mg/L)	66 ±132	0	49 ±99

Notes: Go: Ultimate gas production; Bo: Ultimate methane production.

Experimental run T1 was initiated using R2 on its 208th day of operation. Figure 6.2 shows the temperature and OLR profiles for reactor R2 during its TU run T1, where it took 35 days (from day 208 to 243) to reach 70 °C at a rate of 2.5 °C/week. Reactor R1 and C were kept under thermophilic conditions during T1, and received, as for R2, the same OLR of 1.7 gVS/L/day. Due to variations in the solids content between different lots of substrate, OLR was increased to 3.0 gVS/L/day on day 240 and kept at 2.4 gVS/L/day thereafter. Because reactor R2 suffered an accumulation of VFA and a

reduction in methane production, its OLR was dropped to 1.2 gVS/L/day on day 258 and, after day 261, R2 kept working without feed supply until the end of the 85 day experiment.

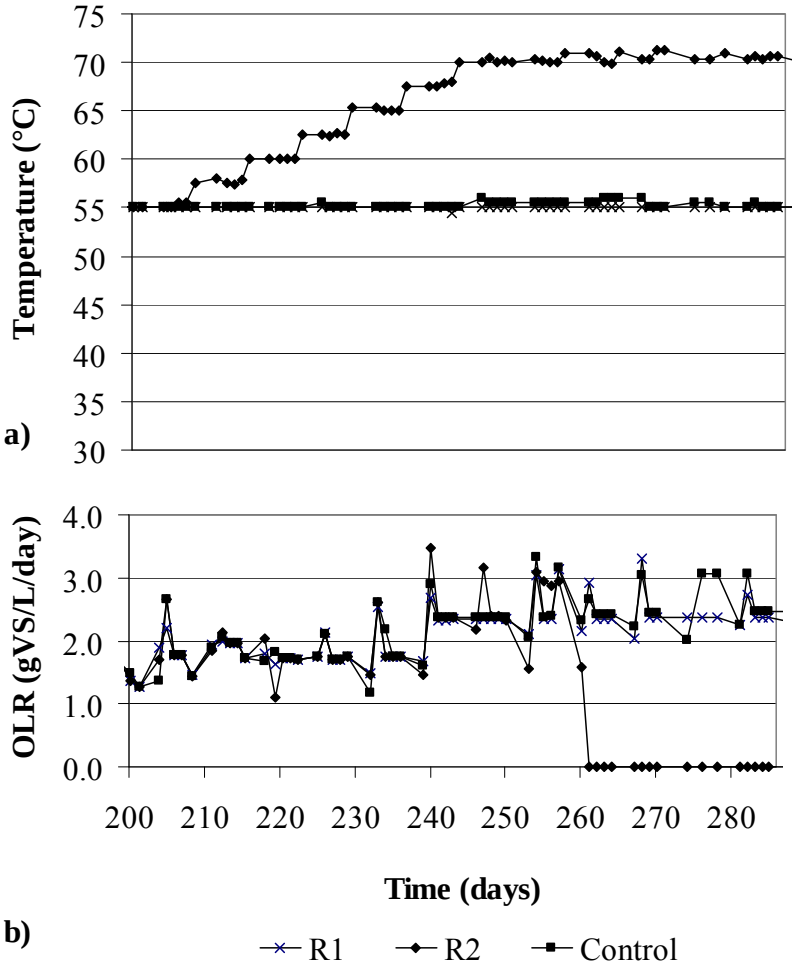


Figure 6.2. Operating conditions for reactors R1, R2 and C, during the temperature upgrade T1.

Figure 6.3 shows the pH, acetate and acetic acid levels of reactors R1, R2 and C, during T1. The pH of reactors R1 and C remained constant at 8.0, while that of R2 dropped to 7.5 on day 236 when reaching a temperature of 67.5 °C. This pH remained constant for

seven days, until reaching a temperature of 70 °C. At that time, the pH of R2 dropped steadily to reach 5.7 on day 260. On day 261, the pH of R2 was manually corrected to 7.5 and maintained at that level until the end of the experiment.

For all three reactors, the evolution of pH and total acetic acid content (ionized or acetate and un-ionized or acetic acid) is illustrated in Figures 6.3a and 6.3b. The drop in pH for R2 occurred in parallel with the accumulation of total acetic acid which started on day 232 when the reactor reached 65 °C. The concentration of total acetic acid rapidly peaked at 19 g/L on day 249, when reaching 70 °C. Since no substrate was supplied as of day 261, acetic acid concentration dropped to a range of 12 to 15 g/L and remained within this range till the end of the experiment.

The dynamic equilibrium between acetate and un-ionized acetic acid, computed using the Henderson-Hasselbach dissociation equation (Figure 6.3c) indicated a shift towards acetic acid (from inhibiting methanogens) when the pH dropped to 7 on day 246. The concentration of un-ionized acetic acid peaked at 1 288 mg/L, at the end of the second week of hyperthermophilic conditions, when the pH dropped to its lowest value of 5.7. After neutralizing the medium of reactor R2, the concentration of un-ionized acetic acid dropped to 50 mg/L and remained constant until the end of the experiment.

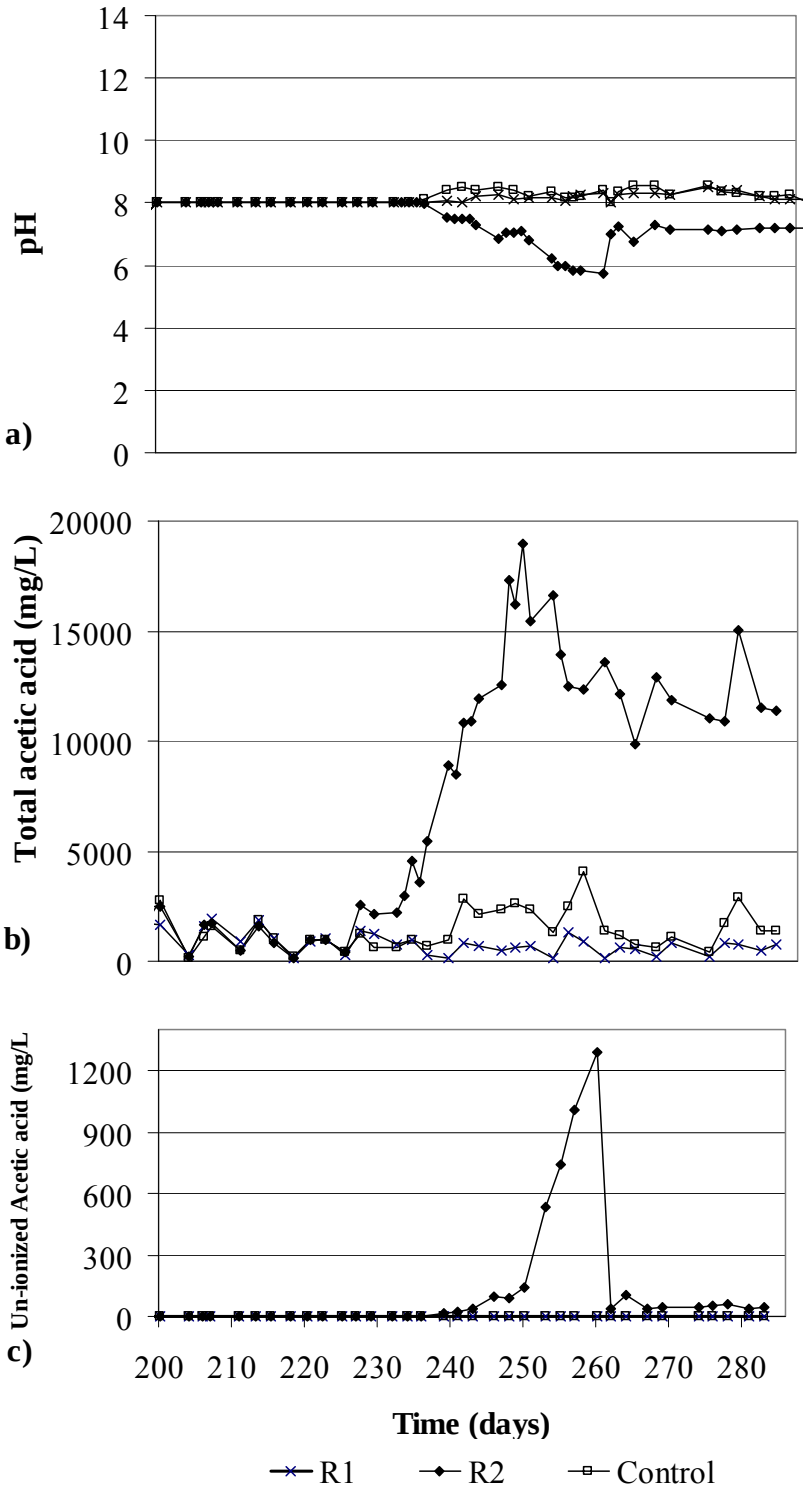


Figure 6.3. Performance profiles for reactors R1, R2 and C, during the temperature upgrade T1.

Figure 6.4 illustrates the volumetric methane production profiles of all reactors and the volumetric production of H_2 appearing solely in reactor R2, during T1. Except for an increase in methane production from day 225 to 228 (1.03 to 1.43 $L_{STP}CH_4$/L/day) for reactor C, all reactors demonstrated a similar gas production profile until day 232, indicating similar response to OLR. Reactors R1 and C kept similar volumetric gas production profiles until the end of the experiment, but R2 showed a significant drop in methane production on day 236 when reaching a temperature of 65 °C and starting to accumulate acetic acid. The production of methane by reactor R2 almost stopped on day 254, while H_2 was produced at a rate of 0.05 and 0.8 $L_{STP}H_2$/L/day, on days 255 and 256, and days 263 and 264, respectively. Since reactor R2 was not fed as of day 261, both CH_4 and H_2 production ceased completely on day 265.

During T1, all three reactors were operated under similar conditions, except for temperature. Only reactor R2 suffering a TU changed its performance when reaching a temperature of 65 °C, at which time gas production dropped and acetic acid started to accumulate. Thus, acetoclastic methanogens were negatively affected by temperatures of 65 °C. Nevertheless and during T1, several factors seemed to cause the failure of reactor R2 which kept producing biogas (CH_4) even two weeks after reaching hyperthermophilic conditions of 70 ºC. Among the causes were extreme temperatures and 1 288 mg/L of un-ionized acetic acid which exceeded inhibitory values of 200 mg/L (Guiot 1991).

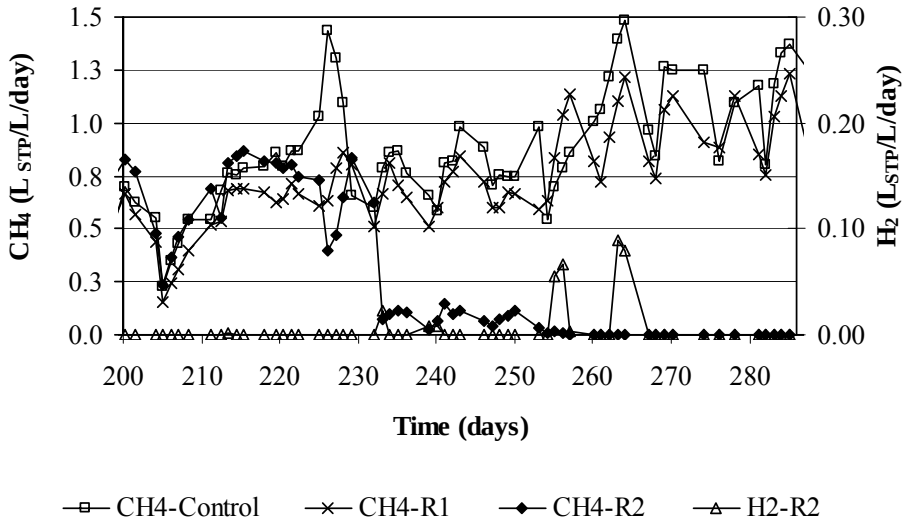


Figure 6.4. Volumetric gas production (methane and hydrogen) during the temperature upgrade T1.

6.3.2 SECOND TEMPERATURE UPGRADING EXPERIMENT (T2)

The TU experiment was repeated using reactor R1, while keeping C as control thermophilic reactor. Test period T2 lasted 87 days, from day 352 to day 439 and to avoid the inhibitory effect of acetic acid, the OLR was decreased in parallel with TU by reducing the volume of substrate fed daily. As well, the pH of reactor R1 was maintained at 8.0 using a saturated bicarbonate solution of Na and K.

Figure 6.5 shows the temperature, OLR, and pH profiles of reactor R1 and C during T2, where the TU of reactor R1 was carried out at a rate of 2.5 °C/week, spanning days 359 to 368, while reactor C was maintained under thermophilic conditions. The starting OLR for both reactors ranged from 2.0 to 3.0 gVS/L/day, but for R1, this rate was reduced to 1.0 gVS/L/day on day 373, and maintained at 0.3 gVS/L/day from day

394 to 411. On day 412, the feeding of reactor R1 was stopped for five days due to substrate supply problems, but was resumed from day 419 till the end of the experiment, at an OLR of 0.4 gVS/L/day. The OLR of reactor C averaged 2.0 gVS/L/day from days 384 to 427, due to variation in substrate solids, and was stopped thereafter till the end of the experiment. The pH profile of both reactors R1 remained unchanged at 7.2 to 8.3 during the full span of T2, because it was artificially controlled. Uncontrolled, reactor C exhibited a constant pH profile of 8.3 for the entire period of T2.

Acetate levels started to increase in reactor R1 as of day 377, when the temperature reached 62.5 ºC (Figure 6.6), and peaked at 9 g/L on day 397 at a temperature of 70 ºC. Since its pH was maintained at 8.0, the concentration of un-ionized acetic acid never exceeded 26.3 mg/L. As with T1, T2 indicated that acetoclastic methanogens were negatively affected by temperatures above 62.5 ºC. Reactor C experienced a concentration of acetate which fluctuated between 250 and 700 mg/L, during T2. Because the pH of reactor C remained slightly alkaline at 8.3, the concentration of un-ionized acetic acid never exceeded 1.0 mg/L.

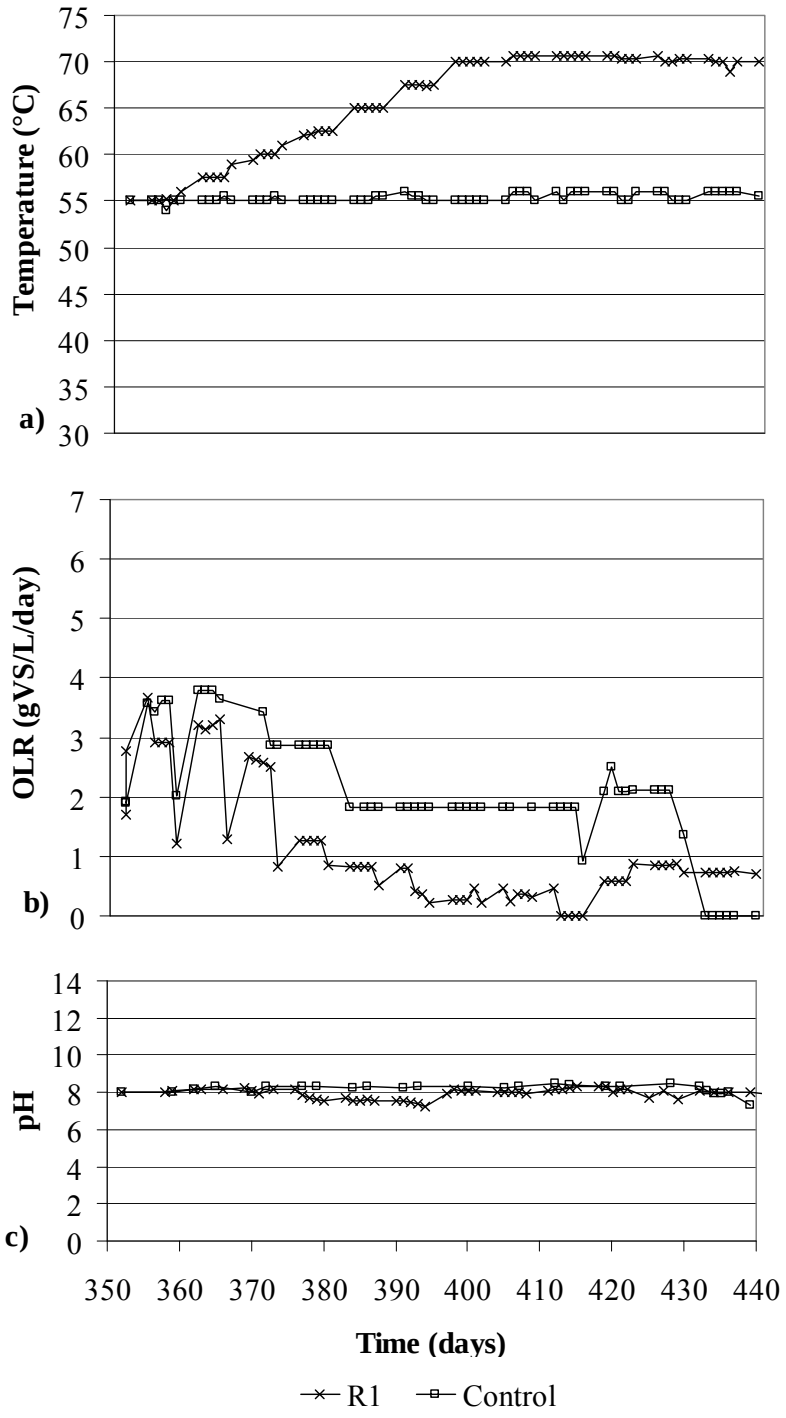


Figure 6.5. Operating conditions for reactors R1 and C, during the temperature upgrade T2.

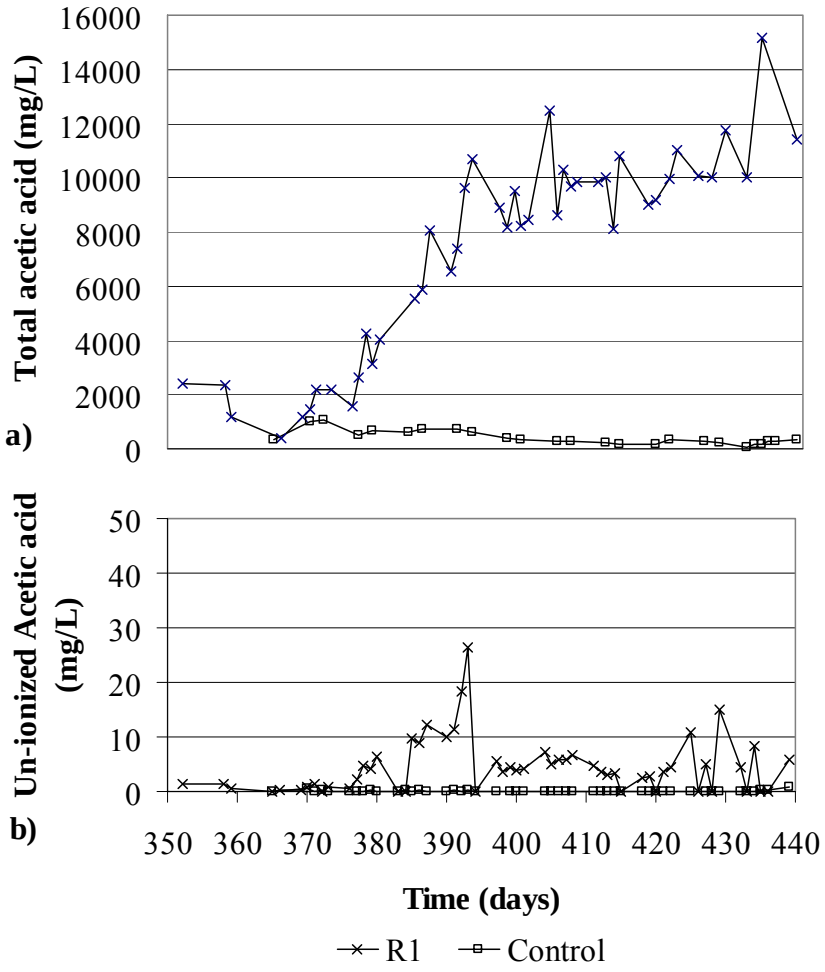


Figure 6.6. Performance profiles for reactors R2 and C, during the temperature upgrade T2.

Because of its low OLR, reactor R1 produced a limited volume of methane which dropped even further to 0.06 $L_{STP}CH_4$/L/day, when reaching a temperature of 62.5 ºC on day 376. Reactor R1 maintained this production until day 393 when its temperature was further increased to 67.5 °C. Acetate started to accumulate as of day 376, along with the drop in methane production. From day 397 to 408, reactor R1 had a volumetric methane

production of 0.005 $L_{STP}CH_4$/L/day which completely stopped on day 409, but in parallel was replaced by H_2 production peaking at 0.02 $L_{STP}H_2$/L/day on day 428. From day 429 till the end of the T2, 0.01 $L_{STP}H_2$/L/day was produced by reactor R1 (Figure 6.7).

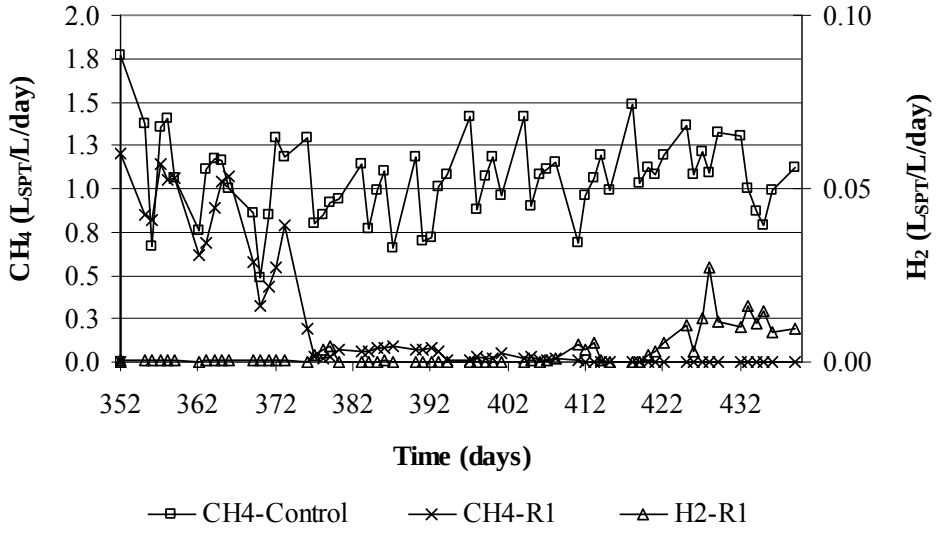


Figure 6.7. Volumetric gas production (methane and hydrogen) during the temperature upgrade T2.

In summary, reactor performance was severely affected by TU, especially when temperatures of 62.5 to 65 °C were reached. These temperatures were accompanied by an accumulation of acetate indicating that acetoclastic methanogens were not able to support such higher temperatures. As observed by Nozevnikova *et al*. (1997), this project failed to adapt hydrogenophilic methanogens to a temperature of 70 ºC because of their low methanogen activity sustained for a short period of time after 65 ºC. Nevertheless, H_2 producing fermentative bacteria managed to support the change in temperature and displaced hydrogenophilic methanogenesis towards a low H_2 production.

6.3.3 THERMOPHILIC AND HYPERTHERMOPHILIC ACTIVITY TESTS (MA AND SA)

Both specific methanogenic activity (MA) and specific substrate activity (SA) tests were conducted on sludge sampled from all reactors, before and after T1 and T2, both at 55 and 70 °C. The tests carried out at 55 °C before each TU measured the activity of the biomass under thermophilic conditions while the test conducted at 70 °C measured the potential activity of the sludge at the targeted temperature, the degree of thermophilic microbial survival and the acclimatization after reaching a temperature of 70 °C.

For T1 and its MA tests (Table 6.2), the sludge from reactor R2 on day 208 (before T1) demonstrated a low but definite thermophilic specific methanogenic activity similar to that of reactor C on day 274. The hyperthermophilic TU had a severe effect on the methanogenic biomass of reactor R2 since no activity was detected, except for a low thermophilic methanogenic activity with 4.5 g COD/L of albumin (0.037 mmol CH_4/gVSS/day).

Table 6.2. MA test results for the sludge before and after the temperature upgrade T1.

	MA (mmol CH_4 / gVSS / day)			
	$R2^t_{55}$	$R2^h_{55}$	$R2^h_{70}$	$Control^t_{55}$
Substrate	(Day 208)	(Day 243)	(Day 243)	(Day 274)
Acetic acid	1.48 ±0.37	0	0	2.19 ±0.26
Albumin	1.11 ±0.19	0.037 ±0.037	0	1.19 ±0.37
Alanine/Glycine	1.48 ±0.37	0	0	3.30 ±0.85
Glycerol	1.86 ±0.37	0	0	0.89 ±0.93
Olive oil	0.74 ±0.26	0	0	0.45 ±0.45
Oleic acid	0.74 ±0.74	0	0	2.38 ±1.19

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the MA test); 70 (temperature of the MA test). A MA negative value indicated a methane production rate which is lower than that of the control bottles with no substrate.

The SA tests conducted on the same sludge (Table 6.3) indicate a negative effect on the trophic groups since both thermophilic or hyperthermophilic conditions produced no activity on day 243. Still, the two weeks of low hyperthermophilic production of CH_4 and H_2 in reactor R2, after the TU (Figure 6.4), indicate that fermentative bacteria and methanogens were able to tolerate and even sustain a moderate activity under hyperthermophilic conditions.

Table 6.3. SA test results for the sludge before and after the temperature upgrade T1.

	SA (mmol Substrate / gVSS / day)			
Substrate	$R2^{t}_{55}$ (Day 208)	$R2^{h}_{55}$ (Day 243)	$R2^{h}_{70}$ (Day 243)	$Control^{t}_{55}$ (Day 274)
Glucose	4.4 ±0.56	0	0	2.2 ±0.22
Propionic acid	-0.01 ±5.7	0	0	0
Butyric acid	0.68 ±0.03	0	0	0.34 ±0.34
Acetic acid	2.0 ±0.67	0	0	2.83 ±0.57
Hydrogen	99.3 ±29.8	0	0	496.3 ±148.8

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the SA test); 70 (temperature of the SA test). Negative values indicated an accumulation and sometimes a production of the specific substrate instead of a degradation throughout the SA test.

As indicated by the MA results for T2 (Table 6.4), samples of R1 collected before TU showed both thermophilic methanogenic and potential hyperthermophilic activity ($R1^{t}_{55}$ and $R1^{t}_{70}$) except for the bottles fed oleic acid under hyperthermophilic condition where no methanogenic activity was observed. Although observed in the R1 sludge on day 357 ($R1^{t}_{70}$), this hyperthermophilic potential was lost after T2 ($R1^{h}_{70}$), and an incomplete pasteurization effect occurred. Methane was produced from all substrates tested under thermophilic conditions ($R1^{h}_{55}$), but at levels under that of the substrate-less control bottles, explaining the negative values of –0.74 to –1.86 mmol CH_4/gVSS/day. The sludge from reactor R1, demonstrated no acetoclastic hyperthermophilic potential before

T2 ($R1^t_{70}$) and no adaptation after T2 ($R1^h_{70}$) (Table 5). While the thermophilic acetate consuming methanogens were unable to acclimatize to the new temperature, the thermophilic hydrogenophilic methanogens were able to endure the change and produce methane for 14 days after TU.

Table 6.4. MA test results for the sludge before and after the temperature upgrade T2.

	MA (mmol CH_4 / gVSS / day)				
	$R1^t_{55}$	$R1^t_{70}$	$R1^h_{55}$	$R1^h_{70}$	$Control^t_{55}$
Substrate	(Day 357)	(Day 357)	(Day 397)	(Day 397)	(Day 306)
Acetic acid	4.60 ±2.49	0.11 ±0.01	-1.86 ±1.48	0	6.31 ±0.93
Albumin	1.93 ±0.26	0.07 ±0.0	-1.48±1.48	0	1.48 ±0.30
Alanine/Glycine	2.86 ±0.30	0.11 ±0.04	-1.48 ±1.86	0	5.83 ±0.45
Glycerol	0.33 ±0.04	0.21 ±0.07	-1.86 ±1.48	0	0.48 ±0.04
Olive oil	0.37 ±0.04	0.07 ±0.04	-0.74 ±1.48	0	0.52 ±0.11
Oleic acid	0.07 ±0.04	0	-1.86 ±1.48	0	-1.37 ±0.19

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the MA test); 70 (temperature of the MA test). A MA negative value indicated a methane production rate which is lower than that of the control bottles with no substrate.

Collected before T2 ($R1^t_{70}$) and subjected to SA tests, the sludge from reactor R1 indicated a reduced population of active microorganisms and an average fermentative potential of 1.11 mmol Glucose/g VSS/day (Table 6.5), but no activity for the other substrates (propionic, butyric and acetic acid, and hydrogen). The thermophilic

fermentation activity remained at 1.67 mmol Glucose/gVSS/day for ($R1^{h}_{70}$) while the activities for propionic, butyric and acetic acid activity were –0.03, 0.03, and 0.08 mmol/gVSS/day, respectively. Thus, TU had an incomplete pasteurization effect on the sludge of reactor R1.

Some microbial activity was maintained during TU of T2, as the thermophilic sludge H_2 activity doubled from 138.9 to 277.9 mmol H_2/gVSS/day, when compared to ($R1^{h}_{55}$) and ($R1^{t}_{55}$), and this activity remained at 4.96 mmol H_2 /gVSS/day, under hyperthermophilic conditions (Table 6.5). This observation was supported by some hyperthermophilic SA of 0.36 mmol Glucose/gVSS/day for ($R1^{h}_{70}$). These findings confirm those observed in reactor R1 after T2 where acetic acid was accumulated and a low volume of H_2 was produced. Nevertheless, acetoclastic methanogens did not acclimatize to the new temperature, as indicated by a SA of -0.03 mmol/gVSS/day and the accumulation of acetate in the bottles.

Table 6.5. SA test results for the sludge before and after T2.

	SA (mmol Substrate / gVSS / day)				
	$R1^{t}_{55}$	$R1^{t}_{70}$	$R1^{h}_{55}$	$R1^{h}_{70}$	$Control^{t}_{55}$
Substrate	(Day 357)	(Day 357)	(Day 397)	(Day 397)	(Day 306)
Glucose	1.67 ±0.83	1.11 ±0.23	1.67 ±0.17	0.36 ±0.03	1.67 ±0.11
Propionic acid	-0.01 ±0.06	0	-0.03 ±0.03	0	0.29 ±0.06
Butyric acid	0.68 ±0.05	0	0.03 ±0.01	0	1.02 ±0.08
Acetic acid	1.33 ±0.1	0	0.08 ±0.05	-0.03 ±0.13	2.33 ±0.23
Hydrogen	138.9 ±26.8	0	277.9 ±29.8	4.96 ±4.96	148.8 ±44.7

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the SA test); 70 (temperature of the SA test). Negative values indicated an accumulation and sometimes the production of the specific substrate instead of its degradation throughout the SA test.

The activity of the sludge from reactor C sampled at the end of the experiment, on day 427, and incubated at 55 °C and 70 °C (Tables 6.6 and 6.7), confirmed the results obtained with reactors R1 and R2. A lower thermophilic MA activity ($Control^{t}_{55}$) on day 427, compared to day 306 ($Control^{t}_{55}$ - Table 6.4) was demonstrated by a drop in biogas production with acetic acid, olive oil, and oleic acid, to 1.41, -0.04 and -1.04 mmol CH_4/gVSS/day. This likely resulted from extending thermophilic conditions as no other change was imposed on the sludge of reactor C. No hyperthermophilic potential ($Control^{t}_{70}$) was detected for any substrates, but methanogenesis was displaced towards H_2 production (Table 6.6) where acetic acid and albumin produced similar amounts of

0.18 and 0.21 mmol H_2/gVSS/day, respectively. Lower H_2 production occurred with glycerol, alanine/glycine, olive oil and oleic acid (Table 6.6).

Table 6.6. Biogas activity test for sludge samples from the control reactor C at the end of the temperature upgrade T2.

	Biogas Activity (Day 427)		
	mmolCH_4/gVSS/day	mmolCH_4/gVSS/day	mmolH_2/gVSS/day
Substrate	Control$^t_{55}$	Control$^t_{70}$	Control$^t_{70}$
Acetic acid	1.41 ±0.82	0	0.18 ±0.53
Albumin	1.78 ±1.60	0	0.21 ±0.21
Alanine/Glycine	3.56 ±1.37	0	-0.32 ±0.35
Glycerol	0.67 ±0.67	0	-0.21 ±0.14
Olive oil	-0.04 ±0.45	0	-0.39 ±0.21
Oleic acid	-1.04 ±0.22	0	-0.46 ±0.28

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the MA test); 70 (temperature of the MA test). A MA negative value indicated a methane or hydrogen production rate lower than that of the control bottles with no substrate.

The SA activity test (Table 6.7) conducted on the sludge of reactor C on day 427, under thermophilic conditions (Control$^t_{55}$) showed a higher fermentative activity for glucose (average 10.4 mmol Glucose/gVSS/day) and for hydrogenophilic methanogenesis (397 mmol H_2/gVSS/day). The syntrophic activity for propionic and butyric acids and the acetoclastic activity remained low at -0.03, 0.7 and 0.67 mmol substrate/gVSS/day,

respectively. Under hyperthermophilic conditions ($Control^{t}_{70}$), the sludge demonstrated a fermentative activity of 1.1 mmol glucose/gVSS/day and a syntrophic activity of 0.01mmol propionic acid/gVSS/day and 0.23 mmol butyric acid/gVSS/day. Hydrogenophilic and acetoclastic methanogenesis persisted but was reduced to 0.17 mmol H_2/gVSS/day and 4.9 mmol Acetic acid/gVSS/day.

Table 6.7. SA test results for sludge samples from the control reactor C at the end of T2.

	SA (mmol Substrate / gVSS /day)	
	$Control^{t}_{55}$	$Control^{t}_{70}$
Substrate	(Day 427)	(Day 427)
Glucose	10.4 ± 4.6^{a}	1.1 ± 0.27^{b}
Propionic acid	-0.03 ± 0.01^{c}	0.01 ± 0.004^{c}
Butyric acid	0.57 ± 0.03^{d}	0.23 ± 0.08^{d}
Acetic acid	0.67 ± 0.03^{e}	0.17 ± 0.25^{e}
Hydrogen	397.0 ± 79.4^{f}	4.9 ± 3.47^{g}

Notes: t (thermophilic sludge); h (hyperthermophilic sludge); 55 (temperature of the SA test); 70 (temperature of the SA test). Negative values indicated an accumulation and sometimes the production of the specific substrate instead of its degradation throughout the SA test. Statistical significance was determined using a *t-test* with paired data (Wardlaw, 1985). Values with the same letter are not significantly different within groups at the level of $P \leq 0.05$.

From the SA and MA tests, the TU of 2.5°C/week, from 55 to 70 °C, did not completely pasteurize the sludge and methanogenic microorganisms (hydrogenophilic at least) were sustained at a reduced activity. Few microorganisms survived TU and even acetoclastic methanogens were practically eliminated at temperatures around 65 °C, resulting in the accumulation of acetic acid from the anaerobic fermentation. Therefore, the complete transformation of organic matter into hydrogen or methane in a single phased hyperthermophilic anaerobic reactor cannot be achieved. Acetic acid accumulation can be prevented using a hydrolytic-hyperthermophilic, hydrogen producing acidogenic reactor coupled to a thermophilic methanogenic system, as explored by Scherer *et al*. (2000). They used a coupled system with an HRT of 4.3 and 14 day, operated respectively at 70 and 60 °C, and loaded with a 6 to 7 % dry solid feed with a 5 % VS concentration. Under these conditions, the hydrolytic and methanogenic reactors were able to remove 6 and 79 % of the VS, respectively, to produce 27 and 540 $L_{STP}/kgSV_{fed}$. The VFA concentration in the methanogenic effluent ranged from 0.25 to 3.0 g/L. A high count of hydrogen-utilizers of $10^8 – 10^9$ /gTS explained the syntrophic acetate oxidation with, as subsequent and last step, autotrophic methanogenesis in the methanogenic reactor.

6.4 CONCLUSIONS

The objective of the present research was to develop a hyperthermophilic seed from acclimatized thermophilic sludge. A 2.5 °C/week temperature upgrading (TU), from 55 °C-thermophilic to 70 °C-hyperthermophilic condition did not completely pasteurize a thermophilic, food-waste anaerobic sludge, but resulted in a low methanogenic stage lasting for two weeks and a low hydrogen producing fermentation stage. Acetoclastic

methanogens survived temperatures below 62.5 °C while hydrogenophilic methanogens survived the full TU and even sustained a low methanogenic activity thereafter. Such activity was displaced towards a low fermentative production of H_2 after 15 days of hyperthermophilic conditions.

Although both CH_4 and H_2 are considered as an alternative source of green energy, their production under hyperthermophilic conditions requires further investigation. A possible solution is the coupling of thermophilic methanogenesis with hyperthermophilic conditions where H_2 is produced from hydrolysis. This system can possibly improve the degradation of complex substrates such as food waste. Another, more challenging possibility is the introduction of hyperthermophilic acetoclastic methanogens, which could survive the transformation of acetic acid into methane at such extreme temperatures. The last alternative would reduce the complexity of the system.

CHAPTER SEVEN

CONCLUSIONS

7.1 SUMMARY

The following conclusions can be drawn from the present experimental work:

1) A mesophilic wastewater-treating anaerobic sludge can be adapted for the treatment of the organic fraction of municipal solid waste (OFMSW) under thermophilic conditions. Despite the loss of thermophilic methanogenic biomass, under batch conditions, the reactor could afterwards sustain a moderate F/M ratio of 1.5 gVS/gVSS while a high F/M ratio of 4.43 gVS/gVSS displaced methanogenesis towards hydrogen production.
2) An instantaneous upgrading procedure from mesophilic to thermophilic conditions positively selected thermophilic fermentative bacteria. The upgrading procedure also reduced thermophilic acetoclastic methanogens and significantly reduced thermophilic hydrogenophilic methanogens. As opposed to temperature, low pH conditions under thermophilic temperatures provoked a shift of the microbial content. Under batch operation, a low pH environment related to a high F/M ratio of 4.43 gVS/gVSS displaced methanogens in favor of thermophilic Clostridium-like hydrogen producing-bacteria.
3) The methane potential methodology (MP) developed in this research project supported that oil hydrolysis did not limit the overall catabolic chain. Instead, the release of long chain volatile acids (oleic acid in this case) decreased and even inhibited the performance of acetoclastic methanogens. On the contrary, the limiting

albumin degradation step was related to the hydrolysis of the protein into peptides, since the mixture of amino acids showed a quick and efficient transformation into acetic acid first and into methane afterwards.

4) Exposure of the thermophilically adapted sludge to a temperature upgrading of 2.5 °C/week, up to hyperthermophilic temperatures (70 °C), negatively affected the micro flora responsible for completing the transformation of organic matter into methane. This temperature upgrading suppressed the activity of acetoclastic methanogens when the sludge reached temperatures of 62.5 to 65 °C. Nevertheless, hyperthermophilic conditions did not completely pasteurize the sludge and hydrogenophilic methanogenesis was sustained for a short period of time and displaced afterwards by hydrogen producing fermentative activity.

7.2 CONTRIBUTION TO KNOWLEDGE

The outcome of this work will prove useful since for the first time, a full description was carried out concerning the transition of a mesophilic sludge all the way through the process of upgrading the process temperature from 35 to 55°C first and from 55 to 70°C afterwards.

As well, for the first time an adaptation of a soluble COD treating granular sludge for the treatment of a high solids (*ca*. 20%) food waste stream was fully described.

This research contributed on gaining knowledge about the effect that temperature changes have over the microbiology of a diverse community of microorganisms such as the case of anaerobic sludge and their interactions. Those interactions may lead to changes as well on the degradation pattern so an important effect was described here

regarding the concentrations of un-ionized acetic acid at which acetoclastic methanogens showed inhibition for producing methane.

Here is shown for the first time as well that hydrolysis is the limiting step for degrading proteins; but in the case of the breakdown of lipids, β-oxidation of long change volatile acids released to the media after oil hydrolysis is the limiting step of the anaerobic degradation sequence.

Additionally, this research showed that although hyperthermophilic methanization of food waste is possible, there is a disconnection between acetoclastic and hydrogenophilic methanogenesis due to the inactivation of the former when the process reached temperatures in the range of 62 to 65 °C. Such disconnection created a large accumulation of acetic acid in the media thus the organic matter removal efficiency was reduced significantly.

This research showed that most changes of the anaerobic process observed here lead to the shift of populations within the sludge. It was shown that either pH or temperature changes positively selected hydrogen producing microorganisms that displaced those methane-producing ones. In this sense, it was shown as well that changes of the process conditions must be carefully planned in order to secure the coupling of trophic associations necessary for the completion of the methanization process.

7.3 FUTURE WORK

The first and most important challenge involving future work on hyperthermophilic digestion of food waste is the necessary coupling of acetoclastic and hydrogenophilic methanogenesis. To divert the flow of COD towards methane production, hyperthermophilic acetoclastic methanogens need to be incorporated within the process biology. This challenge may be met through several approaches which may include: a) genetic engineering of thermophilic acetoclastic methanogens, b) by extensive screening of extreme temperature growing methanogens or c) by forced natural selection.

Another possibility already under evaluation by other researchers is the separation of phases in which a first phase may hyperthermophilically degrade large molecules into acetic acid and hydrogen, among other compounds. To complete the degradation of COD due to the high concentrations of acetic acid, the second process phase could include thermophilic methanization of the acetic acid produced in the first phase. Since previous experiences focused on the acidification of the waste, the challenge in this case is to incorporate the issue of energy production by taking advantage of the naturally occurring production of hydrogen that along with methane production may further increase the energy efficiency and self sustainability of the process.

7.4 GENERAL LIST OF REFERENCES

American Public Health Association/American Water Works Association/ Water Environmental Federation (1995) *Standard methods for the examination of water and wastewater* 19th Ed., Washington, D.C., USA.

Ahring B.K., Ibrahim A.A. and Mladenovska Z. (2001) Effect of temperature increase from 55 to 65 C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. *Wat. Res*., **35** (10), 2446-2452.

Allen Kain Associates and Enviros RIS Ltd. (2001) WDO Study: Implications of different waste feed streams (source-separated organics and mixed waste) on collection options and anaerobic digestion processing facility design, equipment and costs. report propagated for the City of Toronto. www.risltd.com.

Altschul S., Madden T., Schäffer A., Zhang Z., Miller W., and Lipman D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*, **25**, 3389-3402.

Amann R.I., Ludwig W., and Schleifer K.-H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev.,* **59**, 143–169.

Baldasano J. M. and Soriano C. (2000) Emission of greenhouse gases from anaerobic digestion processes: comparison with other municipal solid waste treatments. *Wat. Sci. Tech*., **41** (03), 275.

Battistoni P., Pavan P., Mata-Alvarez J., Prisciandaro M. and Cecchi F. (2000) Rheology of sludge from double phase anaerobic digestion of organic fraction municipal solid waste. *Wat. Sci. Tech*., **41** (03), 51.

Brock T.D. and Madigan M.T. (1991). *Microbiología*. Sixth edition.Prentice Hall Editions. Mexico.

Confer D.R. and Logan B.E. (1998) A conceptual model describing macormolecule degradation by suspended cultures and biofilms. *Wat. Sci. Tech*., **37** (4-5), 231-234.

Cornacchio L., Hall E.R., and Trevors J.T. (1986). Modified serum bottle testing producers for industrial wastewaters. Technology transfer workshop on laboratory scale anaerobic treatability testing technique. Environment Canada -Wastewater Technology Centre. Downsview ON, Canada.

De Baere L. (2000) Anaerobic digestion of solid waste: state-of-the-art. *Wat. Sci. Tech.*, **41** (3), 283.

Fang H.H.P. and Wai-Chung Chung D. (1999). Anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. *Wat. Sci. Tech.,* **40** (1), 77-84.

Ferry James G. (1993) *Methanogenesis: Ecology, Physiology, Biochemistry & Genetics*. Chapman & Hall. USA.

Field J.A. (2002). Limits of anaerobic degradation. *Wat. Sci. Tech*., **45** (10), 9-18.

Ghosh S., Henry M.P., Sajjad A., Mensinger M.C. and Arora J.L. (2000). Pilot-scale gasification of municipal solid wastes by high-rate and two-phase anaerobic digestion (TPAD). *Wat. Sci. Tech*., **41** (03), 101.

Guiot R. S. (1991) Modeling of the Upflow Anaerobic Sludge Bed-Filter System: A case with Hysteresys. *Wat. Res.* **25** (3), pp. 251-262.

Guiot S. R., Safi B., Frigon J.-C., Mercier P., Mulligan C., Tremblay R., and Samson R. (1995) Performances of a full-scale novel multiplate anaerobic reactor treating cheese whey effluent. *Biotech Bioeng*, **45**:398-405.

Gujer W. and Zehnder J. B. (1983) Conversion process in anaerobic digestion. *Wat. Sci. Tech*., **5**, 127.

Hartmann H. and Ahring B. K. (2003) Phthalic acid esters found in municipal organic

waste: enhanced anaerobic degradation under hyper-thermophilic conditions. *Wat. Sci. Tech.* **48** (4), 175-183.

Hongalo W. and Yongfeng N. (2001) Municipal solid waste characteristics and management in China. *Air and Waste Manage. Assoc*. **51**: 250-263.

Huyard A., Ferran B. and Audic J-M. (2000) The two phase anaerobic digestion process: sludge stabilization and pathogen reduction. *Wat. Sci. Tech*., **42** (9), 41.

Kim I.S., Kim D.H. and Hyun S-H. (2000) Effect of particle size and sodium ion concentration on anaerobic thermophilic food waste digestion. *Wat. Sci. Tech*., **41** (03), 67.

Komatsu T., Kimura T., Kuriyama Y., Issihiki Y., Kawano T. Hirao T., Masuda M., Yokohama K., Matsumoto T. and Takeda M. (2002) Anaerobic digestion of organic waste in Japan: the first demonstration plant at Kyoto City. *Wat. Sci. Tech*., **45** (12), 113.

Lalonde Michelle. (2003) Trash talk is turning serious. The Gazette, Montreal. Saturday, April 12, 2003. A14.

Lane D. (1991) *16/23S rRNA sequencing*. Chichester, United Kingdom, John Wiley & Sons.

Li Y.Y., Sasaki H., Yamashita K., Seki K. and Kamigochi I. (2002) High-rate methane fermentation of lipid-rich food waste by a high-solids co-digestion process. *Wat. Sci. Tech*., **45** (12), 143.

Lissens G., Vandevivere P., De Baere L., Biey E.M. and Verstraete W. (2001) Solid waste digesters: process performance and practice for municipal solid waste digestion. *Wat. Sci. Tech*., **44** (8), 91.

Liu H.W., Walter H.K., Vogt G.M., Vogt H.S., and Holbein B.E. (2001) Steam pressure disruption of municipal solid waste Enhances Anaerobic digestion kinetics and biogas yield. *Biotech. & Bioeng*., **77** (2), 121-130.

Maibaum C. and Kuehn V. (1999). Thermophilic and mesophilic operation of an anaerobic treatment of chicken slurry together with organic residual substances. *Wat. Sci. Tech*., **40** (1), 231.

Mata-Alvarez J. (2002). *Biomethanization of the organic fraction municipal solid wastes.* IWA Publishing, UK.

Mata-Alvarez J., Macé S., and Llabrés P. (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology*. **74**, 3-16.

Mayhew M.E., Le M.S. and Ratcliff R. (2002) A novel approach to pathogen reduction in biosolids: the enzymic hydrolyser. *Wat. Sci. Tech*., **46** (4-5), 427.

McCarty P.L. and Mosey F.E. (1991) Modeling of anaerobic digestion processes (A discussion of concepts). *Wat Sci Tech,* **24** (8), 17-33.

Michigan State University -Department of Chemistry. (2007). Lipids. http://www.cem.msu.edu/~reusch/VirtualText/lipids.htm (accessed March 26, 2007)

Muyzer G., de Waal E.C., and Uitterlinden A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rDNA. *Appl Environ Microbiol*, **59**, 675-700.

Muyzer G., Hottenträger S., Teske A., and Wawer C. (1996) Denaturing gradient gel electrophoresis of PCRamplified 16S rDNA – A new molecular approach to analyse the genetic diversity of mixed microbial communities. In: Akkermans ADL, van Elsas

JD & de Bruijn FJ (Eds) *Molecular Microbial Ecology Manual* (3.4.4: pp. 1–23) Kluwer Academic Publishers, Dordrecht, The Netherlands.

Nielsen B. and Petersen G. (2000). Thermophilic anaerobic digestion and pasteurization. Practical experience from Danish wastewater treatment. *Wat. Sci. Tech*., **42** (9), 65.

Nozhevnikova A.N., Holliger C. Ammann A. and Zehnder A.J.B. (1997) Methanogenesis in sediments from deep lakes at different Temperatures (2-70°C). *Wat. Sci. Tech*., **36** (6-7), 57-64.

Nozhevnikova A.N., Kostsyurbenko O.R. and Parshina S.N. (1999) Anaerobic manure treatment under extreme temperature conditions. *Wat. Sci. Tech*., **40** (1), 215.

Ortega L., Barrington S. and Guiot R. S. (2007) Thermophilic Adaptation of a Mesophilic Anaerobic Sludge for Food Waste Treatment. Paper accepted for publication at Journal of *Environmental Management (in press)*.

Ortega L., Barrington S. and Guiot R. S. (2007b) Methanogenic activity test: A tool for evaluating degradation of Albumin and Olive oil under mesophilic and thermophilic conditions. Paper to be submitted to a peer review Journal.

Owen W.F., Stuckey, D.C., Healy J.B., Young Y., and McCarty P.L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Wat. Res*., **13**, 485 – 492.

Pavan P., Battistoni P., Cecchi F. and Mata-Alvarez J. (2000b) Two-phase anaerobic digestion of source sorted OFMSW (organic fraction municipal solid waste): performance and kinetics. *Wat. Sci. Tech*., **41** (03), 111.

Pavan P., Battistoni P., Mata-Alvarez J. and Cecchi F. (2000a). Performance of thermophilic semi-dry anaerobic digestion process changing the feed biodegradability. *Wat. Sci. Tech.*, **41** (03), 75.

Ramsay I.R. and Pullammanappallil C.P. (2001). Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. *Biodegradation*, **12**, 247-257.

Rawn J.D. (1990). *Traité de Biochimie*. Éditions de Renouveau Pédagogique. Belgium.

Reeve J.N., Morgan R. and Nölling J. (1997) Environmental and molecular regulation of methanogenesis. *Wat. Sci. Tech.*, **36** (6-7), 1.

Rhyner C.R., Schwartz L.J., Wenger R.B. and Kohrell M.G. (1995) *Waste management and resource recovery*. Lewis Publishers (CRC Press). First edition. USA.

Robson William. (2003) Toronto's trash a ticking bomb for Canada. National Post. November 6, 2003. A17.

Scherer P.A., Vollmer G.-R., Falkhouri T. and Martensen S. (2000) Development of a methanogenic process to degrade exhaustively the organic fraction municipal "grey waste" under thermophilic and hyperthermophilic conditions. *Wat. Sci. Tech.*, **41** (3), 83-91.

Schieder D., Schneider R., and Bischof F. (2000) Thermal hydrolysis (TDH) as a pretreatment method for the digestion of organic waste. *Wat. Sci. Tech.*, **41** (3), 181-187.

Shelton D.R. and Tiedje J.M. (1984). General method for determining anaerobic biodegradation potential. *Appl. Environ. Microbiol*. **47** (4), 850 – 857.

ten Brummeler E. (2000) Full scale experience with the Biocel process. *Wat. Sci. Tech.*, **41** (03), 299.

Thierbach R.D. and Hanssen H. (2002) Utilization of energy from digester gas and sludge incineration at Hamburg's Köhlbrandhölf WWTP. *Wat. Sci. Tech*., **46** (4-5), 397.

Tresse O., Lorrain M., and Rho D. (2002) Population dynamics of free-floating and attached bacteria in a styrene-degrading biotrickling filter analyzed by denaturing gradient gel electrophoresis. *Appl Microbiol Biotechnol,* **59**, 585-590.

U.S. Environmental Protection Agency. (2003) Landfill Methane Outreach Program. Available from the web via the Internet www.epa.gov/Imop/about.htm#ifgte

U.S. Environmental Protection Agency. (2001) Municipal Solid Waste in the United States: 1999 Facts and Figures. EPA530-R-01-014. Available from the web via the Internet www.epa.gov.

Wardlaw A. C. (1985) *Practical Statistics for Experimental Biologists*. John Wiley & Sons Ltd. Great Britain.

Watanabe H., Kitamura T., Ochi S. and Ozaki M. (1997) Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. *Wat. Sci. Tech*., **36** (6 - 7), 25.

Weimer P. J. and Zeikus J.G. (1977) Fermentation of cellulose and cellobiose by *Clostridium thermocellum* in the absence and presence of *Methanobacterium thermoautotrophicum*. *Appl Environ Microbiol*, **33** (2), 289-297.

Zábranská J., Stepová J., Wachtl P. Jenícek P. and Dohányos M. (2000) The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates. *Wat. Sci. Tech*., **42** (9), 49.

Zábranská J., Dohányos M., Jenícek P., Zaplatílková P. and Kutil J. (2002) The contribution of thermophilic anaerobic digestion to the stable operation of wastewater sludge treatment. *Wat. Sci. Tech*., **46** (4-5), 447.

Zeikus J.G. and Wolfe R.S. (1972) *Methanobacterium thermoautotrophicus* sp. n., an anaerobic, autotrophic, extreme thermophile. *Journal of Bacteriology*, **109**, 707-713.