Thermophilic anaerobic digestion of the organic fraction of municipal solid waste

Ortega, L; Lévesque, M; Barrington, S; Guiot, SR

Proc. (CD) of First International Meeting on Environmental Biotechnology and Engineering (IMEBE), September 6-8, 2004, Mexico City, Mexico. ISBN 970-94112-0-9. Paper 136, 13 pp, 2004

THERMOPHILIC ANAEROBIC DIGESTION OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

Luis Ortega (1,2); Marie-Josée Lévesque (2); Suzelle Barrington (1); Serge Guiot (2)

(1) McGill University, Bioresource Eng. Department. 2111 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.

ABSTRACT

The paper presents results obtained at laboratory scale on the development of a thermophilic anaerobic inoculum for the treatment of the organic fraction of separately collected municipal solid waste (SC-OFMSW). The paper discusses the change in both mesophilic and thermophilic anaerobic microbial groups when temperature is abruptly increased from mesophilic (35°C) to thermophilic (55°C) conditions as well as reactor performance for the treatment of SC-OFMSW under thermophilic conditions.
In order to develop a thermophilic anaerobic biomass, an instantaneous change of temperature from 35 to 55°C did not promote the pasteurization of such a biomass. Also the substrate consumption rate (SA) indicated a thermophilic fermentative activity of 3.58g glucose/gVSS•d and a methanogenic activity related to acetoclastic and hydrogenophilic microorganisms, of 0.47 and 0.26g substrate/gVSS•d, respectively. This inoculum was used to treat SC-OFMSW. During 4 batch stages each of 21 days, a shredded, high solid (17.9%) SC-OFMSW arising from a cafeteria was fed at a Food/Microorganism (F/M) ratio between 0.12 and 4.43 kgVS/kgVSS. Ultimate gas production potential (Go) and ultimate biodegradation potential (Bo) values between 0.2 to 1.4 $m^3/kgVS_{fed}$ and between 0.1 to 0.84 $m^3CH_4/kgVS$ were obtained, respectively. In terms of biomass stabilization, values between 6.7 and 47.9 % of volatile solids reduction were obtained.
This project demonstrated that with OFMSW, the usual time-consuming acclimatization stage for the initiation of thermophilic anaerobic digestion can be avoided, when it is upgraded from mesophilic digestion. In this case, favorable conditions were quickly achieved **(**20 days**)** and the feeding stage could be initiated immediately afterwards. Nevertheless, to increase the amount of thermophilic methanogenic microorganisms, a F/M ratio supply program must be carefully observed.

INTRODUCTION

In most developed countries, the waste arising from municipal activities accounts for up to 9% of the total mass of solid waste generated each year. To improve the use and reuse of municipal solid waste (MSW), researchers and authorities are looking for better and more energy-efficient practices to manage this renewable source of materials and energy.

Brought by solid waste management systems, some of these practices were recycling, reuse of materials, and source sorting and separation practices. Their implementation has reduced landfill masses by nearly 30%. Nevertheless, gas emissions from landfills are blamed for producing 10% of the global, human-related greenhouse gas (GHG) emissions.

At the moment, the best and most energy-efficient way to handle the organic waste coming from the municipality is the use of conventional mixed and heated anaerobic digestion systems. It has been assumed that global GHG emissions arising from landfills could be reduced up to 40% through the use of source separation of MSW, wet gasification, incineration, and landfill burial of ashes (Baldasano and Soriano, 2000). In addition, the potential energy recovery from this type of waste could be improved 22 times if the organic fraction of MSW was treated anaerobically using heated and mixed systems (De Baere, 2000 and US E.P.A., 2003).

The objective of this project was to develop a thermophilic anaerobic inoculum for the treatment of the organic fraction of separately collected MSW. Among other parameters, the paper will discuss biomass activity and performance when the original seed is exposed to an instantaneous increase in temperature, from mesophilic (35°C) to thermophilic (55°C) conditions. The performance and microbial evolution of such inoculum for the treatment of OFMSW under thermophilic conditions will be discussed as well.

METHODOLOGY

Substrate. Substrate (SC-OFMSW$_{BRI}$) was the organic fraction of solid waste arising from the cafeteria of the Biotechnology Research Institute of the National Research Council of Canada (Montreal, Canada). A total amount of 94.5 kg of cafeteria waste matter was collected and manually sorted to eliminate plastic and wood material. Immediately afterwards the waste was shredded using a 2" stainless-steel blade. During the shredding process water was added to obtain a solids content of 17.9%.

The substrate was made up of food scraps (mainly meat, egg, fruit and vegetables, bread, pastries), paper napkins and packaging, plastic packaging, some wood sticks, and plastic table-settings as well. Visibly, the proportion of meat and egg was high and affected the substrate characteristics (**Table 1**). In this sense both protein and total Kjeldahl nitrogen (TKN) showed high values (25.3 g/kg and 17% of TS, respectively). The carbon/nitrogen (C/N) ratio obtained was 3:1 revealing also a high concentration of nitrogenous compounds.

Table 1: Substrate (SC-OFMSW$_{BRI}$) characteristics.

Parameter	Value
Solid (%)	17.9
TS (g/L)	179.13
% VSS	95
COD (g/gVSS)	1.2
TKN (% TS)	17
TP (% TS)	1.2
C/N	3.1
Protein (g/kg)	25.3
Carbohydrates (g/kg)	71.7

Seed. The seed consisted of a mixture of different available granular and non-granular sludge.

Table 2: Physic and chemical characteristics of the seed.

Parameter	Value
Density (kg/L)	1.042
Solids (%)	4.2
TS (g/L)	42.08
VS (% TS)	67
SS (g/L)	38.11
VSS (% SS)	70.0
Total COD (g/L)	42.4
Soluble COD (% TCOD)	28

Table 2 demonstrates sludge characterization after the final mix. Notably, values obtained for total and volatile solids reflect that which is typical for an anaerobic granular sludge.

Reactor set-up. Figure 1 shows a scheme of the 5 L glass reactor used in this study. 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 and the heating system included a 12"-L T-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) surrounding the digester. The pH monitoring system consisted of a epoxy-pH probe (*Cole-Parmer,* Niles, IL) and an *Accumet* pH-meter model 825 MP (*Fisher*, USA). After condensate recovery, the biogas generated was sampled through the biogas sampling port and measured by means of a Wet Tip-like water displacement system.

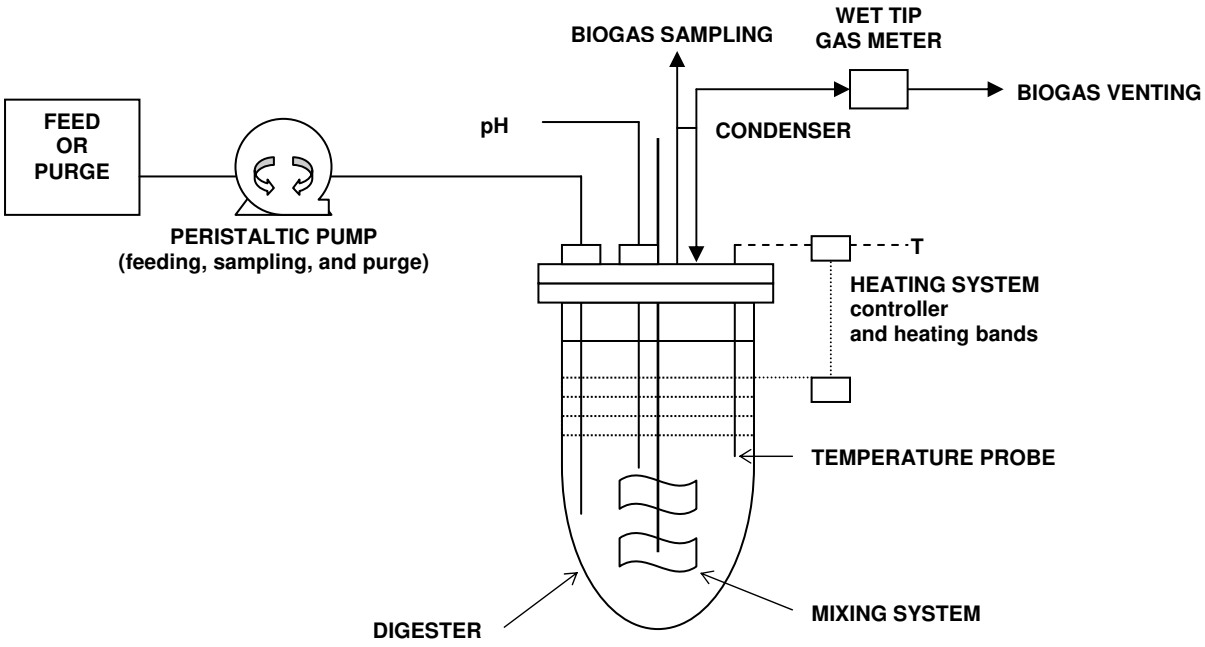

Figure 1: Schematic diagram of the batch reactor system for the thermophilic digestion of SC-OFMSW.

Batch stages. The reactor was operated for 104 days and included five batch stages. The objective of the initial 20 days-stage (C0) was to generate an inoculum adapted to thermophilic conditions (55°C). The reactor used in this experiment was seeded with 4 L of the sludge mixture described in **Table 2**. The system was started-up under mesophilic conditions (35°C) without extraneous substrate supply. On day 13, the process temperature was increased instantaneously up to 55°C. From days 13 to 20 the reactor was kept at 55°C. Thermophilic batch stages one, two, and three (C1, C2, and C3, respectively) were the SC-OFMSW$_{BRI}$-feeding stages and the retention time for each stage was 21 days. A final 21 days-stage (C4) was carried out and the objective was to restore methanogenic conditions; no SC-OFMSW$_{BRI}$ was supplied during the C4 stage.

Analyses. For all five stages, substrate and reactor's effluent were analyzed in terms of total and suspended solids (TS and SS), total and suspended volatile solids (VS and VSS) (all in g/L), pH, total and soluble chemical oxygen demand (COD in g/L). Additionally, the reactor's effluent was analyzed in terms of total alkalinity (mg$CaCO_3$/L) and volatile fatty acids (VFA in g/L). Samples of the SC-OFMSW$_{BRI}$ were further analyzed in terms of total organic carbon (TOC in g/L), total Kjeldahl nitrogen (TKN in g/L), and total phosphorus (TP in g/L). All analysis were carried out in accordance with the techniques of the *Standard Methods* (1995). Protein (g/L) and carbohydrates (g/L) analysis were carried out using the Lowry assay (Lowry *et al*., 1951) and the Dubois assay (Dubois *et al*., 1956), respectively.

Composition of biogas (hydrogen, nitrogen, oxygen, methane, and carbon dioxide) was obtained by means of a HP gas chromatograph 68900 Series (*Hewlett Packard,* Wilmington, DE). Daily determinations of gas production rate (GPR: $m^3/m^3 \cdot d$), ultimate gas production potential (Go in m^3/kgVS), ultimate biodegradation potential (Bo in m^3CH_4/kgVS), and volatile solid reduction (%VSR) were carried out in accordance with the specifications of Mata-Alvarez (2002).

To evaluate the microbial diversity of anaerobic biomass, analysis of substrate consumption rate (SA) was realized (Guiot *et al*., 1995). Additionally, microbial diversity analysis were carried out by the use of DNA-profiling techniques: 16S rDNA amplification was realized through the use of the polymerase chain reaction (PCR) technique. DNA domains (*Bacteria* and *Archaea*) were amplified separately using the primers described in **Table 3**.

Table 3: PCR primers used in this study.

Primer	rDNA Target	Primer sequence (5'-3')	Reference
341f	*Bacteria*	CCTACGGGAGGCAGCAG	Muyzer *et al*. (1996)
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') (Muyzer *et al*., 1993) for DGGE analysis.

Genomic DNA and PCR-DGGE were performed as previously described by Tresse *et al.*, (2002). PCR products were separated in 8% acrylamide gels with a 40 to 60% denaturing gradient. The gels were run in a 1X TAE buffer for 16 hours at 60°C and 80V. Intensely 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). The sequences were submitted for comparison to Genbank databases using the National Center for Biotechnology Information BLASTN program (Altschul *et al.*, 1997).

RESULTS AND DISCUSSION

Figure 2 shows the gas production rate during the C0 stage. As can be seen that, as soon as the reactor started-up the 35°C-operation, the gas production reached its maximum value (1.9 L_{STP}/day) after 24 hrs. By the beginning of the 13th day of operation, the gas production had dropped to 0.32 L_{STP}/day indicating that mesophilic microorganisms consumed all the available substrate. After 13 days at which time the sludge was organically stabilized, the system temperature was instantaneously increased form 35 to 55°C; constant 55°C-thermophilic condition was reached after ten minutes.

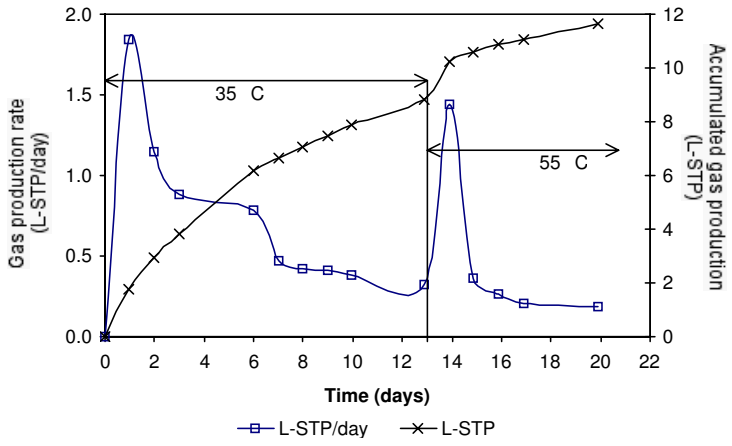

Figure 2: Gas production profile during the C0 stage.

After 2.5 hours of 55°C-operation, the gas production increased from 0 to 0.7 L_{STP}. In the following 1.5 hours, the gas production rate dropped and a final 0.75 L_{STP} gas production was reached. After 22 hours of thermophilic operation, the total volume of gas produced during this stage was 1.4 L_{STP}. These results indicated that only 50% of the gas produced during the temperature changing procedure was related to microbial activity; the remaining 50% was related to an abrupt gas release, during the change of temperature of the liquid phase, caused by a change in solubility of methane and carbon dioxide.

Increasing the temperature of the reactor not only affected gas production rate, but the gas composition as well (**Figure 3**).

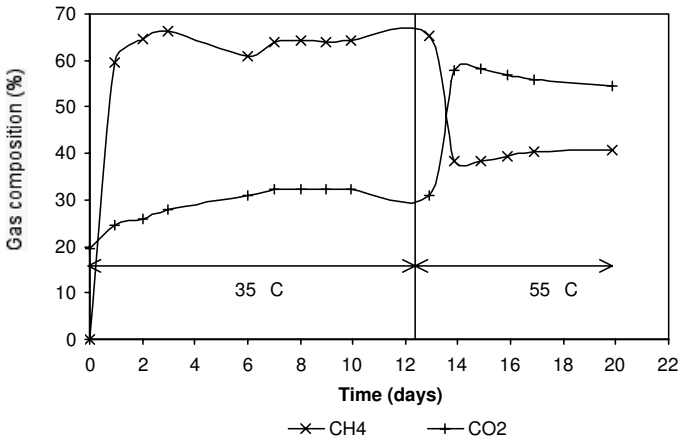

Figure 3: Gas composition profile during the C0 stage.

After one day of 55°C-operation (day 14), the composition of methane dropped from 65 to 38%. The small recovery of a proportion of methane within the gas produced, 38% to 41% from day 14 to day 20, showed that methanogenic microorganisms survived the increment temperature procedure. The C0 stage was therefore concluded.

At the end of the C0 stage the ultimate biogas production (G_0) was 0.1 m^3/kgTVS. During the mesophilic phase (from day 0 to day 13), the volumetric production of methane varied between 0.27 and 0.05 $m^3/m^3/d$. As well, during the thermophilic phase (day 14 to day 20), the volumetric production of methane varied between 0.14 and 0.02 $m^3/m^3/d$. Finally, a volatile solid reduction (%VSR) of 18% was achieved from which 88% corresponded to the digestion of volatile solids contained in the sludge and only 12% was related to soluble substrate degradation.

To evaluate the shift of microbial population within the sludge tests on substrate activity (SA) were carried out at the beginning and at the end of the seeding stage (C0) at both temperatures 35 and 55°C (**Table 3**). The first obvious observation was that increasing the temperature did not produce a pasteurization effect on either bacterial or methanogenic populations.

Table 4: Initial and final sludge specific substrate activity during the C0 stage measured at 35 and 55°C.

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

The results presented in **Table 4** indicated that the abrupt change of temperature from 35 to 55°C on day 13 produced a positive selection of thermophilic fermentative bacteria. Thus, thermophilic glucose consuming bacteria improved their activity almost two fold from 1.85 to 3.58g glucose/gVSS.day. On the contrary, mesophilic glucose consuming bacteria reduced its SA by 58.3%. For propionate consuming bacteria, mesophilic and thermophilic microorganisms reduced their activity from 0.09 to 0.06 and from 0.03 to 0.02g propionate/gVSS.day, respectively. By the end of the 20-days cycle and with activity reductions of 22% and 89.6% for each group, methanogenic activity was related to both acetate and hydrogen consuming thermophilic methanogens (0.47 and 0.26g Substrate/gVSS.day, respectively). Interestingly, mesophilic acetoclastic methanogens were not affected by the abrupt change of temperature, but even improved SA since such a trophic group was in starving conditions. Thus, SA increased two fold, from 0.62 to 1.18g acetate/gVSS.day.

Once the C0 stage was completed, the SC-OFMSW-feeding stage was initiated and included three batch cycles (C1 to C3). The retention time for each stage was 21 days and the average process temperature was 55.2 ±0.28°C. The food/microorganism (F/M) ratio applied varied from 0.12 to 4.43 kgVS/kgVSS (**Table 5**).

Table 5: Operation conditions and reactor performance from C1 to C3.

Stage	F/M kgVS/kgVSS	Average pH	Go m^3/kgVS	Bo m^3CH_4/kgVS	% CH_4	% H_2	% VSR
C1	0.12	-	1.40	0.84	60	0	6.7
C2	1.15	7.3	0.89	0.61	64	0	47.5
C3	4.43	5.17	0.2	0.01	4	14	33.5

Notes: Go and Bo stand for ultimate gas production and ultimate biodegradation potential, respectively (Mata-Alvarez, 2002).

The results presented in **Table 5** showed that the biomass contained in the reactor was able to accommodate low and intermediate F/M ratios (C1 and C2). The initial (C1) F/M ratio was 0.12 kgVS/gVSS, equivalent to an organic loading rate (OLR) of 0.095 kgVS/m^3·d. This OLR was low compared to the typical 3.6 kgVS/m^3·d-organic load suggested by ten Brummeler (2000) for batch reactors. The C2-F/M ratio was increased ten fold (1.15 kgVS/gVSS) which corresponded to an OLR of 0.76 kgVS/m^3·d. The C2-F/M ratio did not cause a negative effect on the reactor, since the production of gas increased significantly, from 11 L_{STP} during the C1 stage to 56 L_{STP} during the C2 stage. The concentration of methane also increased from 60% during the C1 stage to 64% during the C2 stage.

A F/M ratio of 4.43 kgVS/kgVSS applied during the C3 stage produced a complete media acidification as of day two (an average pH of 5.17). This acidification resulted from the accumulation of VFA. At the beginning of the C3 stage, the

concentrations of acetate, propionate and butyrate were 4.58, 0.065 and 7 g/L, respectively. By the end of the C3 stage, only 45 and 40% of acetate and butyrate respectively were consumed, while the concentration of propionate remained unchanged throughout the C3 stage. The F/M ratio applied during this stage also increased the production of gas and the proportion of hydrogen within the gas (up to an average of 14%) as well. As discussed later on, such a production of hydrogen was related to either the consumption of rapidly available carbohydrates by hydrolytic or fermentative bacteria or to the degradation of butyrate by syntrophic bacteria.

According to the gas composition profile during the C3 stage (**Figure 4**), the gas production high in hydrogen content, increase from 0 to 30% after the first day of operation suggesting some fermentative activity. After the second day, the hydrogen content decreased to 0% and then immediately began increasing again reaching 55% by the end the fifth day of operation. Notably, a decrease in total gas production rate from 106.3 to 15.95 L_{STP} /d was observed during this period. Since fermentative bacteria could be inhibited by the high partial pressure of hydrogen at the beginning of the C3 stage, the second stage of hydrogen production could be largely related to syntrophic activity. After day 7, gas and hydrogen production both ceased completely.

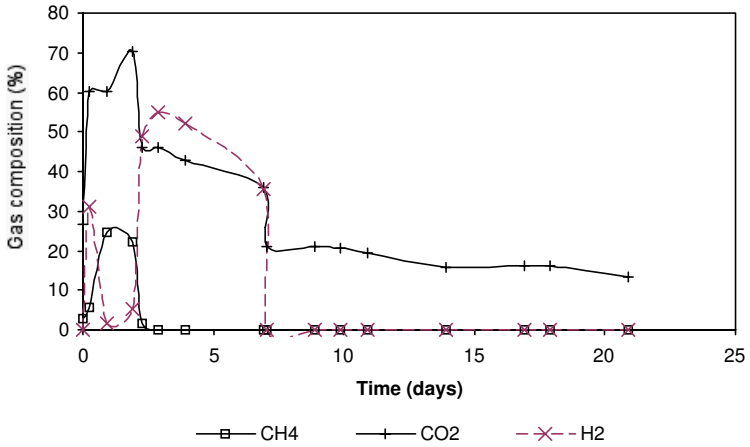

Figure 4: Gas composition profile during the C3 stage.

After the C3 stage a new cycle was started (C4 stage); in this case and in order to restore methanogenic conditions, the reactor received a mixture of 1.6 L of sludge produced at the end of the C2 stage, 0.3 L of the original seed blend, and 0.25 L of a 0.2M $NaHCO_3$ solution pH 8. At the end of the C4 stage, the final pH value was 7.03 and Go, Bo, and CH_4% values of 1.26, 0.8 m^3/kgVS and 64% were obtained, respectively.

Phylogenetic analysis of DNA extracted from the biomass samples at the end of every stage indicated the presence of two types of bacteria (**Table 6**) and several species of methanogens (**Table 7**). Firstly, these results indicated that hydrolytic and fermentative activities and volatile fatty acid oxidation were carried out by Clostridium-like and syntroph-like microorganisms, respectively. Secondly, it was

observed that the application a high F/M ratio favored the activity of bacteria rather than that of methanogens.

Regarding DNA-sequencing and *BLAST* comparison of domain *Bacteria*, several clusters were related to the class of Clostridium sp. Thus, cluster 10 was identified as *Clostridium thermopalmarium* and its presence was relevant during C3 and C4 stages. As well, during C2 and C4 stages, clusters 22 and 23 were related to *Coprothermobacter proteolyticus* and *C. platensis*, respectively, both classified as Clostridium-like microorganisms, belonging to the phylum *Fermicutes*.

Three syntroph-like microorganisms were also identified. Clusters 15 was related to a syntroph uncultured bacteria and it was present during the C0, C1, and C2 phases. In the case of cluster 19 (uncultured bacterium UASB-TL13), the presence of this Cytophaga, syntroph-like microorganism was detected in all samples analyzed (from 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.

Table 6: Results of DNA-profiling analysis and *BLAST* comparison of domain *Bacteria.*

Cluster	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

Regarding DNA-sequencing and *BLAST* comparison of domain *Archaea* (**Table 7**), all clusters analyzed were related to the *phylum* Euryarchaeota of which around 56% were related to the class methanobacteria, 30% to the class methanomicrobia, and 14% were related to non-cultured methanogens.

Table 7: Results of DNA-profiling analysis and *BLAST* comparison of domain *Archaea.*

Cluster	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 the C0 stage, no DNA methanogen-related was recuperated from the biomass sample of the C3 stage. The extreme conditions of pH and H_2-partial pressure during C3 apparently killed all methanogens present in the media, and since methanogenic condition were re-established with the replacement of the acidified biomass with a mixture of the original seed and biomass produced during the C2 stage, all methanogens were completely re-seeded after the C3 stage.

In addition, results presented in **Table 6** and **Table 7**, together with the overall reactor performance (**Table 5**) and the profile of gas composition of the C3 stage (**Figure 4**) a clear picture of the different microbial interactions was obtained for the stages of the experiment. Specifically, these results indicated that hydrolytic activity in general was related to Clostridium-like microorganisms. Meanwhile, fermentative activity responsible for the production of hydrogen, could be related to both Clostridium-like and syntroph-like microorganisms.

Microbial interactions between fermentative bacteria and methanogens are energetically favorable compared to the axenic growth of the bacteria. In this sense, *Clostridium thermocellum*, growing together with *Methanobacterium thermoautotrophicum*, consume glucose producing acetate, carbon dioxide, and hydrogen. This allows larger energy conservation by fermentative organisms, since the acetyl-CoA intermediates in this biochemical pathway can be conserved as adenosine triphosphate (ATP) (Weimer and Zeikus, 1977; Ferry, 1993). During the C3 stage, this condition was present only during the first two days of operation, so the syntrophy between Clostridium sp. and methanogens took place. Afterwards methanogen activity was suppressed by environmental conditions. Thus, it is likely that this interaction was present during the precedent stages (C0 to C2 stages).

As part of the obligate interspecies H_2-transfer chain (McCarty and Mosey, 1991), syntrophic organisms participated as well in the production of hydrogen from day two to day four of stage C3. They partially consumed butyrate producing even more acetate, carbon dioxide, and hydrogen as well.

The presence of these kinds of microorganism within the samples analyzed suggested that during the earliest stage of stage C3, sugars of low molecular weight such as glucose or even cellobiose were consumed by Clostridium-like organisms producing hydrogen and acetate. Thus, the mentioned massive 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 well as. Besides the high H_2-partial pressure in the media, the accumulation of acetate and butyrate lowered the pH from 7 to 5. Consequently, the microbial activity of both bacteria and methanogens was decreased almost completely and the production of biogas was stopped.

CONCLUSIONS

According to the results of this research, it can be concluded that a mesophilic wastewater-treating anaerobic sludge can be easily and quickly adapted for the treatment of the organic fraction of municipal solid waste under thermophilic conditions. In addition, a careful selection of the organic load program must be observed, since methanogenic activity can easily be displaced towards fermentative activity.

This experiment showed that the usual, time-consuming acclimatization stage for the start-up of anaerobic treatment of OFMSW under thermophilic conditions can be avoided. In this case, favorable conditions were quickly achieved **(**20 days**)** and the substrate feeding stage could be initiated immediately afterwards.

The instantaneous upgrading procedure (IUP) from mesophilic to thermophilic conditions did not have a pasteurization effect on anaerobic microorganisms. The IUP provided a positive selection pressure on mesophilic acetoclastic methanogens and thermophilic fermentative bacteria. However, the specific activity of both mesophilic and thermophilic propionate-oxidizing syntrophs and hydrogenophilic methanogens was reduced.

The thermophilic biomass developed was able to adjust to the substrate supplied (SC-OMSW) and could sustain F/M ratios up to 1.15 kgVS/kgVSS, while a F/M ratio of 4.43 kgVS/kgVSS 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.

ACKNOWLEDGEMENTS

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

REFERENCES

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.

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.

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.

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

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., and Smith F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, 28 350-356.

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., and Samson R., 1995. Performances of a full-scale novel multiplate anaerobic reactor treating cheese whey effluent. *Biotech Bioeng*, 45:398-405.

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

Lowry, OH., Rosbrough N.J., Farr A.L., and Randall R.R., 1951. Protein measurement with the Folin-Phenol reagents. *J Biol Chem,* 193: 265-275.

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

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

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.

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

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

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 (U.S.EPA), 2003. Landfill Methane Outreach Program. Available from the web via the Internet www.epa.gov/lmop/about.htm#ifgte

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.

NOTATION In alphabetical order

ATP	adenosine triphosphate
Bo	Ultimate biodegradation potential
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand
C0, C1, C2, C3, C4	Batch cycles
DGGE	Denaturing-gradient gel electrophoresis
F/M	Food to microorganism ratio
GHG	Greenhouse gases

Go	Ultimate gas production potential
MSW	Municipal solid waste
OFMSW	The organic fraction of municipal solid waste
OLR	Organic loading rate
PCR	Polymerase chain reaction
SA	Substrate consumption rate
SC-OFMSW	Separately collected organic fraction of municipal solid waste
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TS	Total solids
VFA	Volatile fatty acids
VRS	Volatile solid reduction
VS	Volatile solids
VSS	Volatile suspended solids