NRC·CNRC

NRC Publications Archive
Archives des publications du CNRC

Degradation performance and microbial population shifts in an anaerobic digester treating thermally pretreated biosolids

Frigon, Jean-Claude; Bruant, Guillaume; Roy, Caroline; Levesque, Marie-Josee; Guiot, Serge R.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

Publisher's version / Version de l'éditeur:

[15th IWA World Conference on Anaerobic Digestion], pp. 333-335, 2017-10

NRC Publications Record / Notice d'Archives des publications de CNRC:
https://nrc-publications.canada.ca/eng/view/object/?id=e1e80fdd-7535-4195-9ca3-0c776556e2d8
https://publications-cnrc.canada.ca/fra/voir/objet/?id=e1e80fdd-7535-4195-9ca3-0c776556e2d8

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at
https://nrc-publications.canada.ca/eng/copyright
READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site
https://publications-cnrc.canada.ca/fra/droits
LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

National Research Council Canada | Conseil national de recherches Canada

Canada

Degradation performance and microbial population shifts in an anaerobic digester treating thermally pretreated biosolids

Jean-Claude Frigon*, Guillaume Bruant*, Caroline Roy*, Marie-Josée Lévesque* and Serge R. Guiot*

* Bioengineering Group, Energy, Mining and Environment, National Research Council Canada, Montreal, Canada, H4P 2R2 (E-mail: jean-claude.frigon@cnrc-nrc.gc.ca, guillaume.bruant@cnrc-nrc.gc.ca, caroline.roy@cnrc-nrc.gc.ca, marie-josee.levesque@cnrc-nrc.gc.ca, serge.guiot@cnrc-nrc.gc.ca)

Abstract
During the construction of an anaerobic plant to process 110 000 wet tons of secondary sludge per year at the site of a Canadian municipality, parallel experimentation took place at lab-scale to validate the design and the operation conditions planned for the full-scale installation. Preliminary tests in bottles confirmed that a thermal hydrolysis pretreatment (55°C, 24-48 hours) increased the biodegradability of the sludge by up to 40%. The lab-scale hydrolyser and anaerobic digester (6L) were assembled and processed secondary sludge from the plant in continuous at an HRT of 20 days and at 37°C during 6 months. The process suffered performance loss on two occasions although digester recovery was successful with partial re-inoculation and strict pH control (< 7.3). Both bacterial and archaeal populations were monitored all along the process through a metagenomics approach. While only limited variations were observed in the archaeal population, major shifts occurred in the bacterial population, with significant differences observed between the various phases of the process (initial, problematic and optimal). Once steady-state conditions were obtained, the sludge was successfully degraded with an average of 56% volatile solids (VS) removal at an organic loading rate of 1.8 gVS/L.d following a one day and 19 days retention time in the hydrolyser and digester, respectively. The methane production stabilized at 279 L STP/kg VS added. Monitoring the microbial population allowed identifying microorganisms possibly directly linked to the optimal performance of the digester, with notably a putative important role of the bacterial phyla *Cloacimonetes* and *Tenericutes* in the recovery and stability of the system. Validation at smaller scale helped reducing the risks of implementing an innovative design at full-scale.

Keywords
Anaerobic digestion; sludge; wastewater treatment plant; thermal hydrolysis; metagenomics; microbial population; *Cloacimonetes, Tenericutes*.

INTRODUCTION

Despite the fact that there are thousands of full-scale anaerobic digesters in operation for the treatment of excess sludge in wastewater treatment plants (WWTP), the start-up of a new installation still presents some risks due to specificity in its design and the highly variable composition of the sludge. Thermal hydrolysis is becoming a common pretreatment for enhancing hydrolysis and biodegradation of municipal sludge prior to anaerobic digestion. Another issue to be considered is that still now, anaerobic digesters in operation are generally not performing optimally. It is now well accepted that the composition, abundance and dynamics of the microbial community are essential elements directly linked to the performance of the digester (Cai *et al.*, 2016). Next generation sequencing-based approaches, such as metagenomics, allow performing a diagnostic of the microbiome of an anaerobic digester. This constitutes a prerequisite for the establishment of a microbial strategy to improve anaerobic digesters' performance.

The main objective of this study was to test at laboratory-scale the exact operating conditions to be applied at full-scale and assess the performance in term of volatile solids degradation and methane production. Monitoring of the microbial population was performed in parallel, in order to characterize the composition and the interactions within the microbial community, and possibly to identify microbial markers of optimal performance or of process failure.

MATERIALS AND METHODS

Process description

The process comprises a first stage for the hydrolysis of the secondary sludge and a 6L CSTR digester as the second stage. The hydrolysis was performed in sealed batch bottles, at 55°C during 24h and

under agitation. The hydrolysed substrate was pumped four times per day in the digester as to obtain an HRT of 19 days. The CSTR was maintained at 37°C with a heating pad, and mechanically stirred with a rectangular impeller spinning at 60 rpm (high-torque motor model #7774, Bellco Glass inc, Vineland, USA).

Microbial community monitoring

Microbial population was characterized and monitored all along the process through 16s rRNA genes sequencing. DNA was extracted from samples taken during the initial phase (day 1), the first period of stable performance (days 15, 30 and 33), the problematic phase corresponding to an increase of VFA and a reduction of methane production (days 44 and 50) and the recovery phase (days 79, 128 and 132). Sequencing of both archaeal and bacterial DNAs was performed using the IonTorrent PGM (Life Technologies inc, Carlbad, USA) system. Sequences were filtered, assembled and clustered through an internal pipeline, which then assigned taxonomy to clusters, generated OTUs tables and performed statistical analyses.

RESULTS AND DISCUSSION

Impact of the thermal hydrolysis

The municipal sludge was received weekly from the WWTP and its organic content varied with time for an average of 34.9 and 34.3 gVS/L for the two phases of the study (Table 1). Figure 1 is showing the variation in the loading rate over time, and related methane production from the digester. A first phase of operation, covering 55 days, showed a stable performance from day 27 to 37. A progressive increase in VFA can be observed during that time, with sudden increase during days 50-55. The combined drastic reduction in methane production and high VFA concentration led to the following recovery steps: reduced agitation to favor solid decantation, addition of water at a 6d HRT during 2 days, addition of 100 gVS of inoculum. Different possibilities were considered for the failure of the digester. The inhibition of methanogenic activity by free ammonia was suspected, as the ammonium concentration (> 2000 mg/L) and pH (7.74 ± 0.03) were high (Table 1), resulting in a free ammonia concentration of over 150 mg/L, above the inhibition threshold (Hashimoto, 1986).

Table 1. Mass balance during the stable performance of the digester

	Parameters	Period #1 (d 20-37)	Period #2 (d 56-116)
Feed	pH	6.10 ± 0.12	6.21 ± 0.08
	total solids (g/L)	43.5 ± 5.5	44.7 ± 4.7
	volatile solids (g/L)	34.9 ± 4.5	34.3 ± 2.9
Digestate	pH	7.74 ± 0.03	7.03 ± 0.10
	total solids (g/L)	31.8 ± 0.8	33.9 ± 0.9
	volatile solids (g/L)	21.0 ± 0.4	23.1 ± 0.5
	sCOD (mg/L)	2729 ± 100	2726 ± 295
	VFA (mg/L)	242 ± 96	117 ± 83
	Ammonium (mg/L)	2245 ± 3	2051 ± 305
	Ammonia (mg/L)	147 ± 21	51 ± 15
Performance	OLR (gVS/L.d)	1.6 ± 0.2	1.80 ± 0.21
	Methane yield (L STP/d)	2.8 ± 0.2	2.9 ± 0.4
	Methane production (L STP/kgVS)	295 ± 33	286 ± 61

After the recovery steps, pretreated sludge loading resumed, while pH was on-line controlled with an upper setpoint of 7.2. Maintaining the pH below 7.2 successfully prevented the free ammonia concentration to increase above its inhibition level (51 ± 15 mg/L, Table 1) during the following 9 weeks of operation. VFA concentration remained low and no sign of inhibition could be observed during that time. The OLR during this period averaged 1.80 gVS/L.d for a resulting methane yield of 286 ± 61 L STP/kgVS added, which is significantly higher than average values of 160 L STP/kgVS reported in the literature (Bolzonella *et al.*, 2004).

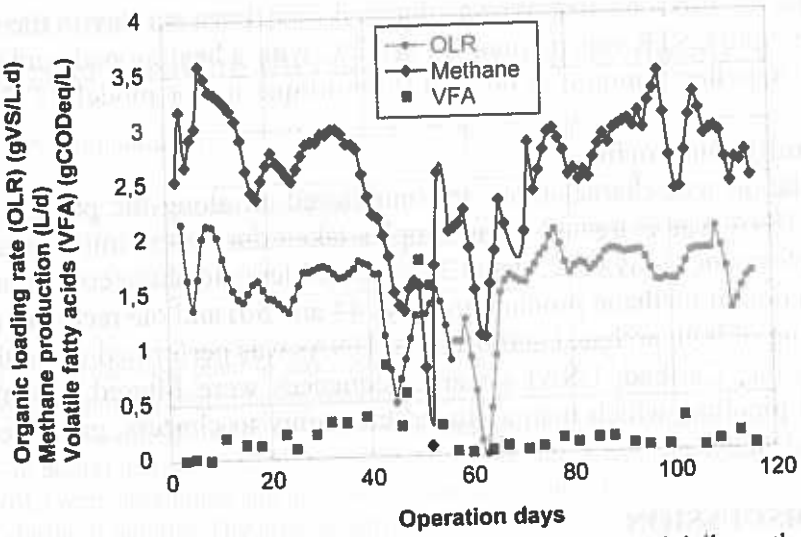


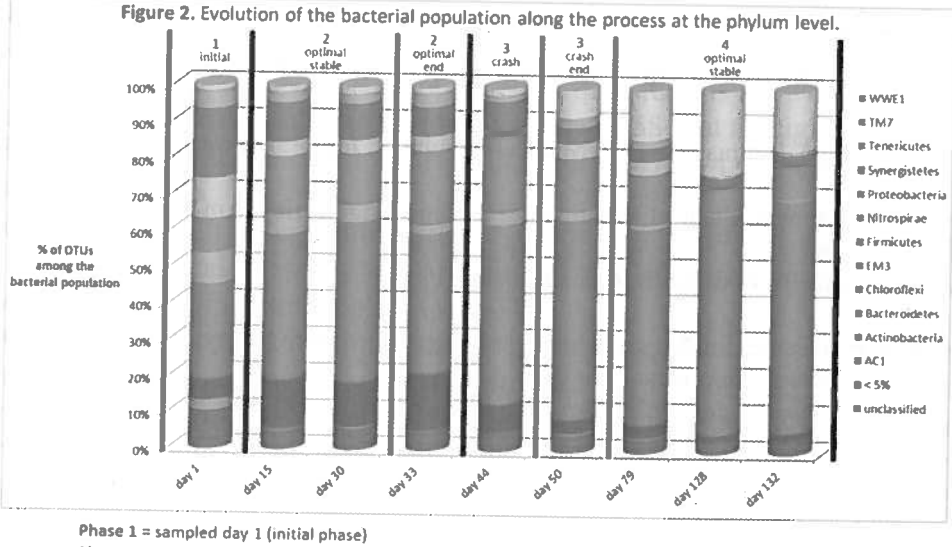
Figure 1. Time-course monitoring of the OLR applied to the digester and daily methane production

Shifts of the microbial population related to the digester performance

Methanomicrobia was the dominant archaeal class all along the process (72.7% to 83.3% of the archaeal population) as typically reported (Nelson et al., 2011), with the obligate acetoclastic methanogen *Methanosaeta* being the most represented archaeal genus (14.0% to 33.4% of the archaeal population). The most remarkable shift was observed during the problematic phase with a decrease of *Methanosaeta* coupled with an increase of the phylogenetic cluster RCII, composed of presumed hydrogenotrophic methanogens. The link between *Methanosaeta* and the digester's good health was confirmed by their increase at the end of the problematic phase and their continued dominance during the recovery phase. The hydrogenotrophic methanogen *Methanobacterium* also appeared to play a role in the recovery of the digester, with its increase at the end of the problematic phase and at the beginning of the recovery phase.

In opposition to Archaea, major shifts occurred in the bacterial population during the study. An heterogeneous initial population evolved towards a strong dominance of four phyla in the final phase, *Bacteroidetes* (representing up to 64.2% of the bacterial population), *Cloacimonetes*, *Firmicutes* and *Tenericutes* (Figure 2). *Clostridiales* and *Actinomycetales*, which include cellulolytic microorganisms, appeared to be linked to performance loss. In effect, they were more numerous just before and/or during the problematic phase, possibly being responsible for the VFA accumulation. On the opposite, *Cloacimonetes*, a newly described bacterial phylum suggested to be composed of cellulose-degraders, *Tenericutes*, already detected in anaerobic digesters and whose members are likely to be involved in lactate and/or acetate production, and members of the family *Rikenellaceae* (from phylum *Bacteroidetes*), whose members are known to produce acetate, propionate and succinate, appeared to have a particular importance in the recovery and stability of the process. Finally, the initially dominant family SB-1 from the phylum *Bacteroidetes*, was progressively replaced by *Rikenellaceae*, probably due to an accumulation of ammonia.

Figure 2. Evolution of the bacterial population along the process at the phylum level.



Phase 1 = sampled day 1 (initial phase)
Phase 2 = sampled days 15, 30 (optimal phase with stable performance) and 33 (end of optimal phase)
Phase 3 = sampled days 44 (problematic phase with crash) and 50 (end of problematic phase)
Phase 4 = sampled days 79, 128 and 132 (optimal phase after recovery, with stable performance)

CONCLUSIONS

This study reported the performance of a CSTR digester fed with thermally pretreated municipal sludge. Despite the relatively high OLR and short HRT compared with similar systems, high methane production could be achieved during steady-state conditions. The main strategy consisted in maintaining a low free ammonia concentration by controlling the pH under 7.2. Significant shifts in the bacterial population observed all along the process suggested an important role of *Cloacimonetes* and *Tenericutes* in the recovery of the digester. Further studies are required to identify potential markers that could be used through a metagenomics approach to prevent digester upset.

ACKNOWLEDGMENTS

The authors would like to thank M. Gilbert Samson, Secretary Treasurer of the Régie de l'Assainissement du bassin La Prairie for co-investment in the project, and Dr Van Kiet Nguyen for his help with the process design and project operation.

REFERENCES

Bolzonella D., P. Pavan, P. Battistoni and F. Cecchi. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochemistry, 2004, 40: 1453-1460.

Cai M., D. Wilkins, J. Chen, S.K. Ng, H. Lu, Y. Jia, and P.K. Lee. Metagenomic reconstruction of key anaerobic digestion pathways in municipal sludge and industrial wastewater biogas-producing systems. Front Microbiol, 2016, 7:778.

Hashimoto A.G. Ammonia inhibition of methanogenesis from cattle wastes. Agricultural Wastes, 1986, 17: 241-261.

Nelson M.C., M. Morrison, and Y. Zhongtang. A meta-analysis of the microbial diversity observed in anaerobic digesters. Bioresour Technol. 2011, 102:3730-3739.