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Metagenomic analysis on thermophilic biogas reactors fed with high load of Long Chain Fatty Acids (LCFA)

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Abstract
In anaerobic digestion systems, the accumulation of long chain fatty acids (LCFA) leads to process instability and decrease of the methane production. This detrimental condition is known to be reversible depending on the concentration of the accumulated LCFAs and mainly on the microbial consortium populating the biogas reactors. Therefore, the understanding of how the microbial communities change in response to LCFA pulses is essential to optimize the overall process. In this study two lab-scale continuously stirred reactors were used to characterize, via 16s rRNA gene analysis, the microbial shifts due to LCFA increase in the feedstock composition. The result shows that the addition of sodium oleate caused a reversible inhibition of reactor. The correlation between the microbial community's profile and the reactors performance indicated that *Syntrophomonas* was the genus most likely involved in sodium oleate degradation. Other genera that were found abundant are *Pseudomonas, Clostridium XI* and *Clostridium III*. The relative abundance of these genera was not significantly affected by the addition of sodium oleate, and this indicates that they are probably involved in later steps of degradation. *Methanoculleus* was the main methanogen that was found in the reactors and its relative abundance was also not significantly affected by the addition of sodium oleate. This indicates that the main methanogenic pathway was not shifted with the addition of sodium oleate.

Keywords
Anaerobic digestion; 16s rRNA gene; LCFA; metagenomic analysis

INTRODUCTION

With the promotion of centralized biogas plant the challenge caused by substrate variation has become a key study subject. Among the organic substrates used in anaerobic digestion, lipids are especially important due to their high methane potential. During degradation, the lipids are first hydrolysed to long chain fatty acid (LCFA) which can cause inhibition to the digestion process (Angelidaki and Ahring 1992, Chen, Cheng et al. 2008). Lalman (2000) reported that LCFA can be oxidized via ß-oxidation and described several possible degradation pathways. However, the microbes involved in such oxidation processes are not fully known. A better understanding of the microbial communities that are involved in LCFA degradation is essential for the optimization of the anaerobic digestion process.

Metagenomic analysis is a practical methodology that is widely applied to characterize the microbial communities from different environments. Different strategies, such as 16s rRNA gene analysis, random sequencing analysis, different fingerprint analyses and etc. have been proposed and applied on environmental samples. Nowadays, 16s rRNA gene analysis is one of the most popular methods for metagenomic surveys. Several studies have utilized this methodology to characterize the microbial communities in anaerobic digestion reactors (Liu, Wang et al. 2009, Kougias, De Francisci et al. 2014). These studies identified members of bacteria and archaea involved in lipid degradation and methane production. More specifically, it has been previously reported that families *Syntrophomonadaceae* (phyla *Firmicutes*) and *Syntrophaceae* (phyla *Proteobacteria)* are often found in lipid-rich anaerobic digestion systems (Sousa, Smidt et al. 2009).

The aim of this study was to elucidate the microbial community dynamics in response to increased LCFA content in the substrate and eventually correlate the substrate composition and reactor performance to the observed community changes. The present abstract contains the results obtained via 16s rRNA gene analysis; random sequencing was also performed on the same samples and are currently under analysis. The results of this study increased our understanding of the microbes' activity in LCFA degradation and opens interesting scenarios for the control and optimization of the anaerobic digestion process when the lipid content in the reactor substrate is increased.

MATERIALS AND METHODS

The present work was conducted in two replicate lab-scale continuously stirred tank reactors (CSTR) (working and total volume, 2L and 1.5L, respectively) operating under thermophilic conditions (54±1°C). The reactors had as Hydraulic Retention Time (HRT) of 15 days. The experiment was divided in two experimental periods; during the first period both reactors were fed with cattle manure as substrate while in the second period the reactor's substrate was supplemented with sodium oleate (12 g/L feed) as representative of LCFA. Methane production, pH values and VFA concentration in the reactors were monitored during the entire experiment. To characterize the microbial community DNA samples from the steady states of the two periods were extracted from both reactors. The genomic DNA was sequenced by Illumina Hiseq 2500 platform. PCR was performed on the V4 region of the rRNA gene using 519f -802r primers (Klindworth, Pruesse et al. 2013). The resulting DNA amplicons were sequenced by Illumina Miseq platform and microbial identity was analyzed with RDP classifier V2.8 (Wang, Garrity et al. 2007).

RESULTS AND DISCUSSION

Process monitoring of biogas reactors

The reactors were operated for a total of 80 days. The VFA concentration and methane yield are shown in Fig. 1.

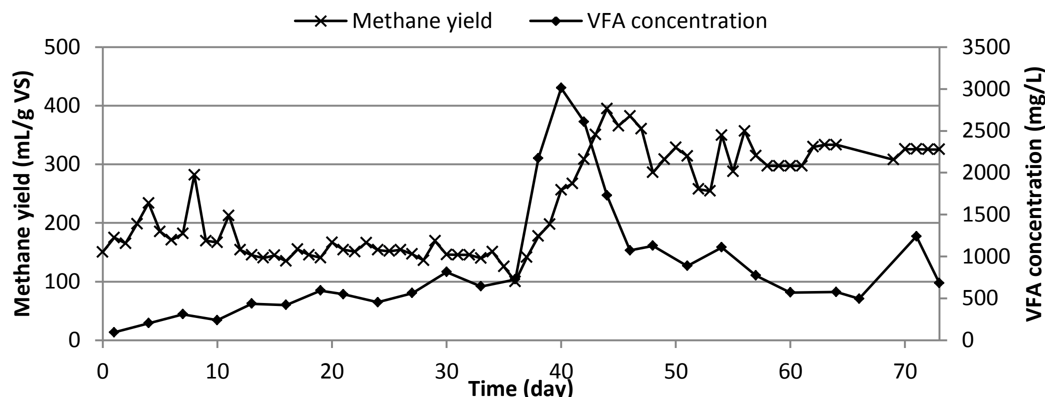

Figure 1 Average methane yields and VFA concentration from both CSTR reactors

The addition of sodium oleate caused an accumulation of total VFA in the reactors from day 40. Acetate was the main VFA produced and accumulation of propionate was also observed in both reactors with a delay of approximately 5 days. The accumulation of VFAs was followed by a decrease of pH. Nevertheless, the pH values of the reactors remained within the range of 7.5-8.5 during the whole experiment, which was favorable to methanogenesis. The average methane yield was 150 ± 4 ml CH_4/gVS for the first steady state and 380 ± 37 ml CH_4/gVS for the second steady state. Based on the calculated theoretical methane potential of sodium oleate, 75% of the added sodium oleate was utilized to produce methane at the second steady state. The addition of sodium oleate caused a slight decrease on the methane yield with a concomitant increase of VFA. The reactors were inhibited for 10 days and recovered afterwards. The VFA slowly decreased to levels

as before the sodium oleate addition and the methane yield increased. The increase of methane yield could be explained by the development of a specialized LCFA degradation microbial consortium.

Microbial community characterization by 16s rRNA gene analysis

To characterize the microbial community before and after the sodium oleate addition, DNA was extracted from samples retrieved at the two steady states of the two reactors. The V4 region of the rRNA gene was amplified and sequenced. In average, 1.6 million sequencing reads were generated from each sample and 20% of the reads were identified using RDP classifier. Among results, genera that had relative abundance higher than 1% or increased more than 2 folds after sodium oleate addition are chosen for discussion (Tab.1).

Table 1 The relative abundance and number of increase folds of selected identified genera

	Genus	**Relative abundance (%)**		**Increase**
		Period 1	Period 2	(fold)
Bacteria	*Syntrophomonas*	0.61	9.81	4.0
	Pseudomonas	1.50	4.34	1.5
	Clostridium XI	0.78	1.95	1.3
	Clostridium III	1.97	1.95	0.0
	Desulfomicrobium	0.01	0.09	2.8
	Marinospirillum	0.01	0.34	5.0
	Aminobacterium	0.00	0.02	3.5
	Paenalcaligenes	0.02	0.16	3.1
	Arcobacter	0.06	0.33	2.4
Archaea	*Methanoculleus*	1.53	1.86	0.3
	Methanothermobacter	0.00	0.08	4.1
	Methanosarcina	0.00	0.09	8.4

Considering Bacteria, 4 genera had relative abundance higher than 1% after the LCFA addition, suggesting that these involving in the LCFA degradation process. The relative abundance of *Pseudomonas*, *Clostridium XI* and *Clostridium III* was not significantly affected (changed less than 2 folds) by the addition of LCFA, suggesting that these genera are not directly involved in the ß-oxidation process but probably in later steps of the degradation process, i.e. the metabolism of VFA. In previous studies, *Pseudomonas* has been reported to have versatility in catabolizing esoteric organic compounds (von Graevenitz 1976), and *Clostridium III* has been found playing important role in the efficient operation of anaerobic reactors (Shiratori, Ikeno et al. 2006). *Syntrophomonas,* on the other hand, increased significantly after the addition of sodium oleate and became the most abundant genus identified in the reactors. Several members of *Syntrophomonas* genus have been proven to be specialized in performing ß- oxidation on LCFA (McInerney, Bryant et al. 1981, Sousa, Smidt et al. 2007, Sousa, Smidt et al. 2009). The significant increase of *Syntrophomonas* found in this experiment confirmed that this genus is a key player in the LCFA degradation under thermophilic anaerobic conditions. Together with *Syntrophomonas, Desulfomicrobium Marinospirillum, Aminobacterium, Paenalcaligenes* and *Arcobacter* were also observed to have increased significantly in relative abundance after the addition of sodium oleate. Cravo-Laureau et al. (2004) characterized *Desulfomicrobium* as a sulfate-reducing bacterium able to use oleate as substrate (Cravo-Laureau, Matheron et al. 2004). Nevertheless, other genera (*Marinospirillum, Aminobacterium, Paenalcaligenes*) that increased in relative abundance are not known for performing ß-oxidation according to the literature.

Regarding Archaea, *Methanoculleus* was found to be the most abundant genus, with no significant difference in relative abundance before and after sodium oleate addition. A significant increase of *Methanosarcina* and *Methanothermobacter* was also observed. However, even with an increase of 4.1 and 8.4 folds, *Methanosarcina* and *Methanothermobacter* still had much lower

relative abundance compared to *Methanoculleus*. The relative abundance of aceticlastic and hydrogenothrophic methanogens remained unchained. This is also expected, as LCFA are fully decomposed to acetate and H2, CO2 and thus provide substrate for both groups of methanogens. This observation is in accordance with Hori et al. (2006), suggesting that the main methanogenesis pathway was not affected by the addition of sodium oleate (Hori, Haruta et al. 2006).

CONCLUSIONS

The results of this study show that addition of LCFA inhibited the process as immediate increase of VFA, pH drop, and decrease in methane yield was observed. The inhibition, however, lasted for a short period and was reversible. Methane yield increased as a result of a change in the microbial population, with the increase in relative abundance of genera specialized in LCFA degradation. The results indicated that *Syntrophomonas* was the main genus that performed ß-oxidation and degraded the added sodium oleate. *Pseudomonas, Clostridium XI* and *Clostridium III* were the most abundant genera found and probably involved in intermediates degradation. *Methanoculleus* was the main methanogen found in the reactors and its relative abundance was not significantly affected by the addition of sodium oleate, indicating that the main methanogenesis pathway did not shift.

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REFERENCES

Angelidaki, I. and B. K. Ahring (1992). "Effects of Free Long-Chain Fatty-Acids on Thermophilic Anaerobic-Digestion." *Applied Microbiology and Biotechnology* **37**(6): 808-812.

Chen, Y., J. J. Cheng and K. S. Creamer (2008). "Inhibition of anaerobic digestion process: A review." *Bioresource Technology* **99**(10): 4044-4064.

Cravo-Laureau, C., R. Matheron, J.-L. Cayol, C. Joulian and A. Hirschler-Réa (2004). "Desulfatibacillum aliphaticivorans gen. nov., sp. nov., an n-alkane-and n-alkene-degrading, sulfate-reducing bacterium." *International journal of systematic and evolutionary microbiology* **54**(1): 77-83.

Hori, T., S. Haruta, Y. Ueno, M. Ishii and Y. Igarashi (2006). "Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester." *Applied and environmental microbiology* **72**(2): 1623-1630.

Klindworth, A., E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn and F. O. Glockner (2013). "Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies." *Nucleic Acids Research* **41**(1).

Kougias, P. G., D. De Francisci, L. Treu, S. Campanaro and I. Angelidaki (2014). "Microbial analysis in biogas reactors suffering by foaming incidents." *Bioresource Technology.***167**: 24-32

Lalman, J. D. A. (2000). Anaerobic Degradation of Linoleic (Clsa), Oleic (C18: i) and Stearic (Clsa) Acids and their Inhibitory Effects on Acidogens, Acetogens and Methanogens, University of Toronto.

Liu, F. H., S. B. Wang, J. S. Zhang, J. Zhang, X. Yan, H. K. Zhou, G. P. Zhao and Z. H. Zhou (2009). "The structure of the bacterial and archaeal community in a biogas digester as revealed by denaturing gradient gel electrophoresis and 16S rDNA sequencing analysis." *Journal of Applied Microbiology* **106**(3): 952-966.

McInerney, M. J., M. P. Bryant, R. B. Hespell and J. W. Costerton (1981). "Syntrophomonas wolfei gen. nov. sp. nov., an Anaerobic, Syntrophic, Fatty Acid-Oxidizing Bacterium." *Appl Environ Microbiol* **41**(4): 1029-1039.

Shiratori, H., H. Ikeno, S. Ayame, N. Kataoka, A. Miya, K. Hosono, T. Beppu and K. Ueda (2006). "Isolation and characterization of a new Clostridium sp. that performs effective cellulosic waste digestion in a thermophilic methanogenic bioreactor." *Applied and environmental microbiology* **72**(5): 3702-3709.

Sousa, D. Z., H. Smidt, M. M. Alves and A. J. Stams (2007). "Syntrophomonas zehnderi sp. nov., an anaerobe that degrades long-chain fatty acids in co-culture with Methanobacterium formicicum." *International journal of systematic and evolutionary microbiology* **57**(3): 609-615.

Sousa, D. Z., H. Smidt, M. M. Alves and A. J. Stams (2009). "Ecophysiology of syntrophic communities that degrade saturated and unsaturated long- chain fatty acids." *FEMS microbiology ecology* **68**(3): 257-272.

von Graevenitz, A. (1976). "Genetics and Biochemistry of Pseudomonas." *The Yale journal of biology and medicine* **49**(3): 308.

Wang, Q., G. M. Garrity, J. M. Tiedje and J. R. Cole (2007). "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy." *Applied and environmental microbiology* **73**(16): 5261-5267.