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ARTICLE

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Methane Production in an UASB Reactor Operated Under Periodic Mesophilic–Thermophilic Conditions

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ABSTRACT: Methane production was studied in a laboratory-scale 10 L anaerobic upflow sludge bed (UASB) reactor with periodic variations of the reactor temperature. On a daily basis the temperature was varied between 35 and 45°C or 35 and 55°C with a heating period of 6 h. Each temperature increase was accompanied by an increase in methane production and a decrease in the concentration of soluble organic matter in the effluent. In comparison to a reactor operated at 35°C, a net increase in methane production of up to 22% was observed. Batch activity tests demonstrated a tolerance of mesophilic methanogenic populations to short-term, 2–6 h, temperature increases, although activity of acetoclastic methanogens decreased after 6 h exposure to a temperature of 55°C. 16S sequencing of DGGE bands revealed proliferation of temperature-tolerant *Methanospirillum hungatii* sp. in the reactor.

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KEYWORDS: periodic operation; temperature; thermophilic–mesophilic consortium; anaerobic degradation

Introduction

Temperature is an important operating parameter of the anaerobic degradation process. The influence of temperature on microbial growth and biodegradation rate can be described by the Arrhenius equation (Batstone et al., 2002; Hao et al., 2002; Siegrist et al., 2002). Operation of anaerobic reactors under thermophilic conditions offers a number of advantages such as increased reaction rates and improved biodegradability of organic compounds (Kim et al., 2002; Rintala, 1997). However, startup and operation of a thermophilic reactor is cumbersome due to the high sensitivity of thermophilic microorganisms to variations in organic loading rate, influent composition, reactor pH, and other factors.

It is generally assumed that a transition from mesophilic to thermophilic conditions is accompanied by a significant (over 80%) and lengthy (over 4 days) decrease in methane production due to adaptation of methanogens to thermophilic temperatures (van Lier et al., 1992; Visser et al., 1993) Nevertheless, mesophilic methanogenic populations were shown to tolerate short-term temperature increases (Ahn and Forster, 2002; Speece and Kem, 1970) or sludge exchange between mesophilic and thermophilic reactors (Song et al., 2004).

Our recent study confirmed a tolerance of mesophilic populations to short-term temperature increases (Morel et al., 2007). In this study, the temperature was increased in response to increasing organic load resulting in improved chemical oxygen demand (COD) removal and increased methane production. In order to avoid reactor failure due to temperature-related inactivation of mesophilic methanogens, duration of the thermophilic phase was limited to 6–8 h. This control strategy resulted in successful stabilization of the effluent COD concentration.

In the present study the concept of mesophilic–thermophilic operation of an anaerobic reactor is further investigated with the aim of developing an anaerobic methane-producing microbial consortium capable of operating in a broad range of temperatures.

Materials and Methods

Chemicals, Media Composition, and Analytical Methods

Yeast extract was obtained from Lallemand (Rexdale, ON, Canada). Chemicals were obtained from Sigma–Aldrich Canada (Oakville, ON, Canada). Stock solution of synthetic wastewater had a COD content of 315 g COD L^{-1} and

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contained (in g–L^{-1}): sucrose 100, yeast extract 60, whey 100, KH_2PO_4 3, K_2HPO_4 3.5, and NH_4HCO_3 34. Stock solution of trace metals contained (in g L^{-1}): $AlK(SO_4)\cdot 12H_2O$ 0.0006; H_3BO_3 0.001; $Ca(NO_3)_2\cdot 4\ H_2O$ 0.5351; $Co(NO_3)_2\cdot 6H_2O$ 0.0075; $Cu(SO_4)$ 0.0003; $Fe(SO_4)\cdot 7H_2O$ 0.0546; $MgSO_4$ 0.1973; $Mn(SO_4)\cdot H_2O$ 0.0151; $Na_2(MoO_4)\cdot 2H_2O$ 0.0023; $NiSO_4\cdot 6H_2O$ 0.0007; Na_2SeO_4 0.0013; and $ZnSO_4\cdot 7H_2O$ 0.0035. Bicarbonate buffer was composed of 1.36 g L^{-1} of $NaHCO_3$ and 1.74 g L^{-1} of $KHCO_3$. Phosphate buffer was composed of (in g L^{-1}) K_2HPO_4 4.0; Na_2HPO_4 2.7; $NaH_2PO_4\cdot H2O$ 1.1.

Analytical measurements of COD, suspended solids (SS), and volatile suspended solids (VSS) were carried out according to Standard Methods (APHA, 1995). Volatile fatty acid (VFA) concentration in the effluent was determined using a gas chromatograph (Sigma, 2000, Perkin-Elmer, Norwalk, CT) equipped with a 91 cm × 4 mm i.d. glass column packed with 60/80 Carbopack C/0.3% Carbopack 20 NH_3PO_4 (Supelco, Mississauga, Ontario, Canada). Gas-phase concentrations of methane, hydrogen, and carbon dioxide were measured by gas chromatography (Sigma, 2000, Perkin-Elmer) equipped with a thermal conductive detector. More details are provided in Morel et al. (2007).

Batch Activity Tests

Batch activity tests were carried out in 60 mL serum bottles maintained under anaerobic conditions. All tests were carried out in triplicates. Anaerobic sludge samples of the inoculum, reactor R-1 (mesophilic), and reactor R-2 (temperature cycles) were used for the tests. At startup, the bottles were inoculated with anaerobic sludge diluted in phosphate buffer to a final volume of 10 mL and a concentration of approximately 5 g VSS L^{-1}. Synthetic wastewater or acetate was added at startup to obtain an initial COD concentration of 4 g L^{-1}. The bottles were flushed with N_2/CO_2 (80%/20%) and then incubated on a rotary shaker (New Brunswick Scientific Co., Edison, NJ) for a period of 26 h at 100 rpm at a constant temperature. To avoid substrate limitation, bottles were spiked after 24 h at the same initial substrate concentration as used in the startup. Concentration of methane in headspace as well as the volume of biogas produced were measured at $t = 2, 6, 24$, and 26 h. The volume of biogas accumulated in the bottle headspace was measured using a burette (gas displacement method). At the end of the test the VSS content of each bottle was determined. Methane production rates were calculated by dividing the quantity of methane produced between the measurements by VSS values and time intervals between the measurements.

Primers, Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE)

Specific archaeal primer GC-931 (primer 931 plus a GC clamp attached at its 5′ end, underlined below) and a reverse universal primer 1392 supplied by MicroArray Lab (Biotechnology Research Institute Montreal, QC, Canada), were used in this study to amplify archaeabacterial 16S rDNA. The nucleotide sequence of the primers were as follows: Primer GC-931: 5′-CGCCCGCCGCGCGCGG-CGGGCGGGGCGGGGGCACGGGGGGAGGAATTGGC-GGGGGAGCA-3′; primer 1392: 5′-ACGGGCGGTGTGT-(G/A)C-3′.

Genomic DNA extraction and PCR conditions were the same as described previously (Tresse et al., 2002) except that the annealing temperature of the touch down PCR was 66–56°C. PCR products were verified on a 1.5% agarose gel after SYBR Safe (Invitrogen, Carlsbad, CA) staining.

DGGE analysis of PCR products was performed with a Bio-Rad D-Code System (Bio-Rad Laboratories, Mississauga, Ontario, Canada). PCR samples were concentrated and 300 ng were loaded onto a 6.5% (w/v) polyacrylamide gel containing a 40–60% gradient of denaturant (80% denaturant correspond to 5.6 M urea and 32% (v/v) deionized formamide). Other procedures were as described in Tresse et al. (2002).

Bands of interest were reamplified, purified and sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing ready reaction Kit (PE Biosystems, Foster City, CA) according to the manufacturer's instruction. Sequences were read with an automated DNA Sequencer (ABI PRISM 377 DNA Sequencer; PE Biosystems) and submitted for comparison to GenBank database using BLAST algorithms.

Reactor Setup and Instrumentation

A 10 L upflow anaerobic sludge bed (UASB) reactor with an external recirculation line was used for the experiments (Morel et al., 2007). The reactor was equipped with a water jacket and a water heating system for temperature control. Synthetic wastewater and trace metals were added to the bicarbonate buffer stream at a rate of 0.4 L day^{-1} each. The total influent flow rate was 10 L day^{-1}, which corresponded to a hydraulic retention time (HRT) of 24 h. The reactor was inoculated with 3 L of a mesophilic anaerobic granular sludge from a wastewater plant (A. Lassonde Inc., Rougemont, QC, Canada), which had an average VSS content of 50 g L^{-1}.

Biogas production and composition were measured on-line using an electronic bubble counter and a methane analyzer (Nova Analytical Systems, Hamilton, ON, Canada), respectively. Twenty milliliter liquid samples were periodically withdrawn from the external recirculation line for COD and VFA analysis. Reactor pH was measured by means of a pH-meter (Cole-Parmer Instrument, Vernon Hills, IL) equipped with a probe, which was inserted in the external recirculation line. Reactor pH was controlled by NaOH addition. TH series temperature sensors (Roctest, Saint-Lambert, QC, Canada) were used for on-line measurements of temperature in the reactor, water jacket, and air. A PC equipped with a PC-1200 acquisition board (National

Instruments, Austin, TX) was used for data acquisition and pump control. The software for reactor monitoring and control was developed in-house using Visual Basic v6 (Microsoft Corporation, Redmond, WA) and MATLAB (MathWorks Inc., Natick, MA).

Results

Reactor Experiments

For comparison purposes, two reactor experiments were sequentially carried out. In the first reactor run (R-1) the effect of an abrupt transition from mesophilic to thermophilic conditions on methane production and COD removal was studied. The second reactor run (R-2) was aimed at evaluating reactor performance under periodic temperature variations between mesophilic and thermophilic conditions (T-cycle). Therefore, R-1 served as a basis for comparison with periodic temperature variations in R-2.

The first run was started up at a temperature of 35°C and an OLR of 2.6 g COD L_R^{-1} day^{-1}. After 4 days of reactor operation methane production stabilized at 6.4 L_{STP} day^{-1} and OLR was increased to 5.2 g L_R^{-1} day^{-1}. On day 13 OLR was again increased to 7.7 g L_R^{-1} day^{-1}. At this organic load methane production reached 12.7 L_{STP} day^{-1}, while total COD (tCOD) and soluble COD (sCOD) concentrations in the effluent stabilized at 1.4 and 0.55 g L^{-1}, respectively. Effluent VFA concentrations were relatively low (61 and 44 mg L^{-1} for acetate and butyrate, respectively). High sCOD concentration suggested that the reactor was operated near its maximal degradation capacity. This performance was considered sufficient for the purpose of the test and on day 20 the reactor temperature was abruptly increased from 35 to 55°C. Three days later methane production drastically declined demonstrating reactor failure due to an abrupt mesophilic–thermophilic transition (Fig. 1).

Following this experiment, the reactor was re-inoculated with the same anaerobic sludge as used for R-1 startup and reactor operation was re-started at the same OLR and temperature as in the first run (Fig. 2A). After a 4-day adaptation period, reactor performance was similar to that observed in R-1 with methane production at 5.5 L_{STP} day^{-1}. Periodic variations of the reactor temperature were started on day 5. The temperature was changed daily from 35 to 45°C (days 5–18), from 35 to 52°C (days 25–34), then from 35 to 55°C (days 35–42). A heating period of 6 h was applied within each cycle. Also, OLR was gradually increased to 7.7 g L_R^{-1} day^{-1} (day 25, Fig. 2A) and then on day 40 to 8.3 g L_R^{-1} day^{-1}.

On-line monitoring of biogas production and composition showed that during each cycle, methane production closely followed temperature variations (Fig. 2B). Dynamics of methane production during a 35–55°C temperature cycle is described in more detail in Figure 3A. Prior to heating, an average methane production rate of 17.3 ± 0.9 L_{STP} day^{-1} was measured. Upon heating for 1.5 h reactor temperature reached 45°C and methane production increased to 25.7 L_{STP} day^{-1} (a momentary drop in methane production at the startup of heating was caused by liquid sampling). While reactor temperature was further increased to 55°C, methane production was unchanged at 25.7 L_{STP} day^{-1}. The onset of temperature decrease at the end of the heating

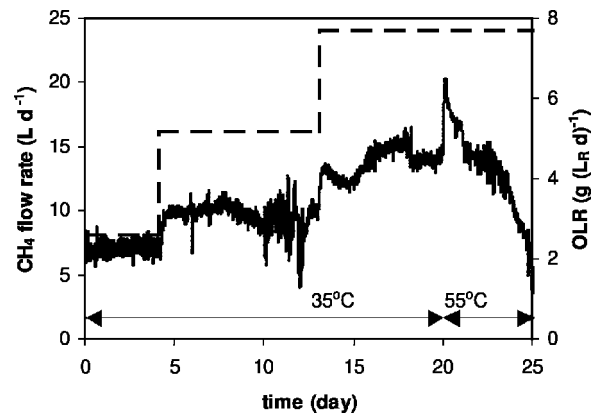

Figure 1. Methane production in R-1 during reactor operation at 35°C followed by an abrupt temperature increase to 55°C on day 20. Dashed line shows applied organic load.

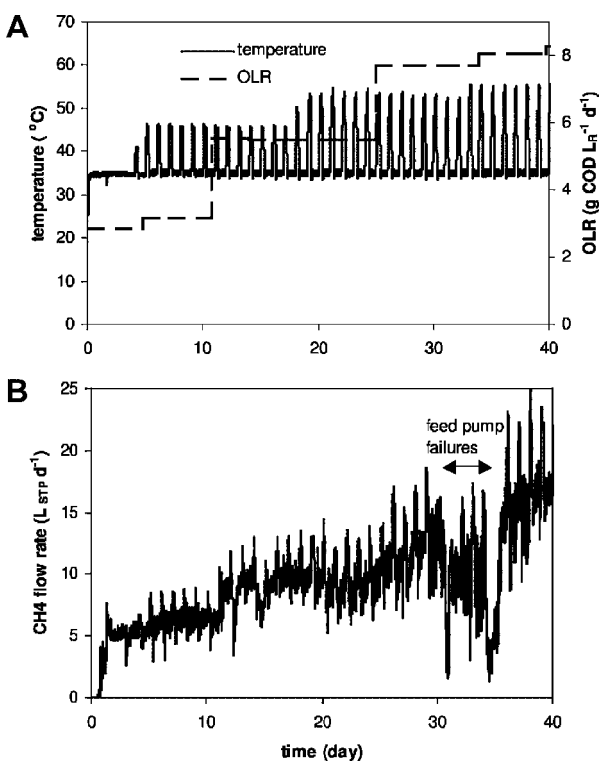

Figure 2. Operating conditions (**A**) and methane production rate (**B**) in R-2 during first 40 days of reactor operation with daily temperature cycles. Feed pump malfunctioned between days 31 and 35 resulting in low feed rates.

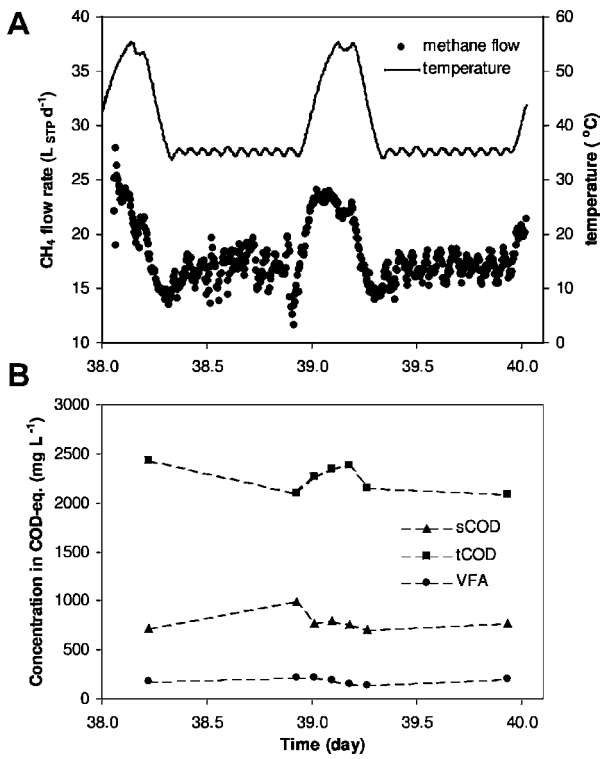

Figure 3. Variations of reactor temperature and methane production (**A**) and total COD, soluble COD and VFA concentrations during a temperature cycle on day 39 (**B**).

period was accompanied by an immediate decrease in methane production, which dipped to 14.6 L_{STP} day^{-1} once reactor temperature was 35°C and then returned to 17.3 L_{STP} day^{-1} after 2 h (Fig. 3A). Analysis of effluent COD and VFA concentrations during several cycles showed that the increase in methane production was accompanied by increased concentrations of total COD, while concentrations of soluble COD and VFAs were decreasing during each heating period (Fig. 3B). In spite of increased solids washout during each heating cycle, visual inspection of granular sludge at the end of the experiment showed that the structure and integrity of the anaerobic granules was not affected. The average VSS of the sludge bed increased from 60.4 to 79.7 g L^{-1} during the 49-day experiment.

In addition to reactor operation with daily temperature cycles, reactor transition to the thermophilic mode of operation was also tested. For this test, the reactor temperature was maintained at 35°C between days 42 and 45. Then on day 45 conditions leading to reactor failure in R-1 were reproduced. The temperature was increased to 55°C and maintained at this level for the next 4 days. An average methane production rate of 16.5 ± 1.1 L_{STP} day^{-1} was observed prior to this temperature increase. After an initial peak in methane production upon temperature increase, on days 48–49 methane production rate was stabilized at 19.4 ± 1.1 L_{STP} day^{-1}.

Batch Tests

To evaluate the impact of temperature variations on microbial populations of the anaerobic reactor, batch activity tests were carried out using samples of the inoculum sludge and sludges withdrawn from R-1 and R-2 reactors on day 20 of each reactor operation (i.e., prior to thermophilic operation of R-1). The tests were carried out at mesophilic (35°C) and thermophilic (55°C) temperatures. At each temperature, both synthetic wastewater (which contained sucrose, whey, and yeast extract) and acetate were used as carbon sources.

In the 35°C tests, methane production was similar for R-1 and R-2 samples at all sampling times and these values were significantly higher than those of the inoculum sludge (Fig. 4A). Also, similar methane production rates were observed in bottles with synthetic wastewater and acetate as a carbon source. By the end of the test methane production slightly increased in all bottles, apparently due to sludge adaptation to test conditions (e.g., pH).

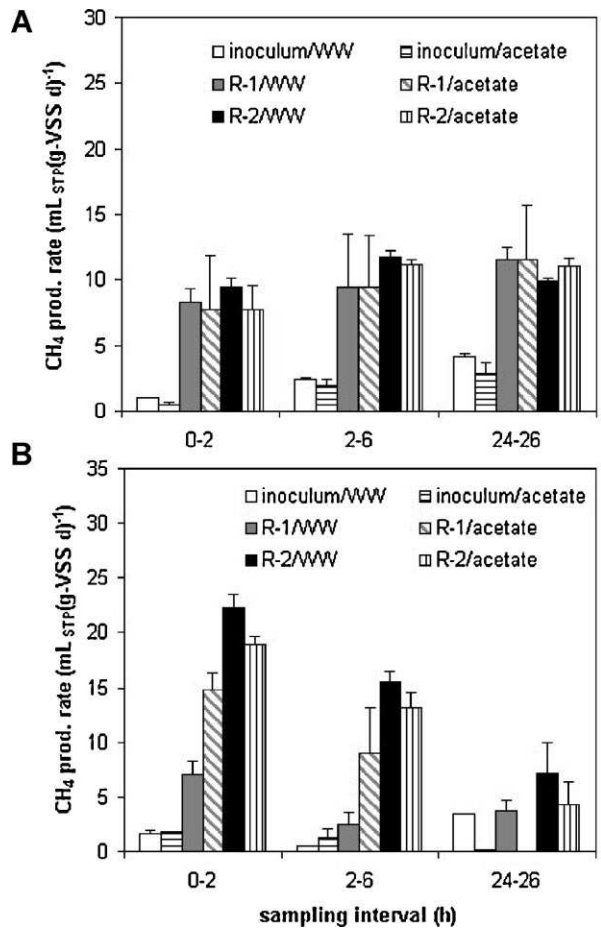

Figure 4. Results of batch activity tests on synthetic wastewater (WW) and acetate at 35°C (**A**) and 55°C (**B**) using inoculum, R-1, and R-2 sludges. In both reactor runs sludge samples were taken on day 20 of reactor operation. Samples are denoted by their origin (inoculum, R-1, R-2) and the type of substrate used for the test (acetate or synthetic wastewater composed of sucrose, whey, and yeast extract).

During the 55°C tests on synthetic wastewater and acetate (Fig. 4B), methane production doubled in all R-2 samples and almost doubled in R-1 samples on acetate at $t = 2$ h as compared to tests carried out at 35°C. However, the next sampling ($t = 6$ h) revealed significant differences between R-1 and R-2 samples. While in R-2 bottles methane production rate remained high, production rate in R-1 bottles became comparable to values observed under mesophilic conditions between 2 and 6 h. Furthermore, methane production on acetate between 24 and 26 h (that is after substrate addition at $t = 24$ h) dropped to zero in all bottles with the exception of R-2. On synthetic wastewater, between 24 and 26 h, methane production in R-1 bottles was low (3.7 mL_{STP} (g-VSS day)$^{-1}$) while in R-2 bottles methane production was 7.2 mL_{STP} (g-VSS day)$^{-1}$, that is, comparable to that under mesophilic conditions (Fig. 4A, 8–10 mL_{STP} (g day)$^{-1}$).

DGGE and 16S Sequencing

The distribution of *Archaeal* populations in two reactor experiments was studied using sludge samples obtained on day 20 of each reactor experiment. In addition, inoculum sludge was analyzed. DGGE profiles were obtained using *Archaeal* primers. A comparison of R1 and R2 profiles against the inoculum showed an overall decrease in the diversity of Archaeal populations and the emergence of few predominant bands in each reactor (Fig. 5). 16S sequencing of band #1, which was visible on all profiles, corresponded to *Methanosaeta* spp. (97% similarity). Band #2 was visible on R-1 and R-2 profiles, but not on the inoculum profile. This band sequence was identified as belonging to *Methanospirillum* spp. (95% similarity). Finally, Band #3 was only visible on R-2 profile and its sequence corresponded to *Methanospirillum hungatii* (97% similarity).

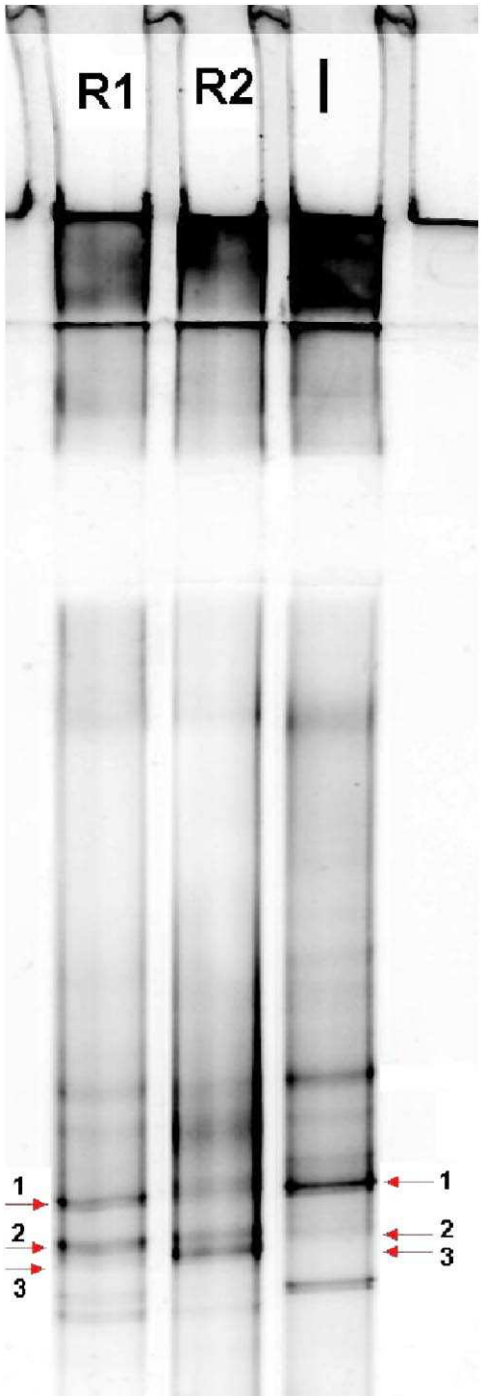

Figure 5. DGGE profiles of Archaea populations in the inoculum sludge (I), mesophilic sludge (R1, day 20), and sludge from a reactor with periodic temperature variations (R2, day 20). Band identification: Band #1—Methanosaeta sp. (97% similarity); Band #2—*Methanospirillum* sp. (95% similarity); Band #3—*Methanospirillum hungatii* (97% similarity).

Discussion

Our previous research demonstrated tolerance of mesophilic methanogenic populations to relatively short thermophilic periods (Morel et al., 2007). A temperature-based reactor control strategy was demonstrated, in which the temperature was increased for a limited time in order to improve reactor performance during organic overloads. Following these results, we hypothesized that periodic variations of reactor temperature to create a sequence of mesophilic and thermophilic conditions might improve the overall reactor performance. Indeed, experimental results presented in this study showed that increased methane production and improved methane yields could be achieved when operating a reactor at moderate to high organic loads.

Importantly, periodic temperature variations did not require a lengthy adaptation of microbial populations to new operating conditions. The onset of temperature cycles did not have an adverse effect on methane production in the reactor, as shown in Figure 2B. Furthermore, an increase to 55°C in R-1 led to reactor failure after 4 days of reactor operation at this temperature. A similar 4 day temperature increase in R-2, which was operated in T-cycle mode for

45 days prior to the temperature increase, did not lead to a failure. Likely, periodic temperature variations may be used as a thermophilic reactor startup strategy.

The observed tolerance of microbial populations to a broad range of temperatures can be explained by the development of a mixed mesophilic–thermophilic consortium of anaerobic microorganisms. This hypothesis was corroborated by a comparison of methane production rates in batch tests. At 35°C, methane production was similar in R-1 and R-2 bottles, that is, mesophilic populations in R-2 were not affected by temperature variations (Fig. 4A). Major differences between R-1 and R-2 bottles were observed in the 55°C tests (Fig. 4B). In both R-1 and R-2 bottles, methanogenic activity almost doubled during the first 2 h of the test. However, between 2 and 6 h, methane production declined in R-1, but not in R-2 bottles. Furthermore, between 24 and 26 h, R-2 bottles maintained methanogenic activity on both substrates, suggesting the presence of thermophilic or at least temperature-tolerant methanogens in the corresponding reactor (Fig. 4B). In R-1 bottles, methane production between 24 and 26 h was only observed on synthetic wastewater, which contained glucose, whey, and yeast extract. Based on this observation, it can be suggested that temperature-tolerant methanogens were also present in R-1 and these were likely hydrogenophilic species.

The differences in microbial populations of the two reactors were confirmed by 16S-sequencing of bands extracted from the DGGE gel. By day 20 both reactors were operated at relatively high organic loads of up to 7.7 L_R^{-1} day^{-1} thus creating substrate non-limiting conditions for acetoclastic and hydrogenophilic methanogens. The inoculum sludge contained a significant amount of acetoclastic methanogens and these species were retained in the reactors as was evidenced by the presence of Band #1, corresponding to *Methanosaeta* spp., in all samples. In addition, hydrogenophilic methanogens proliferated in both reactors. Band #2 identified as *Methanospirillum* spp., was not visible on the DGGE profile of the inoculum sludge but was observed in both R-1 and R-2 samples. Furthermore, Band #3, corresponding to *M. hungatii*, was only visible in R-2 sample. In the literature, it has been demonstrated that the optimal temperature for methane production for at least one strain of this microorganism (*M. hungatei* GF1) is between 40 and 45°C with sustainable methane production up to 55°C (Patel et al., 1976). Thus, proliferation of *M. hungatei* in R-2 can be attributed to its ability to withstand elevated reactor temperatures.

Along with the development of a temperature-tolerant microbial consortium, periodic temperature variations in R-2 resulted in improved performance. Methane production in R-1 and R-2 was comparable at the lowest OLRs only, when both reactors were underloaded. Once OLR increased, the difference became apparent. At an organic load of 7.7 g COD L_R^{-1} day^{-1} methane yields of 0.16 and 0.195 ± 0.07 L_{STP} $(g\text{-}COD)^{-1}$ were obtained in R-1 (mesophilic) and R-2 (T-cycle) runs, respectively, that is an improvement of 22% was achieved (Fig. 6). Notably, the influent stream

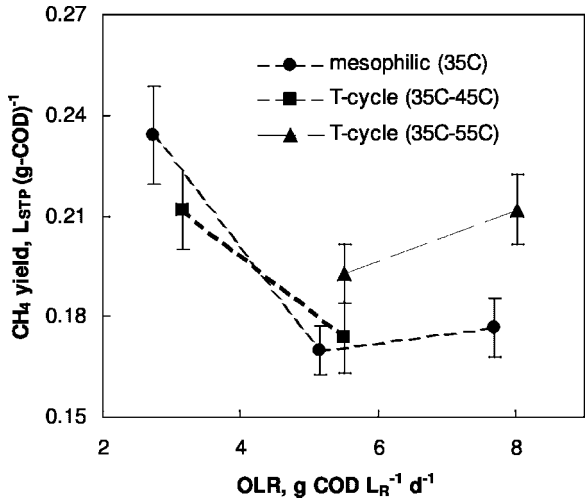

Figure 6. Comparison of methane yields in R-1 and R-2 runs. R-2 was operated either at 35–45 or 35–55°C temperature cycles. The yields were calculated based on the difference between total COD fed to the reactor and soluble COD in the reactor effluent.

contained 24% of solid materials. Methane yield was calculated based on the difference between the amount of total COD fed to the reactor and the amount of soluble COD in the reactor effluent. This method was used to exclude biomass washout from the calculation and resulted in an underestimation of the methane yield, as it excluded non-degraded solids in the effluent stream.

Higher methane production in R-2 was accompanied by slightly higher concentration of sCOD as compared to R-1, while VFA concentrations remained similar. For instance, at an OLR of 7.7 g L_R^{-1} day^{-1} average (per cycle) effluent sCOD concentrations were 0.55 and 0.7 g L^{-1} in R-1 and R-2, respectively. VFA concentrations were at around 0.2 g L^{-1} in both reactors. At the same OLR, average tCOD concentrations were 1.5 and 1.2 g L^{-1} for R-1 and R-2, respectively. Therefore, periodic temperature increases resulted in improved hydrolysis and degradation of organic materials, solids in particular, which were used for methane production. Consequently, methane yields were improved, as shown in Figure 6.

This improvement can be attributed to several factors. As discussed above, proliferation of thermophilic microorganisms with higher substrate consumption rates might play a role. Also an increase of methane production during each heating cycle might be explained by increased enzymatic activity, which follows the Arrhenius dependence (Siegrist et al., 2002). This may have contributed to increased methane production observed during initial hours of each temperature increase. However, the increase in methane production exceeded the observed decrease of sCOD concentration. During each heating period methane production increased by 40–50%, while sCOD concentrations decreased by 30–35%.

Calculations showed that changes in methane solubility due to temperature differences accounted for less than 1% of the observed increase in methane production. It was hypothesized that heating increased hydrolysis rates of solids, such as yeast extract, fed to the reactor and accumulated during the mesophilic part of the cycle. These solids could accumulate during mesophilic reactor operation and hydrolyze at a higher temperature. A peak of methane production was observed immediately after each temperature increase. The increased rate of methane production was followed by either decreasing enzymatic activity or substrate limitation, once all of the surplus substrate was consumed. Overall, the duration of thermophilic periods had to be limited.

An increased methane production during heating periods led to increased lift-off and washout of inert solids and suspended biomass, which was reflected in increased tCOD concentration in the reactor effluent (Fig. 3B). Nevertheless, sludge granulation was not affected. Furthermore, batch tests showed an increase in the volumetric rate of methane production in comparison to the inoculum sludge, that is, the amount of inert organic materials within the granules did not increase. Retention and formation of anaerobic granules should be attributed to hydrodynamic conditions created in the UASB reactor by liquid recirculation. Notably, R-1 and R-2 tests were carried out at a high ($2\ m\ h^{-1}$) upflow velocity, which not only minimized COD and VFA gradients along the reactor height, but also promoted formation and retention of anaerobic granules.

It is worth noting that additional energy input is required to implement the proposed strategy at a large scale. This requirement, however, can be offset by additional production of methane and improved reactor stability. The proposed strategy of reactor operation with periodic temperature variations offers the advantage of flexibility. Mesophilic mode of operation can be used during low load periods while the reactor can be promptly returned to thermophilic conditions, if required. Also, a two-unit reactor setup with liquid and sludge recirculation, as suggested by Song et al. (2004) can be implemented. In this configuration one reactor is operated at mesophilic temperatures while the temperature of the second reactor is maintained within a thermophilic range and controlled as a function of organic load.

Conclusion

This study presents a strategy of anaerobic reactor operation with periodic temperature variations. Application of temperature cycles to a 10 L lab-scale anaerobic reactor resulted in improved overall reactor performance and development of a robust mesophilic–thermophilic consortium of anaerobic microorganisms with near linear response to temperature variations. Consequently, periodic temperature variations can be used to increase the volumetric degradation capacity of a mesophilic anaerobic reactor. Also, the absence of a lag-phase during the mesophilic–thermophilic transitions makes this approach suitable for process control algorithms aimed at avoiding reactor failure due to organic overloads (Morel et al., 2007).

While the experiments demonstrated feasibility of reactor operation with periodic temperature variations, the study was conducted at preset temperature cycle parameters. Optimization of cycle length and temperature amplitude might be required.

Contribution of M.-J. Levesque to DGGE analysis and 16S sequencing of Archaea populations is gratefully acknowledged. NRC publication no. 49099.

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