Anaerobic digestion of domestic wastewater at low temperatures (4, 8 and 15^{o}C) in reactors with psychrophilic inocula.

Evangelos Petropoulos; Jan Dolfing; Emma Bowen; Russell Davenport; Thomas Curtis.
School of Civil Engineering and Geosciences, Newcastle University, Newcastle NE1 7RU, England, UK

Keywords: Low Temperature, Psychrophilic,Cold-adapted, Anaerobic digestion, Extremophiles, Wastewater treatment

Abstract

Low temperature methanogenesis is the most challenging aspect of anaerobic digestion (AD). Thus far attempts to find the lower operating temperature limits were focused on acclimatizing mesophilic sludge to low temperature. This approach may not be appropriate to determine the true limits of AD with respect to temperature and a more representative approach would be the use of distinct psychrophilic communities as seed. Thus 8 batch reactors were inoculated with psychrophilic inoculum (seed:substrate 1:4) collected from the high Arctic to treat raw domestic wastewater (600mgCOD L^{-1}). The temperatures selected were 4, 8 and 15^{o}C. The wastewater was UV-sterilized (110kJ/cm^2) to preclude competition between the indigenous microorganisms from wastewater and those inhabiting the seed. The results showed that wastewater can be treated within 60days achieving an effluent able to meet the UWWTD (91/271/EEC) standards. The removal coefficient was estimated at 0.02 and 0.03day^{-1} for 4 and 15^{o}C, respectively. The CH_4 conversion was lower at lower temperatures indicating that reactors at extreme conditions operate partially as clarifiers. Based on COD removal and VFA production it was concluded that hydrolysis limits the process. Microbiological analysis showed that communities differ at different temperatures for bacteria and archaea. *Methanomicrobiales* and *Methanosaetacea* were equally dominant in methanogenic communities at 15^{o}C. *Methanomicrobiales* were dominant at lower temperatures (4, 8^{o}C) followed by *Methanosaetaceae* suggesting that at low temperature methanogenesis tends to follow the hydrogenotrophic pathway. Communities of *Methanosarcina* were also detected at all temperatures. Specific methanogenic activity at 4, 8 and 15^{o}C were 6.3, 7.6 and 10.3 femtomols CH_4 $cell^{-1}day^{-1}$; hydrolytic activity was estimated at 76.2, 186.6 and 250.9 femtograms COD $cell^{-1}day^{-1}$. The results suggest that inoculating digesters for low temperature operation with psychrophilic communities is a promising way to treat wastewater and appropriate to investigate the limits of the process.

CONCEPT

The results will help to determine if cold-adapted seed can anaerobically treat domestic wastewater to UWWTD standards (COD < 125 mg L^{-1} (UWWTD 91/271/EEC)) at 4, 8, and 15^{o}C. This will tackle the issue of cold-climate countries where ambient temperature decrease causes AD failure, especially nowadays where carbon neutral WW treatment is necessary and oil prices renders financially unsustainable the use of fossil fuels to increase temperature.

RESULTS AND DISCUSSION

Under anoxic conditions COD (chemical oxygen demand) informs about the potential amount of methane generated when waste is converted into methane and carbon dioxide (Heidrich et

al. 2011). COD is one of the most important parameters in wastewater; figure 1 shows that COD has been reduced at levels lower than those suggested from UWWTD (125 mgCOD L^{-1}) at all temperatures after a period of 56 days. This means that in terms of COD the WW effluent can go on to the tertiary treatment or be discharged.

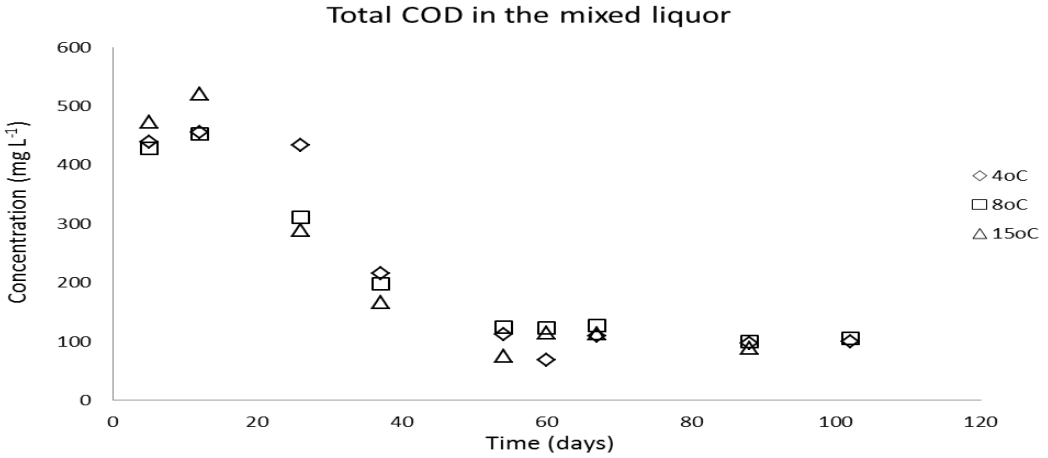

Figure 1 - Total COD in the mixed liquor after 102days of incubation at the particular temperature

The performance in terms of organic load removal and conversion to methane suggests that the inoculum is successful and in 56 days wastewater of 600 mgCOD L^{-1} can be hydrolysed, fermented and converted to methane even at its raw phase (figure 1, 3). The question generated is 'is it really all being hydrolysed?' Comparing the methane production rates and the rates of COD removal from K coefficient (figure 2a, b), both expressed as mgCODday^{-1} a disagreement which is negligible at 15°C but grows larger the lower the temperature gets can be observed. This suggests that the digester acts partially as a clarifier when temperature is decreased. Organic matter settles onto the surface of the reactors and being attached to the biomass so it is impossible to be detected via liquid COD measurements. This difference is approximately 3.2, 1.35 and almost negligible for 4°C, 8°C and 15°C respectively.

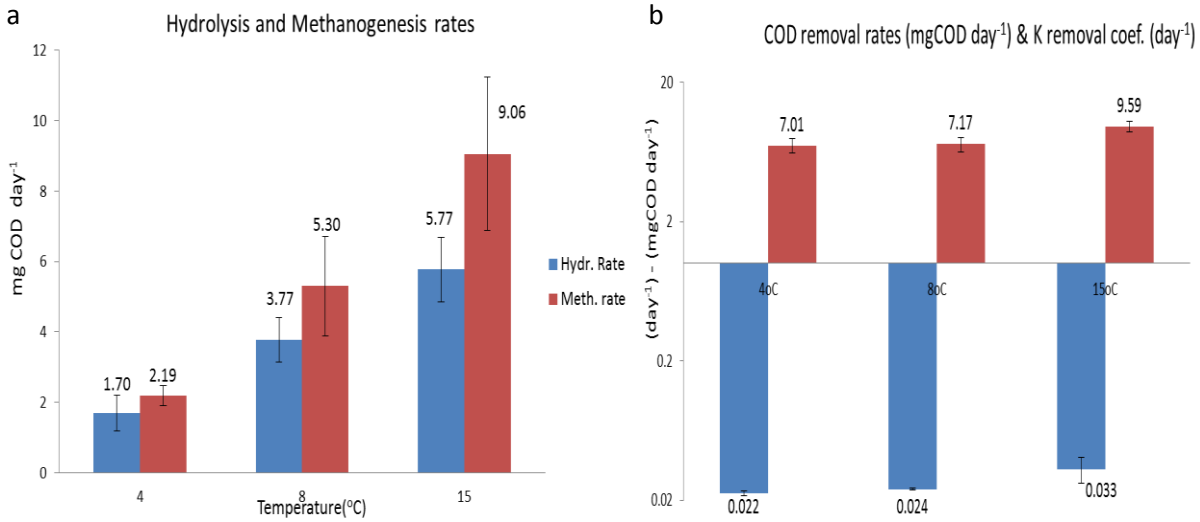

Figure 2 - (a) Hydrolysis and methanogenesis rate for wastewater as substrate at all three conditions; (b) K removal coefficient (day^{-1}) for the reactors seeded with cold adapted microbial communities at 4, 8 and 15°C (day^{-1}); expressed also as removal rate of mgCOD day_{-1}.

After operation of 56 days the amount of CH_4 (via GC-FID) between highest and lowest temperature is only double-folded (figure 3) although the temperature is 3.75 times lower; at 8^{o}C lies in between these two. This suggests that if we increase the ratio 'seed:substrate'>1:4 better performance can be achieved in terms of methane production as the inoculum is cold-adapted and able to treat. The quality of the biogas is significantly high as the percentage of CH_4 was at all 3 temperatures more than 90% (CH_4:(CO_2-CH_4)).

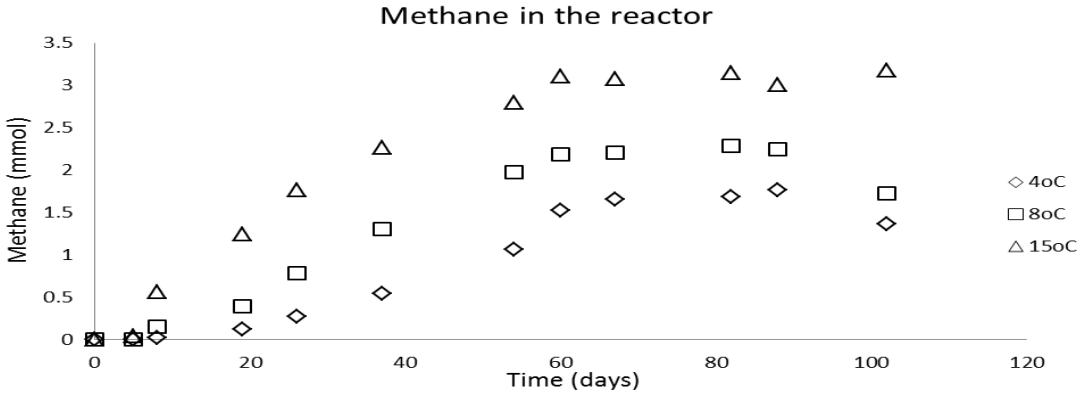

Figure3 - CH_4 formation from wastewater for the particular seed at 3 temperatures for 102 days of incubation

DNA sample was extracted (Q-Biogene Cambridge, UK) on day 56, 16S rRNA gene fragments were PCR amplified, analysed as a DGGE image via Bionumerics (Applied Maths, Austin Texas, US) and Prime6 (Multivariate statistics for ecologists, Lutton, UK) for microbial community analysis at all temperatures. Differentiation between microbial communities was observed between operational temperatures – different dominant bands (data not shown). Statistical analysis of the DGGE image indicates that the bacteria at 4 and 8^{o}C retained a good degree of similarity with a percentage of 85%. This suggests that 4 degrees difference do not cause changes between bacterial communities at these low temperatures. At 15^{o}C the bacterial communities differed from those at 4 and 8^{o}C that formed a cluster, having a lower similarity with the 1st (<75%) (Figure 4b). Similarly for archaea the differences between communities were negligible on the first days of operation according to DGGE analysis. Although the community structure was similar (day 1) differences in intensity advocate that there were differences in abundance of particular taxa (differentiation occurred in previous batch as the experiment was re-fed for 400 days). The differences follow the pattern of temperature differentiation – stronger bands for *Methanosaetaceae* at higher temperature/stronger bands of *Methanomicrobiales* for lower temperature. On the 2nd (day 56) and last one date (day 102) of analysis the differences were even stronger in terms of band intensity. After statistical analysis on day 56 it can be also seen that the seed at 4 and 8^{o}C have a high similarity (>70%) whereas that at 15^{o}C (Figure 4a) have a lower similarity of 60% (P=1). The data above can be supported via qPCR (data not shown) where the dominant taxa at lower temperatures of 4 and 8^{o}C is *Methanomicrobiales*, a strictly hydrogenotrophic methanogen, followed by *Methanosaetaceae*, a strictly aceticlastic methanogen (also in accordance with DGGE pattern). At 15^{o}C the difference between the two taxa was lower than the standard deviation so we cannot be certain of the dominant methanogenic taxon. The trend suggests that lower temperatures are more optimal for hydrogenotrophs compared to acetotrophs.

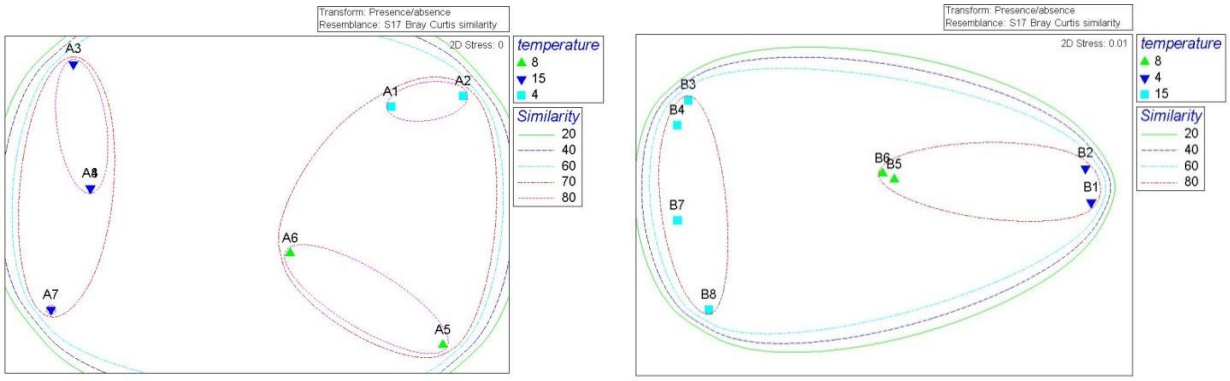

Figure 4 - (a) Similarity test between microbial communities in the reactors for different temperatures; (b) Archaeal test; (b) Bacterial test; both based on presence-absence Bray-Curtis transformation.

Combining the VFA-methane (VFA data not shown) production data with the bacterial cell enumeration estimated by FISH (Coskuner et al. 2005) using Bac338I, II, III probes, and qPCR (Yu et al. 2005) for the methanogens, enabled the specific activity of the cells at these extreme temperature conditions to be estimated (Figure 5a, b):

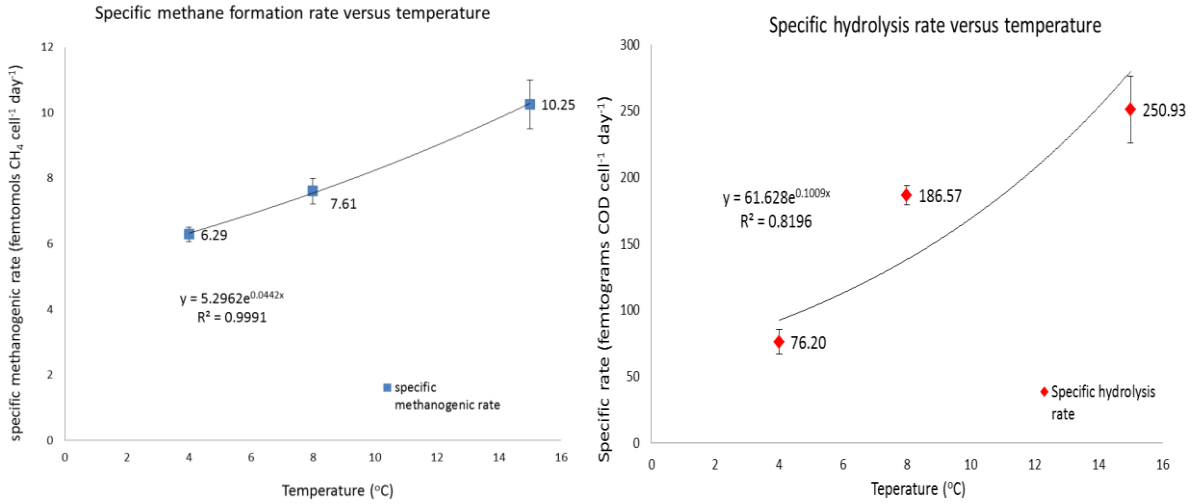

Figure 5 - (a) Specific methanogenic activity per methanogenic cell at all temperatures; (b) specific hydrolytic activity per bacterial cell at all temperatures.

ACKNOWLEDGEMENTS

I would like to thank EPSRC that funded this project (EP/G032033/1) and to acknowledge funding for an RCUK Academic Fellowship (EP/E500501/1).

REFERENCES

Coskuner G., Ballinger S., Davenport R., Pickering R.L., Solera R., Head I.M., Curtis T.P. 2005 Agreement between theory and measurement in quantification of ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* **71** (10), 6325–6334.

Heidrich E., Curtis T.P., Dolfing J. 2011 Determination of the internal chemical energy of wastewater. *Environmental Science & Technology* **45** (2), 827-832.

Yu Y., Lee C., Kim J., Hwang S., 2005 Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering* **89** (6), 670-679.