

# Addition of electron acceptors stimulates methanogenesis from lipids by anaerobic sludge

A.P. Guedes\*, A.J. Cavaleiro\*, S.A. Silva\*, M.M. Alves\*, A.J.M. Stams\*\* and D.Z. Sousa\*

\* IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Portugal (E-mail: [anaguedes@ceb.uminho.pt](mailto:anaguedes@ceb.uminho.pt); [acavaleiro@deb.uminho.pt](mailto:acavaleiro@deb.uminho.pt); [sergiosilva@ceb.uminho.pt](mailto:sergiosilva@ceb.uminho.pt); [madalena.alves@deb.uminho.pt](mailto:madalena.alves@deb.uminho.pt); [dianasousa@deb.uminho.pt](mailto:dianasousa@deb.uminho.pt))

\*\* Laboratory of Microbiology, Wageningen University, The Netherlands (E-mail: [fons.stams@wur.nl](mailto:fons.stams@wur.nl))

## HIGHLIGHTS

Incubation of anaerobic sludge with triolein or oleate in the presence of nitrate or sulphate led to an increased methane production, relatively to incubations without inorganic electron acceptor. Faster methane production was obtained in assays amended with nitrate. Methanogenesis occurred after the reduction of alternative electron acceptors.

## Keywords

LCFAs; Methane; Nitrate; Sulphate

## INTRODUCTION

Lipid-rich wastewaters are attractive substrates for anaerobic digestion to biogas. Lipids are rapidly hydrolysed to glycerol and long-chain fatty acids (LCFA), but LCFA conversion to methane requires interspecies electron transfer via  $H_2$ /formate (Schink & Stams 2006). The presence of alternative electron sinks (such as sulphate and nitrate) in wastewaters streams may impact methane production. Out-competition of methanogens by sulphate-reducers has been previously reported during LCFA degradation, for almost stoichiometric concentrations of sulphate (Sousa *et al.* 2009). Xie *et al.* (2012) studied the effect of nitrate on methanogenesis of wastewater containing high concentrations of volatile fatty acids (VFA). High carbon/nitrate ratios resulted in the simultaneous occurrence of nitrate-reduction and methanogenesis. In this work, we studied the effect of the addition of substoichiometric concentrations of nitrate and sulphate on the anaerobic degradation of lipids and LCFA.

## MATERIAL & METHODS

Anaerobic suspended sludge, collected from a municipal sludge digester (Coimbra, Portugal), was used as inoculum for the batch assays (3gVS L<sup>-1</sup> final concentration). Bottles were prepared with bicarbonate-buffered mineral salt medium and headspace filled with  $N_2/CO_2$  (80/20, 1.7 bar). Triolein (1mM) or sodium oleate (3mM) were used as carbon sources, in independent assays. For each of the substrates, incubations were performed in the presence of either  $KNO_3$  (15mM) or  $Na_2SO_4$  (15mM), or without inorganic electron acceptor. Blank assays (without substrate) were also prepared for each condition tested. Methane was monitored by GC. Nitrate was measured by spectrophotometry and sulphate was quantified using a Hach Lange kit (Hach Lange, Germany).

## RESULTS AND DISCUSSION

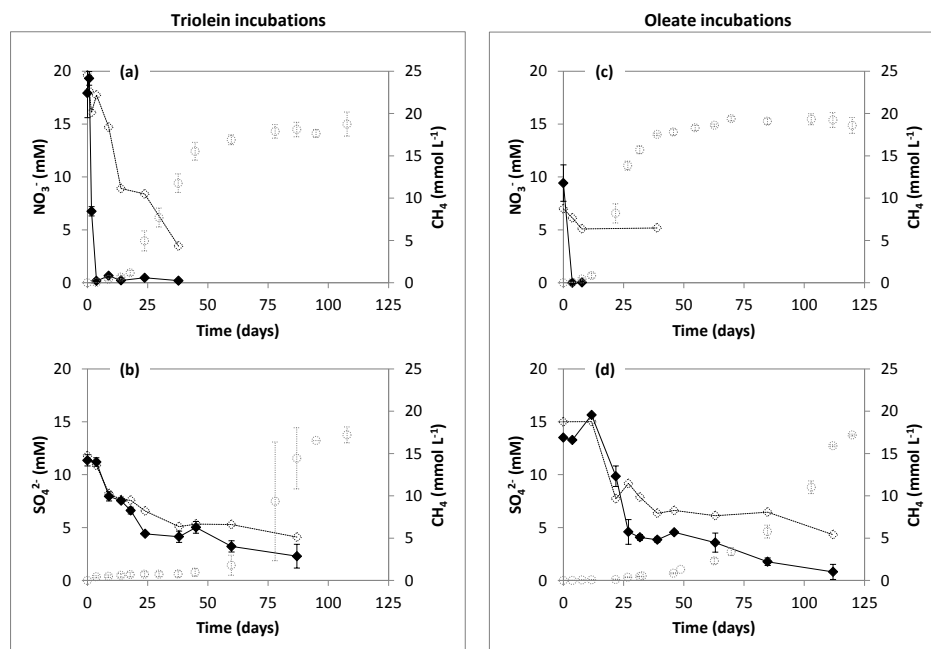
Cumulative methane production from triolein and oleate, in the presence of substoichiometric concentrations of sulphate or nitrate and without inorganic electron acceptor, is shown in Table 1. Methane yields, calculated with basis to the maximum theoretical methane that could be obtained from triolein or oleate in the absence of inorganic electron acceptor, are also presented. In the methanogenic cultures incubated with oleate, methane production was lower than that registered for the blank assay, even after 120 days. This was not observed in triolein assays, where an increment of 17% in the methane production was registered after 57 days of incubation. This could be related to the degradation of glycerol deriving from triolein hydrolysis.

**Table 1.** Methane production in the assays amended with Triolein (1mM) or Oleate (3mM) in the presence and absence of inorganic electron acceptors

|                                      | Triolein  |                           |                       | Oleate  |                           |                       |
|--------------------------------------|---|---------------------------|-----------------------|---|---------------------------|-----------------------|
|                                      | CH <sub>4</sub> production (mmol CH <sub>4</sub> L <sup>-1</sup> ) <sup>a</sup> | CH <sub>4</sub> yield (%) | t (days) <sup>b</sup> | CH <sub>4</sub> production (mmol CH <sub>4</sub> L <sup>-1</sup> ) <sup>a</sup> | CH <sub>4</sub> yield (%) | t (days) <sup>b</sup> |
| NO <sub>3</sub> <sup>3-</sup>        | 18.2±0.2  | 23                        | 46±5                  | 18.9±0.1  | 49                        | 31±2                  |
| SO <sub>4</sub> <sup>2-</sup>        | 15.8±0.9  | 20                        | 85±8                  | 18.2±0.6  | 48                        | 113±7                 |
| no inorganic e <sup>-</sup> acceptor | 13.2±0.8  | 17                        | 57±6                  | 0   | 0                         | n.a.                  |

<sup>a</sup> The background methane production measured in the blank assays was discounted. <sup>b</sup> Time necessary to reach the plateau of the methane production curve. n.a. – non applicable.

Significantly higher methane yields ( $P < 0.03$ ) were obtained in all the assays amended with inorganic electron acceptors relatively to the methanogenic assays. Methane yields observed during triolein and oleate conversion in the presence of sulphate and nitrate were identical, but methane production was faster in assays with nitrate (Table 1, Fig. 1).



**Figure 1.** Methane production (circles) and nitrate and sulphate reduction (black diamonds) in (a,b) triolein and (c,d) oleate assays. Empty diamonds correspond to electron acceptor measured in blank assays.

Nitrate was reduced in less than 5 days in triolein and oleate assays, whereas sulphate reduction was slower. Nitrate and sulphate decrease in blank assays was significant, likely due to the presence of organic material in the inoculum sludge. Onset on methane production occurred after a major part of the inorganic electron acceptor was reduced. Enhancement of methane production in the presence of nitrate and sulphate can be related to incomplete

LCFA oxidation to simpler compounds (e.g. acetate) that are easily converted to methane. Therefore, the addition of alternative electron acceptors, namely nitrate, may be beneficial for improving methanogenesis from lipids or LCFA.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Schink, B., Stams, A.J.M. 2006 Syntrophism among prokaryotes. Dworkin M., Falkow S, Rosenberg E, Schleifer K.-H., Stackebrandt E. The Prokaryotes: an evolving electronic resource for the microbiological community. Ed 3rd, vol 2, 309-335. 2006. New York, Springer-Verlag.
- Sousa, D.Z., Alves, J.I., Alves, M.M., Smidt, H., Stams, A.J.M. 2009 Effect of sulfate on methanogenic communities that degrade unsaturated and saturated long-chain fatty acids (LCFA). *Environ. Microbiol.* **11**(1), 68-80.
- Xie, L., Chen, J.R., Wang, R., Zhou, Q. 2012 Effect of carbon source and COD/NO<sub>3</sub>-N ratio on anaerobic simultaneous denitrification and methanogenesis for high-strength wastewater treatment. *J. Biosci. Bioeng.* **113**(6), 759-764.