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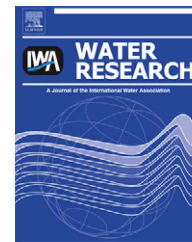

Methane production from oleate: Assessing the bioaugmentation potential of *Syntrophomonas zehnderi*

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ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form
2 July 2010
Accepted 13 July 2010
Available online 21 July 2010

Keywords:
Bioaugmentation
Syntrophomonas zehnderi
Methane
Oleate
Sepiolite

ABSTRACT

The potential for improving long-chain fatty acids (LCFA) conversion to methane was evaluated by bioaugmenting a non-acclimated anaerobic granular sludge with *Syntrophomonas zehnderi*. Batch bioaugmentation assays were performed with and without the solid microcarrier sepiolite, using 1 mM oleate as sole carbon and energy source. When *S. zehnderi* was added to the anaerobic sludge methane production from oleate was faster. High methane yields, i.e. 89 ± 5% and 72 ± 1%, were observed in bioaugmented assays in the absence and presence of sepiolite, respectively. Sepiolite stimulated a faster methane production from oleate and prevented the accumulation of acetate. Acetoclastic activity was affected by oleate in the absence of sepiolite, where methane production rate was 26% lower than in assays with microcarrier.

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1. Introduction

Long-chain fatty acids (LCFA) are produced during the hydrolysis of oils and fats and are commonly present in fatty-wastewaters. These compounds can be efficiently converted to methane in anaerobic bioreactors, if the appropriate technology and feeding strategy are applied (Alves et al., 2009; Cavaleiro et al., 2009). Nonetheless, LCFA accumulation during the degradation of lipid-rich wastewaters has been frequently described. Broughton et al. (1998) reported the accumulation of stearate and palmitate during batch degradation of sheep tallow, and these LCFA were also found in bioreactors fed with milk-oleate synthetic wastewaters (Cavaleiro et al., 2009; Pereira et al., 2002).

Complete LCFA degradation evolves through the coordinated activity of syntrophic bacteria, which convert LCFA to acetate and hydrogen, and methanogenic archaea that utilize these substrates, making the overall conversion energetically possible (Stams, 1994). Presently, there are 7 species of syntrophic bacteria reported as capable of growing on LCFA with more than 12 carbon atoms. Among these bacteria only 3 mesophilic species can utilize unsaturated LCFA, namely *Syntrophomonas sapovorans*, *Syntrophomonas curvata* and *Syntrophomonas zehnderi* (Sousa et al., 2009).

Several authors reported the presence of syntrophic acetogenic bacteria belonging to the Syntrophomonadacea family in microbial communities degrading different LCFA, either in mesophilic enrichment cultures (Hatamoto et al., 2007; Sousa et al., 2007b) or in continuous bioreactors (Sousa et al., 2007a). In the work from Sousa et al. (2007a) *Syntrophomonas*-related microorganisms appear as predominant microorganisms in sludges that were submitted to contact with saturated and unsaturated LCFA, but not in the sludge used as inoculum for the bioreactors. Thus, extended contact with LCFA might stimulate the occurrence of syntrophic bacteria (Sousa et al., 2007a).

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doi:10.1016/j.watres.2010.07.039

It is likely that LCFA degradation relies on the development of syntrophic communities and, thus, the addition of LCFA-degrading bacteria to anaerobic sludge can potentially be used to (1) accelerate bioreactors' start-up phase, which is generally time-consuming, or (2) promote the recovery of disrupted treatment processes when LCFA accumulation/adsorption onto the biomass could not be prevented.

Bioaugmentation has been frequently applied in polluted site or bioreactors to fasten the removal of undesired compounds, to improve the performance of on-going biological processes, and to ease the establishment of specific populations in microbial communities (Bouchez et al., 2000; Jiang et al., 2007). Bioaugmentation of anaerobic waste and wastewater-treating processes, with pure or mixed cultures, has also been reported (Table 1).

Cirne et al. (2006) studied the effects of bioaugmentation as a means of improving the hydrolysis and solubilization of lipids. Batch assays with a model waste containing 10% triolein were bioaugmented with *Clostridium lundense*, a lipolytic strain isolated from bovine rumen. The hydrolysis of the lipid fraction was improved, but LCFA degradation appeared to be the limiting step in the complete conversion of the substrate to methane.

Bioaugmentation success depends on the survival of the inoculated microbial culture, which is influenced by several parameters including phenotypic characteristics of the selected strains, complex microbial interactions and environmental factors (Jiang et al., 2007). Therefore, the applicability and limits of bioaugmentation can be initially tested in microcosms or batch assays, but should be further evaluated in conditions comparable to the real environment where bioaugmentation is to be performed. Batch assays allow effective retention of the inoculated culture in the system, since washout is avoided, and provide more homogeneous environmental conditions. To increase the success of bioaugmentation strategies, some authors used carrier materials such as alginate, agarose and polyurethane, to provide a temporary protective environment to the inoculum (Boon et al., 2002). The use of microcarriers, such as sepiolite and diabase, has also been reported as beneficial for stimulating methanogenic activity (Sanchez et al., 1994).

In this work, the potential for improving methane production from LCFA was studied by bioaugmenting anaerobic sludge with *S. zehnderi*. A direct link between *S. zehnderi* and oleate degradation, either in continuous and fed-batch reactors, has been previously reported (Sousa et al., 2008). This bacterium is able to degrade a wide range of saturated and unsaturated fatty acids (with 4–18 carbon atoms), which makes it suitable for using as a bioaugmenting strain in LCFA degradation (Sousa et al., 2007c).

2. Materials and methods

2.1. Preparation of the bioaugmenting culture

S. zehnderi DSM 17840^T was pre-grown in co-culture with *Methanobacterium formicicum* DSM 1535^T in bicarbonate-buffered mineral salt medium. Anaerobic medium was prepared as described by Stams et al. (1993), dispensed in bottles and sealed with butyl rubber septa and aluminum screw caps. Bottles were pressurized with a mixture of N_2/CO_2 (80:20 vol/vol, 1.7×10^5 Pa) and autoclaved for 20 min at 121 °C. Before inoculation, mineral medium was reduced with 0.8 mM sodium sulfide and supplemented with bicarbonate and salts plus vitamins solutions (Stams et al., 1993). Sodium oleate (≥99%, Fluka) was added to the medium from a sterile stock solution. Inoculation of *S. zehnderi* and *M. formicicum* active cultures, as well as the addition of the solutions, was performed aseptically using sterile syringes and needles. *S. zehnderi* was incubated with 3× 0.5 mM oleate (successive substrate additions) at 37 °C, statically and in the dark. Prior the bioaugmentation assays, *S. zehnderi* co-culture was centrifuged (1600 g, 10 min, 4 °C) and washed (2×) with anaerobic medium. Co-culture was distributed in two bottles under N_2 atmosphere; one of this bottles was heat treated (121 °C, 40 min, 2×) in order to inactivate the culture.

2.2. Microcarrier characterization

Sepiolite, a clay mineral, was the solid microcarrier selected for this work based on the reports of Alves et al. (1999) and

Table 1 – Examples of bioaugmentation studies in anaerobic bioreactors.

Bioreactor	Microorganism(s)	Type of waste/wastewater	Reference
UASBR	*Desulfomonile tiedjei* (as a pure culture and in co-culture)	3-Chlorobenzoate	Ahring et al., 1992
UASBR	*Desulfitobacterium hafniense* strain DCB-2	Pentachlorophenol	Christiansen and Ahring, 1996
UASBR	*Dehalospirillum multivorans*	Tetrachloroethene	Hörber et al., 1998
UASBR	Phenol, *o*- and *p*-cresol degrading enriched microbial consortium	Phenolic compounds	Hajji et al., 2000
AnSBBR	Enriched culture of sulphate reducing bacteria (immobilized in alginate beds)	Sulphate-rich wastewater	Mohan et al., 2005
Batch	*Clostridium lundense* (DSM 17049^T)	Restaurant lipid-rich waste	Cirne et al., 2006
Batch	*Azoarcus* sp. strain DN11	Benzene contaminated groundwater	Kasai et al., 2007
Batch	*Caldicellulosiruptor lactoaceticus* or *Dictyoglomus* sp.	Cattle manure	Nielsen et al., 2007
Two stage CSTR	*Caldicellulosiruptor lactoaceticus*	Cattle manure	Nielsen et al., 2007
Batch	*Ralstonia* sp. HM-1	Cd and Zn contaminated sediment	Park et al., 2008
UASBR	*Sulfurospirillum barnesii* (immobilized in polyacrylamide gels)	Selenate and nitrate or sulphate	Lenz et al., 2009

AnSBBR – anaerobic sequencing batch biofilm reactor;
UASBR – upflow anaerobic sludge blanket reactor; CSTR – continuous stirred tank reactor.

Sanchez et al. (1994). Chemically sepiolite is a hydrated magnesium silicate with general formula $Si_{12}Mg_8O_{30}(OH_2)_4(OH)_4 \cdot 8H_2O$. A wet grain density of 1.64 ± 0.02 g cm^{-3} was determined by water picnometry for the calcinated sepiolite. Average values of equivalent diameter, area and perimeter were determined by image analysis, following the procedure described by Abreu et al. (2007). Average equivalent diameter of 0.64 mm was observed for 99.8% of the sepiolite grains. Average area and perimeter of sepiolite were 0.35 mm^2 and 2.34 mm, respectively. Before addition to the bottles, sepiolite was submitted to calcination at 550 °C for 2 h, washed with distilled water (3×) and sterilized in autoclave (121 °C, 20 min).

2.3. Bioaugmentation potential of S. zehnderi

Bioaugmentation of anaerobic sludge with the pre-grown *S. zehnderi* co-culture was done in bottles with anaerobic granular sludge, in the absence and presence of sepiolite. A scheme of the experimental set-up is depicted in Fig. 1a.

Non-acclimated granular sludge was obtained from a pilot-scale upflow anaerobic sludge blanket (UASB) reactor treating winery effluent (Santiago de Compostela, Spain). Medium was amended with sludge and *S. zehnderi* co-culture to final concentrations of 13% and 0.3% (w/v), respectively. Inactivated *S. zehnderi* co-culture was used in non-bioaugmented controls. Assays with microcarrier were performed with 5 g (dry weight) of sepiolite. Bottles were incubated with 1 mM sodium oleate at 37 °C, statically and in the dark. Blank assays containing no oleate were also performed. In order to warrant uniform hydrogenotrophic activity in bioaugmented and non-bioaugmented assays, 5% (v/v) of an active culture of *M. formicicum* was added to all the bottles. Assays were performed in triplicate. Methane concentration in the headspace of the bottles and volatile fatty acids (VFA) in the liquid medium were monitored during the experiment. LCFA were quantified at the end of the assays. Methane yield was calculated as the ratio between methane concentration measured in bottles' headspace and the theoretical stoichiometric value for complete conversion of 1 mM oleate (i.e. 12.75 mM CH_4).

2.4. Effect of oleate on acetoclastic methanogenesis in bioaugmented assays

Bottles with anaerobic sludge and *S. zehnderi* were supplemented with 16 mM acetate (sodium salt ≥99%, Sigma) and 16 mM acetate plus 1 mM oleate. Experimental procedure and monitoring were similar to the previously described in Section 2.3. Set-up was done according to Fig. 1b. Once more, assays were performed in the absence and presence of sepiolite.

2.5. Analytical methods

Methane was measured using a Pye Unicam GC-TCD gas chromatograph (Cambridge, England), with a Porapack Q (100–180 mesh) column. Helium was the carrier gas (30 mL min^{-1}) and the temperatures of the injection port, column and detector were 110, 35 and 110 °C, respectively. VFA were analyzed by HPLC (Jasco, Japan) using a Chrompack organic analysis column (30 × 6.5 mm) with a mobile phase of 5 mM H_2SO_4 at a flow rate of 0.7 mL min^{-1}. The column temperature was set at 60 °C and the detection was made spectrophotometrically at 210 nm. Mixed liquid and solid phases (cells and microcarrier) withdrawn from the bottles at the end of the experiment were analyzed for total LCFA. Bottles with microcarrier were sonicated for 30 min prior sampling. Saturated and unsaturated LCFA were extracted and quantified as previously described by Neves et al. (2009). Esterification of free fatty acids was performed with propanol, in acid medium (3.5 h at 100 °C). Propyl esters were further extracted with dichloromethane and analyzed in a gas chromatograph (Varian 3800) equipped with a flame ionization detector and a eq.CP-Sil 52 CB 30 m × 0.32 mm × 0.25 μm capillary column (Teknokroma, TR-WAX). Helium was used as carrier gas at

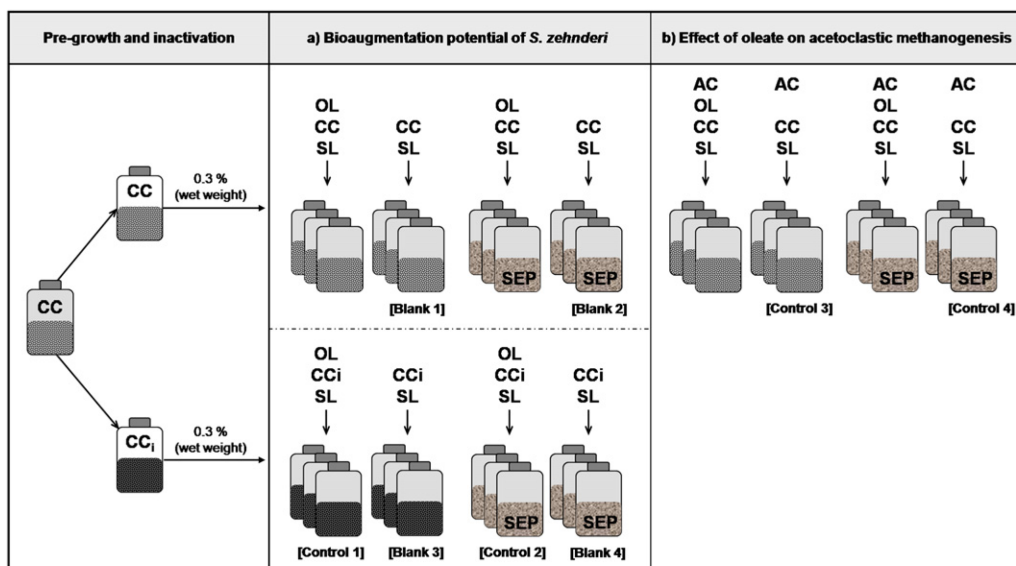

Fig. 1 – Experimental set-up. CC – *S. zehnderi* and *M. formicicum* co-culture; CCi – inactivated co-culture; SL – anaerobic sludge; OL – sodium oleate 1 mM; SEP – sepiolite; AC – sodium acetate 16 mM.

a flow rate of 1.0 mL min^{-1}. Initial oven temperature was set at 50 °C for 2 min and final temperature of 225 °C was attained with a ramp rate of 10 °C min^{-1}. Injector and detector temperatures were 220 °C and 250 °C, respectively.

2.6. Statistical analysis

The statistical significance of the differences detected in methane yields after 15 days of incubation was evaluated using single factor analysis of variances (ANOVA). Statistical significance was established at the $P < 0.05$ level.

The modified Gompertz equation was used to describe the progress of cumulative methane production in batch assays (Zwietering et al., 1990). Methane production rate, maximum methane production and lag phase time were estimated using equation (1).

$$M(t) = P\exp\left[-\exp\left[\frac{R_m e}{P}(\lambda + t) + 1\right]\right] \quad (1)$$

where $M(t)$ = cumulative methane production (mM), P = maximum methane production (mM), R_m = methane production rate (mM day^{-1}), $e = 2.7182818$, λ = lag-phase time (days). For each assay, all the individual measurements performed in the three replicates were utilized independently. R^2 values and the standard errors for each variable were calculated.

3. Results

3.1. Bioaugmentation potential of *S. zehnderi*

Methane production from oleate by *S. zehnderi* bioaugmented and non-bioaugmented sludges, in the absence and presence of sepiolite, is depicted in Fig. 2. The adjustment of the Gompertz modified equation to the experimental cumulative methane values is also shown, as well as acetate concentration measured in the liquid medium.

Methane was produced both by bioaugmented and non-bioaugmented sludges, but faster methane production was achieved in bottles supplemented with active *S. zehnderi* co-culture. After 15 days of incubation, 72 ± 1% of the oleate could be already accounted as methane in bioaugmented assays with sepiolite, contrasting with the 27 ± 1% observed in non-bioaugmented assays (Fig. 2c and d, Table 2). The presence of sepiolite influenced positively methane production rate, as after 15 days of incubation methane yield in bioaugmented sludges grown in the absence of microcarrier was only

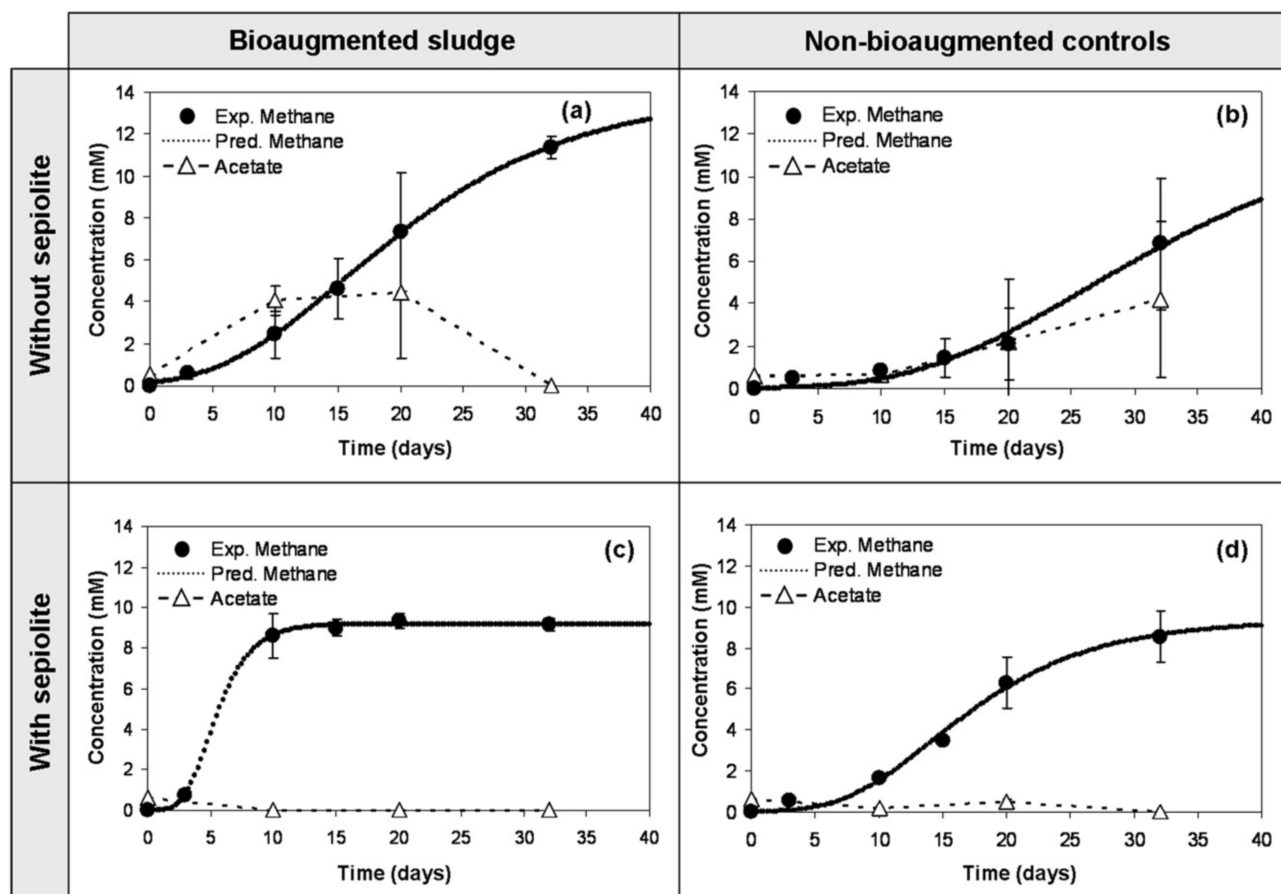

Fig. 2 – Cumulative methane production: (●) experimental values and (········) predicted values according to equation (1). Acetate concentration (△) in the liquid medium. (a) Bioaugmented sludge without sepiolite, (b) non-bioaugmented controls without sepiolite, (c) bioaugmented sludge with sepiolite, and (d) non-bioaugmented controls with sepiolite. The bars indicate standard deviation values calculated at each time point (n = 3).

36 ± 12% (Fig. 2a). In the non-bioaugmented assays without sepiolite a lag phase preceded the onset of methane production and only 54 ± 24% of the added oleate was converted to methane by the end of the trial period (Fig. 2b). Cumulative methane production in blank assays was always lower than 1.6 mM (data not shown).

After 32 days of incubation oleate could not be detected, both in bioaugmented and non-bioaugmented assays. Intermediate LCFA or VFA could not be detected as well, except for acetate and butyrate. *n*-Butyrate was only occasionally detected in concentrations not higher than 0.4 mM. Acetate was the major intermediate measured during oleate degradation, and it was mainly present in assays without sepiolite in which reached a maximum concentration of 5 mM; acetate concentration in assays with sepiolite was residual (<0.6 mM) (Fig. 2).

3.2. *Effect of oleate on acetoclastic methanogenesis in bioaugmented assays*

Methane production from oleate is dependent on syntrophic relations between acetogenic bacteria and methanogenic archaea (Stams, 1994). Successful bioaugmentation is intrinsically dependent on hydrogenotrophic and acetoclastic activities. From these two archaeal groups, acetoclastic methanogens are described as particularly sensitive to LCFA inhibition (Hanaki et al., 1981; Hwu and Lettinga, 1997; Koster and Cramer, 1987; Lalman and Bagley, 2001; Shin et al., 2003) and, therefore, the effect of oleate on acetoclastic methanogenesis was studied using bioaugmented sludge. In the presence of sepiolite, similar methane production rates, i.e. 2.05 ± 0.24 and 2.65 ± 0.21 mM CH_4 day^{-1}, were observed from the degradation of acetate in the absence or presence of oleate, respectively (Table 2). However, this was not the case in the assays without microcarrier, where methane production from the degradation of acetate alone was faster (2.33 ± 0.33 mM CH_4 day^{-1}) than from the mixture of acetate and oleate (0.69 ± 0.08 mM CH_4 day^{-1}).

4. Discussion

Biogas production from waste lipids is a promising technology for sustainable energy production (Alves et al., 2009). In anaerobic environments, lipids are easily hydrolyzed to glycerol and LCFA (Hanaki et al., 1981; Pavlostathis and Giraldo-Gomez, 1991), but further conversion of these substrates is necessary to maximize methane production. LCFA accumulation in anaerobic bioreactors has been ascribed to the low relative abundance of syntrophic bacteria (i.e. 0.01–3% of Syntrophomonadaceae members) (Hansen et al., 1999; Menes and Travers, 2006). Though predominance of syntrophic bacteria can be stimulated after extended contact with LCFA (Sousa et al., 2007a), this requires long reactor set-up periods. Cavaleiro et al. (2009) reported a fed-batch start-up of more than 100 days for an anaerobic reactor converting oleate and skim milk. The addition of syntrophic bacteria to anaerobic bioreactors, operating either in batch- or continuous-mode, could lead to a faster establishment of stable syntrophic communities and decrease start-up times. In this work, we show that bioaugmentation of anaerobic sludge with syntrophic bacteria accelerates methane production from oleate and circumvents the accumulation of intermediates during the conversion of this substrate.

Addition of *S. zehnderi* to granular sludge resulted in a faster oleate conversion to methane, preventing the lag phase observed in non-bioaugmented assays (i.e. $\lambda = 12.8$ days) (Fig. 2a,b; Table 2). Additionally, after 15 days of incubation, methane yield was significantly higher ($p < 0.03$) in the bioaugmented assay than in the non-bioaugmented controls (Table 2). A noticeable improvement in oleate degradation velocity was observed when sepiolite was used as microcarrier. Methane production rates (R_m) of 1.8 ± 0.3 and 0.5 ± 0.1 mM day^{-1} were predicted for the bioaugmented and non-bioaugmented sludges in the presence of sepiolite, respectively, by fitting the modified Gompertz equation to the experimental data (Table 2). Moreover, bioaugmented sludges incubated with sepiolite showed already its maximum

Table 2 – Relevant data concerning methane production during the experiment.

	Without sepiolite		With sepiolite	
	Bioaug.	Non-bioaug.	Bioaug.	Non-bioaug.
Methane yield after 15 days of incubation (%)	36 ± 12[a]	11 ± 7[b]	72 ± 1[c]	27 ± 1[b]
Methane yield after 32 days of incubation (%)	89 ± 5	54 ± 24	72 ± 1	67 ± 10
Parameters from modified Gompertz equation Eq. (1):				
P (mM)	13.9 ± 2.5	[d]	9.2 ± 0.2	9.3 ± 0.8
R_m (mM day^{-1})	0.5 ± 0.1	0.3 ± 0.1	1.8 ± 0.3	0.5 ± 0.1
λ (days)	5.5 ± 1.8	12.8 ± 5.5	2.8 ± 0.3	7.2 ± 1.3
R^2	0.924	0.764	0.989	0.956
Methane production rate (mM CH_4 day^{-1}) from:				
Acetate (16 mM)	2.33 ± 0.33	n.d.	2.05 ± 0.24	n.d.
Acetate (16 mM) + Oleate (1 mM)	0.69 ± 0.08	n.d	2.65 ± 0.21	n.d

n.d. not determined.
a, b, c Different letters indicate statistically significant differences ($p < 0.003$).
d In order to fit equation (1) to this data set a plateau (*P*) value of 12.75 mM was constrained; this value corresponds to the maximum theoretical cumulative methane production from 1 mM oleate.

Table 3 – Possible reactions involved in the syntrophic conversion of oleate to methane (adapted from Sousa et al. (2009)).

Reaction no.	Process	Equation	$\Delta G^{\circ\prime}$ (kJ reaction^{-1}) [a]	$\Delta G'$ (kJ reaction^{-1})[b]
1	Oleate degradation	$C_{18}H_{33}O_2^- + 16H_2O \rightarrow 9C_2H_3O_2^- + 15H_2 + 8\,H^+$	+338	−164
2	Acetoclastic methanogenesis	$C_2H_3O_2^- + H_2O \rightarrow HCO_3^- + CH_4$	−31	–
3	Hydrogenotrophic methanogenesis	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−136	–

a Gibbs free energies (at 25 °C, pH 7) calculated at standard conditions (solute concentrations of 1 M and gas partial pressure of 10^5 Pa).
b Gibbs free energies (at 25 °C, pH 7) for oleate concentration of 1 mM, acetate concentration of 16 mM and H_2 depletion to a partial pressure of 1 Pa.

methane yield (i.e. 72 ± 1%, Fig. 2c; Table 2) after only 15 days of incubation. The differences between bioaugmentation in the presence and absence of sepiolite were also significant ($p < 0.007$), either after 15 or 32 days of incubation.

Comparing bioaugmented assays, one can observe that acetate accumulates in the medium only in the absence of sepiolite (Fig. 2a,c). Differences in acetoclastic activity could be the reason for faster oleate to methane conversion in the bioaugmented assay with sepiolite. Even though energetics of oleate β-oxidation is favorable in the presence of acetate, if hydrogen partial pressure is kept sufficiently low, its conversion to methane is crucial for maximal methane production (Table 3).

A positive effect of sepiolite in methane production from acetate has been previously reported by Sanchez et al. (1994). These authors observed an increase in acetoclastic methanogens numbers in anaerobic sludge incubated with acetate in the presence of sepiolite. In the present study, acetoclastic activity of bioaugmented sludges in the absence and presence of sepiolite, measured through incubation with 16 mM acetate (no oleate added), did not differ significantly. Methane production rates obtained in assays in the absence and presence of sepiolite were similar (i.e. 2.33 ± 0.33 and 2.05 ± 0.24 mM CH_4 day^{-1}, respectively; Table 2). However, when 1 mM oleate was added together with 16 mM acetate, a positive effect of sepiolite in the overall methane production rate was evident ($p < 0.035$). Oleate strongly affected acetoclastic activity in assays without sepiolite lowering methane production rates to 0.69 ± 0.08 mM CH_4 day^{-1} (which corresponds to only 26% of the methane production rate measured in the presence of sepiolite) (Table 2). In fact, acetoclastic archaea are described as highly susceptible to the presence of LCFA; oleate IC_{50} values (concentration that causes a 50% relative methanogenic acetoclastic activity loss) between 0.03 and 3.55 mM (Hanaki et al., 1981, 1983; Hwu et al., 1996; Hwu and Lettinga, 1997; Koster and Cramer, 1987; Shin et al., 2003). The presence of sepiolite seems to prevent acetoclastic activity inhibition as methane production rate was high, i.e. 2.65 ± 0.21 mM CH_4 day^{-1}. This effect might be due to a release of Mg^{2+} ions from the sepiolite to the liquid medium causing the precipitation of oleate in the form of magnesium dioleate. Anionic and cationic surfactants precipitation with Mg^{2+} ions released from sepiolite has been reported (Özdemir et al., 2007; Sabah et al., 2002). Precipitation of LCFA with divalent ions, such as Ca^{2+} and Mg^{2+}, has been previously used in anaerobic bioreactors as a means of reducing its toxicity (Hanaki et al., 1981; Roy et al., 1985). Adsorption of LCFA on sepiolite surface is also plausible, as this carrier is a good adsorbent for polar molecules (Özdemir et al., 2007). LCFA bioavailability in the medium can be reduced by this physical mechanism which potentially decreases toxicity effects.

Cell proximity has been referred as a key factor in syntrophic methanogenesis (Ishii et al., 2006; Stams, 1994; Stams and Plugge, 2009). The use of microcarriers might facilitate interspecies metabolite exchange, enhancing the cooperation between the acetogenic bacteria and the methanogenic archaea. Bioaugmentation assays with sepiolite might also have beneficiated of this effect.

Bioaugmenting anaerobic sludge with syntrophic bacteria or even with LCFA-degrading enriched cultures appears to be a promising strategy for accelerating methane production from LCFA. Further studies are required to evaluate the potential of bioaugmenting continuous systems with LCFA-degrading bacteria, but this work shows the advantage of amending these microorganisms during batch start-up of bioreactors treating fatty wastewaters. The presence of sepiolite had a positive effect on methane formation, but the mechanisms of sepiolite interactions appear to be very complex and require further assessment.

5. Conclusions

The potential for improving methane production from oleate by bioaugmenting anaerobic sludge with *S. zehnderi* was demonstrated. Higher methane yields were attained in the bioaugmented assays, and a faster methane production was recorded in the presence of sepiolite. The positive effect of sepiolite in oleate to methane conversion might be related with: (1) a decrease in LCFA toxicity due to their precipitation with Mg^{2+} ions and physical adsorption; (2) a potential improvement in metabolites transfer between acetogens and methanogens, as cells can grow on the microcarrier and increase proximity between groups.

Bioaugmentation of anaerobic bioreactors with *S. zehnderi* or other syntrophic LCFA-degraders can be potentially useful for faster reactor start-up or recovery of an LCFA-inhibited bioreactor.

Acknowledgements

The authors acknowledge Ângela Abreu for the help with the application of image analysis techniques. The financial

support from Fundação para a Ciência e a Tecnologia (FCT) through the PhD grant SFRH/BD/24256/2005 attributed to A.J. Cavaleiro is gratefully acknowledged.

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