

# New strategies for optimal methane production from long chain fatty acids

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**Abstract** High methane production can be expected from biodegradation of long chain fatty acids (LCFA) in anaerobic bioreactors; however, in practice, this process is limited by LCFA accumulation onto the sludge. To optimize methane production from LCFA-rich wastewater, two novel strategies were tested: (i) bioreactor start-up based on the alternation of continuous-feeding phases with batch-degradation phases, and (ii) bioreactor bioaugmentation with the LCFA-degrading bacterium *Syntrophomonas zehnderi*. Using the first strategy, and after five alternation cycles, continuous bioreactor operation at an organic loading rate (OLR) of 21 kgCOD.m<sup>-3</sup>.day<sup>-1</sup> (50% as oleate) was successfully applied, with an average methane yield of 72%. A specialized microbial community, exhibiting high LCFA-tolerance and high methanogenic activity, was developed. *Methanobacterium*- and *Methanosaeta*-like microorganisms prevailed in this system. Syntrophic activity was also shown to be an important factor for the efficient conversion of LCFA to methane. Anaerobic sludge amended with *S. zehnderi* and incubated in the presence of a solid microcarrier (sepiolite) achieved 1.7x higher methane yields and 4x higher methane production rates than the correspondent non-bioaugmented controls. This effect was more pronounced in bioaugmented assays amended with sepiolite+Fe(OH)<sub>3</sub>. This work opens new perspectives for efficient treatment of LCFA-rich wastewater combined with high methane recovery.

**Keywords** Bioaugmentation, continuous/batch cycles, LCFA, methane, *Syntrophomonas zehnderi*

## INTRODUCTION

Anaerobic treatment of wastewaters containing high concentration of lipids and long chain fatty acids (LCFA) has the potential of combining pollution removal with high biogas production. However, bioreactor operational problems directly caused by lipids/LCFA have limited the use of anaerobic technologies to produce methane from these compounds. Excessive LCFA adsorption onto the biomass, with consequent sludge flotation and washout, and slow degradation rates, are the main problems described. Process failure generally occurs at organic loading rates (OLR) higher than 10 kg COD m<sup>-3</sup> day<sup>-1</sup>.

Several approaches have been attempted to enhance LCFA conversion to methane. Utilization of sieve drums, biomass recirculation or biomass adhesion to supports were some of the strategies proposed for overcoming flotation problems (Hamdi *et al.*, 1992; Hwu, 1997). Periodic reseeded of anaerobic reactors was also suggested by Hwu *et al.* (1998) and Jeganathan *et al.* (2006). Addition of calcium ions (Roy *et al.*, 1985) or inert materials, e.g. activated carbon, bentonite or other clays, was also proposed (Angelidaki *et al.*, 1990; Palatsi, 2010). These materials function as competitive additives preventing the inhibition of the anaerobic sludge by LCFA.

An important fact is that the adverse effects of LCFA on anaerobic sludge are not irreversible. Pereira *et al.* (2003; 2004) have shown that anaerobic sludge overloaded with LCFA can still be metabolically active. This biomass contains a high amount of associated LCFA, which can be converted to methane if no other carbon source is supplemented. After the degradation of the

biomass-associated LCFA, the specific methanogenic activity was even higher (Pereira *et al.*, 2003; 2004). These results suggested that fed-batch systems could be an option for the efficient methane production from LCFA.

Complete LCFA conversion to methane involves the activity of syntrophic bacteria and methanogenic archaea (Schink, 1997). LCFA accumulation in anaerobic bioreactors has also been ascribed to low relative abundance of syntrophic bacteria in the microbial communities (Hansen *et al.*, 1999). Therefore, the addition of LCFA-degrading bacteria to anaerobic bioreactors can possibly lead to a faster establishment of stable syntrophic communities, thus contributing to enhanced LCFA conversion to methane.

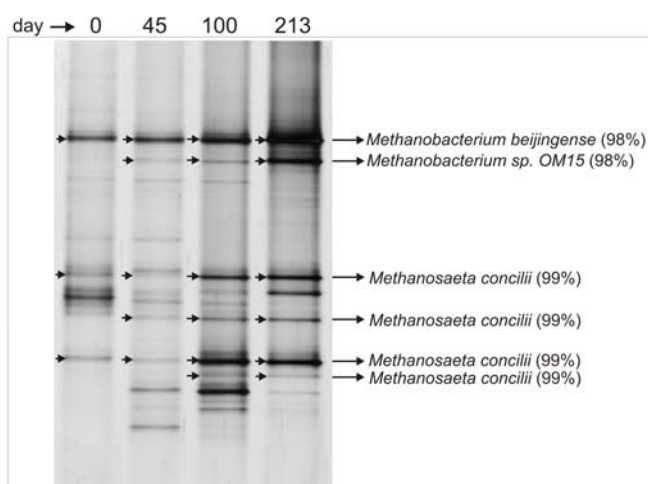
This work reports novel strategies for optimal methane production from LCFA-rich wastewater: (i) bioreactor start-up based on the alternation of continuous-feeding phases with batch-degradation phases, and (ii) reactors bioaugmentation with the LCFA-degrading bacterium *Syntrophomonas zehnderi*.

### **BIOREACTOR START-UP USING CYCLES OF CONTINUOUS/BATCH PHASES**

The start-up of a mesophilic up-flow anaerobic reactor was performed in cycles. Each cycle included a continuous feeding phase and a batch degradation phase. A total of five alternating cycles were applied during 213 days of operation (Period I). An OLR of 4 kg COD m<sup>-3</sup> day<sup>-1</sup> and a hydraulic retention time (HRT) of 1.6 days was applied during cycles 1 to 4; in the 5<sup>th</sup> cycle OLR was increased to 13 kg COD m<sup>-3</sup> day<sup>-1</sup>. Oleate (unsaturated C18 LCFA) and skim milk were used as carbon and energy source in the synthetic wastewater, accounting for 50% of total COD each.

LCFA accumulated onto the sludge only during the first two cycles, and were efficiently biodegraded thereafter, concurrently with a shift in methane production from the reaction to the feeding phases. Moreover, methane yields increased from 67% to 91% during the start-up period, showing an enhancement of the microbial biodegradation capacity. Continuous operation was applied afterwards (Period II, OLR from 5 to 31 kg COD m<sup>-3</sup> day<sup>-1</sup>, during 422 days), and the bioreactor was able to accommodate an OLR of 21 kg COD m<sup>-3</sup> day<sup>-1</sup> while recovering 72% of the COD removed as methane. Specific acetoclastic and hydrogenotrophic methanogenic activity of the sludge also increased during the reactor operation.

Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was used to follow the changes in the archaeal communities present in the sludge samples collected at days 0, 45 (end of cycle 1), 100 (end of cycle 2) and 213 (end of the start-up period); seven sludge samples were also collected during the continuous operation. According to the DGGE profile (Figure 1), the most relevant changes on the archaeal community took place during the first two cycles of Period I, suggesting that the selection of the archaeal microorganisms was based on their tolerance/adaptation to oleate induced by the feeding strategy applied. Alternating periods of high LCFA concentration with batch-degradation phases was the driving force for the selection of the predominant methanogenic microorganisms. After acclimation, archaeal community diversity remained stable until the end of the experiment, despite the change on the bioreactor operation to continuous mode. In both operational periods the sequences obtained by cloning and sequencing were closely related to those of organisms belonging to *Methanobacterium* and *Methanosaeta* genera. These two methanogenic groups exhibited a high tolerance to the increasing LCFA loads applied during the continuous bioreactor operation, contributing to the efficient recovery of LCFA energy in the form of methane.

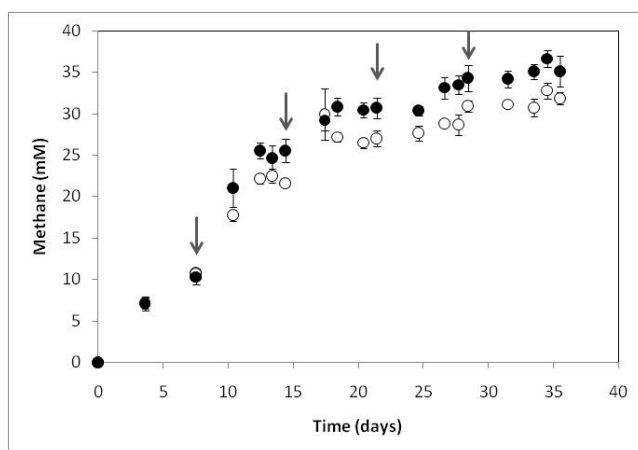


**Figure 1.** DGGE patterns of archaeal 16S rRNA gene fragments obtained from sludge samples collected during reactor start-up (Period I). Numbers 0 to 213 correspond to sampling days. Predominant DGGE bands were identified by cloning and sequencing; the closest relative and percentage of identity of the 16S rRNA gene are indicated in the figure.

### REACTORS BIOAUGMENTATION WITH *SYNTROPHOMONAS ZEHNDERI*

Methanogenic archaea are often reported as sensitive to LCFA, but the endurance of *Methanobacterium* and *Methanosaeta* genera after extended contact with high LCFA loads was demonstrated in the previous work. Therefore, the addition of LCFA-degrading bacteria may constitute an alternative or complementary strategy for optimal methane production from these compounds. *Syntrophomonas zehnderi* was isolated as a co-culture with *Methanobacterium formicicum* from an anaerobic bioreactor treating an oleate-based effluent (Sousa *et al.*, 2007). This mesophilic bacterium was chosen as bioaugmenting strain due to its capacity for degrading straight-chain fatty acids with 4 to 18 carbon atoms, and also unsaturated LCFA, such as oleate (in co-culture with *M. formicicum*). Moreover, the presence of *S. zehnderi* related bacteria in oleate-degrading sludges suggest their direct link to oleate degradation (Sousa *et al.*, 2008).

Addition of *S. zehnderi* to granular sludge resulted in faster oleate conversion to methane, preventing the lag phase (13 days) observed in non-bioaugmented controls. This effect was more pronounced when sepiolite was used as microcarrier, increasing the methane production rate and yield 4x and 1.7x, respectively, comparatively with the non-bioaugmented assays with sepiolite. These results were confirmed in assays amended with four successive pulses of 1 mM oleate. Again, significantly higher methane yields ( $p < 0.001$ ) were observed in bioaugmented vials containing sepiolite, in relation to the corresponding non-bioaugmented controls. Addition of ferric hydroxide at substoichiometric amounts to bioaugmented vials with sepiolite made this effect even more evident (Figure 2).



**Figure 2.** Cumulative methane production in bioaugmented assays incubated in the presence of (o) sepiolite and (●) sepiolite + ferric hydroxide. The arrows (↓) indicate the moments of oleate addition.

Bioaugmenting non-acclimated anaerobic sludge with *S. zehnderi* in the presence of sepiolite or other solid microcarriers/immobilizing matrixes appears to be a promising strategy to enhance the development of syntrophic communities able to degrade LCFA, and consequently can potentially lead to faster bioreactors start-up.

## CONCLUSIONS

Acclimation of anaerobic sludge through repeated cycles of continuous feeding/batch phases is crucial for the development of a specialized microbial community able to efficiently convert LCFA to methane. Despite the reported sensitivity of methanogens to LCFA, *Methanobacterium* and *Methanosaeta* genera are capable of tolerate and endure during prolonged exposure to high LCFA loads. Bioaugmentation of anaerobic sludge with LCFA-degrading bacteria enhance methane production from LCFA, and this effect can be strengthened by the presence of solid microcarriers (e.g. sepiolite) or other additives (e.g. ferric hydroxide).

## Acknowledgments

This work was funded by FEDER, through the COMPETE program, and by Portuguese funds, through Portuguese Foundation for Science and Technology (FCT), in the frame of projects FCOMP-01-0124-FEDER-007087 and FCOMP-01-0124-FEDER-014784. Financial support from FCT and the European Social Fund (ESF) through the PhD grant SFRH/BD/24256/2005 attributed to Ana Júlia Cavaleiro is also acknowledge.

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