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ENDURANCE OF METHANOGENIC ARCHAEA IN ANAEROBIC BIOREACTORS TREATING OLEATE BASED WASTEWATER

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ABSTRACT

Methanogenic archaea are anaerobic microorganisms specialized on methane production from simple substrates such as hydrogen and acetate. These substrates are abundant in anaerobic bioreactors treating long-chain fatty acid (LCFA)-rich wastewater. LCFA are utilized by bacteria and their mineralization can be achieved by the final set of methanogenic reactions that result on methane production. However, methanogens are often reported as sensitive to LCFA concentrations what makes the anaerobic treatment of LCFA- rich wastewaters problematic. Nevertheless, successful anaerobic treatment of LCFA-rich wastewater, both in fed-batch and continuous mode, has been already reported. In the present work, the archaeal community developed during fed-batch and continuous treatment of oleate (unsaturated LCFA), in a highly efficient anaerobic reactor, was studied using molecular tools. Cloning and sequencing results showed that predominant microorganisms are closed related to *Methanobacterium* and *Methanosaeta* species. The presence of these microorganisms in the high rate anaerobic bioreactor proves that it is possible to obtain an archaeal community, with high methanogenic activity, adapted to continuous feeding of LCFA-rich wastewater.

INTRODUCTION

Even though some authors report LCFA as toxic towards microorganisms, in particular to methanogenic archaea, (Lalman & Bagley, 2001; Perle *et al.*, 1995; Hwu & Lettinga, 1997) others considered the adverse effect of LCFA towards microbial communities reversible and mainly attributed to mass transfer limitations (Alves *et al.*, 2001; Pereira *et al.*, 2003; Pereira *et al.*, 2004). The work developed by Cavaleiro *et al.* (2009) opened new perspectives on the anaerobic digestion of LCFA containing wastewaters when these authors demonstrated that continuous high rate treatment of oleic acid rich wastewater is possible if the appropriate conditions for biomass acclimatization to LCFA are applied. In this way, the energy stored in LCFA can be recovered coupling wastewater treatment to bioenergy production. In this work the composition and dynamics of the archaeal community that colonized the anaerobic bioreactor from Cavaleiro *et al.* (2009) was investigated.

MATERIALS AND METHODS

Sludge samples were obtained from an anaerobic bioreactor operated at mesophilic conditions and fed with a synthetic dairy wastewater (50% COD-skim milk and 50% COD-sodium oleate) during 213 days of step-feeding (Period I) and 422 days of continuous operation (Period II), as described elsewhere (Cavaleiro *et al.*, 2009). Organic loading rates (OLR) increased from 4.4 to 8.2 kgCOD m^{-3} day^{-1}during Period I and from 5 to 31 kgCOD m^{-3} day^{-1} during Period II. Fifteen sludge samples were collected during periods I and II.

DNA was extracted from sludge samples and 16S rRNA-genes were amplified by polymerase chain reaction (PCR) with primers targeting conserved domains. DGGE analysis of the amplicons was performed and similarity between DGGE profiles determined by calculating similarity indices of the densitometric curves of the profiles. Cloning was performed by using pGEM-T plasmids. Clones with the same electrophoretic mobility as that of predominant bands of archaeal DGGE patterns were selected for further sequence analysis. Phylogenetic affiliation of 16S rRNA gene sequences was assessed by Blast similarity searches and RDP Classifier.

RESULTS

The analysis of DGGE band-pattern from the sludge samples revealed two major shifts in the archaeal community during the start-up period of the bioreactor operation (Figure 1). The first one was observed between the inoculum and remaining samples (47%

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similarity) and the second between samples collected during the first two cycles and the last 3 cycles. During the continuous operation (Period II), similarity between the seven samples was always higher than 84% and fluctuated from 89 to 98% within the 5 last samples (Figure 2). Archaeal 16S rRNA gene fragments retrieved from the samples collected at the end of periods I and II were used to construct clone libraries.

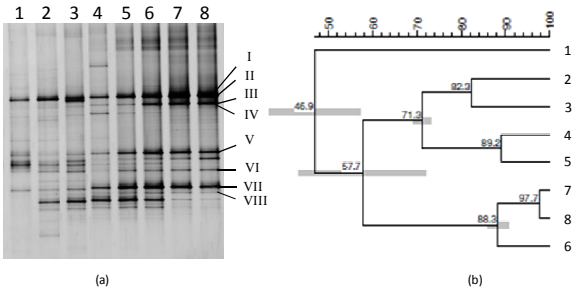

Figure 1: DGGE pattern of archaeal 16S rDNA fragments obtained from samples collected during Period I (a). The numbers in the top of each lane correspond to sample identification. Cluster analysis by the unweighted pairwise grouping method with mathematical averages (UPGMA) of DGGE profiles of archaeal amplicons (b). Numbers I to VIII indicate the bands that were identified by cloning and sequencing.

The retrieved 16S rRNA sequences were affiliated with methanogenic archaea belonging to the phylum *Euryarchaeota* and to the orders *Methanobacteriales* and *Methanosarcinales*. At the end of the start-up period, retrieved sequences showed higher similarity to those of *Methanobacterium* sp. OM15 (99%), *Methanobacterium beijingense* 8-2 (98%) and *Methanosaeta concilii* GP-6 (99%).

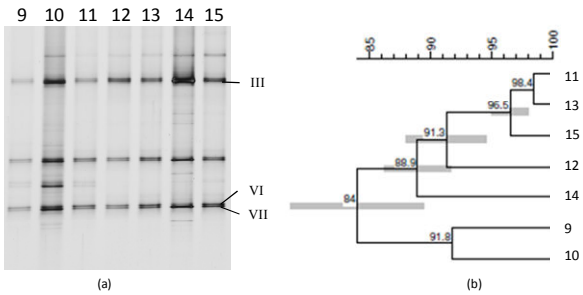

Figure 2: DGGE pattern of archaeal 16S rDNA fragments obtained from samples collected during Period II (a). The numbers in the top of each lane correspond to sample identification. Cluster analysis by the unweighted pairwise grouping method with mathematical averages (UPGMA) of DGGE profiles of archaeal amplicons (b). Numbers I to VII indicate the bands that were identified by cloning and sequencing.

At the end of Period II retrieved sequences were most related with the following sequences: *Methanosaeta concilii* GP-6 (99% of similarity) and *Methanobacterium* sp. OM15 (99% of similarity).

DISCUSSION AND CONCLUSIONS

Cavaleiro *et al*. (2009) showed that anaerobic conversion of LCFA degradation intermediates, namely acetate and hydrogen, to methane is possible either in fed-batch or in continuous bioreactors. By a strategy of cloning and sequencing we were able to identify 16S rRNA sequences corresponding to the most abundant methanogenic players during this bioreactor operation. The sequences identified are mostly related to those belonging to *Methanosaeta* and *Methanobacterium* genera. These results show that microorganisms close related to *Methanosaeta* and *Methanobacterium* species not only survived but were also able to maintain a high methanogenic activity during both operational periods. The results also suggest that these microorganisms are not as sensitive to the adverse effect of LCFA as they are often described. Moreover, these archaea could tolerate high LCFA concentrations in high rate bioreactors showing that in fact they are more resistant that previously thought. Further work should be done in order to evaluate the effect of LCFA on methanogenic archaea and to understand the mechanisms that lead to the tolerance of certain methanogens to LCFA in continuous anaerobic bioreactors.

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