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Advantages of using sequential loading/degradation steps for the anaerobic mineralization of Long Chain Fatty Acids

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Abstract In this work, suspended sludge that was able to accumulate up to 4570±257 mg COD-LCFA/gVSS (83% of palmitic acid) was studied. The specific methanogenic activity, the tolerance of aceticlastic methanogens to oleic acid toxicity and the oleic acid biodegradation capacity, were assessed before and after allowing the mineralization of the biomass-associated LCFA. The capacity of the sludge to mineralize the associated LCFA was proven and the behavior of the sludge before and after that mineralization clearly suggested that sequential loading/degradation steps for the treatment of lipidic/LCFA based effluents have a potential interest.
Keywords Long chain fatty acids (LCFA), methanogenic activity; biodegradation.

Introduction

Recent studies on LCFA anaerobic biodegradation conducted in our research group allowed to obtain new conclusions about biodegradation and toxicity of LCFA in anaerobic processes. It was found that, when oleic acid is continuously fed to anaerobic sludge, palmitic acid is the main LCFA that accumulates onto the sludge (more than 80%) and further β-oxidation is partially inhibited by oleic acid (Pereira *et al*., 2002). If the sludge is removed from the reactor, washed to remove the residual soluble substrate, and incubated in batch assays without any added organic carbon source, β-oxidation proceeds and methane is produced due to degradation of LCFA that remained associated to the biomass by mechanisms of adsorption, precipitation or entrapment. These previous results clearly suggest the interest of using a sequential fed-batch operation, where a first step of LCFA accumulation is promoted, followed by its mineralization. The key feature seems to be the optimal specific LCFA load that can be applied in order to promote the maximal mineralization rate of the biomass-associated LCFA (Pereira *et al.*, 2004). Salminen *et al*. (2001) also observed the batch biodegradation of accumulating materials (mainly LCFA) in semi-continuous anaerobic digesters that previously failed the operation, during the treatment of poultry slaughterhouse waste. The aim of this work was to evidence the advantages of allowing the mineralization of the biomass-associated LCFA accumulated onto the sludge, in terms of methanogenic activity, the tolerance to oleic acid toxicity and the capacity of oleic acid biodegradation.

Materials and Methods

Reactor set-up and operation to produce the sludge under study

In order to obtain sludge containing biomass-associated LCFA, suspended sludge was continuously loaded in a 1 l expanded granular sludge bed (EGSB) reactor at a constant influent concentration of 4 g of chemical oxygen demand (COD)/l (HRT≅1 day), under mesophylic conditions (37±1 ºC). The reactor was equipped with an external settler (vol = 200 ml) from where biomass was recycled at a rate of 4 l/day. During the first 28 days, start-up period, the substrate consisted of skim milk (50% COD) and sodium oleate (50% COD) diluted with tap water and supplemented with macro and micronutrients. To give suitable alkalinity, 5 g $NaHCO_3$/l was added to the feed. From the day 28 on, the carbon source was exclusively composed by sodium oleate and a nitrogen supplement was added

to the macronutrients solution in order to maintain a ratio COD/N/P of 200:5:1.1. During this operation time (day 28 to 75) the reactor presented an average COD removal efficiency of 80.3±5.4%, effluent VSS of 0.88±0.24 g/l and CH_4 production of 0.09±0.01 l/(l.day). A methane yield as low as 33 l CH_4/kg $COD_{removed}$ was achieved, indicating a considerable accumulation of non-mineralized substrate. Prior to reactor inoculation, the suspended sludge was characterized in terms of specific methanogenic activity (SMA) in the presence of acetate, propionate, butyrate, ethanol and H_2/CO_2 and oleic acid toxicity towards the aceticlastic methanogens. The sludge exhibited no detectable activity against propionate and a SMA against acetate, butyrate, ethanol and H_2/CO_2 of 146±15, 80±18, 72±4 and 581±33 mg COD-CH_4/(gVSS.day), respectively. In terms of toxicity limit of aceticlastic methanogens to oleic acid an IC_{50} value of 70±10 mg/l was found.

Batch experiments

Specific methanogenic activity (SMA) tests: SMA was assessed in the presence of acetate, propionate, butyrate, H_2/CO_2, and, in some cases also ethanol, according to a pressure transducer techniques described by Colleran et al., 1992. The basal medium used, made up with demineralized water, was composed of cysteine-HCL (0.5 g/l) and sodium bicarbonate (3 g/l), the pH was adjusted to 7.0-7.2 with NaOH 8N and was prepared under strict anaerobic conditions. No calcium or trace-nutrients were added. The values of biogas production were corrected for the standard temperature and pressure conditions (STP). Blank controls were used for liquid substrates (no added substrate) and for gaseous substrates (pressurized with N_2/CO_2 - 80:20 vol/vol at 1 Bar). SMA values were determined dividing the initial slope of the biogas production curve by the VSS content of each vial at the end of the experiment and multiplied by the methane content determined by gas chromatography, at the end of the experiment. They were expressed in ml CH_4/(gVSS.day), which were then converted to the equivalent COD. Background methane production due to the residual substrate was discounted.

Assessment of SMA before and after allowing the mineralization of biomass-associated LCFA:

The SMA of the sludge before and after degrading the biomass-associated LCFA was compared using parallel assays. In the first set of vials the SMA against acetate, propionate, butyrate, and H_2/CO_2 were determined in duplicate, using the same pressure transducer technique described above. In the other set of vials, no substrate was added but the mineralization of the biomass-associated LCFA was followed until stabilization. After this stabilization, the SMA with the same substrates was measured as described for the first set of vials. The cumulative methane production at the end of the blank control assays, without any added organic carbon source, calcium or nutrients, was considered an indirect measurement of the amount of biomass-associated LCFA. The specific LCFA content was then determined by dividing the maximum plateau achieved in the methane production curve by the VSS content of each vial at the end of the experiment and was expressed as mg COD/gVSS. The same approach of setting parallel assays was used to characterize the sludge, before and after degrading the biomass-associated LCFA, in terms of oleic acid toxicity towards the aceticlastic bacteria and for oleic acid biodegradation capacity.

Toxicity and biodegradability tests: Toxicity tests were performed using the same pressure transducer technique previously described for the methanogenic activity measurements. Oleic acid was the potential individual toxicant at concentrations in the range of 100 to 900 mg/l. Acetate (30 mM) was added, in order to focus the toxicity assays in the aceticlastic methanogens. In the biodegradability experiments, oleic acid was the sole organic carbon source added to the vials at concentrations ranging from 100 to 900 mg/l

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Extraction and GC analysis of LCFA accumulated onto the sludge

After two consecutive washings and centrifugations (4000 rpm, 10 min) with anaerobic basal medium, an aliquot of sludge sample was dried at 105ºC, weighed and placed into separating funnels. A solution of internal standard (pentadecanoic-C15) was added to the sample, and, after acidification

to pH 2, a multiple extraction with 5x1 ml of petroleum ether was applied. The ether phase was transferred to glass vials, immediately capped, and stored at –20ºC. LCFA concentration was determined by a gas chromatograph (CP-9001 Chrompack) equipped with a flame ionization detector (FID) and a split/splitless injector. LCFA were separated on a FFAP-CB 25m x 0,32mm x 0,3µm column (Chrompack), using nitrogen (N_2) as carrier gas at 35KPa, 31:1 split rate. Oven temperature was 40ºC for 2 min, with a 5ºC/min ramp to 250ºC, and a final hold at 250ºC for 15 min.

Results and Discussion

The suspended sludge had a specific LCFA content of 4570±257 mg COD/gVSS, in which palmitic acid represented 83% of the most expected β-oxidation intermediates (C10:0 to C18:0). Before degrading this accumulated LCFA, the sludge exhibited no SMA with acetate, propionate or butyrate as substrates. Only the initial activity against H_2/CO_2 presented a measurable value of 434±12 mg COD-CH_4/(gVSS.day). After allowing the depletion of the biomass-associated LCFA, the SMA exhibited by the same sludge against acetate, butyrate and H_2/CO_2 was increased to 533±95, 224±71 and 2709±38 mg COD-CH_4/(gVSS.day), respectively. The SMA with propionate as substrate was still considerably low after the depletion of the biomass-associated LCFA, i.e. 16±4 mg COD-CH_4/(gVSS.day), suggesting that this particular trophic group can be affected by the long-term contact with LCFA. However, the seed sludge already exhibited a null methanogenic activity with propionate as substrate. When extending this comparison to the other tested substrates, one can not disregard the significantly higher activity with acetate, butyrate and H_2/CO_2 exhibited by the sludge after the depletion of the biomass associated LCFA, when compared to the previously exhibited by the seed sludge. Enhancement of sludge activity in these particular substrates may find a feasible explanation based on the mechanism of LCFA β-oxidation. In fact, during oleate/palmitate β-oxidation both butyrate and acetate are formed (Weng and Jeris, 1976), which may result in the enrichment of both aceticlastic methanogens and acetogenic butyrate-degraders bacteria. The same can occur with hydrogenotrophic methanogens, an important group that acts syntrophically with hydrogen producing acetogenic bacteria during LCFA degradation. On the opposite, being propionate a less probable intermediate of oleate/palmitate β-oxidation, an enhancement of sludge activity in this substrate may, therefore, not be promoted. In terms of toxicity measurement, since the sludge containing the biomass-associated LCFA exhibited no initial methanogenic activity with acetate (control), the toxicity limit (IC_{50}) to oleic acid could not be determined. After depletion of the biomass-associated LCFA, the sludge exhibited a toxicity limit (IC_{50}) to oleic acid of 80±10 mg/l. A similar IC_{50} value (70±10 mg/l) was previously exhibited by the seed sludge before being continuously loaded with oleic acid, which suggests that no significant change on the resistance to oleic acid toxicity occurred by the LCFA contact/degradation. In terms of LCFA biodegradation capacity, the advantage of allowing the mineralization of the biomass-associated LCFA prior to a new oleic acid addition is clearly evidenced in Figure 1. For instance, if oleic acid at 900 mg/l (highest concentration tested) is added to the sludge containing biomass-associated LCFA, methane production started only after 1500 hours and total mineralization lasted about 2700 hours (Figure 1 (e)). By contrast, if the biomass-associated LCFA was allowed to be firstly mineralized, about 900 hours are required and if oleic acid is added afterwards, complete degradation occurs in a total period of 1400 hours (Figure 1 (e)). Phenomena such as LCFA reversible inhibitory effect, transport limitation effects caused by the LCFA accumulated onto the cells or possible enrichment of specific populations in the consortium during the mineralization of the biomass-associated LCFA, can be responsible for this behavior.

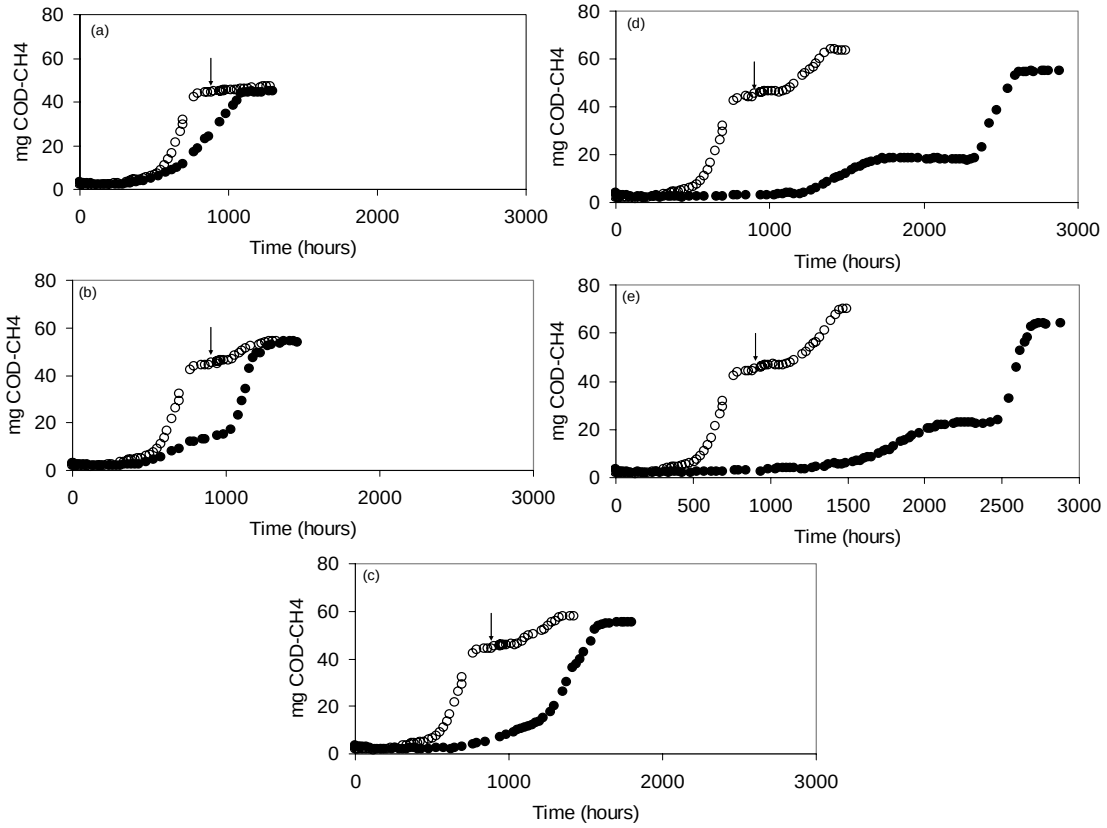

Figure 1 Methane production during oleic acid biodegradation batch experiments exhibited by the sludge, when oleic acid at concentrations of 100 mg/l (a), 300 mg/l (b), 500 mg/l (c), 700 mg/l (d) and 900 mg/l (e), was added before (•) and after (o) allowing the mineralization of the biomass-associated LCFA (arrows indicate moment of oleic acid addition).

CONCLUSIONS

The results presented in this work support the practical interest of using sequential loading/degradation steps for the treatment of lipidic/LCFA based effluents, because besides the sludge ability to mineralize high amounts of biomass-associated LCFA, its activity is enhanced after the degradation step.

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