Anaerobic Biodegradation of Long Chain Fatty Acids: a review

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ABSTRACT

If an effluent with high lipid content is fed to a continuous high rate anaerobic digester, a significant accumulation of Long Chain Fatty Acids (LCFA) onto the sludge aggregates (flocs or granules) is observed, without further mineralization to methane. That accumulation is progressive and, after some time, the sludge becomes surrounded by whitish foam and tends to float, leading, most of times, to a severe washout. This happens not only with granular sludge reactors, but also with fixed bed reactors. This fact, associated to the accepted idea that LCFA were highly toxic to the anaerobic consortia, imposed that lipids had to be removed from any wastewater before the anaerobic treatment step. Therefore, the high methane potential of these compounds was lost. However, it was observed that anaerobic sludge had the capacity to mineralize the LCFA associated to the cells by mechanisms of adsorption, precipitation or entrapment. The mineralization of these biomass-associated LCFA was inhibited by oleic acid present in the feed. Furthermore, after the mineralization of these biomass-associated LCFA, the specific methanogenic activity of the sludge was enhanced, being able to accumulate and mineralize a new LCFA load. These facts contradict the accepted theories about the severe and irreversible toxicity of LCFA and clearly suggest that only a reversible inhibition occurs, probably a simple physical transport limitation effect. The main practical result of this research is that a sequencing batch reactor (SBR) based process will be the most suitable way to efficiently mineralize effluents with high lipids content. The new SBR concept is under development and was recently awarded with the Lettinga Award 2004. A review of the most important results on anaerobic LCFA biodegradation is presented in this paper.

INTRODUCTION

Lipids, abundant in slaughterhouses, edible oil/fat refineries or dairy wastewaters are potentially attractive for biogas production due to their high theoretical methane yield. However, in practice, a physico-chemical pre-treatment is usually applied before the anaerobic treatment, because neutral fats are easily hydrolysed to LCFA, which are reported to exert an acute toxic effect towards the microorganisms involved in the β-oxidation and methanogenic pathways (Koster and Cramer, 1987, Rinzema, 1988, Angelidaki and Ahring, 1992, Hwu, 1997).

In general a severe toxic effect is referred, although granular sludge seems to be more resistant to LCFA toxicity than suspended sludge (Hwu, 1997). Rinzema (1988) reported that LCFA exert a bactericidal effect, and that acetoelastic bacteria do not adapt to LCFA neither upon repeated exposure to toxic concentrations, nor after prolonged exposure to non-toxic concentrations. The recovery after a lag phase usually observed in batch assays, was attributed by this author, to the growth of few survivors. Angelidaki and Ahring (1992) reported a permanent toxic effect which underlines the conclusions of Rinzema (1988). The benefit of adding lipids to anaerobic digesters is, however, reported in the literature (Ahring, 2003) and
therefore the mechanisms of anaerobic lipids/LCFA biodegradation seem to be far from well understood.

In recent studies conducted in our research group, it was observed that anaerobic sludge was able to accumulate and mineralize LCFA in amounts up to 3 gCOD·gVSS⁻¹, provided it was associated to the sludge, but not in the bulk medium, evidencing that the anaerobic consortium remained active in such conditions (Pereira et al., 2002, Pereira et al., 2003). It was also observed that suspended sludge was significantly better for LCFA accumulation/degradation than granular sludge (Pereira et al., 2002). Figure 1 represents an example of a methane production curve in a batch assay, when the sludge taken from a continuous reactor fed with oleic acid was incubated without any extra organic carbon source. However, if oleic acid was added to the vials the methane production was severely inhibited (Figure 2). This put forward the hypothesis that biomass associated LCFA in that conditions was not oleic acid, but any intermediate of its degradation and therefore the extraction and quantification of these LCFA has shown that palmitic acid (C16:0) represented more than 80% of the most expected β-oxidation intermediates (C10:0 to C18:0) (Pereira et al., 2002).

Figure 1. Example of a methane production curve in a batch assay, during the mineralization of the biomass-associated LCFA

Figure 2. Influence of oleic acid concentration on the mineralization of biomass-associated LCFA. o-control (washed sludge no oleate), ■- 100 mg/L, △-300 mg/L, ★-500 mg/L, ▲-700 mg/L, ●- 900 mg/L.

Acumulation of palmitic acid was also reported by other authors. Salminen et al. (2001) reported that during the batch degradation of solid poultry slaughterhouse wastes, stearate was consumed faster than it was produced and also that palmitic acid was the most abundant LCFA detected in the medium. Beccari et al. (1998) applied a two-reactor system with partial phase separation for treating olive oil effluents and reported that in the conversion between oleic acid and palmitic acid, the saturation from oleic to stearic was the limiting step, whereas the first step of β-oxidation (stearic to palmitic) proceeded quickly. Also, Lalman and Bagley (2001) reported that palmitic acid was the primary detected product from oleic and linoleic acids, and that stearic acid was not detected.

This clearly contradicts the generally accepted idea that LCFA adsorption onto anaerobic sludge is closely linked to LCFA inhibition through mechanisms of cell membrane/wall damage, eventually inducing cytolysis and bactericidal effects (Demeyer and Henderickx, 1967, Galbraith et al., 1971). Salminen et al. (2001) observed the batch biodegradation of accumulating materials (mainly LCFA) in anaerobic digesters that previously failed the operation, during the semi-continuous treatment of poultry slaughterhouse waste. These
previous results prompted a renewed interest for a more deep study on the anaerobic biodegradation of LCFA that are mainly associated to the biomass, aiming to optimize its degradation rate. In this regard, the kinetics of anaerobic mineralization of the biomass-associated LCFA was established, the limits of methanogenic activity enhancement after the mineralization of the biomass-associated LCFA were assessed, the effect of individual Volatile Fatty Acids (VFA) on the mineralization of the biomass-associated LCFA and the role of mass transport limitations imposed by the accumulation of LCFA were studied.

**KINETICS OF BIOMASS-ASSOCIATED LCFA DEGRADATION**

Thirty eight (38) sludge samples taken from continuous reactors fed with oleic acid based effluents were individually incubated in batch vials without any added organic carbon source, calcium or nutrients. Methane production due to the mineralization of the biomass-associated LCFA was recorded. The specific methane production rate and the amount of the biomass-associated LCFA per VSS unit (specific LCFA content) were calculated for each sample as exemplified in Figure 1, and are plotted in Figure 3.

![Figure 3. Kinetics of methane production in batch assays due to the degradation of the biomass-associated LCFA. Bars represent the standard deviation.](image)

This graph represents the influence of the available substrate, on the kinetics of its own mineralization. It is not a conventional kinetic plot, as the substrate is not dissolved in the medium, but instead it is associated to the sludge. In spite of that, experimental data were fitted by equation 1, which represents an adaptation of an inhibition model used before by Andrews (1971) to describe the inhibition of volatile fatty acids in the anaerobic digestion process. This model is based on the substrate inhibition enzymatic kinetics developed by Haldane (1930).

\[
q_p = \frac{\hat{q}_p \times S_{ba}}{K_S + S_{ba} + \frac{S_{ba}^2}{K_i}} 
\]

(Eq. 1)
where:
\( q_e \) – specific methane production rate [Msubstrate. Mbiomass\(^{-1}\). t\(^{-1}\)]
\( S_{ba} \) – biomass-associated substrate per VSS unit [Msubstrate. Mbiomass\(^{-1}\)]
\( \hat{q}_p, K_s, K_i \) are model parameters

Based on this model, a value of 1080 mg COD-LCFA.gVSS\(^{-1}\) was found to be the critical biomass-associated substrate per VSS unit (\( S_{ba}^{crit} \)), that allows the maximal mineralization rate, which was found to be 250 mg COD-CH4.gVSS\(^{-1}\).day\(^{-1}\) (eq. 2).

\[
S_{ba}^{crit} = \sqrt{K_s K_i} \tag{Eq. 2}
\]

The low correlation coefficient obtained for the fitting (0.56), imposes a significant uncertainty on the value of \( S_{ba}^{crit} \). Therefore the value around 1000 mg COD-LCFA.gVSS\(^{-1}\), represents just a rough estimate of the specific LCFA load that allows the maximal mineralization rate of the biomass associated LCFA. However, even with precision limitations it has practical interest as far as the mineralization of LCFA is concerned.

The low correlation coefficient obtained by this fitting also suggests that the mechanism of inhibition is more complex than a simple substrate inhibition phenomenon, in part because β-oxidation process involves a sequence of substrates and products that can compete with each other. Furthermore, the method used to measure the amount of the accumulated substrate is based on the measurement of the final product (methane) and thus depends on the metabolic activity of different trophic groups in the consortium. On the other hand, as previously suggested by Pereira et al. (2003), transport limitations may have an important role on LCFA mineralization.

**ENHANCEMENT OF THE SPECIFIC METHANOGENIC ACTIVITY AFTER THE MINERALIZATION OF THE BIOMASS-ASSOCIATED LCFA**

One of the most relevant results obtained concerned the measurement of the specific methanogenic activity before and after the mineralization of biomass-associated LCFA (Pereira et al., 2004). Three sludge samples (A, B and C) with specific LCFA contents of 1221±144, 2838±63 and 4571±257 mg COD-CH\(_4\).gVSS\(^{-1}\), respectively were studied in terms of the methanogenic activity in the presence of individual substrates (acetate, propionate, butyrate, and H\(_2\)/CO\(_2\)) before and after the depletion of the biomass-associated LCFA (Table 3). The specific methane production rates were, in average, 243±28, 87±2 and 35±4 mg COD-CH\(_4\).gVSS\(^{-1}\).day\(^{-1}\) for sludge A, B and C, respectively. In the sludge with a lower specific LCFA content (sludge A), no lag phase was observed preceding the initial methane production, whereas in sludge B and sludge C, lag phases around 100 and 500 hours were respectively detected, which is traduced by a low value in terms of initial methane production rate. However, after these delays, methane production proceeded at rates up to 400 mg COD-CH\(_4\).gVSS\(^{-1}\).day\(^{-1}\).

In general there was a significant increase on the SMA from all the substrates tested, providing a clear evidence that the effect of LCFA in the decrease of the SMA, is a reversible phenomenon in the range of specific LCFA content between 1000 and 5000 mg COD.gVSS\(^{-1}\).
Table 3 – Specific methanogenic activities exhibited by the sludges A, B and C, before and after the mineralization of the biomass-associated LCFA.

<table>
<thead>
<tr>
<th>In the presence of:</th>
<th>Specific methanogenic activity (mgCOD-CH$_4$/gVSS$\cdot$day$^{-1}$)</th>
<th>Sludge A</th>
<th>Sludge B</th>
<th>Sludge C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before (*)</td>
<td>after(**)</td>
<td>before(*)</td>
<td>after(**)</td>
</tr>
<tr>
<td>acetate</td>
<td>143±29</td>
<td>326±13</td>
<td>0</td>
<td>579±4</td>
</tr>
<tr>
<td>propionate</td>
<td>0</td>
<td>43±2</td>
<td>0</td>
<td>25±1</td>
</tr>
<tr>
<td>butyrate</td>
<td>102±28</td>
<td>146±6</td>
<td>0</td>
<td>646±9</td>
</tr>
<tr>
<td>H$_2$/CO$_2$</td>
<td>1462±94</td>
<td>1670±81</td>
<td>1218±1</td>
<td>2817±146</td>
</tr>
</tbody>
</table>

(*) Methane production rates of 243±28, 87±2 and 35±4 mg COD-CH$_4$/gVSS$\cdot$day$^{-1}$ (blank assays) were discounted in the assessment of the SMA in the sludge A, B and C, respectively.

(**) background methane production rates (blank assays) were null.

This is a remarkable result that clearly contradicts the bactericidal or permanent toxic effects of LCFA, previously reported by Rinzema (1988) and by Angelidaki and Ahring (1992) and creates a new challenge for the anaerobic treatment of wastes/wastewater with high lipid contents.

The low or null SMA measured before the depletion of the biomass-associated LCFA may result from a strong effect of transport (diffusion) limitations imposed by the LCFA layer surrounding the cells, which could hamper the access of the added substrates, as well as the subsequent biogas release. This is reinforced by the fact that H$_2$, the smallest substrate used, was, in all cases, easily mineralized to methane, suggesting a fast transport of this molecule through the LCFA layer. Transport limitations phenomena may be responsible by the observed lag phases that have been reported to be ascribed to mechanisms of cell wall damage and bactericidal effect, widely accepted for years. The recovery after a lag phase, attributed to the growth of few survivors (Rinzema, 1988), could be a delay imposed by transport limitations. In a recent work (Pereira et al., 2005), the evidence of transport limitation imposed by the accumulation of LCFA onto the anaerobic sludge was reported.

**EFFECT OF VFA ON THE MINERALIZATION OF THE BIOMASS-ASSOCIATED LCFA**

It was also important to evaluate the ability of anaerobic sludge to uptake VFA when loaded with the optimal amount of biomass-associated LCFA. Figure 4 presents the methane production and VFA depletion when sludge A (loaded with 1221±144 mg COD/gVSS$^{-1}$) was characterized in terms of SMA with acetate, propionate or butyrate (Pereira et al., 2004). Individual VFA were also monitored during the mineralization of the biomass-associated LCFA (“blank” assay), which revealed that, in these conditions, there was no detectable release of VFA to the medium. This was assumed to prevail also in the test vials. During propionate degradation, acetate and butyrate were detected in concentrations not exceeding 140 mg/l, and 74 mg/l, respectively. Acetate was also detected during butyrate degradation, in concentrations lower than 170 mg/l.
From the obtained results it can be observed that, when acetate was added to the LCFA-loaded sludge (Fig 4 (a)) the initial methane production was mainly due to the consumption of the added acetate, since acetate depletion rate was similar to the corresponding methane production. A similar pattern was observed when the added VFA was butyrate (Fig 4 (c)), but concerning the propionate a different behaviour was found (Fig 4 (b)). The VFA concentration decreased only 10% in the first 50 hours, whereas in the same time period, 99.1% and 83.6% of total VFA disappeared from the medium, when acetate and butyrate were added, respectively. Furthermore, when in presence of propionate, the initial specific methane production rate was significantly lower than in the situation where the mineralization of the biomass-associated LCFA was monitored (Figure 4 (b)). In the former situation, methane production seems to be a result of the interaction between both substrates (propionate+LCFA) degradation, since there was no evidence of a sequential consumption pattern.

**CONCLUSIONS**

The results presented in this work point out new insights on the anaerobic biodegradation of biomass-associated LCFA. It is important to highlight the sludge ability to accumulate and mineralize biomass-associated LCFA up to specific contents of 5000 mg COD-LCFA.gVSS-1, as well as the enhancement of sludge activity after the LCFA mineralization. The mineralization kinetics of the LCFA associated to the sludge was established and a specific content of about 1000 mg COD-LCFA.gVSS-1 was found to be the optimal for an efficient methane production rate. For sludge loaded with a LCFA specific content near the optimal value, the presence of acetate, propionate or butyrate in concentration of 30 mM for acetate and propionate and 15 mM for butyrate, did not impair the process, since no delay was observed in terms of overall methane production. From a practical viewpoint the results herein presented clearly support the approach of using sequential accumulation and degradation steps for the treatment of lipid/LCFA based effluents.
Therefore, the main practical conclusion of this research is that a sequencing batch reactor (SBR) based process will be the most suitable way to efficiently mineralize effluents with high lipids content. The new SBR concept is under development and was recently awarded with the Lettinga Award 2004.

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