Biosorption and mineralization of a dairy wastewater under sequential operation mode

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Abstract This work aimed to apply a sequential batch operation mode to promote the biomethanation of a real dairy wastewater enriched in fat. Three cycles were conducted with biomass previously acclimated to oleic acid. Soluble COD, VFA and methane content were analyzed in each cycle at different time intervals. The soluble COD removal efficiency improved slightly in each new feed and the total VFA levels detected decreased successively. The maximum cumulative methane production also improved from cycle 1 to cycle 3. These results contribute to support the idea that the key for the degradation of effluents with high lipid content is to sequence adsorption and degradation steps during the treatment process.

Keywords dairy wastewater; sequential operation; biomethanation

Introduction

When compared with other organic matter, lipids are attractive for biogas production, due to their high theoretical methane production. Lipids are one of the major components of organic matter in wastewater and dairy industries are important contributors to their total emission.

In anaerobic digestion, neutral fats are easily hydrolyzed to Long Chain Fatty Acids (LCFA), which are further converted to acetate and hydrogen through an β-oxidation mechanism (Weng and Jeris, 1976). The adsorption of oleic acid and other LCFA on the surface of anaerobic sludge is described in the literature (Hanaki *et al.*, 1981; Hwu, 1997, Alves *et al.*, 2001). The initial adsorption proceeds fast (within one day), but establishment of the adsorption equilibrium is a relatively slow process (weeks). After starting the methane production a certain amount of oleate was desorbed due to biogas release (Hwu, 1997).

The existing literature suggests that LCFA exert a bactericidal effect on methanogenic bacteria (Rinzema *et al.*, 1994) and that the mechanism of LCFA toxicity is related to the adsorption of the surface active acids onto the cell wall of bacteria, which affects its transport and/or protective functions (Demeyer and Henderickx, 1967; Galbraith and Miller, 1973). However, Pereira *et al.* (2002) observed that even when the methanogenic activity measurements indicated a severe inhibition, the anaerobic sludge was able to efficiently mineralize the LCFA that were mainly associated to the biomass, and that anaerobic consortia remained active even after being loaded with amounts of LCFA up to 5000 mg COD/g VSS. These authors also suggest that the key for the degradation of effluents with high lipid content is to sequence adsorption and degradation steps during the treatment process.

This work aimed to apply a sequential batch operation mode to the biomethanation of a real dairy wastewater enriched in fat.

Methods

Experiments were conducted in three series of vials of 70 mL. In each series, 2 vials were set up per sampling point and more two vials were set up to measure the biogas production. At different time

intervals, two of the vials were sacrificed and the respective content was analyzed for soluble COD and VFA. In parallel the biogas production was recorded. The vials were incubated at 37ºC and 150 rpm. Strict anaerobic conditions were maintained.

Inoculum and substrate

The inoculum was composed of crushed anaerobic granules previously acclimated to oleic acid (3 gVSS/L). The substrate was an emulsion obtained by mixing cream and a real dairy wastewater. The total COD was 3600 mg/L (1.2 g COD/gVSS), being the cream fraction of 83.3 % in terms of COD. The initial Fat-COD/VSS ratio was based on the optimal specific LCFA load previously determined by Pereira *et al.* (2004).

Analytical methods

Chemical Oxygen Demand (COD) and volatile suspended solids (VSS) were determined according to the Standard Methods (1995). The fractions of adsorbed and methanized COD were calculated according to Petruy and Lettinga (1997). VFA were determined by HPLC (Jasco, Japan) using a Chrompack column (6.5 x 30 mm^2); the mobile phase was sulfuric acid (0.01 N) at a flow rate of 0.7 ml/min. The column temperature was set at 40 ºC and the detection was made spectrophotometrically at a wavelength of 210 nm. The methane production in the gas vials was followed by measuring the pressure developed, using a pressure transducer technique (Colleran *et al.*, 1992). The methane content of the accumulated biogas was periodically measured by a Pye Unicam GCD gas chromatograph (Cambridge, England), using a Chrompack column Haysep Q (80-100 mesh). N_2 was used as carrier gas (30 ml/min) and the temperatures of injection port, column and flame ionization detector were 120, 40 and 130 ºC, respectively.

Results and Discussion

The soluble COD removal efficiency was always higher than 90% and in each new feed improved slightly (Figure 1). However, it is clear that in the first cycle most of the COD was not mineralized, but instead it probably accumulated into the sludge, by mechanisms of adsorption, precipitation or entrapment. In the second and in the third feed, the maximum cumulative methane production was much more consistent with the initial COD fed, indicating an efficient mineralization of the substrate.
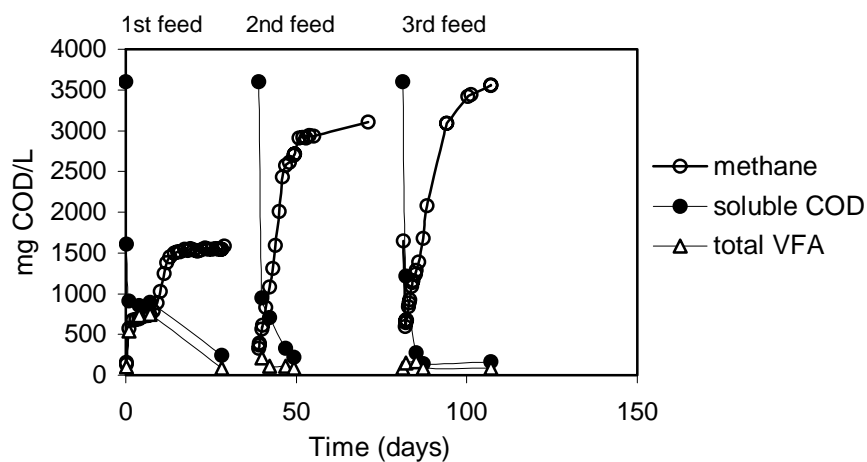

Figure 1 Time course of methane production, soluble COD and total VFA levels in the three successive feedings.

The pattern of VFA evolution along the three cycles was also significantly different in the first cycle than in the subsequent ones (Figure 2). Acetate, that was detected in concentrations up to 600 mg COD/L in the first cycle, did not appeared in concentrations higher than 100 mg COD/L in the second one and was consistently below in the third one.
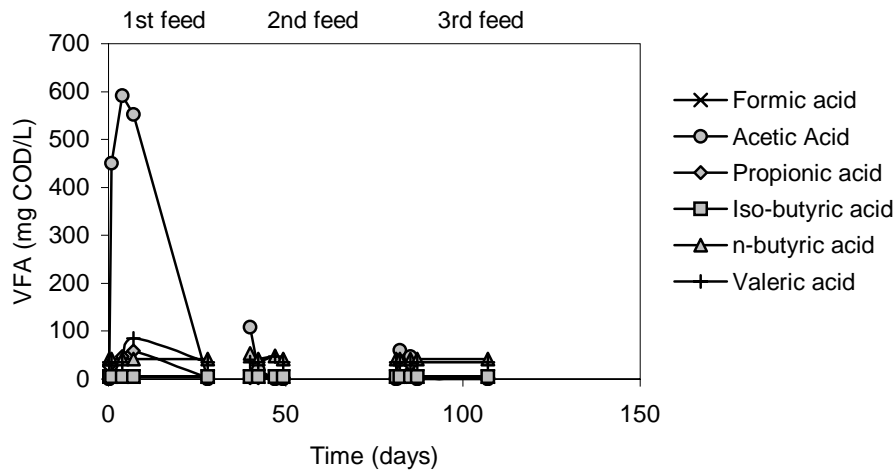

Figure 2 Time course of VFA levels in the three successive feedings.

The maximum cumulative methane production was also improved in every new cycle (Figure 1 and Table 1). A diauxic behavior was observed in the methane production only in the first cycle.

Figure 3 presents the fraction of adsorbed (or precipitated/entrapped) COD and the methanized COD. As previously stated by other authors, adsorption is a very fast process, since in the first point (time 0) already 50-60% of the added COD was adsorbed. After adsorption, a significant increase in the methane production was observed, more evidenced in cycles 2 and 3 than in cycle 1.

Table 1 Maximum cumulative methane production in the three successive cycles.

	Maximum cumulative methane production
	(ml CH_4@PTN/g VSS)
1st feed	$131,5 \pm 10,6$
2nd feed	$265,0 \pm 2,5$
3rd feed	$312,4 \pm 24,6$

Conclusions

Three cycles were run under batch operation mode in order to assess the feasibility of a sequential process to efficiently mineralize a real dairy wastewater rich in fat. In each new cycle the maximum cumulative methane production was improved, and the VFA levels decreased significantly. These results support the suggestion made by Pereira *et al.* (2002) that the key for the degradation of effluents with high lipid content is to sequence adsorption and degradation steps during the treatment process.
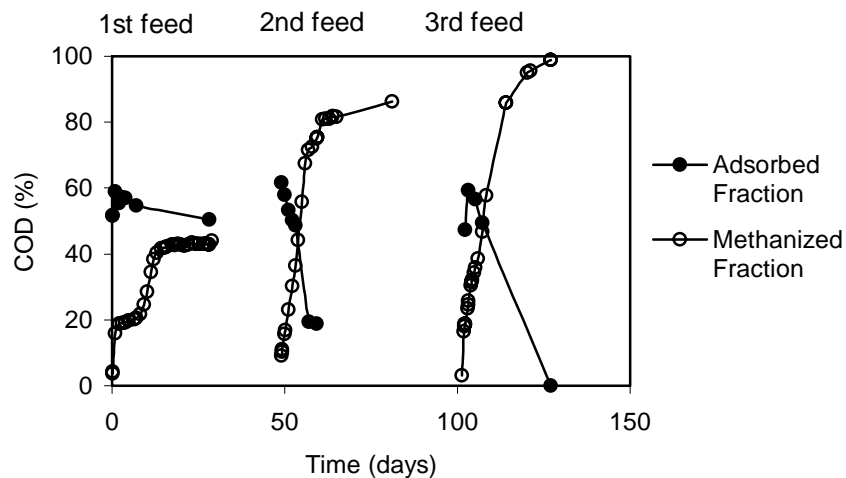
Figure 3 Time course of adsorbed and methanized COD fractions in the three successive feedings.

References
Alves M. M., Mota Vieira J. A., Álvares Pereira R. M., Pereira M. A., Novais J. M., Mota M. (2001). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors – Part II: Oleic acid toxicity and biodegradability". Wat. Res., 35(1), 264-270.

Coates J. D., Coughlan M. F., Colleran E. (1996). Simple method for the measurement of the hydrogenotrophic methanogenic activity of anaerobic sludges. Journal of Microbiological Methods, 26, 237-246.

Colleran E., Concannon F., Golden T., Geoghegan F., Crumlish B., Killilea E., Henry M., Coates J. (1992). Use of methanogenic activity tests to characterize anaerobic sludges, screen for anaerobic biodegradability and determine toxicity thresholds against individual anaerobic trophic groups and species. Wat. Sci. Tech., 25(7), 31-40.

Demeyer D. I., Henderickx H. K. (1967). The effect of C_{18} unsaturated fatty acids on methane production *in vitro* by mixed rumen bacteria. Biochim. Biophys. Acta, 137, 484-497.

Galbraith H., Miller T. B. (1973). Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. Journal of Applied Bacteriology, 36, 647-658.

Hanaki K., Matsuo T., Nagase M. (1981). Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. Biotechnol Bioeng., 23, 1591-1610.

Hwu C.-S. (1997). Enhancing anaerobic treatment of wastewaters containing oleic acid. Ph.D. Thesis, Wageningen Agricultural University, Wageningen , The Netherlands.

Pereira M. A., Pires O. C., Mota M., Alves, M. M. (2002). Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitate as a key intermediate. Wat. Sci. Tech., 45, 139-144.

Pereira, M.A., Sousa, D.Z., Mota, M., Alves, M.M. (2004) Mineralization of LCFA associated to anaerobic sludge: kinetics, transport limitations, enhancement of methanogenic activity and effect of VFA. Biotechnol Bioeng. (accepted).

Petruy R., Lettinga G. (1997). Digestion of a milk-fat emulsion. Bioresource Technology, 61, 141-149.

Rinzema A., Boone M., Van Knippenberg K., Lettinga G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. Wat Environ. Res., 66(1), 40-49.

Standard Methods for the Examination of Water and Wastewater (1995). 19th ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Weng C.-N., Jeris J. S. (1976). Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. Wat Res., 10, 9-18.