Enzymatic hydrolysis of floatable fatty wastes from dairy and meat food-processing industries and further anaerobic digestion

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Abstract

The wastewater from food-processing industries, such as dairy and meat, is heavily charged on lipids and proteins. Flotation process is commonly applied to separate the hydrophobic material phase, producing flotation froth, a waste that has high levels of fats and proteins. Enzymatic hydrolysis may be used to overcome the difficulty of fat biotransformation in a subsequent anaerobic digestion. In the present work wastes from the flotation process of two industries (dairy and poultry slaughterhouse) were hydrolyzed at 30 ^{o}C for 12 or 24 hours. Experiments were conducted without enzyme addition (control) or adding to the reaction medium a commercial lipase. The effect of adjusting the pH at the beginning of the hydrolytic assays was also investigated. The long chain free fatty acids (LCFAs) released were identified (amount and percentage) and 5-d digestion assays were conducted with the hydrolyzed material. The results indicated that the hydrolysis assays conducted with initial pH adjusted to 7.0 promoted a higher increase on LCFAs amounts and, particularly on unsaturated acids. The utilization of a commercial enzyme (0.1% w/w) led to an increase on LCFAs contents for the assays conducted with initial pH adjustment. In most anaerobic digestion assays, the specific methane production ($mL.g^{-1}$ waste) showed a decreasing trend with the increase of unsaturated fatty acids in the medium. The specific methane production was, in most cases, higher when the hydrolysis pH was adjusted at the beginning of the assays. In general, the utilization of a commercial enzyme (lipase) in the hydrolysis process did not contribute to enhance methane production in 5-d anaerobic digestion assays.

Keywords: fatty wastes, lipids, lipase, hydrolysis, anaerobic digestion.

1 Introdution

The wastewater from several food-processing industries such as dairy and meat contain high concentrations of organic matter, mainly lipids and proteins. To remove particulate organic matter, flotation process is commonly applied to remove hydrophobic materials from the aqueous phase, resulting in the recovery of large amounts of fats and greases (fatty waste) from the wastewater. Thus, a generally pasty waste (flotation froth) is generated, whose environmental safe disposal or treatment is required. Anaerobic digestion could be an appropriate technology to reduce the pollution potential of this kind of fatty waste besides producing biomethane, which is a renewable source of energy. Potentially, fats present high theoretical conversion yields to methane (0.99 L g^{-1}) when compared with carbohydrates (0.42 L g^{-1}) and proteins (0.63 L g^{-1}) [1]. However, the hydrolysis of lipids is frequently a limiting-step for the anaerobic process. In general, fats are removed by several techniques to avoid operation problems in biological treatment systems. Problems caused by excessive oil and grease include the reduction in cell-aqueous phase transfer rates, sedimentation hindrance due to the development of filamentous microorganisms, development and flotation of sludge with poor activity, clogging and the emergence of unpleasant odors [2].

The simplest lipids containing fatty acids are triacylglycerols, which are composed of three fatty acids each one bonded to a glycerol molecule by an ester bond. When these substances are hydrolyzed, glycerol and free fatty acids are liberated. Although the splitting of these molecules by hydrolysis seems to be beneficial for the subsequent biodegradation process, some triacylglycerols release long-chain fatty acids (LCFAs) and, these substances may hinder, particularly, anaerobic digestion. The LCFAs have already been reported, some decades ago, as inhibitors of the anaerobic process [3-4]. Meanwhile, the cleavage of triacylglycerols by hydrolysis can increase the biodisponibility of organic molecules in the medium, improving the biodegradation process [5].

So, it seems that a kind of dilemma exists: hydrolysis (mainly enzymatic hydrolysis) of fats is really beneficial for the subsequent anaerobic digestion? or does hydrolysis generate LCFAs that have an inhibitory effect on anaerobic digestion?

A relative abundant literature evidenced that, for industrial wastewaters containing oils and grease (O&G) contents up to 1,500 mg L^{-1}, the previous enzymatic hydrolysis enhanced methane production [6-9]. The successful results obtained with wastewaters were a source of motivation to investigate if enzymatic hydrolysis would be able to improve the anaerobic biodegradation of froth wastes, a very different matrix and, consequently enhance methane production. Thus, the hydrolysis of the floatable fatty wastes from dairy and meat food-processing industries was investigated.

2 Methods

The fatty wastes were collected from flotation units of local industries (dairy and poultry slaughterhouse), and stored at -4.0 ºC until use. The wastes characteristics were determined according to standard procedures of APHA [10]. Lipids were determined according to the adapted method of Postma and Stroes [11]. Proteins were determined by the classical method of Lowry [12].

2.1 Hydrolysis Assays

The enzyme lipase commercialized by the company Biocatalysts was used in the hydrolysis assays. Lipomod MD is a solid commercial lipase preparation, which presents a nominal activity of 11,500 $U.g^{-1}$. The assays were conducted in stirred flasks containing the fatty waste and the enzyme preparation (0.10% w/w). The ion calcium (lipase activator) was added to the reaction medium (0.15% w/w). For a set of experiments, the fatty waste pH was adjusted to 7.0 before performing the hydrolysis. For other set of assays the initial pH was not adjusted. The hydrolysis was conducted for 12 or 24 hours under agitation at the temperature of 30 ^{o}C. The control assays were always performed without enzyme and with calcium addition under the same conditions above described. The parameter used to establish the best conditions of hydrolysis was the acidity index (IA), which was determined according to the procedure named Free Fatty Acids Method Ca 5a-40 [13].

Samples were withdrawn from the flasks at the end of the reaction and the determination of the liberated long chain fatty acids (LCFA) was made by high resolution gas chromatography – mass spectrometry (GC-MS) using the following equipment: HRGC-MS Agilent Technologies, model 7890A-5975C, with a CTC PAL automatic injector. The column used was a DB-FFAP (15m x 0.10mm, 0.10µm). The detector temperature was maintained at 240°C. The sample injection volume was 1.0 µL and the injection flow rate was 0.5 mL min^{-1}, with a split ratio of 1:100. The helium was used as the entrainment gas at a flow rate of 1.0 mL min^{-1}. The selective monitoring of the specific ions of each fatty acid in the mass spectrometer detector was performed in scanning mode with a mass range of 40-400 m/z. The performance evaluation of the method followed the DOQ-CGCRE-008 [14]. The free fat acids fractions from the liquid-liquid extraction phase were transesterified before chromatographic analysis. The chromatographic technique was validated for 12 organic acids, including 8 LCFAs (C12:0; C14:0; C14:1; C16:0; C16:1; C18:0; C18:1; C18:2).

2.2 Anaerobic digestion assays

The hydrolyzed wastes were named hydrolyzed 0.1%, when lipase was used and, control, when no commercial enzyme was used. The anaerobic digestion of the hydrolyzed wastes was carried out using sealed 100-mL penicillin-type flasks and 60 mL working volume of a blend of anaerobic sludge and waste. The proportion of sludge and waste added in each anaerobic digestion assay was 1.4:1 (w/w). The flasks were sealed with rubber stoppers and aluminum seals, connected to plastic syringes to collect the produced biogas. This procedure was based on the work published elsewhere [8].

Anaerobic sludge from an industrial digester treating poultry processing wastewater was used as inoculum, contained a concentration of volatile suspended solids of 57 g mL^{-1} and specific methanogenic activity of 2.72 mL_{CH4} $g^{-}VSS^{-1}h^{-1}$. The pH of the digestion medium was adjusted to 7.0 before feeding the medium to the flasks. Incubation was conducted at 35 ^{o}C, without agitation, for 5 days.

The volume of biogas produced in the anaerobic biodegradability assays was measured through the dislocation of the embolus of plastic syringes connected to the penicillin flasks.The methane content of the biogas was determined by gas chromatography (Agilent 7890A with flame ionization detector).

3 Results and Discussion

The waste from the dairy industry (FW-D) is a flotation froth having *mousse* aspect, with low capacity of phase separation. The waste from the poultry slaughterhouse (FW-P) do not have such aspect, it was more aqueous and presented sedimentable solids. Both wastes have a characteristic unpleasant odor. The main physical and chemical characteristics of the wastes are presented in Table 1.

The two wastes have different properties. FW-D has a high level of fats, almost two folds higher than that found in the waste FW-P. The levels of chemical oxygen demand (COD) and O&G are higher in the flotation froth from the dairy industry (FW-D), which also has a lower humidity. These differences will certainly affect the hydrolysis and the anaerobic digestion of these wastes.

3.1 Hydrolysis Assays

The increase on the acidity index in relation to the initial index (IA/IAo) for assays that lasted 12 and 24 hours with and without initial pH adjustment and with 0.1% w/w of commercial enzyme addition and control (no enzyme addition) is reported in Table 2. The increase of IA was moderate, 2.35 folds was the maximum result, and the best results were observed for the poultry fatty waste (FW-P). A slight increase on the ratio (IA/IAo) was observed when the initial pH was adjusted to 7.0, as well, when the reaction time was higher (as expected). Although, the acidity increase in the control assays was significant, in most assays the values obtained were lower than those achieved in the corresponding assays with enzyme addition. The acidity increase in the control assays can be attributed to the autochthonous microorganisms adapted to the substrates (mainly fats and proteins) that are able to produce hydrolytic enzymes, including lipases.

Table 1. The main physical and chemical characteristics of FW-P (poultry fatty waste) and FW-D (dairy fatty waste).

Charateristics	**FW-P**	**FW-D**
Density, kg L^{-1}	0.990 ± 0.008	0.939 ± 0.004
Humidity, %	90.27 ± 0.82	84.54 ± 0.41
pH	5.7 ± 0.1	5.3 ± 0.1
Alkalinity, g$CaCO_3$ L^{-1}	0.79 ± 0.01	1.27 ± 0.02
Proteins, g L^{-1}	13.2 ± 0.4	10.2 ± 0.3
COD, g L^{-1}	41 ± 5	73 ± 8
O&G, %	7.1 ± 0.5	12.5 ± 0.8
Lipids, g L^{-1}	15.1 ± 0.6	54.8 ± 0.8
Total LCFAs, g L^{-1}	0.76 ± 0.13	5.85 ± 0.45
IA, mg L^{-1}	4.99 ± 0.001	10.22 ± 0.03

Table 2. Relative increase on the acidity index (IA/IAo) observed in the hydrolysis assays conducted during 12 and 24 h, employing enzyme contents of 0.1% (w/w) or not.

	pH No Adjustment				pH Adjusted			
Wastes	Control		Hydrolyzed 0.1%		Control		Hydrolyzed 0.1%	
	12h	24h	12h	24h	12h	24h	12h	24h
FW-P	1.44	1.54	1.94	1.93	1.78	1.92	2.27	2.35
FW-D	1.09	1.12	1.10	1.14	1.13	1.22	1.14	1.25

In the present work, the term LCFA corresponds to free fatty acids. Only 8 LCFA were found at detectable levels at the end of the hydrolysis reactions that lasted 12 and 24h. The relative amount of each LCFA and the total concentration of saturated and unsaturated fatty acids are shown in Tables 3 and 4, to FW-P and FW-D respectively.

Oleic acid was the predominant LCFA (unsaturated) in the reaction media of both fatty-wastes (FW-D and FW-P). It was found in percentages comprised between 55 and 72% of the LCFAs detected. The original content of this LCFA in the raw waste was 61.3 and 54.7% for FW-P and FW-P, respectively. Palmitoleic acid was the second unsaturated acid found in hydrolysis experiments, but it was detected at low percentages (below 9%) for both wastes. Linoleic acid (unsaturated) was also found in low percentages, below 2% for FW-D and below 7% for FW-P.

The concentration of unsaturated LCFAs increased during the hydrolysis process (from 12 to 24 h) in 50% of the experiments with FW-D. For the poultry waste (FW-P) that increase was observed in 100% of the

experiments (control and with enzyme addition, with and without pH adjustment). A slight increase on the percentage of unsaturated acids was observed in most experiments. They were predominant in the reaction media, corresponding to 61 to 79% of the LCFAs in the experiments with the dairy fatty-waste (FW-D) and 59 to 87% for the poultry fatty waste (FW-P).

Table 3. LCFAs composition of the raw waste (FW-P) and determined at the end of the hydrolysis reaction (12 and 24 h). Experiments with or without initial pH adjustment.

		Control				Hydrolyzed (0.1%)			
		No pH adjustment		pH adjusted		No pH adjustment		pH adjusted	
%LCFA	Raw	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
Lauric [C12:0]	0.4	0.3	0.2	0.4	0.2	0.6	0.2	0.4	0.3
Myristic [C14:0]	3.1	7.5	3.9	1.9	1.1	1.7	3.6	2.0	1.0
Myristoleic [C14:1]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Palmitic [C16:0]	19.3	21.8	17.0	12.1	12.2	16.3	18.3	15.5	8.3
Palmitoleic [C16:1]	4.5	0.2	3.0	5.8	7.6	5.7	3.7	4.5	8.4
Stearic [C18:0]	9.0	10.9	7.7	5.7	5.2	6.2	8.2	6.9	3.7
Oleic [C18:1]	61.3	59.0	66.4	70.6	68.3	63.7	62.4	67.1	71.8
Linoleic [C18:2]	2.0	0.2	1.9	3.4	5.5	5.8	3.5	3.6	6.5
Saturated LCFA (mg L^{-1})	240	450	410	665	615	340	550	380	840
Unsaturated LCFA (mg L^{-1})	515	660	1015	1890	2680	1040	1270	1580	5480
Total LCFA (mg L^{-1})	755	1110	1425	2555	3295	1380	1820	1960	6320

n.d not detected – below the detection limit of the method.

Table 4. LCFAs composition of the raw waste (FW-D) and determined at the end of the hydrolysis reaction (12 and 24 h). Experiments with or without initial pH adjustment.

		Control				Hydrolyzed (0.1%)			
		No pH adjustment		pH adjusted		No pH adjustment		pH adjusted	
%LCFA	Raw	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
Lauric [C12:0]	1.5	1.3	1.4	0.9	0.7	1.1	1.4	0.7	0.6
Myristic [C14:0]	7.8	7.1	7.1	5.1	4.2	6.0	7.1	4.0	3.5
Myristoleic [C14:1]	0.1	0.7	1.0	0.7	0.9	0.5	0.9	0.8	0.7
Palmitic [C16:0]	21.9	21.3	20.1	15.4	14.4	18.4	20.2	13.0	11.8
Palmitoleic [C16:1]	4.3	4.4	4.2	5.2	6.4	4.9	4.7	5.4	5.4
Stearic [C18:0]	9.6	9.7	8.7	7.2	7.2	8.5	9.0	6.5	5.8
Oleic [C18:1]	54.7	56.0	58.2	65.8	65.5	60.5	57.0	69.8	71.3
Linoleic [C18:2]	0.1	0.2	0.2	0.4	1.6	0.6	0.6	0.7	1.6
Saturated LCFA (mg L^{-1})	2390	2630	2790	2920	2400	2650	2690	2510	2620
Unsaturated LCFA (mg L^{-1})	3455	4040	4670	7270	6660	5150	4440	7880	9480
Total LCFA (mg L^{-1})	5845	6670	7460	10190	9060	7800	7130	10390	12100

The most abundant saturated LCFAs were palmitic, stearic and myristic in hydrolysis media of both wastes (FW-D and FW-P). The percentages of those acids did not change appreciably during hydrolysis (from 12 to 24 h), the same occurred, in general, with the concentration of the saturated LCFAs. The most prominent increase of saturated LCFAs concentration occurred in the first 12 h of hydrolysis, as can be observed from raw waste data in Tables 3 and 4. Saturated acids corresponded to 32% and 41% of the total LCFA concentration for FW-P and FW-D, respectively. The distribution of LCFA had a small change when the commercial lipase was used. For FW-P there was a relative increase of the oleic acid content and a reduction of the palmitic acid content. For FW-D this same trend was observed.

Hydrolysis was conducted under non-sterile conditions and for long time (24 h) at the temperature of 30°C. Thus, hydrolysis of triglycerides was occurring catalyzed by enzymes produced by the autochthonous microorganisms, as well, by the commercial enzyme (in the experiments with enzyme addition). The microorganisms may also be able to promote the degradation of the fatty acids liberated from the triglycerides. Thus, there is a balance between LCFAs liberation by hydrolysis and occasional transformation of these substances by autochthonous microorganisms.

The pH adjustment carried with a concentrated solution of NaOH, before the beginning of the assays, promoted an increase on the total LCFA concentration, caused by basic hydrolysis. As hydrolysis proceeded the total LCFAs content always increased for the waste FW-P. An increase of this parameter was also observed for the waste FW-D until 12 h of reaction. After that, a small decrease was observed in some experiments.

The pH adjustment at the beginning of the reaction was an important factor affecting the hydrolysis process. Figure 1 illustrates this effect for both wastes comparing data obtained after 24 h of reaction for the control experiments and the assays conducted with commercial enzyme addition (0.1% w/w).

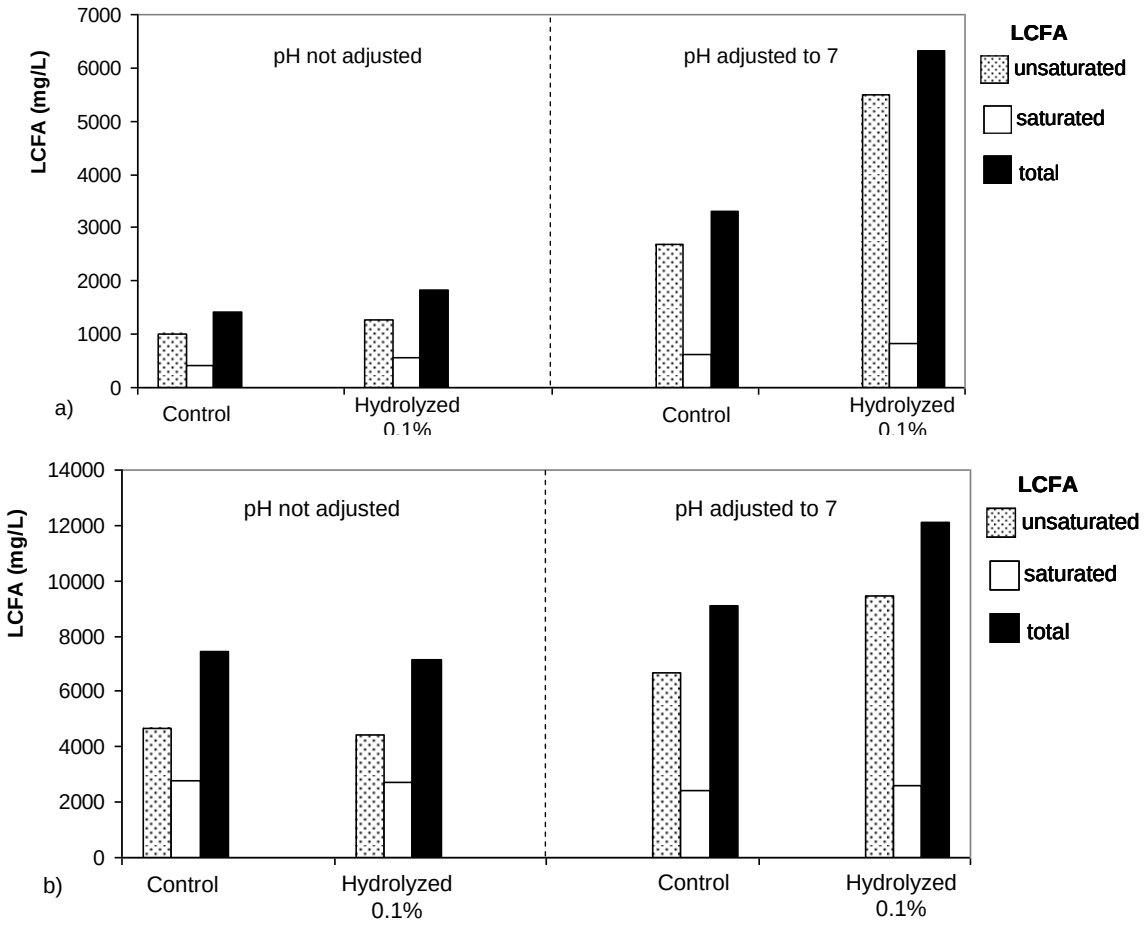


Figure 1. Variation of LCFAs concentration in experiments with and without initial pH adjustment. a) FW-P, b) FW-D. Time of hydrolysis considered: 24 h.

The utilization of commercial enzyme (lipase 0.1%) effectively promoted an increase on LCFAs concentrations, in comparison with control assays, when the initial pH was adjusted. In such case, the augmentation on total LCFAs concentration was much more pronounced for unsaturated acids. In a study with wastewater from a poultry slaughterhouse presenting 800 mg L^{-1} of O&G, during enzymatic hydrolysis, using

lipase, the LCFAs concentration decreased in the reaction period of 4 to 8 h. This decline continued until 24 h and was attributed to the consumption of LCFAs by microorganisms [15]. Thus, despite the differences between wastewater and flotation froth properties, the consumption of LCFAs by microorganisms seems to have occurred in some experiments with both wastes (FW-P and FW-D).

3.2 Biodigestion assays

The specific methane production (mL g^{-1} waste) was calculated based on the volume of methane produced in 5 days and is represented as SMP-5d. Data is shown in Table 5. In general, this parameter was higher for the waste from the poultry slaughterhouse (FW-P). Production of methane was lower for the dairy waste (FW-D). The specific methane production results obtained with the hydrolyzed wastes (lipase addition) were lower than those achieved in the control assays for the poultry slaughterhouse (FW-P). Thus, the utilization of the commercial enzyme was not effective in this case. For the fat waste from the dairy industry (FW-D) the beneficial effect of enzymatic hydrolysis was only observed when the hydrolysis was carried for 24 h with initial pH adjustment. The effect of adjusting the waste pH before hydrolysis on SMP-5d was slightly positive for almost all digestion assays.

Table 5. Specific methane production (SMP-5d) determined for all digestion assays.

	Control				Hydrolyzed (0.1%)			
	No pH adjust.		pH adjusted		No pH adjust.		pH adjusted	
	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
FW-D								
Specific Methane Production ($mL.g^{-1}$ waste)	1.32	0.40	1.29	1.34	0.28	1.15	1.30	2.46
FW-P								
Specific Methane Production ($mL.g^{-1}$ waste)	2.28	2.30	3.10	2.20	2.19	1.77	2.64	1.90

Figure 2 illustrates the methane production during the biodigestion assays conducted with the two wastes previously submitted to hydrolysis under the following conditions: waste pH adjusted to 7.0 just before the hydrolysis and time of reaction of 24 h. For the fat waste (FW-P) the assays named control and hydrolyzed had almost the same profile of CH_4 production. For the waste (FW-D) the profiles of CH_4 production showed different patterns. The assay named control presented a lag time and the volume produced increased very few in the interval 50 - 120 h. It is worth to note that three of the methane production profiles in that figure show an increasing trend at the end of the assay (120 h or 5 d). Certainly, to have a more accurate evaluation long term assays should be performed in future experiments.

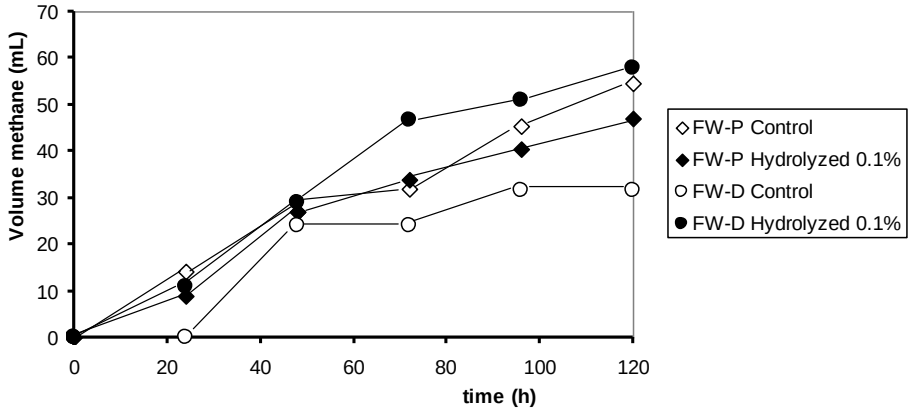


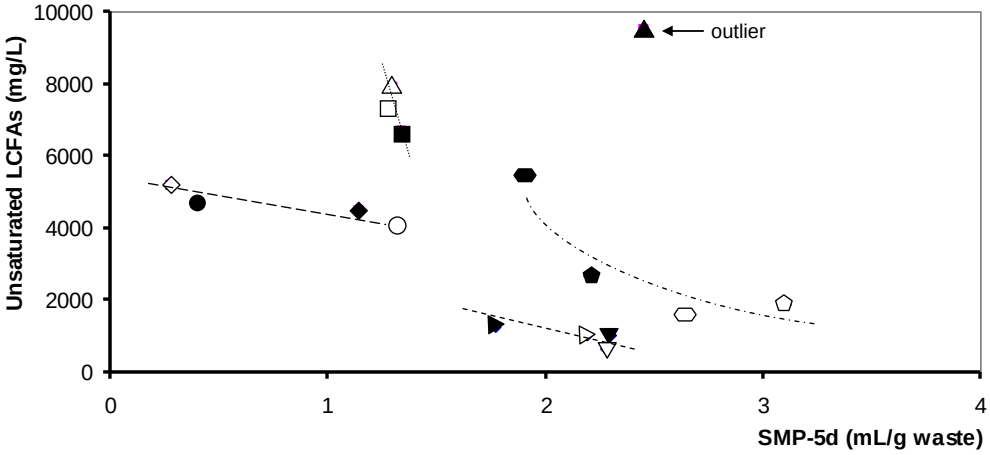
Figure 2. Production of methane during anaerobic digestion of hydrolyzed wastes: control (without enzyme addition) and hydrolyzed (with commercial enzyme addition 0.1% w/w). Hydrolysis was conducted for 24 h and the initial pH of the wastes was adjusted to 7.0 at the beginning of the reaction.

The profiles of methane production from complex fatty-wastes may present an interesting behavior. In long term experiments (63-d) of anaerobic digestion of the flotation froth from a dairy industry conducted in an

automatic system (AMPTS) by members of our research group, a significant increase on methane production was observed after 16 days of incubation [16]. In that work, for the material hydrolyzed without addition of commercial lipase (control), the specific methane production increased from 0.56 to 33.4 mL g^{-1} waste in the period of 8 to 63 days. For the material hydrolyzed with lipase (0.1% w/w) the methane production raised from 0.81 to 34.4 mL g^{-1} waste in the same period. Thus, it is possible that high yields of methane can be achieved with the two tested wastes in long term digestion assays, since in 5 days expressive specific methane production values were attained in comparison with those reported in the cited work for 8 days [16].

The inhibition of anaerobic digestion by LCFAs, mainly unsaturated LCFAs, was reported in many works with wastewaters and synthetic solutions containing triglycerides [17,18]. However, the inhibition phenomenon is complex, because the adaptation of microorganisms may occur and the inhibition intensity seems to dependent on the anaerobic sludge characteristics, in particular its biological diversity, since some methanogens are more sensitive to LCFAs than others [19]. A severe inhibition by unsaturated LCFAs was reported [20], in particular, by linoleic and oleic acids.

In Figure 3 the concentration of unsaturated LCFAs was plotted against the specific methane production (SME-5d), using all data obtained. A different symbol was used for each experimental condition investigated. Taking in consideration all experiments, SMP-5d was lower when the concentration of unsaturated LCFAs was higher in the hydrolyzed waste (with or without commercial enzyme addition).



FW-D No pH adjust: Control 12 h (○), Control 24 h (●), Hydrolyzed 12 h (◇), Hydrolyzed 24 h (◆)
FW-D pH adjusted: Control 12 h (□), Control 24 h (■), Hydrolyzed 12 h (△), Hydrolyzed 24 h (▲)
FW-P No pH adjust: Control 12 h (▽) , Control 24 h (▼), Hydrolyzed 12 h (▷), Hydrolyzed 24 h (▶)
FW-P pH adjusted: Control 12 h (⬠), Control 24 h (⬟), Hydrolyzed 12 h (⬭), Hydrolyzed 24 h (⬬)

Figure 3. Effect of unsaturated LCFAs in the hydrolyzed waste (with and without enzyme addition) on the specific methane production (SMP-5d).

The lines in Figure 3 are mere illustrations of the variation trend of SMP-5d with unsaturated LCFAs concentration for a particular hydrolyzed waste, which pH was adjusted or not in the initial step of hydrolysis. Just one experiment had a very different result and was considered an outlier.

Comparing the two wastes, it is clear that the one from the dairy industry showed worse results in terms of SME-5d. However, in anaerobic degradation assays lasting long times, several tens of days, adaptation of microorganisms can overcome inhibitory effects caused by LCFAs, as already observed [18; 20]. For wastes with very high content of organic material, including fats and proteins and, presenting a pasty consistence, like FW-D, long times of digestion will be necessary to reach high methane production yields.

The hydrolysis step, with or without the addition of commercial lipase, promoted an increase on the concentration of LCFAs. For the waste richer in fats (FW-D), the larger amount of LCFAS liberated (mainly unsaturated acids) led to smaller methane yields, at least, in 5-d incubation assays. For the waste FW-P, presenting lower fat content, lower LCFAs concentrations were attained at the end of the hydrolytic step and, higher methane production yields were observed.

4 Conclusion

The step of waste hydrolysis (with or without addition of a commercial enzyme) promoted increase of the acidity index in most experiments and enhanced the formation of LCFAs. In comparison with the raw wastes, the concentration of LCFAs increased in the hydrolysis step and more intensely when the initial pH of reaction

was adjusted to 7.0. When this occurred, the utilization of the commercial enzyme led to higher LCFAs concentrations for the two wastes studied. The free fatty acids composition (in percentage) did not change appreciably during hydrolysis. Oleic acid was the preponderant LCFA in the reaction media of both wastes. Unsaturated LCFAs were always found in higher concentrations in all hydrolysis experiments.

The specific methane production, determined in 5-d period, was higher for the waste FW-P, for which the amount of liberated unsaturated fatty acids was smaller, in function of its lower fat content. The amount of unsaturated LCFAs seems to promote some inhibition on methane production. A trend of reduction of the specific methane production with unsaturated LCFAs concentration was observed. Finally, concerning methane production, the addition of commercial lipase in the hydrolysis step was only beneficial for the waste from the poultry industry (FW-P). The potential of the autochthonous microorganisms can not be neglected, because, adequately stimulated they can produce enzymes, making unnecessary the utilization of commercial enzymes. With respect of the dilemma before mentioned, it is possible to state that the positive effect of hydrolysis on methane production is limited when the waste presents a high fat content. In this case, the inhibition effect on anaerobic digestion seems to surpass the occasional benefits of fat hydrolysis.

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