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Toxicity and Biodegradability of Olive Mill Wastewaters in Batch Anaerobic Digestion

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ABSTRACT

The anaerobic biodegradability and toxicity of olive mill wastewaters (OMW) were studied in batch anaerobic digestion experiments. Anaerobic digestion of OMW or the supernatant of its centrifugation, the methane production was achieved at up to 5–15% (V/V) dilution corresponding to only 5–20 g/L COD. The washed suspended solids of OMW were toxic at up to 80 g/L COD; however, the kinetic of biodegradability of OMW or the supernatant was faster than for suspended solids, which are constituted meanly of cellulose and lignin. The darkly colored polyphenols induce the problem of biodegradation of OMW, whereas the long chain fatty acids (LCFA), tannins and simple phenolic compounds are responsible its toxicity for methanogenic bacteria.

Index Entries: Anaerobic digestion; methane; olive mill wastewaters; phenolics; inhibition.

INTRODUCTION

Olive oil extraction is mainly carried out by the traditional discontinuous press process or by the more recent continuous solid-liquid centrifuge system. During this extraction, the olives were ground and pressed

to separate the residual solids (husk), which contain oil to be recovered by means of solvent extraction from emulsion of oil and vegetation waters. Finally, the oil was separated from the vegetation waters by centrifugation. The washing waters were added to the vegetation waters to give the olive mill wastewaters (OMW).

In the olive growing countries of the Mediterranean area, the OMW production is more than 30 million m^3/y, inducing considerable pollution (*1*). Several experiments have been carried out to valorize this effluent. However, no work has reached the industrial scale for economic reasons.

Anaerobic digestion offers significant advantages in lowering energy consumption and sludge production. Moreover, the anaerobic digestion of OMW is possible with higher performance in comparison with other food industrial wastewaters (*2*). However, the experimental results were not satisfactory because of methanogenic bacteria inhibition (*3–5*). Indeed, in all cases, this effluent had to be diluted for anaerobic treatment to reduce the inhibitory effect of OMW.

The objective of this work was the study of toxicity and biodegradability of OMW in batch anaerobic digestion and to discuss an appropriate pretreatment that can decrease the inhibitory effect of OMW to decrease the toxicity of this waste and improve its biodegradability.

METHODS

Inoculum and Culture Media

The urban digestor sludge was obtained from an anaerobic mixed digester treating the sludge coming from the aerobic treatment of urban wastewater. After concentration by sedimentation, the dry weight and volatile suspended solids (vss) were, respectively, 59.6 and 44.2 g/L. Ten mL of sludge and 0.2 mL of Na_2S, $9H_2O$ (25% w/v) were loaded under O_2-free nitrogen in 60 mL serum bottles stoppered with black butyl rubber closures (Bellco Glass Inc., Vineland, NJ). These bottles were kept at 4°C.

At the time of utilization, 10 mL of OMW supplemented with urea (COD:N, 50) were added; the pH was adjusted to 7.5 with $Ca(OH)_2$, and the bottles were incubated at 35°C.

Analytical Methods

Total solids (TS) were obtained by drying the sample overnight at 105°C. Total suspended solids (TSS) were obtained by centrifugation at 4000 g during 15 min; the settled solids were then dried overnight at 105°C. The ash content was determined after calcination of the dry sludge at 600°C for 1 h. The difference between total suspended solids and ash content was defined as volatile suspended solids (VSS). The

Table 1
Composition of Different Fractions of OMW

Concentration, g/L	OMW	After centrifugation	
		Suspended solids	Supernatant
Chemical oxygen demand	157	105	128.4
Reducing sugars	16.7	2.7	17.2
Glucose	7	0.8	7.5
Fats	3.6	-	3.9
Proteins	2.15	3.8	1.3
Condensed tannins	2.3	1.8	0.94
Hydrolysed tannins	7	3.8	7.8
Monomeric flavoid	1.5	0.57	1.6
Simple phenolics	6.4	2.84	7.1

chemical oxygen demand (COD) was estimated by the economical method described by Knechtel (*6*).

Methane and volatile fatty acids (VFA) were analyzed by the same chromatograph apparatus. Gas samples were taken with a syringe and analyzed by gas chromatography with a flame ionization detector (DELSI 30, Delsi-Nermag, Argenteuil), fitted with a 80-cm stainless steel column packed with 4% H_3PO_4 on Porapack Q (80–100 mesh). N_2 was used as the carrier gas at 28 mL min^{-1} with H_2 and air flows of 25 and 30 mL min^{-1}, respectively. The oven, injector, and detector temperature was 200°C.

The total phenolics, which are hydrolyzable tannins, condensed tannins, monomeric flavoids, and simple phenolics were first determined according to the method used for vegetable extracts (*7*) and adapted to raw olive mill effluent and prefermented effluent with few modifications (*8*). Gel filtration on Sephadex G-50 was used to analyze the polyphenolic compounds present in raw and treated OMW. Three mL of product were filtered and placed on a Sephadex coarse G-50 column (3×50 cm) previously equilibrated with distilled water. This column was washed with 400 mL of distilled water at a flow rate of 0.33 mL/min. The effluent was collected on the basis of 3 mL/tube. These fractions were measured spectrophotometrically at 280 nm.

RESULTS AND DISCUSSION

OMW Analysis

Analysis of OMW (Table 1) show that this effluent contains easily fermentissible products, such as reducing sugars and glucose on the one hand, and compounds diffically biodegradable as long chain fatty acids (LCFA) and phenolic compounds, on the other hand.

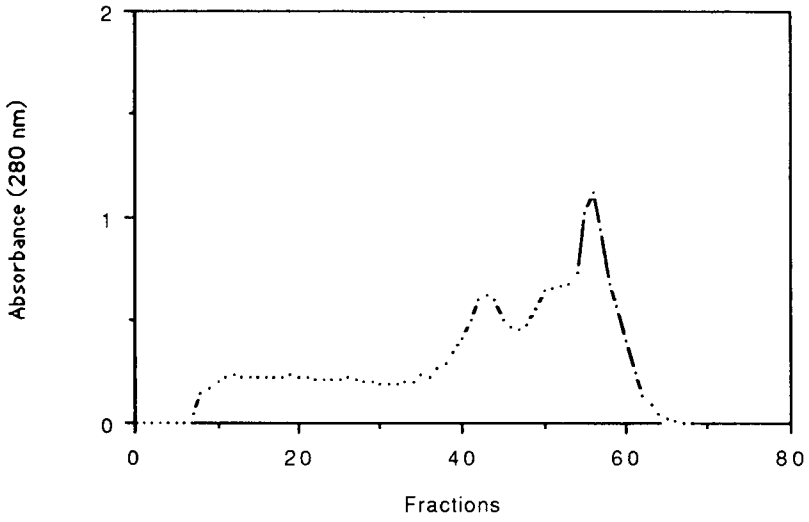

Fig. 1. Gel filtration of OMW on sephadex G-50.

Gel filtration chromatography showed that phenolic compounds contained in OMW can be divised mainly in two groups (Fig. 1). The phenolic compounds of the first group eluated in 40–80 fractions contain simple phenolic compounds, not autoxided tannins (low MW tannins), anthocyanins (*9*), and a class of compounds giving a yellow color. The main phenolic acids are syringic acid, *p*-hydroxyphenylacetic acid, vannilic acid, veratric acid, caffeic acid, protocathechuic acid, *p*-coumaric acid, and cinnamic acid (*10,11*). The polyphenols of the second group correspond to 7–40 fractions, which contain darkly colored polymers and result from the polymerization and autoxidation of phenolic compounds of the first group. The phenomenon of polymerization by autoxidation of tannins into darkly colored polymers was demonstrated (*12*).

The color of the OMW depends on the ratio between the two groups of polyphenols. It was observed that OMW becomes blacker when it has been stored for some time. This change in color might be a result of the oxidation and subsequent polymerization of like tannins, giving darkly colored polyphenols.

Methane Formation from Anaerobic Digestion of OMW

The sludge incubated without OMW addition (control) evolved an average of 300 μM of methane. When the substrate added to the sludge contained toxic compounds, the methane production decreased; when the level of toxic compounds was low, the methane production could be increased by the biodegradation of OMW.

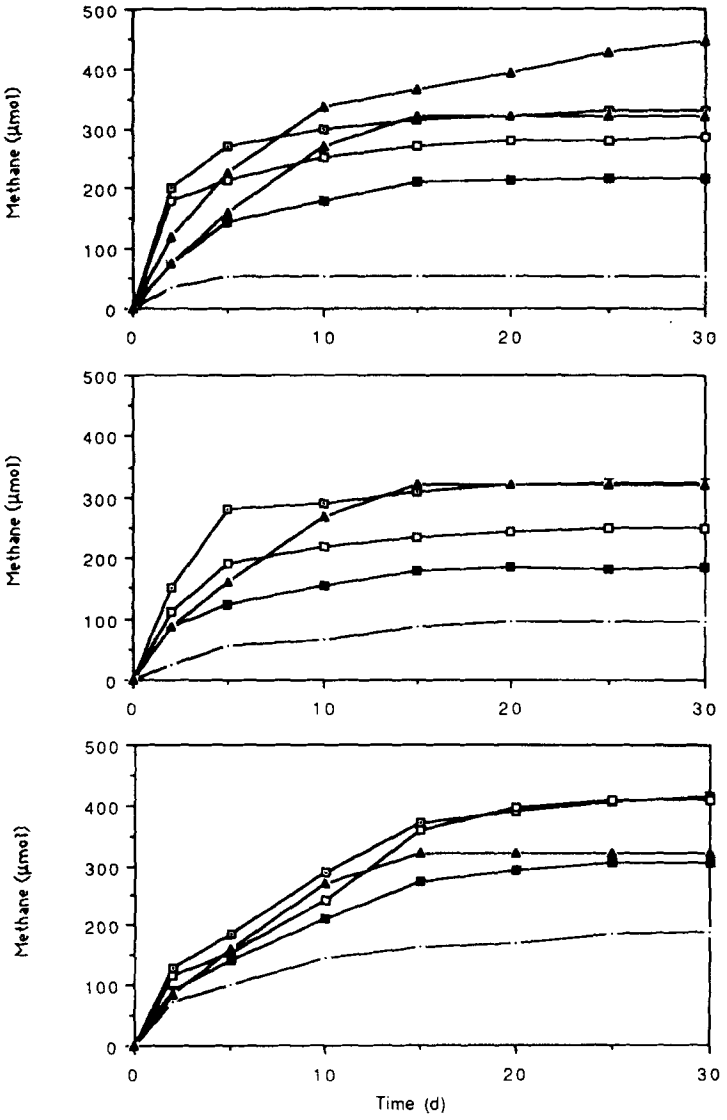

Fig. 2. Methane production in batch cultures containing various concentrations of crude OMW (a), supernatant of OMW (b) and suspended solids (c) at various concentrations. (▲), 5 g/L. (□) 20 g/L. (□), 40 g/L. (■), 60 g/L. (·), 80 g/L. (△), Control.

Batch cultures fed with proportions of OMW ranging from 5 to 80 g COD/L were studied. Figure 2a summarizes the methane production from these batch cultures for a 30-d incubation period. At concentrations greater than 60 g COD/L, the methane production was 50% that of the control experiment. The methane production obtained in batches with

the supernatant of OMW was a little different from that of unmodified OMW (Fig. 2b). Methanogenic bacteria activities were inhibited by oils and phenolic compounds. Indeed, methanogenic bacteria inhibition was caused by some simple phenolic acids of OMW at low concentrations (*13*). Moreover, tannins, the phenolic compounds, and oleic acid were toxic to methanogenesis from VFA in anaerobic digestion only at an average of 2 g/L (*14*), 1 g/L (*15*), and 5 m*M* (*16*), respectively. The main LCFA contained in olive oil were unsaturated, and oleic acid was the more plentiful (65%). Unsaturated LCFA were inhibitors of microbial formation of methane from acetate rather than saturated LCFA. Indeed, the toxicity level of oleic acid for *Methanosarcina* sp. was 2.4 m*M*. The toxicity of a mixture for LCFA could be enhanced significantly by synergism of individual LCFA (*16*). The addition of $CaCl_2$ reduced the inhibitory effect of LCFA in the anaerobic digestion process (*17*). Calcium hydroxide, which precipitates phenolic compounds and long chain fatty acids toxic to methanogenic bacteria, improves the total alkalinity and is better for adjustment of the pH of olive mill wastewaters (OMW) than NaOH (*5*).

This phenomenon of polymerization by autoxidation of tannins into darkly colored polymers was demonstrated, but these polymers cause resistance to degradation (*12*). Against the problem of toxicity, anaerobic degradation of aromatic compounds and long-chain fatty acids comes up against a thermodynamical barrier (*18*), and these compounds can be removed only by acetogenic bacteria associated with hydrogen oxidizing bacteria (*19,20*).

Of batches receiving washed suspended solids contained in OMW, four concentrations were studied (Fig. 2c). The two concentrations with 20 and 40 g/L of COD were not toxic and biodegradable. This result proves that toxic compounds of OMW are not contained in TSS but in the liquid fraction. However, the kinetic of methane productions were weaker than for unmodified OMW. This is because suspended solids of OMW (pulp) are constituted of 50–90% cellulose and lignin (*21*), that bioconversion into methane is slow (*22*). As shown in Table 1, this fraction of OMW that contains a weak concentration of phenolic compounds can be toxic only with high concentrations of COD.

Kinetic of VFA and Ethanol Formation in Anaerobic Digestion of OMW

Kinetics of VFA and ethanol accumulation in batch cultures receiving OMW 20 g/L COD were reported in Fig. 3. Although the VFA formation, especially acetate and H_2/CO_2 (data unreported) show the activities of acidogenic bacteria, the methane performed is not higher than control. This proves that OMW is partially biodegradable but is very toxic for

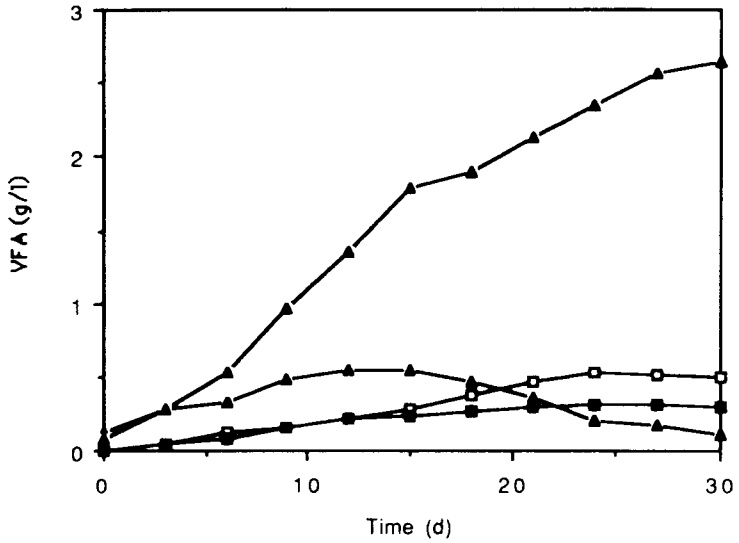

Fig. 3. Time course of acetate (▲), propionate (■), butyrate (□) and ethanol (△) production and consumption in batch cultures receiving unmodified OMW at 20 g/L of COD.

methanogenic bacteria. The ethanol is slightly converted into acetate because the high partial pressure of hydrogen and acetate accumulation depend on thermodynamical considerations (*18*).

The anaerobic digestion of OMW is very complicated because it contains compounds such as carbohydrates and others easily fermentissible, and aromatic compounds and LCFA toxic that are difficult to biodegrade. Acidifying microorganisms grow easily on the carbohydrates dissolved in the waste. The methanogenesis, which represents the limiting step in the anaerobic digestion of solubles compounds, is severely hindered by the combined inhibition caused by the high concentration of aromatic compounds and the buildup of volatile acids (*3*). Phenolic compounds and fatty acids inhibit methanogenic bacteria inducing at an accumulation of hydrogen and acetate. High partial pressure of hydrogen inhibits the oxidation of VFA, LCFA, and aromatic compounds (*23*) and involves the accumulation of reduced fermentation products (ethanol, propionate, and butyrate) and the diminuation of acetate production (*24*) as explained in Fig. 4. So, anaerobic digestion of OMW can be improved by the increase of its biodegradability and the reduction of methanogenic bacteria inhibition caused by VFA, phenolic compounds, and LCFA. It was reported that anaerobic digestion of lignin and tannins is as well as efficient when their mol wt decreases (*14,25*). The chemical or biological detoxification step is indispensable to facilitate anaerobic digestion of OMW. In addition, the use of anaerobic fixed film reactors was found to be a means of limiting the toxicity of inhibitory compounds (*5,26*).

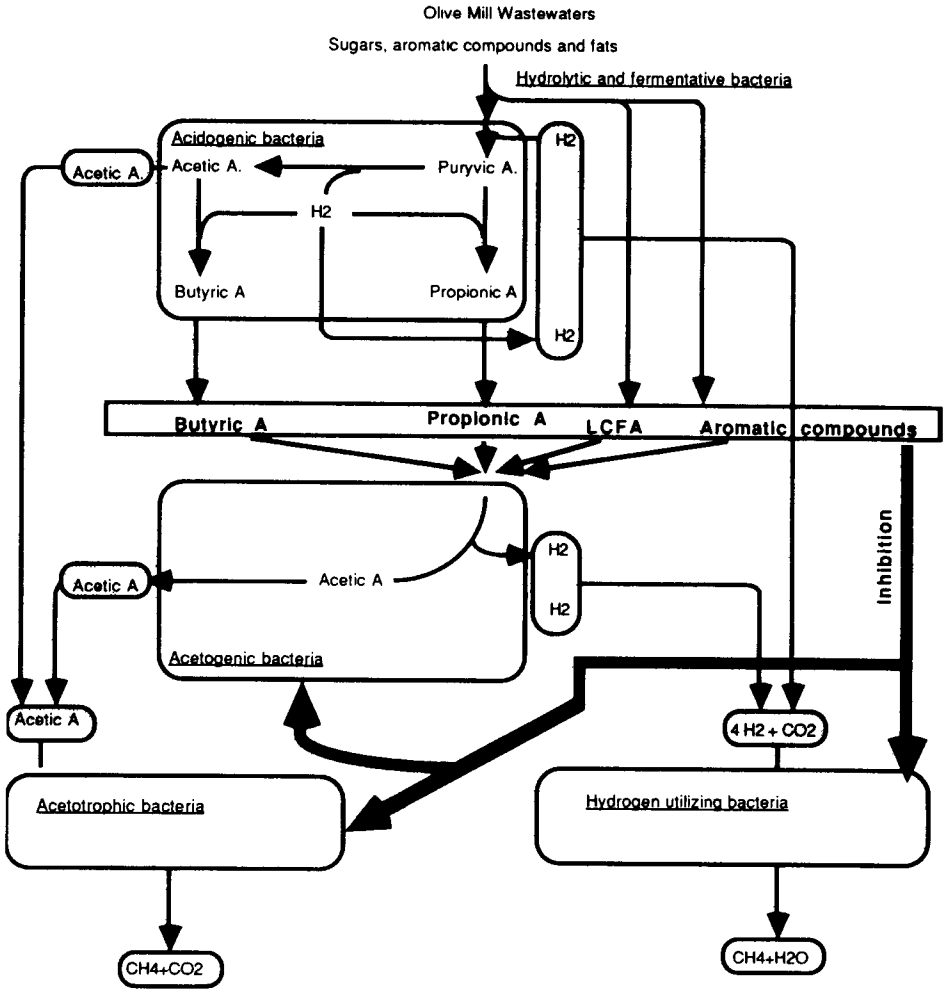

Fig. 4. Schematic representation of the substrate-linked redox process operative during the microbially mediated conversion of OMW to methane.

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