Biodegradation **13:** 401–410, 2002.

Removal of molecular weight fractions of COD and phenolic compounds in an integrated treatment of olive oil mill effluents

M. Beccari, G. Carucci, A.M. Lanz, M. Majone* & M. Petrangeli Papini
*Department of Chemistry, University of Rome "La Sapienza", Piazzale A. Moro 5, 00185 Rome, Italy (*author for correspondence: e-mail: mauro.majone@uniroma1.it)*

Accepted 19 December 2002

Key words: olive oil mill effluent, bentonite, adsorption, anaerobic digestion, aerobic post-treatment

Abstract

Previous works (Beccari et al. 1999b; Beccari et al. 2001a; Beccari et al. 2001b) on the anaerobic treatment of olive oil mill effluents (OME) have shown: (a) a pre-treatment based on the addition of $Ca(OH)_2$ and bentonite was able to remove lipids (i.e. the most inhibiting substances present in OME) almost quantitatively; (b) the mixture OME – $Ca(OH)_2$ – bentonite, fed to a methanogenic reactor without providing an intermediate phase separation, gave way to high biogas production even at very low dilution ratios; (c) the effluent from the methanogenic reactor still contained significant concentrations of residual phenolic compounds (i.e. the most biorecalcitrant substances present in OME). Consequently, this paper was aimed at evaluating the fate of the phenolic fractions with different molecular weights during the sequence of operations (adsorption on bentonite, methanogenic digestion, activated sludge post-treatment). The results show that a very high percentage (above 80%) of the phenolic fraction below 500 D is removed by the methanogenic process whereas the phenolic fractions above 1,000 D are significantly adsorbed on bentonite; the 8-day activated sludge post-treatment allows an additional removal of about 40% of total filtered phenolic compounds. The complete sequence of treatments was able to remove more than the 96% of the phenolic fraction below 500 D (i.e. the most toxic fraction towards plant germination). Preliminary respirometric tests show low level of inhibition exerted by the effluent from the methanogenic reactor on aerobic activated sludges taken from full-scale municipal wastewater plants.

Introduction and aims

In the olive growing countries of the Mediterranean area, difficulties in the anaerobic treatment of olive oil mill effluents (OME) are mainly connected with high organic concentrations (with COD up to 200 g l^{-1}), seasonal operation (requiring storage, often impossible in small mills), high territorial scattering (e.g., in Italy there are about 6,000 oil mills) and, first and foremost, the presence of biorecalcitrant and/or inhibiting substances (essentially phenolic compounds and lipids, respectively).

Typically, OME can be degraded with high biomethanation yields (about 80%) provided that they are diluted enough (down to 10 g COD l^{-1}), whereas remarkable inhibition of methanogenesis occurred at higher concentrations (Beccari et al. 1996). Several authors suggested that main responsible for methanogenesis inhibition in OME are lipids, and namely oleic acid (Beccari et al. 1999a; Koster & Cramer 1980; Hanaki et al. 1981), even though these compounds are easily and fully degraded under non-inhibiting conditions.

OME also contain a large variety of phenolic compounds in a range of different molecular weights (MW), starting from low-MW substituted phenols (one single aromatic ring with one or more substituting groups) to complex high-MW phenolic compounds (usually condensed aromatic rings with several substituting groups), (Montedoro et al., 1992). Phenolic compounds are also of concern in anaerobic treatment of OME because they contribute to the overall inhibition effect, even though to a less extent than lipids. Moreover, this phenolic fraction is partially recalcitrant being its degradation not complete also under non-inhibiting conditions (Beccari et al. 1996; Beccari et al. 1999a).

These evidence suggested the opportunity of a physical-chemical pre-treatment carried out to remove lipids and phenolic compounds, as more selectively as possible, before anaerobic digestion. In this regard, addition of $Ca(OH)_2$ up to pH 6.5 and bentonite at 15 g l^{-1}, followed by centrifugation, turned out to remove lipids by 99.5% and phenolic compounds by about 45%. Such a pre-treatment allowed to achieve high bioconversion into methane of the residual OME, even at very low dilution ratios (1:1.5) (Beccari et al. 1999b). Furthermore, the highest biomethanation yields were obtained when no solid/liquid separation before the biological process was performed, thus showing that adsorption of COD through the addition of lime and bentonite was able to decrease inhibition effects also in the presence of the biomass and that at least a fraction of the removed COD was biodegraded when slowly released back from the solid phase during the biological test (Beccari et al. 1999b). Moving to continuous lab-scale experiments, further work has confirmed that the mixture OME–$Ca(OH)_2$–bentonite, fed to a methanogenic reactor, without providing an intermediate phase separation, gave way to high COD removal and high biogas production even at very low dilution ratios (Majone et al. 2000; Beccari et al. 2001a; Beccari et al. 2001b).

Despite the encouraging results resumed above, the problem remained, in that the effluent from the methanogenic reactor still contained significant concentrations of residual phenolic compounds. Consequently, in order to better understand and describe the feasibility of phenolic compounds removal in the proposed treatment scheme, this paper was aimed at evaluating the fate of the phenolic fractions with different molecular weights during the sequence of operations (adsorption on bentonite and methanogenic digestion). Moreover, the capacity of a biological post-treatment using aerobic activated sludges for removing the residual phenolic compounds was evaluated in order to allow agricultural applications, directly or through composting (Paredes et al. 2001).

Materials and methods

OME characterization

Fresh OME were obtained from an olive oil continuous centrifuge processing plant located in Itri (Lazio, Italy). Table 1 shows OME characterization. A range of values is reported because of the variability in the composition of OME from one storage batch to another.

All the experimentation was performed by using diluted and modified OME. This was prepared twice a week by mixing and diluting in distilled water known amounts of OME (to a final volume of two parts per one part of OME, dilution ratio 1:2), urea (to a final concentration of 0.45 g/l) , and $Ca(OH)_2$ (to a final pH of 6.5).

Continuous lab-scale methanogenic reactor

The experimental apparatus (Figure 1) was constituted of a stirred physical-chemical pre-treatment tank (1,500 ml liquid volume), and a stirred glass methanogenic reactor (New Brunswick scientific Co., 2,200 ml liquid volume). The system also included a slowly stirred (1 rpm) cylindrical settler (1,500 ml liquid volume) by which the sludge was thickened. The reactor was inoculated with acclimatised sludge from previous batch tests. In order to assure anaerobic conditions, N_2 gas was fluxed into the reactor and into the settler during start up and occasionally during maintenance operations. The pretreatment tank was periodically filled (twice a week) with modified diluted OME (1:2 dilution ratio, see Material and Methods section) and bentonite. According with previous research, bentonite concentration was fixed at 10 g l^{-1} with respect to undiluted OME (Beccari et al. 2001a). The methanogenic reactor was continuously fed by a peristaltic pump taking the mixed slurry (bentonite plus modified OME) from the pretreatment tank. The liquid level in the methanogenic reactor was controlled by a conductivity probe commanding excess liquid to be dragged out into the settler by means of a second peristaltic pump. The pH in the reactor was controlled at 8.0 by NaOH addition. The thickened slurry from the settler was continuously recirculated into the methanogenic reactor by means of a third peristaltic pump. The recirculation ratio was 1. Excess sludge was periodically taken from the recycle flow, in order to control the sludge age (assumed to be equal to the solid retention time) at the chosen value (also considering loss of solids in the effluent and analytical samples). At

Table 1. OME characterization

Parameter	OME	Filtered OME	Parameter	OME	Filtered OME
pH	4.3 ± 4.4		Oleic acid (g l^{-1})	0.24 ± 2.08	
TSS (g l^{-1})	35.1 ± 40.3		Phenolic compounds (g l^{-1})	7.24 ± 8.38	4.51 ± 7.25
VSS (g l^{-1})	33.8 ± 37.9		Acetic acid (g l^{-1})		0.49 ± 3.23
COD (g l^{-1})	109 ± 153	67.9 ± 75.9	Propionic acid (g l^{-1})		Traces
Lipids (g l^{-1})	10.1 ± 16.6		Butyric acid (g l^{-1})		Traces

regular intervals, liquid and or gas samples were taken from the different units and lines (feed, pre-treatment tank, methanogenic reactor, effluent) for subsequent analytical determinations. Gas production in the methanogenic reactor and in the settler was continuously recorded by means of on line gas counter, based on liquid displacement by gas and automatic refilling in a chamber of known volume.

Aerobic post-treatment

200 ml activated sludge from a waste water treatment plant were diluted up to 500 ml with nutrient solution (NH_4SO_4 0.5 g/l, $FeCl_3*6H_2O$ 0.002 g/l, NaEDTA 0.003 g/l, Tiourea 0.02 g/l, K_2HPO_4 0.223 g/l, KH_2PO_4 0.173 g/l, $MgSO_4*7H_2O$ 0.05 g/l, $CaCl_2*2H_2O$ 0.1 g/l, concentration values referred to the final volume). The sludge was kept under continuous aeration in a batch reactor at 25 °C. Afterwards, 100 ml of effluent from the methanogenic reactor were added. Care was taken in continuously keeping the pH value at 7.5 by adding NaOH 0.5 M or H_2SO_4 0.5 M, as required. 80 ml each samples were taken at time 0, and at 2 and 8 days. Immediately after sampling, aliquots of the mixed liquor was filtered through 0.45 μm cellulose fibre filters (ALBET) and ultra-filtration membranes (DIAFLO AMICON), and stored for subsequent analysis.

Ultra-filtration tests

Pre-filtered OME (0.45 μm cellulose fibre filter) was ultra-filtered (AMICON model 8050) through DIAFLO AMICON membranes (10,000 D, 1,000 D and 500 D). Each filtration stage was performed on a volume of 50 ml. Ultrafiltration was run under a pressure of 4 bar. At each filtration experiment, the first 10 ml of filtrate were disposed of, whereas next 15 ml of filtrate were kept for COD and phenolic compounds analysis.

Table 2. Summary of experimental conditions in the methanogenic reactor

Temperature (°C)	35
pH	8.0
Flow rate (l d^{-1})	0.220
Liquid volume (l)	2.20
HRT (d)	10.0
Sludge age (d)	60.1
Organic load (kg COD m^{-3} d^{-1})	6.7
Total suspended solids[1] (g l^{-1})	29.8
Fixed suspended solids[2] (g l^{-1})	12.7
Volatile suspended solids (g l^{-1})	17.1

[1] Including bentonite.
[2] Mainly bentonite.

Table 3. Experimental results from bench-scale plant

Parameter	Feed[2]	Final effluent
Total COD (g COD l^{-1})	67.1	8.1
Filtered phenolic compounds (g l^{-1})	2.4	1.1
Biogas (l l^{-1} d^{-1} at STP)[1]		2.48
(% CH_4)		72

[1] Data refer to total biogas production (in the methanogenic reactor and, to a limited extent, also in the settler).
[2] OME modified and diluted 1:2 , as reported in Materials and Methods.

Table 4. Process efficiencies

Parameter	(%)
Total COD removal	88
Filtered phenolic compounds removal	55
Methane yield	
g CH4 (as g COD)/g total COD)	0.76
g CH4 (as g COD)/g removed COD)	0.86

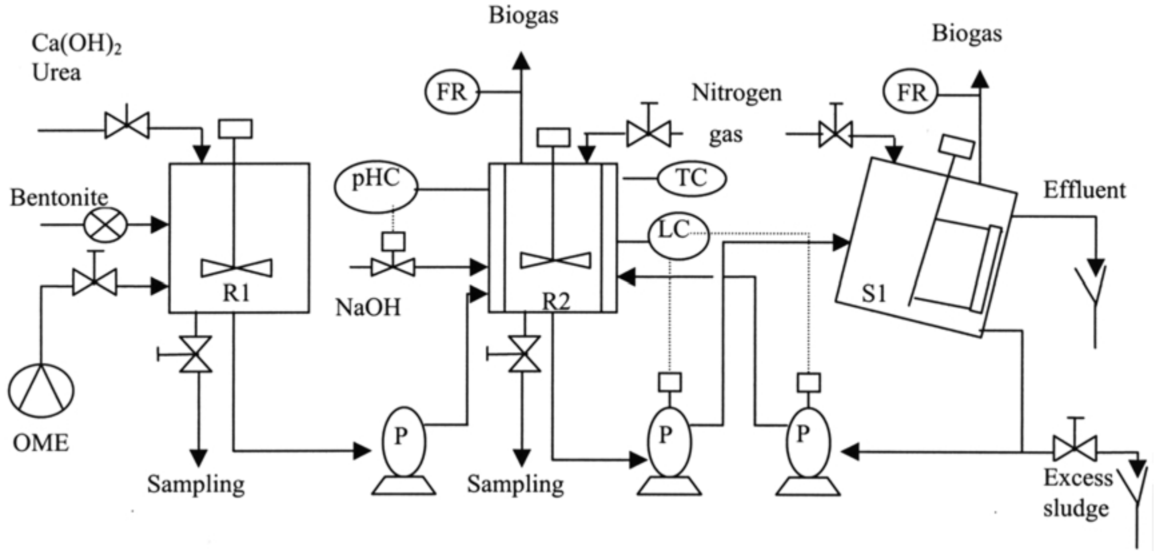

Figure 1. Bench scale plant for physical-chemical/anaerobic integrated treatment of olive oil mill effluents (OME). R1: feeding tank and physical-chemical reactor; R2: Methanogenic reactor; S1: Settler; P peristaltic pump, FR: gas counter for flow rate measurement; TC: temperature controller; LC: level control; pHC: pH controller.

Inhibition tests

Inhibition tests were performed by mixing 100 ml of the methanogenic reactor effluent (undiluted or diluted) to 100 ml of aerated sludge (from sewage aerobic treatment plant of Roma Nord) in batch reactors (300 ml of volume), and measuring the related oxygen uptake rate (OUR). OUR was determined by intermittently stopping aeration of the slurry and measuring the oxygen concentration decrease by means of a polarographic probe (Oxyliquid 22). After several determinations during the tests, maximum slope was taken and converted to specific OUR by taking into account the concentration of total suspended solids. Endogenous OUR of the aerated sludge before methanogenic reactor effluent addition was also measured and subtracted in calculating specific OUR. In order to determine at which concentration OME exerted an inhibitory effect, tests were replied in the same day with different aliquots of the sludge by using different dilution ratios for methanogenic reactor effluent (5, 10, 25, 50 or 100 ml of anaerobically pre-treated OME to 100 ml).

Analytical methods

Phenolic compounds were determined spectrophotometrically according to the Folin–Ciocalteu method (Folin & Ciocalteu, 1927), which is sensitive to any compounds containing the phenolic ring(s). Because method sensitivity is depending on the structure of phenolic compounds, it was necessary to calibrate the method by using a reference compound (para-hydroxibenzoic acid) and to consider the resulting "lumped" concentrations in terms of equivalents amounts of the chosen reference compound (Hamdi 1991; Martin et al. 1994; Beccari et al. 1999a). Lipids were determined gravimetrically after petroleum ether extraction (IRSA 1985a), oleic acid by gas cromatography (GC 3400 Varian with FID detector, 15 m $*$ 0.53 mm wide bore column Nukol, He carrier at 48 kPa, 110–200 °C at 8 °C min^{-1}) after ethyl ether extraction, and other parameters according to IRSA (1985a). More detailed description of the analytical methods used is reported elsewhere (Beccari et al. 1999a).

Results and discussion

Performance of the continuous process

The continuous bench-scale plant (Figure 1) coupling adsorption on bentonite (first step, feeding and pre-treatment tank) and methanogenesis on the OME-bentonite mixture in a contact process (second step, CSTR plus settler with sludge recycle) was operated at very low dilution ratio of influent OME (1:2, see Materials and Methods). Reactor operating conditions are

Table 5. Effect of adsorption and methanogenesis on removal of the filtered phenolic fractions with different molecular weights

Fraction	Concentration (mg l^{-1})			Removal (%)		
	Influent [1]	Effluent from		By adsorption on bentonite	By methanogenesis with reference to	
(D)		Adsorption tank	Methanogenic reactor		Influent the	Effluent from adsorption tank
>10,000	542 (22.5%)	243 (13.1%)	299 (27.3%)	55.2	44.8	–
10,000 ÷ 1,000	571 (23.7%)	376 (20.3%)	385 (35.2 %)	34.1	32.6	–
1,000 ÷ 500	326 (13.5%)	319 (17.2%)	223 (20.4%)	2.1	31.6	30.1
<500	973 (40.3%)	915 (49.4%)	187 (17.1%)	6.0	80.8	79.6
Total	2,412 (100%)	1,853 (100%)	1,094 (100%)	23.2	54.6	41.0

[1] Influent: OME modified and diluted 1:2 , as reported in Materials and Methods.

summarised in Table 2. In this run sludge age and organic load were changed with respect to the ones used in a previous research (Beccari et al. 2001a) whereas remaining operating conditions were at comparable values. Indeed, in order to verify possible increase of COD removal, sludge age was brought from 48 days up to 60 days and correspondingly the organic load was decreased from 8.20 to 6.82 kg COD m^{-3} d^{-1}.

After a short start up period (about 20 days of operation), main parameters measured in the effluent of the methanogenic reactor (e.g. COD and VFA in the liquid phase, total suspended solids and daily methane production) were constant for the remaining operation time (about 30 days). Thus, we judged a steady state had been reached and calculated average steady state data for the latter period. Average steady-state performance is reported in Table 3 whereas efficiencies are reported in Table 4. The process performance was quite satisfactory achieving total COD removal of 88% and methane yield of 0.76 g CH4 (as g COD)/g total COD). However, the effluent from the methanogenic reactor had still a relatively high concentration of total COD (about 8 g/l) and soluble COD (about 4 g/l). Although these results confirmed the performance achieved in previous runs (Beccari et al. 2001a), a longer sludge age did not produce the expected decrease in the effluent COD.

Role of molecular weight in fate of phenolic fraction

To provide more information on removed and residual COD, each stream influent and/or effluent the different steps of the process was separated into several molecular-weight fractions by ultrafiltration and characterised with respect to content of phenolic compounds and COD. Table 5 shows how phenolic compounds are distributed in the different fractions as function of molecular weight and how they are depleted from any fraction by adsorption on bentonite and methanogenesis. In influent diluted OME, main fraction is the one under 500 D (about 40%). By considering also the intermediate fraction between 500 and 1000 D, phenolic compounds less than 1000 D are more than 50% of total soluble phenolic compounds. Both fractions increase as percentage after pre-treatment on bentonite (49.4 and 17.2%, respectively). Indeed, adsorption on bentonite acts essentially on the fractions above 1,000 D, by removing 55.2 and 34.1% of fractions above 10,000 D and between 10,000 and 1,000 D, respectively. This removal corresponds to removing about 23% of total filtered phenolic compounds. On the other hand, methanogenesis acted essentially on the fractions below 1,000 D, and especially on the fraction below 500 D (30.1 and 79.6% removal with respect to effluent from adsorption tank, respectively). As a whole, the combined physical-chemical-biological treatment was able to remove about 55% of filtered phenolic compounds contained in the untreated OME.

Table 6. Effect of adsorption and methanogenesis on removal of the filtered COD fractions with different molecular weight

Fraction (D)	Influent[1] mg COD l^{-1}	Effluent from methanogenic reactor mg COD l^{-1}	COD removal %
>10,000	4,352 (13.2%)	1,086 (24.4%)	75.0
10,000 ÷ 1,000	4,821 (14.6%)	1,374 (30.8%)	71.5
1,000 ÷ 500	6,897 (20.9%)	924 (20.7%)	86.6
<500	16,937 (51.3%)	1,074 (24.1%)	93.7
Total	33,007 (100%)	4,458 (100%)	86.5

[1] OME modified and diluted as reported in Materials and Methods.

As for COD (Table 6), its removal was high (at least 70%) for all fractions, even though increasing as molecular weight decreased; for the fraction below 500 D, COD removal was 93.7%.

Comparison of phenolic compounds/COD ratio before and after the anaerobic treatment (Figure 2) showed that it increased in all MW fractions. By assuming a conversion factor of 2.34 mg COD/mg phenolic compounds (corresponding to phenol) it was estimated that most residual COD was due to the phenolic compounds. This result confirms that phenolic compounds are more recalcitrant than average COD in OME. However, the minimum phenolic compounds/COD ratio after anaerobic treatment was found in the fraction at less than 500 D, so confirming that phenolic compounds in this fraction are more biodegradable than at higher MW.

Aerobic post-treatment

In order to verify the effectiveness of an aerobic post-treatment, batch tests using activated sludge from a full-scale plant were performed on effluent of anaerobic treatment. Table 7 shows the removals of the various fractions of filtered phenolic compounds obtained after 2 and 8 days of treatment in the batch activated sludge reactor. An 8-day post-treatment allowed an additional removal of about 40% (about 73% with reference to filtered phenolic compounds in the untreated OME); the highest percentage of removal concerned the fraction below 500 D, similarly to what occurred in the methanogenic reactor. Removal after 2-days treatment was quite less than after 8 days, so confirming the slowly biodegradable nature of residual phenolic compounds from anaerobic digester.

As for the soluble COD (Table 8), removal after 8 days of post-treatment was about 28%. Like the phenolic compounds, the COD removal increased as the treatment time increased, with the exception of the COD fraction below 500 D that increased in concentration from 2 to 8 days of treatment, probably due to hydrolysis of the COD components at higher molecular weight. The fractions above 10,000 D of both phenolic compounds and COD were not biodegradable under the post-treatment conditions.

As for the phenolic compounds ratio/COD ratio, the phenolic compounds removal is a bit higher than COD removal, so indicating that aerobic post-treatment is preferentially active on residual phenolics from anaerobic treatment than on residual COD of different chemical nature. In particular, Figure 2 shows that aerobic post-treatment is most effective in decreasing back the phenolic compounds/COD ratio for the fraction at less than 500 D.

In a parallel research, the same effluent was treated by means of an aerobic co-culture of Ralstonia sp. LD34 and Pseudomonas putida DSM1868 immobilised on two different fixed bed biofilm reactors (Bertin et al. 2001) and best removal was 68.6% for phenolic compounds and 52.4 for COD. Even though effectiveness was better when utilising specialised bacteria bi-culture, still activated sludge performance was encouraging, considering possible advantage of directly performing the aerobic post-treatment with non specialized sludge in municipal full scale facilities.

For this reason, preliminary respirometric tests were performed in order to ascertain possible inhibition of activated sludge as function of the organic load of the effluent from the methanogenic reactor. Figure 3 shows the low level of inhibition exerted by the anaerobic effluent on aerobic activated sludge.

Preliminary technical and economic analysis

According to experimental results of this paper and previous research (Beccari et al. 2001a), an integrated process for OME treatment can be proposed, based on (1) chemical physical pre-treatment with bentonite, (2) anaerobic digestion, and (3) aerobic post-treatment in activated sludge plant. A preliminary technical and economic analysis of the process has been performed according to the scenario reported in Table 9. Capital and running costs were evaluated according to IRSA (1985b) and updated to 2000 according to the National Institute of Statistics (ISTAT 2000), as also

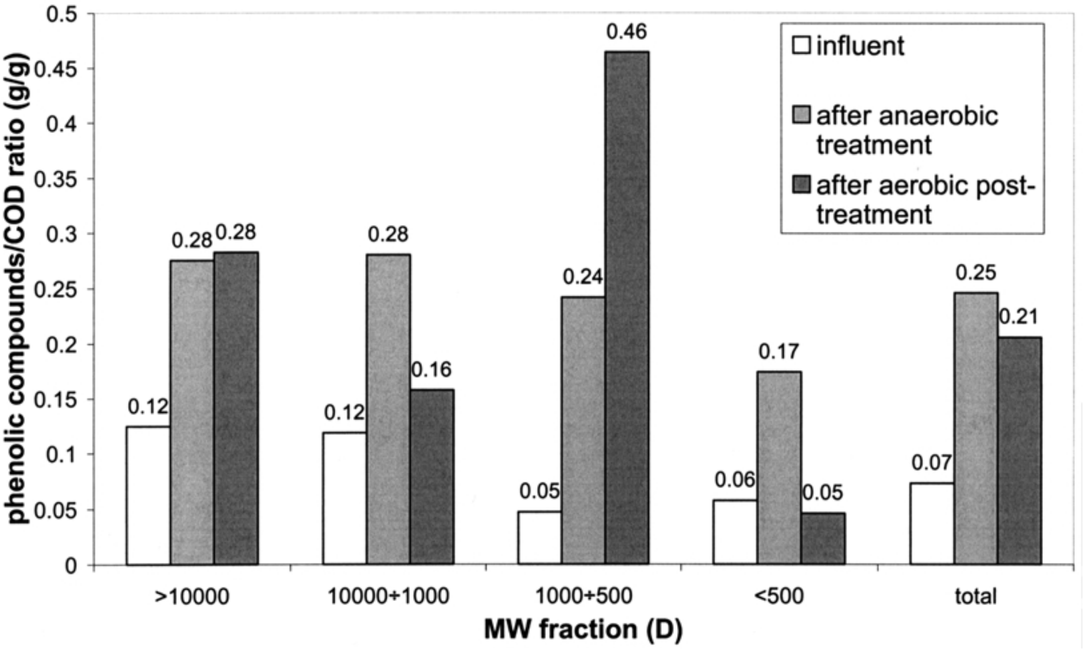

Figure 2. Phenolic compounds/COD ratio in fractions at different molecular weights as resulting from different steps of OME treatment.

Table 7. Effect of activated sludge post-treatment on removal of the filtered phenolic fractions with different molecular weights

Fraction (Dalton)	Removal (%)			
	After 2 days of post-treatment		After 8 days of post-treatment	
	With reference to the effluent from the methanogenic reactor	With reference to the influent untreated OME	With reference to the effluent from the methanogenic reactor	With reference to the influent untreated OME
>10,000	0	44.8	0	44.8
10,000 ÷ 1,000	26.6	50.5	59.5	72.7
1,000 ± 500	13.5	40.8	30.0	52.1
<500	67.7	93.8	80.7	96.3
Total	23.0	65.1	39.7	72.6

reported in Table 9. Total capital and running cost (excluding staffing) of combined physical chemical and anaerobic treatment is 20.97 Euro/m^3 raw OME, including storage tank (7.04 Euro/m^3), adsorption tank (3.36 Euro/m^3), anaerobic digester (6.45 Euro/m^3), and settler (4.12 Euro/m^3); considering staffing cost (8.61 Euro/m^3) and biogas revenue (4.65 Euro/m^3), net total cost is 24.93 Euro/m^3. By summing cost of sludge disposal, including conditioning, dewatering and disposal in controlled landfill (4.85 Euro/m^3), and aerobic post-treatment of anaerobic effluent in activated sludge process (8.78 Euro/m^3), an overall cost of 38.56 Euro per m^3 is obtained. It can be estimated that 38.56 Euro m^{-3} is about 20–25% less than actual cost of direct treatment in municipal activated sludge plants (with reference to the cost of unit of COD removed in municipal wastewater treatment plants). Moreover, calculated cost could be substantially decreased by increasing the volume of OME to be treated, so increasing the size of the anaerobic plant.

Table 8. Effect of activated sludge post-treatment on removal of the filtered COD fractions as function of molecular weight.

MW fraction (Dalton)	Removal (%)			
	After 2 days post-treatment		After 8 days post-treatment	
	With reference to the effluent from the methanogenic reactor	With reference to the influent untreated OME	With reference to the effluent from the methanogenic reactor	With reference to the influent untreated OME
>10,000	0	75.0	0	74.6
10,000 ÷ 1,000	4.8	72.9	27.9	79.5
1,000 ÷ 500	30.5	90.7	63.6	95.1
<500	57.5	97.3	27.4	95.4
Total	21.3	89.4	28.0	90.3

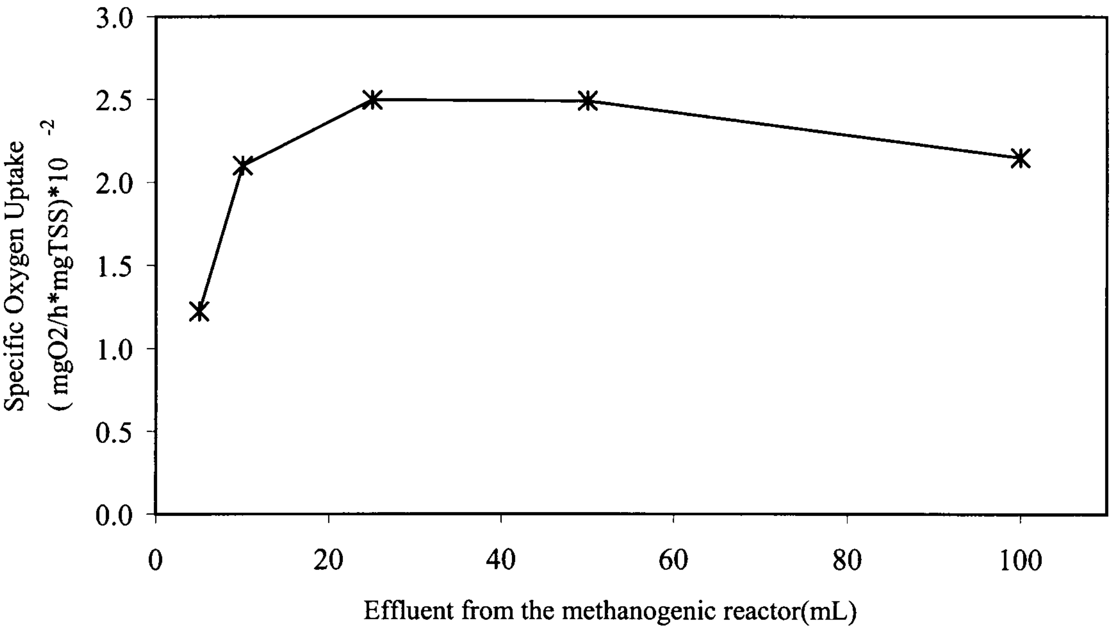

Figure 3. Influence of increasing percentages of the effluent from the methanogenic reactor on specific oxygen uptake rates (100 mL of activated sludge and (100 mL − XmL) of dilution water were mixed with XmL of effluent).

Conclusions

The experimentation carried out in a continuous lab-scale plant has confirmed that pretreating OME by means of adsorption on bentonite under optimized conditions allows high biomethanation efficiencies even at very low dilution ratios. Such a result depends on the double role of bentonite that adsorbs inhibitors (essentially lipids) during the pre-treatment and then is able to release slowly the adsorbed biodegradable matter during the biological process. A very high percentage (about 80%) of the phenolic fraction below 500 D is removed by the methanogenic process whereas a significant percentage of the phenolic fractions above 1,000 D is adsorbed on bentonite.

The post-treatment of this effluent by using an aerobic batch activated sludge reactor turned out to remove a significant fraction (40%) of the residual filtered phenolic compounds. The post-treatment was particularly active towards the phenolic fraction below 500 D (percentage of removal: about 81%). Consequently, the final effluent from the post-treatment contained less than the 4% of the phenolic fraction be-

Table 9. Data for technical and economic analysis of the process

OME to be treated	Overall process
Number of mills: 3 Mill capacity: 10,000 kg/d Production period: 4 months OME production: 50 l/100 kg of olives OME total production:15 m^3/d = 1,800 m^3/y Equivalent population (on daily basis): – 5,000 inhabitants (as COD, at 0.12 kg COD/d inhabitant); – 60 inhabitants (as volumetric rate, at 0.25 m^3/d inhabitant)	Process operation: 6 months/year OME pre-treatment: HRT = 5 d Anaerobic digestion: – HRT = 12d, – Recycle ratio = 0.8 Settling: hydraulic load = 2 m^3 m^{-2} d^{-1} Aerobic post-treatment: activated sludge municipal plant Sludge disposal: dewatering and landfill disposal
Basic data for cost evaluation	Specific running costs
Amortization period: – 10 years (electromechanical equipment) – 25 years (civil works) Annual interest of capital: 5% Maintenance (annual cost): – storage tank and adsoption tank: 1% of the total plant cost – anaerobic digester and settler: 3% of the electromechanical equipment cost	Power: 0.137 Euro/kWh Urea : 258 Euro/t $Ca(OH)_2$: 83 Euro/t Bentonite: 5 Euro/t Biogas revenue: 0.145 Euro/Nm^3 Staffing: – Number of operators: 1 (6 month/year) – Salary cost: 30987,4 Euro/year

low 500 D initially present in the untreated OME. Taking into account that the lower the molecular weight of phenolic compounds the stronger the toxicity on plant germination (Aliotta et al. 2000; Capasso 1997), the effluent either from the anaerobic digestion or, to a greater reason, from the aerobic post-treatment, is likely to be spread on agricultural soils without any risks.

A preliminary technical economic analysis has shown that the process based on 1) chemical physical pre-treatment with bentonite, 2) anaerobic digestion, and 3) aerobic post-treatment in activated sludge plant, can be interesting as an alternative to direct treatment in municipal activated sludge plant.

Acknowledgements

This work was supported by a Ministry of University and Scientific/Technological Research grant (National Research Project). The authors also like to thank Mr. Nino Astorino and Dr. Lucia Torrisi for the help in the economical assessment of the process.

References

Aliotta G, Cafiero R, De Feo V, Di Blasio B, Iacovino R & Oliva A (2000) Allelochemicals from Rue (*Ruta graveolens* L.) and Olive (*Olea europaea* L.) oil mill waste waters as potential natural pesticides, Current Topics in Phytochemistry 3: 167

Beccari M, Bonemazzi F, Majone M & Riccardi C (1996) Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Wat. Res. 30: 183–189

Beccari M, Carucci G, Majone M & Torrisi L (1999a) Role of lipids and phenolic compounds in the anaerobic treatment of olive oil mill effluents. Environ. Technol. 20: 105–110

Beccari M, Majone M, Riccardi C, Savarese F & Torrisi L (1999b) Integrated treatment of olive oil mill effluents: effect of chemical and physical pretreatment on anaerobic treatability. Wat. Sci. Tech. 40: 347–355

Beccari M, Petrangeli Papini M, Majone M & Torrisi L (2001a) Enhancement of anaerobic treatability of olive oil mill effluents by addition of $Ca(OH)_2$ and bentonite without intermediate solid/liquid separation, Wat. Sci. Tech. 43: 275–282

Beccari M, Carucci G, Lanz AM, Majone M & Petrangeli Papini M (2001b) Fate of the phenolic fractions in an integrated treatment of olive oil mill effluents (adsorption on bentonite, methanogenesis, activated sludge post-treatment). In: AIDIC (Ed), Proceedings of IcheaP-5, the fifth Italian Conference on Chemical and Process Engineering, Florence, May, 20–23, Vol. 1 (pp. 357–362)

Bertin L, Majone M, Di Gioia D & Fava F (2001) An aerobic fixed-phase biofilm reactor system for the degradation of the low molecular weight aromatic compounds occurring in the effluents of anaerobic digestors treating olive mill wastewaters. Journal of Biotechnology 87: 161–177

Capasso R (1997) The chemistry, biotechnology and ecotoxicology of the polyphenols naturally occurring in vegetable wastes. Current Topics in Phytochemistry 1: 145

Folin O & Ciocalteu V (1927) On tyrosine and tryptofan determinations in protein. J. Biol. Chem. 73: 627–650

Hamdi M (1991) Effects of agitation and pretreatment on the batch anaerobic digestion of olive mill waste water. Bioresource Technology 36: 173–178

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

IRSA (1985a) Metodi analitici per i fanghi. Vol. 3. Parametri chimico-fisici. Quaderni Istituto di Ricerca sulle Acque, 64, CNR, Roma (in Italian)

IRSA (1985b) Quaderni Istituto di Ricerca sulle Acque, 70, CNR, Roma (in Italian)

ISTAT (2000). Indice di rivalutazione della lira, in: Annuario 2000, Ed. ISTAT, Roma (in Italian)

Koster IW & Cramer A (1980) Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. Appl. Environ. Microbiol. 53: 403–409

Majone M, Beccari M, Petrangeli Papini M & Torrisi L (2000) Processo integrato chimico-fisico e biologico per il trattamento di reflui di frantoi oleari (Acque di vegetazione, AV). European Patent no. 01115896.1-1213

Martin A, Borja R & Banks CJ (1994) Kinetic model for substrate utilization and methane production during the anaerobic digestion of olive mill wastewater and condensation water waste. J. Chem. Tech. Biotechnol. 60: 7–16

Montedoro G, Servili M, Baldioli M & Miniati E (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. Journal of Agricultural and Food Chemistry 40(9): 1571-1576

Paredes C, Bernal MP, Roig A & Cegarra J (2001) Effects of olive mill wastewater addition in composting of agroindustrial and urban wastes. Biodegradation 12: 225–234