

## Ozone pretreatment of olive mill wastewaters (OMW) and its effect on OMW biochemical methane potential (BMP)

E. Tsintavi, N. Pontillo, M. A. Dareioti and M. Kornaros

### ABSTRACT

The possibility of coupling a physicochemical pretreatment (ozonation) with a biological treatment (anaerobic digestion) was investigated for the case of olive mill wastewaters (OMW). Batch ozonation experiments were performed in a glass bubble reactor. The parameters which were tested included the ozone concentration in the inlet gas stream, the reactor temperature and the composition of the liquid medium in terms of raw or fractionated OMW used. In the sequel, ozone-pretreated OMW samples were tested for their biochemical methane potential (BMP) under mesophilic conditions and these results were compared to the BMP of untreated OMW. The ozonation process alone resulted in a 57–76% decrease of total phenols and a 5–18% decrease of total carbohydrates contained in OMW, depending on the experimental conditions. Nevertheless, the ozone-pretreated OMW exhibited lower chemical oxygen demand removal and methane production during BMP testing compared to the untreated OMW.

**Key words** | anaerobic digestion, BMP, olive mill wastewater, ozonation, pretreatment

E. Tsintavi  
N. Pontillo  
M. A. Dareioti  
M. Kornaros (corresponding author)  
Department of Chemical Engineering,  
University of Patras,  
1 Karatheodori str.,  
University Campus,  
26500 Patras,  
Greece  
E-mail: [kornaros@chemeng.upatras.gr](mailto:kornaros@chemeng.upatras.gr)

### INTRODUCTION

The liquid by-products of olive oil production using the three-phase centrifugation process, i.e. olive mill wastewaters (OMW), are recognized in the whole Mediterranean, Aegean and Marmara region as a severe environmental problem because of their high organic content and their recalcitrance to biodegradation, which is particularly due to the presence of phenolic compounds. OMW are mainly composed of sugars, lipids, proteins and phenols (Beccari *et al.* 1999). The total concentration of phenols, which contribute to a high toxicity and antibacterial activity (Capasso *et al.* 1995), can reach up to 24 g/L (Borja *et al.* 2006; Paraskeva & Diamadopoulou 2006). Phenols are high molecular compounds, which are mainly responsible for the antioxidant characteristics of olive oil but their recalcitrance to biological degradation as well as their phytotoxicity results in environmental deterioration of ecosystems in the cases where direct disposal of raw or inefficiently treated OMW takes place.

Due to their high phenolic content, treatment of OMW with physicochemical methods, such as oxidation by ozone (O<sub>3</sub>), prior to anaerobic digestion (best available technology according to the 96/61/EC directive) is considered to be a promising pretreatment

process, in the aim to improve the biodegradability of OMW by removing the phenolic inhibitors to a great extent and thus enhancing the yield of the following anaerobic degradation.

Combination of chemical and biological treatment is often the way to optimize the overall process. Each waste should be considered separately, to decide which chemical and biological treatments are more suitable, in which order, and to what extent to operate one treatment before starting the other one.

Anaerobic digestion is a proposed process for treatment of wastes with high organic content because of its obvious advantages: low operating costs, due to its low-energy and nutrient demands, and anaerobic biotransformation of most of the organic substances present in the waste into a renewable energy source: methane. However, some organic compounds like phenols are toxic for methanogenic bacteria and inhibit the final and most critical step of the process. The inhibition of methane production is caused mainly by the presence of lipids and phenolic compounds, like p-hydroxybenzoic acid, in OMW (Beccari *et al.* 1999). So a chemical pretreatment process, like ozonation, which is able to remove phenolics compounds, may decrease the

toxicity for methanogenic bacteria and facilitate the anaerobic digestion (Benitez *et al.* 1997).

Ozone is a powerful oxidant and one of the most effective disinfectants that may degrade OMW containing phenolic compounds (Andreozzi *et al.* 1998). One of ozone's characteristics is that it is rather selective towards double bonds. Theoretically, it would leave intact the proteins and the sugars of OMW, which are biodegradable anyway, and attack selectively the double bonds of unsaturated fatty acids and phenols. In this way, the total chemical oxygen demand (COD) would vary to a less extent, because the toxic compounds are present in minor concentrations and the biomass potential to feed in the anaerobic reactor would not be lost. The increase in biodegradability after ozonation has already been proved for 28 substituted aromatic compounds under aerobic conditions (Gilbert 1987) and for some phenolic compounds under anaerobic ones (Wang 1990). Several studies (Benitez *et al.* 1997; Andreozzi *et al.* 1998) have shown that the extent of COD removal achieved during OMW ozonation even at the most favorable conditions could not exceed 20–30%.

The aim of the present work was to investigate the behavior of raw OMW in addition to various OMW fractions during the ozonation process in different conditions and, afterwards, compare their methane production yields and rates using biochemical methane potential (BMP) assays.

## MATERIALS AND METHODS

### Olive mill wastewaters

OMW was obtained from a local olive-oil mill in the area of Patras (Western Greece) that used a three-phase centrifugation decanter. Immediately after sampling, the OMW sample was stored in the freezer at  $-18^{\circ}\text{C}$  until further experimental use. A detailed physicochemical characterization of raw OMW (R-OMW) was carried out (Table 1). In this study, ozonation of centrifuged OMW (C-OMW) and filtered OMW (F-OMW) was also tested. Centrifuged OMW was prepared by centrifugation of raw OMW for 15 min at 4,000 rpm followed by re-centrifugation of the supernatant at the same conditions. Filtered OMW was produced via filtration of raw OMW through Whatman glass microfiber filters, Grade GF/F. The physicochemical characteristics of filtered OMW were similar to the dissolved ones measured in raw OMW (Table 1), i.e. the total COD of F-OMW was  $37.4 \pm 8.10 \text{ g O}_2/\text{L}$  etc. The average characteristics of the centrifuged OMW were: TSS =  $19.80 \pm 0.55 \text{ g/L}$ , VSS =

**Table 1** | Physicochemical characteristics of raw OMW

Parameter	Units	Value
pH	–	$5.19 \pm 0.10$
Total suspended solids (TSS)	g/L	$31.0 \pm 5.00$
Volatile suspended solids (VSS)	g/L	$28.9 \pm 4.50$
Total COD	g O <sub>2</sub> /L	$79.9 \pm 13.20$
Dissolved COD	g O <sub>2</sub> /L	$37.4 \pm 8.10$
Total organic carbon (TOC)	g/L	$32.2 \pm 5.00$
Dissolved organic carbon (DOC)	g/L	$11.5 \pm 1.67$
Total carbohydrates	g equiv. glucose/L	$16.0 \pm 1.00$
Dissolved carbohydrates	g equiv. glucose/L	$12.8 \pm 0.95$
Total phenols	g equiv. syringic acid/L	$3.0 \pm 0.80$
Total Kjeldahl nitrogen (TKN)	g/L	$0.60 \pm 0.02$
Ammonium-nitrogen	g/L	$0.19 \pm 0.01$
Total phosphorus	g/L	$0.28 \pm 0.003$
Dissolved phosphorus	g/L	$0.19 \pm 0.002$

$17.23 \pm 0.53 \text{ g/L}$ , COD =  $62.10 \pm 2.61 \text{ g O}_2/\text{L}$ , total organic carbon (TOC) =  $25.59 \pm 1.61 \text{ g/L}$ , total carbohydrates  $12.95 \pm 0.62 \text{ g equiv. glucose/L}$ .

### Analytical methods

The pH was measured using an electrode (Orion 3-Star), while total and volatile solids and COD were determined according to *Standard Methods* (1995). For the determination of carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan, sulfuric acid and boric acid, which was subsequently measured colorimetrically at 520 nm (Joseffson 1983). Dissolved phenolic compounds were determined spectrophotometrically according to the Folin–Ciocalteu method (Waterman & Mole 1994). Organic carbon (OC) was determined using a Shimadzu analyzer. The biogas produced was measured using glass syringes, while gas composition was measured as reported by Dareioti *et al.* (2010).

### Experimental setup

All batch ozonation experiments were conducted in a glass bubble reactor. A schematic diagram of the experimental set-up is presented in Figure 1. The total reactor volume was 1 L, whereas the liquid working volume was 600 mL.

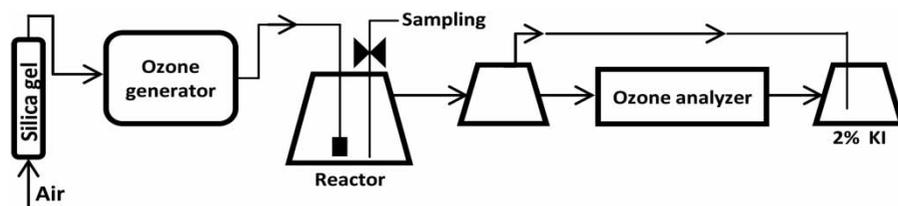


Figure 1 | Experimental set-up of ozonation.

The ozone gas produced by an ozone generator (ANSEROS GM) was supplied to the liquid at a flow-rate of 21.5 L/h through a fritted glass dispenser creating fine bubbles facilitating the dissolution of ozone gas in the liquid medium. The ozone concentration in the feeding and outlet stream from the reactor was measured using an ozone gas analyzer (Ozomat GM-6000-PRO from ANSEROS GmbH). To ensure ozone decomposition, a 2 L Erlenmeyer flask with 2% KI was attached to the gas outlet from the analyzer. Seven experiments using different OMW samples and conditions and the respective control experiments using deionized water were carried out at least in duplicate.

Laboratory-scale BMP tests were carried out to assess the BMP of ozone-treated and raw OMW samples. All BMP tests were conducted in serum vials (total volume of 160 mL), sealed with a butyl rubber septum secured in place with an aluminum crimp seal. Prior to sealing, all samples (100 mL) were flushed for 5 min with nitrogen gas. All vials were shaken throughout the BMP testing period in an orbital shaking water bath (Grant OLS200) at 80 rpm at constant temperature (37 °C). Ozone-pretreated and raw OMW were used in duplicate as substrate in low-strength concentrations according to Owen *et al.* (1979). Acclimated methanogenic anaerobic sludge from an anaerobic continuous stirred tank reactor fed with a mixture of OMW/cheese whey/liquid cow manure (55%:40%:5%)

was used as seeding sludge (control) at 20% v/v, and defined media containing nutrients and vitamins for mixed anaerobic cultures were also added (Owen *et al.* 1979). All reported gaseous volumes hereafter refer to STP conditions.

## RESULTS AND DISCUSSION

### Ozonation of OMW

Ozonation of OMW in this study was investigated by modifying the ozone concentration in the reactor's gas inlet stream ( $C_{O_3,gi} = 6, 12$  and  $18 \text{ g O}_3/\text{Nm}^3$ ), the reaction temperature ( $T = 25, 30$  and  $40 \text{ }^\circ\text{C}$ ) and OMW characteristics in the liquid phase (R-OMW, C-OMW, and F-OMW diluted with water). The results from ozonating various OMW samples at different operating conditions are presented in Table 2, in terms of reduction percentage for every measured parameter during each conducted experiment. Taking into account the ozone concentration variation in the gas inlet stream as well as the duration of each experiment and the liquid working volume, the equivalent ozone doses used were calculated (Table 2) in  $\text{g ozone}/\text{m}^3$  of treated OMW for each experiment. A significant decrease in both total phenols and suspended solids was achieved, especially at almost ambient temperature (25 °C). When the inlet ozone

Table 2 | Ozonation of OMW samples at different ozone doses. The reduction of measured parameters during each experiment is given as a percentage

Tested sample <sup>a</sup>	$C_{O_3,gi}$ ( $\text{g O}_3/\text{Nm}^3$ )	Ozone dose ( $\text{g O}_3/\text{m}^3$ )	Phenols (%)	Total COD (%)	Dissolved COD (%)	TOC (%)	DOC (%)	Total CH (%)	Dissolved CH (%)	TSS (%)	VSS (%)
1	6	2,150	66.54	–	26.03	–	1.66	–	5.26	–	–
2	12	3,010	68.08	–	37.77	–	1.45	–	7.89	–	–
3	18	5,910	76.43	–	23.78	–	0.25	–	9.78	–	–
4	18	6,770	64.52	8.70	21.10	4.10	3.05	7.69	12.7	49.7	51.1
5	18	6,770	67.11	5.38	24.87	8.18	3.15	17.65	22.26	45.8	47.6
6	18	6,770	57.87	10.00	23.05	4.91	2.68	11.14	14.92	32.6	35.5
7	18	6,770	57.44	5.41	19.64	0.74	0.62	6.04	10.5	15.5	17.4

<sup>a</sup> 1: F-OMW diluted (1:2), 25 °C 2: F-OMW diluted (1:2), 25 °C 3: F-OMW diluted (1:2), 25 °C 4: C-OMW, 25 °C, 5: R-OMW, 25 °C 6: R-OMW, 30 °C 7: R-OMW, 40 °C.

concentration ( $C_{O_3,gi}$ ) increased, the phenols degradation also increased, with phenol reduction reaching 66.5, 68.1 and 76.4% at 6, 12 and 18 g  $O_3/Nm^3$ , respectively. On the other hand, the phenols removal decreased as temperature increased at constant  $C_{O_3,gi}$  (see Table 2, tests 5, 6, 7) in the case of raw OMW. The ozone concentration in the outlet gas stream ( $C_{O_3,go}$ ) was also measured during each experiment. In the first minutes of ozonation,  $C_{O_3,go}$  decreased (in some cases zeroed) and gradually increased and reached a steady value, lower than the inlet ozone concentration, indicating that chemical reactions (and thus ozone consumption) were still taking place. The difference between both values,  $C_{O_3,gi}$  and  $C_{O_3,go}$ , is directly related to the amount of ozone reacted. Furthermore, a noticeable reduction in carbohydrates (CH) was measured (18% at maximum), although not expected, but it did not reach the levels of phenols degradation. Figure 2 depicts the evolution of the most important parameters in the experiment with raw OMW ( $C_{O_3,gi} = 18 \text{ g } O_3/Nm^3$  at temperature  $25^\circ\text{C}$ ), taken as an example. As can be observed an important removal of total phenolic compounds (67.11%) was reached after 9.5 hours of ozonation, whereas the total COD

reduction was only 5.38%. This low conversion can be attributed to the fact that, although the organic compounds are very reactive towards ozone, they generate, because of the ozonation reactions taking place, numerous intermediates, mostly smaller molecules, still having a high COD. This behavior is in agreement with Benitez et al. (1997), who reported 6.2% COD reduction in their experiments, and other researchers stating that during ozonation of OMW the observed COD reduction is rather low.

### Biochemical methane potential

The cumulative methane production during the BMP tests of untreated and differently ozone-treated OMW samples is shown in Figure 3(a). During methanogenesis, degradation of phenols and carbohydrates was observed in all tested samples along with a direct removal of COD and methane production. Methane yield was calculated by dividing the net volume of methane produced (volume produced minus endogenous production from seeding sludge) by the amount of COD added in each sample. Figure 3(b) illustrates the net methane production and yield for each

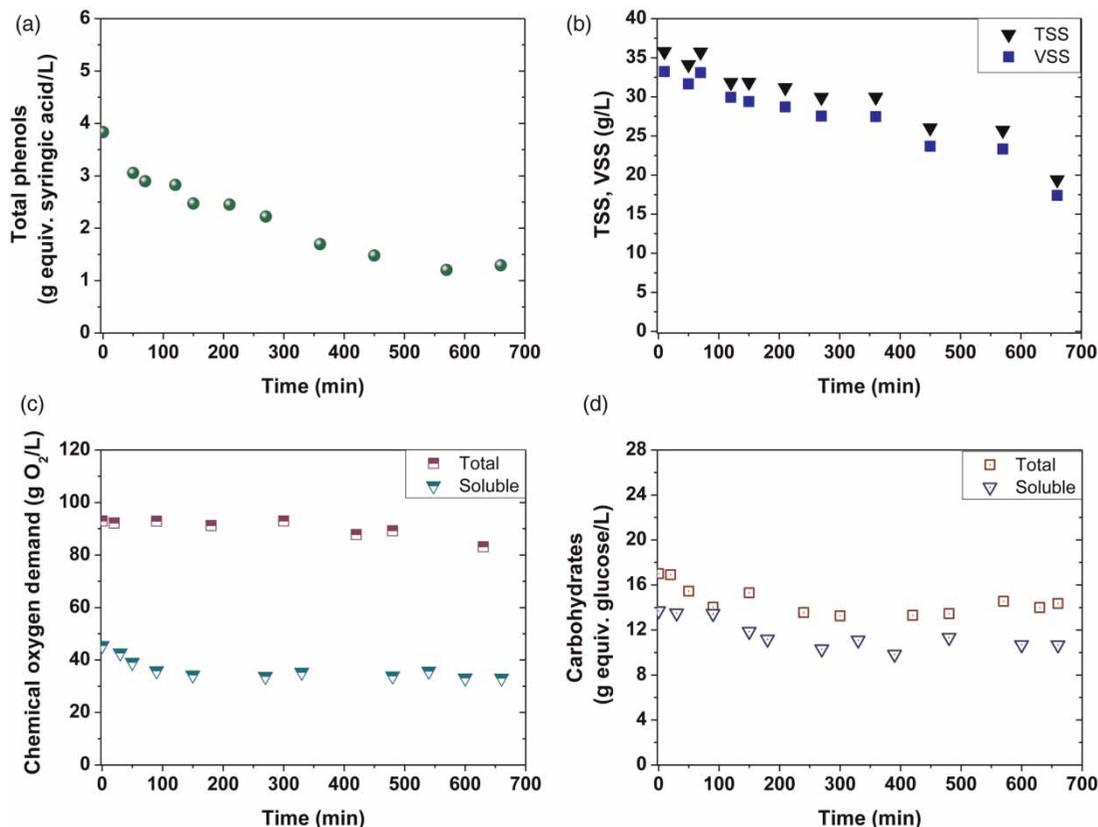
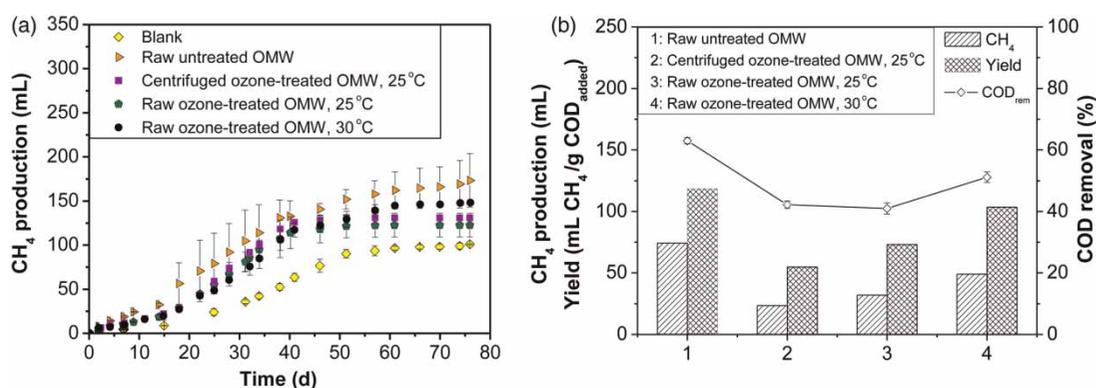


Figure 2 | (a) Total phenols, (b) TSS and VSS concentration, (c) chemical oxygen demand and (d) carbohydrates reduction during ozonation of raw OMW ( $C_{O_3,gi} = 18 \text{ g } O_3/Nm^3$  at  $25^\circ\text{C}$ ).



**Figure 3** | (a) Cumulative methane production at STP conditions during BMP testing and (b) net methane production, methane yield per g of added COD and percentage of COD removal for each tested substrate. Comparison between untreated and ozone-treated samples.

untreated and ozone-pretreated OMW sample. The COD removal measured in each sample at the end of BMP assay is also presented in Figure 3(b).

The highest production of methane (74.3 mL CH<sub>4</sub> at STP conditions) and highest COD reduction ( $63.0 \pm 1.0\%$ ) were obtained in the untreated OMW sample. Hence, ozonation of OMW samples exhibited detrimental inhibitory effects on the methanogenic stage of digestion since methane production from all ozonated samples roughly exceeded the production of the control and in all cases was lower than methane production from untreated OMW, possibly due to the fact that ozonation products are more inhibitory than the original substrate (Andreozzi *et al.* 1998). As also stated by Wang (1990) the ozonation of aromatic compounds may lead to products which inhibit the methanogenesis and whose toxicity increases as the pH of the solution decreases. In all ozonation experiments that were conducted during this study, pH was kept manually constant at 5.20 (pH of R-OMW) but there was a continuous trend for pH decrease during each ozonation experiment, which may have affected the type of ozonation products and their potential toxicity to methanogens.

OMW are very complicated wastes and thus the additional inhibition on methanogens due to ozonation is probably the combination of different factors. For example, it can be ascribed to the products of oleic acid ozonation, i.e. azelaic and nonanoic acid, which are proved to be more inhibitory than the original compound (oleic acid). Moreover, the main ozonation products from various phenolic compounds present in OMW may cause inhibition by different pathways (Andreozzi *et al.* 1998). On the other hand, an improvement of the anaerobic biodegradation of OMW was observed by Benitez *et al.* (1997) due to ozonation pretreatment, which, however, could be attributed

either to their well-acclimated anaerobic sludge or to the dilution of ozonated inhibitory products due to low COD loadings tested in their anaerobic digestion unit or the high percentage of phenols removal (>90%) achieved via ozonation. In the work of Bougrier *et al.* (2007) the effect of ozone treatment on waste activated sludge solubilization and anaerobic biodegradability was reported. For most of the ozone doses tested, they observed not only an increase in sludge biodegradability but also an acceleration of biogas production. However in their work no polyphenols or lipids were involved.

## CONCLUSIONS

The pretreatment of OMW with ozone reduces considerably the total phenolic compounds concentration, which is the main source of toxicity to methanogenic bacteria caused by these wastes. Nevertheless, ozonation of OMW, as pretreatment, exhibited inhibitory effects on subsequent methane production due to the fact that ozonation products are more inhibitory than the original substrate. A substantial decrease of carbohydrates present in the raw OMW was also observed due to ozonation, especially when it took place at close to ambient conditions, thus additionally decreasing the potential of the treated substrate for methane production. Therefore, it can be concluded that despite the effective and selective oxidation of inhibitory compounds in OMW, the ozone treatment cannot be used yet to improve the performance of anaerobic methanogenesis. However, further study is required to test the level of toxicity caused by ozonated OMW products in relation to the extent and the exact conditions under which ozonation takes place (pH, temperature, etc.) as these seriously affect the type of

produced compounds and their toxicity to methanogenic bacteria.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support from the European Commission project LIFE08/ENV/GR578-INTEGRASTE.

## REFERENCES

- Andreozzi, R., Longo, G., Majone, M. & Modesti, G. 1998 Integrated treatment of olive oil mill effluents (OME): study of ozonation coupled with anaerobic digestion. *Water Research* **32** (8), 2357–2364.
- Beccari, M., Carucci, G., Majone, M. & Torrisi, L. 1999 Role of lipids and phenolic compounds in the anaerobic treatment of olive oil mill effluents. *Environmental Technology* **20** (1), 105–110.
- Benitez, F. J., Beltran-Heredia, J., Torregrosa, J. & Acero, J. L. 1997 Improvement of the anaerobic biodegradation of olive mill wastewaters by prior ozonation pretreatment. *Bioprocess Engineering* **17** (3), 169–175.
- Borja, R., Rincón, B. & Raposo, F. 2006 Anaerobic biodegradation of two-phase olive mill solid wastes and liquid effluents: Kinetic studies and process performance. *Journal of Chemical Technology and Biotechnology* **81** (9), 1450–1462.
- Bougrier, C., Battimelli, A., Delgenes, J.-P. & Carrere, H. 2007 Combined ozone pretreatment and anaerobic digestion for the reduction of biological sludge production in wastewater treatment. *Ozone: Science and Engineering* **29** (3), 201–206.
- Capasso, R., Evidente, A., Schivo, L., Orru, G., Marcialis, M. A. & Cristinzio, G. 1995 Antibacterial polyphenols from olive oil mill waste waters. *Journal of Applied Bacteriology* **79** (4), 393–398.
- Council Directive 96/61/EC of 24 September 1996 concerning integrated pollution prevention and control. *Official Journal of the European Communities* **L 257**, 26–40.
- Dareioti, M. A., Dokianakis, S. N., Stamatelatos, K., Zafiri, C. & Kornaros, M. 2010 Exploitation of olive mill wastewater and liquid cow manure for biogas production. *Waste Management* **30** (10), 1841–1848.
- Gilbert, E. 1987 Biodegradability of ozonation products as a function of COD and DOC elimination by example of substituted aromatic substances. *Water Research* **21** (10), 1273–1278.
- Joseffson, B. 1983 Rapid spectrophotometric determination of total carbohydrates. In: *Methods of Seawater Analysis* (K. Grasshoff, M. Ehrhardt & K. Kremling, eds). Verlag Chemie, Berlin, pp. 340–342.
- Owen, W. F., Stuckey, D. C., Healy, J. B., Young, JR. L. Y. & McCarty, P. L. 1979 Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research* **13** (6), 485–492.
- Paraskeva, P. & Diamadopoulos, E. 2006 Technologies for olive mill wastewater (OMW) treatment: a review. *Journal of Chemical Technology and Biotechnology* **81** (9), 1475–1485.
- Standard Methods for the Examination of Water and Wastewater* 1995 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Wang, Y.-T. 1990 Methanogenic degradation of ozonation products of biorefractory or toxic aromatic compounds. *Water Research* **24** (2), 185–190.
- Waterman, P. G. & Mole, S. 1994 Analysis of phenolic plant metabolites. In: *Methods in Ecology* (J. H. Lawton & G. E. Likens, eds). Blackwell Scientific Publications, Oxford.

First received 11 July 2013; accepted in revised form 16 September 2013. Available online 25 October 2013