Anaerobic Digestion of olive mill wastewater after detoxification using Fenton reagents

A. Vlyssides*, G. Lamprou, A. Vlysidis, C. Tsiodra, G. Seintis and K. Bampalioukas

[1]School of Chemical Engineering, National Technical University of Athens, 15780, Athens, Greece

*Corresponding author: E-mail: avlys@tee.gr, Tel +30 210 7723268, Fax: +30 210 7723269

Introduction

Mediterranean countries produce more than 98% of the world's olive oil, which is estimated at over 2.5 million metric tons per year. About 75% is produced in the European Union (EU). Treatment of OMWW is complicated by the seasonal and geographically diffuse nature of olive oil production. Olive production varies significantly from year to year (trees typically bear the most fruit every second year). Furthermore, olives are collected and oil is extracted in the winter months (primarily from early November to late February). Finally, the oil is typically extracted in a large number of small olive mills scattered throughout the olive oil-producing countries.

OMWW has a very high organic load. Chemical Oxygen Demand (COD) typically ranges from 50 to 150 g l^{-1}, or about two orders of magnitude higher than municipal wastewater). OMWW also contains high levels of phytotoxic and microbially inhibitory compounds, such as phenolics and long-chain fatty acids [1]. The main organic constituents of OMWW are sugars and polyphenols. Due to presence of phenolics, the OMWW are not appropriate for irrigation. The complex composition of OMWW coupled with the seasonal nature of olive production and the wide geographical dispersion of mills poses considerable technical and economic barriers for efficient effluent treatment and disposal. [2]

Because of the content of those effluents, the environmental problems and potential hazards caused by olive mill wastewater has prompted many countries to limit its discharge. They also promoting research in order to develop new technologies in their treatment.

The combination of advanced oxidation processes (AOPs) and biological treatments can be a suitable solution for the removal of toxic compounds from OMWW. AOPs are based in the use of the highly and non-specific reactive hydroxyl radicals that make them potentially useful for degradation of a wide range of organic compounds. [3] Aim of the procedure is to apply an AOP to a toxic and/or non-biodegradable effluent and generating an intermediate sample that is fully biodegradable. Detoxification takes place in a short time. Furthermore, optimization in chemical and energy consumption is achieved. At the end of chemical oxidation, follows a subsequent biological treatment for the complete removal of organic matter. Among these technologies, Fenton oxidation could appear to be very promising to achieve high conversion of organic pollutants and allows performing oxidation reaction at ambient conditions, limiting the investment costs.

In this context, this study aimed to investigate the potential of OMWW detoxification and susceptibility to anaerobic degradation by the application of a Fenton oxidation pretreatment step. The effect of 4 operational parameters on Fenton oxidation and aerobic degradation performance was investigated.

MATERIALS AND METHODS

2.1 Chemicals

The chemicals used in the experiments, $FeSO_4 \cdot 7H_2O$ (Fulka), H_2O_2 (Merck, Perhydrol, 30% w/w) were applied as reagent grade. All the solutions were daily prepared in deionized water.

2.2 Wastewater

The wastewater used in this study was olive mill wastewater (OMWW) that was collected from a typical three- phase olive mill. The characteristics of OMWW were: COD 36,20±3,1 g L^{-1}, TOC 18,14±4,2 g L^{-1}, TKN 613,6±5,2 g L^{-1}, phenolic compounds 2,86±0,39 g L^{-1} expressed as gallic acid, oil 22,95±1,9 g L^{-1} and pH 4,5±0,2.

2.3 Inoculum

Seed sludge for the anaerobic degradation process was sludge cultivated on wastewater from a potato processing factory. The sludge was seeded from a UASB bioreactor (Upflow Anaerobic Sludge Blanket), which operates under a loading of 0,05 g COD g VSS^{-1} d^{-1}.

2.4 Methodology

Fenton oxidation procedure

The OMWW was initially subjected to Fenton oxidation treatment. The oxidation was carried out batchwise at 20°C in an agitated (100 rpm) glass reactor of 500mL capacity for 30 minutes. Firstly, the Fenton oxidation reagent was added. As ferrous salt, $FeSO_4 \cdot 7H_2O$ was used and the hydrogen peroxide was of 30% concentration. After oxidation, a sample was collected and analysed in terms of TOC, COD, TKN and oil according to the Standard Methods for Examination of Water and Wastewater, whereas total phenolic compounds (TPC) were determined with Folin-Ciocalteu method [4]. Each experiment was performed in duplicate and the results presented are the mean values.

Anaerobic digestion procedure

In a 500mL beaker, 100 mL of the pretreated wastewater (or the raw OMWW) were added as well as 100 mL of inoculant. The mixture was vigorously stirred (180rpm), in order to achieve a satisfactory oxygen transfer. Every 1 hour, a sample was taken and analyzed for TOC and VSS. For each experiment, the performance data mentioned above were collected until steady-state conditions were obtained. The steady-state condition in the biological system implied that the changes in the TOC were not more than ±16%. The biological degradation procedure was conducted to all chemically treated samples. Each experiment was repeated three times.

Factorial design

The aim of the experimental procedure was to determine the influence of some basic process parameters on the effectiveness of the oxidation treatment in terms of % TOC, TKN, TPC and fats removal and on the effectiveness of the biological treatment in terms of the biokinetic constant maximum substrate uptake rate k_{max}. These parameters were the coagulation/ flocculation, the ferrous sulphate heptahydrated concentration, the hydrogen peroxide concentration and the presence of oil [5]. The term “coagulation/ flocculation” refers to the process of neutralization with lime, coagulation with a weak anionic polyelectrolyte (Praestol 2540, 0,1%) and flocculation. The parameters mentioned above are referred to as “controlling parameters” of the system. The effect of the controlling parameters on the optimization parameter was estimated by performing a 2^3 factorial experiment.

Table 2. Controlling parameters and their levels in the factorial experiment

Controlling parameters				
	Coagulation	**$FeSO_4 \cdot 7H_2O$ (g/L)**	**H_2O_2 30% (mL/L)**	**Oil**
+	Yes	10	20	Yes
-	No	1	2	No

Estimation of biokinetic constants

The biological degradation of the organic substrate of a wastewater in an aerobic activated sludge biological system, can be expressed by the generally accepted model of Lawrence –McCarty that is another version of the Monod model.

$$\frac{dS}{dt} = \frac{k_{max} \cdot S}{K_S + S} \cdot X \quad \text{and} \quad \frac{dX}{dt} = Y \cdot \frac{dS}{dt} - b \cdot X \qquad (1)$$

where: S = biodegradable substrate concentration, $mg \cdot L^{-1}$

X= activated sludge concentration, $mg \cdot L^{-1}$

k_{max}= maximum substrate uptake rate, d^{-1}

b = biomass inactivation rate, d^{-1}

Y = yield coefficient

K_s = substrate inhibition coefficient, $mg \cdot L^{-1}$

A way to estimate the course of the detoxification of a wastewater, with various treatment methods, is to monitor the empirical kinetic constants of the model mentioned above. As the wastewater gets more biodegradable, the parameter k_{max} increases.[6]

In order to estimate the biokinetic constants of the examined substrates (pretreated and raw olive mill wastewater), the experimental data of all sets of experiments (time evolution of substrate and biomass concentrations) were used as input in suitable software (Polymath 6.1) for the solution of the differential equation system. Given the short duration of aerobic degradation experiments (1-2 days), it was assumed that biomass decay could be neglected. Furthermore, it was assumed that the yield coefficient and the substrate inhibition coefficient were the same for all the experiments, since the same inoculum as well as the same type of substrate were used. Thus, apart from Y and

K_s, the maximum substrate uptake rates of all the experiments of the factorial design and of the raw wastewater were estimated.

3. Results

In Table 3, the results of the factorial experiment regarding $\%TOC_r$, $\%TKN_r$, $\%TPC_r$, %fats removal efficiencies as well as the maximum substrate uptake rate k_{max} are presented. The biomass yield coefficient Y was found equal to 0.6 and the substrate inhibition coefficient K_s 200mgC/L.

Table 3. %TOC, %TKN, %TPC, %fats removal efficiencies and k_{max} for the experiments of the factorial design

Parameters					**Response factors**				
Trial	**Coagulation**	**$FeSO_4 \cdot 7H_2O$**	**H_2O_2**	**Oil**	**% TOC_r**	**$\%TKN_r$**	**% TPC_r**	**% $fats_r$**	**k_{max} (d^{-1})**
	X1	X2	X3	X4	Y1	Y2	Y3	Y4	Y5
1	+	+	+	+	43,42	19,75	78,12	73,03	1,09
2	-	+	+	+	29,43	18	76,26	30,5	7,76
3	+	-	+	+	31,83	78,37	82,04	87,97	3,32
4	-	-	+	+	0	5,74	57,04	72,35	6,22
5	+	+	-	+	25,43	44,26	70,19	87,41	8,55
6	-	+	-	+	0	9,69	25,59	76,60	3,18
7	+	-	-	+	19,48	40,59	70,33	97,06	1,72
8	-	-	-	+	8,85	25,44	30,79	75,12	4,43
9	+	+	+	-	30,08	44,56	66,67		1,11
10	-	+	+	-	40,77	0,70	68,73		1,39
11	+	-	+	-	26,37	52,06	63,42		3,84
12	-	-	+	-	13,67	0	0		8,43
13	+	+	-	-	29,38	49,35	55,35		5,46
14	-	+	-	-	26,01	4,41	3,13		2,05

15	+	-	-	-	30,52	32,64	52,39		1,88
16	-	-	-	-	6,21	19,75	9,69		5,11

It is worth noticing that the Fenton oxidation process was in some cases quite effective, since the %TOC removal efficiency reached 43,42%, the %COD removal efficiency 34,32%, the %TKN removal efficiency 78,37%, the %TPC removal efficiency 82,04% and last but not least the % fats removal efficiency reached the high percentage of 97,06%. From the experimental results, it is clear that Fenton oxidation managed to oxidise fats either partially by converting them to soluble organic compounds or totally by full mineralization. The concentrations of the other parameters such as TOC, COD, TKN and TPC may have presented a slight increase which is also in accordance with the assumption that complex chemical components, insoluble in water, broke down into water soluble fragments. By all the facts mentioned above, the beneficial effect of fenton oxidation is obvious.

The maximum substrate uptake rate k_{max} of the untreated OMWW was $1.09d^{-1}$. This value is lower than the respective values estimated for the pretreated samples. This implies that pretreatment by Fenton oxidation improves the following aerobic process. The reason for this may be attributed to the fact that the chemicals added oxidize the fats as well as the phenolic compounds which are inhibitors of anaerobic microorganisms.

3.1 TOC removal

Oxidation process includes two different steps, which can be performed either sequential or in parallel. One step is the breaking down of complex organic substances to smaller molecules and the other one is the mineralization of organic compounds. TOC removal expresses the mineralization efficiency of the oxidation process.

According to the results of the factorial experiment and by following a specific analytical procedure, the following linear model was estimated, interrelating the %TOC removal efficiency (Y1) with the controlling parameters of the system:

$$Y1 = 21,89 + 7,67 \cdot X1 + 6,17 \cdot X2 - 3,66 \cdot X3 - 3,48 \cdot X4 - 3,66 \cdot X1X2 - 0,29 \cdot X1X3 + 3,96 \cdot X1X4 + 4,2 \cdot X2X3 - 0,01 \cdot X2X4 + 1,3 \cdot X3X4 - 2,89 \cdot X1X2X3 + 1,88 \cdot X1X2X4 + 2,68 \cdot X2X3X4 - 2,58 \cdot X1X2X3X4 \quad (5)$$

The significance of the linear coefficients and their interactions was checked through statistical analysis. It was proved that the most significant linear parameters ($p < 5\%$) were the coagulation (X1), the ferrous sulphate heptahydrated concentration (X2), the hydrogen peroxide concentration (X3) and the presence of oils (X4). The minus (-) in the above equation indicates that an increase of the controlling parameter leads to a lower % TOC efficiency and, consequently, to a less effective oxidation. It was shown through statistical analysis that the interaction of coagulation with ferrous sulphate, as well as with the presence of oil should be accounted for. So, the model was simplified to the following:

$$Y1 = 21,89 + 7,67 \cdot X1 + 6,17 \cdot X2 - 3,66 \cdot X3 - 3,48 \cdot X4 - 3,66 \cdot X1X2 + 3,96 \cdot X1X4 + 4,2 \cdot X2X3 - 2,89 \cdot X1X2X3 + 2,68 \cdot X2X3X4 - 2,58 \cdot X1X2X3X4 \quad (6)$$

The adequacy of the mathematical model was checked by the Fisher criterion.

Adequacy of the simplified model: $F_{exp} = 1,44 < F_{tab} = 8,76$.

Where F_{exp} = the experimental value of the Fisher criterion

F_{tab} = the value of the Fisher criterion from the statistical tables

As it is obvious, the simplified model is adequate and thus could be used for TOC removal prediction in the controlling parameters examined range.

In the experimental range studied, the higher % TOC efficiency measured was 43,4% in the experimental point (X_1, X_2, X_3, X_4) = (with coagulation, 10g/L $FeSO_4.7H_2O$, 20mL/L H_2O_2, with oil).

3.3 TKN removal

The mathematical model describing the TKN removal (Y2) is the following:

$Y2 = 25,61 + 19,59 \cdot X1 - 1,77 \cdot X2 + 0,97 \cdot X3 + 4,62 \cdot X4 - 3,95 \cdot X1X2 + 2,5 \cdot X1X3 - 4,07 \cdot X1X4 - 4,06 \cdot X2X3 - 5,53 \cdot X2X4 - 0,74 \cdot X3X4 - 6,75 \cdot X1X2X3 - 2,49 \cdot X1X2X4 - 0,22 \cdot X2X3X4 - 4,54 \cdot X1X2X3X4$ (9)

The analysis for the statistically significant linear coefficients and their interactions revealed the following:

$Y2 = 25,62 + 19,59 \cdot X1 - 1,77 \cdot X2 + 4,62 \cdot X4 - 3,95 \cdot X1X2 + 2,51 \cdot X1X3 - 4,07 \cdot X1X4 - 4,06 \cdot X2X3 - 5,53 \cdot X2X4 - 6,75 \cdot X1X2X3 - 2,49 \cdot X1X2X4 - 4,54 \cdot X1X2X3X4$

It was proved that the most significant linear parameter was by far the coagulation (X1) followed by the presence of oils (X4) and the ferrous sulphate heptahydrated concentration (X2). It was proved that only the interactions X3X4 and X2X3X4 as well as the hydrogen peroxide concentration (X3) were not statistically significant. When the organic substrate contains heteroatoms, mineralization often leads to the formation of inorganic acids (HCl, HNO_3, NH^{+4} , H_2SO_4, etc.). Nitrogen-containing compounds may form HNO_3 exclusively or a mixture of NH^{+4} and HNO_3 . Redox interconversion of NH^{+4} and NO^{-3} during HO·-intiated reactions involve a number of intermediate steps and species (e.g., NH_2OH, $NH\cdot_2$, NO·) whose importance is governed by pH, and presence of electron, proton, or hydrogen donors or acceptors and O_2 [7].

The adequacy of the mathematical model was $F_{exp} = 2,72 < F_{tab} = 8,76$.

Thus, the simplified model is adequate.

In the experimental range studied, the higher % TKN efficiency measured was 78,4% in the experimental point (X_1, X_2, X_3, X_4) = (with coagulation, 1g/L $FeSO_4.7H_2O$, 20mL/L H_2O_2, with oil).

3.4 TPC removal

Phenolic compounds removal (Y3) could be described according to the factorial experiment results analysis by the following mathematical model:

$Y3 = 50,59 + 16,71 \cdot X1 + 4,9 \cdot X2 + 10,91 \cdot X3 + 10,69 \cdot X4 - 4,64 \cdot X1X2 - 5,67 \cdot X1X3 - 2,84 \cdot X1X4 + 6,03X2X3 - 3,66 \cdot X2X4 + 1,15 \cdot X3X4 - 6,46 \cdot X1X2X3 + 2,38 \cdot X1X2X4 - 3,45 \cdot X2X3X4 + 2,93 \cdot X1X2X3X4$ (10)

Through statistical analysis it was proven that all coefficients are statistically significant.

Generally phenolic compounds removal was high reaching 82 % at the experimental point (X1, X2, X3, X4) = (with coagulation, 1g/L $FeSO_4.7H_2O$, 20mL/L H_2O_2, with oil).

The coagulation parameter has the highest linear coefficient, reflecting its crucial role in phenolic compounds removal. Apart from coagulation, effective breakdown of the phenolic compounds can be achieved by radical produced by Fenton reagents. This was also reported by [4] who studied the oxidation of one of the most representative phenolic compounds present in industrial wastewater (gallic acid) using Fenton's reagents. It was proved that the % degradation of gallic acid may reach a maximum of 95,5±1,3%.

3.5 Fats removal

According to the results of the factorial experiment and by following a specific analytical procedure [6, 7], the following linear model was estimated, interrelating the fats removal (Y4) with the controlling parameters of the system:

$$Y4 = 75 + 11{,}37 \cdot X1 - 8{,}12 \cdot X2 - 9{,}04 \cdot X3 + 1{,}97 \cdot X1X2 + 3{,}18 \cdot X1X3 - 6{,}07 \cdot X2X3 + 4{,}75 \cdot X1X2X3 \quad (12)$$

Apart from coagulation, all other controlling parameters were not statistically significant. The model was turned to the following:

$$Y4 = 75 + 11{,}37 \cdot X1 \quad (13)$$

The adequacy of the simplified model was checked by the Fisher criterion: $F_{exp} = 2{,}9 < F_{tab} = 3{,}3$.

As it is obvious, the simplified model is adequate. Nevertheless, the high standard errors that were observed in the fats removal parameter should be pointed out. This fact could be responsible for the statistical insignificance of most parameters. The crucial role of coagulation on fats removal has also been reported in literature. A study of the effect of lime treatment on various OMW after a classic coagulation/flocculation/sedimentation/filtration process, revealed that lime dosing corresponding to a pH increase to 12 gave the optimal performance, resulting in 95% oil and grease removal. [20,35]

.In the experimental range studied, the higher % fats removal efficiency measured was 97,1% in the experimental point (X_1, X_2, X_3, X_4) = (with coagulation, 1g/L $FeSO_4.7H_2O$, 2mL/L H_2O_2).

3.6. Maximum substrate uptake rate k_{max}

The maximum substrate uptake rate k_{max} (Y5) could be described according to the factorial experiment results analysis by the following mathematical model:

$$Y5 = 4{,}22 + 0{,}59 \cdot X1 - 1{,}08 \cdot X2 + 1{,}39 \cdot X3 + 0{,}31 \cdot X4 - 0{,}14 \cdot X1X2 - 0{,}27 \cdot X1X3 + 0{,}26 \cdot X1X4 - 0{,}46 \cdot X2X3 + 0{,}54 \cdot X2X4 + 0{,}53 \cdot X3X4 + 0{,}31 \cdot X1X2X3 + 0{,}76 \cdot X1X2X4 + 0{,}08 \cdot X2X3X4 + 0{,}76 \cdot X1X2X3X4 \quad (10)$$

The significance of the linear coefficients and their interactions was checked through statistical analysis. It was proven that the only significant linear parameter (p<5%) was the hydrogen peroxide concentration. Thus the final simplified model is:

$$Y5 = 4{,}22 + 1{,}39 \cdot X3 \qquad (11)$$

The adequacy of the simplified model was: $F_{exp} = 2{,}04 < F_{tab} = 8{,}76$.

So, the simplified model that derived is adequate.

The higher maximum substrate uptake rate k_{max} that was observed was $8{,}6d^{-1}$ at the experimental point (X1, X2, X3, X4) = (without coagulation, 1g/L $FeSO_4.7H_2O$, 20mL/L H_2O_2, with oil).

4. Conclusions

Olive mill wastewater is high-strength wastewater, containing high concentrations of phenols and fats. Subsequently, the need for the management of such wastewater becomes urgent because not only are there many olive mills operating, producing large volumes of wastewater, but also their wastewater are not amenable to conventional biological treatment.

In this study, OMWW was treated by a Fenton oxidation procedure in order to either to break down the large molecules of fats and polyphenols or to mineralise organic pollutants. Mineralization efficiency measured as total organic carbon removal reached 43,42%. On the other hand, in some cases the %TPC and %fats removal efficiencies were much higher, reaching 82,04 and 97,1%, respectively. Thus, the fenton oxidation step seems to meet its main goal: to break down complex chemical components. The optimum conditions achieved for the oxidation of olive mill wastewater in terms of most parameters were found to be: (with coagulation, 1g/L $FeSO_4.7H_2O$, 20mL/L H_2O_2, with oil) and TOC reduction was 31,8%, TKN 78.4%, TPC 82% and oil removal 88%.

By the mathematical and statistical analysis of the factorial experimental data, the effect of the parameters studied on the response factors was revealed (Table 3). Coagulation with lime had a positive effect on the removal of all pollution parameters examined. In literature coagulation has been studied as a physicochemical pretreatment of olive mill wastewater with promising results regarding lipids, polyphenols and COD removal. In this study, coagulation was coupled with Fenton oxidation and it was proved that the synergistic effect was positive. The biokinetic constant of maximum substrate uptake rate reflects the biodegradability of the substrate. The concentration of H_2O_2 that defines the intensity of oxidation was proved to be the governing factor in the biodegradability improvement.

Table 3. Summarized effects of parameters examined on the response factors of the factorial experiment (% TOC_r, % TKN_r, % TPC_r, % $fats_r$, k_{max}).

Response factor	Positive effect (+)	Negative effect (-)
% TOC_r	Coagulation $FeSO_4 \cdot 7H_2O$	H_2O_2 Oil
% TKN_r	Coagulation Oil	$FeSO_4 \cdot 7H_2O$
% TPC_r	Coagulation H_2O_2 $FeSO_4 \cdot 7H_2O$ Oil	
% $Fats_r$	Coagulation	
k_{max}	H_2O_2	

Conclusively, Fenton oxidation pretreatment proved to be an effective method for the detoxification of the OMWW since it resulted to higher maximum substrate uptake rates k_{max} rendering OMWW susceptible to anaerobic biological treatment. The feasibility of the pretreatment method by its incorporation in an integrated plant could be the aim of a future work. Technical and economical aspects should be evaluated.

References

[1] A. Rozzi and F. Malpei, “Treatment and disposal of olive mill effluents,” *Int. Biodeterior. Biodegradation*, vol. 38, no. 3–4, pp. 135–144, 1996.

[2] A. Vlyssides, E. M. Barampouti, and S. Mai, *Industrial Pollution*, First. Athens, Greece: J. Sideris, 2015.

[3] D. Mantzavinos and N. Kalogerakis, “Treatment of olive mill effluents: Part I. Organic matter degradation by chemical and biological processes - An overview,” *Environ. Int.*, vol. 31, no. 2, pp. 289–295, 2005.

[4] A. Vlyssides, E. M. Barampouti, S. Mai, M. Sotiria, and N. Eleni, “Degradation and Mineralization of Gallic Acid Using Fenton’s Reagents,” *Environ. Eng. Sci.*, vol. 28, no. 7, pp. 515–520, 2011.

[5] A. G. Vlyssides, E. M. Barampouti, and S. Mai, “Influence of ferrous iron on the granularity of a UASB reactor,” *Chem. Eng. J.*, vol. 146, no. 1, pp. 49–56, Jan. 2009.

[6] A. Vlyssides, E. M. Barampouti, and S. Mai, “Effect of ferrous ion on the biological activity in a UASB reactor: Mathematical modeling and verification,” *Biotechnol. Bioeng.*, vol. 96, no. 5, pp. 853–861, Apr. 2007.

[7] M. R. Gonçalves, J. C. Costa, I. P. Marques, and M. M. Alves, “Strategies for lipids and phenolics degradation in the anaerobic treatment of olive mill wastewater,” *Water Res.*, vol. 46, no. 6, pp. 1684–1692, 2012.