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Integrated aerobic biological treatment and chemical oxidation with Fenton's reagent for the processing of green table olive wastewater

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Abstract

Table olive processing wastewater (TOPW) is unsuitable for disposal at municipal or industrial wastewater treatment plants due to its high organic and phenol content. Aerobic biological treatment using an *Aspergillus niger* strain in a bubble column bioreactor in combination with chemical oxidation was studied for the management of TOPW to a quality which corresponds to the input standards for wastewater treatment plants (COD $<$ 1200 ppm, BOD $<$ 500 ppm). After 2 days of biological treatment COD was reduced by 70%, while the total and simple phenolic compounds were decreased by 41 and 85%, respectively. In the chemical treatment step, the effect of different H_2O_2 concentrations on the patterns of COD and phenol reduction was studied. The main effect of the chemical oxidation step was the elimination of persistent phenolic compounds during the biological treatment of total phenolic compounds. Coagulation with CaO significantly improved the efficiency of the process.

Keywords: Table olive wastewater; *Aspergillus niger*; Biological treatment; Phenolic compounds; Fenton's reagent; Hydrogen peroxide

1. Introduction

Table olive processing wastewater (TOPW) causes an important local environmental problem, as it is characterised by seasonal peaks, very high organic load and high concentrations of phenolic compounds, which are known to cause toxic effects to living organisms. In Mediterranean countries, this type of wastewater is usually discharged untreated to small streams or directly to the sea. In the best cases, it is transported to evaporation ponds, where malodours are a common nuisance, while the risk of polluting surface or ground waters is not always avoided [1–3]. For green olive preparation, the characteristics of the wastewater which arises from the several treatment stages (cleaning, debittering using NaOH, washing after debittering, fermentation brines and general use water) fluctuate as follows—pH: 3.6–13.2; suspended solids: 0.03–0.4; dissolved solids: 0.2–80; BOD_5: 0.1–6.6; COD: 0.3–16.2; chloride: 0.0–48.5 and sodium chloride 0.0–80.0 g/l [1]. The stages of debittering and subsequent washing constitute the largest and most heavily polluted fraction of the wastewater, seasonally produced from September to November.

In the last few years, as environmental regulations and enforcement have become more stringent, a growing interest in the development of new treatment methods for this type of wastewater has emerged. Biological treatment methods have been recognized as overall economical and effective processes [3–6]. However, the presence at high concentration of aromatic, phenolic and polyphenolic compounds, which are toxic to many microorganisms (especially those found in municipal wastewater treatment plants), inhibits the efficiency of biodegradation processes [3,7].

In order to facilitate the degradation of toxic or non-biodegradable organic substances, many researchers have proposed combined methods comprising of chemical and biological treatment steps. A common approach refers to the oxidation of the wastewater using a strong oxidative agent, such as ozone [8], Fenton's reagent, a mixture of hydrogen peroxide and ferrous or ferric iron [9,10], a combination of UV radiation and hydrogen peroxide as well as photo-Fenton [7]. These methods are based on the creation

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of very reactive oxidizing free radicals, especially hydroxyl radicals.

In Fenton's reaction, the ferrous and/or ferric cation decomposes catalytically hydrogen peroxide to generate powerful oxidizing agents, capable of degrading a number of organic and inorganic substances. Fenton's oxidation is a complicated system that involves a large number of reactions, including redox reactions, complexation, precipitation equilibrium, etc. Also, there is an uncertainty in the nature of oxidizing species generated during the process (formation of radical species or generation of aquo- or organocomplexes of high valence iron). A model of Fenton's reagent processing of brines from the table olive industry, a wastewater relatively similar to that resulting from the olive debittering stage, which was studied in this work, is described by Rivas et al. [10].

In this study, wastewater from the debittering process of green table olives and the subsequent washings were treated aerobically using *Aspergillus niger* [11]. The *Aspergillus* genus has been used successfully for the bioremediation of olive oil mill wastewater, which is also characterized by high organic and phenolics content and satisfactory removal efficiencies have already been reported [12–14]. Fenton's reagent was used as a secondary chemical treatment step for the oxidation of the recalcitrant organic compounds or metabolites of those that could not be oxidized biologically. The use of chemical oxidation as a secondary treatment process offers the advantage of reducing the amount of the required oxidants and improves the economic feasibility of the treatment process.

2. Materials and methods

2.1. Wastewater

Fresh washing and debittering wastewater (TOPW, pH 12.1–12.5, conductivity 24–44.3 mS/cm) was obtained from the industrial plant of the Agroindustrial Cooperation of Stylida (Lamia, Central Greece). Before biological treatment, the pH was adjusted to 4.5–4.8 using an average of 5.06 ml/l conc. H_2SO_4. COD and conductivity of TOPW after pH correction ranged from 6.50 to 13.55 g/l and 12.5 to 22.2 mS/cm, respectively.

2.2. Inoculum

A strain of *A. niger* isolated from undiluted TOPW was used for the biological treatment. The strain was maintained on potato-dextrose agar slants. For inoculum preparation, a spore suspension in Ringer's solution was made from a 2 week culture of the fungus, cultivated at 30 °C. Spore suspension was vortexed to separate clumped spores resulting in a final inoculum concentration of 10^8 spores per ml. The bioreactor was inoculated with 1% from the above-described inoculum at the beginning of the fermentation process.

2.3. Aerobic biological treatment

Aerobic biological treatment was performed in a bubble column bioreactor under non-sterile conditions. The bioreactor was constructed of a central tube of Pyrex glass, of 140 cm height and 12 l total volume. Air was supplied continuously through the bottom of the bioreactor at a flow rate of 12 l/min throughout the fermentation, while the dissolved oxygen concentration never fall below the level of 3 ppm. Experiments were carried out at ambient temperature (25 °C). The bioreactor was operated in a 3 day batch mode followed by continuous operation with a 2 day hydraulic retention time of the wastewater and a biomass recycling ratio of 80%. The supernatant of the effluent was collected and kept at −20 °C for further treatment or analysis.

2.4. Chemical treatment—Fenton's reaction

The effluent from the biological treatment (supernatant after natural sedimentation) was further treated using Fenton's reaction and subsequent lime flocculation. For Fenton's reaction, Fe^{2+} was added in the form of $FeSO_4{\cdot}7H_2O$ (GR for Analysis, MERCK) and the H_2O_2 was a 30% extra pure stabilized solution, (Riedel-de Haen).

Four different concentrations of H_2O_2 were tested (treatments A–D): 0.2, 0.4, 0.6 and 0.8 g/l (keeping constant, at 0.5 g/l, the concentration of Fe^{2+}). Samples were taken during the reaction at 15, 30, 60 min, 2 and 3 h time intervals after H_2O_2 addition and they were analyzed for residual H_2O_2, Fe^{2+} ions, COD and phenols. Experiments were carried out at ambient temperature (25 °C) while conductivity, pH and temperature of the reacting mixture were continuously monitored.

At the end of the chemical oxidation step, the reaction products were treated with CaO (10% water suspension) up to pH 12 and left to settle in settling cones for 2 h. Samples were taken from the supernatants for COD and phenol measurement. Effluent from the biological treatment, which was not chemically oxidized was also flocculated with CaO and used as control.

2.5. Analytical methods

Total phenolics (simple phenolic and polyphenolic compounds) were measured according to the Folin–Ciocalteau method [15]. Results are expressed as ppm of gallic acid. The procedure for the analyses of the simple phenolic compounds and organic acids has been described elsewhere [16]. In order to gather more information on the concentration of specific important phenolic compounds and organic acids present in TOPW both the Folin–Ciocalteau as well as an analytical procedure developed in our laboratory has been used.

COD measurements, were performed according to standard methods [17]. In the case of samples taken during

Table 1
Total solids, suspended solids, dissolved solids, volatile suspended solids (g/l) and oxygen consumption rate (ppm/h) during aerobic biological treatment

	TS	SS	VS	VSS	OCR
Initial (pH 12.5)	15.12 (0.13)	0.81 (0.07)	4.08 (0.3)	0.46 (0.02)	–
Influent (pH 4.7)	20.33 (0.16)	3.54 (0.54)	5.25 (0.17)	2.37 (0.06)	0.00
Batch operation					
Day 1	19.25 (0.13)	1.65 (0.00)	4.02 (0.15)	0.94 (0.01)	40.02 (0.93)
Day 2	18.55 (0.03)	1.76 (0.05)	3.49 (0.07)	0.9 (0.01)	17.3
Day 3	18.8 (0.01)	1.95 (0.12)	3.31 (0.07)	1.01 (0.04)	10.8 (1.01)
Continuous operation					
Day 5	16.46 (0.05)	1.9 (0.02)	3.03 (0.09)	1.15 (0.04)	14.4 (1.01)
Day 10	18.23 (0.05)	2.46 (0.61)	3.24 (0.22)	1.79 (0.02)	24.48 (1.01)
Day 15	20.97 (0.04)	4.05 (0.08)	6.44 (0.27)	3.47 (0.03)	48.24 (0.51)

Numbers in parenthesis are the standard deviation of the means.

Fenton's reaction, where the presence of Fe^{2+} ions and residual H_2O_2 could interfere with the COD measurements, the pH of the samples was raised to above 10 with NaOH 6N, prior to the analysis, in order to precipitate Fe^{2+} ions and remove residual H_2O_2.

Total solids (TS), suspended solids (SS), volatile solids (VS), volatile suspended solids (VSS) and oxygen consumption rate (OCR) were measured according to standard methods [17]. An increase to the volatile solids value was noticed after pH correction, which is attributed to interferences caused by the presence of sulphate [17]. Addition of sulphate makes the samples hygroscopic and apparently an amount of water that was retained during evaporation at 105 °C was removed at 550 °C and measured as volatile solids. This interference introduces a systematic error in TS and VS measurements (Table 1) in all the samples obtained after pH correction.

During Fenton's reaction, the residual H_2O_2 concentration of the samples was determined according to the Ce(IV) method [18] and the presence of Fe^{2+} ions was estimated using analytical test strips (3–500 ppm Fe^{2+}, MERCK).

3. Results and discussion

3.1. Aerobic biological treatment of green table olive wastewater

Fig. 1 shows the COD, pH and VSS evolution during the initial 3 day batch culture and the subsequent continuous culture, during which the hydraulic retention time of the wastewater was 2 days. After 1 day of batch operation, the COD removal was 56% and reached 71 and 74% on the second and the third day, respectively. During the continuous operation phase, the average organic loading rate was 5.4 g [COD]/l per day with a standard deviation of 1.1 g/l per day and the mean COD removal was approximately 70%. The high variation in the organic loading rate is indicative of the high fluctuation in the COD values of the influent. This fluctuation is characteristic of this type of wastewaters and should be anticipated in real scale plants. The mean pH value of the influent was adjusted at 4.6 and was further reduced approximately to 3.6 as a result of the activity of *A. niger*, a fungus known for the production of acidic metabolic products.

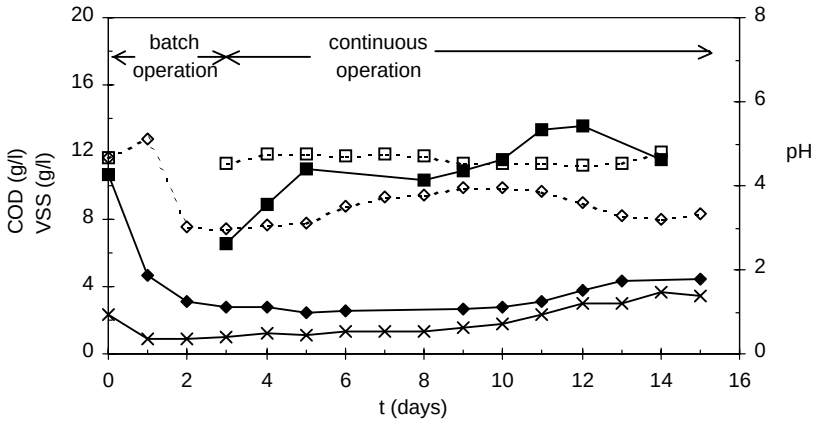

Fig. 1. COD of the influent (■) and effluent (◆), pH of the influent (□) and the effluent (◇) and VVS (×) evolution during the aerobic biological treatment of TOPW with *A. niger*.

The basic characteristics of the initial wastewater, the influent (i.e. after pH correction) and the mixed liquor during batch and continuous operation of the biological treatment are presented in Table 1. The VS/TS ratio of the initial wastewater indicates that a large portion of the total solids (>70%) represents inorganic matter. Salts in the influent were further increased due to the H_2SO_4 addition. The biodegradation efficiency did not appear to be affected by the salinity of the wastewater. High salts concentration constitutes an important limitation for the biological treatment of saline wastewaters and especially brines (i.e. wastewater containing at least 3.5% total dissolved solids) [19]. However, the salts content of the wastewater used, as estimated from the non-volatile solids (Table 1) did not exceed 1.6% (w/v), a level well tolerated by a wide range of microorganisms [19].

It should be noticed that the values of all the solids measuring parameters were increased after the pH correction. There was a four-fold increase of the SS value due to the pH alteration and this was also connected to a visible increase of the turbidity and a five-fold increase of the VSS. It is likely that the pH alteration induces a chemical coagulation of organic and inorganic compounds, while also the added SO_4^{2-} may form various salts of different points of volatilization. The suspended and volatile suspended solids of the influent were reduced in the first day of the batch culture, probably due to their re-dissolution and/or their consumption by the microorganisms. Their subsequent increase during continuous operation is mainly attributed to the growth of the biomass, which was also apparent by eye and microscopic observation. Biomass constituted mainly of *A. niger* mycelium while a few yeasts were occasionally observed during microscopic examination. The presence of bacteria reported by other researchers during the fermentation of olive mill wastewater using *A. niger* [20], was not observed probably due to the low pH of the medium (Fig. 1), which provides a selective advantage to fungal growth. The oxygen consumption rate was high, exceeding 40 ppm/h during the exponential growth of the fungus (first day of batch culture), declined gradually to about 11 ppm/h at the end of the batch phase due to substrate consumption, to increase again to almost 50 ppm/h during the continuous operation phase, in correspondence to substrate enrichment.

In the period between the 5th and the 15th day of the continuous operation phase the biomass loading rate decreased from 42.7 to 18.4 g COD_{in}/g per day VSS and the biomass production increased from 0.18 to 0.48 g VSS/g $COD_{removed}$. The ratio of VSS/SS was increased from 0.60 to 0.86, which indicates an increase of biomass production.

During the continuous phase of the biological treatment, total phenolic removal was 41% while simple phenolic removal was more efficient and reached 85% (Table 2). This was attributed to the fact that phenolic compounds with high molecular weights are not easily biodegradable. In contrast, simple phenolics are highly toxic, but biodegradable [21]. The sum of the measured simple phenolic compounds concentrations was very low (26 ppm) compared to the total phenolic concentration (320 ppm) indicating that the main fraction of the phenolic substances was bound in complex forms.

3.2. Chemical treatment of the effluent

Four different concentrations of H_2O_2 were tested: 2, 4, 6 and 8 g/l (treatments A–D) using the same concentration of Fe^{2+} (0.5 g/l). As illustrated in Fig. 2, all treatments are characterized by a high initial decomposition rate, as determined by the COD value (Fig. 2a), accompanied by a high H_2O_2 consumption rate (Fig. 2b), which tends to slow

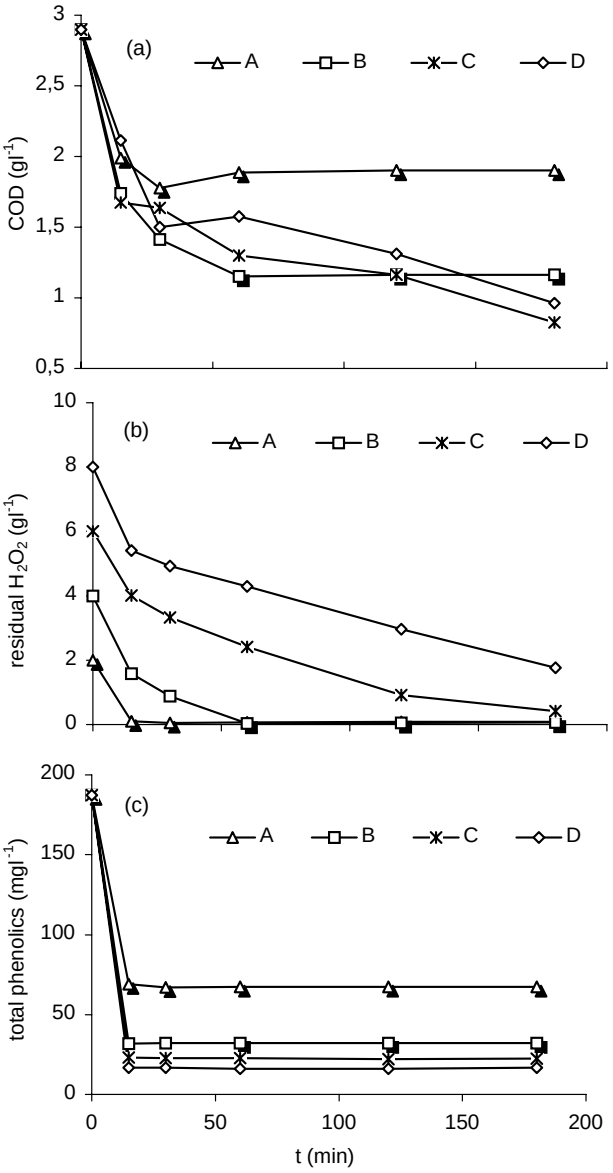

Fig. 2. COD (a), residual H_2O_2 (b), and total phenolics (c) evolution, during chemical oxidation (Fenton's reaction) of the effluent of biological treatment. All the treatments (A–D) carried out using 0.5 g/l Fe^{2+} and 2 g/l H_2O_2 (A), 4 g/l H_2O_2 (B), 6 g/l H_2O_2 (C), 8 g/l H_2O_2 (D). Shadowed symbols (in treatments A and B) indicate the appearance of Fe^{2+} ions.

Table 2
Total phenolics (as ppm gallic acid) and some characteristic phenolic compounds and organic acids (ppm) measured during the successive steps of biological and chemical treatment of the waste

	Biological treatment		Fenton's reaction				CaO flocculation				
	Influent[a]	Effluent[a]	A[b]	B[b]	C[b]	D[b]	Effluent + CaO[c]	A + CaO[d]	B + CaO[d]	C + CaO[d]	D + CaO[d]
(1) Total phenolics	319.6	187.2	67.2	32.1	22.57	16.7	51.4	16.1	9.7	6.94	5.5
(2) Simple phenolic compounds											
Trans-cinnamic acid	0.1955	0	0.127	0.14	0	0.266	0.196	0	0	0	0.213
Veratric acid	1.246	0	0	0	0.554	0	0	0	0	0	0
Vanillic acid	8.1665	0.79	0.217	0.404	0.449	0.314	2.475	0.154	0.166	0.122	0.159
Protocatechuic acid	0.067	0.141	0.299	0.272	0.4	0	0.192	0.441	0.206	0.178	0
Syringic acid	0.449	0.547	0	0.168	0	0.17	0.348	0	0	0	0.15
p-Coumatric acid	9.3255	0.472	0	0	0	0	0.688	0	0	0	0
Gallic acid	0.33	0.761	0.171	0.115	0.274	0	0.794	0	0	0	0
Ferulic add	0.1725	0.16	0.095	0.294	0.413	0.086	0.155	0.506	0.134	0.152	0
Caffeic acid	0.311	0.163	0.219	0.196	0	0	1.309	0	0.093	0	0
Tyrosol	5.9385	0.929	0	0	0	0.15	1.048	0	0	0	0
Sum	26.2015	3.963	1.128	1.589	2.09	0.986	7.205	1.101	0.599	0.452	0.522
(3) Simple organic acids											
Oxalic acid	0.591	0.496	14.204	3.641	6.494	8.619	0.414	1.154	0.423	0.441	6.865
Cyclohexane carboxylic add	0	0	0	0	0	0	0	0	0	0	0
D-L-Lactic acid	0.8115	0.709	3.583	1.619	1.855	0.63	3.131	3.135	1.728	1.788	1.845
D-L-Malic acid	0.4285	0.38	0.485	0.427	0.338	0.425	0.324	0.357	0.405	0.226	0.445
Citric acid	1.361	2.353	4.98	4.993	3.494	1.983	4.274	1.941	2.292	1.002	3.118
Benzoic acid	0.3225	0.087	0.077	0	0	0	0.09	0	0	0.137	0
D-3-Phenyllactic acid	0	0.129	0	0	0	0	0.206	0	0	0	0
Dibutyl phthalate	0.8985	0.902	1.037	3.772	5.303	0.968	1.17	3.41	1.202	1.038	0.404
2-Phenoxyethanol	0.4035	0.226	0.214	0.205	0.151	0.185	0.153	1.055	0.214	0.172	0.168
Sum	4.8165	5.282	24.58	14.657	17.64	12.81	9.762	11.052	6.264	4.804	12.845

[a] Mixture of several days' liquid.
[b] After Fenton's reaction using 0.5 g/l Fe^{2+} and 2 g/l H_2O_2 (A), 4 g/l H_2O_2 (B), 6 g/l H_2O_2 (C), and 8 g/l H_2O_2 (D).
[c] Supernatant, after 2 h sedimentation of the effluent that was not subjected Fenton's reaction.
[d] Supernatant, after 2 h sedimentation of the products of the treatments A–D.

down after the first 15 min. This was more pronounced in treatments B–D, while in treatment A the oxidant was depleted during the first 15 min. In this time period, the phenolic oxidation has already been completed for all treatments (Fig. 2c), as no change in phenolic concentration was observed thereafter. It seems that Fenton's reaction exhibits a selectivity towards phenolic substances, as their oxidation is completed first, while the global organic compounds oxidation, as measured by the COD reduction, continues for as long as the hydrogen peroxide is not depleted. The fraction of the COD attributed to phenolic compounds is considerably lower after the reaction: the phenolic compounds comprised 6.6% of the COD after the biological treatment while they were reduced to 2.3, 1.1, 0.8 and 0.6% at the end of the treatments A–D, respectively).

The observation of the slow phase of the COD decomposition could be attributed to the selectivity of Fenton's reaction: a part of the organic carbon is easily oxidized while other substances may form several intermediates before their complete oxidation. It could also be attributed to Fe^{2+} depletion, caused by Fe-organic complexes formation [22]. The accumulation of oxalic and maleic acid as products of phenols decomposition during Fenton's reaction has been reported [23]. In the current study, an increase in the concentration of organic acids after Fenton's reaction has also been observed (Table 2). Some of them, such as the oxalate, are reported to deactivate iron by forming stable complexes [24]. It has also been reported that the formation of a dark color during the progress of the reaction is attributed to these complexes of organic acids with iron [24]. In treatment A, the residual concentration of Fe^{2+} at the end of the reaction was approximately 250 ppm, while in treatment B, only 100 ppm Fe^{2+} were recovered. In treatments C and D iron in the form of Fe^{2+} was not recovered in any appreciable amount. Provided that the amount of iron used is adequate to saturate the chelators that exist or are created during the reaction, the concentration of iron in the reacting mixture will only affect the reaction kinetics, while the final extent of oxidation will be limited by the hydrogen peroxide concentration [24].

During Fenton's reaction, the pH value typically decreases from 3.3 after the biological treatment step, to 2.0–2.7 according to the H_2O_2 concentration. In lower concentrations, the pH was stabilized sooner (15 min), compared to higher concentrations of H_2O_2 (120 min, Fig. 3a). The pH variation showed a highly significant correlation with the COD ($r =$

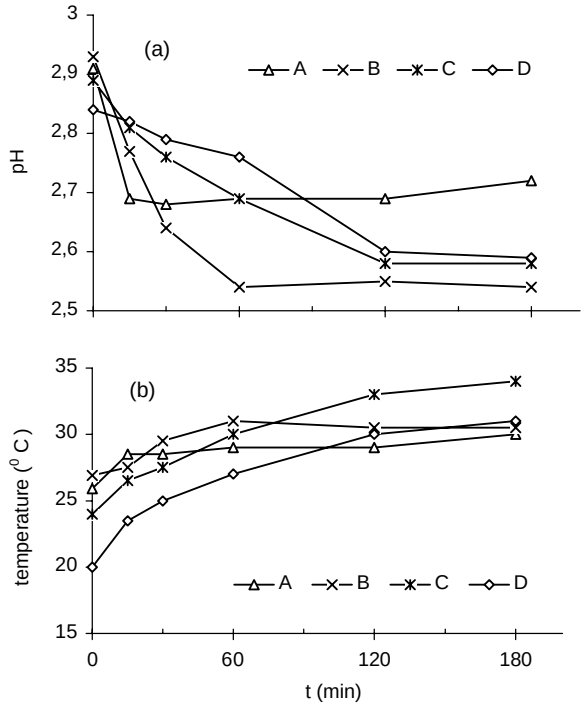

Fig. 3. The pH (a) and temperature (b) evolution during Fenton's reaction. All the treatments (A–D) carried out using 0.5 g/l Fe^{2+} and 2 g/l H_2O_2 (A), 4 g/l H_2O_2 (B), 6 g/l H_2O_2 (C), 8 g/l H_2O_2 (D).

0.857, $P < 0.001$) and its stabilization denoted the end of the reaction, making it suitable as a simple indicator of the progress of the Fenton system, which can be continuously and on-line monitored. The differences in the pH values observed among the treatments at $t = 0$ min are attributed to the spontaneous nature of the Fenton's reaction, especially at higher concentrations of H_2O_2. The expected increase in temperature was also observed, with final temperatures not exceeding 34 °C (Fig. 3b).

The yield of the Fenton's reaction was in terms of COD removal between 34 and 72% and in terms of total phenolic reduction between 64 and 91%, respectively for H_2O_2 concentrations ranging from 2 to 8 g/l (Table 2).

However, parallel inefficient decomposition routes may contribute to the wastage of H_2O_2 (i.e. $H_2O_2 \xrightarrow{\Delta H/\text{catalyst}} 1/2\,O_2 + H_2O$). For that reason, the efficiency coefficients were determined after 3 h of reaction, as ppm $COD_{removed}$/ppm $H_2O_{2\,(consumed)}$ or as ppm $phenol_{removed}$/ppm $H_2O_{2\,(consumed)}$ in the case of total phenolics (Fig. 4). As the H_2O_2 concentration increases, the efficiency of COD and phenols removal declines linearly and exponentially, respectively. This provides an indication that as the ratio of H_2O_2 to the organic matter increases the peroxide wastage routes become more prevalent and/or the remaining carbon is more resistant to oxidation. The efficiency coefficient of the phenol levels off at H_2O_2 concentrations above 6 g/l, denoting the higher selectivity of the Fenton's system for these compounds.

Despite the significant reduction of total phenolics during Fenton's oxidation, the sum of simple phenolic compounds

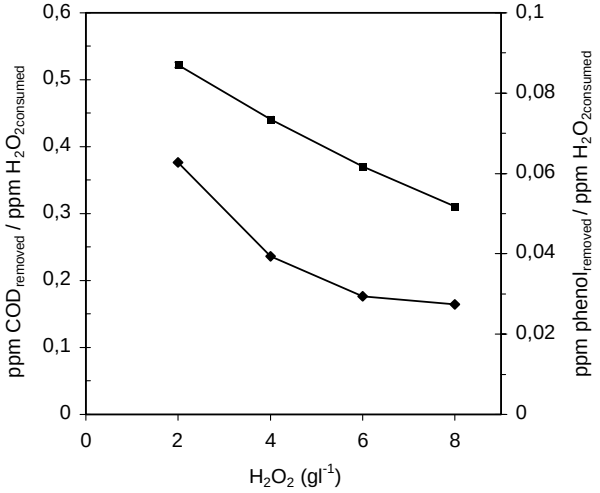

Fig. 4. Efficiency coefficients for the different H_2O_2 concentrations (treatments A–D) in COD (■) and phenolic (◆) removal.

practically remains unchanged, as some of them are declining while others are formed. Simultaneously, a variety of organic acids are produced. A similar behavior has been reported by Rivas et al. [23], who observed the generation of dihydroxybenzenes (catechol and hydroquinone mainly) through decarboxylation of *p*-hydroxybenzoic acid and the final accumulation of oxalic and maleic acid.

After chemical oxidation, COD removal was highly enhanced by coagulation with CaO (Table 3). Coagulation with CaO resulted in over 60% COD removal with the exception of treatment C, where COD removal was 38%, possibly because COD removal during chemical oxidation was already high. The final COD ranged from 370 to 580 ppm, rendering the effluent acceptable to municipal wastewater treatment plants in Greece, which typically have a limit of 1200 ppm COD. In contrast, coagulation with CaO of the control (wastewater after biological treatment only) was not very effective (29% COD removal). This observation offers a confirmation to the position that Fenton's oxidation is particularly suited as a pre-treatment for subsequent flocculation,

Table 3
COD after CaO sedimentation of the waste and CaO quantity necessary to elevate the pH of the waste up to 12

	Effluent (control)[a]	Treatments[b]			
		A	B	C	D
COD in (g/l)	2.90	1.90	1.16	0.83	0.96
COD out (g/l)	2.06	0.58	0.37	0.51	0.37
COD removal (%)	28.92	69.26	68.33	38.01	61.75
CaO (g/l)	2	3.4	4.4	5.8	5.1
Sludge (ml)/waste (l)	26	290	210	170	160

[a] Supernatant, after 2 h sedimentation of the effluent that was not subjected to Fenton's reaction.

[b] Supernatant, after 2 h sedimentation of the product of Fenton's reaction of treatments: 2 g/l H_2O_2 (A), 4 g/l H_2O_2 (B), 6 g/l H_2O_2 (C), and 8 g/l H_2O_2 (D).

improving removal efficiencies [25]. After the flocculation with CaO (Table 2), total phenolic removal ranged from 76 to 67% for treatments A–D respectively, while the value for the control was 73% indicating that coagulation with CaO, was very effective in phenols removal [26].

4. Conclusion

The aerobic biological treatment of TOPW with *A. niger* constitutes an effective method for the reduction of the organic and phenolic load of this type of wastewater. In this study, the biological treatment stage yielded a 70% COD reduction, 41% total phenolic reduction and 85% simple phenolic reduction, with a hydraulic retention time of 2 days. Biological treatment of industrial wastewaters of special composition, such as TOPW, requires a prolonged acclimatization period for the biomass, even if a pre-treatment stage precedes [2,8,27]. A significant advantage for the use of a selected species, such as the *A. niger*, for the biological treatment is the immediate initiation of the process, which may be very important in the case of seasonal production of large quantities of wastewater.

Addition of a subsequent step of chemical oxidation using Fenton's reaction allows the effective control of the organic load and phenols content of the final effluent, to achieve the levels required for disposal to the final receiver and meet the various regulatory restrictions. Control is achieved by adjusting the H_2O_2 concentration, relative to the COD load after the biological stage, while iron concentration will determine the time required for the completion of the reaction. Since Fenton's reagent corresponds stoichiometrically to the COD content, possibilities to improve the efficiency of the reaction are limited, although important economy in reagents use may be achieved in combination with the sedimentation process. On the other hand, improvement of the biological stage seems to be feasible and will result in a significant reduction of the required chemicals, improving the overall feasibility of the integrated process.

Acknowledgements

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