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Estimating the bioremediation of green table olive processing wastewater using a selected strain of *Aspergillus niger*

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ABSTRACT

Green table olive processing wastewater (TOPW) constitutes a notoriously polluting and difficult to treat wastewater, mainly due to its high polyphenol and organic content. This study reports on the laboratory development of an aerobic biological treatment method for TOPW, using a selected strain of *Aspergillus niger*. Two duplicated treatments from a single green table olive producing plant were examined in order to assess the bioremediation potential of the selected strain of *Aspergillus niger*. The wastewater arising from two different production processes was examined: (a) the typical debittering protocol, using dilute NaOH solution, and (b) an alternative protocol, using dilute KOH solution. Trials were carried out using cultures in flasks, and were monitored for changes in the pH values, electrical conductivity, oxygen uptake rate, chemical oxygen demand (COD), total solids, and total phenols, for 118 hours. A total of 5 dilutions (100%, 85%, 70%, 55%, and 40%) of wastewater were inoculated with *Aspergillus niger*. The COD removal efficiency varied in the range of 60–87% and 50–87% for the NaOH and KOH treatment, respectively. Substituting NaOH with KOH seems a promising option, as the latter wastewater may be beneficially added to the soil after the step of the biological treatment.

Keywords: Wastewater; Table olives; Spanish type olives; Biological treatment; *Aspergillus niger*

1. Introduction

Green and black table olive processing wastewaters (TOPW) constitute a major polluting factor mainly due to their high phenolic content and organic load. Specifically in the Mediterranean region, this factor could be transformed to a real threat since high volumes of the TOPW are seasonally produced and usually disposed untreated to streams or to the sea [1]. At the best case, they are stored in evaporation ponds [2], where anaerobic conditions are quickly established leading to malodors, breading of insects and risks of surface and groundwater contamination. This does not quite reflect the trend that is noticed to the scientific literature: plenty of papers study and suggest methodologies for the treatment of TOPW [3]. Certainly, the given situation does not comply with the current and the forthcoming environmental regulations.

Referring to green table olives (so-called "Spanish type"), the production processing involves crop selection, cleaning, debittering using dilute NaOH solution, washing, fermentation and canning. The largest and the most heavily polluted fraction of TOPW is produced through the debittering and washing stages. Up to date, various treatment methods have been evolved, such as aerobic and anaerobic biological processes, advanced oxidation

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processes, and the applicable combinations of them. Biological treatment methods have been introduced as overall economical and effective processes [4], but the composition of the TOPW (i.e., polyphenols) often inhibits the biodegradation ability of microorganisms and thus, reduces the biodegradation efficiency of the treatment. An alternative approach to the minimization of the polluting burden of TOPW is the substitution of NaOH with compounds beneficial to soil, such as KOH.

This study explores the laboratory development of a biological treatment method of the wastewater that derives from the debittering process of "Spanish type" green table olives, using a selected strain of *Aspergillus niger*. Two duplicated treatments from a single green table olive producing plant were examined in order to assess the bioremediation potential of the selected strain of *A. niger*, under aerobic conditions. The wastewater arising from two different production processes was examined: (a) the typical debittering protocol, using dilute NaOH solution, and (b) an alternative protocol, using dilute KOH solution, given that K is beneficial to soil while Na is known for its detrimental effect. Trials were carried out using cultures in flasks. Variations in the pH values, electrical conductivity, oxygen uptake rate, chemical oxygen demand (COD), total solids, and total phenols, for 118 h, were reported. In order to investigate the influence of the wastewater concentration to the fungi biodegradation ability a total of five dilutions (100%, 85%, 70%, 55%, 40%) were tested.

2. Materials and methods

2.1. Wastewater

Green table olive processing wastewaters (TOPW) consisted of fresh debittering wastewater and washing effluents as they arise in the industry of green table olive processing. The wastewaters originated from the Agricultural Cooperation of Rovies in Greece during the harvesting period of 2007. In particular, they were derived from two different production lines: one that followed the typical debittering process protocol, using diluted NaOH solution as a debittering agent (S), and another adjusted to an alternative protocol, whereby NaOH is replaced by KOH (P). TOPW from both lines were stored separately at –20 °C, within 24 h after sampling to avoid auto-oxidation and the subsequent polymerization of phenolic compounds and tannins [5,6], which would inhibit the biodegradation.

Table 1
Physical and chemical properties of the reacting wastewater solutions, before pH adjustment (COD_0: Chemical oxygen demand of the solution at 0 h. CODred: the % reduction of COD. Nomenclature: S stands for treatment with NaOH; P for treatment with KOH; The 2- or 3-digited number indicates the concentration of wastewater in the flask)

Experiment	COD_0 (g/l)	CODred (%) (±SD) 28 h	CODred (%) (±SD) 118 h	pH	E.C. (mS/cm)
S1-100	21.6	27.08 (± 6.87)	72.96 (± 4.70)	11.95	10.67
S1-85	17.6	40.15 (± 31.07)	69.38 (± 0.88)	12.15	9.92
S1-70	15.6	42.11 (± 0.00)	69.55 (± 4.57)	11.92	8.77
S1-55	11.2	42.86 (± 5.05)	61.43 (± 3.03)	11.50	7.42
S1-40	6.6	37.14 (± 6.00)	68.00 (± 4.78)	11.40	5.46
S2-100	14.5	74.65 (± 0.70)	77.49 (± 3.90)	12.09	9.09
S2-85	14.4	63.89 (± 2.36)	77.50 (± 5.89)	11.91	7.82
S2-70	7.9	74.67 (± 3.30)	90.23 (± 4.37)	11.73	–
S2-55	9.1	73.92 (± 2.17)	82.62 (± 0.15)	11.85	5.53
S2-40	4.9	59.83 (± 1.21)	69.66 (± 6.65)	11.71	4.03
P1-100	18.4	68.76 (± 6.15)	87.57 (± 0.49)	11.32	8.75
P1-85	12.0	52.71 (± 2.30)	71.17 (± 0.24)	11.24	7.69
P1-70	10.4	57.50 (± 1.36)	64.42 (± 1.36)	11.18	6.43
P1-55	7.9	59.56 (± 0.92)	77.57 (± 0.54)	11.21	5.23
P1-40	2.9	17.87 (± 1.37)	51.41 (± 0.99)	11.16	4.10
P2-100	14.5	47.03 (± 3.44)	73.11 (± 10.89)	11.55	9.01
P2-85	10.2	42.40 (± 11.88)	73.76 (± 0.00)	11.53	7.72
P2-70	9.5	48.33 (± 1.21)	81.71 (± 8.25)	11.50	6.52
P2-55	7.1	27.49 (± 0.00)	66.31 (± 1.1)	11.26	5.34
P2-40	5.8	48.48 (± 0.00)	76.85 (± 3.64)	11.28	3.88

Prior to the biological treatment, the pH value of the TOPW was adjusted to 4.6 (± 0.3) adding conc. H_2SO_4, in order to retain selectivity for the dominant proliferation of *A. niger.* The biological treatment of TOPW was conducted twice per processing line, here denoted by the symbols S1 & S2 for TOPW from the NaOH process and P1 and P2 for TOPW from the KOH process. In order to estimate the effect of the organic load and the suspended solids concentration on the performance of the biological treatment system, five duplicated dilutions (100%, 85%, 70%, 55% and 40%) of S1, S2, P1 and P2 were tested. Physical and chemical properties of the S1, S2, P1 and P2, before pH adjustment, are shown in Table 1.

2.2. Inoculum and aerobic biological treatment

TOPW was treated aerobically using a specific strain (B) of the acidophilic and acid producing filamentous fungi *A. niger* (Harokopio University collection) [7]. The inoculum preparation is explicitly described elsewhere [2,7]. The aerobic biological treatment of TOPW was carried out at a laboratory scale, in a non-sterile system using 2 l Erlnemeyer flasks containing 400 ml of acidified TOPW. The inoculated flasks were continuously horizontally shaken on a rotary incubator (180 rpm, at 25 °C) for 118 h. All cultures were performed duplicated. During the biological treatment, samples of approximately 10 ml were withdrawn from each flask at regular intervals (0, 6, 10, 22, 28, 34, 46, 70, 94 and 118 h), to analyze the biodegradation of the reacting media.

2.3. Analytical methods and calculations performed

Electrical conductivity (EC), pH values, COD, total suspended solids (TSS), and volatile suspended solids (VSS), were determined according to standard procedures [8]. The efficiency of the biological treatment was assessed in terms of COD reduction (CODred % = 100 × (COD_i – COD_t)/COD_i where COD_i: the COD value at 0 h and COD_t: the COD value at the indicated sampling time). The respiration rate, measured as oxygen consumption rate (OCR – mg O_2/l/h) was calculated from the rate of change in the dissolved oxygen concentration of the sample (YSI probe, model 5718, and meter, model 52CE). Total phenols (TP) were determined using the Folin–Ciocalteau method [9], results being expressed as mg/l of caffeic acid.

3. Results and discussion

As it was pronounced by the design of this work, two types of green TOPW were used to examine the effect of substituting NaOH with KOH on their biodegradability by *A. niger*, aiming at full-scale systems exploitation. Within this frame, factors relevant to the biological activity of *A. niger*, as well as biodegradation indices were monitored.

In general TOPW are characterized by alkaline pH values (11.0–12.5), as well as a high content of organic compounds such as sugars and phenolic compounds. In all treatments, the initial pH values were adjusted to 4.7 (± 0.3) in order to promote the preferential growth of *A. niger*. Alternatively, the favorable selectivity for the proliferation of *A. niger* could be achieved by wastewater sterilization. However, this option was rejected because it would not be economically feasible for full-scale systems. At this point, worth mentioning is the observation that the aforementioned pH adjustment with conc. H_2SO_4, resulted to an increase of suspended solids and decrease of COD values (Table 2). The rise in suspended solids values has been reported in previous studies [2] and seems to feature a systematic error in TS and VS measurements.

Biological activity was followed through pH variation and respiration rate (Fig. 1). In previous studies [7], the dependency of pH values on the acid production capacity of *A. niger* has been indicated. In the present study, independently of the trial, the pH values remained almost steady during the first 10–22 h of the treatment and then decreased (Fig. 1(a)). The invariability of pH during the first 10–22 h of the treatment could be attributed to the lag phase of the strain, which seems unaffected by both the wastewater concentration and the debittering treatment.

Respiration rate forms a global indicator of biological activity, regardless the type of the active microorganisms [10]. At Fig. 1b, the respiration rates (OCR) of the undiluted S1, S2, P1 and P2 are shown. Among all four treatments, the S1-100 sample exhibited the highest OCR. In terms of fungi proliferation interesting is that samples from NaOH processes exhibited the highest OCR value at 34 h, while samples from the KOH processing lines exhibited peak OCR values as early as at 22 h. However, OCR values were peaked within the temporal space of the growing phase of *A. niger* (10–34 h). Thereafter, an exponential decrease was noticed, followed by a slight fleeting increase. This increase could be taken as indication of a second phase of growth on substances produced by the biodegradation, but this claim calls for further investigation.

The maximum OCR value achieved for each culture flask was typical for batch cultures, demonstrating an initial phase of exponential indicator of the amount of the available substrate. Considering the concentration of the examined solution, the OCR values were decreasing following the amount of the dilution. Furthermore,

Table 2
Evolution of several parameters in experiments S1-100, S1-40, P1-100 and P1-40, taken as an example of the biodegradation process (nomenclature: S stands for treatment with NaOH; P for treatment with KOH; The two or three digits number following indicates the concentration of wastewater in the flask. Samples taken at –0.1 h were not pH adjusted)

Expt.	Time (h)	COD (g/l)	CODred (%) (± SD), 118 h	E.C. (mS/cm)	S.S. (g/l)	V.S.S. (g/l)
S1–100	–0.1	21.6	–	10.67	1.18	0.925
	0	17.3	19.83 (± 17.57)	11.40	2.86	2.300
	6	15.9	26.62 (± 0.98)	11.55	1.98	1.950
	10	15.1	30.02 (± 4.68)	11.61	2.64	2.013
	22	15.6	27.78 (± 2.62)	11.40	3.30	2.756
	28	15.8	27.08 (± 6.87)	11.52	3.64	2.744
	34	11.0	49.17 (± 10.61)	11.64	4.14	3.375
	46	8.8	59.07 (± 4.98)	12.17	4.16	3.569
	70	7.6	65.00 (± 3.40)	12.39	4.29	3.856
	94	6.9	67.96 (± 4.98)	12.07	5.18	4.175
	118	5.8	72.96 (± 4.71)	12.17	3.75	3.475
S1–40	–0.1	6.6	–	5.46	0.55	0.575
	0	5.3	20.96 (± 5,46)	5.71	1.36	1.206
	6	5.0	26.68 (± 30.89)	5.77	1.23	1.138
	10	–	–	5.46	1.42	1.256
	22	4.7	31.65 (± 13.76)	5.80	1.53	1.213
	28	4.3	37.14 (± 5.99)	5.59	1.70	1.513
	34	4.2	38.47 (± 6.14)	5.71	1.81	1.588
	46	3.1	–	5.70	1.87	1.731
	70	3.1	56.59 (± 0.72)	5.81	1.94	1.400
	94	2.6	–	5.77	2.02	1.694
	118	2.4	68.00 (± 4.78)	5.74	1.98	1.788
P1–100	–0.1	18.4	–	8.75	0.30	0.287
	0	9.9	46.20 (± 0.00)	9.30	1.93	1.319
	6	7.7	58.33 (± 0.98)	9.75	2.55	1.988
	10	6.2	66.10 (± 8.33)	9.52	2.08	1.488
	22	6.2	66.35 (± 6.39)	9.53	2.51	2.044
	28	5.7	68.76 (± 6.15)	9.63	2.36	1.963
	34	5.2	71.69 (± 10.24)	9.65	2.48	3.194
	46	5.0	72.98 (± 1.44)	9.90	2.64	2.188
	70	3.2	82.45 (± 5.61)	10.23	3.03	2.356
	94	4.0	78.20 (± 2.77)	9.63	3.27	2.663
	118	2.3	87.57 (± 0.49)	9.43	2.91	2.513
P1–40	–0.1	29.3	–	4.10	0.38	0.300
	0	22.7	22.5 (± 7.97)	4.27	0.86	1.525
	6	28.0	–	4.42	0.81	–
	10	23.7	19.28 (± 0.62)	4.30	0.74	0.669
	22	22.3	23.94 (± 7.97)	4.36	1.05	0.863
	28	24.1	17.87 (±1.37)	4.45	0.97	0.863
	34	19.4	33.86 (± 5.90)	4.50	1.04	1.469
	46	16.3	44.37 (± 2.99)	4.58	1.28	1.013
	70	14.1	52.11 (± 27.99)	4.76	1.28	1.153
	94	15.1	48.59 (± 18.92)	4.52	1.17	0.969
	118	14.3	51.59 (± 0.99)	4.55	1.30	1.206

a delay at the peak values was noted at the 40% solutions (results not shown), which could be attributed to the insufficient density of organic substances to feed the fungal population.

Referring to COD (Fig. 2), it should be noted that debittering solutions even when they are derived from the same processing plant during the same harvest period, are characterized of intense heterogeneity.

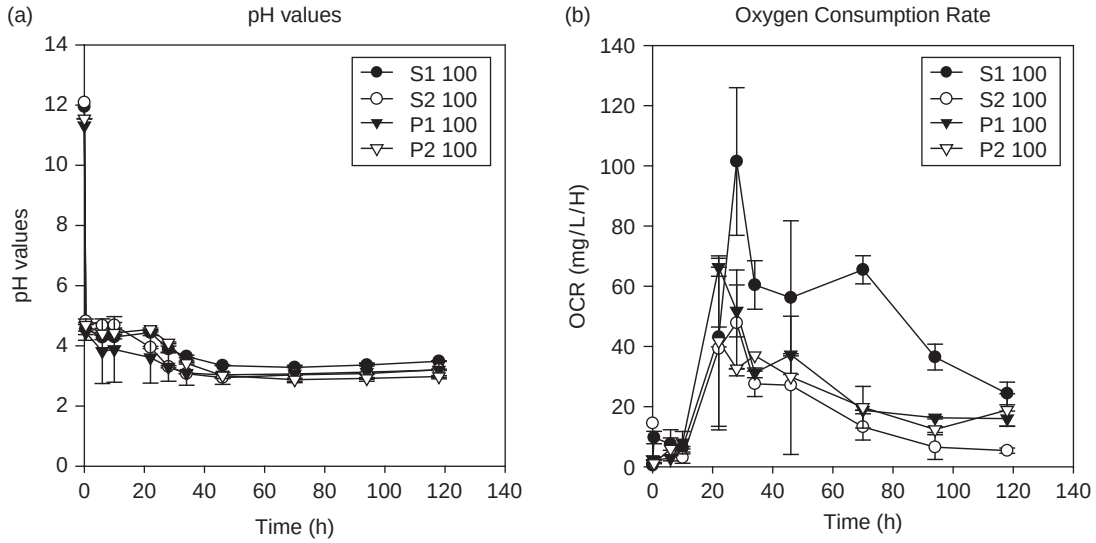

Fig. 1. Variation of (a) pH values; and (b) oxygen consumption rate, during the biological treatment of undiluted wastewater with *A. niger.* Nomenclature: S stands for treatment with NaOH; P for treatment with KOH.

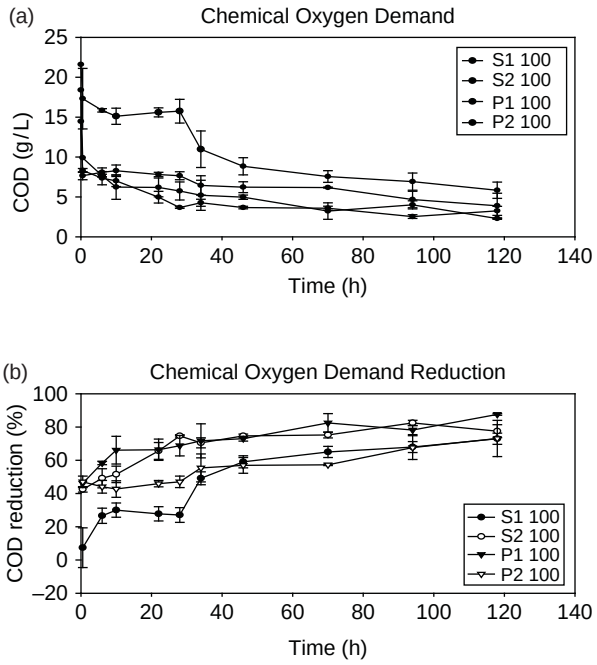

Fig. 2. Variation of (a) chemical oxygen demand; and (b) chemical oxygen demand reduction, during the biological treatment of undiluted wastewater with *A. niger.* Nomenclature: S stands for treatment with NaOH; P for treatment with KOH.

The values of COD of the S1 and S2 effluents showed an elevated standard deviation (S1-100: 21.6g/l; S2-100: 14, 5 g/l, St Deviation: 5.02). Still by estimating the COD reduction of all five dilutions (ranged from 60% to 87%), it can be deduced that even the 40% dilution showed an important organic load removal capacity. The initial COD of the wastewater for the KOH treatment ranged from about 3.0 to 18.4 g/l, with a COD removal efficiency varying from 50% to 87%. In spite of the variation in the initial COD values, it does not affect the COD reduction, which casts a parameter independent of the inherent variation of initial COD value.

Total phenolics (results not shown) increased slightly after acidification, which is in accordance with the findings of Lasaridi et al. [5]. During the biological treatment, they were decreased. The same pattern was noticed by Kotsou et al. [1].

4. Conclusions

A large fraction of the organic load was removed from TOPW through the biological treatment with *A. niger*. Despite of the high COD removal capacity (87% in some trials), the use of further treatment should be performed, in order to achieve the limits for acceptance in wastewater treatment plants (1.2 g/l). Substituting NaOH debittering solution with KOH, is proved to be promising and feasible approach, which could offer important advantages as it could allow utilization of the treated wastewater for irrigation purposes. The biodegradation of polyphenolics and their potential contribution to the inhibition of the biodegradation requires further investigation.

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