Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.812985

52 (2014) 6009–6015
September

Taylor & Francis
Taylor & Francis Group

Impact of cultivation conditions on olive mill wastewater pretreatment by means of *Aspergillus niger* van Tieghem

F. Hanafi[a,*], A. Belaoufi[a], M. Mountadar[b], O. Assobhei[a]

[a]*Laboratoire de Biotechnologies Marine et de l'Environnement, Faculté des Sciences, Université Chouaib Doukkali, B.P 20, El Jadida 24000, Morocco*
Tel. +212 523 373 254/212 663 351 889; email: hanafifatiha@yahoo.fr
[b]*Unité de Chimie Analytique et Sciences de l'Environnement, Faculté des Sciences, Université Chouaib Doukkali, El Jadida, Morocco*

Received 31 October 2012; Accepted 1 June 2013

ABSTRACT

An *Aspergillus niger* van Tieghem strain was used to treat olive mill wastewater (OMW) in an investigation aimed at exploring their dephenolization and discoloration ability and, consequently, the feasibility of using this strains in a pretreatment step in the processing of OMW. The results show that a peak COD reduction of 76% was reached at 28˚C after 8 days with the following conditions: 4.57 initial pH, 0.5 g/l inoculums size, 3.4 g/l $(NH_4)_2SO_4$, 1.2 g/l KH_2PO_4, and 2.0 g/l NaCl. We conclude that *A. niger* could be effectively used in the pretreatment step of a combined aerobic/anaerobic process to solve the environmental problems caused by OMW in Mediterranean countries.

Keywords: Aspergillus niger van Tieghem; OMW; Phenolic compounds; Pretreatment; COD

1. Introduction

Olive oil is a product of the Mediterranean region. The biggest olive oil producer worldwide is Spain, followed by Italy, Greece, and Turkey, as well as Tunisia, Portugal, Morocco, and Algeria. Also outside the Mediterranean area, olives are cultivated in the Middle East, the USA, Argentina, and Australia. As a result of the increase in olive trees plantation and hence the expansion in olive milling industries in Morocco, the amounts of generated wastewater augmented. OMW are dark liquid effluents, which pose a critical problem for olive oil-producing countries. The OMW basic characteristics are as follows: strong offensive smell; extremely high degree of organic pollution (COD values up to 220 g/l) and a COD/BOD_5 ratio between 2.5 and 5 (hardly degradable); pH between 3 and 5.9; high content of phenolic compounds (up to 80 g/l) which are not easily biodegradable and toxic to most micro-organisms and high content of solid matter (total solids up to 20 g/l). In terms of pollution effect, 1 m^3 of OMW is equivalent to 100–200 m^3 of domestic sewage. The phytotoxic effects as well as the antibacterial activity of OMW have been associated with the monocyclic phenolic components and tannins [1]. The reduction of the organic and phenolic load of OMW through the use of micro-organisms capable of growing in OMW has attracted considerable interest. The most strains used in OMW remediation were *Phanerochaete chrysosporium* [2], *Pleurotus ostreatus* [3–5], and Geotrichum *candidum*

*Corresponding author.

[6,7]. The cultivation periods of white-rot fungi have been as much as 25 days in biodegradation studies for initial total phenolic compound concentrations ranging between 0.8 and 6.8 g/l. *Aspergillus niger*, which has been used for some time now in citric acid production [8], possesses extracellular enzymes such cellulolytic, pectinolytic enzymes, xylanase [9], tannase, protease [10], and glucose oxydase [11]. *Aspergillus* strains have also been studied for their ability to biodegrade products in OMW—in particular, *A. niger* and *Aspergillus terreus*. *Aspergillus* spp. have shorter cultivation periods (2–7 days) than white-rot fungi [12,13].

The aims of this study were to explore the dephenolization and discoloration ability of *A. niger* van Tieghem strains, to identify the factors impact such as inoculums size, nutrient, NaCl, and pH on the fungus growth and efficiency of removing organic matter in the OMW and to propose a feasible pretreatment step for application in the processing of OMW.

2. Methods

2.1. Characteristics of olive mill wastewater

The OMW used was collected from local olive oil production plant (South Morocco). The typical composition and characteristics of OMW are shown in Table 1. The concentration of total phenolic compounds was 4.5 g/l. The OMW contained high organic loading and had a 28 g/l COD.

2.2. Inoculum preparation

Pure cultures of new strains were isolated from Moroccan pomace using the agar plate technique and classified by DSMZ (Braunschweig, Germany) as *A. niger* van Tieghem (DSM 24787). Fungi were maintained through periodic transfer at 4°C on potato-dextrose (2.4%) agar plates in the presence of 0.5% yeast extract. Cultures of *A. niger* were conducted in 250-ml Erlenmeyer flasks containing 50 ml sterilized stored OMW supplemented with 0.35% $(NH_4)_2SO_4$ and 0.065% KH_2PO_4 (w/v). The fungus was grown in the form of pellets.

Table 1
Typical composition and characteristics of OMW (all values, except pH, in mg/l)

Subjects	Value
Chemical oxygen demand (COD)	28,000 ± 237
Phenolics	4,500 ± 178
Suspended solids	2,300 ± 97
Orthophosphates	22.13 ± 0.3
Ammonium	32.07 ± 0.5
pH	4.57 ± 0.24

2.3. Cultivation conditions

Based on the cultural conditions, the parameters studied include initial pH, nitrogen, phosphorus, inoculum sizes, and NaCl levels. The inoculums size was carried on by addition of culture in the form of pellets at a concentration of 0.3, 0.4, 0.5, 0.6, and 0.9 g dry weights/l OMW. The nitrogen concentration was calculated by the addition of $(NH_4)_2SO_4$ at a concentration of 0.6, 1.4, 3.4, 7.0, and 14.0 g/l, whereas the phosphorus concentration was calculated by the addition of KH_2PO_4 at a concentration of 0.2, 0.6, 1.2, 2.0, and 3.0 g/l. The experiments for the salt concentration was carried out by the addition of NaCl at a concentration of 1.0, 2.0, 3.0, 4.0, and 5.0 g/l. Initial experiments for varying the pH were conducted at pH 2.0, 4.5, 6.0, and 8.5 adjusted by adding 2 M NaOH or HCl. All the experiments were conducted in 250-ml shake flasks containing 50 ml OMW. The medium was sterilized at 121°C for 15 min. The temperature of cultivation was set up at 28°C. Shaker speed was set up at 150 rpm.

2.4. Analytical methods

Growth represented by pellets dry weights obtained by filtration of the culture medium on glass microfibres (GF/A Whatman Inc.), and the biomass was washed twice with distilled water. The biomass evolution was estimated by measuring the dry weight after 24 h at 105°C. The analyses of pH, COD, and dark color were carried out according to standard methods [14]. The soluble COD was measured on the centrifuged OMW at $4000 \times g$ during 15 min. OMW discoloration was assayed by measurement of the absorbance at 395 nm using a cary 1E varian UV–vis spectrophotometer.

2.5. Phenolic analysis

Phenol (with respect to gallic acid) concentrations were determined spectrophotometrically according to the Folin–Ciocalteu method [15] using a cary 1E varian UV–vis spectrophotometer. Total phenol content was measured using the Folin–Ciocalteu's phenol reagent involving the successive additions of 5 ml sodium carbonate (200 g/l) and 2.5 mL Folin–Ciocalteu's phenol reagent to 50 mL of properly diluted sample. After 60 min at 20°C, the absorbance was measured at 725 nm against distilled water.

All fermentations were carried out in duplicate flasks, and the experimental results represent the mean of two identical fermentations. Reproducibility was satisfactory. The standard deviation of the duplicate data never exceeded 10% of the mean throughout this work. The tools used to examine and interpret the data consist of analysis of variance (ANOVA).

3. Results and discussion

In preliminary experiments, different strains have been isolated from OMW and pomace. Strain *A. niger* was selected for its ability to better grow in the acidified OMW (pH 4.5), to tolerate high phenolics content (4.5 g/l), and to their OMW-decolorizing potential. *A. niger* is an acid producing organism, therefore changes in the pH of the medium constitute a measure of its activity.

3.1. Inoculum concentration effect

A sufficient quantity of inoculum ensures rapid proliferation and biomass synthesis in cultivation. The influence of inoculums size on the growth of *A. niger* and efficiencies of the organic removal was investigated by varying the inoculums size at the range of 0.3–0.9 g dry wt/l (Fig. 1). The generated biomass was 2 g dry wt/l, when the inoculums concentration was 0.3 g dry wt/l, whereas for the other inoculums concentration ranged between 0.4 and 0.9 g dry wt/l, the generated biomass was ranged between 5.6 and 6.2 g dry wt/l, respectively. When the inoculums size increased, the pellet diameter decreased and pellets became smoother. The lower biomass yield at the low inoculums concentration might be related to low growth rate because of the nutrient and oxygen limitation inside large pellets. However, increasing the inoculums size above 0.5 g dry wt/l did not improve much biomass yield as well as efficiencies of the organic removal. An increase in inoculums size generally improves the growth and growth-related activities of the organism up to a certain level and with further increase in inoculums size, there could be a reduction in microbial activity due to nutrient limitations. Cultures with higher inoculums concentration were more efficient in dark color, phenolic compounds, and COD removal, which attained about 76% COD removal, for 0.5 g dry wt/l inoculums concentration after 4 days incubation, while no significant differences were observed after doubling this amount. Moreover, the pH reduction was more rapid for the high inoculums concentration, indicating a higher rate of growth and microbial activity. An inoculums size of 0.5 g dry wt/l was appropriate for *A. niger* growing in shake flasks and used for further studies.

3.2. Nutrients correction effect

A. niger van Tieghem strain was incubated with different concentrations of ammonium sulfate $(NH_4)_2SO_4$ and potassium phosphate $(KH_2)PO_4$ to determine the influence of nitrogen and phosphorus on biomass production and reduction of all parameters analyzed (color A395, phenolic compounds and

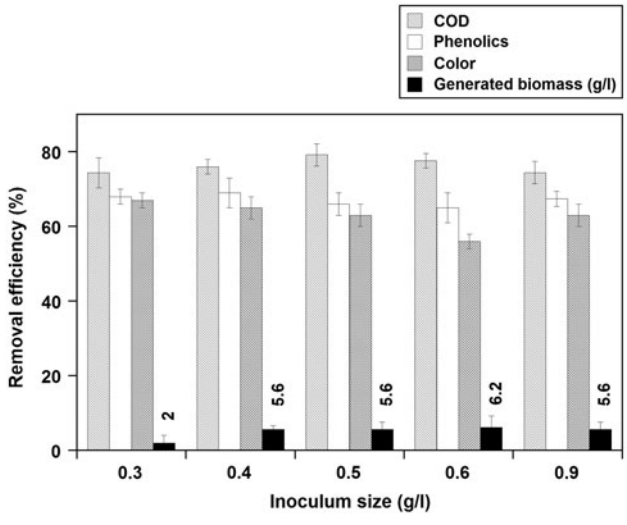

Fig. 1. Effect of inoculum size on reduction of COD, phenolics and color during *A. niger* growth on OMW. Data are the means of two parallel experiments ± standard deviation.

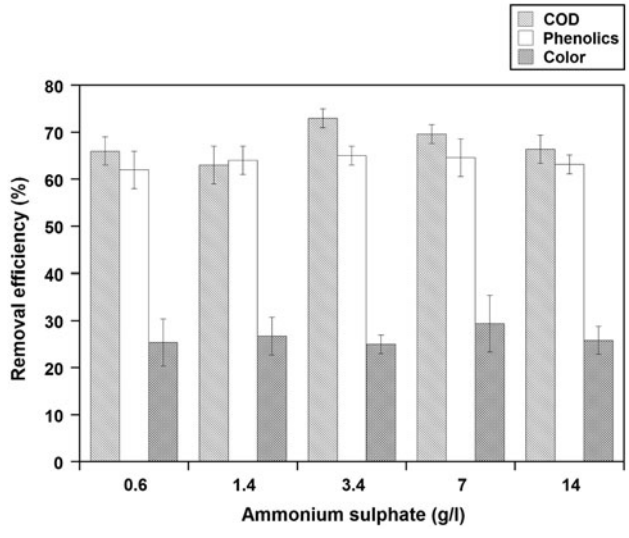

Fig. 2. Effect of nitrogen concentration on reduction of COD, phenolics and color during *A. niger* growth on OMW. Data are the means of two parallel experiments ± standard deviation.

COD). Phenolic compounds and COD removal efficiency increased with increase in ammonium sulfate concentration and leveled off beyond 3.4 g/l (Fig. 2). The COD removal percentages, using *Geotrichum* sp., *Aspergillus* sp. and *Coriolus tropicalis*, of the media containing only OMW was too low when compared with the removal percentages of reactors containing OMW, nitrogen, phosphorous, and sulfate [12]. Phenolic compounds and COD reduction was found to increase from 64 to 66% and 68 to 74.4%, respectively, when the phosphorus concentration was increased from 0.2 to 1.2 g/l and leveled off beyond that (Fig. 3). The maximum phenolic compounds and COD reduction was obtained at the ammonium sulfate and potassium phosphate concentration of 3.4 and 1.2 g/l, respectively, with a generated biomass of 4–4.4 g dry wt/l. In the cassava starch processing (CSP), wastewater characterized by the poorness in nitrogen sources, the supplementation of nitrogen sources improved significantly in the efficiency of biomass production. On the other hand, the supplementation of ammonium sulfate increased the efficiencies of *Aspergillus oryzae* for the elimination of organic matter in the artificial CSP wastewater [16]. Fadil et al. [12] using three micro-organisms (*Geotrichum* sp., *Aspergillus* sp. and *C. tropicalis*) for aerobic biodegradation of OMW indicated that nitrogen and phosphorous must be supplied at a corrected C/N/P ratio of 100/5/1 + $(NH_4)_2SO_4$ (1 g/l) to provide the best growth of micro-organisms and achieved the highest total phenol and COD removal value. The pH obtained at the end of the culture for all medium supplemented with nitrogen and phosphorus exceeded six indicating that surplus nitrogen or phosphorus exerts a depressive effect on citric acid production, probably by inducing fungal growth that uses citric acid for the production of energy and cellular constituents. Therefore, the discoloration was too low (25–35%) when compared with the removal percentages of the culture at low final pH.

3.3. Salt concentration effect

Considering the existence of salinity in OMW effluents, it is important to assess the biodegradation of OMW at high salt concentration by strain *A. niger*. Fig. 4 shows the effects of NaCl on the removal efficiency of dark color, phenolic compounds, and COD of OMW by *A. niger*. Under low NaCl concentration (0–2 g/l), discoloration rate of OMW was enhanced with increasing salinity. It was reported that the discoloration of Reactive Brilliant Blue by *Penicillium terrestre* was improved as salinity increased from 0 to 2% [17]. The possible reason was that the fungal pellets became smaller under higher NaCl concentrations, which produced more surface areas for adsorption. However, higher salinities (3–5 g/l) could inhibit removal efficiency of dark color, phenolic compounds, and COD, although there was still about 55% removal. It was probably because the solubility of some phenolic compounds decreases when the salt concentration increases [18]. Furthermore, the addition of sodium chloride had a negative effect on glucose oxidase activity and glucose conversion to gluconic acid [11]. Therefore, controlling appropriate salinity could obtain better OMW biodegradation efficiency.

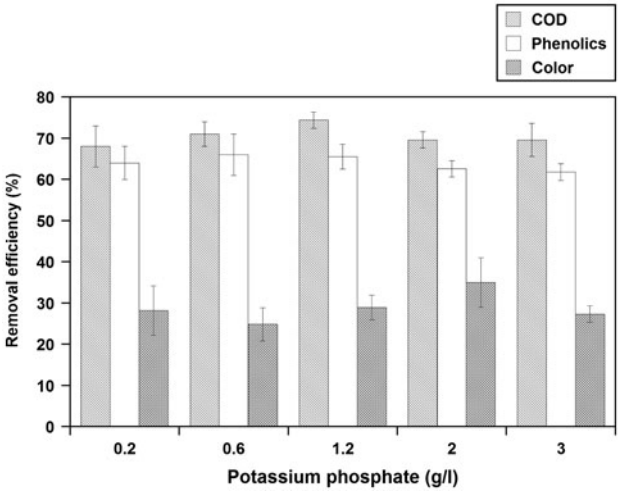

Fig. 3. Effect of phosphorus concentration on reduction of COD, phenolics and color during *A. niger* growth on OMW. Data are the means of two parallel experiments ± standard deviation.

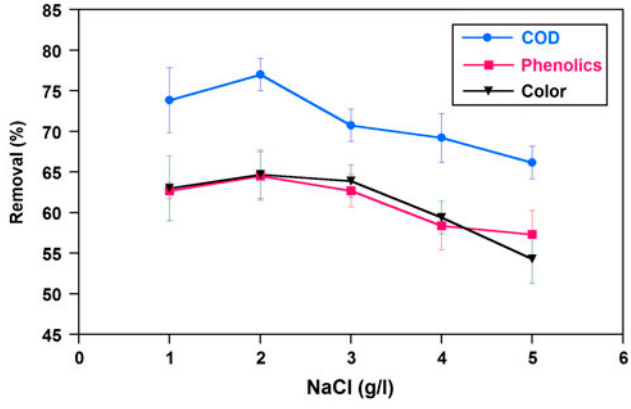

Fig. 4. Effect of varying concentrations of NaCl on reduction of COD, phenolics and color during *A. niger* growth on OMW. Data are the means of two parallel experiments ± standard deviation.

Table 2
Effect of initial pH on reduction of COD, phenolics and color during *A. niger* growth on OMW

Initial pH	COD (%)	Phenolics (%)	Color (%)	Final pH
2	60 ± 3.2	60.8 ± 1.9	66.9 ± 3.7	1.70 ± 0.21
4.5	64 ± 2.9	60.8 ± 3.6	64.6 ± 2.5	3.14 ± 0.15
6.1	66 ± 5.1	61.22 ± 2.4	64.4 ± 3.8	3.21 ± 0.17
8.55	58 ± 2.4	56 ± 1.6	45.86 ± 2.1	4.41 ± 0.44

Note: Values are means of duplicate ± standard deviation.

3.4. pH effect

The initial pH of any substrate is of physiological importance for growth and multiplication and viability as well as the metabolism of microorganisms. Accordingly, the variation of the pH for OMW biodegradation by *A. niger* strain was investigated at the pH values ranging between 2.0 and 8.5 at 28˚C for 4 days. The removal rate of dark color, phenolic compounds, and COD is presented in Table 2. Maximum COD and phenolic compounds reduction (64–66 and 60.8–61.22%, respectively) was obtained after 4 days at initial pH 4.5–6.0. The maximum biomass production was 4.2–4.4 g/l. During OMW biodegradation, the pH value was further reduced as a result of the activity of *A. niger*, a fungus known for the production of acidic metabolic products. Sharma et al. [19] studied the effect of pH on activity and stability of tannase produced by *A. niger* van Tieghem and reported that the enzyme had a broad pH profile with an optimum at pH 6.0 and a secondary peak at pH 4.5. Under initial pH in the range of 4.0–5.0 in the CSP wastewater, *A. oryzae* was satisfiable for the production of fungal biomass and the reduction of TOC (88–91%) and COD (84–86%) [16]. Highest COD reduction (66.10–79.75%) and biomass production (4.55 g/l) was recorded at pH 6 for treating potato chips industry wastewater using a mixed culture of *Aspergillus foetidus* and *A. niger* [20]. In our case, the removal efficiency of dark color increased with acidic pH. At initial pH 2, (the final pH was 1.7) the reduction of color was 67%, and the generated biomass was 3.8 g/l, while at initial pH 8.5, (the final pH was 4.4) the reduction of color was 45% and the generated biomass was 3.8 g/l. The low pH obtained at the end of the culture may be one of the major systems responsible for discoloration of this effluent. In fact, Qin et al. [21] noticed that the phenolics color of green tea (catechins) changed from light brown to dark brown with pH increases. Moreover, they are more stable and not oxidized in acid solutions. In fact, when the pH exceeds 6, phenolic compounds polymerize and give dark polymers which adsorb strongly to proteins [22].

3.5. Kinetics of biodegradation of OMW

A. niger van Tieghem have shown a specific pattern of COD and phenolics reduction and biomass production from OMW. With increase in time, there was increase in biomass and high reduction in COD and phenolics. Maximum COD and phenolics reduction (76 and 68%, respectively) was obtained in 192 h (8 days) and after this no significant increase in COD reduction and biomass was observed (Fig. 5). The initial pH value of the OMW was 4.57 and was further reduced approximately to 2.5 as a result of the activity of *A. niger*, a fungus known for the production of acidic metabolic products. A correlation was found between color removal and the reduced pH, maximum discoloration was attained in 4 days (Fig. 6) with concomitant reduction in phenolic compounds. The color and phenol reductions could be due to secretion of phenol-oxidizing enzymes, to an acidic hydrolysis and/or to acidic precipitation of phenolic compounds [23], [3], [24]. Ergül et al. [25] showed that after 16 days incubation, the adapted *Trametes versicolor* was able to remove 78% of total phenolics in shake flask experiments and 39% in static culture using undiluted OMW medium with 5.2 g/l as initial phenolic content. Asses et al. [26] reported that *G. candidum* needed 7-day incubation period for removing 60% COD and

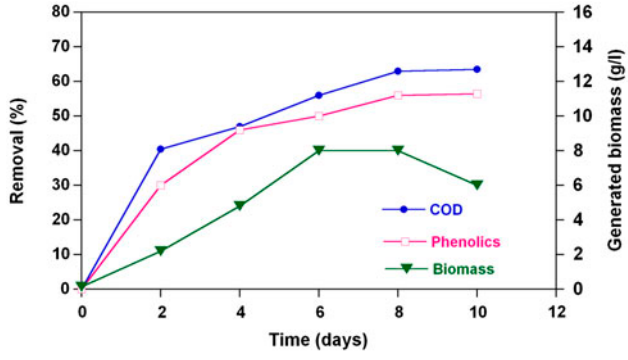

Fig. 5. Time course of phenolics, chemical oxygen demand reductions and generated biomass in OMW treated with *A. niger*.

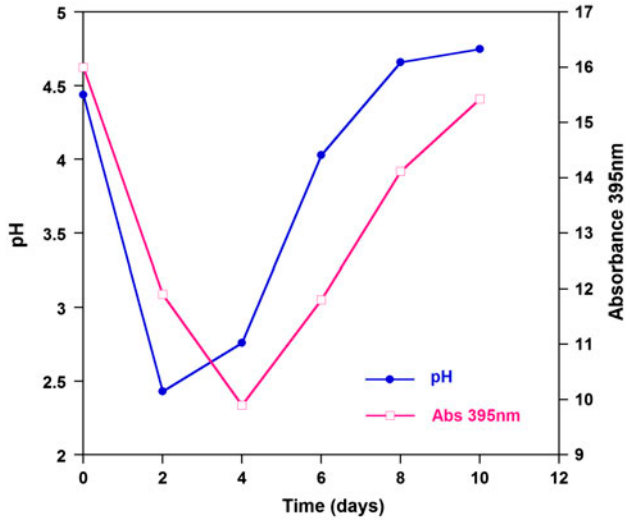

Fig. 6. Time course of change in pH and discoloration (absorbance at 395 nm) of OMW treated with *A. niger* van Tieghem.

50% phenolic content from OMW. This high removal efficiency could be attributed to both degradation and conversion of the phenolic compounds. In fact, the phenolics concentrations did not inhibit the microorganism, and the degradation time was enough to allow the fungus to decolorise OMW.

4. Conclusion

In most biological systems, nutrient availability is extremely important in determining good growth of microorganisms. Additional supply of the nutrients to the OMW as substrates will help to stabilize and enhance the fungal growth, synthesis of intracellular and extracellular proteins and enzymes and subsequently purifying the effluent. Supplementation of the nutrients especially the nitrogen sources will enhance the fungal growth cycle. *A. niger* van Tieghem is very efficient in reduction of organic and phenolic load if the OMW is supplemented with additional nitrogen and phosphorus sources. The results of this study confirmed the potential application of the *A. niger* van Tieghem for OMW treatment. The best conditions found was as follows: 4.57 initial pH, 0.5 g/l inoculums size, 3.4 g/l $(NH_4)_2SO_4$, 1.2 g/l KH_2PO_4 and 2.0 g/l NaCl. The fungus seems to be highly promising for development of effective pretreatment process for OMW effluents that contain high phenolic compounds.

References

[1] A. D´Annibale, M. Ricci, D. Quaratino, F. Federici, M. Fenice, *Panus tigrinus* efficiently removes phenols, color and organic load from olive-mill wastewater, Res. Microbiol. 155 (2004) 596–603.

[2] M.S.Y. Haddadin, J. Haddadin, O.I. Arabiyat, B. Hattar, Biological conversion of olive pomace into compost by using *Trichoderma harzianum* and *Phanerochaete chrysosporium*, Bioresour. Technol. 100 (2009) 4773–4782.

[3] G. Aggelis, D. Iconomou, M. Christou, D. Bokas, S. Kotzailias, G. Christou, Phenolic removal in a model olive oil mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process, Water Resour. 37 (2003) 3897–3904.

[4] G. Olivieri, A. Marzocchella, P. Salatino, P. Giardina, G. Cennamo, G. Sannia, Olive mill wastewater remediation by means of *Pleurotus ostreatus*, Biochem. Eng. J. 31 (2006) 180–187.

[5] E. Kalmis, N. Azbar, H. Yildiz, F. Kalyoncu, Feasibility of using olive mill effluent (OME) as a wetting agent during the cultivation of oyster mushroom, *Pleurotus ostreatus*, on wheat straw, Bioresour. Technol. 99 (2008) 164–169.

[6] L. Ayed, N. Assas, S. Sayadi, M. Hamdi, Involvement of lignin peroxidase in the discoloration of black olive mill wastewaters by *Geotrichum candidum*, Letters App. Microbiol. 40 (2005) 7–11.

[7] N. Assas, L. Ayed, H. Bouallagui, S. Sayadi, M. Hamdi, Biodegradation of different molecular-mass phenolic compounds derived from olive mill wastewaters by Geotrichum candidum, Int. Biodeterior. Biodegrad. 63 (2009) 407–413.

[8] I. Haq, A. Sikander, M.A. Qadeer, I. Javed, Citric acid production by selected mutants of *Aspergillus niger* from cane molasses, Bioresour. Technol. 93 (2004) 125–130.

[9] R. Pyć, J. Sójka-Ledakowicz, H. Bratkowska, Biosynthesis of enzymes by *Aspergillus niger* IBT-90 and an evaluation of their application in textile technologies, Fibre. Text. Eastern Eur. 11(4) (2003) 43.

[10] M. Cruz-Hernández, C. Augur, R. Rodríguez, R. Contreras-Esquivel, C.N. Aguilar, Evaluation of culture conditions for Tannase production by *Aspergillus niger* GH1, Food Technol. Biotechnol. 44 (2006) 541–544.

[11] L. Jianguo, C. Zhanfeng, Optimization of operating conditions for glucose oxidation in an enzymatic membrane bioreactor, J. Membr. Sci. 302 (2007) 180–187.

[12] A. Fadil, A. Chahlaoui, A. Ouahbi, A. Zaid, R. Borja, Aerobic biodegradation and detoxification of wastewaters from the olive oil industry, Int. Biodeterior. Biodegrad. 51 (2003) 37–41.

[13] H. Aissam, F. Errachidi, M.J. Penninckx, M. Merzouki, M. Benlemlih, Production of tannase by *Aspergillus niger* HA37 growing on tannic acid and olive mill waste waters, J. Microbiol. Biotechnol. 21 (2005) 609–614.

[14] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.

[15] F. Hanafi, M. Mountadar, O. Assobhei. Combined electrocoagulation and fungal processes for the treatment of olive mill wastewater. Fourteenth International Water Technology Conference, IWTC 14 (2010), Cairo, Egypt.

[16] Q.T. Truong, N. Miyata, K. Iwahori, Growth of *Aspergillus oryzae* during treatment of cassava starch processing wastewater with high content of suspended solids, J. Biosci. Bioeng. 97 (2004) 329–335.

[17] B.P. Xin, Q.M. Zou, Y.Y. Zhuang, Z.H. Jin, G.C. Hu, W.H. Song, Biosorption and discoloration of reactive brilliant blue KN-R by a specific strain GX2, Acta Sci. Circumst. 20 (2000) 97–102.

[18] A. Noubigh, M. Abderrabba, E. Provost, Temperature and salt addition effects on the solubility behaviour of some phenolic compounds in water, J. Chem. Thermodyn. 39 (2007) (2009) 297–303.

[19] S. Sharma, T.K. Bhat, R.K. Dawra, Isolation, purification and properties of tannase from *Aspergillus niger* van Tieghem. W, J. Microbiol. Biotechnol. 15 (1999) 673–677.

[20] B.K. Mishra, A. Arora, G.G. Lata, Optimization of a biological process for treating potato chips industry wastewater using a mixed culture of *Aspergillus foetidus* and *Aspergillus niger*, Bioresour. Technol. 94 (2004) 9–12.
[21] Y.Z. Qin, Z. Anqi, T. David, H. Yu, C. Zhen-Yu, Stability of green tea catechins, J. Food Chem. 45 (1997) 4624–4628.
[22] J.A. Field, G. Lettinga, Treatment and detoxification of aqueous spruce bark extracts by *Aspergillus niger*, Water. Sci. Technol. 24 (1991) 127–137.
[23] S. Crognale, A. D'Annibale, F. Federici, M. Fenice, D. Quaratino, M. Petruccioli, Olive oil mill wastewater valorisation by fungi, J. Chem. Technol. Biotechnol. 81 (2006) 1547–1555.
[24] F. Hanafi, A. Belaoufi, M. Mountadar, O. Assobhei, Augmentation of biodegradability of olive mill wastewater by electrochemical pre-treatment: Effect on phytotoxicity and operating cost, J. Hazard. Mater. 190 (2011) 94–99.
[25] F.E. Ergül, S. Sargın, G. Öngen, F.V. Sukan, Dephenolisation of olive mill wastewater using adapted *Trametes versicolor*, Int. Biodeterior. Biodegrad. 63 (2009) 1–6.
[26] N. Assas, L. Ayed, H. Bouallagui, I. Ben Rejeb, M. Gargouri, M. Hamdi, Use of *Geotrichum candidum* for olive mill wastewater treatment in submerged and static culture, Bioresour. Technol. 100 (2009) 2182–2188.