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Improvement of green table olive processing wastewater decolorization by *Geotrichum candidum*

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ABSTRACT

In the current paper, olive processing wastewater (TOPW) decolorization by *Geotrichum candidum* was investigated. For environmental factor optimization, a 2^3 factorial experimental design was employed, wherein three factors, namely glucose, diammonium tartrate, and pH were varied simultaneously. Then, the interaction between the factors was analyzed using MINITAB 16 statistical software. Glucose and diammonium tartrate and their interactive effect influenced the decolorization yield. Regression models were developed to study the interaction between color removal variables. The effect of various carbon (glucose, glycerol and lactic acid) and nitrogen sources (diammonium tartrate, ammonium sulfate, ammonium nitrate and yeast extract) on the decolorization was further determined. *G. candidum* showed 63% TOPW decolorization with optimized medium containing glucose (5 g/L) and yeast extract (5 mM nitrogen) at pH 6, with significant reduction of phenolic compounds (60%) and COD (71%). HPLC and FTIR study suggests that decolorization can be attributed to adsorption to biomass and to certain phenolic compound biodegradation. Manganese peroxidase (MnP) and lignin-peroxidase (LiP) are the two enzymes responsible for the TOPW decolorization. With optimized culture conditions, *G. candidum* had maximum LiP and MnP activities of 58.4 and 78 U/L respectively.

Keywords: *Geotrichum candidum*; Table olive processing wastewater; Decolorization; Lignin peroxidase; Manganese peroxidase

1. Introduction

Table olives have been always the traditional fermented vegetable of the Mediterranean countries. In fact, the most significant industrial preparations are the green Spanish style, with about 60% of the production [1]. The procedure of table olives preparation consists in treating the fruits with dilute NaOH solution to hydrolyze their natural bitterness (oleuropein), followed by one or two water washes to remove the alkali excess and, finally, a spontaneous lactic acid fermentation in brine for several months [2].

Table olive processing wastewater (TOPW) consists of many complex substances, which represent a serious ecological problem in the Mediterranean countries [3]. The main organic constituents of TOPW are sugars and phenolic compounds, particularly tyrosol, hydroxytyrosol, ferulic acid, 4-hydroxycinnamic acid and caffeic acid; some nitrogenous compounds, organic acids,

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tannins, pectin and oil residues compounds [3,4]. The inorganic fraction consists of both high concentrations of sodium chloride and sodium hydroxide, which are used for olive debittering and fermentation, as well as trace amounts of various metals [3–5]. TOPW's phytotoxicity is due to the phenolic substances, which confer a sharp characteristic odor and make TOPW toxic and resistant to biodegradation. Environmental contamination problems can be resolved thanks to numerous technologies.

Currently, the TOPW decolorization is based mainly on chemical methods, which use a strong oxidative agent, such as ozone and Fenton's reagent [6,7]. However, the implementation of these methods has some disadvantages such as high cost, hazardous by-products formation, and exhaustive energy consumption [8].

The biological treatments have proved to be sustainable, non-hazardous, safe, and environment-friendly methods [9]. In fact, biodegradation can take place under both aerobic and anaerobic conditions. Anaerobic biological treatment is a promising alternative; however, the effectiveness of this technology is not always satisfactory as some compounds such as phenolics and tannins may contribute to the inhibition of methanogenic bacteria [10–13]. Borja et al. [14] showed that the toxic effects were related to phenolic compound structure and that the inhibitory impact was greater for the ortho-diphenols than for their corresponding monophenolic compounds. On the other hand, aerobic processes may reduce the wastewater toxicity and improve the biodegradability in anaerobic digestion [10].

Several fungal species have proved to be effective for the aerobic wastewater treatment under controlled operating conditions [15]. Previous studies suggest that nutrient supplementation enhances biodegradation by increasing microbial biomass [16,17]. However, biodegradation which is based on cultivation of filamentous fungi requires long-lasting fermentation. Besides, their application on a large scale was limited by the difficulty of achieving continuous culture because of the formation of filamentous pellets and mycelia. To overcome this limitation, the use of yeasts could be a promising way. Moreover, yeasts are resistant to high concentrations of phenols [18]. *Geotrichum candidum* has been reported to efficiently decolorize various wastewaters [19–22]. *G. candidum* decolorization ability is essentially attributed to ligninolytic enzymes [22,23].

For optimization of environmental factors affecting decolorization, a 2^3 factorial experimental design is used, in which three factors were varied simultaneously. The advantage of factorial experiments was not only to study the effect of individual factors but also to study the combined effect of more than one factor and the interaction between them [24].

The major objective of this study is to investigate, for the first time, the effects of various environmental factors on TOPW decolorization efficiency by *G. candidum*. A 2^3 full factorial design was used to evaluate the joint effect of three selected parameters, namely initial pH, glucose, and diammonium tartrate concentrations on color removal. To this end, we have examined the effect of different carbon and nitrogen sources, and the enzymes involved in the decolorization process.

2. Material and methods

2.1. Used wastewaters

The wastewater consisted in debittering and washing water resulting from green TOPW. Fresh TOPW has been stored at −20°C to avoid the auto-oxidation of phenolic compounds (Table 1).

2.2. The biological material and culture procedures

G. candidum was isolated from olive mill wastewater [21]. *G. candidum* was maintained at 4°C on potato dextrose agar plates. Arthroconidia of *G. candidum* were inoculated on PDA containing 20% TOPW. After 4 d at 30°C, the surface of the slant was covered with white arthroconidia. Arthroconidia were then suspended in sterile distilled water and used as inoculums.

TOPW was adjusted to pH 6 by using concentrated HCl and sterilized by autoclaving. Experiments were carried out in 500 ml conical flask containing 90 ml of

Table 1
Main characteristics of fresh TOPW

Characteristics	Fresh TOPW
pH	15.9 ± 0.9
TSS (g/L)	1.14 ± 0.3
CODs (g/L)	15.4 ± 2.4
Color (OD 390 nm)	2.54 ± 0.2
Reducing sugars (g/L)	3.0 ± 0.2
TKN (g/L)	0.66 ± 0.1
TP (mg gallic acid/L)	488.5 ± 22

Note: Data are reported as mean ± standard deviation of results carried out in triplicate.

TS: total solids; TSS: total suspended solids; CODs: soluble chemical oxygen demand; TKN: total Kjeldahl nitrogen; TP: total phenolic compounds.

the TOPW. After inoculation with 10 ml of arthroconidia suspension (10^7 arthroconidia/mL), cultures were placed in a rotary shaker at 150 rpm and 30°C for 6 d.

Then, the effect of varying the concentration and the nature of carbon (0, 2, 5, 7, 10 g/L) and nature of ammonium sources on the TOPW decolorization were studied. In each experiment only one factor, has changed while the other factors remain constant.

2.3. The factorial design

A 2^3 factorial design was adopted to investigate the effects of three factors on TOPW decolorization: glucose (A), pH (B), and diammonium tartrate (C) each at two levels. The factor low and high levels were selected according to some preliminary experiments.

Batch experiment was conducted in a series of 500 ml conical flasks containing 90 ml TOPW supplemented with carbon and nitrogen source. 10 mL of inoculum was transferred into each flask and incubated in a rotary incubator shaker at 150 rpm for 6 d.

The factorial design and the decolorization measured in each factorial experiment are shown in Table 2. The decolorization was determined as average of three parallel experiments.

The impact of each factor on response was evaluated by the determination of b_i relating to each of the three factors and their interactions are given by the following equation:

$$b_i = 1/8(\Sigma A_i X_i) \quad (1)$$

A_i means either high (+) or low (−) level in experimental run i, X_i is the TOPW decolorization (%).

The behavior of the system is explained by the following equation:

$$Y = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7ABC \quad (2)$$

where b_0 represents the global mean and b_i represents the regression coefficient corresponding to the main factors effects and interactions.

The results of experimental design were studied and interpreted by MINITAB 16 statistical software to estimate the response of dependent variable (% color removal).

2.4. Analytical methods

The growth was measured by absorbance at 600 nm using a spectrophotometer 63200 UV/vis (Jenway, Essex, UK). Chemical analyses were carried out in triplicate according to standard methods [25].

2.5. Decolorization assay

The supernatants of the culture were adjusted to pH 6.0 by concentrated HCl, and the OD at 390 nm was measured against distilled water using a spectrophotometer 63200 UV/vis (Jenway, Essex, UK).

2.6. HPLC analysis

A reversed-phase high performance liquid chromatographic technique has been developed to identify and quantify the major phenolic compounds contained in the ethyl acetate extracts of fresh and treated TOPW by *G. candidum*. The HPLC chromatograph was performed on a Shimadzu apparatus (Shimadzu Italy, Milan) composed of a LC-10 ATVP pump and a SPD-10 AVP detector. Elutes were detected at 280 nm. The column was (4.6 × 250 mm) model Shimpach

Table 2
2^3 full factorial design with observed response values for TOPW decolorization by *G. candidum* after 6 d of culture

Experiments		Glucose (g/L)	pH	Diammonium tartrate (mM nitrogen)	
Low level		0	6	5	
High level		2	7	20	Color removal (%)
1	+	−	−	−	35 ± 1.5
2	+	+	−	−	61 ± 2.2
3	+	−	+	−	39 ± 3.2
4	+	+	+	−	56 ± 1.7
5	+	−	−	+	42 ± 1.5
6	+	+	−	+	35 ± 4.5
7	+	−	+	+	42 ± 3.3
8	+	+	+	+	28 ± 3.1

VP-ODS and its temperature was maintained at 40˚C. The flow rate was 0.5 mL/min. The mobile phase used was 0.1% phosphoric acid in water (*A*) vs. 70% acetonitrile in water (*B*) for a total running time of 40 min. The gradient was charged as follows: solvent *B* started at 20% and increased immediately to 50% in 30 min. After that, elution was conducted in the isocratic mode with 50% solvent *B* within 5 min. Finally, solvent *B* decreased to 20% until the end of running [22].

2.7. Fourier transform infra-red (FTIR) spectroscopy

A quantity of 2 mg of sample was compressed under vacuum with 250 mg of KBr. The pellets obtained were analyzed with an Equinox 55 (Bruker, Wissembourg, France) FTIR spectrophotometer equipped with Attenuated Total Reflectance (ATR) accessory. Spectra were recorded from 600 to 4,000 cm^{-1}, at a rate of 16 nm/s. Spectra were analyzed with Opus NT 3.1 software (Bruker) [26].

2.8. Enzyme assays

Supernatants from culture medium were analyzed for lignin-peroxidase (LiP) and manganese peroxidase (MnP) activities.

LiP activity has been determined colorimetrically with the method of Tien and Kirk [27] by measuring the absorbance increase at 310 nm (ε_{310} = 9,300/M cm). The reaction mixture (2 mL) containing 4 mM veratryl alcohol in 10 mM sodium tartarate buffer (pH 3) incubated with 100 µl of the culture at 30˚C. The reaction was initiated by addition of the suitable amount of 0.2 mM H_2O_2. The blanks contained buffer in place of veratryl alcohol. One unit of LiP activity was defined as the amount of enzymes catalyzing the formation of 1 µmol of veratraldehyde per minute under the assay conditions.

MnP activity has been measured by monitoring the oxidation of 1 mM $MnSO_4$ in 50 mM sodium malonate buffer (pH 4.5) in the presence of 0.1 mM H_2O_2 [28], via the increase in absorbance at 468 nm (ε_{468} = 49.600/M cm). One unit of MnP activity was defined as the amount of enzyme required to produce an absorbance increase of one per minute per milliliter of the reaction mixture. The blanks contained all reagents except $MnSO_4$.

2.9. Statistical analysis

All data presented are the average of triplicate measurements ± S.D. A variance analysis (ANOVA) has been conducted by using performed SPSS version 16.0 software (SPSS Inc., Chicago, IL). The statistical software used to evaluate the experimental design results was Minitab 16 (Minitab Inc., PA, USA). Effects were considered significant when the *p*-value was less than 0.05.

3. Results and discussion

3.1. Determination of factors affecting TOPW decolorization by G. candidum

In a biological decolorization process, there are various influencing parameters. They are mainly related to microbial growth conditions and to the wastewater characters. Hence, to achieve maximum TOPW decolorization by *G. candidum*, three factors: pH, glucose, and diammonium tartrate were tested using 2^3 factorial designs.

The results are displayed in Table 2. The estimated effects, coefficients, and standard deviation of each coefficient and probability for the full 2^3 factorial designs are presented in Table 3. The regression coefficient significance was determined by applying a Student's *t*-test. The *p*-values were used as a tool to check the significance of each of the interactions between the variables. In other words, the coefficient term is significant, if *T*-value is larger and *p*-value is smaller [29]. From the coefficient of individual variables, it is proved that the increase in the glucose concentration (*A*) and the decrease in diammonium tartrate concentration (*C*) increased the decolorization. The effect of the concentration of diammonium (*C*) and glucose concentration (*A*) was found to be highly significant (p = 0.001, 0.002 respectively) on decolorization. pH with *p*-value > 0.05 is considered insignificant.

The decolorization yield (*Y*) can be predicted using the coefficients given in Table 3:

$$Y = 43.5 + 4.875A + 0.5B - 6.37C - 0.25AB - 2.05AC - 0.875BC + 0.25ABC \quad (3)$$

Previous studies have shown that carbon and nitrogen sources are essential for decolorization [22–30]. The increase in glucose level has shown a positive effect on decolorization. This result confirms the necessity of glucose addition as an energy source. In fact, the fungus consumes and grows on readily available carbon sources at the initial stages of growth and then produces secondary metabolites and extracellular enzymes for biodegradation of phenolic compounds at an appropriate concentration of carbon [19,22,31]. Kim

Table 3
Estimated Effects and Coefficients, *T*, *P* and standard deviation for TOPW decolorization

Term	Effect	Coef	SE Coef	*T*	*P*
Constant		43.50	1.035	42.05	0.000
Glucose (*A*)	9.77	4.875	1.035	4.71	0.002
pH (*B*)	1.00	0.5	1.035	0.48	0.642
Diammonium tartrate (*C*)	−12.37	−6.37	1.035	−6.16	0.001
Glucose * pH (*AB*)	−0.5	−0.25	1.035	−0.19	0.854
Diammonium tartrate * Glucose (*AC*)	−12.75	−2.05	1.035	−9.12	0.000
Diammonium tartrate * pH (*BC*)	−1.75	−0.875	1.035	−0.85	0.422
Glucose*diammonium tartrate * pH (*ABC*)	0.5	0.25	1.035	0.55	0.598

Note: $S = 3.09233$; $R^2 = 96.84\%$; $R^2_{adj} = 94.08\%$.

and Shoda [19] showed that a supply of sugars allows the maintenance of the *G. candidum decolorization activity*.

The *G. candidum* decolorization activity was stimulated at low diammonium tartrate concentration, suggesting nitrogen repression of some part of the *Geotrichum* enzymatic system. Kim and Shoda [19] reported that in presence of 0.5 g/L of ammonium tartrate, the dye decolorization using *G. candidum* was higher than those in presence of 2 g/L, and showed that the higher nitrogen concentration caused dye adsorption onto the fungus surface. Ikehata et al. [32] showed that the peroxidase enzyme production by *Phanerochaete chrysosporium* was promoted when the nitrogen concentration was limited.

The interactive effect of glucose concentration and diammonium tartrate concentration (*AC*) was found also significant ($p < 0.01$). However, the interactive effect of glucose concentration and pH, and diammonium tartrate concentration and pH was found to be insignificant.

In order to develop the regression model that is statistically significant insignificant terms in the Eq. (3) are eliminated. The combined factors (*AB*), (*CB*), and (*ABC*) are insignificant ($p > 0.05$) and hence removed. As a result, the statistically significant regression model obtained is:

$$Y = 43.5 + 4.875A - 6.37C - 2.05AC \quad (4)$$

3.2. Effect of different carbon sources on TOPW decolorization

In order, to find the most suitable carbon source, glucose, glycerol, and lactic acid were used in decolorization experiments. The color removals are listed in Table 4. When *G. candidum* was incubated with TOPW without carbon source, the decolorization was limited when compared to the media supplemented with the carbon sources. However, color removal has not been as effective as that obtained with glucose when glycerol and lactic acid were used as the carbon source.

Glucose provided the highest decolorization, especially at 5 g/L (63%). Swamy and Ramsay [33] showed that Amaranth decoloration by *Trametes versicolor* required glucose and that glucose-2-oxidase may be a major source of H_2O_2. On the other hand, a gradual increase of glucose concentration led to the decrease of color removal because glucose is used more easily as a carbon source than phenolic compounds [34]. For glycerol and lactic acid, the decolorization is improved by increasing their concentration because they are poor carbon sources. In fact, the efficiency of the treatment is influenced by the C/N ratio. These results are converged to those of El Hajjouji et al. [15].

These analyses showed that color removal was statistically significant ($p < 0.05$) between all tested carbon sources, and between all concentrations tested for different C-sources, glucose seemed to be the most suitable carbon source for TOPW decolorization.

3.3. Effect of different nitrogen sources on decolorization of TOPW

The additional nitrogen sources have stimulated the growth of the fungi, and decolorization. Four different nitrogen sources (diammonium tartrate, ammonium nitrate, ammonium sulfate and yeast extract) were tested at the same concentration (5 mM nitrogen) (Fig. 1).

Compared to inorganic N sources, decolorization was increased with organic N sources. Galhaup et al. [35] reported that both the nature and the concentration of the nitrogen source employed had a considerable importance. Yeast extract and diammonium tartrate were found to be the most effective supplement for supporting higher decolorization. However,

Table 4
TOPW decolorization by *G. candidum* under different carbon source concentrations (pH 6, diammonium tartrate 5 mM nitrogen) after 6 d of culture

TOPW decolorization Carbon source (g/L)	Glucose	Glycerol	Lactic acid	*p*-value
0	29 ± 3.1	29 ± 3.1	29 ± 3.1	1
2	47 ± 0.9	33 ± 4.1	35.3 ± 1.8	<0.05
5	63 ± 2	36 ± 3.2	42 ± 2.4	<0.01
7	47 ± 2.9	40 ± 1.1	49 ± 2.1	<0.01
10	37 ± 2.2	44 ± 3	59 ± 1.7	<0.01
p-value	<0.01	<0.01	<0.01	

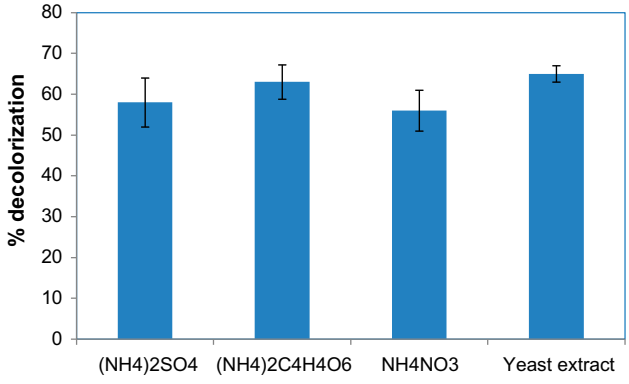

Fig. 1. Effect of ammonium source (5 mM nitrogen) on TOPW decolorization fermented with *G. candidum* after 6 d of culture ($p < 0.05$).

ammonium sulfate and ammonium nitrate led to a color reduction lower than 60%. Hence, yeast extract was selected as the best nitrogen source. It was reported that yeast extract was considered essential medium supplement for the NADH regeneration that acts as the electron donor for the azo dyes reduction by microorganisms [36].

Moreover, yeast extract degradation released ammonium and increased the pH, and it was reported that at neutral or alkaline pH, polyphenols are transformed into phenolates, losing part of their antimicrobial activity and becoming a suitable carbon source for microorganisms [37].

Statistical analysis of the data revealed that there was a highly significant ($p < 0.05$) difference between different decolorization treatments.

3.4. Influence of optimal conditions on TOPW decolorization

The optimized factors previously set up were used for the study of the growth, COD, color, and phenol

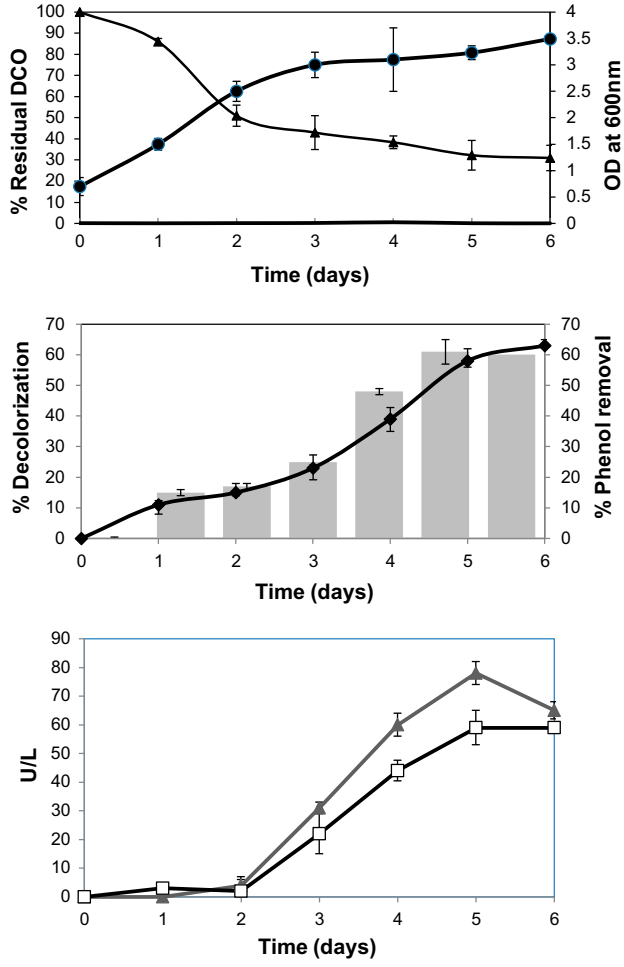

Fig. 2. Time course of biomass (●), % residual COD (▲), % decolorization (♦), % phenol removal (■), Lignin peroxidase (□) and manganese peroxidase (▲) during the TOPW pretreatment by *G. candidum*.

removals of TOPW by *G. candidum*. The obtained results are presented in Fig. 2. *Geotrichum* grows mainly in the first three days metabolizing sugars and other organic compounds.

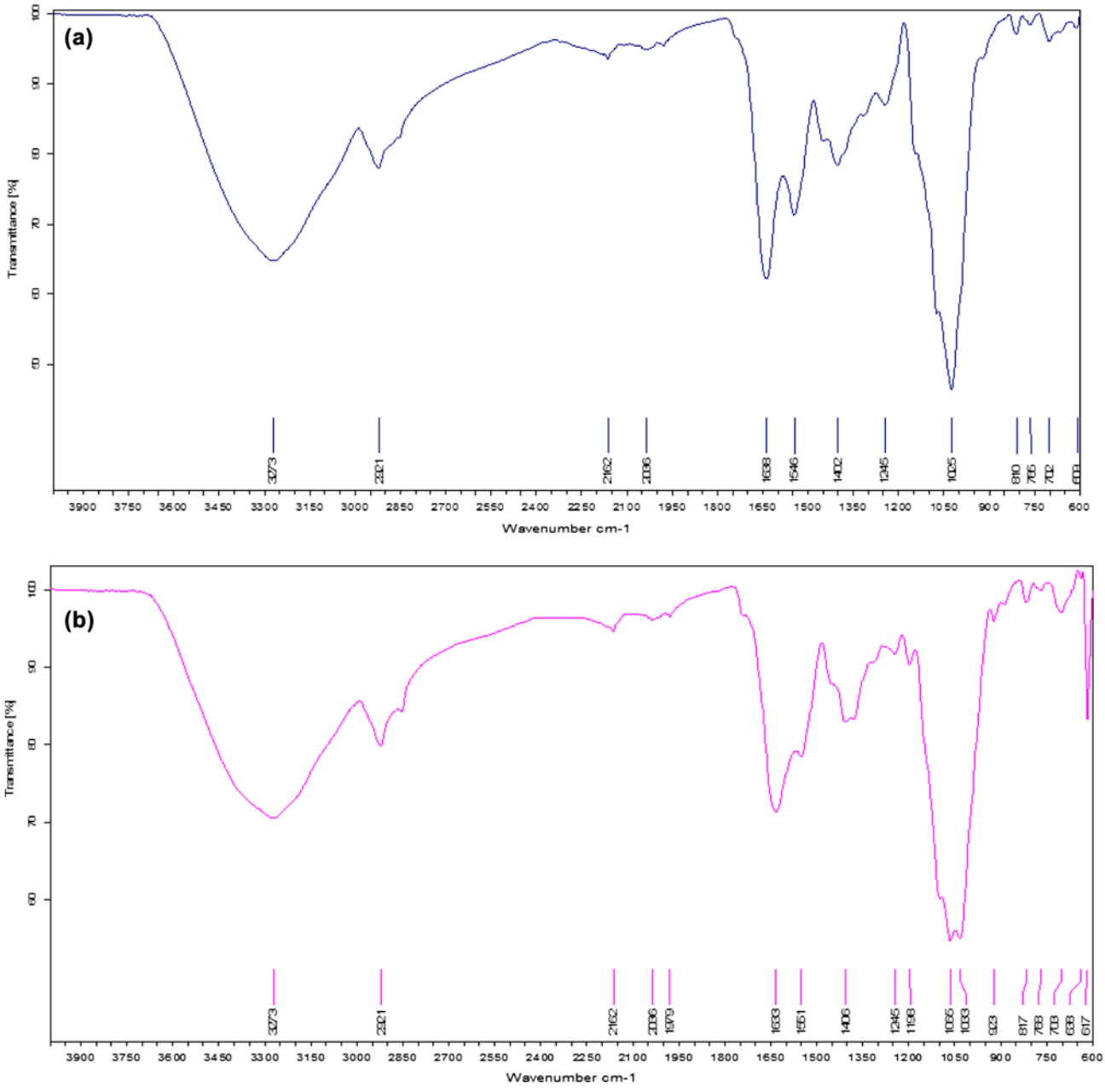

Fig. 3. Spectrum of *G. candidum* before (a) and after the TOPW pretreatment (b).

A slight color reduction was noted at the beginning despite the lack of enzyme which may be the result of adsorption phenomenon. Moreover, a biomass darkening was observed after incubation, confirming this hypothesis. In order to elucidate the nature of the functional groups responsible for the biosorption, FTIR analysis of the biomass was carried out. A comparison of biomass FTIR spectra before and after sorption (Fig. 3) showed the appearance of new peaks in the region of 1,633 and 1,000 cm^{-1} and confirmed the adsorption of the phenolic compounds on the biomass. Peak intensities in the region between 3,600 and 3,000 cm^{-1} decreased, which indicated that free OH and NH groups probably get bound with phenolic compounds. In addition, some changes happened in the characteristic peak location from 1,638, 1,546, 1,402, and 1,025 cm^{-1} in the spectrum (a) to 1,633, 1,551, 1,406, and 1,033 cm^{-1} in the spectrum (b) confirm the participation of –C=O–, –OH, and NH in adsorption. Similar FTIR results were reported for the

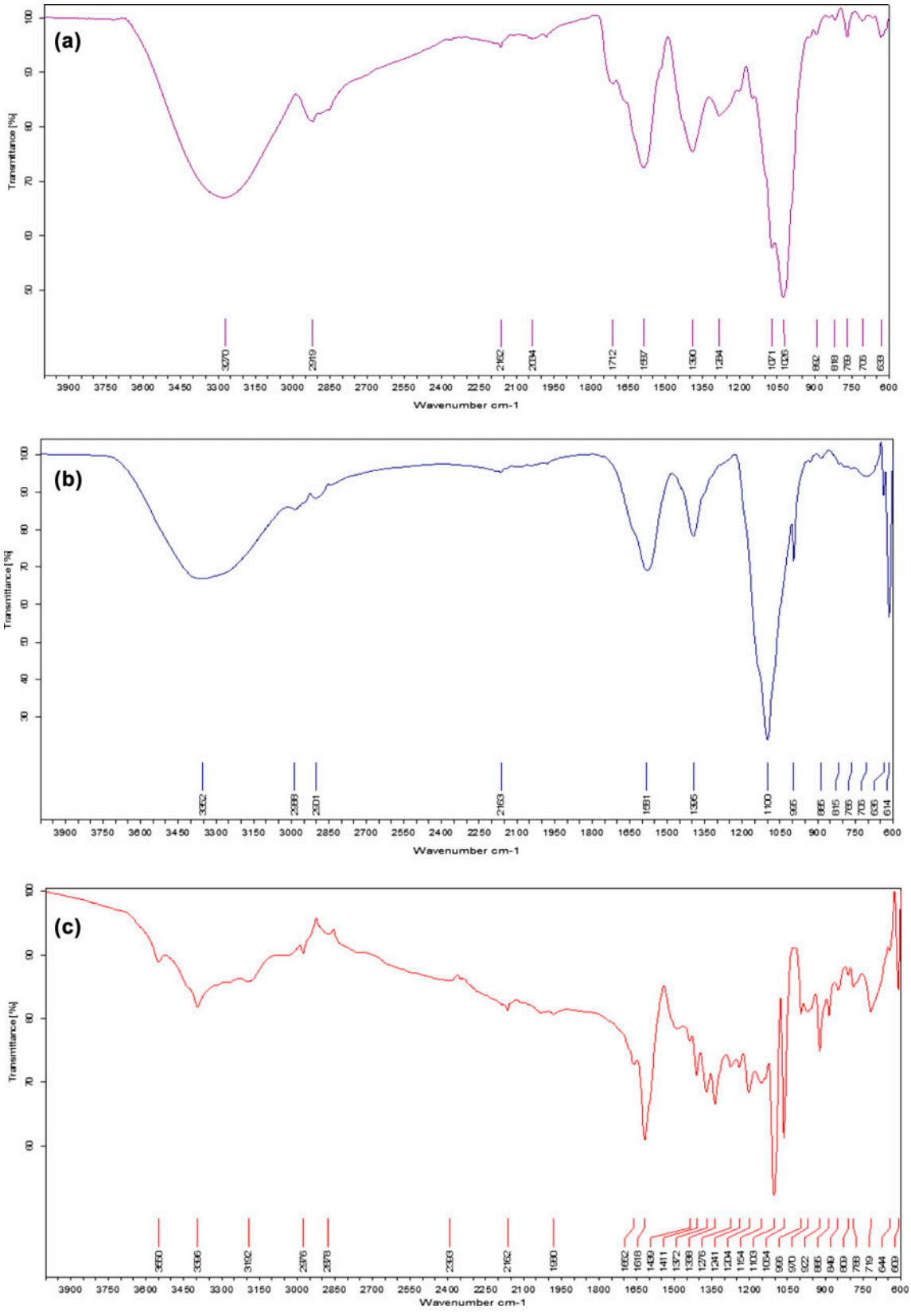

Fig. 4. FTIR spectra of the untreated TOPW (a), the non-supplemented treated TOPW (b) and the optimized treated TOPW by *G. candidum* (c).

Table 5
Main phenolic compounds present in untreated and treated TOPW by *G. candidum* after 6 d of culture

Phenolic compounds	Concentration in TOPW (mg/L)	Concentration in treated TOPW (mg/L)
Gallic acid	4.52	2.50
Tyrosol	170.2	42.5
Hydroxytyrosol	12.5	1.35
Cafeic acid	5.80	2.43
Coumaric acid	1.52	0
Vanillic acid	370	165.91
Ferulic acid	1.89	1.32
Oleuropein	35.2	3.6

phenolic biosorption on various fungus biomasses [38–40].

The color removal increased when the biomass remained constant, because the decolorization was corroborated with the extracellular peroxidase production as indicated in Fig. 2. Ligninolytic system is produced during the secondary metabolism. LiP and MnP activities were remarkable initially on the third day, but they didn't reach a maximum activity only after 5 d of incubation. LiP and MnP activities obtained were 58.4 and 78 U/L, respectively. It has been reported that ligninolytic enzymes are responsible for the decolorization of olive mill wastewater [41,42].

Geotrichum's growth induced 71, 63, and 60% of COD, color and phenolic compound removals respectively after 6 d of culture. As it is known, TOPW aerobic pretreatment by fungi has not yet been well studied unlike the olive mill wastewater pretreatment. The only works which were published are those of Lasaridi et al. [43] and Ayed et al. [44]. They showed that *Aspergillus niger* can reduce the toxicity and TOPW dark color. The COD removal efficiency was in the range of 60–87%. The use of *G. candidum* has the advantages over filamentous fungi, in that *Geotrichum* is less sensitive to shear stress. The decolorization was enhanced when TOPW is supplemented with nutriments, thereby, increasing the pretreatment cost. The addition of a low-value material in order to enrich TOPW can overcome this drawback. Indeed, preliminary tests on TOPW decolorization in the presence of brewery by-products appeared a promising approach (unreported data).

The TOPW biodegradation by *G. candidum* was monitored also using FTIR Spectroscopy. The FTIR spectrum of control TOPW (Fig. 4(a)) showed a peak at 3,270 cm^{-1}, which may be assigned to free OH bond of alcohol, phenol, or carboxyl groups (COOH). A peak at 2,940 cm^{-1} is due to C–H stretching vibration of aliphatic compounds. The bands between 1,581 and 1,397 cm^{-1} are attributed to the aromatic ring C=C stretching vibrations. The bands of peaks at 1,098 and 995 cm^{-1} are generally assigned to OH deformation and C–O stretching in phenolic, to C–H deformation of CH2 and CH3 groups and to COO^{-} anti-symmetric stretching. The peaks between 600 and 900 cm^{-1} correspond to C–H deformation vibration of aromatic compounds. The principal absorption bands in the FTIR spectra and their corresponding assignments are based on the literature [15–45].

The FTIR spectra of TOPW and the non-supplemented treated TOPW by *G. candidum* (Fig. 4(a) and (b)) showed the same band pattern indicating that unnoticeable qualitative changes have occurred during the biodegradation. Changes affected only band intensities, indicating that *Geotrichum*'s growth does not involve significant changes in the TOPW composition. However, the intensity of some peaks has been increased, in particular, the ones between 3,700 and 3,270 cm^{-1}. In addition to the above observations, the peak at 2,919 cm^{-1} has decreased, corresponding to a preferential biodegradation of aliphatic structures. Furthermore, the peaks between 1,000 and 1,200 cm^{-1} have decreased, showing the polysaccharide degradation.

Compared to the non-supplemented treated TOPW, the spectrum of optimized treated one (Fig. 4(c)) showed, a decrease in the –OH groups at 3,400 and at 1,072 cm^{-1}. Another decrease was detected at –CH groups at around 2,924 and at 1,546 cm^{-1} explaining the peptide structure degradation.

Besides, an increase in the peak at 1,638 cm^{-1} was observed, this can be attributed to C=C. Several peaks have also appeared between 670 and 900 cm^{-1} which can be attributed to aromatic C–H out of plane.

A HPLC analysis has been carried out to identify the phenolic monomers present in TOPW before and after biodegradation (Table 5). Vanillic acid and tyrosol were found to be the most abundant components in untreated TOPW. Whereas oleuropein,

hydroxytyrosol and different phenolic acids were present at lower concentrations. Coumaric acid was completely removed. Oleuropein, tyrosol, and vanillic acid were then removed at 89, 75, and 55% respectively after 6 d of culture and a relevant removal in the concentration of the other simple phenolic compound's content was shown. However, Ferulic acid is maintained at the same level as in the untreated TOPW.

4. Conclusion

Pretreatment of TOPW by *G. candidum* has been studied via experiment statistical design in order to identify the effective factors and to optimize decolorization. In fact, glucose and diammonium tartrate were identified as significant factors. Since synthetic carbon and nitrogen sources are very expensive, cheap and abundant locally available agro-residues can be tested as co-substrates. Moreover, mixing TOPW with other effluents can offer several advantages such as COD and phenolic concentration reductions.

The COD, color, and phenolic removals under optimized conditions are 71, 63, and 60%, respectively. The decolorization efficiency was due both to enzyme's secretion of LiP and MnP and to adsorption on biomass.

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