Polyvinyl acetate processing wastewater treatment using combined Fenton's reagent and fungal consortium: Application of central composite design for conditions optimization

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The Fenton reaction as an oxidative degradation process was used for industrial chemical wastewater (ICW) pretreatment. The biodegradation of pretreated ICW was performed, in aqueous environment under aerobic condition, by a defined fungal consortium. The central composite design (CCD) was used to study the effect of nitrogen and phosphorus addition and the concentration of the pollution on the removal of polyvinyl alcohol (PVA) and organic compounds. The interaction between parameters was modeled using the response surface methodology (RSM). Results of optimization showed COD, PVA and color removal yields of 97.8%, 98.5% and 99.75%, respectively with a supplementof 1.4 gL^{-1} of $(NH_4)_2SO_4$, 1.2 gL^{-1} of KH_2PO_4 and 75% of concentrated ICW. Enzymatic analysis proved that laccase and lignin peroxidase were involved in the biodegradation with 45 UIL^{-1} and 450 UIL^{-1}, respectively. Furthermore, the analysis of metabolic products using Fourier transforms infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{1}HNMR) showed clearly the mineralization of organic compounds and the formation of formic acid and ethanol. Therefore, the effective treatment of ICW was achieved by developing an integrated chemical and biological process which met the requirement for a safety effluent respectful for environment without risks for public health.

Keywords:
Industrial wastewater treatment
Polyvinyl alcohol
Fenton's reagent
Fungal consortium
Response surface methodology

1. Introduction

Rapid industrialization has resulted in extensive synthetic polymer and plastics pollutions, which are resistant to degradation, and consequently their disposal is fuelling an international drive for the development of biodegradable polymers [1]. They are very stable and are not readily enter in the natural cycle of biodegradation [2]. The pollution generated by these kinds of synthetic polymers is recognized as the source of many problems [3,4].

The development of research for PVA, a synthetic biodegradable polymer, in the environment was initiated for its application in pulp and paper industries [5,6]. PVA is produced by the hydrolysis of polyvinyl acetate (PVAC) which is mainly used in textile industries [7]. The effluent generated is characterized by high chemical oxygen de-mand (COD), intense color and high total suspended solids (TSS), im-plying the presence of recalcitrant organic matter. In fact, PVA is one of the most biodegradable of vinyl polymers in wastewater treatment by activated sludge process [8].

There are various physical-chemical methods for the treatment of PVA, such as coagulation, flocculation, chemical oxidation [9], Fenton process [10], electro-coagulation [11,12], photo-catalytic degradation [13] and adsorption on power activated carbon [14]. Also, advanced oxidation process have been promoted efficient results but need to be run under specific conditions [15,16]. However, these methods are expensive and often produce toxic. Therefore, biological processes has been developed to find an eco-friendly system for the removal of these compounds operated at ambient temperature, and without the necessity of the regulation of many parameters [17,18]. Various microorganisms have been identified for PVA biodegradation [17,19]. Biodegradation of PVA with mixed cultures of bacteria strains was also studied [20]. It was described that the degradation of PVA required the synergy of two strains for the entire biodegradation [21]. *Fusarium lini* was the first known microorganism capable of PVA degradation, producing carbon dioxide and water [22]. However, due to the complexity of the effluent, the biological degradation of PVA needs a long term of microbial population acclimation [2]. In some cases it needs to be degraded in

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Published Journal Article available at: https://doi.org/10.1016/j.jhazmat.2018.06.050

denitrifying conditions [23]. In fact, Magdum et al. [24] showed that treatment of effluent with high value of COD from 10,000 to 20,000 mg $L^{-1}O_2$ with 0.5 to 1% PVA concentration need a defined microbial consortium with optimization of the process's condition.

Thus, the use of chemical pretreatment of industrial wastewaters was necessary. Several studies evaluated the effectiveness of chemical oxidation of PVA, as a pretreatment, combined to the biological treatment. In fact, the effective oxidation of PVA by persulfate activated with heat Fe^{2+} and zero-valent iron was suggested as an option for improving the biodegradability of PVA [25].

In spite of the optimization of the conditions of treatments for wastewater [26,27], the poor composition of the industrial wastewater can limit the growth of the microorganisms and their abilities to biodegradation. Biostimulation was widely studied in order to improve the activities of microorganisms [28,29]. The most used nutrients to increase the degradation of pollutants were nitrogen, phosphorus and yeast extract [30]. Moreover, biostimulation can enhance the color removal of dyes and the production of effective products [31,32].

The present work aims to study the importance of the pre-treatment with FR to accelerate the biodegradability of ICW by a consortium of fungi. ICW was first pre-oxided with FR before its incubation with the fungal consortium. RSM was used to define the optimal conditions for the growth by varying the composition of the media. The produced metabolites during the degradation were predicted by FTIR spectroscopy and ^{1}HNMR. In addition, UV–vis 317 nm and COD analyses were employed to evaluate the performance of combined process.

2. Materials and methods

2.1. Effluent collection and Fenton reagent preoxidation

ICW was used as a culture medium. It was collected from the effluent treatment plant from the factory MPC PROKIM, Tunis, Tunisia. Table 1 summarizes a representative characterization of ICW and the regional standard limits for effluent discharge on public sewage. The pH of each PVA solution was adjusted to 4.2 with 0.1 mol L^{-1} HCl.

The FR treatment was carried out in 250 ml glass beaker, at 25 ± 2 °C. Ferrous sulfate hepta-hydrate (FeSO4.7H2O, 99.98%purity) and hydrogen peroxide (H_2O_2) were used for Fenton reaction. The FR reaction was prepared by mixing simultaneously equal volumes of H_2O_2 (2.8 mol L^{-1}) with $FeSO_4$ (0.10 mol L^{-1}) in the presenceof PVA [33]. 0.2 ml of FR was added to beaker containing 20 ml of a sterile PVA solution (0.5% w/v) and the preparations were incubated after mixing. The Fenton reaction was stopped after 24 h with the adjustment of pH at 6 with 0.1 mol L^{-1} of NaOH to avoid the production of hydroxyl groups HO [34]. The entire reagents were purchased from Sigma Al-drich.

Table 1
Characteristics of ICW and maximum discharge limits.

Parameter	Units	Minimum	Discharge limit value[a]
pH		7.11 ± 0.5	6.5-8.6
TSS	mg L^{-1}	3531 ± 5	400
TS	mg L^{-1}	9200 ± 2	400
COD_{Total}	mg O_2 L^{-1}	23,480 ± 4.5	1000
$COD_{Soluble}$	mg O_2 L^{-1}	17600 ± 4.5	No data
BOD_5	mg O_2 L^{-1}	3160 ± 5	400
BOD_5/COD		0.134	0.35
Conductivity	(ms/cm)	3.1 ± 0.2	No data
Turbidity	NTU	3644 ± 2.5	No data
Color		Milky -White	No data

[a] Tunisian Standard (NT106.002) limits for effluent discharge on public sewage.

2.2. Culture conditions

ICW was supplemented with medium components in the following ways: 0.1 g L^{-1} NaCl, 0.2 g L^{-1} $MgSO_4$, g L^{-1} K_2HPO_4. It was sterilized, inoculated with the fungi and incubated at 28 °C for 10 days, at 150 rpm. ICWwas considered such a carbon source for the growth of fungi after correction of the pH to 5.5. During the culture run, a sample from the broth medium was filtered to remove the fungal pellets, and then centrifuged at 9000 rpm for 25 min. The supernatant obtained was used to all the assays.

2.3. Microorganisms and growth conditions

The consortium of fungal strains was composed by three fungi species, *Chaetomium globosum IMA1*, *Aspergillus niger* and *Rhizopus oryzae*. The filamentous fungi species were selected based on their capacity for pollutant removal via the biodegradation mechanism and synergetic effect of their enzymes. In fact, these fungi have known for their important production of ligninolytic enzymes and other enzymes such as lipase and cellulolytic activities. Furthermore, Pajak et al. [22] showed that LiP was involved in the mechanism of degradation of PVA.

One of the several advantages of fungal process is the enzyme-mediated activity that provides solution to the treatment of wastewaters containing hazardous organic pollutants. In fact, the fungi are used thanks to their superior aptitudes to produce a large variety of extracellular enzymes [35]. Moreover, the fungi are recognized for their abilities to adapt to severe environmental constraints [36,37].

The fungal strain previously isolated from ligninolytic decaying materials was *Chaetomium globosum IMA1 KJ472923*. Identification of fungal strain was done by microscopic morphology and analysis of the nucleotide sequence of the ITS1-5.8-ITS2 ribosomal RNA region [38]. *Rhizopus oryzae ATCC 1230* and *Aspergillus niger ATCC 6275* were purchased from Leibniz-Institut DSMZ German collection of Microorganisms and cell cultures. The monitoring of strains purity was done by microscopy. The strains were cultivated periodically on potato dextrose agar (PDA) at 28 °C and preserved at 4 °C. The PDA or the potato dextrose broth (PDB) was used for routine culture of the fungi at 28 °C with shaking for liquid cultures. Before the inoculation of fungi for the different experiences, each fungus was individually pre-cultured in Erlenmeyer flasks containing 20 ml of PDB for 3 or 4 days at 28 °C at 150 rpm. ICW was inoculated with 20% of inoculum size for the total volume of the culture. From each pre-culture of fungus, the inoculation was done by adding 5 ml of spore suspension having a spore con-centration $1.44*10^5$ ml^{-1}. The combination of each fungal strain was in the same proportion (33%) in the culture medium of ICW.

2.4. Analytical methods

The supernatant obtained after centrifugation was used for further analytical studies like FTIR, ^{1}HNMR, color OD at 317 nm and PVA concentration analysis.

2.4.1. Fourier transform infrared spectroscopy

FTIR was used to characterize the presence of specific chemical groups in the samples. FTIR spectra, using attenuated total reflectance (ATR) technique, were recorded using Bruker (Alpha-P) with a resolution of 4 cm^{-1}, in ranges of 600 to 4000 cm^{-1}, with a sample scan time 16 scans at room temperature. Samples of the reaction product were lyophilized at −50 °C and at a pressure of 0.5 mbar for 24 h. A baseline correction was performed on the spectra each time before integration with Bruker software. FTIR spectrums were used for the ICW, ICW treated with FR and ICW preoxided with FR and after treatment with the fungal consortium.

2.4.2. Proton nuclear magnetic resonance analysis

All ^{1}HNMR spectra were recorded with a Varian Gemini 200

instrument. The spectra analyses were made in 5–10% by weight of Deuterated Dimethyl Sulfoxide (DMSO-*d*6) solution in tubes of 5 mm at room temperature. The ^{1}HNMR measurements were carried out at a frequency of 200 MHz, by using the following experimental conditions: 64 numbers of scans, acquisition time 2 s, and number transient from 1 to 128. The chemical shifts of protons were determined with a precision of at least 0.1 ppm. ^{1}HNMR studies were used for the ICW, ICW treated with FR and ICW preoxided with FR and after treatment with fungal consortium. The lyophilized samples were dissolved in DMSO-*d*6.

2.4.3. Analytical measurements

The analysis of pH, total solids (TS), TSS, conductivity, turbidity, biochemical oxygen demand (BOD_5) and COD were performed according to APHA standard methods [39]. The effluents were stored at 4 ° C for any following uses.

2.5. Expression of color and PVA removal efficiency

Decolorization was monitored by UV–vis spectroscopic analysis (Jasco V-650 UV visible spectrophotometer). For each sample, it was followed by monitoring the changes in the absorption spectrum (300 nm–800 nm). The color of the ICWwas measured at the maximum absorbance λ_{max} (317 nm). The decolorization was determined by the following equation.

$$D = \frac{(\mathrm{Ai}-\mathrm{At})}{Ai} \times 100$$

Where D is the decolorization of effluent (in %), A_i is the initial absorbance of the effluent and A_t is the effluent absorbance along the time.

Additionally, decolorization study was carried out to confirm that it was not related to non- biological activity like adsorption mechanism. Jiranuntipon et al. [40] developed in his study the NaOH extraction method that consist to disperse the cell's fungal with equal volume of NaOH (0.1 mol L^{-1}) to extract color substances adsorbed to cell surface. The extracts resulted were centrifuged and OD was measured at 317 nm.

The concentration of PVA was determined by using the colorimetric technique described by Finely by the reaction of the chain of PVA with iodine [41]. PVA medium purchased from Sigma (5 g L^{-1} PVA) was used as the standard for PVA solution to determine the calibration curve. This was diluted to 0.5 ml with H_2O, and then 0.75 ml of 4%boric acid and 0.15 ml of I_2-KI (12.7 g of I_2 and 25 g of KI in 1 L) were added. The solution was mixed well and equilibrated for 30 min at room temperature. Then, the mixture was diluted to a volume of 2.5 ml with H_2O and analyzed at 690 nm [17]. In order to avoid the inter-ferences of the residuals hydroxyls groups with boric acid and iodine, the blank received the distilled water instead of the tested sample containing PVA. The blank assay was used before each determination of PVA concentration for all experiments. The measurement of absorbance was made with spectrometer UV–vis. The PVA removal was determined by the following equation.

$$C = \frac{(\mathrm{Ci}-\mathrm{Ct})}{Ci} \times 100$$

Where$C$ is the elimination of PVA from effluent (in %), C_i is the initial concentration of the effluent and C_t is the effluent concentration along the time.All assays for color and PVA removal were carried in triplicate.

2.6. Enzymatic activity assays

Supernatants from the final optimum decolorization medium were analyzed for lignin-peroxidase (LiP), manganese peroxidase (MnP) and laccase activities. In all cases, the reaction mixture contained 100 µl crude enzyme extract in a final volume of 2 ml.Laccase activity was determined in the reaction mixture containing 2 mmol L^{-1} of 2,2-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in 1 ml of 100 mmol L^{-1} sodium succinate buffer (pH 4.5) at 30 °C and monitored by measuring the increase of the oxidation at 420 nm (ε_{420} = 36,000 (mol L^{-1}) $^{-1}$cm^{-1}) [42]. The blanks received the buffer in place of ABTS. One unit of enzyme activity was defined as the amount of enzyme re-quired to produce an absorbance increase of one unit per minute per milliliter of the reaction mixture.

MnP activity was measured by monitoring the oxidation of 1 mmol L^{-1} $MnSO_4$ in 50 mmol L^{-1} sodium malonate buffer (pH 4.5) in the presence of 0.1 mmol L^{-1} H_2O_2, and monitored by measuring the increase of the oxidation at 468 nm (ε_{468} = 49,600 (mol L^{-1})$^{-1}$cm^{-1}). The blanks contained all reagents except $MnSO_4$. One unit of MnP activity was defined as the amount of enzyme required to produce an absorbance increase of one unit per minute per milliliter of the reaction mixture [43].

LiP activity was determined by monitoring the formation of veratraldehyde by measuring the absorbance increase at 310 nm (ε_{310} = 9300 (mol L^{-1})$^{-1}$cm^{-1})) in the reaction mixture containing 4 mmol $L^{-1}$of veratryl alcohol in 10 mmol L^{-1} sodium tartarate buffer (pH 3) incubated at 30 °C. The reaction was initiated with addition of 0.2 mmol L^{-1} H_2O_2 [44]. The blanks contained buffer in place of veratryl alcohol. One unit of LiP activity was defined as the amount of enzyme catalyzing the formation of 1 µmol of veratraldehyde per minute under the assay conditions. The presented data are the average of triplicate measurements.

2.7. Statistical analysis

RSM is an effective tool for optimizing the process, which combines independent variables and their interactions [45,46]. In this study, it was used to optimize three response variables namely color removal, COD and PVA removal. Each independent variable was coded at three levels between -1 and +1, where the variables (g L^{-1}) in this experiment $(NH_4)_2SO_4$ (X1), KH_2PO_4(X2) and ICW concentration (%)(X3) were changed in the ranges shown in Table 2.

The critical ranges of selected parameters were determined based on preliminary experiments and the literature review. Sixteen experiments were augmented with two replications at the design center. The statistical software package Statistica version 10.01 was used for regression analysis of experimental data and to plot response surface. The experimental data can be represented by the following equation:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 - a_{13}X_1X_3 + a_{23}X_2X_3 - a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2$$

whereX_1, X_2 and X_3 are the variables, Y is the predicted response correlated to the set of regression coefficients (a): the intercept (a_0),linear (a_1, a_2, a_3), interaction (a_{12}, a_{13}, a_{23}), and quadratic coefficients (a_{11}, a_{22}, a_{33}).

2.8. Validation of experiment

The mathematical model generated during RSM implementation was validated by conducting on given optimal medium setting in triplicate with the determination of kinetic of enzyme's activities, COD removal, decolorization, FTIR and PVA removal.

Table 2
Independent variables and their levels in the experimental design.

Independent Variables	Symbol	Coded variable levels		
		−1	0	1
$(NH_4)_2SO_4$(g L^{-1})	X1	0.4	0.95	1.5
KH_2PO_4 (g L^{-1})	X2	0.4	0.95	1.5
ICW concentration (%)	X3	25	75	100

3. Results and discussion

3.1. The physical-chemical characterization of ICW

Physical and chemical characteristics of the ICW were summarized in Table 1. Results showed considerably high values of total COD, soluble COD, TS and TSS, which are above the prescribed values of regulation. It is characterized with high conductivity due to the presence of different salts and an important value of absorbance equal to 29.94 at λ = 317 nm (Fig. 1 in Supplementary material). In addition, the total COD was close to soluble COD showing that the majority of pollution was soluble in ICW. Other studies of different kinds of industrial wastewaters showed high values of COD. In fact, sulphate containing yeast industry wastewaters showed a rate of 14400–25700 mg $L^{-1}O_2$ [47]. Moreover, ICW showed low rate of BOD_5 (3160 mg $L^{-1}O_2$) comparing to COD (23,480 mg L^{-1} O_2).The corresponding ratio of BOD_5 to COD showed a very low value, equal to 0.13 (< 0.35), indicating that ICW contained non-biodegradable organic matter and need specific treatment [48]. In this study, the concentration of ICW on PVA was in adequacy with other studies with 1.265 g $L^{-1}$of PVA. Kim et al. [17] used a 5 g L^{-1} of PVA concentration from textile factories to screen PVA degrading bacteria. Another study of Magdum et al [24]. used a desizing effluent from textile industry with 0.531% of PVA.

3.2. Optimization of medium composition for PVA, color and COD removal

The complete design matrix with the values of responses based on the experimental runs is presented in Table 3. The first three columns show run numbers and their experimental conditions as arranged by the CCD. The last column shows the results of the different responses. The regression model equations are as follows:

$$Y1_{PVA\ removal}(\%) = 99.71 + 5.95X_1 + 6.67X_2 + 2.29X_3 + 10.31X_1X_2 - 1.33X_1X_3 - 3.19X_2X_3 - 9.70X_1^2 - 7.64X_2^2 - 4.10X_3^2$$

$$Y2_{decolorization}(\%) = 95.29 + 0.09X_1 + 0.021X_2 + 12.24X_3 - 1.88X_1X_2 - 0.09X_1X_3 - 0.28X_2X3 + 1.81X_1^2 + 1.82X_2^2 - 7.96X_3^2$$

$$Y3_{COD\ removal}(\%) = 88.73 + 6.64X_1 + 6.34X_2 + 0.26X_3 + 12.14X_1X_2 - 1.55X_1X_3 - 1.86X_2X_3 - 4.50X_1^2 - 4.32X_2^2 - 2.65X_3^2$$

The coefficient of determination (R^2) was found to be 79.97%, 90.93% and 74.27% for PVA, color and COD removal, respectively. It shows a high correlation between observed and predicted values for decolorization. The accuracy of the models and the effect of factors on each response were checked using ANOVA analysis (Table 4). The obtained p-values for interaction models were of 0.000 for the three responses showing that the three models could predict well COD, color and PVA removal from ICW. For the PVA removal the quadratic effect of nitrogen and the interaction of nitrogen and phosphorous showed a p-value less than 0.05. Therefore, according to ANOVA analysis, ni-trogen concentration and the combined effect of nitrogen and phos-phorous has a significant effect on PVA removal with a p-value of 0.044 and 0.046, respectively. Close results were found for COD response, where the interaction of nitrogen and phosphorous factors gave a sig-nificant effect on COD removal with a p-value of 0.033. However, for color removal, dilution factor has the most important effect. This factor has a significant effect with a p-value of 0.0004 and 0.017 for linear and quadratic dilution factor, respectively. Consequently, the obtained models equations using the most affecting parameters are as follows:

$$Y1_{\ PVA\ removal}\ (\%) = 99.71 + 10.31\ X_1X_{\ 2}\ -9.70\ X_1^{\ 2}$$

$$Y2_{\ decolorization}(\%) = 95.29 + 12.24\ X_3\ -7.96\ X_3^{\ 2}$$

$$Y3_{\ COD\ removal}\ (\%) = 88.73 + 12.14\ X_1\ X_2$$

3.3. Effect of ICW and nitrogen concentration on the COD removal

The contour plot of CCD was determined for the responses. Only the data of COD removal were presented in this work for clarity of purpose. Fig. 1 shows COD removal against two factors (dilution and phosphorus concentration). Nitrogen variations showed an important effect on COD removal efficiency at high concentrations.

Fig. 2 shows COD removal against nitrogen and phosphorous concentrations. Dilution values variation didn't give significant changes and confirms that only phosphorous and nitrogen concentrations affect COD removal. In fact, according to RSM, the yield of COD, color and PVA removal increased when the proportion of nitrogen and phosphorus were between 0.95 and 1.5 g $L^{-1}$and with a 70–80% of concentration. However, low concentration of ICW was ineffective to

Table 3
The coded values designed by CCD and the responses.

Run No.	Codified values			Experimental values			Response		
	X1	X2	X3	$(NH_4)_2SO_4$	KH_2PO_4	Dilution(%)	PVA(%)	Color(%)	COD(%)
1	−1	−1	−1	0.4	0.4	25	82.40	70.86	86.64
2	−1	−1	+1	0.4	0.4	100	91.12	99.76	85.55
3	−1	+1	−1	0.4	1.5	25	74.04	79.84	75.20
4	−1	+1	+1	0.4	1.5	100	67.14	99.95	63.87
5	+1	−1	−1	1.5	0.4	25	91.77	78.87	92.31
6	+1	−1	+1	1.5	0.4	100	94.39	99.93	95
7	+1	+1	−1	1.5	1.5	25	96.90	72.93	94
8	+1	+1	+1	1.5	1.5	100	97.42	99.97	99.55
9	−1	0	0	0.025	0.95	50	56.70	99.95	52
10	+1	0	0	1.87	0.95	50	96.17	99.95	96
11	0	−1	0	0.95	0.025	50	90.34	99.95	88
12	0	+1	0	0.95	1.87	50	94.07	99.99	97
14	0	0	−1	0.95	0.95	5	80.20	60.46	79
13	0	0	+1	0.95	0.95	125	100	99.85	98
15	0	0	0	0.95	0.95	50	99.5	89.73	99
16	0	0	0	0.95	0.95	50	99.5	89.37	99

Table 4
Analysis of variance of PVA, Color and COD removal for the selected linear and interaction model for ICW.

PVA removal (%)	Sum of Squares	Df*	Mean Square	F value	P value
X_1	467.541	1	467.541	3.441	0.113
X_1^2	866.956	1	866.96	6.380	0.044
X_2	587.289	1	587.29	4.322	0.082
X_2^2	538.632	1	538.63	3.964	0.094
X_3	67.457	1	67.46	0.496	0.507
X_3^2	114.035	1	114.035	0.839	0.395
X_1X_2	850.781	1	850.781	6.261	0.046
X_1X_3	14.725	1	14.725	0.108	0.753
X_2X_3	85.376	1	85.376	0.628	0.458
Error	815.280	6	135.880		
Total sum of squares	4072.065	15			
Colorremoval (%)	Sum of Squares	Df*	Mean Square	F value	P value
X_1	0.098	1	0.098	0.002	0.962
X_1^2	30.191	1	30.191	0.756	0.418
X_2	0.580	1	0.580	0.015	0.908
X_2^2	30.458	1	30.458	0.763	0.416
X_3	1930.727	1	1930.727	48.337	0.0004
X_3^2	431.117	1	431.117	10.793	0.0167
X_1X_2	28.388	1	28.388	0.711	0.432
X_1X_3	0.066	1	0.066	0.002	0.969
X_2X_3	0.679	1	0.679	0.017	0.901
Error	239.657	6	39.943		
Total sum of squares	2643.889	15			
CODremoval (%)	Sum of Squares	Df*	Mean Square	F value	P value
X_1	582.327	1	582.327	3.706	0.103
X_1^2	186.823	1	186.823	1.190	0.317
X_2	530.622	1	530.622	3.377	0.116
X_2^2	172.360	1	172.360	1.097	0.336
X_3	0.882	1	0.882	0.006	0.943
X_3^2	47.776	1	47.776	0.304	0.601
X_1X_2	1179.037	1	1179.037	7.504	0.034
X_1X_3	19.993	1	19.993	0.127	0.734
X_2X_3	28.800	1	28.800	0.183	0.683
Error	942.706	6	157.118		
Total sum of squares	3663.840	15			

* Degrees of freedom.

improve the action of fungal consortium. These observations could be mainly attributed to the metabolism of fungi, which need imperatively a definite ratio of carbon and nitrogen to achieve the highest grow using ICW like a source of carbon.

Several studies focused on the importance of the nutrients supplements to improve biodegradation efficiency [30–46]. Khelifi et al. [31] showed that at optimum carbon and nitrogen sources, fungi reached a rate of 98.6% and 98% of color removal obtained for Indigo and Congo red dyes, respectively. According to Djelal et al. [49], the effect of the consortium increased COD removal yields from 55% to 75%. Moreover, bioaugmentation with fungi has a significant reduction on the non biodegradable COD by increasing the ratio of COD on BOD_5 from 451%–1111% to 257%-153%.

The ^{1}HNMR spectrum of ICW (Fig. 3a) shows the presence of typical signals of PVAC and PVA. The chemical shift corresponding to the range of signal of 1.9–2 ppm corresponds to methyl group. Signals at 1.2–1.75 ppm are corresponding to protons of methylene group. Signal at 2.5 ppm is attributed to DMSO-d6. The 3.35–4.0 ppm range contains the signal of alcohol group CH2—CH—OH. The 4.7–4.8 ppm range contains the signal of CH related to acetate group of PVAC [CH (OCOCH3)].

The preoxidation of ICW (Fig. 3b) leads to the reduction of the intensity of the majority of the signals. Methylene is only present with a signal at 1.7 ppm. There is a huge reduction in the amount of methylene and methyl group after pre-oxidation with FR. The signal corresponding to 4.5 ppm of hydroxyl group of PVA is totally absent. Apparition of signal at 5.8 ppm, could be the formic acid, a product of the oxidation of ICW. The biodegradation of pretreated ICW with the consortium of fungi (Fig. 3c) led to the depolymerization of the polymeric chain in ICW. Only, signals at 1.06 ppm, 3.44 ppm and 4.35 ppm corresponding to CH3, CH2 and OH, respectively, persisted in the final effluent. These signals were attributed to ethanol. Similar study from Szczyrba et al.[50] determined the enzymes involved in vinyl acetate degradation and proposed a pathway of vinyl acetate degradation that involved a reduction of acetaldehyde to ethanol as a result of the reduction by al-cohol dehydrogenase.

Fig. 4 exhibits FTIR spectrum of different samples of ICW before and after treatment. The right section (< 1500 cm^{-1}) of the infrared spectrum refers to its "fingerprint", it can reveal many bands that differs by their shape and nature which were related to the structure of ICW. There is no significant difference in the right part between Fig. 4.a and b, they were found in the same place and relative intensities. Besides, the left section of the spectra included most of the characteristic bands of functional groups. The large bands observed between 3550 and 3200 cm^{-1}were linked to the stretching of O—H from the intermolecular and intramolecular hydrogen bonds (Fig. 4.a). The hydroxyl group O—H at 3351 cm^{-1} presented a broad absorption peak. The vibrational band observed between 3000 and 2872 cm^{-1} refers to the stretching C—H from alkyl groups. Additionally, the peak at 1728 cm^{-1} which is presented as a strong absorber are due to the stretching C═O and C—O from acetate group from the copolymer of PVA and PVAC from ICW. Infrared spectra of ICW confirmed the theoretical important presence of the PVA and PVAC.

The intensity of the 1728 cm^{-1} is more important for ICW treated with Fenton (Fig. 4.b), indicating that oxidation of ICW led to the formation of ketone groups. The intensity of the 3550 and 3200 cm^{-1} is weak comparing to one of Fig. 4.a. The reaction of the ICW with the FR results in a considerable reduction of the intensity of the O—H peaks, indicating a possible interaction between ·OH and O—H from ICW. It seems that one ·OH is formed in solution it can extract a hydrogen from the polymeric backbone to generate free H_2O and to attack carbon of the polymeric chain of ICW to generate ketone groups.

For instance, FTIR spectra of the pretreated ICW after the treatment with the fungal consortium(Fig. 4.c) revealed two important bands at ν = 1102 and 937 cm^{-1} of C—H stretching related to carbonic chain with peaks attributed to the alkene chain. These bands are overlapping and broadening in the new treated ICW. Also, new band appears at 1635 cm^{-1} for alkene group (C═C at ν = 1700–1561 cm^{-1}). The peak observed at 1644 cm^{-1}is due to the bending mode of water absorbed at the sample surface which also contributes to the weak peak at 3295 cm^{-1}of the stretching of O—H. We can make an hypothesis that in the treated ICW with FR and fungi, there is no more PVA and PVAC. We can estimate the presence of small compounds of carbon. The FTIR spec-trum of treated ICW after 10 days shows a significant change in the band positions compared to control ICW spectrum. It seems that pro-ducts of Fenton's reaction are not toxic to the consortium of fungi.

3.4. Validation of the model

In order to determine the optimal range of biodegradation with the different variables, the analysis of RSM of the full quadratic model between the response and the variables shows that from the graphic's surface, the relation between the percentage of degradation and the factor can be given. In fact, the optimized ranges for each factor leading to the highest percentage of degradation were extracted from these surfaces [51]. The optimized ranges for both KH_2PO_4 and $(NH_4)_2SO_4$ are 0.95 to 1.87 g L^{-1}. ICW showed an optimized concentration be-tween 70–80%. Middle point of each optimum range was after de-termined. Three repetitive experiments were performed under these conditions (Figs. 5 and 6).These results were in agreement with the obtained optimized ranges and confirmed that these conditions led to the highest values of degradation.

The kinetic of the FTIR was determined after 3, 5 and 10 days (Fig. 5).The disappearance of the peak at1728 cm^{-1}which was

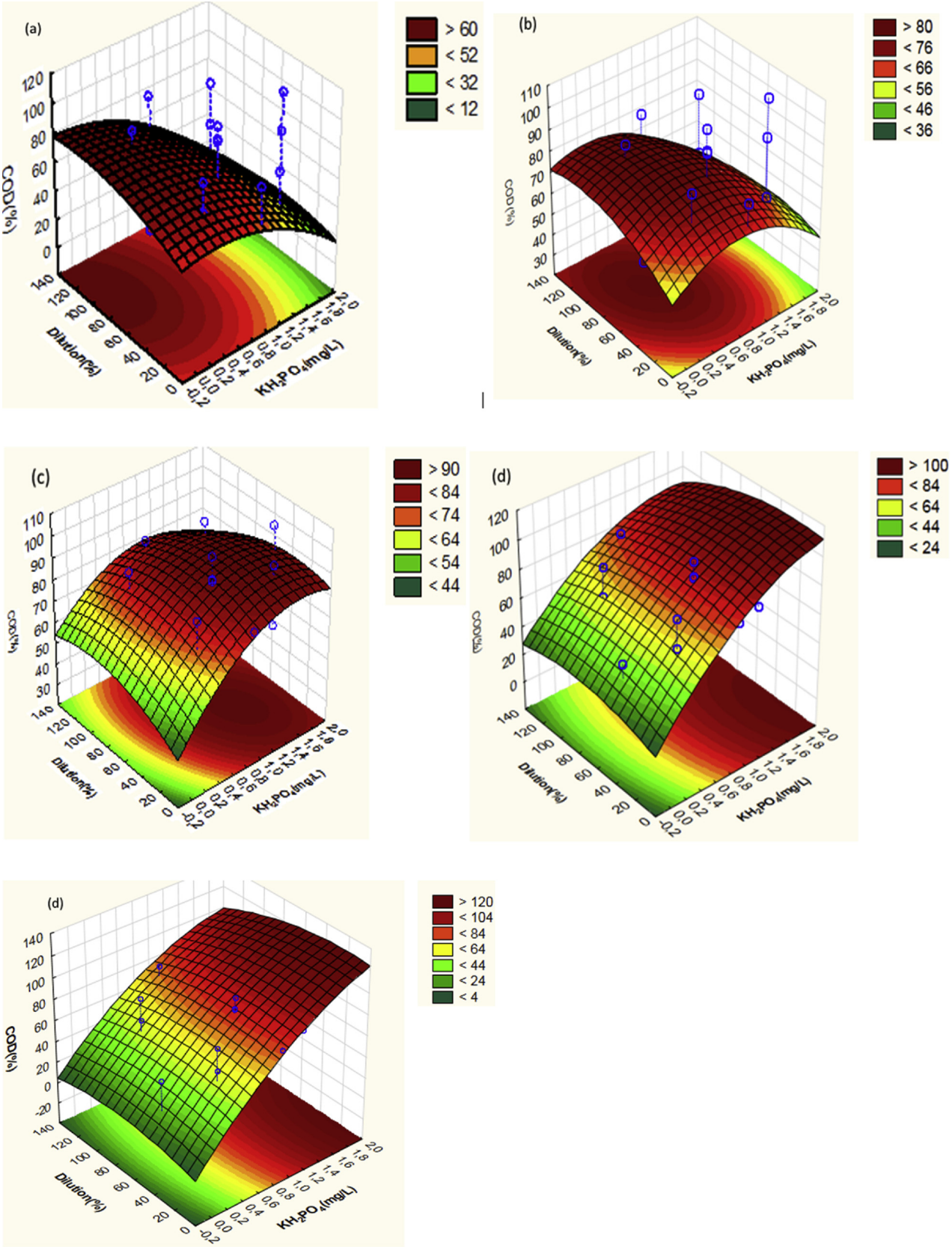

Fig. 1. Response surface plot described by the model, showing the effect of the concentration of $(NH_4)_2SO_4$on COD removal with 0.025 g $L^{-1}$of $(NH_4)_2SO_4$ concentration (a), 0.4 g L^{-1} of $(NH_4)_2SO_4$ concentration (b), 0.95 g L^{-1} of $(NH_4)_2SO_4$ concentration (c), 1.5 g L^{-1} of $(NH_4)_2SO_4$ concentration (d) and 1.87 g $L^{-1}$of $(NH_4)_2SO_4$ concentration (e).

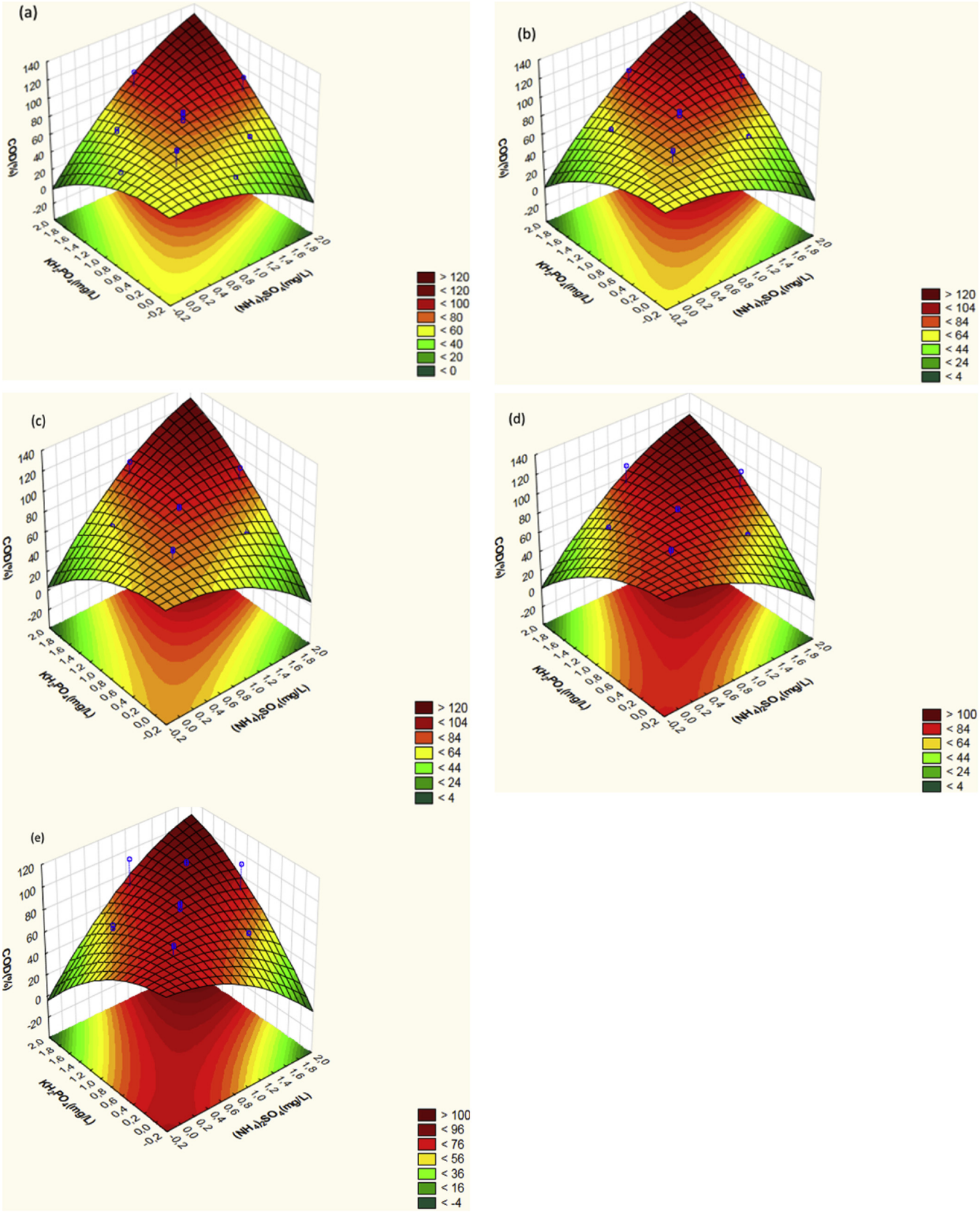

Fig. 2. Response surface plot described by the model, showing the effect of the concentration of the concentration on COD removal with 5% of ICW concentration (a), 25% of ICW concentration (b), 50% of ICW concentration (c), 100% of ICW concentration (d) and 125% of ICW concentration (e).

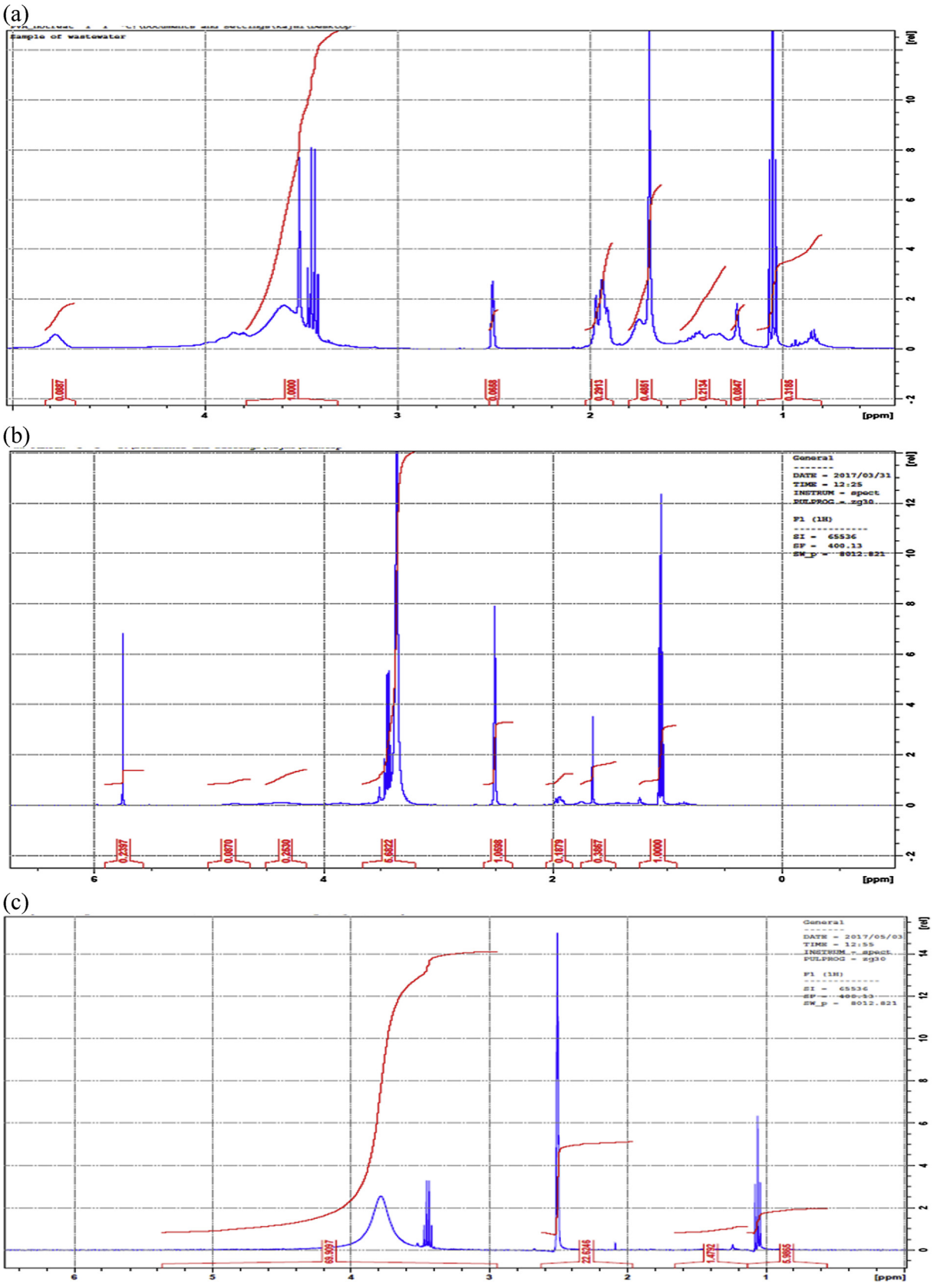

Fig. 3. ^{1}HNMR spectrum of ICW (a) of ICW after preoxydation with FR (b) and of ICW after biodegradation with consortium of fungi and preoxydation with FR (c).

presented the stretching C═O and C–O from acetate group was from the third day of the biodegradation. The hydroxyl group O–H at 3351 cm^{-1} presented a very large absorption peak (Fig. 5a). In the fifth day (Fig. 5b), there was no more hydroxyl group. The stretching vibration at 1644 cm^{-1} is due to the presence of water. The FTIR spectrum after 10 days showed a significant change in the positions compared to the

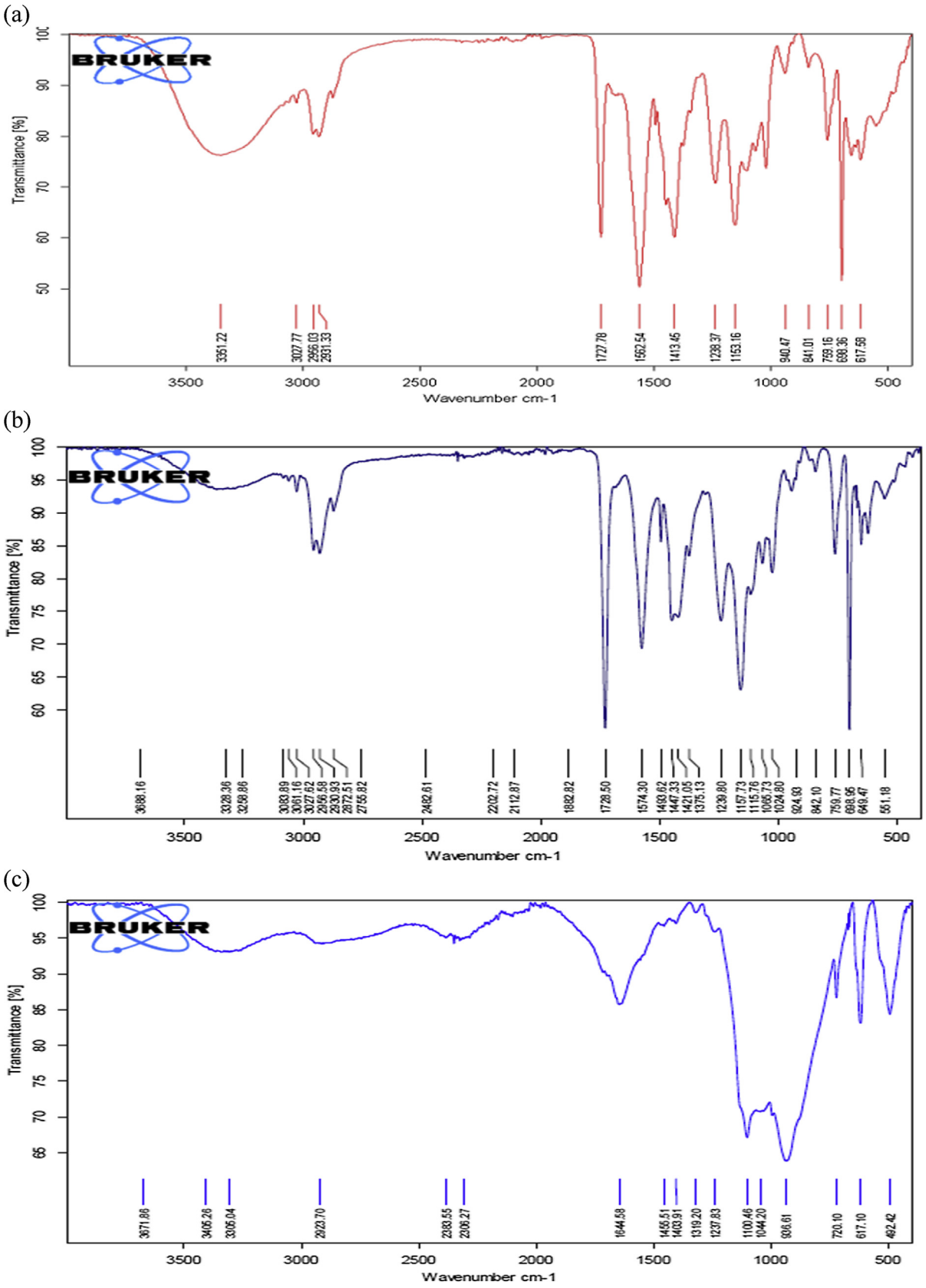

Fig. 4. FTIR spectrum of ICW (a) of preoxidation of ICW with FR (b) and of ICW after biodegradation and peroxydation with FR (c).

untreated effluent (Fig. 5c). Therefore, the combination of the two processes could make a total biodegradation after only 5 days with a total disappearance of the polymer structure of PVA and PVAC. So, the preoxidation of ICW can avoid the long time that need the fungi to make their acclimation in the medium.

Kinetics for PVA, color and COD removal were determined for 10

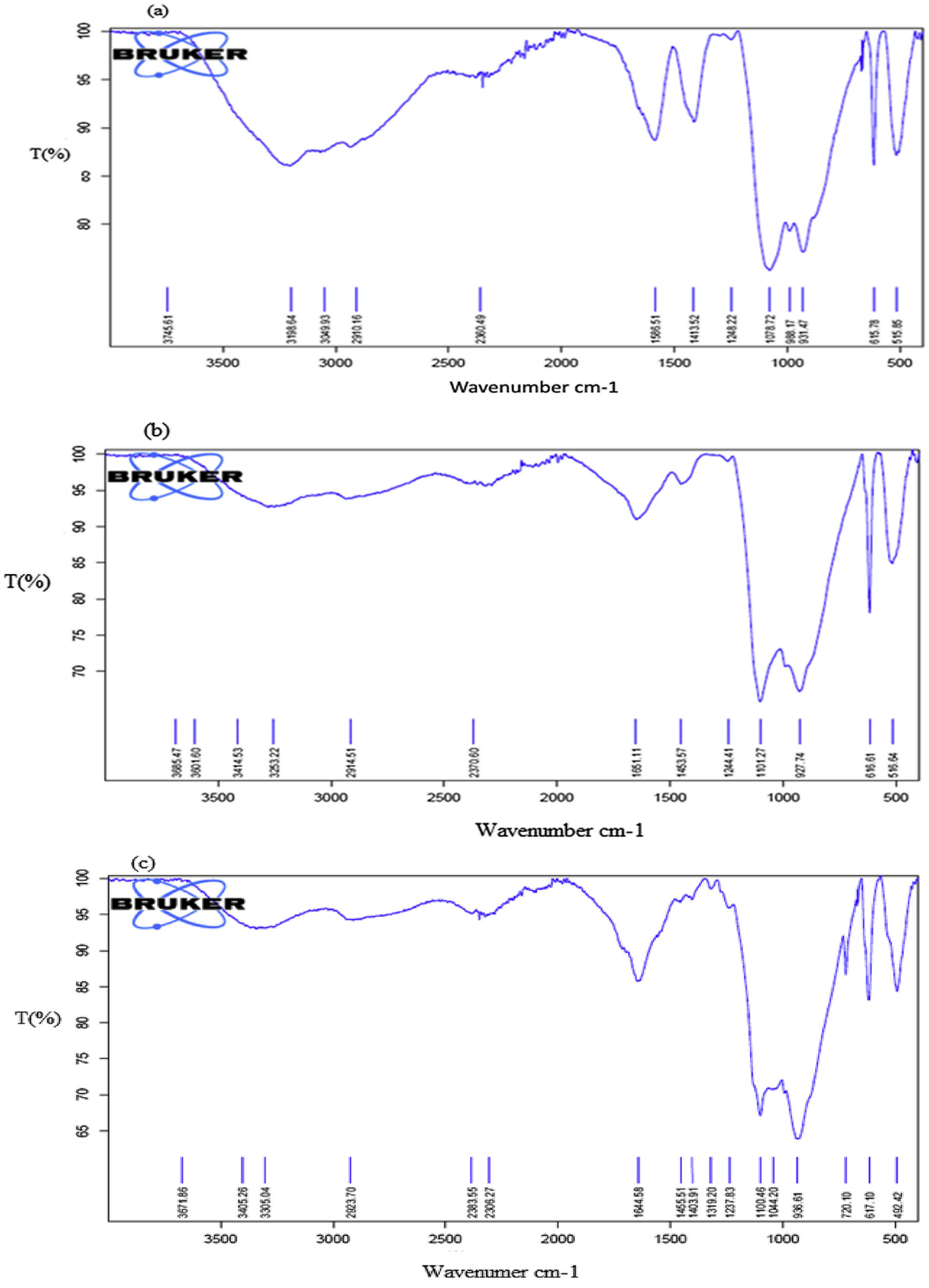

Fig. 5. FTIR spectra kinetic of ICW treated in optimal conditions (a) after 3 days(b) after 5 days and (c) after 10 days.

days (Fig. 6). The spectrophotometricanalysis of culture supernatant after oxidation with FR and biological degradation with fungi showed significant reduction in absorbance, COD and concentration of PVA (Fig. 6a). In fact, the final absorbance of the supernatant was about 0.18 ± 0.01 (Fig. 1. in Supplementary material). The maximum of the absorbance was detected at λ = 317 nm in the UV–vis spectra. The

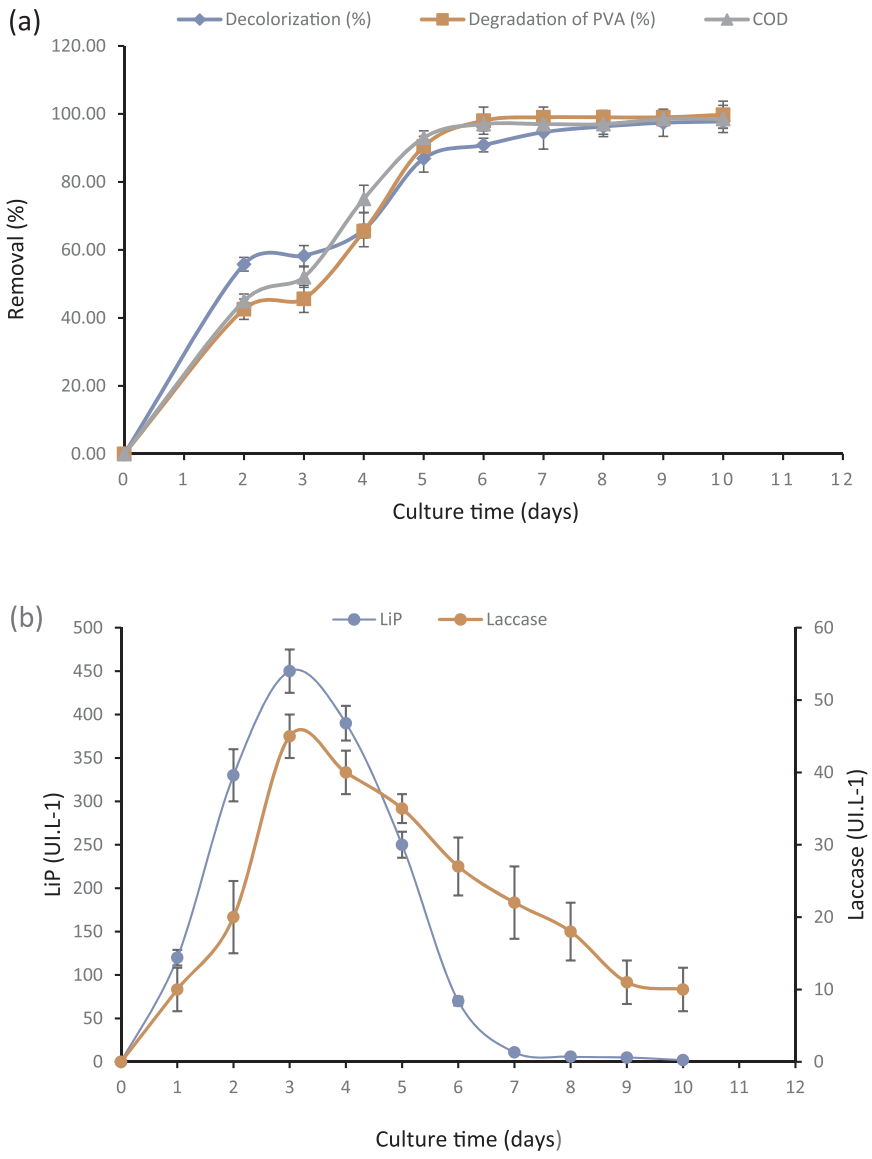

Fig. 6. Evolution of (a) COD, color and PVA removal and (b)LiP and laccase activities produced by the consortium of fungi.

peak decreases as the day progresses, which shows the decolourization of ICW using the FR process combined to the fungal consortium. At the end of the 10th day of operation, the peak at 317 nm attains nearly zero, which shows almost complete decolourization of the ICW.

The validation test confirmed that the supplemented nutrients nitrogen and phosphorus in medium of culture should be1.4 g $L^{-1}$and 1.2 g L^{-1}, respectively. The higher color, PVA and COD removal were observed after the fifth day. The degradation rate for COD, PVA and color removal increased to 97.8%, 98.5% and 99.75%, respectively. The increased biodegradation rate might be due to the presence of nutrients in medium of culture, which allowed to the fungi of the consortium, cellular growth and production of enzymes. Generally, the biodegradation of PVA containing wastewater is characterized by its limited rate and extent of degradation due to the higher value of COD and the lack of nutrients why the correction of the medium with nutrients and the process optimization were generally adopted [24]. Therefore, the optimization of nutrients is necessary to avoid economic lost. The optimized concentration of nitrogen allowed an important cellular growth and high rate of biodegradation. Our results are consistent with the predicted information from RSM which confirm that $(NH_4)_2SO_4$ is a significant factor.

Besides, using NaOH extraction method showed that decolorization is linked to biodegradation effect. In fact, UV–vis results showed that NaOH-extractable color substance were negligible with an absorbance of 0.058 at 317 nm. Thus, decolorization is due to biological activities of enzymes and non-to adsorption mechanism.

3.4.1. Enzymatic analysis

Adequacy of the model was also checked by means of the kinetic of the linolytic enzymes. The enzymatic activities of the fungalconsortium showed that the consortium was able to grow in the supplemented media of ICW.As shown in Fig. 6b, the important production of laccase and LiP were in the third day of the culture with highest activities of 45 UI L^{-1} and 450 UI L^{-1}, respectively and decreased in the fourth day. We can suggest that LiP and laccase catalyzed the oxidative breakdown of ICW. However, the MnP activity was not detected in the culture. The absence of MnP suggested that the experimental conditions did not induce the enzyme activity and inhibited its secretion.

Huang et al. showed that the degradation of the solution of PVA by a combined FR and *Phanerochaete chrysosporium* treatment was only 728% [34]. Also, Larking et al. [33] indicated that the PVA remaining was less than 20% for the degradation of a solution containing PVA after preoxidation with FR and biological treatment. These results were in accordance with the analysis made in this study with 985% of PVA

removal. In fact, the effectiveness of this process comparing to others could be related to the mixed culture that overcomes the limitations of pure cultures. This study suggested the presence of synergy between the fungi that composed the consortium. Other studies involved the effectiveness of microbial consortium in order to overcome the shortcoming of pure culture. Ayed at al [52]. used a developed consortium of bacteria which achieved a higher reduction in color (92.22%) and COD removal (84.83%) in less time (48 h) comparing to the biodegradation with pure cultures. They also suggested that laccase and LiP were mostly involved in the process of decolorization of the azo dye.

In the present study, the induction of LiP and laccase were strongly indicated that they could use the ICW like a source of carbon to be degraded and cleaved into simple biodegraded metabolites. Several studies indicated that laccase was involved in the oxidation of PVA. In fact, Larking et al. [33] showed that the preoxidation of the PVA resulted in greateroverall degradation compared to the degradation obtainedwithout an FR treatment. However, others studies reported that only Manganese peroxidase was involved in the degradation of PVA and neither laccase nor LiP were detected [34].

As previously reported, oxidation of PVA could be a good alternative to have biodegradable molecules. The oxidation of PVA by persulfate activated with heat, Fe^{2+}, andzero-valent iron was very efficient to enhance the biodegradability of PVA [25]. In fact, vinyl acetic acid was detected by GC–MS after oxidation of PVA. Xiao et al. [53] observed that the pretreatment of PVA processing wastewater by FR permitted an increasing removal of COD equal to 61.4%, and an enhancing ratio of biodegradability (BOD_5/COD) from 9.7% to 68.3%. Therefore, FR is suitable for the pretreatment ofthe refractory PVA wastewater to enhance its biodegradability.

Previous works indicated that the effective biodegradability of PVA containing wastewater was improved by the application of ionizing radiation pretreatment [54]. The overall degradation efficiency of textile and chemical wastewater was generally achieved by the combination treatment. FR showed large advantages, however, the application of the process meets numerous limitations like the low pH value and the large amounts of iron-containing sludge produced in the treated effluent.

4. Conclusion

In this study, the CCD selected as a RSM proved to be suitable for performing biodegradation in complex medium like ICW by more than one microorganism in hostile medium. This consortium was effective in the bioremediation of ICW by the production of effective enzymes and had a maximum growth with 1.4 g L^{-1} of $(NH_4)_2SO_4$ and 1.2 g L^{-1} of phosphorus after 5 days of growth's culture at 28 °C and an optimum pH of 5.5. The combination of FR with biological process is suitable for wastewater showing a very low biodegradability. A better understanding of the mechanisms used by the fungi will help to apply this process for a large scale.

Acknowledgements

The authors wish to acknowledge the Ministry of Higher Education and Scientific Research in Tunisia, which has facilitated the carried work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.jhazmat.2018.06.050.

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