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Phenol Removal by a Sequential Combined Fenton-Enzymatic Process

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ABSTRACT

A two-stage process for the treatment of phenol by Fenton reaction coupled with enzymatic polymerization was investigated. The study was conducted on synthetic and industrial wastewaters containing phenol. The pretreatment of this effluent was carried out by the Fenton's reaction followed by enzymatic treatment with immobilized turnip peroxidase. Results showed that enzymatic treatment was an efficient complementary process to eliminate toxic compounds that were generated by the Fenton radical oxidation. Both processes were performed under optimal conditions, starting from a concentrated phenol solution of 100 mg L^{-1} which was pretreated by the Fenton reaction during 120 minutes at pH 3 and 40°C, in the presence of iron(II) (5 mg L^{-1}) and hydrogen peroxide 9mM. Phenol concentration after the Fenton treatment decreased to 40 mg L^{-1}. Other compounds such as hydroquinone, catechol and benzoquinone were also present. Residual phenol, catechol and hydroquinone were eliminated by the immobilized turnip peroxidase treatment at pH 7 and 40°C, during 165 minutes by adding H_2O_2 (10.6 mM) and 5 U of immobilized peroxidase. Benzoquinone was eliminated by coagulation-precipitation with chitosan (4g L^{-1}). The global phenol removal by the combined process was 99.7% with almost total elimination of catechol, hydroquinone and benzoquinone. The application of the combined treatment to a pharmaceutical effluent containing initially 56 mg L^{-1} of phenol was also successful. More than 99.3% of phenol was eliminated after 120 and 165 minutes of Fenton and enzymatic processes, consecutively; and more than 72% decrease in COD and 66.7% in BOD_5 were obtained.

INTRODUCTION

Wastewaters resulting from the widespread use of phenols in chemical, coke, paper, and pharmaceutical industries pose serious threat to public health, since this type of compounds is considered as carcinogenic and dangerous even when they are present at trace levels in the environment (Kujawski et al. 2004, ATSDR 2009). Various technologies for the elimination of phenols from water can be implemented. Separation techniques such as distillation (Galceran et Santos 1988), liquid-liquid extraction (Palmaa et al. 2007), adsorption (Anisuzzaman et al. 2016, Girodsa 2009, Jie Ren 2013, Jia-Qian Jiang 2012), membrane pervaporation and membrane-solvent extraction (Busca et al. 2008) were thoroughly studied. Destruction technologies such as thermal oxidation, catalytic oxidation, photocatalytic destruction and biofiltration (Busca et al. 2008) as well as biological techniques have been also considered (Mohsina & Khalil-ur-Rehman 2009, Bansal & Kanwar 2013). However, these technologies have some limitations in terms of efficiency, flexibility or cost effectiveness (Busca et al. 2008).

The Fenton technique is an advanced oxidation process, which has been widely used for the elimination of phenols from wastewaters (Bautista et al. 2008, Babuponnusami & Muthukumar 2014). Iron and hydrogen peroxide react together to generate some hydroxyl radicals as shown in the following equations:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad ...(1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}OOH + H^+ \quad ...(2)$$

Hydroxyl radicals react with the pollutants to be oxidized. The efficiency of the Fenton's process depends on H_2O_2, Fe^{2+} concentrations and the pH of the reaction. Typical Fe:H_2O_2 ratios are 1:5-10 wt/wt, though when iron levels are in the range 25-50 mg L^{-1}, excessive reaction times (10-24 hours) are required. Values of pH should be in the range 3 to 5 to avoid iron precipitation. The Fenton's reagent is

most effective as a pretreatment tool, where CODs are greater than 500 mgL^{-1} (Babuponnusami & Muthukumar 2014, Kos et al. 2010).

Nevertheless, aromatic compounds such as hydroquinone, catechol and benzoquinone could be generated during phenol oxidation, which gives toxicity levels much higher than those generated by phenol (Ibtihage 2009) as indicated in Fig. 1.

This disadvantage can be diminished by the help of an enzymatic process. Indeed, phenol could be polymerized to biphenols via oxydoreduction by enzymes, such as peroxidase (Gaspar et al. 1982). In the same way, as indicated in Fig. 2, it has the capacity to catalyse the oxidation of hydroquinone to p-benzoquinone (Manjeet & Mani 1985, Noritomi et al. 1988) and catechol to semiquinone (Mason et al. 1961, Kalyanaraman & Sealy 1982) or o-benzoquinone (Sadler 1988, Kang et al. 2002) respectively. The latter could be removed by physical means such as coagulation-precipitation onto chitosan (Beeta 2011).

The aim of this study was, therefore, to develop effective process for phenol degradation by a combination of the Fenton reaction and enzymatic polymerization in order to obtain the highest yield of removal with minimum residual toxic by-products. Experiments were conducted on synthetic and industrial wastes containing different phenol concentrations under optimal conditions. The Fenton treatment constituted the first step and was followed by enzymatic polymerization involving immobilized peroxidise, which finally gave a precipitate that was separated by microfiltration (Fig. 3). The efficiency of the process was evaluated through the estimation by HPLC analysis of phenol and by-products after treatment.

MATERIALS AND METHODS

Chemical Products

Turnip Peroxidase (TP) was isolated from Turnip. Hydrogen peroxide (30% w/v) and crystallized phenol were purchased from Panreac, the iron source, ferrous sulphate from Fluka, sodium alginate from Sigma-Aldrich (Norway), and hexahydrated calcium chloride from BDH (UK). Potassium chloride buffer (10 mM, pH 2), potassium biphthalate buffer (10 mM, pH 3.0-5.0), potassium phosphate buffer (10 mM, pH 6.0 and 7.0), potassium chloride and boric acid buffer (10 mM, pH 8.0-10.0), citric acid and disodium phosphate buffer (0.5 M, pH 2.0 and 7 respectively) were purchased from Sigma-Aldrich. 4-aminoantipyrine (Am-NH_2) and potassium ferricyanide were purchased from Fluka Chemika. Chitosan, formic acid, oxalic acid, catechol, hydroquinone and p-benzoquinone were purchased from Sigma-Aldrich. All other chemical were of analytical grade and used without further purification.

Materials

A laboratory centrifuge type 422Mark, Mini Chopper Mixer-Grinder-commercial type Clearline, Electronic water bath type Memmert winb 7-45, A pH meter type HANNA equipped with a combined glass electrode were used. Calibration buffer solutions pH 4.00, 7.00 and 10.00 were from BDH Inc. (Toronto, Ontario). The heating bath was a Memmert model-Type WNB 29). Magnetic stirrers Micro V (-1100 rpm, 4805-00 model) and Mag VWR Stirrers (100-1500 rpm, model 82026-764) were from VWR International Inc. Magnetic stir bars of different sizes were obtained from Cole-Parmer from Canada Inc. (Montreal, QC). PerkinElmer Series 200HPLC Systems were equipped with a C_{18} column.

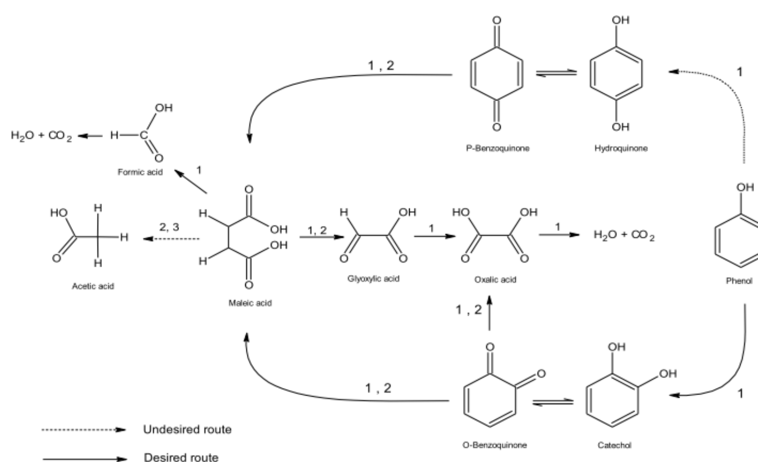

Fig. 1: Mechanism of phenol degradation by Fenton oxidation with reaction steps including 1. Oxidation 2. c-c bond cleavage. 3. Transfer hydrogenation.

BOD_5 measurements were made using an OxiTop Box: Thermostat box with forced air circulation 230V 208432.

Methods

Extraction and partial purification of peroxidase from turnip roots: Fresh turnip roots were peeled, minced and then mixed with distilled water (1:2 ratio turnip mass in g/distilled water in mL). The mixture was filtered through double-layered cotton gauze to remove large particles, namely poorly ground. The filtrate was labelled as crude extract turnip peroxidase. Partially purified turnip peroxidase (TP) was obtained by precipitation with cold acetone (-20°C; 2:1 ratio acetone/crude extract). The precipitate was collected by centrifugation at 6,000 rpm for 10 min, redissolved in 10 mM potassium phosphate buffer (pH 7.0). The semi-purified TP was stored at 4°C and warmed to room temperature immediately prior to use (Cutler 1996).

Immobilization by entrapment method: Immobilization was achieved by dissolving 0.15g of sodium alginate in 10 mL of soluble TP. The solution was agitated for 1 to 1.5 hours until obtaining a homogeneous viscous mixture. The mixture was pillowed for 20 min to avoid any air bubbles. Calcium alginate capsules were prepared by extrusion using a simple one-step process similar to that described by (Guisan Jose 2006, Nigma et al. 1988). The entrapped TP beads were prepared by pouring the mixture drop-wise through a syringe needle (20 G) in 50 mL of a $CaCl_2$ solution (0.05M) under constant magnetic agitation. Beads were left in $CaCl_2$ solution for 1 hour, then washed with distilled water and stored in a buffer solution (pH 7.0) at 4°C.

Activity measurement of immobilized enzyme: The enzyme activity (EA) was assayed by the Am-NH_2 method (Nicell & wright 1997) as follows: 0.2 mL of soluble enzyme (approx. 25 beads of immobilized TP) was added to a mixture of phenol (0.1 M), Am-NH2 (0.01M), H_2O_2(0.1mM) and potassium phosphate buffer (10 mM, pH 7.0) in a 50 mL flask. The active enzyme concentration is proportional to the colour development rate measured at 517nm (Sadasivam & Manickan 2004).

Phenol degradation by Fenton and enzymatic processes: All experiments were carried out in closed flasks at constant temperature. The reaction mixture for Fenton batch process consisted of:1 mL of hydrogen peroxide (4-24 mM), 1 mL of phenol solution (20-140 mg L^{-1}), 1 mL of ferrous sulfate (1-720 mgL^{-1}) and 3 mL of buffer solution (0.5 M). Enzymatic degradation was carried out at fixed activity of

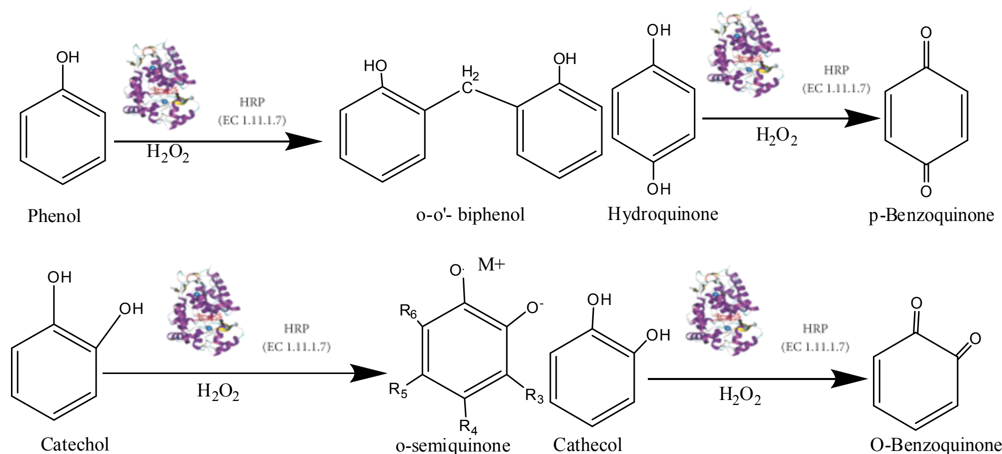

Fig. 2: Enzymatic transformations of phenol, hydroquinone and catechol by horseradish peroxidase (HRP).

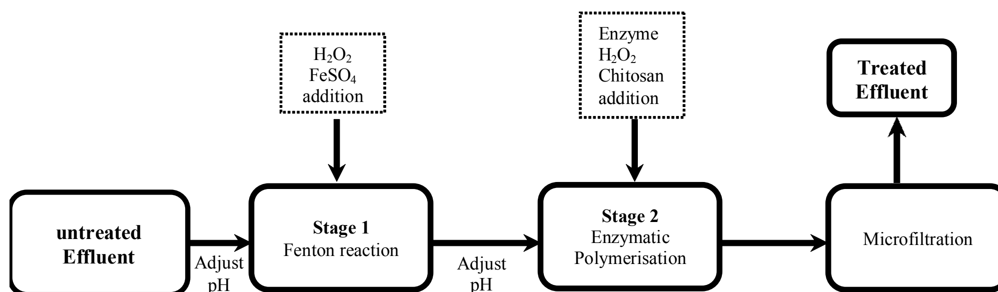

Fig. 3: Schematic representation of the Fenton-enzymatic combined process for phenol degradation.

alginate immobilized TP (1-10 U), in a mixture of 1 mL of hydrogen peroxide (1-15.4 mM), 1 mL of phenol (20- 140 mg L^{-1}) and 2.8 mL of buffer solution (0.01 M). The impact of the different factors was studied by varying one parameter at a time. The obtained optimal value was then selected for the subsequent experiment.

Organic compounds analysis: Reaction samples were immediately analysed to avoid any further evolution. Phenol and aromatic by-products were identified and quantified immediately after sampling by means of HPLC (Perkin Elmer 200) in isocratic mode. A Microsorb C_{18} column (MV 100, 15 cm long, 4.6 mm diameter) was used as the stationary phase and 1 mL min^{-1} of 4 mM aqueous sulphuric solution as the mobile phase. A UV detector was used at a wavelength of 210 nm to quantify phenol, catechol, and hydroquinone and at 246 nm for p-benzoquinone. Short-chain organic acids were analysed by ion chromatography with chemical suppression using a conductivity detector. A Metrosep A supp 5-250 column (25 cm long, 4 mm diameter) was used as the stationary phase and 0.7 mL min^{-1} of an aqueous solution containing 3.2 mM of Na_2CO_3 and 1mM of $NaHCO_3$ as the mobile phase. Values of 0.01 and 0.1 mg L^{-1} detection limits were established for aromatic compounds and short-organic acids, respectively. Phenol and organic compounds removal was calculated by the following equation:

$$R(\%)=\frac{C(0)-C(t)}{C(0)}\times 100 \qquad ...(3)$$

Where, R: removal yield (%)

C(0): initial concentration of untreated solution (mg L^{-1})

C(t): concentration of the treated solution at a given time t (mg L^{-1})

COD and BOD analysis: Chemical oxygen demand (COD) was measured according to the standard protocol (APHA 1999) with an automatic COD analyser of LoviBond (Dortmund, Germany). Biochemical oxygen demand (BOD) was measured using a Box OxiTop system.

Phenol index: Phenols react with Am-NH_2 at pH 10 in the presence of potassium hexacyanoferrate (III) forming a coloured complex with the Am-NH_2. This colour complex is extracted from the aqueous phase with chloroform and the absorbance is then measured at 460 nm. The phenol index is expressed as milligrams of phenol per litre (ISO 1990).

Combined Fenton-enzymatic process treatment: To obtain the highest conversion yield, a two-stage process was employed (Fig. 3). In stage 1, phenol degradation by the Fenton process was carried out under the optimal conditions as described in Table 2. The reaction was conducted for a controlled time in order to reach the optimal phenol concentration required for enzymatic treatment by free or immobilized TP. The pH value was adjusted between stages from 3.0 to the optimal value (7.0) for the enzymatic treatment by adding sodium hydroxide. Hydrogen peroxide and TP were added at the optimal levels which are mentioned in Table 2. The influence of the chitosan mass to be added in the second stage was studied in order to minimize the final concentration of benzoquinone. In the final stage, the polymerisation compound was filtered through a 0.45µm membrane.

Application of the combined process to industrial wastewater sample: Phenol enters in the formulation of a wide variety of drugs and, therefore, could be present as a major or a minor component in pharmaceutical wastewaters (Rajender Singh Rana et al. 2014). A sample of non-treated wastewater from an antibiotic industrial complex (Saidal, Medea, Algeria) was withdrawn and filtered in order to analyse the presence of phenol by HPLC as described earlier. A phenol solution (200 mg L^{-1}) was used as a standard.

RESULTS AND DISCUSSION

Optimal Conditions of Phenol Elimination by the Fenton Reaction

Phenol removal by Fenton and enzymatic processes was studied by varying different parameters, such as initial concentrations (hydrogen peroxide, ferrous ion) and contact time at constant phenol concentration (100 mg L^{-1}), pH3 and temperature 40°C (Babuponnusami & Muthukumar 2014). The choice of optimal values of these parameters was guided by obtaining the maximal rate and the highest removal yield of phenol at the end of the treatment.

Hydrogen peroxide concentration: Hydrogen peroxide concentration was varied between 1 and 15mM, while keeping all other parameters constant: phenol (100 mg L^{-1}); $FeSO_4$ (23 mg L^{-1}), pH 3 and contact time (2 hours). The obtained results are summarized in Fig. 4. Maximum removal yield under the specified conditions was 54% after two hours of treatment. No significant variation of the removal yield was observed above 1 mM H_2O_2. Taking into account loss and decrease of reactants during the Fenton oxidation, an excess of H2O2 was considered for the rest of the optimization process, namely an initial concentration of 9mM, as also discussed thereafter, and which corresponded to a ratio of $[H_2O_2]/[phenol]$ of 8.2.

Ferrous ion concentration: The effect of ferrous ions (Fe^{2+}) on the reaction yield was studied in a concentration range between 1 and 20 mg L^{-1}. From Fig. 5, it could be noticed that maximal phenol reduction (60%) was obtained for Fe^{2+}

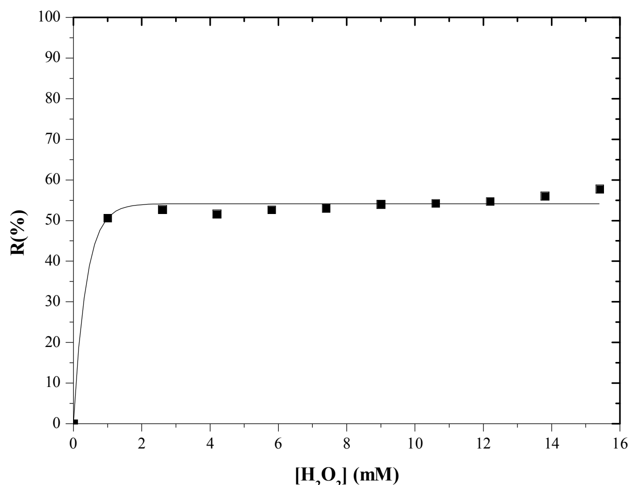

Fig. 4: Effect of the initial concentration of H_2O_2 on phenol removal by the Fenton reaction. pH = 3, T = 40°C, [Phenol]=100 mg L^{-1}, ;[$FeSO_4$] = 23 mg L^{-1}, Contact time = 2 hours.

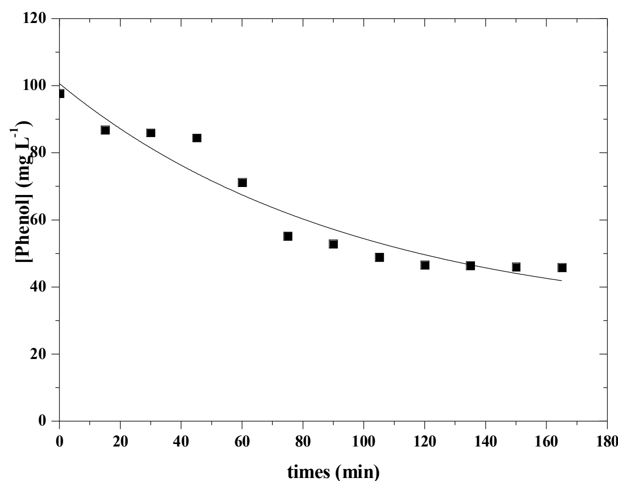

Fig. 6: Kinetics of phenol degradation by the Fenton process under optimal conditions. [H_2O_2] = 9 mM, [Phenol] = 100 mgL^{-1}, pH = 3, T = 40°C, [$FeSO_4$] = 5 mg L^{-1}.

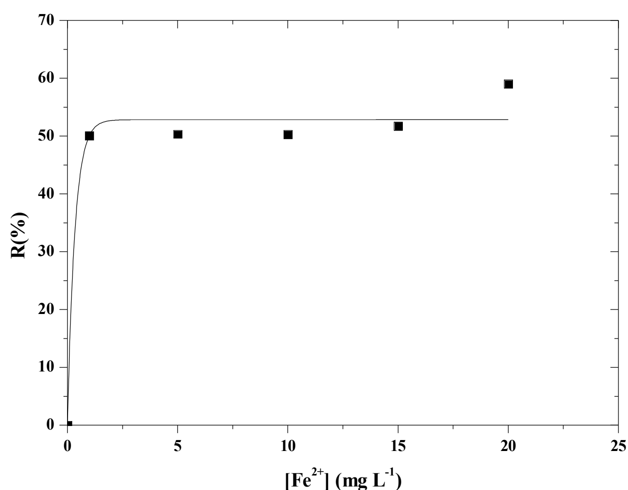

Fig. 5: Effect of ferrous ion concentration on phenol removal by the Fenton reaction. pH = 3, T = 40°C, [Phenol] = 100 mg L^{-1}, [H_2O_2] = 9 mM, contact time = 2 hours.

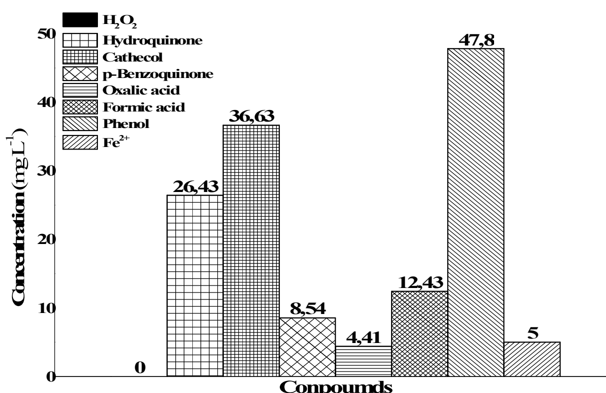

Fig. 7: Composition after Fenton reaction under optimal conditions. pH = 3, T = 40°C, [H2O_2] = 9 mM, [Phenol] = 100 mg L^{-1}, [$FeSO_4$] = 5 mg L^{-1}, contact time = 2 hours.

above 5 mg L^{-1}. Nevertheless, for environmental considerations (OJAR 2006), the concentration of ferrous ions selected for the subsequent experiments was 5 mg L^{-1} following the standards of industrial wastewater. The molar ratio [Fe^{2+}]/[phenol] was therefore 0.081.

Kinetics of phenol degradation and by-products generation by the Fenton reaction: In the first stage, the Fenton reaction was carried out under optimal conditions at pH 3.0, a temperature of 40°C, for the following ratio of phenol (1.1 mM): Fe^{+2}:H_2O_2, 1:12:8.5 and a reaction time of 120 minutes. The oxidation kinetics was analysed by determining the concentrations of phenol and the generated compounds at the end of the process. Fig. 6 shows that more than 50% of phenol was transformed after 2 hours under optimal conditions; no further significant evolution was noticed up to 165 minutes. From HPLC analysis, about 54% of the initial phenol was converted to a mixture of catechol (36.63 mg L^{-1}), hydroquinone (26.4 mg L^{-1}) and benzoquinone (8.54 mg L^{-1}) as major components, while oxalic (4.41 mg L^{-1}) and formic acids (12.43 mg L^{-1}) at lower concentrations (Fig. 7).

Optimal Conditions of Phenol Elimination by Alginate Entrapped TP

A one factor at a time experimental strategy was conducted to optimize phenol degradation by alginate entrapped TP. The optimized parameters were: pH, total enzyme activity, initial concentration of hydrogen peroxide and contact time.

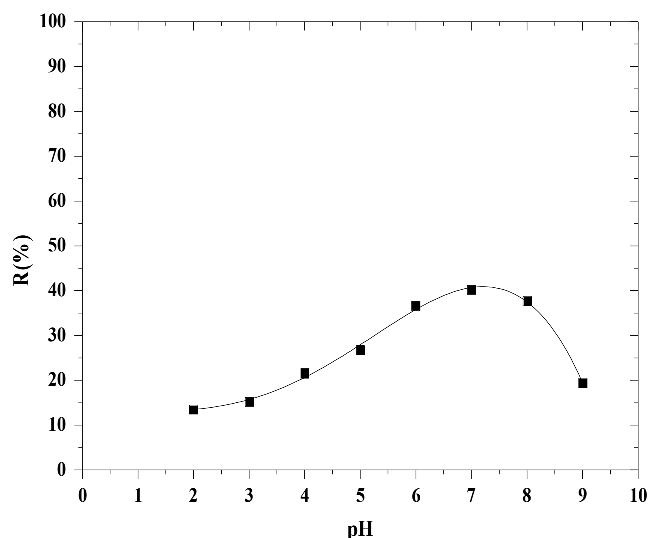

Fig. 8: Effect of pH on phenol removal by alginate entrapped TP. T = 40°C, [Phenol] = 40 mg L^{-1}, EA = 5.064 U, [H_2O_2] = 13 mM, contact time = 2 hours.

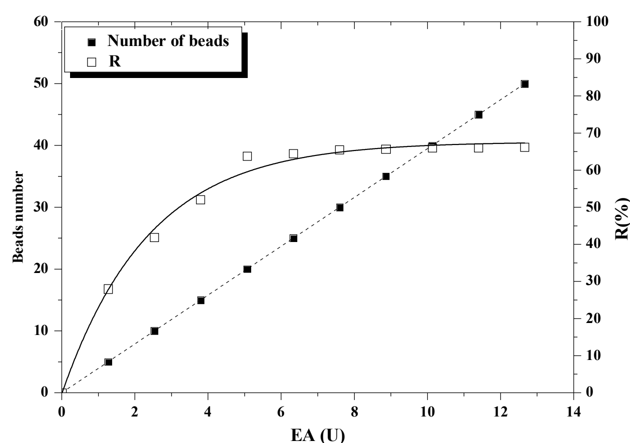

Fig. 9: Effect of enzyme activity on phenol removal by alginate entrapped TP. pH = 7, T = 40°C, [Phenol] = 40 mg L^{-1}, EA = 5 U, [H_2O_2] = 13 mM, contact time = 2 hours.

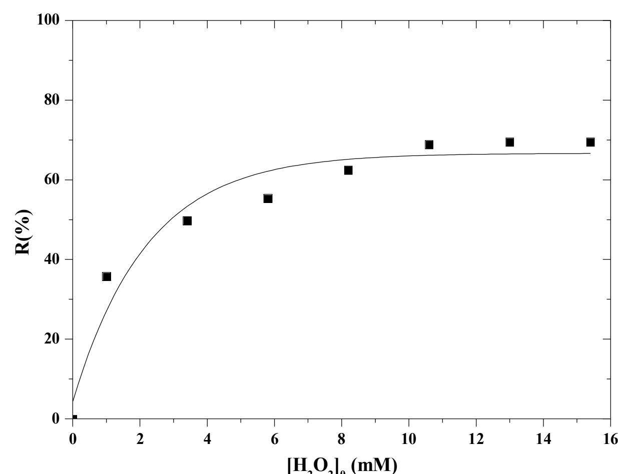

Fig. 10: Effect of hydrogen peroxide concentration on phenol removal by alginate entrapped TP. T = 40 °C, pH = 7, [Phenol] = 40 mg L^{-1}, Contact time = 2 hours.

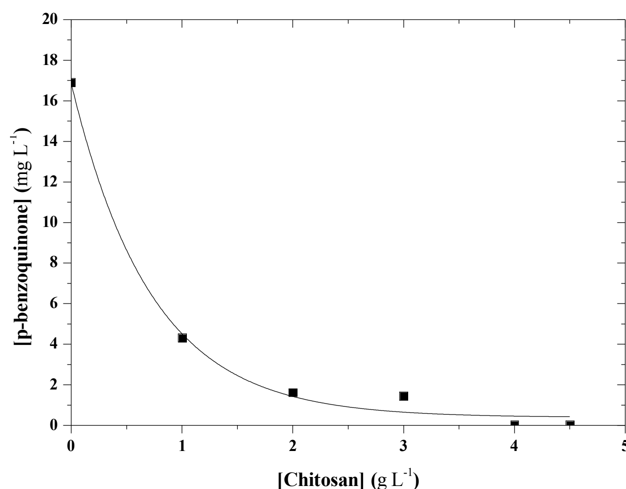

Fig. 11: Effect of chitosan concentration on p-benzoquinone removal during the second stage of the Fenton/immobilized TP combined treatment. pH = 7, T = 40°C, [H_2O_2] = 10.6 mM, [Phenol] = 40 mg L^{-1}, EA = 5.0 U, contact time = 165 min.

Temperature was kept constant at an optimal value of 40°C (Yasha & Qayyum 2006). Since the main objective of the study was the design of a combined Fenton-enzymatic process, the initial phenol concentration was kept at the level which was obtained at the end of the Fenton oxidation 40 mg L^{-1}.

Effect of pH: The pH is a critical parameter to be taken into account during phenol degradation process by a peroxidase. This parameter acts both on the enzyme and on the ionization state of phenol (Fersht 1984, Illanes 2008, Seyhan et al. 2002, Siva 2009). The corresponding results are displayed in Fig. 8, showing that the highest removal yield was obtained for pH values between 6 and 8; at pH 7, more than 40% of the initial phenol was removed.

Effect of the enzyme activity: As shown in Fig. 9, an increase of the removal yield with total enzyme activity was observed. This result was expected, since the availability of enzyme site induces the attraction of the substrate molecules and subsequently the number of molecules converted per unit time would increase. The best degradation yield (63.7%) was obtained when total enzyme activity was about 5 U which corresponds to approximately 20 beads (Fig. 9). Above this value, the variation in removal yield was not significant.

Effect of hydrogen peroxide concentration: After determining the optimal values of pH and enzyme activity, the effect of hydrogen peroxide concentration on phenol degradation was studied. The corresponding results are shown

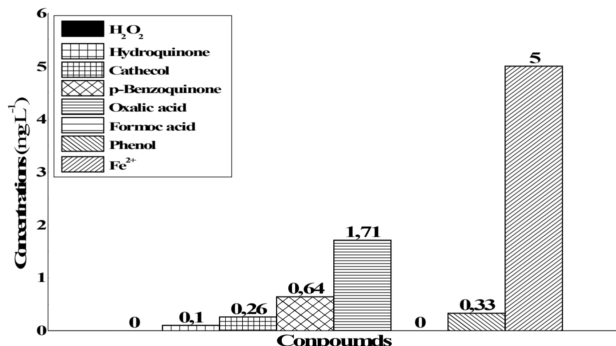

Fig. 12: Composition after enzymatic treatment by entrapped TP under optimal experimental conditions. pH = 7, T = 40°C, $[H_2O_2]$ = 10.6 mM, [Phenol] = 40 mgL^{-1}, EA = 5.0U, [Chitosan] = 4 g L^{-1}, contact time = 165 minutes.

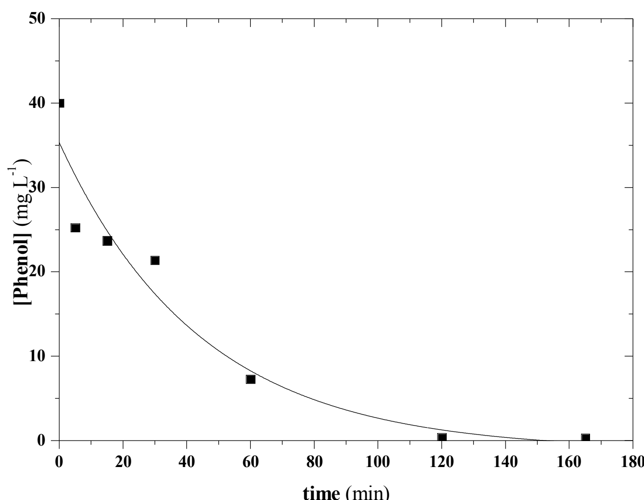

Fig. 13: Kinetics of phenol removal by alginate entrapped TP. T = 40°C, pH = 7, [Phenol] = 40 mgL^{-1}, $[H_2O_2]$ = 10.6 mM, EA = 5.0U, [alginate] = 15 mgL^{-1}, [Chitosan] = 4 g L^{-1}.

in Fig. 10. It can be noticed that phenol biodegradation increased when the concentration of hydrogen peroxide was varied from 2 to 10mM. A maximal degradation yield of 69% was obtained at 10.6 mM, which corresponded to an optimal ratio of H_2O_2 to phenol of 22.

Optimal conditions for phenol degradation by the proposed combined process coupling Fenton and enzymatic processes are summarized in Table 1.

Optimal Chitosan Concentration for p-Benzoquinone Coagulation-Precipitation

Benzoquinone was separated by coagulation-precipitation in the presence of chitosan. In order to increase the process efficiency, the effect of chitosan concentration was studied. Fig. 11 shows that optimal chitosan concentration, namely giving the highest yield of benzoquinone was close to 4.0 g L^{-1}. At the end of the enzymatic process treatment (165 minutes), more than 99% of p-benzoquinone was removed from the medium. Therefore, an optimal chitosan concentration of 4 g L^{-1} could be selected for the combined treatment.

Kinetics of Phenol Degradation by Combined Fenton/ Immobilized TP Process Under Optimal Conditions

Regarding the complementary aspects of the two processes, the combined Fenton/enzymatic system was also explored to determine its effectiveness for phenol degradation and elimination of toxic by-products (hydroquinone, benzoquinone, catechol) generated by the Fenton oxidation. For this, a 100 mgL^{-1} phenol solution was pretreated by the Fenton process under optimal conditions, namely pH 3, T=40°C, [phenol] =100 mgL^{-1}, $[H_2O_2]$ = 9 mM, $[Fe^{2+}]$ = 5 mgL^{-1}. The iron sludge was allowed to settle and the supernatant of the mixture was used for the enzymatic treatment, after pH adjustment. Enzymatic treatment was performed under optimal conditions: pH 7, T = 40°C, $[H_2O_2]$ = 10.6mM, EA = 5U.

Being outside the scope of the enzymatic treatment due to its chemical structure, benzoquinone was removed by a coagulation-precipitation process involving chitosan. The treatment of the residual compounds by entrapped TP in the second stage was conducted by adding hydrogen peroxide at a phenol to H_2O_2 molar ratio of 1:25. Chitosan was also added at a concentration of 4 gL^{-1} at the start of the enzymatic process. The process was conducted for a total duration of 120 (Fenton process) + 165(enzymatic treatment) minutes.

At the end of each batch process, samples were filtered and analysed for phenol, hydrogen peroxide and residual organic compounds concentrations by HPLC.

At the end of the first stage, the oxidation by the Fenton process under optimal conditions resulted in the degradation of 54% of phenol after 120 minutes, with the generation of hydroquinone, benzoquinone and catechol 26.4, 8.54 and 36.63 mg L^{-1} respectively, and lower concentrations of

Table 1: Optimal parameters for phenol degradation by alginate entrapped TP and Fenton reaction.

Parameter	Unit	Fenton reaction	Alginate entrapped TP
pH	-	3	7
Temperature	°C	40	40
$[H_2O_2]$	mM	9	10.6
$[FeSO_4]$	mgL^{-1}	5	-
EA	U	-	5
[Phenol]	mgL^{-1}	100	40
$[H_2O_2]$/[Phenol]	mM mM^{-1}	8.5	22
$[Fe^{2+}]$/[Phenol]	mM mM^{-1}	0.08	-
Removal	%	54	77
Contact time	min	120	165

Table 2: Results of the treatment of synthetic phenol solution by the combined Fenton-enzymatic process.

Characteristics of the wastewater	Unit	At the end of Fenton process	At the end of enzymatic process	Standards for industrial wastewater	References for the standard values
Phenol	mg L^{-1}	40	0.33	0.1	IRIS 2002
BOD_5	mg L^{-1}	140	40	40	OJAR 2006
COD	mg L^{-1}	132	20	130	OJAR 2006
DBO_5/DCO	-	1.06	2	3	-
Phenol index	mg L^{-1}	4.27	0.3	0.5	OJAR 2006
Suspended solid	mg L^{-1}	0	40	40	OJAR 2006
pH	-	3	7	7	OJAR 2006
Temperature	°C	40	40	40	OJAR 2006
Hydroquinone	mg L^{-1}	0.29	0.1	0.1	-
Catechol	mg L^{-1}	7.47	0.26		-
p-Benzoquinone	mg L^{-1}	7.73	0.64	< 3	OJAR 2006
Iron	mg L^{-1}	5	5	5	OJAR 2006

Table 3: Characteristics of pharmaceutical wastewater before and after combined Fenton-enzymatic treatment.

Characteristics of the wastewater	Unit	Before coupled treatment	After coupled treatment	Removal (%)	Standards for industrial wastewater	References for the standard values
Phenol	mgL^{-1}	56	0.39	99.3	0.1	IRIS 2002
BOD_5	mg L^{-1}	15	5	66.7	35	OJAR 2006
COD	mg L^{-1}	44	12	72.7	120	OJAR 2006
BOD_5/COD	-	0.34	0.42	-	0.2	-
Phenol index	mgL^{-1}	4.27	0.28	81.3	0.3	OJAR 2006
pH	-	5.45	7.05	-	6.5 - 8.5	OJAR 2006
Temperature	°C	40	23	-	30	OJAR 2006
Total iron	mg L^{-1}	5	5	-	10	OJAR 2006
Hydroquinone	mg L^{-1}	0	1.49	-	1	-
Catechol	mg L^{-1}	0	1.93	-	3.5	-
p-Benzoquinone	mg L^{-1}	0	0	-	0.1	OJAR 2006
Oxalic acid	mg L^{-1}	0	14.75	-	-	OJAR 2006
Formic acid	mg L^{-1}	0	0	-	-	IRIS 2002
Suspended solid	mg L^{-1}	0	40	-	40	OJAR 2006

formic and oxalic acids (Fig. 7). With the combined process, a maximum phenol degradation yield of 99.7% was obtained (Fig. 13). Besides, 92.5% benzoquinone, 99.6% hydroquinone and 99.3% catechol removal yields were obtained (Fig. 12). The corresponding results are collected in Table 2.

Table 2 summarizes the characteristics of the supernatant of a phenolic solution treated by combined Fenton and alginate entrapped TP.

Treatment of Pharmaceutical Effluent Containing Phenol by Fenton and Entrapped TP Process

The feasibility of the combined Fenton/enzymatic process for a pharmaceutical wastewater containing phenol was tested. A volume of 50 mL pharmaceutical effluent containing 56 mg L^{-1} of phenol was subjected to Fenton treatment under optimal conditions (pH = 3, T = 40 °C, $[H_2O_2]$ = 9 mM, $[FeSO_4]$ = 5 mgL^{-1}) for a duration of 120 minutes. After pH adjustment and separation of the precipitate, the resulting solution was treated by alginate entrapped TP under optimal conditions (T = 40°C, $[H_2O_2]$ = 10.6 mM, EA = 5.0 U) and with the optimal amount of chitosan (4 g L^{-1}) was added. The enzymatic process was then conducted for 165 minutes. Results are resumed in Table 3. A global abatement percentage of phenol of 99.3% was achieved. The remaining concentrations of by-products (hydroquinone, benzoquinone and catechol), BOD_5, COD and the phenol index in the supernatant were below the discharge standards as indicated in Table 3. COD and BOD_5 were reduced by more than 72% and 66.7% respectively. Phenol index decreased by 95%, showing a final value in agreement with the environmental standards (OJAR 2006). Total elimination of benzoquinone was also achieved, which shows the efficiency of the separation on chitosan.

CONCLUSION

The feasibility of a sequential combined Fenton-enzymatic process for phenol removal from synthetic and industrial wastewater was proved. The process combined a Fenton oxidation, which was followed by polymerization with alginate-entrapped peroxidase.

The Fenton process was conducted in a controlled way in order to obtain the optimal phenol concentration for the subsequent enzymatic treatment. Nevertheless, pH adjustment, hydrogen peroxide addition and chitosan injection were necessary before the implementation of the second step.

The enzymatic treatment was a complementary process principally directed towards the oxidative polymerization of the residual components (phenol, catechol, hydroquinone) issued from the Fenton process. Benzoquinone which could not be a substrate for the enzymatic reaction was removed by a coagulation-precipitation process with an optimal amount of chitosan added. Thus, the disadvantages of the Fenton process during phenol treatment were totally overcome by a successful enzymatic treatment as illustrated by the obtained results. The relevance of the sequential combined process was also confirmed on an industrial sample of pharmaceutical wastewater. Indeed, analysis showed that the characteristics of the treated effluent were in accordance with the environmental standards.

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