Chloroperoxidase mediated oxidation of chlorinated phenols using electrogenerated hydrogen peroxide

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Abbreviations: 2,4,6-TCP: 2,4,6-trichlorophenol
2,4-DCP: 2,4-dichlorophenol
4-CP: 4-chlorophenol
ABTS: 2,2-azino-bis[3-ethyl-benzothiazoline]-(6)-sulphonic acid
AE: auxiliary electrode
CC C: cathodic chamber control
CP: chlorinated phenols
CPO: chloroperoxidase
DA: direct addition
EG AC: anodic direct oxidation bioelectrochemical processes
EG CC: bioelectrooxidation at the cathodic chamber
EG: electrogeneration
HLT: half-life time
HRP: horse radish peroxidase
P: phenol
PCP: pentachlorophenol
RDE: rotary disk electrode
SCE: saturated calomel electrode
WE: working electrode

Chloroperoxidase (CPO) from *Caldariomyces fumago* catalyses the oxidation of several chlorinated phenols (CP) commonly found in industrial waste waters in the presence of hydrogen peroxide. This study compares the direct addition of hydrogen peroxide (DA) with its continuous electrogeneration (EG) during the enzymatic oxidation of CP. Reaction mixtures were studied containing chemically modified CPO, hydrogen peroxide and the phenolic substrates: phenol (P), 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP),

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2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP), in 100 mM sodium-potassium phosphate buffer pH 6.0, at 25ºC. Results were compared in terms of residual phenol concentration (oxidation efficiency), precipitate formation (removal) and residual enzyme activity (stability). With the electrochemical system evaluated at -620 mV$_{SCE}$, and continuous aeration the maximum H$_2$O$_2$ concentration of 1.2 mM was obtained. Under this conditions and after 4 hrs using EG, no phenol or 4-CP were detected, and 97%, 93% and 88% of 2,4-DCP, 2,4,6-TCP and PCP were degraded, respectively. The use of EG improves enzyme half-life time in comparison to the results obtained by DA.

Non toxic phenols can result from plant degradation into tannin-like compounds which are responsible for flavour and colour of potable water and have natural occurrence in the environment. In contrast, phenolic compounds specially chlorinated phenols are unusual, being very toxic and caustic, for that reason they are considered highly pollutants (Atlow et al. 1984; Carmichael et al. 1985). These compounds are present in the wastewaters generated from industrial activities as such petrochemical, coal-conversion, pharmaceutical, wood preserving, plastic, rubber proofing, pesticide use, iron steel and paper and cellulose bleaching. Large scale coal gasification and carbonization plants are also generators of wastewaters containing large quantities of high toxic phenolic compounds. The United States Environmental Protection Agency regulates the phenol content in the wastewater from less than 1 mg/l to the several thousand mg/l (Denizli et al. 2004).

Conventional methods for phenol removal of industrial wastewater include chemical and photo oxidation, adsorption on activated charcoal, solvent extraction and microbial degradation (Atlow et al. 1984; Rivas et al. 1999). The application of these methods can be easily hindered by their cost, detoxification efficiency in relation to the phenol features and concentration as well as the formation of hazardous by-products. Furthermore, geographic and climate characteristics also affect the efficiency for wastewater treatment processes. This is particularly true concerning biological processes that are more sensible to the aforesaid factors.

Nowadays, environments in which contaminants are merely transferred from one medium to another are no longer acceptable. It is no surprising that biomolecules with oxidizing activity such as ozone or hydrogen peroxide should be so widely used. Both can act individually at high concentrations or in conventional biological treatments including as pre-treatment to degrade toxic, refractory or bio-inhibitory organics, make them more amenable to biodegradation. Hydrogen peroxide can be also used at low concentration in a polishing step with enzymatic reactions using peroxidases. In this approach, hydrogen peroxide can destroy trace levels of recalcitrant organics pollutants that could easily pass through biotreatments (Annachatre and Gheewala, 1996; Yee and Wood, 1997; Wagner and Nicell, 2002).

In the literature there are an extended list of works where the direct addition of hydrogen peroxide is combined with the enzymatic oxidation of phenol and other pollutants. Some of these examples are the biocatalytical oxidations achieved in the case of dyes as methylene blue (Ferreira-Leitão et al. 2003) and phenolic compounds using horseradish peroxidase (HRP) (Nicell et al. 1993; Vasudevan and Li, 1996; Laurenti et al. 2003) or chloroperoxidase (CPO) (La Rotta and Bon, 2002). And in the oxidation of polyaromatic hydrocarbons (PAH's) using manganese peroxidase (Baborová et al. 2006; Eibes et al. 2006).

Nevertheless, the use of hydrogen peroxide in biocatalysis as co-substrate for peroxidase activity is limited due to its inhibitory effect at high concentrations above 25 mM and slow deleterious effects at long time with lower concentrations (Nicell and Wright, 1997). One solution for this problem is the use of very low and controlled hydrogen peroxide supplementation, necessary just for the maintenance of an effective oxidation level, with no inhibitory effects. This can be achieved using direct pulsated addition (DA) or by *in situ* electrogeneration (EG). The electroenzymatic approach provides a significantly lower and easily controllable hydrogen peroxide formation rate than any other so far. EG was already applied to some biocatalytical oxidations, such as, in the asymmetric oxidation of thioanisole by CPO (Lütz et al. 2004), the oxidation of PCP (Kim and Moon, 2005), and the oxidation of dimethylaniline by HRP (Chen and Nobe, 1993).

The purpose of this study was to compare the conventional enzymatic degradation of phenolic compounds yet reported for HRP (Nicell at al. 1993) and CPO (La Rotta and Bon, 2002) with the electroenzymatic oxidation. Results were compared in terms of hydrogen peroxide production, electrochemical conditions, residual phenol concentration (oxidation efficiency), precipitate formation (removal efficiency), and residual enzyme activity after each treatment.

MATERIALS AND METHODS

Biocatalyst features

Chloroperoxidase (EC. 1.11.1.10) from C*aldariomyces fumago* CMI 89362 was produced, purified and chemically modified by cross linking with glutaraldehyde in accordance to previous reports (La Rotta and Bon, 2002; La Rotta et al. 2005). The enzyme concentration was given in mmol·L^{-1} from its extinction coefficient at pH 6.0 (A$_{403\,nm}$= 75.3 mmol^{-1}·L cm^{-1}) (Morris and Hager, 1966). Both initial and residual peroxidase activities were determined based on the oxidation of 2,2-azino-bis[3-ethyl-benzothiazoline]-(6)-

sulphonic acid diamonium salt (ABTS) in the presence of hydrogen peroxide 100 mmol·L^{-1} sodium-potassium phosphate pH 6.0, at 25ºC. The change in absorbance was determined at 405 nm (ε_{405} = 18600 mol^{-1}·cm^{-1}). One unit of peroxidase activity catalyses the oxidation of one μmoles per millilitres of ABTS per minute at pH 6.0 and 25ºC (Zhang and Nicell, 2000).

Materials

Phenol was purchased by Fluka Chemie GmbH (Buchs - Switzerland); 4-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,2'-azino-bis[3-ethyl-benzothiazoline-(6)-sulphonic acid diamonium salt (ABTS), 4-aminoantipyrine (4-AAP) and Chitosan, medium molecular weight 85% DA were purchased from Sigma-Aldrich Co. (St. Louis - USA); Pentachlorophenol, potassium hexacyanoferrate (III) and 30% aqueous hydrogen peroxide solution were supplied by Merck Co. (Damnstag - Germany) this last one, was standardized by permanganometry. Graphite felt (0.6 mm thickness) and reticulated vitreous carbon foam (3% density and 100 ppi) from Duocel - ERG Co. (Oakland – USA). Electrolytic copper web (27 cm^2) from Haver & Boecker (Westfalen - Germany).

Equipment

A spectrophotometer Shimadzu Multispect 1501 with temperature-controlled cell (Shimadzu Co. Japan) was used for the enzymatic and colorimetric assays. The electrochemical measurements during the hydrogen peroxide electrogeneration and bioelectrooxidation of CP were performed using a potentiostat/galvanostat Omnimetra PG 19 model (Brazil) and VoltaLab PGZ 301 Radiometer (Denmark) was using during the voltammetry studies.

Voltammetry studies

Voltammetry curves were obtained using as working electrode a rotary disk electrode (RDE) constituted by a cylindrical copper rod (Johnson - Maltey) with 0.2 cm^2 of cross-sectional area, a scan rate of 150 mV·s^{-1} was used and different disk speeds from 100 to 1000 rpm were applied. The copper electrode surface was polished with a silicon carbide emery cloth grade 1000, then rinsed with ethanol and finally dried under hot air. A platinum wire was used as auxiliary electrode (AE) and a saturated calomel electrode (SCE) as reference.

Hydrogen peroxide electrogeneration

The arrangement for the electrochemical bicompartmented reactor used in this study is presented in Figure 1. The electrochemical system was composed by a platinum wire as AE and saturated calomel electrode as reference. Graphite felt (GF) and reticulated vitreous carbon foam (RVCF), both with 2.0 cm^2 of apparently surface area and an electrolytic copper web of 27 cm^2 were evaluated as working electrodes (WE). Cathodic potentials from -120 to -820 mV, were evaluated in terms of the hydrogen peroxide

production as well as two strategies for air supplementation: Discontinuous aeration by 1 hour with pure O_2 (5.0 mL·min^{-1}) and continuous aeration with pure O_2 (5.0 mL·min^{-1}) along the 4 hrs reaction. Continuous magnetic stirring at 200 rpm was maintained in all cases. To evaluate H_2O_2 generation, samples during 4 hrs from the cathodic chamber were taken, and then H_2O_2 was quantified using the methodology described bellow.

Enzymatic oxidation of CP using direct addition (DA) and electrogeneration (EG) of H_2O_2

DA experiments were evaluated using reaction mixtures containing 0.6 U·mL^{-1} of CPO; 0.5 mmol·L^{-1} of the chlorinated phenol and 0.5 to 1.0 mmol·L^{-1} H_2O_2 at 100 mmol·L^{-1} potassium phosphate buffer pH 6.0 25ºC and magnetic stirring at 200 rpm. Reactions were started by single or step-wise addition of 3 and 5 equal pulses of hydrogen peroxide up-to the desired final hydrogen peroxide concentration. The bioelectrochemical oxidation of CP was performed using the reaction mixture described above. Reactions were started by the addition of CPO and turning on the system at a specific potential. Control experiments were also carried out where the effect of the anodic oxidation and the chemical oxidation due to hydrogen peroxide were determined.

Hydrogen peroxide production and residual phenol quantifications

A spectrophotometric method for microquantification of hydrogen peroxide was used, employing hydrogen peroxide reduction by a peroxidase followed by simultaneous oxidation of 2,4-dichlorophenol and coupling with 4-aminoantipyrine. Reaction mixtures contained peroxidase (4.0 nmoles·L^{-1}), 2,4-dichlorophenol (1.0 mmol·L^{-1}) and 4-aminoantipyrine (0.5 mmol·L^{-1}) in 100 mmol·L^{-1} potassium phosphate buffer pH 6.0, at 30ºC. Variable concentrations of hydrogen peroxide were used as reaction starters. Absorbance readings were performed at 510 nm during three minutes and a pre-plotted in a calibration curve of hydrogen peroxide concentration *vs.* absorbance. The residual phenol quantification was performed using the modified red-phenol method (Metelitza et al. 1991; Fiamegos et al. 2002) based on the oxidation of the phenolic compound by potassium hexacyanoferrate (III), followed by simultaneous coupling with 4-aminoantipyrine in an alkaline 1:1 ethanol/water medium. Reaction mixtures of 2.0 mL containing 20 μL of potassium hexacyanoferrate (III) (8%), variable concentrations of CP from 0.05 to 1.0 mmol·L^{-1} 100 μL of ammonium hydroxide (2%) and 50 μL of 4-aminoantipyrine (40 mmol·L^{-1}) were used to build the calibration curves of concentration *vs.* absorbance for each CP.

CPO stability

Residual peroxidase activity was determined from DA and EG experiments using reaction mixtures containing 0.6

U·mL^{-1} of un-modified or cross-linked CPO, 0.5 mmol·L^{-1} of 4-CP in 100 mmol·L^{-1} sodium-potassium phosphate buffer pH 6.0. For DA a maximum hydrogen peroxide concentration of 1.0 mmol·L^{-1} was assumed. Determination of the isolated effect of hydrogen peroxide as well as the effect of both phenol oxidized derivatives and hydrogen peroxide over the CPO activity were performed. Positive control for CPO activity contained only 0.6 U·mL^{-1} of CPO in 100 mmol·L^{-1} sodium-potassium phosphate buffer pH 6.0 in the absence of hydrogen peroxide and phenol. Similar negative control used a boiled CPO solution.

RESULTS AND DISCUSSION

The hydrogen peroxide electrogeneration

The electrochemical production of hydrogen peroxide results from the dissolved oxygen reduction present in the reaction mixture. This oxygen reduction produces, as final product, water, with the consumption of 4 electrons. However, depending on the material utilized as working electrode, this reaction could occur in two steps producing the stabilization of the hydrogen peroxide in a determined range of polarization. As literature recent showed, carbonaceous materials such as graphite felts or reticulated vitreous carbon have a good efficiency for hydrogen peroxide electrogeneration (Lütz et al. 2004). However, in preliminary studies (data not shown) was also demonstrated a severe increase over adsorption up to 40% of phenol initial concentration can be expected, as well as over polymeric by-products formed after the enzymatic oxidation, which can difficult further separation operations required during the phenol removal.

Figure 2 shows the voltammograms obtained with copper RDE from aerated and un-aerated buffer phosphate solutions (pH 6.0) containing 4-CP. Clearly it can be observed that in the presence of oxygen, the cathodic current increases showing two limited regions (gray-dark line). First region at approximately -600 mV$_{SCE}$ corresponds to the hydrogen peroxide production from the cathodic reduction of the dissolved oxygen, and the second region at - 900 mV$_{SCE}$ corresponds to the formation of water from the reduction of the hydrogen peroxide previously formed. The presence of 4-CP (gray-light line) did not affect the profile observed for the un-aerated solution (black line), this result demonstrates that phenol is not electrochemically active in this polarization rage and did not suffers cathodic reduction.

According to Figure 3, using a copper cathode, the H$_2$O$_2$ production increases following the decrease of potential up to -620 mV$_{ECS}$. The highest hydrogen peroxide generation rates were observed during the first 30 min of reaction with accumulative maximum concentrations after one hour. After this point, the H$_2$O$_2$ production decreased, in part due to the use of a discontinuous aeration that limited the oxygen availability, and also from the drop in the oxygen concentration caused by the electrochemical process itself.

The highest hydrogen peroxide generation under these conditions reached 0.77 mmol·L^{1}. But when a continuous air supplementation was applied, hydrogen peroxide concentration increased up to 1.2 mmol·L^{-1} (Figure 4). Nevertheless, the choice for pre-aeration instead continuous air stripping it was justified in part by the lowest air-drag effect to be caused over the phenolic solution during the process.

Degradation and removal using hydrogen peroxide direct addition (DA)

Figure 5 shows the enzymatic oxidation of CP using direct addition of hydrogen peroxide. The maximum degradation levels were observed for P and 4-CP, when a multiple hydrogen peroxide addition of 5 pulses was used. Lower degradation levels with this addition of 88, 66 and 59% for 2,4-DCP, 2,4,6-TCP and PCP, were observed respectively. Hydrogen peroxide addition also caused chemical oxidation up to 12% depending on the phenolic compound. This observation corroborates the previous studies where the oxidation of 4-CP was studied (La Rotta and Bon, 2002).

The formation of precipitates was observed during the first 2 hrs. In consequence, 80% of both P and 4-CP, were removed. In the case of 2,4,6-TCP and PCP only an increase in turbidity was observed with no precipitate formation. Since the simultaneous addition of several coagulants such as chitosan gels was already successfully used with other peroxidases during the enzymatic oxidation of phenols (Zhang et al. 1997; Lai and Lin, 2005), we decided to evaluate its addition in a final concentration of 2% (w/v). Consequently, the complete removal of phenol and 4-CP was achieved and increments in removal of 12 and 30% were observed for 2,4-DCP and 4-CP, respectively. Removal quantification was not possible for 2,4,6-TCP and PCP due in part to their solubility features. However, residual CP values (data not shown) indicated the incidence of phenol removal from the non polymerized products generated during the enzymatic oxidation and the addition of chitosan.

Bioelectrodegradation of CP

Figure 6 shows the effect of the cathodic potential over the 4-CP bioelectrochemical degradation. As it can be seen, 4-CP bioelectrooxidation at the cathodic chamber (EG CC) increased with the cathodic potential. As a consequence of this, no 4-CP was detected after 4 hrs at -620 mV$_{SCE}$. And using -420 and -220 mV$_{SCE}$, 7 and 25% of residual 4-CP was detected, respectively. These results demonstrate the dependence of the applied potential over the electroezymatic degradation. It was also observed anodic direct oxidation (EG AC) up to 30% during the bioelectrochemical processes. Cathodic chamber controls (CC C) with no CPO addition showed 18% of chemical oxidation due to the electrogenerated hydrogen peroxide.

In Figure 7 is showed how the hydrogen peroxide electrogeneration plus the enzymatic oxidation of chlorinated phenols, provided excellent oxidation levels after 4 hrs for P and 4-CP, were no residual phenol was detected, and good oxidation levels of 77%, 69% and 59% for 2,4-DCP, 2,4,6-TCP and PCP, respectively. Additionally, controls for anodic oxidation levels from 10 to 30% were observed depending on the phenolic compound.

Electrodegradation also droves the precipitation only at the cathodic chamber. As a consequence, when a higher H_2O_2 level was produced, the highest precipitate formation occurred. Maximum precipitation was observed for phenol and 4-CP. And only increases in turbidity were observed for 2,4-DCP, 2,4,6-TCP and PCP, with no precipitation. On the other hand, when chitosan was added, removal levels achieved 91,5 and 91,3%, for 4-CP and 2,4-DCP, respectively.

Although the chitosan addition improves removal levels during EG, could also promotes the electrode passivation, since the flocculated product could remains adhered on the electrode, promoting mass transfer problems and decreasing the electrode life-time. This result shows that application use of chistosan or other coagulants during EG should be analysed, probably in a discontinuous processes or with a different reactor arrangement.

As showed in Table 1, initial oxidation rates were higher for DA than EG. As such, an increment of two fold-times was observed when DA was used during the oxidation of 4-CP. In the case of PCP and 2,4-DCP increments of 4 and 6 fold-times, were respectively observed. For TCP, the lowest initial oxidation rate was achieved. This observation can be related to the hydrogen peroxide availability at the reaction medium, especially for EG where an effective hydrogen peroxide concentration is very small and depends on the dissolved oxygen concentration.

Aiming to improve the bioelectrooxidation process in terms of efficiency and reaction rates, experiments of electrochemical generation of hydrogen peroxide and simultaneous enzymatic oxidation of CP were evaluated in a single chamber reactor with continuous aeration. As it can be seen in Figure 8 same oxidation levels were obtained for P and 4-CP. Improvements up to 97%, 93% and 88% for 2,4-DCP, 2,4,6-TCP and PCP, were observed respectively. As it can also be observed in Table 1, reaction rates obtained using EG in a single chamber reactor showed to be quite similar to those obtained using a 5-pulsated addition of hydrogen peroxide.

Evaluation of residual peroxidase activity

This parameter indicated the real advantages between EG and DA in terms of enzyme stability. Figure 9 shows the residual peroxidase activity observed after 4 hrs in experiments using single and a 5-pulsated direct addition

(DA) of hydrogen peroxide. Also, the electrogeneration (EG) in a single-chamber reactor was compared with a control with no CPO addition.

Although, both methodologies were similarly efficient in terms of phenol oxidation and precipitate formation, it was observed that CPO loses activity rapidly when the direct addition was employed. This fact is not just related to the presence of hydrogen peroxide, but is even more remarkable when both oxidized products and hydrogen peroxide were simultaneously present during the reaction with DA or EG, indicating a possible suicide inactivation already observed for oxidases such as tyrosinase (Haghbeen et al. 2004). Thus, up to 60% of initial activity was lost in DA experiments, followed by 40% using EG after 4 hrs. Free and pure CPO in 100 mmoles·L^{-1} potassium phosphate buffer pH 6.0 remained stable up-to 4 hrs at 25ºC. In terms of inactivation rates (Table 2), CPO was more rapidly inactivated with the direct addition of hydrogen peroxide rather than with its electrogeneration. On the other hand, CPO presented half-life and work-life times two fold-times higher when EG were used instead DA.

CONCLUDING REMARKS

The continuous EG and pulsated DA of the hydrogen peroxide offer the possibility to conciliate effective an hydrogen peroxide availability to undisruptive concentrations in the reaction mixtures containing phenol, 4-CP, 2,4-DCPl, 2,4,6-TCP and PCP, selected as targets for enzymatic oxidation in batch reactions using chloroperoxidase. With EG system it was possible to obtain variable hydrogen peroxide concentrations depending on the working electrode material, the applied cathodic potential and aeration mode. Working electrode materials of graphite felt, reticulate vitreous carbon foam and an electrolytic copper web were evaluated, in terms of hydrogen peroxide generation, efficiency and phenol adsorption. All materials showed excellent results in terms hydrogen peroxide electrogeneration, however the carbonaceous materials showed the highest phenol adsorption. When a bicompartmented cell was used, bioelectrooxidation of the CP was comparable to the conventional enzymatic oxidation of CP using direct addition. In both cases a low chemical oxidation due to the hydrogen peroxide was observed. On the other hand, when EG was performed in a single-chamber reactor a better accumulative effect of chemical, anodic and enzymatic oxidations of CP was attained. DA and EG systems also led CP removal by the precipitation derived from the polymerization of these phenolic compounds. The use of coagulant agents was advantageous in both cases. Even thought, DA was relatively more efficient in terms of enzymatic oxidation than the electrochemical approach, this last one showed a lower inactivation rates, and longer half-life and work-life times.

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7

APPENDIX

TABLES

Table 1. Comparison between the effects of DA in pulsed addition up-to 5X and electrogeneration of hydrogen peroxide in bicompartmented cell and single-chamber reactor over the initial oxidation rates of CP.

	1x Vo $\mu M\ mL^{-1}\ min^{-1}$	5x Vo $\mu M\ mL^{-1}\ min^{-1}$	EG (Bicomp.) Vo $\mu M\ mL^{-1}\ min^{-1}$	EG (Single chamber) Vo $\mu M\ mL^{-1}\ min^{-1}$	Increment Bicomp. vs Single chamber %
P	0.00961	0.00980	0.00790	0.00920	14,1
4-CP	0.01063	0.01300	0.00710	0.00820	13,4
2,4-DCP	0.00783	0.01350	0.00240	0.00740	67,5
2,4,6-TCP	0.00431	0.00580	0.00060	0.00420	85,7
PCP	0.00243	0.00410	0.00110	0.00390	71,8

Table 2. Inactivation rates for CPO using DA by single and 5X pulsed additions and electrogeneration (EG) of hydrogen peroxide. Half-life time (HLT) and work-life time (WLT) for CPO were estimated in all cases.

	k_{inact} $UI\ mL^{-1}\ min^{-1}$	WLT h	HLT h
EG	0.0092	11.2	5.7
DA 1X	0.0162	6.2	3.1
DA 5X	0.0146	6.9	3.5
CPO	0.0001	998.0	498.0

FIGURES
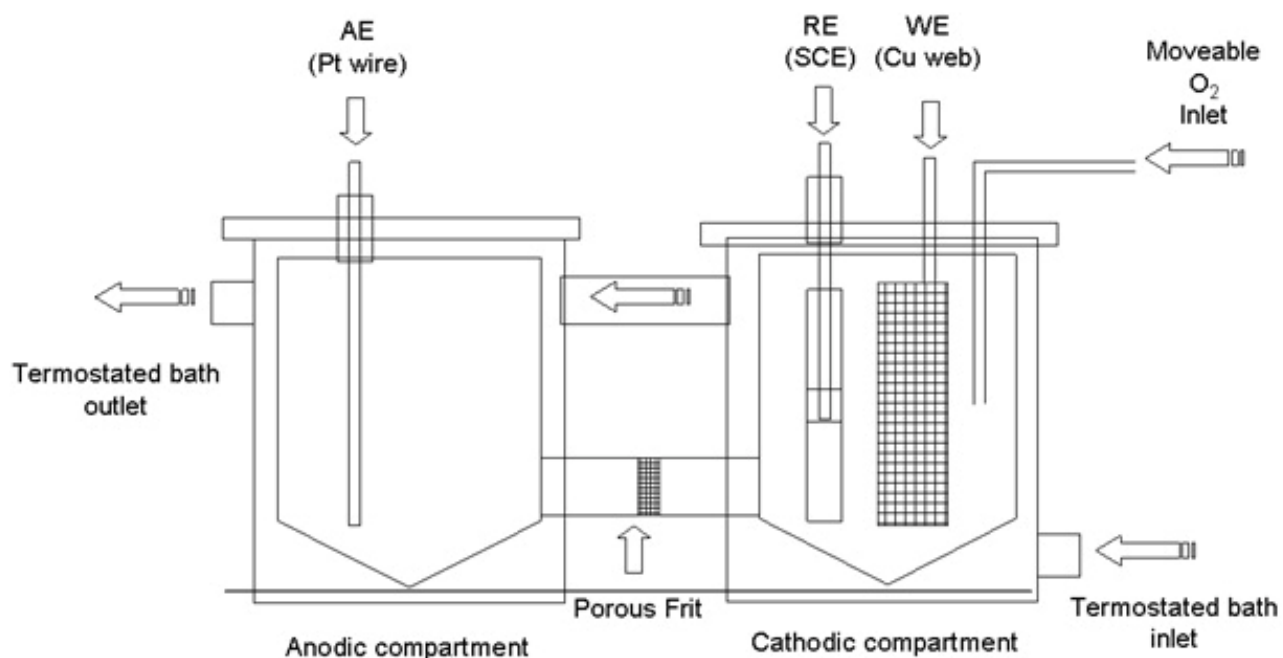
Figure 1. Diagram for the bicompartmented electrochemical cell used during the experiments for hydrogen peroxide generation and bioelectrochemical oxidation of chlorinated phenols. AE: auxiliary electrode; RE: reference electrode; We: working electrode.
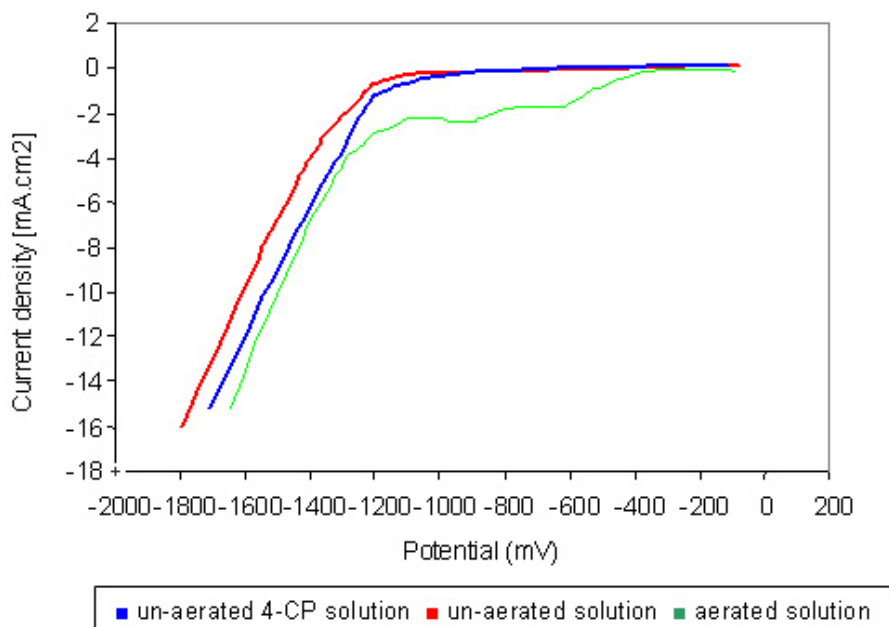

Figure 2. Voltammetry profiles for copper RDE (0.2 cm^2) obtained at 1000 rpm using in 100 mmol·L^{-1} sodium-potassium phosphate buffer pH 6.0 at 25ºC with a 150 mV·s^{-1} scan rate.
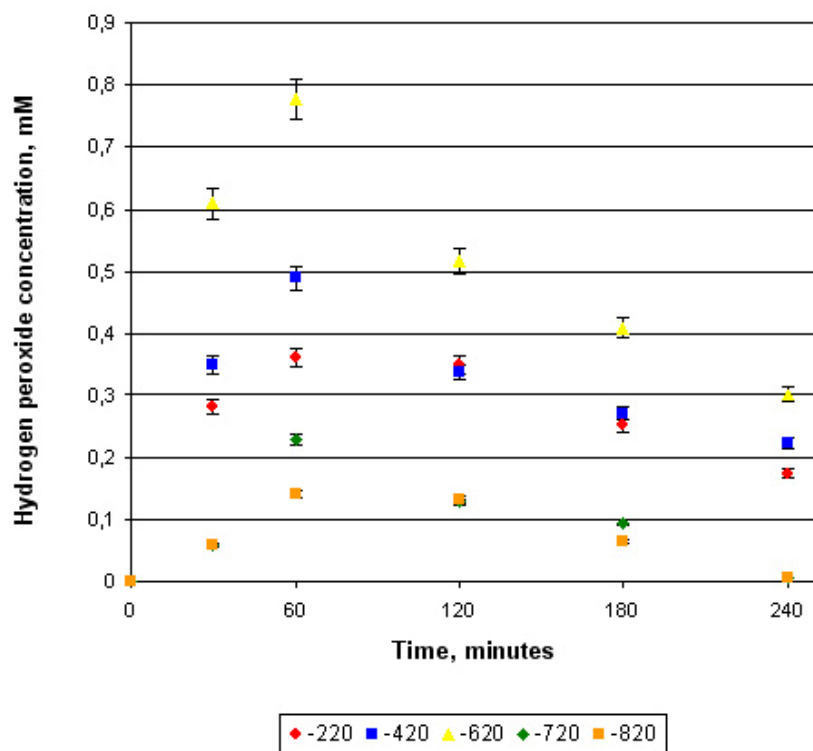
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Figure 3. Effect of the applied potentials over the hydrogen peroxide electrogeneration. Bicompartmented cell using an electrolytic copper web of 27 cm^2 (WE); pre-aeration of 1 hr (O$_2$ 0.5 vvm), in 100 mmol·L^{-1} sodium-potassium phosphate buffer pH 6.0, at 25ºC.
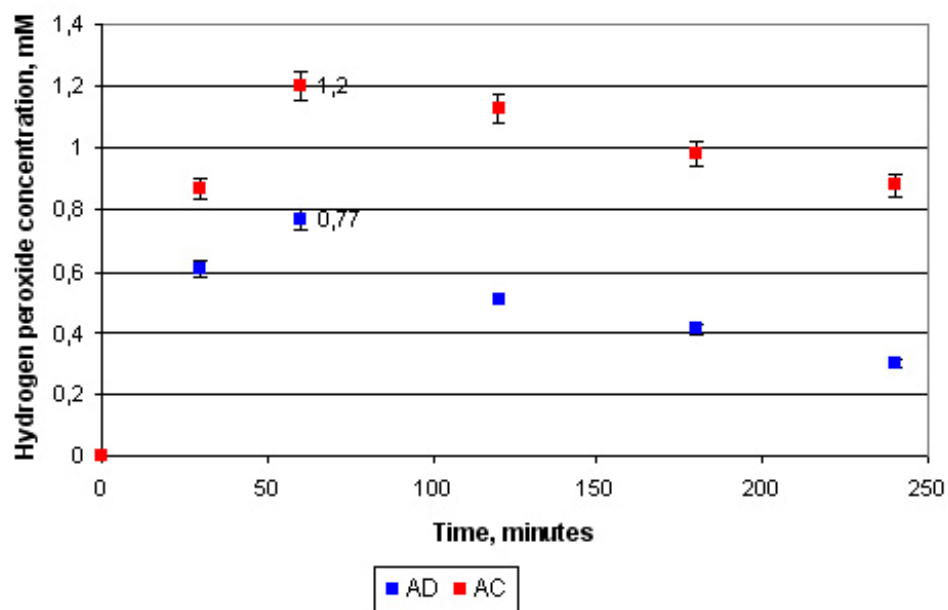
Figure 4. Effect of the aeration mode over the hydrogen peroxide electrogeneration. Pre-aeration (O$_2$ 0.5 vvm) of 1 hr (■); and continuous aeration (O$_2$ 0.5 vvm), during 4 hrs (■). In a 100 mmol·L^{-1} sodium-potassium phosphate buffer pH 6.0 at 25ºC
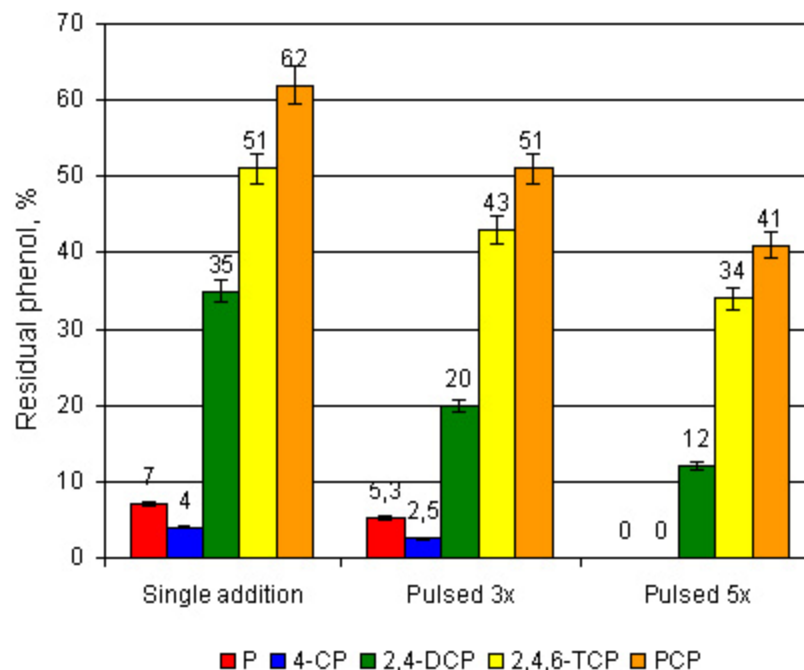
Figure 5. Effect of hydrogen peroxide direct addition: single addition, pulsed three and five additions, over the enzymatic oxidation of chlorinated phenols by CPO.
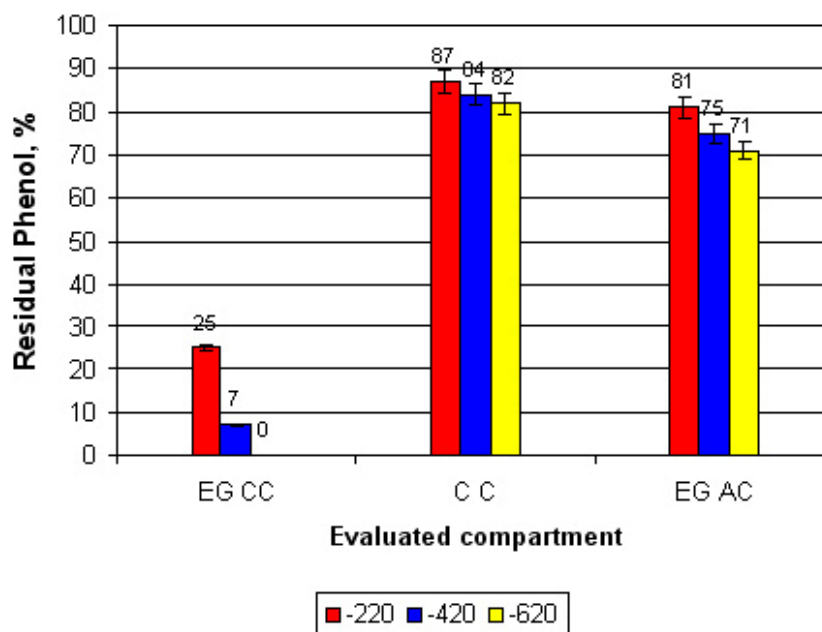

Figure 6. Effect of the potential over the bioelectrodegradation of 4-CP. Reaction mixture containing 6 UI/mL CPO, 0.5 mM 4-CP in 100 mM sodium-potassium phosphate buffer pH 6.0, at 25ºC. EG CC: bioelectrodegradation at the cathodic chamber; EG AC: Anodic oxidation in the anodic chamber; CC C: Cathodic chamber control.
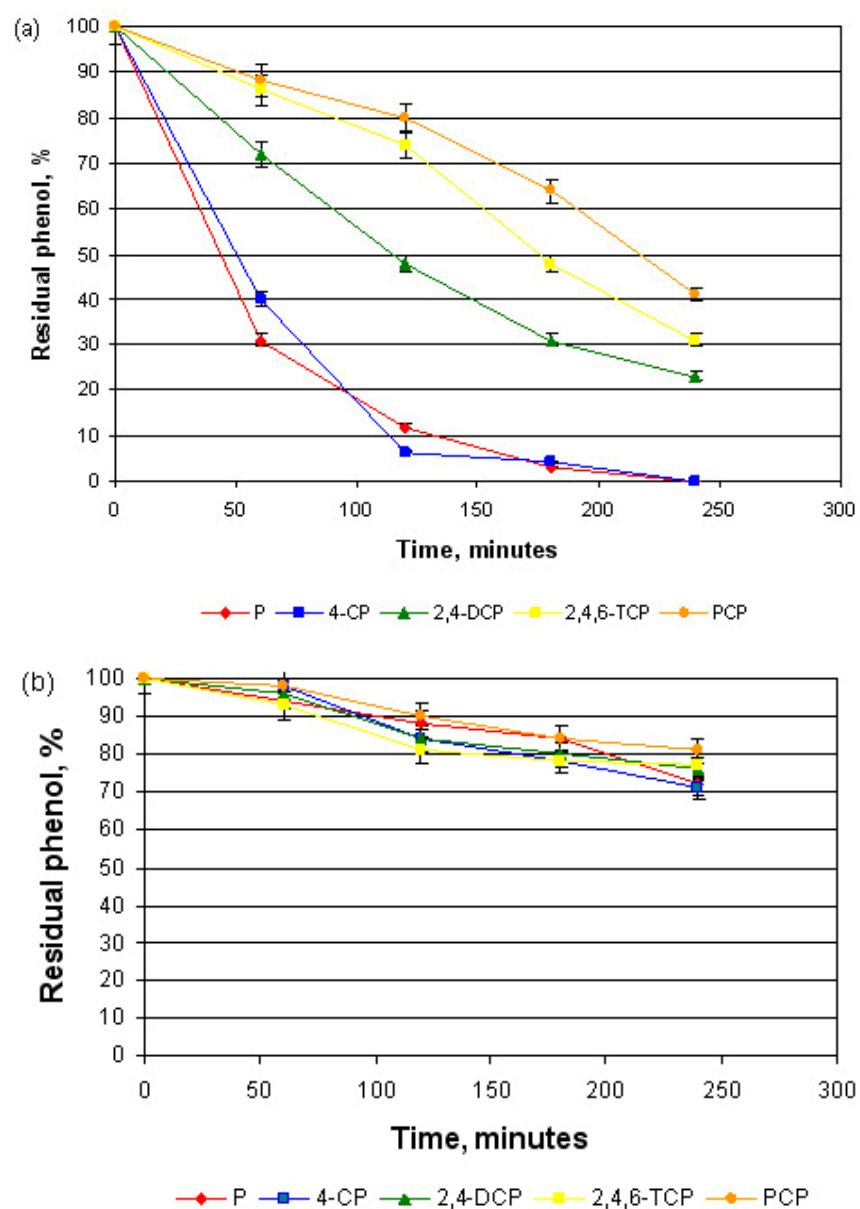
Figure 7. Bioelectrodegradation of CP during the electrolysis at -620 mV in:
(a) Cathodic chamber EG CC.
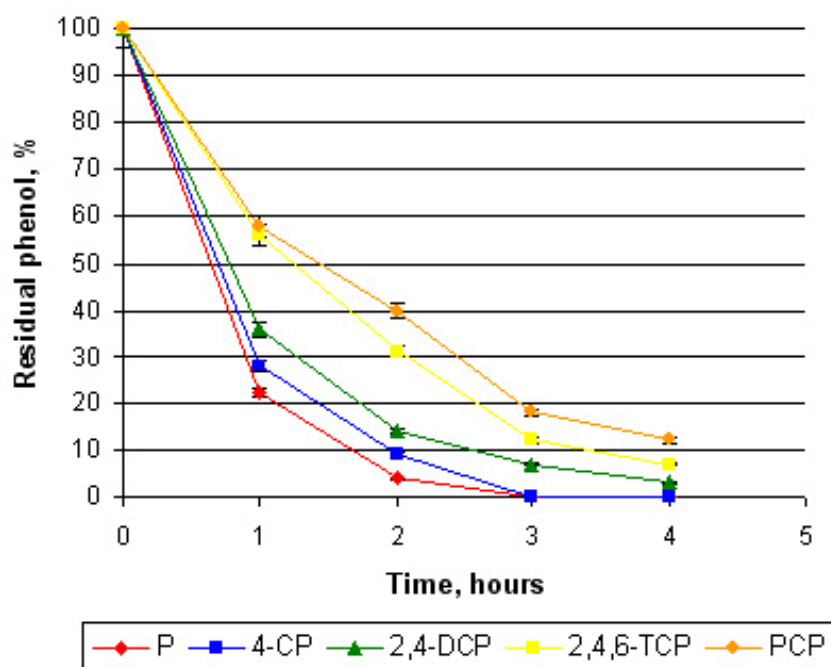**(b) Anodic chamber using discontinuous pre-aeration of 1 hr.**

Figure 8. Bioelectrodegradation of CP during the electrolysis at -620 mV in single camber reactor. Using continuous aeration during 4 hrs (O_2 0.5 vvm). Reaction mixtures containing 6.0 UI/mL of CPO, 0.5 mM of CP in 100 mM sodium-potassium phosphate buffer pH 6.0 at 25ºC and 200 rpm.
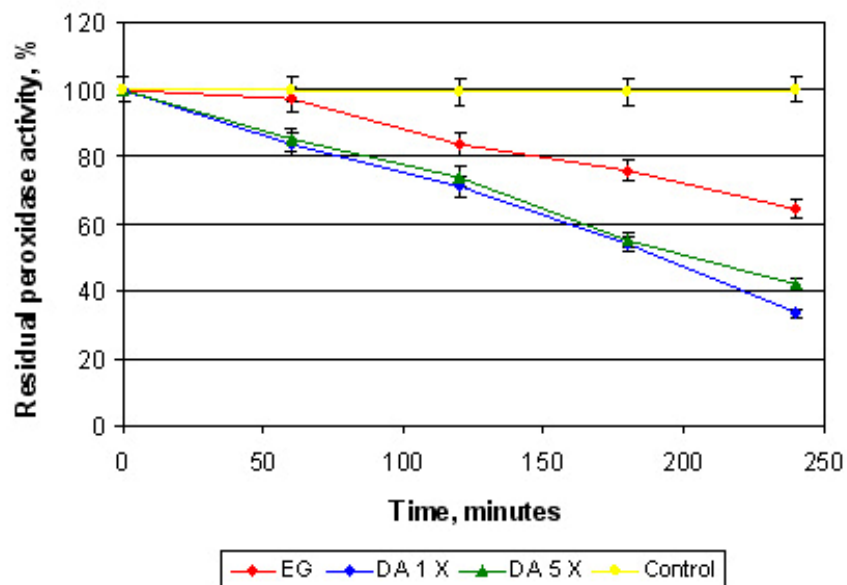

Figure 9. Comparison of CPO residual activities during 4 hrs using direct addition (DA by single and 5X pulsed additions, bioelectrochemical (EG) process with continuous aeration (EG -620mV$_{SCE}$) and controls with only CPO. Reaction mixtures contained 6.0 UI/mL CPO, 0.5 mM CP, 1.0 mM peroxide concentration for DA in 100 mM sodium-potassium phosphate buffer pH 6.0 at 25ºC and 200 rpm.