Revised Manuscript
Click here to view linked References

© <2021>. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
This document is the Accepted Manuscript version of a Published Work that appeared in final form in [Journal of Hazardous Materials]. To access the final edited and published work see [https://doi.org/10.1016/j.jhazmat.2009.03.059].

Comparison of alternative treatments for 4-chlorophenol removal from aqueous solutions: Use of free and immobilized soybean peroxidase and KrCl excilamp

M. Gomez[1], G. Matafonova[2], J.L. Gomez[1*], V. Batoev[2], N. Christofi[3]

[1]Department of Chemical Engineering, Murcia University, 30071 Murcia, Spain

[2]Baikal Institute of Nature Management, Siberian Branch of Russian Academy of Sciences, Sakhyanova 6, Ulan-Ude 670047, Russia

[3]Pollution Research Unit, School of Life Sciences, Edinburgh Napier University, Merchiston Campus, Edinburgh EH10 5DT, Scotland UK.

[1*]Corresponding author: J.L. Gomez, Tel: (34) 968367351, Fax: (34) 968364148; E-mail: carrasco@um.es

Keywords: 4-chlorophenol, soybean peroxidase, aqueous solution, excilamp, immobilization, photodegradation

Abstract

The removal of 4-chlorophenol (4-CP) from industrial wastewater continues to be an important environmental issue and some interesting results have been obtained using oxidoreductive enzymes such as peroxidases and UV, generated by novel excilamps. In this study enzyme (free and immobilized soybean peroxidase) and UV (produced by a KrCl excilamp) were used to treat 4-CP solutions at concentrations ranging from 50 to 500 mg L^{-1}. It was shown that the excilamp can facilitate higher removal efficiencies in all cases with complete 4-CP elimination taking place between 5 and 90 min. The enzyme removed ~80% of the 4-CP concentrations in both the free and immobilized state up to concentrations of 250 mg L^{-1}. At 500 mg L^{-1} the immobilised

system shows much higher removal efficiency due to increased enzyme stability in the presence of higher formation of by-products.

1. Introduction

Chlorophenols (CPs) are considered as priority pollutants since they are harmful to organisms even at ppb levels. Environmental contamination with these chemicals occurs from industrial effluents, such as those generated by high-temperature coal conversion, petroleum refining and the manufacture of plastics, resins, textile, iron, steel and paper [1-3].

The problem of phenolic compound removal from wastewater is a current and important issue. Physical, chemical and biological methods, including incineration, adsorption on activated carbon, chemical or enzymatic oxidation, solvent extraction, microbial degradation and others have been proposed for removing or degrading several CPs from waste waters [4-11]. Despite extensive research, convenient, robust and cost effective treatment of the pollutants has still to be implemented, and, there is a continued need to delineate effective systems.

Some disadvantages of conventional treatment methods can be overcome by adopting an enzymatic method, which has a high degree of specificity and a minimal environmental impact. The application of oxidoreductive enzymes, such as peroxidases, to catalyse the removal of aromatic compounds from wastewater has been widely investigated [12-19]. These enzymes catalyse the oxidation of aqueous CPs by hydrogen peroxide to produce free radicals that spontaneously interact to form

polymers and oligomers of high molecular weight and low solubility [20, 21]. A significant treatment cost, due to the price of the purified enzyme, is the main disadvantage of enzymatic methods [22]. Also, the use of free enzymes means that peroxidases are susceptible to permanent inactivation by various undesirable side reactions of the treatment process, such as the suicide-peroxide inactivation. In addition, physical phenomena come into play as the enzyme is sequestered by coprecipitation with the reaction products, oligomers and polymers. The use of additives, such as polyethylene glycol, can minimize some of these disadvantages [13].

The enzymes can be immobilized on various supports and this can confer some advantages over the soluble forms, including enzyme reuse and stabilisation, control of product formation and easy separation from the reaction medium [18-19]. However, the use of immobilized enzymes has often been shown to have the important disadvantage that enzyme activity can be lost due to the immobilization process and, additionally, the partial precipitation of reaction products, oligomers and polymers, over the surface of catalytic particles, diminishes the activity of the immobilized enzyme [23].

Among other methods considered for the removal of these toxic organic pollutants from wastewater, Fenton's oxidation [24-26] and photodegradation with UV mercury lamps [27, 28], at a principal wavelength of 254 nm, have been widely used, and, frequently, photocatalysts have been used to improve the efficiency of the photolytic process, titanium dioxide being the most common [29, 30].

Attention has been focusing recently on the development and use of novel sources of UV radiation. Excimer lamps and excilamps [31] are a class of spontaneous radiation sources based on transitions of exciplex (rare gas halides) or excimer molecules (rare gas or halogen dimers). They emit UV radiation in a narrow band of high intensity. Excilamps are an attractive alternative to commonly used mercury lamps and lasers for microbial inactivation and applications in pollution control technology because of the absence of elemental mercury, long lifetime, geometric freedom, high photon flux and other advantages [32-34].

A potentially viable solution is the integration of photochemical and biological treatment processes [35, 36]. The basic concept behind this technology lies in the initial photolysis of target phenolic compounds with powerful UV lamps and subsequent biodegradation of more degradable products. In other words, the photochemical process would be used as a pre-treatment in order to increase the biodegradability of the wastewater. In the case of 4-CP photodegradation, a previous study carried out by the authors using GC-MS analysis shows that two major photoproducts, benzoquinone (BQ) and hydroquinone (HQ), are formed [37]. Also, small amounts of others hydroxylated compounds, such as phenol, catechol, resorcinol, 1,2,4-trihydroxybenzene and even some chlorinated oxidation by-products, such as 4-chlorocatechol, and products from the cleavage of the aromatic ring, such as phthalic acid, have been identified [37-39].

In the present work, two different methodologies are used and compared for 4-CP removal. These methods involve enzymatic treatment using free and immobilized soybean peroxidase (SBP) [40, 41] and photodegradation treatment using a KrCl

excilamp [37]. Their comparative study is a first step in the further development of combination photochemical-enzymatic treatment, similar but essentially different from the commonly and widely used photochemical-biological treatments. This combination treatment benefits from enzyme use in that there is a high degree of specificity and minimal environmental impact. Excilamps have a narrow band of high intensity that offsets disadvantages of price of the purified enzyme used to reduce significant concentrations of non degraded by-products found when high initial 4-chlorophenol concentrations are used in the UV treatments.

2. Materials and Methods

2.1. Chemicals and equipment

Soybean peroxidase (E.C.1.11.1.7), (SBP), (lyophilized powder, 90 Umg^{-1}), catalase (E.C.1.11.1.6) (lyophilized powder, 2200 Umg^{-1}), 4-CP (purity 99%) and hydrogen peroxide (35% w/v) reagent were purchased from Sigma-Aldrich Fine Chemicals. Immobilization reagents and support, γ-APTES ((3-aminopropyl) trietoxysilane), glutaraldehyde (25%) and uncoated controlled pore glass PG-75-400 (200–400 mesh particle size), were also from Sigma. Other chemicals were of analytical grade and were used without further purification.

A KrCl excilamp with output power of 5 W was purchased from Institute of High Current Electronics of Russia and used for photodegradation experiments. The typical spectrum of this lamp emitting UV radiation shows a narrow band of high intensity at 222 nm (the band of the KrCl molecule). The excilamp was of cylindrical geometry

covered by a metal case having an UV exit window with an area of 75 cm^2 [34]. The output power of the excilamp was measured with a H8025-222 photodetector (Hamamatsu Photonics KK) and was tested using an electrochemical actinometer. The average radiation intensity delivered to the solution was 2.47 mW cm^{-2}.

2.2. Excilamp and enzymatic treatment

All enzymatic assays were carried out in a batch reactor of 50 ml reaction media, where SBP catalyses the oxidation of 4-CP in the presence of hydrogen peroxide. The enzyme is used in the soluble form or immobilized onto porous glass. Immobilized derivatives were prepared by covalent coupling between the amine groups of the protein and the aldehyde groups of the porous glass treated with (3-aminopropyl) triethoxysilane and glutaraldehyde, as indicated in a previous study [41]. Immobilized derivatives were stored at 4 ºC in 0.1M phosphate buffer, pH 7. After 60 days of storage, immobilized SBP maintained 96% of its initial activity, in good agreement with the results obtained in a previous study [41]. Two experimental series were carried out with the enzyme: The first series utilised different 4-CP concentrations ranging from 50 to 500 mg L^{-1} and a fixed enzyme concentration of 5 mg L^{-1}, an effective value utilized in previous studies [14, 40]. The second series of experiments used a fixed 4-CP concentration of 250 mg L^{-1} and variable enzyme concentrations from 5 to 20 mg L^{-1}. Samples were taken at various time intervals during treatment and analyzed for residual 4-CP concentration and by-products. In the assays with free SBP, catalase was added to each sample in order to stop the progress of the reaction as in previous work [40]. In the case of immobilized systems, a nylon mesh (10 μm)

was placed at the extreme of the syringe used to take samples to prevent passage of the glass beads with the enzyme, or, the polymer into the aliquots for analyses.

As for the photodegradation process, the excilamp exit window was oriented vertically at a distance of 2.2 cm from the quartz tube that had an operating length of 22 cm and external diameter of 2.6 cm. The UV dose reaching the centre of the tube was determined using actinometry as in previous studies [34]. 4-CP at the required concentrations was dissolved in distilled water, placed into the quartz tube covered with a reflector and irradiated at room temperature (23–25 ^{0}C) under static conditions for different time periods.

Replicate analyses were made for both enzymatic and photodegradation assays giving a mean standard deviation of 3.98%, 2.42% and 2.49% for the assays carried out using the excilamp, the free SBP and the immobilized SBP, respectively.

2.3. Analysis

4-CP, at concentrations ranging from 50 to 500 mg L^{-1}, was tested for both photodegradation and enzymatic oxidation. 4-CP, HQ and BQ concentrations were determined by HPLC analysis using a Varian Prostar 210 chromatograph with UV-VIS detector and a C18 reverse phase column. The mobile phase was a mixture of methanol, acetic acid and water (60:2.5:37.5 v/v) with a flow rate of 1 ml min^{-1}. Simultaneous determination of 4-CP and HQ was carried out at a wavelength of 283 nm, where 4-CP and HQ present strong absorption bands at retention times of 6.2 and

2.2 min, respectively. BQ was determined, independently, at wavelength of 237 nm and retention time of 2.4 min.

3. Results and discussion

3.1. Immobilization results

The results obtained for the immobilization of SBP are shown in Table 1. The percentages of immobilized protein, protein content for the immobilization support and for the storage suspension, and, activity yield are presented as in a previous study [41]. It is seen that an activity yield (65%) was obtained, indicating that the immobilization process does not have a significant negative effect on enzyme activity.

3.2. Comparison of treatments varying 4-chlorophenol concentration

Figure 1 shows the experimental results for the series where 4-CP concentration was varied from 50 to 500 mg L^{-1}, and the enzyme concentration was fixed in 5 mg L^{-1} (see Materials and Methods). In Figure 1, the temporal removal efficiencies attained by the different treatments are expressed as the percentage of 4-CP removed relative to the initial solution concentration.

All 4-CP concentrations tested were completely converted to by-products by UV delivered from the excilamp between 5 and 90 min (Figure 1). This conversion pattern can be explained by the fact that the radiation flux was constant in all the assays and, as a consequence, the higher the 4-CP concentration, the lower intensity of radiation

is available for each chlorophenol molecule, thus increasing the total degradation time. Additionally, for the highest 4-chlorophenol concentrations tested, the high concentration levels of benzoquinone attained in the photodegradation process seems to indicate a partial adsorption of this compound on the wall of the quartz tube. Yellow colouration appearing on the tube wall can diminish the efficiency of the UV penetration and ultimate compound treatment. In other words, the shielding effect was observed when irradiating concentrated 4-CP solutions. To further illustrate this, UV dose levels, calculated as the product of radiation intensity relative to the exposure time, are shown in Figure 2 for all the assays carried out with the excilamp. Here, high UV doses (more than 10 J cm^{-2}) are required to achieve high percentages of total removal when high 4-CP concentrations are used.

In Figure 3, the percentage changes with time of the two main by-products, HQ and BQ, compared to the initial concentration of 4-chlorophenol, are shown. If only the residual 4-chlorophenol and the by-products HQ and BQ concentrations are considered, the mass balance fails and does not relate to the initial 4-chlorophenol concentration. This is due to the presence of small amounts of some of other by-products formed (see Introduction) not taken into account in this study.

Also, it can be seen in Fig. 3 that at the beginning of the degradation process, that the two main intermediates of the 4-CP photodegradation process, HQ and BQ, showed an increase in concentration reaching a maximum value after the first half-life stage of 4-CP degradation (0.7, 1.6, 5.1, and 14.4 min from the lowest to the highest 4CP concentration). They then decreased in all but the assays using the higher 4-CP concentration, where the photoproduct concentration had yet to reach the maximum.

HQ concentrations were higher than BQ's that were completely removed in the assays with low initial 4-CP concentration indicating low stability and, consequently, more favoured degradation. In another study [29] BQ was also shown to be more photodegradable than HQ. Furthermore, HQ/BQ redox processes and interconversions between HQ and BQ in aqueous solutions are known to be very pH dependent [29]. The pH value of the non-buffered solutions in our study decreased to 2.4. Under acidic conditions the reduction rate of BQ to HQ can be higher than the oxidation rate of HQ to BQ. Thus, it may also contribute to increase of HQ concentration.

Additionally, Figure 3 shows that with the exposure time used in the present work, the apparent residual organic load, corresponding to the residual percentages of HQ and BQ, remains between 20 and 30% of the initial 4-CP concentration The total residual organic load can be higher because small amounts of others by-products, can be present and, as a consequence, additional treatments may be necessary.

In the case of enzyme treatment, removal efficiencies of about 80% are attained up to 4-CP initial concentrations of 250 mg L^{-1}, with reaction times also increasing with the increase of the initial concentrations. However, removal efficiencies are lower for the highest concentration tested (500 mg L^{-1}). The behaviour of both free and immobilized SBP is very similar for all the substrate concentrations tested, except at the highest concentration, where the immobilized derivative shows much better removal (70% removal efficiency for the immobilized form versus 50% for the free enzyme). It is known that enzyme activity can be inhibited by reaction products and that this is enhanced by increasing the initial concentrations [15]. Immobilized SBP is

more stable and resistant to these effects, so it attains higher conversions as the enzyme is protected from high concentrations of products [42].

The enzyme reaction products are precipitated from the solution and can be easily removed by filtration or sedimentation [12-15], which represents an advantage compared to the soluble products of the phototreatment. However, some inconvenience appears for the reuse of the immobilized enzyme when it is used in a batch reactor. After treatment, both the catalytic particles and the reaction products, oligomers and polymers, precipitate at the bottom of the reactor and the immobilized enzyme is covered by a polymeric layer. As a consequence, the enzymatic activity diminishes, and the reuse of the enzyme is not practical for further removal of 4-chlorophenol in a second cycle. This inconvenience can be minimized by using the immobilized derivative in a continuous process, where the reaction products partially leave the reactor with the effluent stream, increasing the operational life of the immobilized derivative [43].

With regard to the nature of polymers, five dimers and a trimer were identified in the reaction solution by Yu et al. [20] working with the horseradish peroxidase/phenol system. They showed that three of these dimers are also peroxidase substrates and can be oxidized similar to phenol, following first-order kinetics obtained by simplifying a bisubstrate model. The dimers are *p*-phenoxyphenol, *p,p*-bisphenol and *o,o*-bisphenol, the oxidation rate of the first being higher than the phenol oxidation rate. According to these authors, the extra-consumption of hydrogen peroxide that takes place above the stoichiometric relationship can be explained by the oxidation of the mentioned compounds.

3.3. Effects of different SBP concentrations on 4-chlorophenol removal

Figure 4 shows the results of treating a fixed 4-CP concentration of 250 mg L^{-1} using four different enzyme concentrations (5, 10, 15 and 20 mg L^{-1}). When SBP concentrations of 5 and 10 mg L^{-1} are used the removal efficiencies are less than those attained by the excilamp. However, at the higher enzyme concentrations tested (15 and 20 mg L^{-1}) the removal efficiencies are practically the same with both enzymatic and photodegradation treatment. Once again, there is no significant difference between free and immobilized SBP.

4. Conclusions

Photodegradation and enzymatic oxidation of 4-chlorophenol has been carried out using a KrCl excilamp and SBP enzyme in its free and immobilized forms, respectively. In the whole range of substrate concentration tested, 50–500 mg L^{-1}, and with a fixed enzyme concentration of 5 mg L^{-1}, the excilamp showed higher removal efficiencies, leading to complete 4-CP removal in all cases, with reaction times between 5 and 90 min. For the main photoproducts, benzoquinone and hydroquinone, the removal efficiency is higher at low 4-CP concentrations, and residual concentration, equivalent to about 20–30 % of initial 4-CP amount, remains, which can be a limitation of the phototreatment.

The results obtained with free and immobilized SBP are similar and reach up to 80% 4-CP degradation for all concentrations tested, except for 500 mg L^{-1}. The immobilized SBP is more stable and is less subject to inhibition by reaction products, allowing up to 70% removal efficiencies while free SBP attains 50%. The resulting

products from the enzymatic treatment are polymers of low solubility that can be removed, so a clear effluent can be obtained.

Enzyme concentrations of 15 mg L^{-1} are required for the enzymatic treatment to achieve the same removal efficiency as the photodegradation process. This concentration is high (and would incur high costs) for a batch process like the one developed in this study. On the other hand, high UV dose levels (more than 10 J cm^{-2}) are also required for total photodegradation of 4-CP at high initial pollutant concentrations and, additionally, attention must be focused on the resultant photoproducts which need to be removed too.

From the obtained results, a combined photochemical-enzymatic treatment integrating the main advantages from both methods, with an initial photodegradation process followed by an enzymatic treatment, appears to be a promising alternative for 4-CP removal.

Acknowledgements

This study was made possible with funding from the Royal Society of Edinburgh to G. Matafonova and from a postdoctoral grant to M. Gómez from Fundación Séneca (Comunidad Autónoma de la Región de Murcia, Spain).

References

[1] I. Gellman, Environmental effects of paper industry wastewater – an overview, Water Sci. Technol. 20 (1988) 59–65.

[2] C.W. Bryant, J.J. Avenell, W.A. Barkley, R.N. Thut, The removal of chlorinated organic from conventional pulp and paper wastewater treatment systems, Water Sci. Technol. 26 (1992) 417–425.

[3] M. Czaplicka, Sources and transformations of chlorophenols in the natural environment, Sci. Total Environ. 322 (2004) 21–39.

[4] O. Milstein, A. Haars, A. Majcherczyk, J. Trojanowski, D. Tautz, H. Zanker, A. Huettermann, Removal of chlorophenols and chlorolignins from bleaching effluent by combined chemical and biological treatment, Water Sci. Technol. 20 (1988) 161–170.

[5] R.C. Wang, C.C. Kuo, C.C. Shyu, Adsorption of phenols onto granular activated carbon in a liquid-solid fluidized bed, J. Chem. Technol. Biot. 68 (1997) 187–194.

[6] G.J. Wilson, A.P. Khodadoust, M.T. Suidan, R.C. Brenner, C.M. Acheson, Anaerobic/aerobic biodegradation of pentachlorophenol using GAC fluidized bed reactors: optimization of the empty bed contact time, Water Sci. Technol. 38 (1998) 9–17.

[7] M. Caldeira, S.C. Heald, M.F. Carvalho, I. Vasconcelos, A.T. Bull, P.M.L. Castro, 4-chlorophenol degradation by a bacterial consortium: development of

a granular activated carbon biofilm reactor, Appl. Microbiol. Biot. 52 (1999) 722-729.

[8] C.D. Wu, X.H. Liu, D.B. Wei, J.C. Fan, L.S. Wang, Photosonochemical degradation of phenol in water, Water Res. 35 (2001) 3927–3933.

[9] Z.P.G. Masende, B.F.M. Kuster, K.J. Ptasinski, F.J.J.G Janssen, J.H.Y. Katima, J.C. Schouten, Platinum catalysed wet oxidation of phenol in a stirred slurry reactor. The role of oxygen and phenol loads on reaction pathways, Catal. Today 79 (2003) 357–370.

[10] B.K. Korbahti, A. Tanyolac, Continuous electrochemical treatment of phenolic wastewater in a tubular reactor, Water Res. 37 (2003) 1505–1514.

[11] V.B. Batoev, G.G. Nimatsyrenova, G.S. Dabalaeva, S.S. Palitsyna, Chlorinated phenols in Selenga river basin, Chemistry for Sustainable Development 13 (2005) 31–36.

[12] A.M. Klibanov, T.M. Tu, K.P. Scott, Peroxidase catalyzed removal of phenols from coal-conversion wastewaters, Science 221 (1983) 259–260.

[13] J.N. Wu, K.E. Taylor, J.K. Bewtra, N. Biswas. Optimization of the reaction conditions for enzymatic removal of phenol from wastewater in the presence of polyethylene glycol, Water Res. 27 (1993) 1701–1706.

[14] N. Caza, K.E. Taylor, N. Biswas, J.K. Bewtra, Removal of phenolic compounds from synthetic wastewater using soybean peroxidase, Water Res. 33 (1999) 3012–3018.

[15] C. Kinsley, J.A. Nicell, Treatment of aqueous phenol with soybean peroxidase in the presence of polyethylene glycol, Bioresource Technol. 73 (2000) 139–146.

[16] P.S. Gonzalez, E. Agostini, S.R. Milrad, Comparison of the removal of 2,4-dichlorophenol and phenol from polluted water, by peroxidases from tomato hairy roots, and protective effect of polyethylene glycol, Chemosphere 70 (2008) 982-989.

[17] S. Ghasempur, S.F. Torabi, S.O. Ranaei-Siadat, M. Jalali-Herabi, N. Ghaemi, K. Khajeh, Optimization of peroxidase-catalyzed oxidative coupling process for phenol removal from wastewater using response surface methodology, Environ. Sci. Technol. 41 (2007) 7073-7079.

[18] M.H. Siddique, C.C. St Pierre, N. Biswas, J.K. Bewtra, K.E. Taylor, Immobilized enzyme catalyzed removal of 4-chlorophenol from aqueous solution, Water Res. 27 (1993) 883–890.

[19] K. Tatsumi, S. Wada, H. Ichikawa, Removal of chlorophenols from wastewater by immobilized horseradish peroxidase, Biotechnol. Bioeng. 51 (1996) 126–130.

[20] J. Yu, K.E. Taylor, H. Zou, N. Biswas, J.K. Bewtra, Phenol conversion and dimeric intermediates in horseradish peroxidase-catalyzed phenol removal from water, Environ. Sci. Technol. 28 (1994) 2154–2160.

[21] J.A. Nicell, Kinetics of horseradish peroxidase-catalyzed polymerization and precipitation of aqueous 4-chlorophenol, J. Chem. Technol. Biot. 60 (1994) 203–215.

[22] G. Busca, S. Berardinelli, C. Resini, L. Arrighi, Technologies for the removal of phenol from fluid streams: A short review of recent developments, J. Hazard. Mater. 160 (2008) 265-288.

[23] J.L. Gomez, A. Bodalo, E. Gomez, J. Bastida, A.M. Hidalgo, M. Gomez, A covered particle deactivation model and an expanded Dunford mechanism for

the kinetic analysis of the immobilized SBP/phenol/hydrogen peroxide system, Chem. Eng. J. 138 (2008) 460-473.

[24] P. Cañizares, L. Martínez, R. Paz, C. Sáez, J. Lobato, M.A. Rodrigo, Treatment of fenton-refractory olive-oil-mills wastes by electrochemical oxidation with boron doped diamond anodes, J. Chem. Technol. Biotechnol. 81 (2006) 1331-1337.

[25] J.A. Zazo, J.A. Casas, A.F. Mohedano, M.A. Gilarranz, J.J. Rodríguez, Evolution of ecotoxicity upon Fenton´s oxidation of phenol in water, Environ. Sci. Technol. 39 (2008) 9295-9302.

[26] P. Bautista, A.F. Mohedano, J.A. Casas, J.A. Zazo, J.J. Rodríguez, An overview of the application of fenton oxidation to industrial wastewaters treatment, J. Chem. Technol. Biotechnol. 83 (2008) 1323-1338.

[27] M. Pera-Titus, V. García-Molina, M.A. Baños, J. Giménez, S. Esplugas, Degradation of chlorophenols by means of advanced oxidation processes: A general review, App. Catal. B–Environ. 47 (2004) 219–256.

[28] M. Czaplicka, Photo-degradation of chlorophenols in the aqueous solution, J. Hazard. Mater. 134 (2006) 45–59.

[29] J. Theurich, M. Lindner, D.W. Bahnemann, Photocatalytic degradation of 4-chlorophenol in aerated aqueous titanium dioxide suspensions: A kinetic and mechanistic study, Langmuir 12 (1996) 6368–6376.

[30] M.A. Barakat, G. Schaeffer, G. Hayes, S. Ismath-Shah, Photocatalytic degradation of 2-chlorophenol by Co-doped TiO_2 nanoparticles, Appl. Catal. B-Environ. 57 (2005) 23–30.

[31] G. Baum, T. Oppenlander, Vacuum-UV-oxidation of chloroorganic compounds in an excimer flow-through photoreactor, Chemosphere 30 (1995) 1781–1790.

[32] E.A. Sosnin, T. Oppenlander, F.V. Tarasenko, Applications of capacitive and barrier discharge excilamps in photoscience, J. Photochem. Photobiol. C 7 (2006) 145–163.

[33] N. Christofi, G.G. Matafonova, V.B. Batoev, M.A. Misakyan, E.M. Barkhudarov, I.A. Kossyi, UV treatment of chlorophenols in water using uv microwave lamps and XeBr excilamp coupled with biodegradation, In: Water Resources Research Progress (ed. L.N. Robinson) 2008a, Nova Science Publishers Inc, NY, pp. 101–126.

[34] N. Christofi, M.A. Misakyan, G. Matafonova, E.M. Barkhudarov, V.B. Batoev, I.A. Kossyi, J. Sharp, UV treatment of microorganisms on artificially-contaminated surfaces using excimer and microwave UV lamps, Chemosphere 73 (2008) 717-722.

[35] B. Guieysse, G. Viklund, A. Toes, B. Mattiasson, Combined UV-biological degradation of PAHs, Chemosphere 55 (2004) 1493–1499.

[36] T. Essam, M.A. Amin, O. El Tayeb, B. Mattiasson, B. Guieysse, Sequential photochemical-biological degradation of chlorophenols, Chemosphere 66 (2007) 2201–2209.

[37] G. Matafonova, N. Christofi, V. Batoev, E. Sosnin, Degradation of chlorophenols in aqueous media using UV XeBr excilamp in a flow-through reactor, Chemosphere 70 (2008) 1124–1127.

[38] V. R. Jr. Thomas, A. F. Schreiner, T. Xie, Ch. Chen, J. S. Gratzl, Photolytic dechlorination of 4-chlorophenol in O_2-saturated aqueous solutions in the

absence of photocatalysts or additives using ArF (193 nm) and KrF (248 nm) excimer lasers, J. Photochem. Photobiol., A 90 (1995) 183-191.

[39] E. Lipczynska-Kochany, J. R. Bolton, Flash photolysis/HPLC applications. 2. Direct photolysis vs. hydrogen peroxide mediated photodegradation of 4-chlorophenol as studied by a flash photolysis/HPLC technique, Environ. Sci. Tecnol. 26 (1992) 259-261.

[40] A. Bodalo, J.L. Gomez, E. Gomez, A.M. Hidalgo, M. Gomez, A.M. Yelo, Removal of 4-chlorophenol by soybean peroxidase and hydrogen peroxide in a discontinuous tank reactor, Desalination 195 (2006) 51–59.

[41] J.L. Gomez, A. Bodalo, E. Gomez, J. Bastida, A.M. Hidalgo, M. Gomez, Immobilization of peroxidases on glass beads: An improved alternative for phenol removal, Enzyme Microb. Tech. 39 (2006) 1016–1022.

[42] A. Bodalo, J. Bastida, M.F. Maximo, M.C. Montiel, M. Gomez, M.D. Murcia, A comparative study of free and immobilized soybean and horseradish peroxidases for 4-chlorophenol removal: protective effects of immobilization, Bioproc. Biosyst. Eng. 31 (2008) 587-593.

[43] J.L. Gomez, E. Gomez, J. Bastida, A.M. Hidalgo, M. Gomez, M.D. Murcia, Experimental behaviour and design model of a continuous tank reactor for removing 4-chlorophenol with soybean peroxidase, Chem. Eng. Process. 47 (2008) 1786-1792.

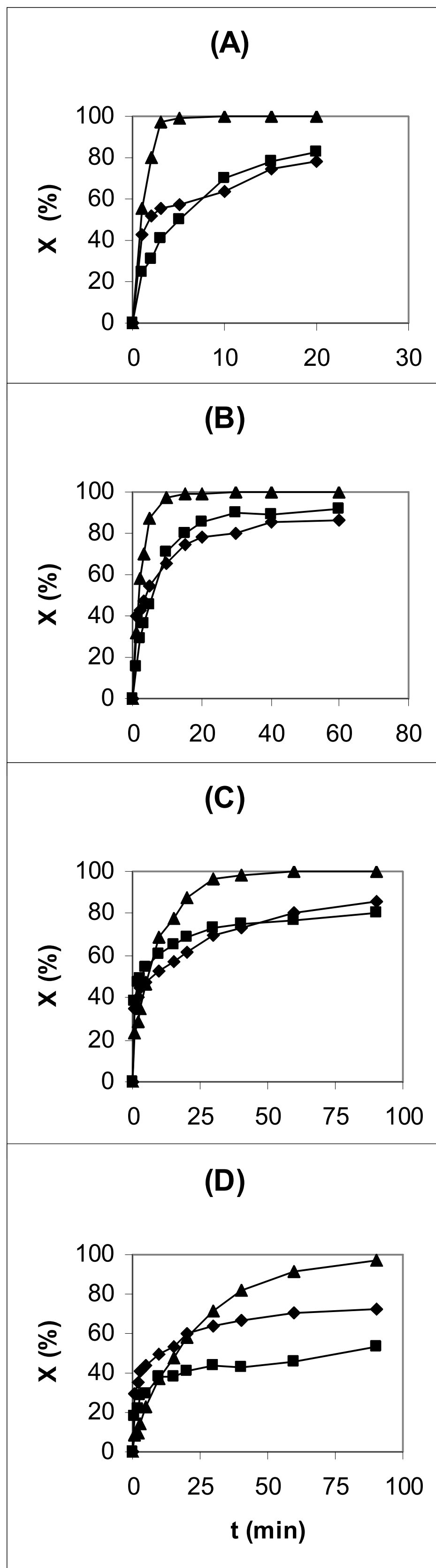

Figure 1

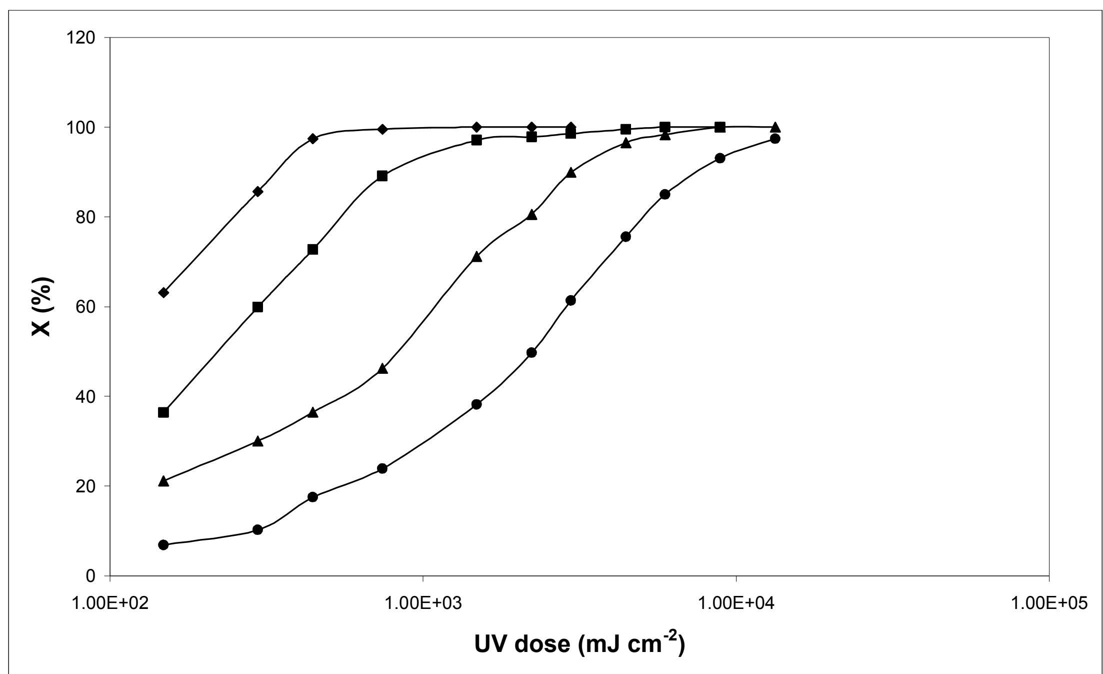

Figure 2

Figure3

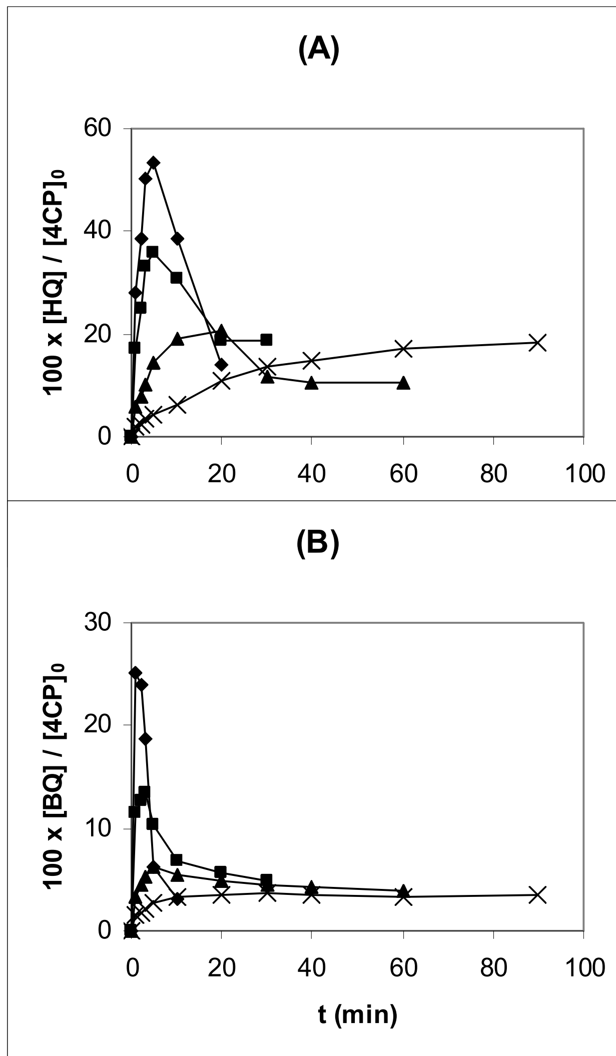

Figure 3

Figure4

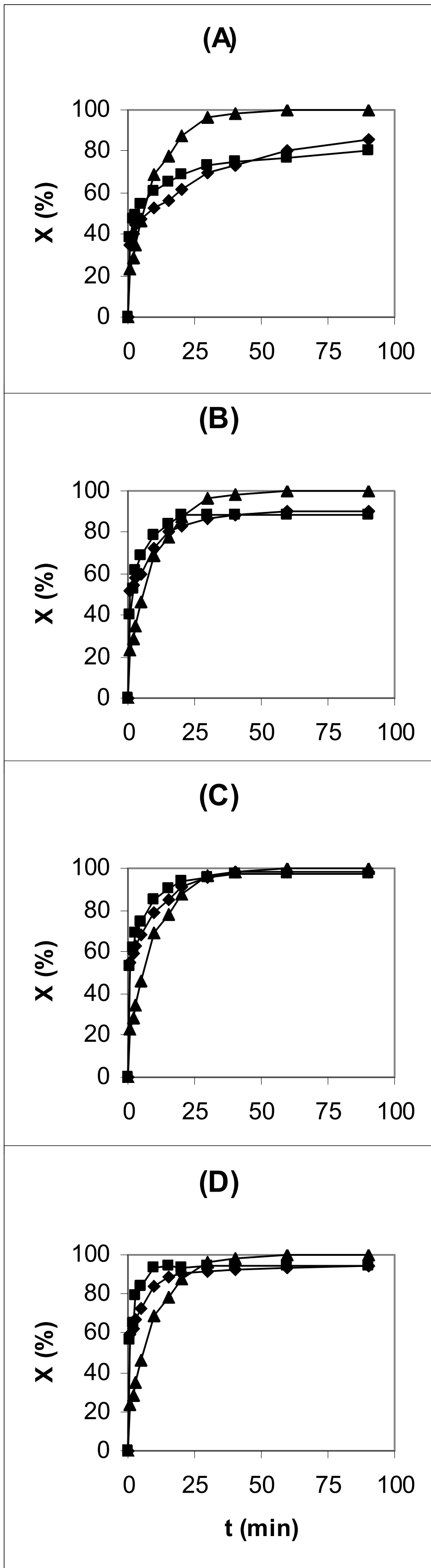

Figure 4

Figure captions

Figure 1. Comparison of treatments at a fixed enzyme concentration of 5 mg L^{-1} and the different 4-chlorophenol concentrations tested: A) 50 mg L^{-1}; B) 100 mg L^{-1}; C) 250 mg L^{-1}; D) 500 mg L^{-1}. ♦ immobilized SBP; ■ free SBP; ▲ excilamp.

Figure 2. UV dose required for the different 4-chlorophenol concentrations tested in the excilamp treatment: ♦ 50; ■ 100; ▲ 250; ● 500 mg L^{-1}.

Figure 3. Residual percentages of two main photoproducts along time, (A) hydroquinone and (B) benzoquinone, for the different 4-chlorophenol concentrations tested: ♦ 50 mg L^{-1}; ■ 100 mg L^{-1}; ▲ 250 mg L^{-1}; X 500 mg L^{-1}.

Figure 4. Comparison of treatments at a fixed 4-chlorophenol concentration of 250 mg L^{-1} and four different SBP concentrations: A) 5 mg L^{-1}; B) 10 mg L^{-1}; C) 15 mg L^{-1}; D) 20 mg L^{-1}. ♦ immobilized SBP; ■ free SBP; ▲ excilamp.

Table 1. Enzyme immobilization and activity

Immobilized protein (%)	*Protein concentration (mg g^{-1} support)*	*Protein concentration (mg L^{-1} suspension)*	*Activity yield (%)*
28	13.3	270	65