REMOVAL OF PHENOLS FROM AQUEOUS SOLUTIONS IN THE PRESENCE OF HORSERADISH PEROXIDASE AND CYCLODEXTRIN DERIVATIVES

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ABSTRACT

The hydroxylated aromatic compounds, present in the industrial waste waters, constitute a great environmental problem and many different processes, such as chemical or enzymatic oxidation, were reported in order to solve this problem. In this preliminary work we studied the influence of some cyclodextrin derivatives on the oxidation of phenols by horseradish peroxidase in presence of H_2O_2. We found that the cyclodextrin presence promote phenols removal from aqueous solution for a large set of compounds and we started to study the kinetic of the highly reactive 2,4,6-tribromophenol.

1. INTRODUCTION

Horseradish peroxidase (HRP), for its relatively low cost and its high reactivity towards many different organic and inorganic compounds, is an enzyme largely used in industrial and laboratory applications. In particular, the oxidation of some phenols and aromatic amines by the system HRP/H_2O_2 was reported in literature [1-4], and recently the relative production of insoluble polymers was used to remove phenols from industrial waste waters [5, 6].

The phenols low solubility in water and the presence of emulsions with organic solvents, which deplete enzyme activity, constitute the greatest problems in the enzymatic

treatment of phenol derivatives. Furthermore it is well known that cyclodextrin derivatives give inclusion compounds with substrates of suitable size and polarity, such as many hydroxylated aromatic compound, and this property is largely used in inverse transfer catalysis [7]. For these reasons we started to study the effect of the presence of β-cyclodextrin (β-CD) and of two cyclodextrin derivatives (methyl-β-cyclodextrin, Me-β-CD and hydroxypropyl-β-cyclodextrin, HP-β-CD) on the enzymatic oxidation of water solutions containing some different phenol derivatives. In this communication we report some qualitative preliminary results on the phenols degradation and the kinetic data on the oxidation of the highly reactive 2,4,6-tribromophenol (TBP).

2. MATERIALS AND METHODS

2.1 Materials

β-CD was a gift from Roquette Italia (Cassano Spinola, Italy); HP-β-CD and Me-β-CD were kindly supplied from Wacker Chemie (Germany). Horseradish peroxidase (E.C. 1.11.1.7) was aquired from Sigma Chemical Co. (USA) (Type VIA, RZ = 3.0, 1100 Sigma units/mg solid). Hydrogen peroxide (30%, ACS grade) was from Merck (Germany). All phenols, from Aldrich (USA), were on analytical grade and used without further purification. UV-visible spectra and kinetic measurements were recorded on a double beam UVIKON 930 spectrophotometer (Kontron Instruments, Italy).

2.2 Methods

The solutions of cyclodextrin derivatives were obtained by diluition of a stock solution of 2.5 wt % of CD in water. These solutions were saturated with the selected phenols and equilibrated overnight under magnetic stirring at room temperature; subsequently the excess of phenol was removed by filtration. UV-visible spectra and kinetics measurements were obtained introducing in a quartz cuvette 1 ml of CD solution, 1 ml of bidistilled water, 20 μl of HRP (1.19×10^{-5} M) and 80 μl of H_2O_2 (7.4×10^{-2} M). The reaction rate was followed by the increase of the absorbance of the reaction product at 359 nm.

3. RESULTS AND DISCUSSION

In Table 1 we report the results of our catalytic preliminary studies: the phenols were organized on the basis of their relative reactivity towards HRP/H_2O_2 system in the presence and in the absence of cyclodextrin derivatives.

TABLE 1.

phenols NON-reactive	phenols more reactive without CD	phenols more reactive with CD
2,6-di-*tert*-butyl-4-methylphenol	4-hydroxydiphenyl	2,4,6-tribromophenol
3-nitrophenol	2,4,6-tri-*tert*-butylphenol	2,4,6-trichlorophenol
dodecylgallate		3-chlorophenol
		1-naphtol
		2-naphtol

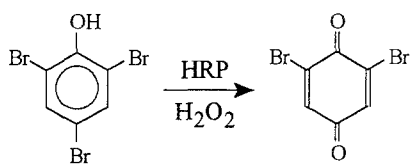
The first column accounts for the phenols unreactive towards HRP both in the presence or in the absence of the CD derivatives. These molecules seems to be not suitable as substrate for HRP under our experimental conditions.

The second group show an higher reactivity in the absence of CDs. We suppose that this behaviour could be ascribed to the protective action of the CD cavity which gives the substrate less available for the enzyme.

The third one (the most numerous) reports the phenols that have higher reaction rate in the presence of the CDs. In particular with 1- and 2-naphtol, insoluble polymeric materials were obtained, which can be easily removed from waste waters by filtration.

With the aim to study in detail the CD influence on these oxidations, we made a series of measures with TBP using different cyclodextrins and various experimental conditions.

The oxidative process of TBP in absence and in presence of β-CD, Me-β-CD or HP-β-CD was followed spectrophotometrically and in all the cases we obtained the same spectral pattern. In agreement with a reported oxidation mechanism [8] we suggest that TBP (**1**) was been oxidated to 2,6-dibromo-1,4-benzoquinone (**2**) as shown in the figure:



1 **2**

In Table 2 we report the reaction initial rates obtained for TBP in presence of different concentrations of the CDs. From these preliminary quantitative data we can argue that

the oxidation initial rate seems depend on the CD concentration and that the catalytic efficiency of the three CDs is quite similar, but with a slight prevalence of the Me-β-CD promotion.

TABLE 2: TBP oxidation initial rate (M/min)

% of CD	β-CD	Me-β-CD	HP-β-CD
0.05	$3.4 \cdot 10^{-4}$	$5.2 \cdot 10^{-4}$	$3.8 \cdot 10^{-4}$
0.10	$4.3 \cdot 10^{-4}$	$6.3 \cdot 10^{-4}$	$4.4 \cdot 10^{-4}$
0.15	$4.7 \cdot 10^{-4}$	$6.4 \cdot 10^{-4}$	$4.8 \cdot 10^{-4}$
0.25	$7.4 \cdot 10^{-4}$	$7.7 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$

We could suppose that the cyclodextrin derivatives effect on the peroxidatic oxidation of TBP depends principally on the substrate solubility increase due to its favourite inclusion in the CD cavity.

In order to completely clarify this problem, further studies concerning the calculation of the inclusion constants and the investigation of the kinetic properties of a series of phenols are in progress.

4. REFERENCES

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