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A covered particle deactivation model and an expanded Dunford mechanism for the kinetic analysis of the immobilized SBP/phenol/hydrogen peroxide system

José L. Gómez *, Antonio Bódalo, Elisa Gómez, Josefa Bastida, Asunción M. Hidalgo, María Gómez

Departamento de Ingeniería Química, Universidad de Murcia, Campus de Espinardo, 30071 Murcia, Spain

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Abstract

An expanded version of the Dunford mechanism, which extends the initial peroxidase cycle to the reaction products, was developed and applied to the kinetic analysis of the immobilized soybean peroxidase/phenol/hydrogen peroxide system. At the same time, an enzyme deactivation model, based on the gradual covering of the catalytic particles by the products originated during the reaction (radicals and end-products, oligomers/polymers), was proposed. From the reaction mechanism and deactivation model, the kinetic equations for phenol, dimeric compounds and hydrogen peroxide were obtained and applied to the design of a batch reactor.

In order to check the mechanism, an immobilized derivative of the enzyme on PG-glutaraldehyde, which retains 74% of the free enzyme activity and which was characterized in a previous work, was used. In a batch reactor, and without adding protective agents, several series of experiments were carried out, and the influence of operational variables on the conversion was studied. Phenol removal percentages of more than 90% were obtained in some of the tested situations.

Using a method for initial rate estimation, three of the model parameters were calculated. In order to determine the remaining parameters, half of the experimental data series and a program for error minimization, based on the Simplex algorithm of Nelder and Mead, were used. A good fitting between the data and the model was obtained, and the typical deviation was 3.27%. Using the data from the remaining series, which had not been used for determining the parameters, the model was checked and even better agreement, with 2.72% typical deviation, was obtained, which confirms the validity of the proposed model.

Keywords: Soybean peroxidase; Phenol; Hydrogen peroxide; Mechanism; Kinetic model; Deactivation model; Covered particle model

1. Introduction

1.1. Removal of phenolic compounds with peroxidases

Phenolic compounds are present in a wide range of concentrations in the wastewaters of oil refineries and numerous other industries, including the plastics, resins, textiles, iron, steel and forestry industries [1–5]. Most of these compounds are toxic, and cannot easily be removed by conventional physical–chemical or biological techniques.

Some disadvantages of conventional treatment methods can be avoided by adopting an enzymatic method. These methods generally have a high degree of specificity, low energy requirements, mild operation conditions, a high reaction rate which reduces processing costs and a catalytic ability over wide ranges of pH, temperature and substrate concentration. Also, they have a minimal environmental impact.

The application of free or immobilized oxidoreductive enzymes to catalyze the oxidation of aromatic compounds from wastewater has been widely investigated. Horseradish peroxidase (HRP) and soybean peroxidase (SBP) catalyzes the oxidation of aqueous phenols by hydrogen peroxide to produce free radicals that spontaneously interact to form oligomers and polymers of high molecular weight and low solubility. These

* Corresponding author. Tel.: +34 968 367351; fax: +34 968 364148.
E-mail address: carrasco@um.es (J.L. Gómez).

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Nomenclature

α_i	parameter defined by a group of constant ($i = 1, 2, 3,\ldots$)
E	enzyme
E_{active}	active enzyme
$E_{inactive}$	inactive enzyme
E_0	total enzyme
EO	enzyme Compound I
$EO\Phi H_2$	intermediate addition complex of Compound I and Phenol
EOHΦ-ΦH	intermediate addition complex of Compound I and dimer
*EOH	enzyme Compound II
$*EOH\Phi H_2$	intermediate addition complex of Compound II and Phenol
*EOHHΦ-ΦH	intermediate addition complex of Compound II and dimer
ΦH_2	phenol
* ΦH	free radical of phenol
*Φ-ΦH	free radical of dimer
HΦ-ΦH	dimer of phenol
HΦ-Φ-Φ-ΦH	tetramer of phenol
k_i	rate constant of an irreversible step i ($i = 1, 2, 3,\ldots$)
k_{cat1}	enzyme catalytic constant in the phenol-oxidizing reaction
k_{cat2}	enzyme catalytic constant in the dimer-oxidizing reaction
k_{CR}	constant defined in Eq. (46)
k_d	enzyme deactivation constant
$k_{H_2O_2}$	proportionality constant for total peroxide consumption
k_n	proportionality constant defined in Eq. (36)
k_R	proportionality constant defined in Eq. (41)
K_i	equilibrium constant of a reversible step i ($i = 1, 2, 3,\ldots$)
K_{Mi}	generic Michaelis constants in the kinetic equation ($i = 1, 2, 3, 4$)
N_{active}	total number of enzyme active centres at time t
N_0	total number (active and inactive) of enzyme catalytic centres
N_{R*}	total number of radical molecules at time t
$r_{\Phi H_2}$	consumption rate of phenol
$r_{H\Phi\text{-}\Phi H}$	consumption rate of dimer
r_{dimer}	overall reaction rate of dimer
r_0	initial reaction rate of phenol
$R*$	generic radical of a phenolic compound
t	time
Δt	time increment
V_{max}	maximum reaction rate
V_R	reactor volume
$[X]$	concentration of the species X in the bulk reaction

products are precipitated from the solution and can be removed by filtration or sedimentation [6–10].

Enzyme immobilization has many advantages, including enzyme reuse and stabilization, the control of product formation and easy separation from the reaction medium [11].

There are several methods for enzyme immobilization as well as support materials. The methods and supports used are chosen to ensure the highest retention of activity and their stability. Conventional methods include physical adsorption, covalent binding, crosslinking, inclusion or encapsulation [12].

In the literature, numerous studies have proposed the mechanism and kinetic equations for peroxidase/phenolic compounds/hydrogen peroxide systems [13–24]. Some of them are described below.

1.2. Kinetics of the enzymatic reaction

1.2.1. Mechanisms

Usually, the oxidation of aromatic compounds with hydrogen peroxide, catalyzed by peroxidase, has been described through the mechanism postulated by Chance–George, also known as the Dunford mechanism [25,26], which is referenced in most of the papers found in the literature.

The steps of this mechanism are the following:

$$E + H_2O_2 \rightarrow E_1$$

$$E_1 + AH_2 \rightarrow E_2 + AH^{\bullet}$$

$$E_2 + AH_2 \rightarrow E + AH^{\bullet}$$

$$AH^{\bullet} + AH^{\bullet} \rightarrow HA{-}AH$$

The native enzyme, E, in the presence of hydrogen peroxide, forms a compound, E_1, called Compound I that, in turn, accepts an aromatic compound, AH_2, oxidizing it and giving the free radical $AH^{\bullet}$. This radical is released to the reaction bulk and the enzyme changes to the E_2 state, Compound II, which is able to oxidize another molecule of AH_2, giving another free radical and returning to the native state, E, so that the cycle is closed. The overall reaction is: $H_2O_2 + 2AH_2 \xrightarrow{E} 2AH^{\bullet} + 2H_2O$

Stoichiometric studies [25] have shown that the species E_2 only contains one of the two oxidation equivalents of the hydrogen peroxide molecule and that it is a covalent compound. However, the definitive structure of this species has not been well established until now and, as indicated in one of the consulted papers [27], "the sequence of steps involved in this catalysis is not, really, very well known", and is usually described by the above mentioned Chance–George mechanism.

As has been described in the literature [25,28,29], in excess of hydrogen peroxide E_2 can be oxidized to E_3, which appears as an inactive form of the enzyme:

$$E_2 + H_2O_2 \rightarrow E_3 + H_2O$$

This is not an irreversible deactivation because E_3 breaks down spontaneously to the native form [30], although with a