ENZYMATIC ELIMINATION OF PHENOL IN STIRRED AND TORUS REACTOR.

Laura Pramparo\textsuperscript{a}, Irama Sancheza\textsuperscript{a}, Frank Stübera\textsuperscript{a}, Josep Font\textsuperscript{a}, Agustí Fortunyb\textsuperscript{b}, Azael Fabregat\textsuperscript{a}, Christophe Bengoaa\textsuperscript{a}

\textsuperscript{a}Universitat Rovira i Virgili, ETSEQ, DEQ, Països Catalans, 26, 43007 Tarragona, España, Tel: 977558619, Fax: 977559621, laura.pramparo@urv.net; \textsuperscript{b}Universitat Politècnica de Catalunya, EUPVG, DEQ, Víctor Balaguer, s/n, 08800 Vilanova i la Geltrú, España

Abstract.
Phenols are priority pollutants that are found in a large number of industrial wastewaters, e.g., petroleum refinery, resin manufacture, and coal processing. Aromatic compounds such as phenol and its derivatives are the major class of pollutants commonly found in industrial wastewaters. Phenol removal through enzyme-catalyzed polymerization is an alternate detoxification method. This method can be defined like the oxidation of phenol using \( \text{H}_{2}\text{O}_2 \) in the presence of an enzyme such a catalyst. The process has many advantages over conventional biological treatment and also potential advantages over chemical/physical processes.

A number of previous work have demonstrated that oxidation of phenolic pollutants is feasible and can be catalyzed by several enzymes [1], including horseradish peroxidase HRP [2], chloroperoxidase CPO [3], laccase from several microbial sources, lignin peroxidase from that white-rot fungus LIP [4,5] and polyphenol oxidase from mushrooms (e.g. tyrosinase) [6]. The torus reactor, which can be considered as a loop reactor, presents some advantages over other stirred tank reactors including efficient mixing of reactants, easy scale-up and design ensured by the absence of dead volume, low power consumption [7], high heat transfer capacity [8], prevention of deposition of polymer or biomass on the reactor wall and efficiency [7,9,10].

The purpose of this work was to study the enzymatic reaction and to determine the kinetic parameters. In the first step, enzymatic elimination of phenol was carried out in a stirred glass reactor of 50-100 ml. Conditions were as follows, phenol concentration (13.8 to 53.0 mM), HRP concentration (4.9\times10^{-5} to 4.9\times10^{-3} mM) and \( \text{H}_{2}\text{O}_2 \) (10 to 192 mM) in 50 or 100 ml of potassium phosphate buffer (0,1 M, pH=7). The phenol and HRP dissolution were allowed to reach the operating temperature (\( T=20^\circ\text{C} \)) before adding the corresponding amount of hydrogen peroxide. The reaction time started when hydrogen peroxide addition began. The initial reaction rate was determined from the consumption of phenol as a function of time. Phenolic compound was quantified by HPLC (High Performance Liquid Chromatography) and gas chromatography (GC). Also, the operation conditions were optimized to determine the optimum concentration of reagents to use.

The kinetic data obtained as a function of \( \text{H}_{2}\text{O}_2 \) concentration are presented in figure 1 and 2. As shown in these figures, the initial reaction rate increases with the increase of \( \text{H}_{2}\text{O}_2 \) initial concentration. The initial rate (\( V_i \)) vs. substrate concentration was fitted to the Michaelis-Menten equation and the parameters were determined by non-linear regression. Apparent Michaelis-Menten parameters for the reaction of HRP with Phenol and \( \text{H}_{2}\text{O}_2 \) are: \( V'_\text{max} \) (M\cdot min\(^{-1}\)) = 2.11, \( K'_m \) (mM) = 17.6 for [Phenol]\(_0\) = 13.8 mM and \( V'_\text{max} \) (M\cdot min\(^{-1}\)) = 5.01, \( K'_m \) (mM) = 42.4 for [Phenol]\(_0\) = 19.1 mM. These results show that both \( V'_\text{max} \) and \( K'_m \) increase when initial concentration of phenol increases and confirm the trend of Michaelis-Menten equations, where it is predicted that \( V'_\text{max} \) and \( K'_m \) will increase with an increase of the phenol initial concentration.

In a second step, the enzymatic elimination will be carried out in a torus reactor of 100 ml. The operation conditions will be the same than the used in the stirred reactor in order to have
the possibility to compare the performances of both reactors. This work is currently on progress.

Figure 1. Steady-state initial rates of oxidation of phenol as a function of H$_2$O$_2$ initial concentrations. 2.48x10$^{-3}$ mM HRP, 13.8 mM phenol, pH 7, 20°C.

Figure 2. Steady-state initial rates of oxidation of phenol as a function of H$_2$O$_2$ concentrations. 2.48x10$^{-3}$ mM HRP, 19.1 mM phenol, pH 7, 20°C.