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Mechanistic insight into sono-enzymatic degradation of organic pollutants with kinetic and thermodynamic analysis

Ritesh S. Malani [a], Swati Khanna [b], Sankar Chakma [a], Vijayanand S. Moholkar [a,b,*]

[a] Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India
[b] Center for Energy, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India

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ABSTRACT

In this paper, we have attempted to get a physical insight into process of sono-enzymatic treatment for degradation of recalcitrant organic pollutants. Decolourization of an azo dye has been used as model reaction with different experimental protocols that alter characteristics of ultrasound and cavitation phenomena in the system. Experimental data is analyzed to determine kinetic and thermodynamic parameters of decolorization process. The trends observed in kinetic and thermodynamic parameters of decolourization are essentially manifestations of the dominating mechanism of the decolorization of the textile dye (or nature of prevalent chemical reaction in the system), viz. either molecular reaction due to enzyme or radical reaction due to transient cavitation. The activation energy for sonochemical protocol is negative, which indicates instantaneity of the radical reactions. The frequency factor is also low, which is attributed to high instability of radicals. For enzymatic and sono-enzymatic protocols, activation energy is positive with higher frequency factor. Enthalpy change for sonochemical protocol is negative, while that for enzymatic and sono-enzymatic protocols is positive. The net entropy change for sonochemical protocol is more negative than enzymatic or sono-enzymatic protocol due to differences in prevalent chemical mechanism of dye decolorization. Due to inverse variations of frequency factor and activation energy, marginal rise in reaction kinetics is seen for sono-enzymatic protocol, as compared to enzymatic treatment alone. Due to inverse variations of enthalpy and entropy change, net Gibbs energy change in all experimental protocols shows little variation indicating synergism of the mechanism of ultrasound and enzyme.

1. Introduction

Sono-enzymatic treatments have been investigated in the recent past for effective degradation of recalcitrant organic pollutants appearing in industrial wastewater discharge. This process has been reported to give enhanced degradation of the recalcitrant organic pollutants, as compared to only enzymatic treatment with conventional techniques such as mechanical stirring [1–5]. Sonolysis (or ultrasound irradiation) itself is also a known technique for degradation of recalcitrant organic pollutants, which works on production of highly reactive oxidation radicals like ·O, ·OH and $HO_2^{\cdot}$ through transient cavitation [6]. The mechanism of enzymatic treatment is completely different. The common enzymes employed for wastewater treatment are peroxidases (examples being lignin peroxidase, manganese peroxidase, laccases and horseradish peroxidase). These enzymes are most effective in decolorization of textiles dyes (which are an important category of the recalcitrant organic pollutants). Peroxidases catalyze the oxidation of a variety of the substrate utilizing H_2O_2 as an electron acceptor. The net action of these enzymes is either precipitation of the dye or transformation into other harmless products. Among the peroxidases mentioned above, the horseradish peroxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7 HRP) is perhaps the most popular enzyme with potential application in chemical, environmental, pharmaceutical and biotechnology industries. Peroxidases can contain a heme cofactor in their active sites, or redox-active cysteine or selenocysteine residues. The binding site for the aromatic substrate oxidation by HRP C is the exposed heme edge. Horseradish peroxidase isoenzyme C (HRP C) contains a single polypeptide of 308 amino acid residues [7]. A general equation for reaction catalyzed by HRP C (and also other isoenzymes) is given as follows [3]:

* Corresponding author at: Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781 039, Assam, India. Tel.: +91 361258 2251; fax: +91 361258 2291.
E-mail address: vmoholkar@iitg.ernet.in (V.S. Moholkar).

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$$H_2O_2 + 2AH_2 \xrightarrow{HRPC} 2H_2O + 2AH^{\cdot} \quad (1)$$

Notation: AH_2 – a reducing substrate (i.e. organic pollutant molecule); $AH^{\cdot}$ – its radical product. The $AH^{\cdot}$ radicals can combine (or dimerize) and precipitate.

Our earlier work [3,4] revealed that transient cavitation has adverse effects on enzymes due to denaturation caused by shock waves generated during transient collapse of the bubble [3] and that immobilization of the enzymes on a suitable support (such as polyurethane foam) can reduce this adverse effect [4]. Moreover, it was also shown that application of high static pressure to the reaction mixture practically eliminates transient cavitation [3].

In this present study, the mechanistic facets of the sono-enzymatic degradation of organic pollutants have been investigated using kinetic and thermodynamic analysis. Decolourization of an azo textile dye Acid Red 14 has been chosen as the model reaction. Essentially, we have tried to find the interactions or interlinks between the individual mechanisms of sonolysis and enzymatic treatment, when both of these techniques are simultaneously applied. The decolorization of Acid Red 14 has been studied in different experiments protocols that vary the characteristics of ultrasound and cavitation phenomena in the medium. With Arrhenius analysis of kinetic data of dye decolorization in these experiments, pseudo 1st order kinetic constant, activation energy, and frequency factor for sono-enzymatic dye decolorization have been deduced. In addition, the thermodynamic properties of the decolourization process, i.e. change in enthalpy, entropy and Gibbs free energy for different protocols have been studied with the help of Eyring equation. Variation in the numerical values of these kinetic and thermodynamic parameters gives a mechanistic insight into the physical mechanism of the sono-enzymatic decolourization process. We would like to clarify here that in our analysis, we have not made an attempt to establish the chemical mechanism or pathway of degradation of Acid Red 14 dye by identifying the intermediates of degradation. This matter is beyond the scope of this study. Earlier published literature has been extensively treated this matter; for example Jadhav et al. [8] have discussed microbial degradation pathway, whereas Rehorek et al. [9] have discussed sonochemical degradation pathway for azo dye. We would suggest interested readers to refer to these papers for greater details on chemical mechanism or pathway of degradation. Our analysis is based on time histories of the (residual) dye concentration in the reaction mixture during different experimental protocols.

2. Materials and methods

2.1. Materials

Horseradish peroxidase (HRP) in the form of lyophilized powder (140 U/mg) was procured from Sigma Aldrich, Germany. Acid Red 14 dye ($C_{20}H_{12}N_2Na_2O_7S_2$) was purchased from local market. All other chemicals (analytical grade) have been procured from Merck (India), and have been used as received, without further any pretreatment.

2.2. Experimental setup

A custom-fabricated test tube (20 mL) made of borosilicate glass was used for all experiments. This test tube has a screw cap with side neck that can be connected to a gas cylinder for raising the static pressure in the tube. An ultrasound bath (Elma, Germany, Model: T460, frequency: 35 kHz, power: 35 W dimensions 25 × 15 × 10 cm) was used for sonication of the dye solution. The bath was filled with 1.5 L of water. The temperature of water during sonication was maintained practically constant (with slight variation of ±2 °C) using a refrigerated circulator (Lab Tech, Korea, Model: RW0525G). The actual power dissipation of the ultrasound bath was characterized calorimetrically. With this procedure, the pressure amplitude of the acoustic wave generated by the transducers (attached to the bottom of the bath) was determined as 150 kPa [10]. The position of the test tube in the central region of the ultrasound bath was carefully maintained same in all experiments. This is due to significant spatial variation of the ultrasound intensity and local acoustic pressure amplitude in the bath [11]. For experiments with elevated static pressure (200 kPa), the test tube was connected to a N_2 gas cylinder having a double stage regulator. The control (or base case) experiments were carried out with mechanical shaking of reaction mixture in the custom-fabricated glass test-tube at 120 rpm in a refrigerated orbital shaker incubator (Jeiotech, South Korea).

2.3. Experimental categories or protocols

The decolourization of Acid Red 14 dye was carried out in four categories or protocols, viz. (1) sonication; (2) enzymatic treatment with mechanical shaking at 120 rpm; (3) enzymatic treatment with sonication at atmospheric static pressure (P_o) = 101.3 kPa; (4) enzymatic treatment with sonication at elevated static pressure (P_o) = 200 kPa. The experiments in each category have been conducted at four different temperatures, i.e. 288, 293, 298 and 303 K. 15 mL dye solution with initial concentration of 20 ppm has been used in all experiments. The characterization of the enzyme (in terms of enzyme activity, optimum pH, and Michaelis–Menten kinetic parameters) has been reported by us in an earlier paper [4]. For all experiments involving HRP, the reaction mixture consisted of 2.4 mL phosphate buffer (pH = 7, 10 mM), 200 μL H_2O_2 solution, 300 μL enzyme solution (2 U/mL), with 15 mL of 20 ppm dye solution, as mentioned earlier. All experiments were conducted for 1 h, and samples of the dye solution were withdrawn every 15 min for analysis. The residual concentration of dye in these samples was determined using UV–Visible spectrophotometer (Model: UV-2300, Make: Thermo Fischer, India) to get the time history of decolourization, which could later be used for kinetic analysis of decolourization. All experiments have been carried out in triplicate to assess reproducibility of the results.

2.4. Experimental data analysis

The time history of decolourization was fitted to different kinetic models to determine the order of decolourization with respect to dye. On the basis of regression coefficients (R^2) for different models, the decolourization was essentially found to follow pseudo 1st order kinetics ($R^2 \geqslant 0.95$). The Arrhenius analysis of the decolourization reactions with different protocols was done using these kinetic constants to determine the activation energy and frequency factor. Arrhenius equation is expressed as: $k = A\exp(-E_a/RT)$, where k is kinetic constant and A is frequency factor or pre-exponential factor for the reaction. E_a is activation energy, which is defined as the energy barrier to be crossed for occurrence of a chemical reaction. R is the universal gas constant and T is the temperature. This equation can be rewritten as: $\ln(k) = \frac{-E_a}{RT} + \ln(A)$. The plot of $\ln(k)$ vs. $1/T$ (termed as the Arrhenius plot) gives the value of $-E_a/R$ as slope and $\ln(A)$ as the y-intercept.

The thermodynamic analysis of kinetic data was done using the Eyring equation coupled with basic relations between thermodynamic properties as follows [12].

$$\ln\frac{k}{T} = -\frac{\Delta H}{R}\frac{1}{T} + \ln\frac{k_b}{h} + \frac{\Delta S}{R} \quad (2)$$

$$\Delta H = E - R\cdot T \quad (3)$$