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Investigations in sono-enzymatic degradation of ibuprofen

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ABSTRACT

The drug ibuprofen (IBP) appears frequently in the wastewater discharge from pharmaceutical industries. This paper reports studies in degradation of IBP employing hybrid technique of sono-enzymatic treatment. This paper also establishes synergy between individual mechanisms of enzyme and sonolysis for IBP degradation by identification of degradation intermediates, and Arrhenius & thermodynamic analysis of the experimental data. Positive synergy between sonolysis and enzyme treatment is attributed to formation of hydrophilic intermediates during degradation. These intermediates form due to hydroxylation and oxidation reactions induced by radicals formed during transient cavitation. Activation energy and enthalpy change in sono-enzymatic treatment are lower as compared to enzyme treatment, while frequency factor and entropy change are higher as compared to sonolysis. Degradation of IBP in sono-enzymatic treatment is revealed to be comparable with other hybrid techniques like photo-Fenton, sono-photocatalysis, and sono-Fenton.

1. Introduction

Wastewater discharge from various process industries has been a major source of water pollution. Many organic pollutants appearing in the wastewater discharge are bio-recalcitrant. Wastewater discharge from pharmaceutical industries is of special concern, due to presence of numerous drugs in it, which are potentially harmful to ecosystem. A common drug that appears in wastewater discharge from pharmaceutical industries is ibuprofen (IBP) or 2-(4-isobutyl phenyl) propanoic acid ($C_{13}H_{18}O_2$). This is a non-steroidal, anti-inflammatory, antipyretic drug, which is widely used for treatment of inflammatory disorders. This drug has several adverse impacts on the ecosystem [1–3]. Advanced oxidation techniques (AOTs) such as Fenton, photo-Fenton, photocatalysis and ozonation are required for complete mineralization of pharmaceutical wastewater containing IBP [4–7]. More recently, sonolysis [8] and sono-hybrid techniques, in which sonication is coupled with AOTs like photocatalysis and Fenton process [9–14], have been employed for degradation of IBP. Ultrasound and cavitation have several physical and chemical effects [15–18], which support the mechanism of conventional advanced oxidation techniques. Sonolysis (or ultrasound irradiation) alone is not effective in degradation as well as complete mineralization of organic pollutants. This is attributed to formation of hydrophilic intermediates during degradation. These intermediates form by hydroxylation/oxidation reactions induced by radicals formed during transient cavitation. Hence, sono-hybrid techniques have been applied for degradation/mineralization of the IBP.

A common sono-hybrid technique for degradation of bio-recalcitrant organic pollutants is sono-enzymatic treatment [19–24], in which sonolysis is combined with enzymatic treatment. This technique has especially been applied for degradation/decolorization of dyestuffs in wastewater from textile industries. Peroxidase enzymes (such as lignin peroxidase, manganese peroxidase, laccase and horseradish peroxidase) that utilize H_2O_2 as electron acceptor have been used in sono-enzymatic technique. A summary of literature in area of sono-enzymatic degradation of variety of pollutants is given in Table S1 in the Supplementary material.

In this paper, we have reported studies in sono-enzymatic degradation of IBP using horseradish peroxidase (HRP) enzyme. We have also attempted to shed light on the mechanistic synergism between the enzymatic treatment and sonolysis during IBP degradation. For this purpose, we have used Arrhenius and thermodynamic analysis of the experimental data, as reported in one of our earlier study [25]. Moreover, identification of the intermediates of degradation also gives an insight into the chemical mechanism of degradation. To the best of our knowledge, this study is the first investigation in IBP degradation using sono-enzymatic technique.

2. Materials and methods

2.1. Materials

Ibuprofen, horseradish peroxidase (EC 1.11.1.7, 140 U/mg) enzyme and pyrogallol ($C_6H_6O_3$) were procured from

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Sigma–Aldrich India Ltd. Mono-potassium phosphate (KH_2PO_4), di-potassium phosphate (K_2HPO_4) and hydrogen peroxide (H_2O_2, 30% v/v) were procured from Merck India Ltd. All solutions were prepared in ultrapure Milli-Q water (resistivity $\geqslant$ 18 MΩ cm at 298 K).

2.2. Activity and Bradford assay for HRP enzyme

Activity of HRP enzyme was determined as per standard protocol (Sigma–Aldrich, EC 1.11.1.7) [26] with pyrogallol as substrate. The following reaction determines the activity of HRP enzyme:

$$\text{Pyrogallol (donor)} + H_2O_2 \xrightarrow{\text{HRP}} 2H_2O + \text{Purpurogallin (oxidized donor)}$$

The assay composition was as follows: ultrapure water 2.1 mL, phosphate buffer (pH 7, 100 mM) 0.32 mL, hydrogen peroxide solution (diluted to 60:1) 0.16 mL, pyrogallol solution 0.32 mL, enzyme (HRP) 0.1 mL. After mixing of above solutions, the final concentrations in 3 mL mixture were: 14 mM of phosphate buffer, 0.027% (w/w) H_2O_2, 0.5% (w/v) pyrogallol. These concentrations were maintained same in all experiments of IBP degradation. The absorbance of purpurogallin was recorded every 20 s at 420 nm using UV–Vis spectrophotometer (ThermoFischer, Model: UV-2300). The activity of the enzyme was calculated using the following formula:

$$\text{Unit/mL Enzyme} = \frac{(\Delta A_{420}/20s) \times V_t \times D_f}{\varepsilon \times v_S}$$

Notation: V_t – total volume of reaction mixture (3 mL), D_f – dilution factor, ε – extinction coefficient (12 for purpurogallin at 420 nm), v_s – volume of sample (0.1 mL).

The protein content of the HRP enzyme was also determined by Bradford assay. Bovine serum albumin (BSA) was used as the standard protein. 3 ml of Bradford reagent and 0.1 ml HRP enzyme were mixed to form Bradford assay mixture. The mixture was kept in incubation for 15 min and the absorbance in mixture was measured at 595 nm. With this procedure, the protein content of HRP was determined as 0.0228 mg/mL.

2.3. Experimental setup and procedure

Purely enzymatic reactions were carried out under well stirred conditions using mechanical stirring with a small magnetic bar (length = 1 cm, diameter = 0.5 cm) at 150 rpm. A schematic of the experimental setup is given in Supplementary material (Fig. S1). Experiments were carried out in a custom built (20 mL) test tube made of borosilicate glass (O.D. = 16 mm, length = 150 mm). The experiments with sonication were conducted in an ultrasound bath (Elmasonic, Germany, Model: P-30H). The ultrasound bath could operate at two frequencies, viz. 37 kHz (130 W) and 80 kHz (100 W). Approximately 2/3rd volume of the bath was filled with water that acted as medium for transmission of ultrasound waves. The test tube with reaction mixture was placed exactly at the center of the ultrasound bath and it was immersed to approx. 75% of its height. This ensured that entire reaction mixture in the test tube was exposed to ultrasound waves. The position of test tube in the bath was maintained carefully same in all experiments to avoid the artifacts in experimental results due to spatial variation of acoustic intensity [27,28]. The actual power input to the ultrasound bath and to the reaction mixture inside the test tube was determined using calorimetric technique [29,30]. On this basis, the exact volumetric energy dissipation for 37 and 80 kHz were as follows: 0.0174 W/mL (for 37 kHz) and 0.0116 W/mL (for 80 kHz). The corresponding pressure amplitudes of ultrasound waves in the bath for frequencies of 37 and 80 kHz were calculated as 200 kPa and 170 kPa, respectively. Moreover, the pressure amplitudes of ultrasound waves in the bath and the reaction mixture inside the reaction tube was found to be same, which indicated negligible attenuation of ultrasound waves through the wall of reaction test tube. Due viscous dissipation of energy of ultrasound waves during propagation, the temperature of the water in the bath rises. This rise was controlled by circulation of water from a temperature controlled cooling water circulating bath (Amkette Analytics, Model: WB2000). The temperature of the reaction mixture in the test tube was same as that of the water in the bath.

The IBP degradation experiments were conducted in four categories, viz. (1) enzymatic treatment with mechanical stirring, (2) sonolysis, (3) sono-enzymatic treatment at atmospheric static pressure, and (4) sono-enzymatic treatment at elevated static pressure of 200 kPa. In categories 2, 3 and 4 involving sonolysis, experiments were conducted at two frequencies (either 37 or 80 kHz). In category 4 experiments, the test tube containing reaction mixture was connected to a nitrogen cylinder through a side nozzle. The mouth of the test tube was sealed with threaded cap (please refer to the schematic of experimental set-up provided in Supplementary material). During withdrawal of aliquots of reaction mixture, the pressure in reaction test tube was released. The static pressure was restored to original level, as the sonication was resumed. The rationale underlying the technique of elevating static pressure of reaction mixture will be explained subsequently. The actual pressure amplitude of ultrasound waves reaching the reaction mixture is a strong function of wall thickness of the test tube holding reaction mixture. In order to keep this important parameter constant, same test tube has been used in all experiments.

The total treatment time was 60 min and the total reaction volume in a typical experiment was 15 mL. In this volume, concentrations of different components were as follows: IBP = 10 ppm (or 4.76×10^{-2} mM), H_2O_2 = 8.55 mM, HRP = 0.08 U/mL, phosphate buffer (pH 7) = 14 mM. The pH of the reaction mixture was maintained at 7 in all experiments. This pH was optimum for the highest activity of enzyme (for results of optimization, refer to the Supplementary material, Fig. S2). To monitor the time profile of IBP degradation, aliquots of reaction mixture (200 μL) were withdrawn at regular intervals and were analyzed for the residual IBP. After completion of the treatment, aliquotes of reaction mixture were analyzed for identification of intermediates of IBP degradation.

2.4. Analytical methods

The concentration of IBP in the aliquot of the reaction mixture was analyzed using High Performance Liquid Chromatography (Shimadzu, Model: SPD-20A) equipped with C-18 reverse phase column (250 mm × 4.6 mm, 5 μm) and UV detector at 220 nm. The mobile phase was a mixture of acetonitrile and aqueous acetic acid 0.1% (60/40, v/v) with an isocratic flow of 1.5 mL/min. In order to determine the mineralization, the total organic carbon (TOC) in the IBP reaction solution was measured with Aurora TOC analyzer (O-I-Analytical, Model: 1030). The intermediates formed during sono-enzymatic degradation of IBP were identified using GC–MS (Varian 240-GC) after liquid–liquid extraction with dichloromethane. 1 μL of extracted sample was injected with a split ratio of 10:1. Helium gas was used as a carrier and the mass detector worked in scan mode with m/z range of 50–600. The GC–MS spectrums are given in Supplementary materials (Figs. S5–S9).

2.5. Experimental data analysis

The kinetic constants of IBP degradation were determined by fitting pseudo 1st order model to the kinetic data of IBP degradation. Using these kinetic constants, activation energy for IBP degradation