Journal of Water Process Engineering 9 (2016) e1–e6

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Ultrasound assisted enzymatic degradation of diclofenac sodium: Optimization of process parameters and kinetics

Rahul S. Sutar, Virendra K. Rathod*

Chemical Engineering Department, Institute of Chemical Technology, Matunga, Mumbai 400019, India

ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form 1 December 2014
Accepted 6 December 2014
Available online 26 March 2015

Keywords:
Diclofenac sodium
Laccase
Ultrasound irradiation
Degradation

ABSTRACT

Diclofenac sodium is the most commonly detected nonsteroidal anti-inflammatory drug in aquatic environment. Considering the various adverse effects of diclofenac, its degradation with novel approach of combination of ultrasound and enzyme laccase has been investigated. Effect of various process parameters including enzyme concentration, temperature, power, duty cycle and speed of agitation has been investigated. The enzyme concentration, temperature, power, duty cycle and speed of agitation which executed maximum extent of degradation were 0.025% (w/v), 50 °C, 100 W, 50% and 300 rpm, respectively. Under these optimized operating parameters, maximum extent of degradation achieved was 96%. Also, the kinetics parameters were investigated from the kinetic study. It has been observed that the combination of ultrasound with enzyme results in improved degradation results over the conventional method along with lower reaction time.

1. Introduction

Recently, pharmaceuticals are evolved as 'emerging contaminants' due to their existence in surface water. Although pharmaceuticals are detected at trace concentrations, i.e. from nanograms per litre to micrograms per litre, considering forthcoming negative influence of pharmaceuticals on the environment and human health, the scientific and public concern has been increasing tremendously for their treatment. Some pharmaceuticals can be detrimental with likely synergistic effects even at trace levels attributed to elicitation of biological response [1–3]. Even though conventional wastewater treatment processes are used for the removal of pharmaceutical compounds present in wastewater effluent, they enter into surface waters due to their partial removal in such processes which is prime source of origin of pharmaceuticals into the wastewater along with pharmaceutical manufacturing plants, landfills, hospitals, human excretion as well as graveyards [4–6].

The nonsteroidal anti-inflammatory drugs (NSAIDs) are the groups of pharmaceutical compounds of extreme environmental concern due to their enormous production, consumption as well as occurrence in wastewater [7]. Diclofenac sodium is the characteristic example of commonly used anti-arthritic, anti-rheumatic and analgesic which is most frequently detected pharmaceutical in aquatic environment. Globally, annual consumption of diclofenac is nearly about 940 tonnes [8]. After the human consumption, nearly about 15% of drug is excreted as unchanged [9]. Due to lower removal efficiency (only 20–30%) in domestic treatment processes, the presence of diclofenac sodium has been detected in surface water, wastewater and also drinking water samples [10].

For the removal of various classes of pollutants, advanced oxidation processes are mostly studied. However, novel advanced treatment processes have also been reported for removal of diclofenac sodium [11,12]. These techniques possess various disadvantages such as slower removal kinetics as well as high operational costs along with hazardous byproducts including 5-hydroxydiclofenac and diclofenac-2,5-iminoquinone in ozone-based methods [10]. Even at trace concentrations such as 5 μg/L, the toxicity study of diclofenac sodium comprises various adverse effects such as cytotoxicity to kidney, gill cells, liver and also renal lesions. In Pakistan and on the Indian subcontinent, the enormous deterioration (95%) of vulture population has been reported due to diclofenac making it critically endangered [8,13,14]. With this background, in order to avoid the hazardous effects, its removal is necessary.

Considering the demand of water quality standards for discharge of industrial wastewater and water decontamination, the attention has been increased significantly towards the use of enzymes for the degradation of pollutants found in the wastewater. The benefits of enzymes over the conventional chemical oxidation and/or biological treatment comprise absence of toxic effects, easy and simple process control, lack of unforeseen products generation

* Corresponding author. Tel.: +91 22 33612020; fax: +91 22 24145614.
E-mail address: vk.rathod@ictmumbai.edu.in (V.K. Rathod).

http://dx.doi.org/10.1016/j.jwpe.2014.12.003
2214-7144/

because of their high specificity, lack of acclimatization period, less energy requirement as well as operability over a wide temperature, pH and salinity at low or high concentrations of pollutants which exhibit attractive replacement for conventional wastewater treatment [15–18].

The use of oxygen by enzyme laccase as a non-limited electron acceptor and its extensive substrate specificity can be attributed to fact that laccase possesses substantial concern for the degradation of pollutants [19–21]. Enzyme laccase, (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) which is found in the fungi, few bacteria and in higher plants, belongs to the polyphenol oxidases, a group of enzymes. With the concomitant reduction of oxygen to water, a laccase containing four copper atoms in its catalytic site catalyzes the oxidation of several aromatic and inorganic substances such as endocrine disrupters and polycyclic aromatic hydrocarbons, phenols, trichlorophenols, organophosphorus pesticides and azo dyes [22–25]. Hence, due to such transformations, less toxic and benign products are formed. Owing the oxidizing property of laccase, its application for the degradation of various pharmaceuticals has been reported in the literature. However, conventional methods of degradation of pharmaceuticals suffer from slower rate reaction leading higher reaction time as well as lesser degradation. These shortcomings of conventional method can be overcome by concomitant utilization of ultrasound with enzymes.

The phenomenon of the generation, subsequent growth and collapse of cavities due to ultrasound irradiation is known as cavitation [26]. The formation of liquid circulation (microstreaming) and turbulence in the cavitation attributes to significant improvement in the mass transfer. Also, thermal and mechanical energy associated with ultrasound (vibration and stirring) apart from cavitation has also enhanced the mass transfer rate [27]. With the use of ultrasound, the increase in the enzyme stability, catalytic activity, and longevity of enzyme biocatalysts has been reported for the enzyme based applications [18].

In the literature, several methods have been reported for the degradation of diclofenac. However, to the best of our knowledge, none of the methods had ever reported in the literature about using combination of enzyme with ultrasound. Therefore, a novel incorporation of concomitant application of ultrasound with laccase has been reported for degradation of diclofenac sodium. Optimization of various process parameters affecting the degradation of diclofenac sodium has been carried out along with determination of the kinetic parameters.

2. Materials and methods

2.1. Materials

The HPLC grade acetonitrile was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. Pharmaceutical diclofenac sodium (pure, 98%) was given by Research Lab Fine Chemicals Ltd., Mumbai as a gift sample while enzyme laccase produced from the strain *Aspergillus oryzae* was gifted by Advanced Enzymes Pvt. Ltd., Thane.

2.2. Laccase-catalyzed degradation of diclofenac sodium

The experiments for the degradation of diclofenac sodium were conducted in the ultrasonic thermostatic water bath with the contemporaneous implementation of enzyme laccase with ultrasound. The ultrasonic thermostatic water bath having capacities of 4.5 L with four transducers located at the base of the water bath was designed by Dakshin India Pvt. Ltd., with Model No. 6-SL200H/DTC/DF. For the experimental reactions, a baffled glass reactor having flat base with capacity of 50 mL and ID 4.5 cm was used. For agitation, the reactor was linked to electric motor with a four-blade turbine glass impeller along with reflux condenser and control system. The complete reactor set-up was positioned in ultrasonic water bath having temperature control system with accuracy of ±1 °C. The reactor was located in the ultrasonic bath at the place of utmost effectiveness of ultrasonic irradiation through all the experimentations [28].

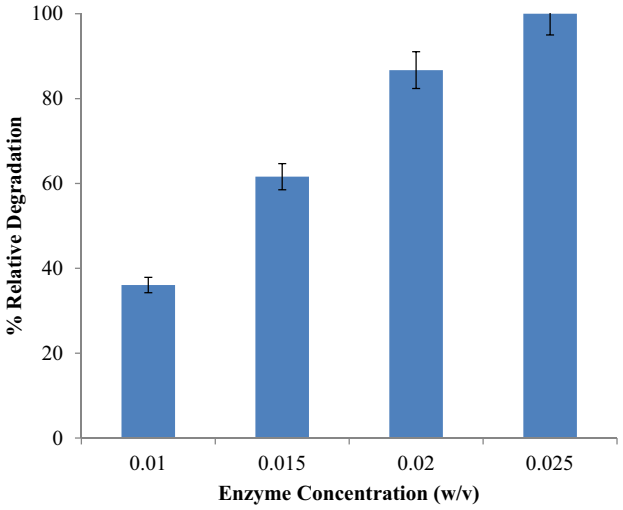

Fig. 1. Effect of enzyme concentration (reaction conditions: diclofenac sodium (40 mg/L) in phosphate buffer of pH 6.0; total reaction volume 20 mL; speed of agitation 200 rpm; enzyme concentration 0.01–0.025% (w/v); temperature 60 °C; under ultrasound waves with frequency 22 kHz; power 50 W; 50% duty cycle).

Experiments were performed in a reactor containing phosphate buffer (50 mM) with 40 mg/L diclofenac sodium and enzyme laccase 0.01% (w/v). Maintaining 60 °C temperature, the agitation was given to reaction mixture for 7 h keeping the speed of agitation 200 rpm with 50 W power at 22 kHz frequency and with 50% duty cycle. Taking into account the enzyme addition as 0 min sample, the other samples from the reaction mixture were removed at time interval of 1 h. The samples removed were analyzed by using high pressure liquid chromatography (HPLC).

2.3. Analytical method

2.3.1. High pressure liquid chromatography

Samples drawn out recurrently from the experiments were analyzed with the help of high pressure liquid chromatography. For the analysis, Agilent Technologies HPLC equipment was used with 1260 Quat pumps VL and 1260 VWD VL as a UV detector. At the wavelength of 290 nm with acetonitrile:water (30:70) as a mobile phase, sample analysis was done using AGILENT C18 Column of 4.6 ID × 100 mm dimensions at 1 mL/min flow rate and for each sample analysis, injection volume of 20 mL was used.

3. Results and discussion

3.1. Effect of enzyme concentration

The exact amount of enzyme required for maximum extent of degradation is foremost feature of enzyme catalyzed degradation reactions considering the commercial aspect. Under ultrasonic irradiation, in order to investigate effect of enzyme concentration on the maximum extent of degradation, enzyme concentration was varied over the range of 0.01–0.025% (w/v) keeping agitation speed of 200 rpm, power 50 W, temperature 60 °C, frequency 22 kHz and duty cycle 50%. Fig. 1 illustrates the effect of different enzyme concentrations on the degradation of diclofenac sodium depicted in terms of percent relative degradation which is defined as the ratio