Chemosphere 92 (2013) 1356–1360

Contents lists available at SciVerse ScienceDirect

Chemosphere

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

Technical Note

Bisphenol A degradation in water by ligninolytic enzymes

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HIGHLIGHTS

- Higher degradation of BPA (90%) by encapsulated ligninolytic enzymes.
- Low degradation of bisphenol A with free enzyme (26%).
- Pectin in the formulation enhanced significantly ($p > 0.05$) the activity of enzymes.

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 26 February 2013
Accepted 27 February 2013
Available online 10 May 2013

Keywords:
Bisphenol A
Ligninolytic enzymes
Encapsulation
Hydrogel

ABSTRACT

Many endocrine disruptor compounds, such as bisphenol A (BPA) are used today and released into the environment at low doses but they are barely degraded in wastewater treatment plants. One of the potential alternatives to effectively degrade endocrine disruptor compounds is based on the use of the oxidative action of extracellular fungal enzymes. The aim of this work is to study the ability of free and encapsulated enzymes (manganese peroxidase, lignin peroxidase and laccase) to degrade BPA. Higher degradation of BPA (90%) by ligninolytic enzymes encapsulated on polyacrylamide hydrogel and pectin after 8 h was obtained. The degradation of BPA while using the free enzyme (26%) was lower than the value obtained with encapsulated enzymes. The presence of pectin in the formulation significantly ($p > 0.05$) enhanced the activity of enzymes. Kinetics of BPA degradation showed an increase in V_m, while K_m remained constant when enzymes were encapsulated. Hence, encapsulation protected the enzymes from non-competitive inhibition.

1. Introduction

Phenolic compounds are one of the major classes of organic pollutants generated by various sources, such as pesticides and phenol-containing products, such as slimicides, plastics, disinfectants, antiseptics and medicinal preparations (de Araujo et al., 2006). Bisphenol A (BPA) is one such toxic organic compound whose global consumption has increased at an annual rate of 7–8% during 2006–2011 (Burridge, 2003; Greiner et al., 2007). BPA was discharged into the environment through a number of routes, including discharge of wastewater and process water generated from BPA production facilities, discharge of effluent from wastewater treatment plant, leaching from consumer products containing BPA at hazardous waste landfill sites and others. In October 2008, the Government of Canada prohibited the importation, sale, and advertising of polycarbonate baby bottles that contain BPA (CDEH, 2008). Conventional processes for removal of phenols from industrial wastewaters include extraction, adsorption on activated carbon, bacterial and chemical oxidation, electrochemical techniques and irradiation, among others (Bratkovskaja et al., 2004). All these methods suffer from serious shortcomings, such as higher cost, incompleteness of purification, formation of hazardous by-products, low efficiency and applicability to a limited concentration range (Regalado et al., 2004).

The degradation of toxic substances, namely endocrine disrupter compounds (EDCs) by enzymes has several advantages: high specificity to substrates, use on a wide range of environmental conditions, simplicity and ease of process control (Gianfreda and Rao, 2004). Nevertheless, the remediation methods using the enzymes have some limitations, such as higher cost of production and purification of enzymes and lower stability of these catalysts (Aitken and Heck, 1998). The higher production cost of enzymes is attributed to the substrates used to produce them (40–60% of production cost). Solid-state fermentation (SSF), in this case is the most economical process for the production of higher activity of ligninolytic enzymes by fungi (Iwashita, 2002). White rot fungi in wood are higher producers of ligninolytic enzymes which are essential for degradation of toxic organic compounds (Gianfreda and Rao, 2004). *Phanerochaete chrysosporium* is one of most studied white rot fungi, which mainly produces manganese peroxidase (MnP) and lignin peroxidase (LiP) (Pandey, 2003). The ligninolytic

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http://dx.doi.org/10.1016/j.chemosphere.2013.02.071

enzymes comprise LiP, MnP, and laccase. Due to the complex structure of lignin, its biodegradation system is considered highly non-specific. Ligninolytic enzymes, therefore, have attracted attention as possible degraders of structurally different environmental pollutants (Dec and Bollag, 1994). Thus, these enzymes have been used in environmental clean-up of EDCs by oxidative degradation.

The principal aim of this study was the degradation of BPA in contaminated water by encapsulated ligninolytic enzymes. Ligninolytic enzymes were produced by SSF of apple pomace waste *by P. chrysosporium*. Ligninolytic enzymes were extracted from fermented solid wastes and encapsulated, using various polymers in order to increase the stability of these enzymes. These enzymes were further used to treat the contaminated water (fortified with 10 ppm of BPA).

2. Materials and methods

2.1. Solid state fermentation

Ligninolytic enzymes were produced using SSF of apple pomace by *P. chrysosporium*. The fermentation was carried out in a 12-L rotating drum solid-state fermenter (Terrafor, Infors HT, Switzerland). About 4.5 kg of sterilized medium was transferred into the sterilized bioreactor under aseptic conditions. For the fermentation, apple pomace was treated with inducers, such as copper sulfate (2 mM), veratryl alcohol (2 mM), and Tween-80 (0.1% v/v), and the pH was adjusted to 4.5. The inoculation was carried out using the spore suspension. The fermentation was carried out in a controlled environment at 37 ± 1 °C, rotational speed of 2 rpm and aeration rate of 2 vvm. After fermentation, the enzymes were extracted by centrifugation by adding 10 mL of 50 mM sodium-phosphate buffer at pH 6.5 to 1 g of fermented substrate.

2.2. Enzymes formulation

Different formulations were produced which depended on the type of process involved. About 2.85 g acrylamide, 0.15 g bisacrylamide (Sigma–Aldrich), 10 mg ammonium persulphate and 1 mL tetra methyl ethylenediamine were added in 50 mL of phosphate buffer solution (5×10^{-2} M, pH 7.2) while stirring. The suspensions were sonicated for 15 min at room temperature using a probe type sonicator (Sonifier 250, Branson, CT, USA). After sonication, the suspensions were filtered through 0.45 μm (vinylidene fluoride) filters (Millex-HV, Millipore, MA, USA) to remove the dust. Finally, clear suspensions were obtained. The next step was the encapsulation of ligninolytic enzymes in polyacrylamide (PA) microgel. For encapsulation, polymers (gelatin, pectin and carboxy methylcellulose (CMC)) at different concentrations (0.01, 0.02, 0.05% w/v) was added to an extract of ligninolytic enzymes (538 U of MnP g^{-1} dry solids (ds), 90 U of laccase g^{-1} ds and 53 U of LiP g^{-1} ds) in a phosphate buffer solution (5×10^{-2} M, pH 7.2). Later, 20 mL of the enzyme preparation and 30 mL acrylamide gel solution were mixed. The mixture was incubated for 1 h at 25 °C and PA microgel-enzyme complex was obtained.

2.3. Extraction of bisphenol from the matrix

To degrade BPA, enzymes encapsulated in hydrogel were added to contaminated water. When the contaminated water was treated with the encapsulated enzyme (PA gel and polymer (pectin, gelatin and carboxy-methy-cellulose), the separation of BPA was not complete and other contaminants present in the samples also reduced the signal of BPA in the laser diode thermal desorption (LDTD) chromatogram. When the samples were injected into LDTD instrument (LDTD–APCI, Phytronix, Canada) to determine BPA concentrations after enzymatic degradation, there was signal suppression. Solid phase extraction method was used for clean-up and pre-concentration of extract. Glass cartridges (OASIS HLB 5 cc 200 mg LP) were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of water at a flow rate of 1 mL min^{-1}. Water extract (100 mL) was passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (−103) kPa. The elution was performed by adding 2.4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 mL prior to LDTD–APCI analysis.

2.4. Bisphenol A degradation and quantification in water

The degradation of BPA was carried out at 25 ± 1 °C at 100 rpm. In all treatments, BPA was degraded by 60 U L^{-1} of MnP, 50 U L^{-1} of laccase and 5 U L^{-1} of LiP. After extraction of enzymes from solid substrates, ligninolytic enzyme activities in the extract were measured (Tien and Kirk, 1984; Collins and Dobson, 1997; Xu et al., 2001). The enzyme extract was diluted to obtain the enzyme activities used in this study.

LDTD is a new approach to analyze emerging contaminants, such as BPA, where the entire sample is introduced in the mass spectrometer by a rapid laser thermal desorption process and is ionized by atmospheric pressure chemical reactions (LDTD–APCI). LDTD combines the ultra-fast thermal desorption of a dry sample with the ionization of the produced neutral molecules in the gas phase without solvent, mobile phase or external matrix. A low sample size thermal desorption and APCI in a "dry" environment provides LDTD its tremendous analytical speed and analytical performance. LDTD ion source works with less than 10 μL of sample (typically 2 μL) and it has the option of simultaneous analysis of 96 samples. This analytical method is able to analyze the environmental concentrations as low as ng kg^{-1}. Quantification of BPA was performed by the internal standard method based on peak areas and relative retention time using BPA d16 as internal standard. Standard working solutions of BPA in the range 0.05–20 μg mL^{-1} were prepared by diluting aliquots of the stock solution (10 μg mL^{-1}) in methanol. About 2 μL volume of the standard BPA solution was injected into the LDTD–APCI system. Calibration curves for concentrations (0.05–20 μg mL^{-1}) were obtained from a linear regression program. The correlation coefficients of peak height to concentration was >0.998.

2.5. Statistical analysis

All the experiments were assayed in three replicates, and an average of replicates was calculated along with the standard deviation. Database was subjected to an analysis of variance (ANOVA). One-way ANOVA followed by student's *t*-test was used to determine significant differences among treatment groups. For all analysis, differences were considered to be significant at $P < 0.05$ (Gassara et al., 2010).

3. Results and discussion

3.1. Bisphenol A degradation by ligninolytic enzymes

Enzymes were produced by SSF of apple pomace and the encapsulation in different hydrogels containing PA and gelatin or pectin