Biochemical Engineering Journal 99 (2015) 1–15

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

A model of the transient kinetics of laccase-catalyzed oxidation of phenol at micromolar concentrations

Stoyan Rangelov, Jim A. Nicell*

Department of Civil Engineering and Applied Mechanics, McGill University, 817 Sherbrooke Street West, Montreal, Quebec H3A 0C3, Canada

ARTICLE INFO

Article history:
Received 29 November 2014
Received in revised form 21 February 2015
Accepted 26 February 2015
Available online 27 February 2015

Keywords:
Enzymes
Biocatalysis
Kinetic parameters
Modeling
Laccase
Phenol

ABSTRACT

Oxidoreductase enzymes have the potential to be used to catalyze the oxidation of aromatic trace contaminants in wastewaters. The feasibility of this approach can be explored by modeling transient kinetics, particularly in the low substrate concentration range, where the model may be used to predict the quantity of enzyme and time required to achieve a particular level of conversion of a target compound. Laccase from *Trametes versicolor* was selected as a candidate enzyme due to its wide substrate specificity and its use of molecular oxygen as an oxidant. Phenol was selected as a target substrate. A four-parameter kinetic model was developed based on the known reactions of laccase. The model was applied to 30 sets of data collected from batch reactions conducted at pH 5 and 25 °C over a three hour period. Initial phenol concentrations ranged from 0.5 to 50 μM and applied enzyme concentrations ranged from 0.12 to 2.5 μM. The model demonstrated its utility for predicting the quantities of enzyme and reaction times required to achieve desired levels of oxidation of phenol for varying initial concentrations. A three-parameter simplified version of the kinetic model was also developed to facilitate calibration and mathematical solution of its equations.

1. Introduction

Aromatic compounds such as polycyclic phenols, alkyl phenols, pesticides and endocrine disruptors are frequently detected in surface water streams that receive effluents from municipal wastewater treatment plants, industries as well as agricultural runoff [1,2]. These compounds are typically present in trace quantities that are measured in parts per billion range ($\mu g\,L^{-1}$) [3,4]. Many of them are known toxins with carcinogenic or estrogenic properties [5] and can coexist in many environmental matrices [6–9] resulting in potentially significant impacts in the environment [10], even when present in extremely low concentrations [5].

Due to their low concentrations, these contaminants are especially difficult to quantify using conventional analytical techniques [4,11] and, hence, it is not surprising that it is challenging to develop effective treatment processes for their removal from wastewaters to environmentally-acceptable levels [12]. This is especially true since conventional wastewater treatment plants are typically designed to process bulk substances (e.g., solids, dissolved organic matter and nutrients) and are unable to specifically target aromatic contaminants as they exist in very low concentrations and/or are resistant to degradation in complex waste matrices [1,3,4,13–15]. As a result, many of these contaminants are not effectively removed using current treatment technologies and have been observed in the surface or ground waters [1,14,16–20]. In addition, when treated biologically, hydrophobic aromatic compounds tend to accumulate in sludges [21,22]. This may be problematic when the sludges are subsequently applied to land since these contaminants can threaten crop yields, long term soil quality, wildlife and eventually human health [23–25]. Research has shown that these compounds can be treated using advanced oxidation processes, but improvements in effluent quality comes at the considerable expense of increased materials and energy consumption [26]. For this reason, alternative processes are sought that can target these compounds for treatment in a more efficient and effective manner.

Enzymatic wastewater treatment is a promising alternative to conventional treatment processes for trace aromatic contaminants [27–31]. In such processes, oxidoreductase enzymes that have been isolated from their parent organisms are used to catalyze the oxidation of targeted pollutants. Some potential advantages of enzymatic treatment compared to chemical methods are high selectivity toward individual or groups of substrates, rapid kinetics, and high catalytic activity under mild reaction conditions [27,30]. Moreover, enzymes are immune to issues that frequently upset biological wastewater treatment systems such as shock loading effects, inhibition of microorganisms due to the presence of toxins, unstable reaction conditions, and stop/start regime delays [27]. In general, it has been suggested that enzymatic wastewater treatment may

* Corresponding author. Tel.: +1 514 398 7251; fax: +1 514 398 7379.
E-mail address: jim.nicell@mcgill.ca (J.A. Nicell).

http://dx.doi.org/10.1016/j.bej.2015.02.034
1369-703X/

be most appropriate as either a pre-treatment or polishing step when targeting specific pollutants in combination with a biological process [30] or with physical treatment, such as membrane filtration [32]. Although enzymes offer several advantages over existing treatment methods, their application is still limited due to their current high cost and the specificity of reaction conditions under which they exhibit acceptable activity and stability [33]. In some cases, the application of enzymatic treatment may also be limited due to conditions that result in the inactivation of the enzyme over time. This can arise either due to undesirable side reactions in the reacting mixtures, inhibition by other mixture components or reaction products, or irreversible conformational changes resulting from the reaction environment [30].

Peroxidases and laccases are two of the most promising candidate oxidoreductase enzymes for targeting the removal of aromatic compounds in wastewaters. These enzymes have been shown to catalyze the oxidation of a wide variety of aromatic compounds in aqueous solutions including phenols, alkyl phenols, polyaromatic hydrocarbons, dyes, and pesticides, among others [31,34–36]. Furthermore, relatively low concentrations of endocrine disruptors have been removed using either free or immobilized enzymes in water matrices in batch and continuous flow reactors [37–44]. Peroxidases, such as horseradish peroxidase, are characterized with higher redox potentials than laccases, with optimal activity [41] demonstrated within the typical domestic wastewater pH range of 7–7.4 [45]. However, they experience fairly rapid inactivation during the oxidation of aromatic substrates [46–48]. In contrast, laccases, such as that from *Trametes versicolor*, have optimal activity in the acidic range but can function well at or around neutral pH [49] and also exhibit greater stability than peroxidase [49–51]. Also, in comparison with peroxidases, a very important advantage associated with the use of laccases is that they use freely-available and inexpensive molecular oxygen as an oxidant, rather than hydrogen peroxide (H_2O_2). This could translate into significant economic benefits relative to the use of peroxidase due to the lower costs associated related to the production, transportation, storage and handling of the oxidant. Furthermore, H_2O_2 can be unstable in contaminated water matrices and, as a result, excess quantities will therefore, be required to satisfy the peroxide demand of other waste components that may inadvertently be oxidized by side-reactions with the oxidant. Additionally, the excess peroxide that is required to meet this demand can negatively impact peroxidases by producing catalytically-inactive forms of the enzyme [52,53]. Such inactivation processes are likely to be particularly significant when catalyzing the oxidation of substrates at low concentrations where reactions are slower and where the need for excess hydrogen peroxide is likely to be greater.

Although many studies have explored the potential for the environmental applications of oxidoreductase enzymes including laccase [31,34,36,54–56], some key issues must be addressed before pursuing implementation on a larger scale. Most importantly, from the perspective of trace aromatic contaminants, while many studies have focused on demonstrating the enzyme's ability to oxidize a variety of substrates and evaluating its stability under variety of reaction conditions, these studies were usually performed using relatively high substrate concentrations and large quantities of the biocatalyst [44,57,58]. Further study is required to determine if enzymes can be used to target aromatic substrates at environmentally relevant concentrations using practical quantities of the biocatalyst. In addition, the transient kinetics of the reactions must be studied in order to determine the quantity of enzyme and time required to achieve a desired level of oxidation of the substrate. The investigation of transient kinetics in the lower concentration range is also important in order to reveal the influence of various phenomena, such as mass transfer limitations, which may became significant in this concentration range. And finally, it is essential to be able to predict the transient kinetics of the reaction at various enzyme and substrate concentrations as a basis for reactor design.

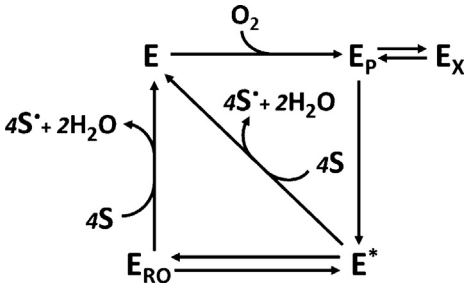

Fig. 1. Catalytic cycle of laccase as it reacts with oxygen, O_2, and a substrate, *S*, to produce phenoxy radicals, *S*·. Laccase involved in the main catalytic cycle in its native and oxidized states is denoted as *E* and E^*, respectively. Also shown are catalytically-slow (E_{RO}) or non-productive (E_P, E_X) states of laccase involved in side reactions. (Source:[66]).

Therefore, the objective of this research is to develop a kinetic model that reflects the phenomenon that govern the catalytic reactions of laccase and can accurately predict the transient kinetics of the oxidation of aromatic substrates at low concentrations. Ultimately, the intent is to apply this model to evaluate the feasibility of using laccase to catalyze the oxidation of aromatic substrates at low concentrations. While the main purpose is to apply this model in support of waste treatment applications of laccase, it is anticipated that it could also be used in support of the development of numerous other applications of laccase including bio-bleaching, bio-pulping, synthetic chemistry, food production, biosensors and biofuel cells, cosmetics production, among others [36].

In this study, laccase from *T. versicolor*, which is part of the multicopper oxidases family, was selected as a model enzyme for assessing the potential and the limitations of enzymatic processes to oxidize trace aromatic compounds. As shown in Fig. 1, laccase catalyzes the four electron reduction of O_2 to H_2O with four single-electron oxidations of molecules of a reducing substrate, *S*, resulting in the formation of phenoxy radicals, *S*·. During its catalytic cycle, the enzyme in its reduced state, E, is first oxidized by oxygen and then passes through several states, E_P and E^*, before returning to the reduced state. The radicals formed during the process react with each other to produce polymers of varying molecular weights [59–62]. Side reactions that can temporarily sequester the enzyme from the main catalytic cycle have been identified [63–65] or proposed recently [66] and are also shown in Fig. 1.

Phenol was selected as a model substrate because it is one of the most common industrial pollutants [1]. It is also a simple aromatic molecule with only one phenolic group that donates one electron during the cascade of four single-electron oxidation steps of reduced laccase producing a phenoxy radical, as shown in Fig. 1. It also provides an excellent basis for comparison with numerous other studies of laccase-catalyzed oxidation of this particular substrate and especially because it was previously used in the development of a kinetic model of laccase applied to the oxidation of phenol concentrations in the range of 500–3000 μM [51], which is much higher than those used in the present study.

2. Materials and methods

2.1. Materials

Laccase (ES 1.10.3.2) from *T. versicolor* of high purity (nominal activity of 23.1 U mg^{-1} as per supplier), phenol crystals (99.5%), and [2,2′-azino-bis-(3ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS, 98%) were obtained from Sigma–Aldrich (St. Louis,