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Critical review

Enzymatic approach to phenol removal from wastewater using peroxidases

T Chiong[1], S Y Lau[1], E H Khor[1], M K Danquah[1*]

Abstract

Introduction

Phenol and its derivatives are present in various industrial effluents and possess a high level of toxicity even at minute concentrations. This could cause serious ecological problems if phenol-containing effluents are discharged untreated or partially treated into water bodies or land sites.

Method

Conventional wastewater phenol removal methods, which include biological treatment, adsorption and chemical oxidation such as ozonation and Fenton reaction, have significant challenges especially for large-scale applications. These methods are either time-consuming, require high energy consumption, non-specific or environmental unfriendly as they result in high greenhouse gas emissions or toxic by-products. Enzymatic treatment is appreciated as an alternative wastewater phenol removal approach.

Discussion

The advantages of enzymatic treatment include highly selective, operate under mild conditions and shorter reaction times. Several techniques to further enhance the performance of enzymatic treatment have been introduced.

Conclusion

This article discusses peroxidase based enzymatic treatment as an effective approach for continuous phenol removal from wastewater. It also presents in-depth analysis of various conventional approaches in juxtaposition with peroxidase based methods for effective phenol removal from wastewater.

Introduction

Effective treatment of industrial liquid effluents continues to be a challenging task in environmental science and engineering. Industrial effluents, if discharged untreated or partially treated, could cause serious ecological problems to aquatic lives and human health. Effluents from a wide variety of industries such as coal conversion, petroleum refinery, resins and plastics, wood preservation, metal coating, dyes and other chemicals, textiles, mining and dressing, and pulp and paper may contain significant concentrations of aromatic compounds which include phenols and aromatic amines. These aromatic compounds constitute one of the major classes of pollutants heavily regulated in many countries. Most aromatic compounds are toxic and therefore must be treated before the containing effluent is discharged into the environment[1]. Phenol, a commonly known aromatic compound, is a toxic organic pollutant even at low concentrations[2]. Its presence in natural water bodies can lead to the formation of secondary or derivatized compounds during disinfection and oxidation processes[3], and these compounds may be harmful and/or difficult to separate from waste streams. Phenol and its derivatives have been defined as priority pollutants by the United States Environmental Protection Agency (USEPA) [4].

*Corresponding author
Email: mkdanquah@curtin.edu.my

[1] Department of Chemical Engineering, Faculty of Engineering and Science, Curtin University Sarawak, CDT 250, 98009 Miri, Sarawak Malaysia.

Overview of conventional phenol removal methods

Conventional processes for phenol removal from wastewater can be divided into three main categories; biological, physical and chemical processes. Biological treatment is appreciated as an inexpensive technique to treat effluents containing phenolic compounds due to the availability of microorganisms capable of degrading organic compounds[5]. Many aerobic bacteria are capable of using aromatic compounds as the sole source of carbon and energy. A typical pathway for metabolizing aromatic compounds is to dehydroxylate the benzene ring to form catechol derivatives and further ortho or meta oxidative cleavage to open the aromatic ring[6]. Tziotzios et al.[7] studied the performance of indigenous bacteria from olive pulp for phenol removal via suspended growth and packed bed reactors. The packed bed reactor was found to be more resistant to high phenol concentrations and led to significantly higher removal rates than the suspended-growth reactor. Marrot et al.[5] demonstrated that it is possible to treat effluents containing high phenol concentrations (up to 1.0 g/L-1) by activated sludge at typical biomass concentrations of ~10 g/L-1 in membrane bioreactors. Bajaj et al.[8] reported that a high phenol removal rate of up to 2.3 g/(L d) at a phenol/COD ratio of 0.8 and influent phenol concentration of 4.9 g/L was achieved in a fixed bed reactor with stepwise increments of influent phenol concentration. The biological removal of phenol from concentrated wastewater using a moving-bed sequencing batch reactor (MSBR) has been reported by Moussavi et al.[9]. The optimum hydraulic retention time (HRT) achieved for the MSBR is 40h at a critical phenol loading rate of 83.4 g phenol m-3h-1, giving a phenol removal efficiency of 99%. The moving bed contributed 28.1% phenol removal efficiency at the critical phenol

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loading rate. Biological treatment of phenolic compounds has several limitations such as lengthy start-up for microbial acclimatization[10], slow rates of microbial degradation due to pollutant toxicity[11], and the potential for microbial growth inhibition due to high concentrations of phenolic compounds [12].

Physical process based on adsorption onto the surface of activated carbon is one of the most effective and widely used techniques in treating high concentrations and low volumes of phenolic wastewaters[13]. A comparison between granular activated carbon and other resins for residual phenol removal from coke wastewater showed that granular activated carbon demonstrated higher adsorption capacities [14]. It has been demonstrated that some phenol removal adsorbents can maintain high adsorption capacities for a specific number of regenerations [15]. The usage of commercial activated carbon has been limited by its high cost [13,16]. Attempts have been made in producing low-cost activated carbons from agricultural by-products such as palm seed coat [16], rice husk ash [17], bagasse ash and wood charcoal [18] and oil palm shell [13,19], with encouraging results. Regeneration of activated carbon, which is challenging due to irreversible adsorption of phenols, is crucial to ensuring that the adsorption process is economically attractive. Thermal regeneration is time consuming and expensive, and progressive regeneration involving repetitive heating and cooling damages activated carbon through loss of carbon [3].

Phenol removal can also be achieved through chemical processes. Treatment of phenol-containing water via ozonation has been studied with high removal rate and ozone mass transfer[20,21]. A comparison of the performances of O3 with radiation (UV-Vis) and/or Titanium oxide (TiO2) was conducted by Gimeno et al. [22], and the combination of ozone and radiation showed the best efficiency in terms of phenol removal and also COD and TOC decay rates. As the initial cost of ozone production is high [23], ozonation is less favorable. Fenton reaction, which uses hydrogen peroxide in conjunction with an iron (II) salt, is recognized as the most economically favorable oxidation alternative due to the simplicity of equipment and the mild operation conditions [3]. In their work on the effect of chloride on Fenton process for phenol removal, Maciel et al. [24] demonstrated that phenol was completely oxidized by Fenton process in saline media (50000mg NaCl L-1) when appreciable concentrations of reagents were used (200mg H2O2 L-1 and 55mg FeSO4 L-1). Although Fenton reaction can effectively remediate phenol, acidic pH and stoichiometric excess of hydrogen peroxide are required for efficient reaction and this usually means that significant quantities of ferric salts need to be disposed of after the reaction[3]. The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed.

Enzymatic method for phenol removal

Due to the significant challenges associated with conventional phenol removal methods, research studies have been focused on finding an alternative method that is versatile for a wide range of reaction conditions with little or no environmental impacts post-treatment. Enzymatic treatment is seen as a potential alternative to conventional methods for phenol removal. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products. They also can change the characteristics of a given waste to render it more amenable to treatment or aid in converting waste material to value-added products [1]. The enzymatic method is suitable for treating high concentration phenol solution because the process can be completed without dilution within a short time. This is advantageous in instances where space cannot be secured for wastewater treatment plants based on biological oxidation [25]. Some other potential advantages of enzymatic treatment as compared to conventional treatment include application to bio-refractory compounds, operation at high and low contaminant concentrations, operation over a wide range of pH, temperature and salinity, absence of shock loading effects, absence of delays associated with the acclimatization of biomass, reduction in sludge volume (no biomass generated) and the ease and simplicity of controlling the process [26]. Enzymes function with high specificity, are very efficient in removing targeted compounds, and are easier to handle and store [27].

Mechanism of action of peroxidase for phenol removal

Amongst the various types of enzymes, peroxidases have been identified as a suitable candidate for the treatment of phenolic contaminants and related compounds. Figure 1 illustrates the catalytic cycle of peroxidase and its postulated side reaction [28].

The one-electron oxidation of aromatic substrate (AH2) catalyzed by peroxidase is usually depicted by the following mechanism [26]:

$$E + H_2O_2 \rightarrow E_i + H_2O \quad (1)$$

$$E_i + AH_2 \rightarrow E_{ii} + {}^{\bullet}AH \quad (2)$$

$$E_{ii} + AH_2 \rightarrow E + {}^{\bullet}AH + H_2O \quad (3)$$

Native horseradish peroxidase enzyme (E) reacts with peroxide to form an oxidized unit designated as compound I (Ei). Compound I accept an aromatic molecule into its active site and oxidizes it. The oxidized aromatic unit, now a free radical, is released from the catalytic site leaving the enzyme in the compound II state (Eii). The catalytic cycle is completed when compound II oxidizes a second aromatic molecule, resulting in the release of a second free radical into the solution and returning the enzyme to its native state. The free radicals generated diffuse from the enzyme into solution where they spontaneously react to form polyaromatic products. These polymers are less soluble than their monomeric precursors and tend to precipitate from solution. If the polymer fails to precipitate, it may be further oxidized through the catalytic action of HRP

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resulting in the formation of higher molecular mass polymers with even lower solubility[26]. Permanent inactivation can result from the return of a free radical to the active center of the enzyme where a bond may form at or near the active site. Such a bond blocks the active site or changes the critical geometric configuration of the enzyme, thus eliminating the enzyme's catalytic ability. Additionally, in the presence of excess hydrogen peroxide, compound II can be oxidized to compound III (Eiii) according to:

$$E_{ii} + H_2O_2 \rightarrow E_{iii} + H_2O \quad (4)$$

Compound III is catalytically inactive but its formation does not represent a terminal inactivation of HRP since compound III decomposes spontaneously to native peroxidase according to:

$$E_{iii} \rightarrow E + O_2^- \quad (5)$$

The decomposition of compound III to the native state is significantly slow that, once compound III is formed, the enzyme is severely hampered in carrying out the catalytic oxidation of aromatic substrates. Therefore, any accumulation of HRP in the compound III state represents a loss in catalytic efficiency [26].

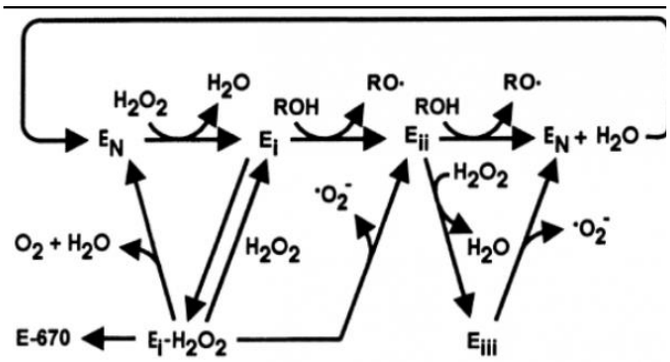

Figure 1: The catalytic cycle and side reactions of peroxidase; EN is native peroxidase; Ei, Eii and Eiii are compounds I, II and III, respectively; E-670 is verdohaemoprotein, and Ei-H2O2 is an intermediate enzyme-peroxide complex; ROH is a phenolic substrate and RO• is a phenoxy radical[28].

Peroxidase-based phenol removal

Klibanov and co-workers are among the pioneers who proposed the use of horseradish peroxidase (HRP) to remove various aromatic compounds from aqueous solutions. Over 30 different phenols and aromatic amines were removed from water using enzymatic approach [29]. Another study by Klibanov and Morris[30] showed that enzymatic treatment resulted in a nearly complete precipitation of carcinogenic aromatic amines from water due to enzymatic crosslinking. The enzymatic removal of carcinogens from water was confirmed by both chemical and toxicological assays. Since the initial demonstration of the potential of horseradish peroxidase by Klibanov's team, a substantial effort has been devoted to making enzymatic treatment economically feasible [31]. The optimum operating conditions such as pH, temperature, reaction incubation time, hydrogen peroxide dose and enzyme concentration have been investigated to obtain more than 95% phenol removal [32,33,34,35]. Nicell et al. [33] also demonstrated that enhanced removal of hard-to-remove compounds could be accomplished by co-precipitation with other substrates of HRP, which was in agreement to the findings by Klibanov et al. [29]. The high cost of HRP has encouraged the search for less expensive sources of this enzyme for commercial applications [36]. Soybean seed hull, a waste product of the food industry, has gained much attention due to its abundance. Soybean peroxidase (SBP) has proven to be effective in removing phenolic compounds from wastewater [27, 28, 37, 38]. Fungal peroxidases produced by Coprinus cinereus[39] and Coprinus macrorhizus[40,41] have also been reported to remove phenol with a comparable efficiency to plant peroxidases. Peroxidases from radish [31, 42] and potato[31] have also been tested to determine their potential in treating wastewater containing phenols.

In the treatment of foundry wastewater using pure and crude HRP, Cooper and Nicell[36] found out that total phenols removal was independent of enzyme purity. It was also noted, prior to maximum removal, that crude extract achieved better total phenols removals than pure HRP for the same enzyme concentrations. Their explanation that the crude enzyme is protected from inactivation was later supported by Wilberg et al.[27]. Another study on foul condensate from Kraft mill by Wagner and Nicell [43] revealed that the condensate contained species that protect the HRP from inactivation by the reaction products. Hence, the wastewater composition had an effect on the efficiency of phenol removal and the rate of enzyme inactivation. Nicell and colleagues[26, 32, 44] also looked into the choice of reactor configuration for continuous-flow wastewater. Their work showed that treatment of wastewater in a continuous stirred tank reactor (CSTR) relatively improves catalytic efficiency and reduces the retention time.

A major drawback of enzymatic wastewater treatment process is that it requires a large amount of enzyme to achieve high removal efficiency due to enzyme inactivation. Inactivation is believed to be caused by interactions between the polymers produced and the enzyme's active sites[29,30]. In this regard, some researchers [25, 45, 46] have studied the use of additives such as high-molecular-weight polyethylene glycol (PEG) and gelatin to suppress enzyme inactivation. The studies also showed that the use of additives greatly reduced the amount of enzyme needed. Another approach which has been used to increase the potential use of enzymes in wastewater treatment is immobilization. Enzyme immobilization is usually targeted at improving operational stability, while preventing contamination of the solution being treated. In addition, immobilized enzymes can be easily separated from the solution for reuse or for use in continuous

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reactors. Several enzyme immobilization methods, such as physical adsorption, covalent bonding, crosslinking, inclusion and encapsulation, have been developed [47]. Also, a significant body of work has been reported on the performance of immobilized peroxidase in treating phenolic compounds. This includes peroxidases immobilized on magnetite[48], glass beads[47, 49,50,51,52], and different polymers with diverse configurations[53,54,55,56,57] and capsules[58,59]. The results from these studies are promising in terms of better enzyme stability [50, 51,52,53,54], comparable or higher removal efficiency than free enzymes[47,48,49,50,51,52,53,54,55,56,57], reusability and less susceptibility to inactivation [54, 55, 57, 58].

Application of peroxidase for continuous phenol removal

The amount of work reported on immobilized peroxidase for continuous removal of phenol is minimal. The biodegradation of phenol and chlorinated phenol by immobilized white-rot fungi in trickling packed-bed reactors employing sequencing batch operation was studied by Ehlers and Rose[60]. The immobilized system provided a greater degree of stability for the fungi and high tolerance for elevated pollutant concentrations because sequencing batch reactor (SBR) operation ensured a concentration gradient was established with subsequent recycling inflows. The removal of phenols was greater than 98% in 24-30h batch cycles. On the other hand, Trivedi et al.[61] utilized a liquid-solid circulating fluidized bed (LSCFB) system for continuous polymerization of phenol by immobilized SBP. Their experimental results showed that an enzymatic reaction and the regeneration of the biocatalysts can be carried out simultaneously and independently in the LSCFB system. Apart from investigating the effects of fluidized bed height, feed flow rate and substrate concentrations on phenol removal, Gomez et al. [62] also developed a model to predict the system's behaviour both in steady and transient state (Figure 2).

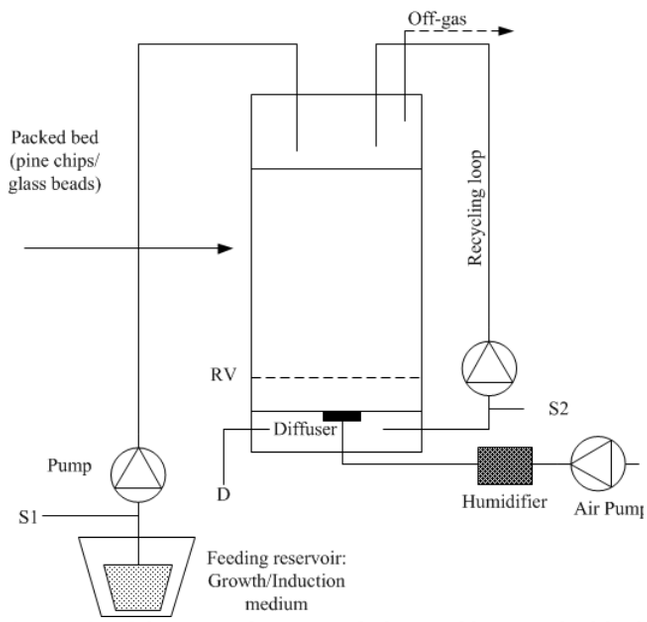

Figure 2: Schematic diagram of the trickling packed-bed reactor. S1 and S2, sampling points 1 and 2; D, drain; RV, recirculation volume[60].

Conclusion

Peroxidase-based enzymatic approach has shown significant potential and capacity for treating phenol-containing aqueous solutions and wastewaters especially at the lab-scale. For full-scale application, future work should be focused on developing optimal and commercially-viable processes for continuous phenol removal. Such studies could cover the characterization of immobilized peroxidases for phenol removal in either fluidized bed or packed-bed reactors to enable application of this enzymatic approach to industrial-scale processes.

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