Appl Water Sci (2015) 5:105–112
DOI 10.1007/s13201-014-0176-8

REVIEW ARTICLE

Biological removal of phenol from wastewaters: a mini review

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Received: 1 September 2013 / Accepted: 5 March 2014 / Published online: 25 March 2014

Abstract Phenol and its derivatives are common water pollutants and include wide variety of organic chemicals. Phenol poisoning can occur by skin absorption, inhalation, ingestion and various other methods which can result in health effects. High exposures to phenol may be fatal to human beings. Accumulation of phenol creates toxicity both for flora and fauna. Therefore, removal of phenol is crucial to perpetuate the environment and individual. Among various treatment methods available for removal of phenols, biodegradation is environmental friendly. Biological methods are gaining importance as they convert the wastes into harmless end products. The present work focuses on assessment of biological removal (biodegradation) of phenol. Various factors influence the efficiency of biodegradation of phenol such as ability of the microorganism, enzymes involved, the mechanism of degradation and influencing factors. This study describes about the sources of phenol, adverse effects on the environment, microorganisms involved in the biodegradation (aerobic and anaerobic) and enzymes that polymerize phenol.

Keywords Phenol · Aerobic treatment · Anaerobic treatment · Enzymes · Wastewater · Industrial pollutant · Toxicant

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Introduction

One of the most important environmental problems faced by the world is management of wastes (Anupama et al. 2013). Industrial processes generate a variety of molecules that may pollute air and waters due to negative impacts such as toxicity, carcinogenic and mutagenic properties (Busca et al. 2008). Phenols are the major organic constituents found in effluents of coal conversion processes, coke ovens, petroleum refineries, phenolic resin manufacturing, herbicide manufacturing, fiberglass manufacturing and petrochemicals (El-Ashtoukhy et al. 2013; Veeresh et al. 2004; Jadhav and Vanjara 2004). Phenol and its derivatives are a major source of environmental pollutants (Said et al. 2013; Varma and Gaikwad 2008). Phenol, a waste product of industrial processes that is introduced into aquatic ecosystems, adversely affects the indigenous biota, including algae, protozoa, invertebrates, and vertebrates (Babich and Davis 1981). The concentrations of these compounds can range from one to several hundred mg/L (Moussavi et al. 2008). Industrial wastewaters associated with the manufacture of halogenated organics characteristically have concentrations as high as hundreds of mg/L (Annachatre and Gheewala 1996). Water pollution by organic and inorganic compounds is of great public concern (Pradeep et al. 2014). Their fate in the environment is

of great importance as they are toxic, recalcitrant and bioaccumulating in organisms (Annachatre and Gheewala 1996). As it adversely affects the aquatic biota, phenol is one of the 129 specific priority chemicals that is considered toxic under the 1977 Amendments to the Clean Water Act and for which the US Environmental Protection Agency (EPA) has issued water quality criteria (Singh et al. 2013; Babich and Davis 1981). Phenol at concentration as low as 5 mg/L imparts typical smell upon chlorination and the World Health Organization (WHO) prescribed 1 mg/L as the maximum permissible concentration of phenol in drinking water (Saravanan et al. 2008).

Effects of phenol

Phenol and its derivatives penetrate ecosystems as the result of drainage of the municipal or industrial sewage to surface water. The pollution of the aquatic environment by phenols could modify the biota of this environment because most of these compounds exhibit a high degree of toxicity (Lika and Papadakis 2009). Acute exposure to phenol is known to cause skin irritation, gastrointestinal discomfort, and headaches. Phenol is toxic to the nervous system, the heart, the kidneys, and the liver and is readily absorbed through skin and mucosa (Wang et al. 2011). Toxicity of phenol towards plants has been proved. The willow trees exposed to 1,000 mg/L phenol wilted and eventually died (Wang et al. 2011). Phenol can also inhibit synthesis and replication of DNA in cells. A study revealed that phenol stopped preparation of DNA in diploid human fibroblasts (Michalowicz and Duda 2007).

Biological removal of phenol

Biological treatment transforms the wastes into simple end products (Pradeep et al. 2011). For this reason the interest in the use of biological methods is increasing (Lika and Papadakis 2009). Although the rate and extent of biodegradation of a chemical compound largely depends upon its structure and the environment in its vicinity, halogens containing organic compounds in particular are found to be biochemically resistant (Annachatre and Gheewala 1996). Biological removal can be classified into microbial and enzymatic methods.

Microbial removal of phenol

Microbial degradation is a useful strategy to eliminate organic compounds and detoxify wastewaters and polluted environments (Gallego et al. 2003). Phenol is degraded by diverse microorganisms including yeasts, fungi and bacteria (Table 1). Because of widespread occurrence of phenol in the environment, many microorganisms utilize phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms (Basha et al. 2010).

Table 1 Various microorganisms capable of degrading phenol

Microorganism	References
Pseudomonas putida	Seker et al. (1997)
Corynebacterium sp., *S. aureus*, *Proteus* sp., *B. subtilis* and *Staphylococcus* sp.	Ajaz et al. (2004)
Kelibsiella, *Citrobacter* and *Shigella*	Kafilzadeh et al. (2010)
Pseudomonas aeruginosa and *Pseudomonas fluorescence*	Sgountzos et al. (2006), Agarry et al. (2008), Wang et al. (2011)
Acinetobacter baumannii	Prasad et al. (2010)
Alcaligenes faecalis	Thomas et al. (2002)
Candida species	Ehrhardt and Rehm (1985), Varma and Gaikwad (2008), Jiang et al. (2010)
Aspergillus sp.	Passos et al. (2010)
Rhodococcus erythropolis	Zidkova et al. (2012)
Pseudomonas stutzeri	Viggiani et al. (2006)
Bacillus brevis	Arutchelvan et al. (2006)

Aerobic biodegradation of phenol

Aerobic biodegradation of phenol, resulting in its complete mineralization, is generally preferred in wastewater clean-up. Phenol is recognized as an inhibitory substrate at relatively low concentrations (100 mg/L) and is a convenient model for studying the kinetics of aromatic molecule degradation (Christen et al. 2012). Since microbial growth is inhibited by higher concentrations of phenol, various approaches were used to overcome this substrate inhibition. Microorganisms can be adapted to higher phenol concentration by stepwise increasing the phenol concentration (Banerjee 1997), cell immobilization (biofilm formation) or procedures of genetic manipulations. Industrial strain improvement has currently moved from simple adaptation and selection to a targeted metabolic engineering (Zidkova et al. 2012).

Chakraborty et al. (2010) studied the phenol degradation using native microorganisms isolated from coke-oven wastewater. Initial phenol concentration of 200 mg/L was given as sole carbon source. Phenol removal was 76.76 % at optimal conditions. Glucose addition up to a specific low concentration could improve the degradation rate, but

impeded the degradation process at higher concentrations. Paraskevi and Euripides (2005) used enriched mixed culture from samples of petroleum-contaminated soil in Denmark for the isolation of microorganisms, which were capable of growing on phenol as a sole source of carbon and energy. Above 300 mg/L of initial phenol concentration no considerable depletion was recorded. Maximum degradation rates for phenol were recorded at 30 °C. Seker et al. (1997) carried out phenol degradation studies using pure culture of *Pseudomonas putida* at 30 °C. The adaptation of species to phenol was done using mineral salt medium (except glucose and yeast extract). Continuos phenol degradation was carried out in a fermentor. Dual-substrate growth kinetics, Haldane kinetics for phenol and Monod kinetics for oxygen were derived with high correlation coefficients. Baek et al. (2003) isolated and characterized the bacteria capable of degrading phenol and reducing nitrate under low-oxygen conditions. The bacteria were isolated from natural and contaminated environments under low-oxygen conditions and were grown using phenol as sole carbon source. Isolated bacteria were classified according to the best similarity with the sequences of the 16S rRNA in GenBank database. The phylogenetic relationships of the phenol-degrading isolates were determined by comparison of the sequences of the 16S rRNA genes. The order of *Rhizobiales* has not been well-known for its ability to degrade aromatic compounds. This study showed that *Rhizobiales* can degrade phenol. Ajaz et al. (2004) carried out a study on phenol degradation using microorganisms isolated from garden soil. The identification, characterization and genetical studies of these strains were carried out. The microorganisms were identified as *Corynebacterium* sp., *S. aureus*, *Proteus* sp., *B. subtilis* and *Staphylococcus* sp. Microorganisms were able to degrade phenol without any adaptation period. This is due to the fact that the microorganisms were able to sustain and use phenol as carbon which was present in the rhizosphere environment. Kafilzadeh et al. (2010) isolated and identified phenol-degrading bacteria from Lake Parishan and they assayed their kinetic growth. The ability of bacteria to degrade different concentrations of phenol was measured using culturing bacteria in different concentrations of phenol from 0.2 to 0.9 g/L. Most of the isolated bacteria showed a good ability of degradation of phenol, where *Pseudomonas* and *Acinetobacter* showed 0.8–0.9 g/L, and *Kelibsiella*, *Citrobacter* and *Shigella* showed 0.6–0.7 g/L and the rest showed 0.2–0.3 g/L of phenol degradation. Agarry et al. (2008) studied the bioremediation potential of an indigenous binary mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* in batch culture using synthetic phenol in water in the concentration range of 100–500 mg/L as a model limiting substrate. The effect of initial phenol concentration on the degradation process was investigated. Prasad et al. (2010) isolated *Acinetobacter baumannii* and tested for phenol degradation. This bacterium had high phenol degradation activity and high tolerance to phenol. The biodegradation assays were performed in liquid medium with phenol as single substrate. Different concentrations of phenol samples ranging from 125 to 1,000 mg/L were taken for observations. Phenol was completely degraded at different cultivation times with different initial phenol concentrations. Sameer et al. (2002) studied the biodegradation of synthetically prepared phenol wastewater in a single stage, bench-scale rotating biological contactor (RBC). The effect of process variables, namely rotational speed (40–175 rpm), input phenol loading and temperature of wastewater (20–30 °C) on the amount of phenol removed in the system was investigated. It was observed that an increase in the speed of rotation significantly improved the performance. An increase in the hydraulic loading rates caused a reduction in the phenol removal rate, while an increase in the organic loading rate resulted in an improvement in performance. An increase in temperature caused an increase in the microbial activity and, therefore, gave better performance.

Anaerobic biodegradation of Phenol

During anaerobic digestion of organic waste, both energy-rich biogas and a nutrient-rich digestate are produced. The digestate can be used as a fertilizer in agricultural soils if the levels of hazardous compounds and pathogens are low. Chemical analysis of digestate from bioreactors operating at thermophilic temperature has detected higher content of phenols compared to mesophilic bioreactors, verifying the degradation results. Digestate with the highest phenol content has the greatest negative impact on soil microbial activity (Leven et al. 2012).

Veeresh et al. (2004) used upflow anaerobic sludge blanket (UASB) for treatment of phenolic wastewater. Phenol concentrations greater than 500 mg/L was effectively treated with acclimatization of inocula. Bench-scale studies showed that phenol and cresols were degraded with and without co-substrate in a UASB process with a suitable operational strategy. Maintenance of substrate concentration within their inhibitory range in the reactor is the utmost control strategy in the treatment of phenolic wastes. Azbar et al. (2009) studied phenol removal using anaerobic hybrid reactor. Phenol removal efficiency ranged from 39 to 80 % at different conditions. Biogas was obtained during this process. Shibata et al. (2006) examined microbial degradation of various phenol and its derivatives under both aerobic and anaerobic conditions in seven Japanese paddy soils. Under flooded and anaerobic conditions, 4-*n*-propylphenol would be degraded as well as phenol and *p*-cresol while alkylphenols with long and branched alkyl chains were hardly degraded.

Phenol can also be degraded in the absence of oxygen and it is less advanced than the aerobic process. It is based on the analogy with the anaerobic benzoate pathway proposed for *Paracoccus denitrificans* in 1970. In this pathway, phenol is carboxylated in the para position to 4-hydroxybenzoate which is the first step in the anaerobic pathway. Here, the enzyme involved is the 4-hydroxybenzoate carboxylase. The anaerobic degradation of several other aromatic compounds has been shown to include a carboxylation reaction. The organisms capable of degrading phenol under anaerobic conditions were *Thauera aromatica* and *Desulphobacterium phenolicum* (Basha et al. 2010).

Enzymatic polymerization of phenol

Enzymes are proteins and are found in all types of living cells. A given cell will characteristically contain hundreds of enzymes. Enzymes are biological catalysts that increase the rate of chemical reactions taking place within living cells. Although an enzyme increases the rate of reaction, it does not affect the reaction equilibrium. The acceleration achieved by enzymatic catalysis is often tremendous, with some reaction rates propelled to one million times faster than the rate in the absence of enzymes (Bailey and Ollis 1986).

Enzymes catalyze specific reactions and mostly act under moderate conditions (temperature, pH, solvents and ionic strength). Hence enzymes represent a promising tool for the selective removal of pollutants from waste streams (Demarche et al. 2011). Enzyme-based processes act with high specificity and are very efficient in removing targeted compounds. The use of enzyme-based techniques to remove organic compounds from aqueous solution was first proposed by Klibanov and colleagues and has been continuously improved since then. Peroxidases are the most widely reported enzymes for phenol polymerization when compared with other enzymes. Authors have reported the use of purified horseradish peroxidase (HRP) to remove 30 different phenols and aromatics amines (Wilberg et al. 2000; Cooper and Nicell 1996).

Phenol conversion is activated by H_2O_2; the enzyme catalyzes the oxidation of aromatic compounds, forming free radicals which undergo spontaneous polymerisation (Wilberg et al. 2002). Soybean seed hulls have been identified as a rich source of peroxidase, the soybean peroxidase (SBP), and being a by-product of soybean food industry, they provide a cheap and abundant source of peroxidase (Wilberg et al. 2002; Hejri and Saboora 2009). Radish roots contain peroxidase enzyme and can be used for the removal of phenol from wastewaters (Naghibi et al. 2003).

Enzyme (peroxidases) mechanism for degradation of aromatic compounds is as follows:

One-electron oxidation of aromatic substrates (AH_2) catalyzed by peroxidase is depicted by the Chance-George mechanism as the following:

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

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

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

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

The native enzyme (E) is oxidized by hydrogen peroxide (H_2O_2) to an active intermediate enzymatic form called compound I (E_i). Compound I accepts an aromatic compound (AH_2) into its active site and carries out its oxidation. A free radical (AH·) is produced and released into solution leaving the enzyme in the compound II (E_{ii}) state. Compound II oxidizes a second aromatic molecule, releasing another free radical product and returning the enzyme to its native state, thereby completing the cycle. The overall peroxidase reaction consists of the reactions described by Eqs. (1)–(3).

In the presence of excess hydrogen peroxide, the reaction of Eq. (4) becomes important because compound III (E_{iii}) is a reversibly inactivated form of the enzyme. This implies that enzyme is inhibited by H_2O_2 in excess. On the other hand, lack of hydrogen peroxide during the reaction step limits the rate of reaction. The semi-batch addition of H_2O_2 to maintain an optimized ratio between hydrogen peroxide and enzyme concentrations was found to suppress this inhibition (Kulkarni and Kaware 2013; Wilberg et al. 2000).

In the process of reducing the enzyme, phenol and its derivatives are transformed to phenolic-free radicals. Phenolic-free radicals catalytically generated during the peroxidase cycle will either combine with each other to form phenolic polymers or undergo radical transfer with phenolic monomers or polymers present in solution converting them to radical species (Zou and Taylor 1994). Phenolic polymers may take part in the peroxidase cycle and be converted to polymer radicals. Polymer radicals may react with other radical species to form larger polymers or undergo radical transfer reactions. The end result is a solution containing polymers of various sizes (Dec and Bollag 1990). Since the phenolic polymers are less soluble than their precursors, they precipitate out of solution and can be physically removed by either gravity settling or filtration, aided in some cases by the use of coagulants (Wright 1995). Various enzymes used for phenol polymerization and their sources are given in Table 2.

Potential advantages of enzyme-based treatment (Wright 1995; Duran and Esposito 2000)

- Selective treatment, only phenolic and similar reducing substrates removed.

Table 2 Various microbial and plant enzymes and their sources involved in biodegradation of phenol

Enzyme	Source	References
Phenol hydroxylase	*Bacillus stearothermophilus*	Gurujeyalakshmi and Oriel (1989)
Polyphenol oxidase	Mushroom	Burton et al. (1993)
Polyphenol oxidase	*Trametes trogii*	Garzillo et al. (1998)
Polyphenol oxidase	Transgenic tomato	Steffens and Li (2002)
Polyphenol oxidase	Potato	Loncar et al. (2011)
Phenol oxidase	*Lentinula edodes LE2*	Okeke et al. (1997)
Catechol 2,3 dioxygenase	*Bacillus* sp.	Ali et al. (1998)
Laccase	*Rhizoctonia praticola*	Bollag et al. (1988)
Laccase	*Aspergillus oryzae*	Schneider et al. (1999)
Laccase	*Pleurotus ostreatus*	Hublik and Schinner (2000)
Laccase	*Chalara paradoxa*	Robles et al. (2000)
Phenol oxidase	*Termitomyces albuminosus*	Johjima et al. (2003)
Horseradish peroxidase	Horseradish	Cooper and Nicell (1996), Wu et al. (1998), Zahida et al. (1998), Ghioureliotis and Nicell (1999); Wilberg et al. (2000); Stanisavljevic and Nedic (2004), Lai and Lin (2005), Mossallam et al. (2009), Iran and Siamak (2009)
Soybean peroxidase	Soybean	Caza et al. (1999), Wright and Nicell (1999), Ghioureliotis and Nicell (1999), Hejri and Saboora (2009)
Radish peroxidase	*Raphanus sativus*	Naghibi et al. (2003)
Peroxidase	*Momordica charantia*	Akhtar and Husain (2006)
Turnip peroxidase	*Brassica napus*	Guerrero et al. (2008)

- Faster reaction velocity, reduced residence time.
- Action on substances which are toxic to microbes.
- Operation over a wide range of substrate concentrations.
- Operation over a wide range of pH, temperature, and salinity.
- No shock loading effects, no acclimatization required.
- Process control is simpler, reliable and predictable.

Wilberg et al. (2000) used an enzymatic process for phenol removal comprising a reaction step in which phenol was polymerized in the presence of an HRP enzyme. Experimental results showed the potential of the proposed technique having a conversion higher than 99 %. Cooper and Nicell (1996) used HRP for the oxidation of phenols by hydrogen peroxide which resulted in the formation of water-insoluble polymers. The feasibility of the enzyme process to treat a foundry wastewater containing 3.5 mM of total phenols (330 mg/L as phenol) was examined. Two enzyme stocks of different purities were used but total phenols removal was independent of enzyme purity. For both stocks, 97–99 % of the phenolic contaminants were removed, despite the presence of other contaminants such as organic compounds and iron in the waste matrix. Lai and Lin (2005) reported the development and application of an immobilized HRP system with porous aminopropyl glass (APG) beads. After 3 h of reaction with the addition of hydrogen peroxide a maximal removal efficiency of 25 % was observed at pH 7.5. The addition of polyethylene glycol (PEG) significantly enhanced the removal efficiency presumably via the formation of a protective shield in the vicinity of the active site of HRP from the free radicals formed during polymerization. The reusability of the HRP immobilized APG was also demonstrated. Mossallam et al. (2009) conducted phenol degradation studies using horseradish peroxidase (HRP) enzyme. Wastewater from petroleum oil reservoirs was used for this study which contained 4.5 mg/L phenol, 300 mg/L petroleum oil, Cl-62 mg/L and pH 6.0. It was found that petroleum oil has strong depressing effect on the activity of HRP at concentrations up to 1 g/L of petroleum oil. The most effective concentrations for treating wastewater were peroxidase at 2 U/ml and 0.5 mM hydrogen peroxide (H_2O_2) at neutral pH. Stanisavljevic and Nedic (2004) reported a process for phenol removal comprising a reaction step in which phenol was polymerized in the presence of an enzyme HRP. Experimental results showed the potential of the proposed

technique. A phenol conversion higher than 90 % was observed using this polymerization process. Wright and Nicell (1999) studied phenol removal using SBP enzyme, activity was optimal at pH 6.4, with significant activity observed between pH 3 and 9. SBP was very stable at 25 °C at neutral and alkaline conditions but experienced rapid inactivation below pH 3. SBP was most effective when used to treat phenolic solutions between pH 6 and 9. In comparison with HRP, the activity of SBP was only slightly more sensitive to pH, more stable at elevated temperatures, and less susceptible to permanent inactivation by hydrogen peroxide.

Iran and Siamak (2009) reported the use of encapsulated HRP in calcium alginate for the purpose of phenol removal. The ratio of hydrogen peroxide/phenol at which highest phenol removal obtained was found to be dependent on initial phenol concentration and in the solution of 2–8 mM phenol it was 1.15 and 0.94, respectively. Loncar et al. (2011) reported an inexpensive immobilized enzyme for the removal of phenols. Partially purified potato polyphenol oxidase (PPO) was immobilized onto different commercial and laboratory produced carriers. 45 % removal of 4-bromophenol was achieved, while the 4-chlorophenol and phenol were 35 and 20 %, respectively. After eight repeated tests, the efficiency of 4-chlorophenol removal had decreased to 55 %.

Caza et al. (1999) conducted experiments to investigate the efficiency of using SBP to remove phenol and its derivatives from unbuffered synthetic wastewater. A removal efficiency of 95 % was observed during this study. Hejri and Saboora (2009) treated synthetic wastewater containing phenol, *o*-cresol and *m*-cresol, with SBP. The results showed that, an increase in hydrogen peroxide up to the optimum amount leads to an elevated removal of phenol and its derivatives. Higher concentrations of H_2O_2 inhibited the reaction. Treatment in the presence of PEG as an additive increased the effect of enzymatic removal. Wilberg et al. (2002) studied the application of low-purity soybean peroxidase (LP-SBP), obtained from wasted seed hulls, as a catalyst for phenol polymerizations in an aqueous solution in the presence of hydrogen peroxide. At all phenol concentrations tested, a retention time of about 100 min was sufficient to achieve good results.

Naghibi et al. (2003) conducted phenol removal using synthetic wastewater buffered at pH 7.4 containing 0.9 mM phenol which was treated with cut *Raphanus sativus* root and its juice. More than 90 % of phenol was removed in both cases.

Plant materials have been found useful in decontamination of water polluted with phenol and its derivatives. The detoxification effect is due to peroxidases contained in plant tissue. Enzyme-mediated oxidative coupling of phenol is followed by precipitation of the formed polymer and its removal from the aqueous phase (Naghibi et al. 2003).

Conclusive comments

The data summarized in this mini review paper provide research efforts carried out for the treatment of phenolic wastewaters. Degradation of phenol and its derivatives using various microorganisms has been the topic of scientific interest for a number of decades. Practical applications of microorganisms for the degradation of phenol are presently used exclusively for treatment of industrial wastewaters, both pure and mixed cultures of microorganisms and enzymes can serve the purpose. The findings of this review suggest that microbial and enzymatic methods can be robust and promising for effectively treating wastewaters containing phenol and its derivatives. Aerobic and anaerobic microbial treatment methods can be used for phenol biodegradation. Pure culture and mixed cultures can be used in different reactors for phenol degradation. Enzymatic methods have been widely reported the polymerization of phenol. These enzymes can be used in free and immobilized forms. Immobilized enzymes can be reused which will reduce the operating costs.

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