# Optimisation of soybean peroxidase treatment of 2,4-dichlorophenol

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## Abstract

In the presence of hydrogen peroxide ($H_2O_2$), peroxidase enzymes (PE) catalyse the oxidation of various chlorinated phenols to free radicals, which then combine to form insoluble polymers that precipitate out of solution. This study systematically characterises the treatment of 2,4-dichlorophenol (2,4-DCP) using soybean peroxidase (SBP) as an oxidising catalyst. The effects of pH, SBP concentration, polyethylene glycol (PEG) additive and initial chlorophenol concentration on 2,4-DCP treatments are reported. Optimum pH for removal of 2,4-DCP without PEG was pH 8.2. The pH operating range of SBP was from 2.5 to 9.4 which is wider than reported for horseradish peroxidase (HRP). A general equation is presented that describes the units of SBP required (without PEG) to treat a given amount of 2,4-DCP at the optimum pH of 8.2. Addition of PEG increased the effectiveness of SBP by factors of 10 and 50 for PEG-3350 and PEG-8000 respectively. A new pH optimum of 6.2 was also found when SBP was used with PEG. Batch and semi -batch enzyme delivery has also been identified as a crucial parameter for the SBP treatment process. The most effective addition scheme was based on five equal concentrations of SBP and $H_2O_2$ over 15 min and 30 min intervals respectively compared to a single batch addition. This protocol was the most effective as it took advantage of limiting the amount of SBP and $H_2O_2$ available at each step. This reduces the possible chance of SBP inactivation by excessive $H_2O_2$ when using a single batch concentration. Average 2,4-DCP removals achieved were 83.5%, 75.5% and 71.5% for 100, 200 and 300 mg/l 2,4-DCP concentrations respectively compared to 62%, 52% and 58% for the single batch addition control.

## Introduction

In the presence of hydrogen peroxide ($H_2O_2$), which acts as an electron acceptor, peroxidase enzymes (PE) catalyse the oxidative polymerisation of phenols, anilines and other aromatics to insoluble oligomers (Dunford and Stillman, 1976). These insoluble oligomers can then be removed through a simple sedimentation or filtration system (Klibanov et al., 1980,1983; Dordick et al., 1980). The kinetics of the peroxidase cycle has been previously described (Dunford and Stillman, 1976; Banci 1997). To date, the majority of the experiments performed have used horseradish peroxidase (HRP) in the treatment of wastewater contaminated with phenols, cresols and chlorinated phenols (Aitken, 1993). However, researchers are currently studying PE from various sources in an effort to study the characteristics of the process and to test the validity of other PE sources (Aitken, 1993). Recently, peroxidase from soybean has been suggested as an alternative to horseradish (Al-Kassim et al., 1993a b, 1995; Nicell and Wright, 1997; Caz et al., 1999, Kinsley and Nicell, 2000). Soybean peroxidase (SBP) is derived from the soybean plant's seedcoat, which is economically advantageous because the seedcoat is a waste by-product of the soybean processing industry. Concomitantly, using SBP would help to convert a waste into a value-added product (Taylor et al., 1998).

While application of SBP is still in its infancy, exploratory studies have been reported. Taylor et al. (1996) provided a limited comparison of HRP and microbial peroxidase to SBP for treatment of phenols and reported that SBP was an effective alternative. This work was followed by two reports that studied the removal of a variety of phenols from wastewater using SBP and a comparative cost analysis of phenols treated individually and separately by SBP, HRP and microbial peroxidase (Taylor et al., 1996,1998, Caza et al., 1999). McEldoon and Dordick (1996) reported that SBP demonstrated unusually high thermal stability that could expand its industrial applications. Recently studies by Wright and Nicell (1999) and Kinsley and Nicell (2000) have also compared the benefits of using SBP over HRP for treatment of phenols as well as demonstrating the benefits of polyethylene glycol (PEG) for the protection of SBP activity.

The SBP treatment process is still in the experimental stage so researchers are continually studying and optimising treatment efficiency while studying other characteristics of the process. The influence of pH, initial chlorophenol concentration, type of chlorophenol, application of protective additives mode of addition and temperature are all factors that influence the applicability of this technology (Al-Kassim et al., 1993a b, 1994a, 1994b,1995; Caza et al., 1999). It is hypothesised that the particulate that forms and precipitates out of solution, entraps the SBP and thereby renders it inactive. To prevent such entrapment, high-molecular mass additives such as PEG can be used to bind with the forming polymers and prevent the PE from becoming entrapped (Wright and Nicell,1999 and Kinsley and Nicell, 2000). It has been reported that PEG with a molecular mass less of than 1 000 is ineffective at protecting HRP when treating phenol and that PEG with a molecular mass of 7 500 (PEG-7500) is more efficient than PEG-1000 (Nakamoto and Machida, 1992). Other researchers have continued this research using PEG-3350 (Wu et al., 1993; Ibrahim et al., 1997). However, there is no supporting documentation that suggests that PEG-3350 is more suitable than PEG-7500. A recent study has shown that SBP in the presence of PEG with higher molecular masss than 7 500 can achieve better efficiency in the removal of phenol from solution (Kinsley and Nicell, 2000).

The effect of pH on HRP catalysing phenol in the presence of PEG has been documented (Bewtra et al., 1995; Dec and Bollag, 1994a,b). The effect of pH on HRP catalysing different chlorinated

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