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Laccase mediated biodegradation of 2,4-dichlorophenol using response surface methodology

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ABSTRACT

The effects of different environmental parameters, i.e., pH, temperature, time and enzyme concentration on the biodegradation of 2,4-dichlorophenol (2,4-DCP) in aqueous phase was evaluated with laccase from *Pleurotus* sp. using response surface methodology (RSM) in the present investigation. The Box–Behnken design of experiments was used to construct second order response surfaces with the investigated parameters. It was observed that the maximum degradation efficiency of ~98% was achieved at pH 6, temperature of 40 °C, time 9 h and an enzyme concentration of 8 IU ml^{-1}. The adequacy of the model was confirmed by the coefficient of multiple regression, R^2 and adjusted R^2 which were adjudged to be 87.9% and 73.6%, respectively indicating a reasonably good model for practical implementation. Despite the fact that many successful attempts have been taken in the past for biodegradation of 2,4-DCP using whole cells, the present study emphasizes the fastest biodegradation of 2,4-DCP, a potent xenobiotic compound.

1. Introduction

Chlorophenols present a serious threat to the environment owing to their toxicity and environmental persistence attributed to their Xenobiotic origin. Pollution from aromatic hydrocarbons such as phenolic compounds severely impact the ecology, health and economy of a region due to high toxicity, recalcitrance, bioaccumulation, strong odour emission, persistence in the environment and suspected carcinogenic and mutagenic effects. The environment is seriously threatened by these toxic, recalcitrant compounds present in the effluents from paper mills, chemical and textile industries. 2,4-DCP is used for the production of germicides and soil strerilizants as well as in the manufacture of methylated chlorophenols used in the production of antiseptics and disinfectants. Due to their persistence and inherent toxicity to a broad spectrum of naturally present organisms, chlorophenols pose a serious threat to the environment (Stoilova et al., 2006). US Environmental Protection Agency includes 2,4-DCP together with other chlorophenols among 129 priority pollutants in 65 classes (Vroumsia et al., 2005).

In order to cope up with the situation, various treatments methods have been developed for the reduction of phenol content in wastewaters. The technologies for the treatment of wastewater containing phenol, bisphenol, chlorophenol can broadly be classified into physicochemical and biotechnological methods. Among the physicochemical processes, the prominent ones are ozonation, adsorption, precipitation, solvent extraction, reverse osmosis, ultrafiltration etc. (Lu et al., 2006; Yasman et al., 2006; Hu and Wang, 2007). However, owing to the huge cost incurred upon by physicochemical methods, they are more often than not practically infeasible. As a result, biological alternatives for the degradation of the chlorophenols are being explored. The degradation of chlorophenols present in the environment by several microorganisms has already been reported (Gallizia et al., 2003; Xiangchun et al., 2003; Vroumsia et al., 2005).

Many investigators have reported biodegradation of toxic compounds in the presence of non-toxic easily biodegradable organic compounds (Loh and Wang, 1998; Fakhruddin and Quilty, 2005; Uysal and Türkman, 2005). Several researchers have investigated biodegradation of 2,4-dichlorophenol, using different bacterial species and fungi. *Micrococcus* sp., *Chrysosporium* sp. and *Mucor* sp. have been found capable of degrading 2,4-DCP (Gallizia et al., 2003; Vroumsia et al., 2005). Immobilized cells of *Achromobacter* sp. in an air-lift bioreactor have also been used for the degradation of 2,4-DCP (Xiangchun et al., 2003, 2004). The removal of 2,4-DCP in a conventional activated sludge by bioaugmentation has also been reported (Chen et al., 2006). However, despite their capacity to degrade chlorophenols, their usage for the degradation is being limited due to their lack of resistance to chlorophenols at a higher concentration.

Chlorophenols at high concentrations usually inhibit cell growth. These toxic effects can be alleviated to some extent by subsequent adaptation of microorganisms to chlorophenols and by

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addition of conventional growth substrates in the medium (Van der Meer et al., 1992; Andretta et al., 2004). Despite acclimatization of the microorganisms capable of degrading chlorophenols, most of them are unable to thrive in 2,4-DCP contaminated effluents where the concentration levels varies from 0.15 μg ml^{-1} to 100–200 μg ml^{-1} (Valo et al., 1990). As living cells are susceptible to such chlorophenols, the degradation of such compounds can alternatively take place by using some biocatalysts, i.e., enzymes, that catalyse the cleavage of such molecules resulting in mineralization.

Some of the potential enzymes that can be used for the degradation of 2,4-DCP are laccases. Owing to the toxicity and recalcitrant nature of 2,4-DCP, enzymatic treatment of the compound forms a viable alternative. However, for an enzymatic process to be carried out successfully, the optimization of its degradation conditions is of foremost importance.

Response surface methodology is an experimental approach to identify the optimum conditions for a multivariable system (Box and Wilson, 1951; Trupkin et al., 2003). To achieve the maximum degradation of 2,4-DCP with minimum contact time, a series of experiments were performed to investigate the role of various parameters and their interactions on the biodegradation of 2,4-DCP. Though quite a few reports are available on the biodegradation of 2,4-DCP by whole cell, hardly any report was found by the authors during rigorous literature review on the enzyme mediated biodegradation of 2,4-DCP. The present article, thus focuses on the enzymatic degradation of 2,4-DCP, the findings of which clearly emphasizes their advantages over whole cell biodegradation.

2. Materials and methods

2.1. Fungal strains

A hyperactive strain of *Pleurous* sp. was isolated locally from the forests of Kharagpur. It was maintained on the slants of Potato Dextrose Agar. Subculturing was done on every 9th day to maintain its viability.

2.2. Chemicals

All the solvents and reagents used in the present study were purchased from Merck Chemicals, Germany.

2.3. Softwares

In the present study, Minitab® (Release 13.1) and Design Expert 7® (trial version) were used for the design of experiment and analysis of the obtained data.

2.4. Biodegradation conditions

1 mM solution of 2,4-DCP was prepared in distilled water and the degradation was monitored at different conditions of pH, temperature, time and enzyme concentration. The optimal conditions for biodegradation of 2,4-DCP was established using response surface methodology.

2.5. Laccase assay

Laccase assay was carried out by monitoring the oxidation of 2,2′-azino-bis-(3-ethylbertzthiazoline-6-sulphonic acid (ABTS) in a reaction mixture containing 1 mM ABTS in 0.1 M sodium acetate buffer (pH 4.5) and 5–50 μl of enzyme sample. Oxidation was monitored at 436 nm. One unit of enzyme activity (IU) was defined as 1 μM of ABTS oxidized per minute under the assay conditions (ε_{436} = 29 300 M^{-1} cm^{-1}).

2.6. Response surface methodology

Response surface methodology is an empirical modeling technique used to evaluate the relationship between a set of controllable experimental factors and observed results. This optimization process involves three major steps: (i) performing statistically designed experiments, (ii) estimating the coefficients in a mathematical model and (iii) predicting the response and checking the

Table 1
The actual design of experiments and response for 2,4-DCP degradation

Run order	pH	Temp (°C)	Time (h)	Activity (IU/ml)	Degradation (%) (experimental)	Degradation (%) (predicted)	*R*-studentized residual
1	5.50	2.5	10.0	7.5	58.70	59.76	−0.31499
2	6.25	25.0	8.5	5.0	75.50	73.07	0.43885
3	6.25	32.5	7.0	10.0	82.40	75.94	1.23627
4	5.50	5.0	8.5	7.5	83.00	79.12	−0.71038
5	6.25	40.0	8.5	5.0	90.00	93.02	−0.55144
6	6.25	25.0	8.5	10.0	77.00	79.80	−0.51041
7	6.25	25.0	10.0	7.5	37.00	46.02	−1.85623
8	6.25	32.5	8.5	7.5	88.00	87.73	0.03785
9	7.00	32.5	8.5	5.0	83.00	80.60	0.43348
10	7.00	32.5	7.0	7.5	63.70	67.79	−0.75360
11	6.25	32.5	8.5	7.5	88.00	87.73	0.03785
12	7.00	40.0	8.5	7.5	96.50	90.83	1.06868
13	6.25	32.5	10.0	10.0	89.00	73.19	5.47268
14	7.00	25.0	8.5	7.5	71.80	63.27	1.72364
15	6.25	40.0	10.0	7.5	70.50	71.21	−0.12861
16	5.50	32.5	7.0	7.5	81.30	79.74	0.28046
17	5.50	32.5	8.5	5.0	80.50	79.1	0.25101
18	6.25	40.0	8.5	10.0	97.00	105.26	−1.65868
19	7.00	32.5	10.0	7.5	43.00	50.39	−1.44914
20	6.25	32.5	7.0	5.0	75.80	82.06	−1.19542
21	5.50	32.5	8.5	10.0	95.00	101.08	−1.15788
22	7.00	32.5	8.5	10.0	72.50	77.58	−0.95043
23	5.50	40.0	8.5	7.5	98.00	96.98	0.18265
24	6.25	40.0	7.0	7.5	92.40	87.07	0.99887
25	6.25	32.5	8.5	7.5	87.20	87.73	−0.07572
26	6.25	25.0	7.0	7.5	63.88	66.85	−0.54194
27	6.25	32.5	10.0	5.0	51.20	48.12	0.56127