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A new combined green method for 2-Chlorophenol removal using cross-linked *Brassica rapa* peroxidase in silicone oil

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HIGHLIGHTS

- A new technique for 2-CP removal coupling absorption to biodegradation was used.
- The partition coefficient air-silicone oil of 2-CP was determined.
- Silicone oil (47V20) allowed the reduction of 2-CP concentration in gas phase five times lower.
- 70% of 2-CP degradation in silicone oil was achieved using cross-linked enzyme aggregates of BRP peroxidase (BRP-CLEAs).

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ABSTRACT

This study proposes a new technique to treat waste air containing 2-Chlorophenol (2-CP), namely an integrated process coupling absorption of the compound in an organic liquid phase and its enzymatic degradation. Silicone oil (47V20) was used as an organic absorbent to allow the volatile organic compound (VOC) transfer from the gas phase to the liquid phase followed by its degradation by means of Cross-linked *Brassica rapa* peroxidase (BRP) contained in the organic phase. An evaluation of silicone oil (47V20) absorption capacity towards 2-CP was first accomplished by determining its partition coefficient (H) in this solvent. The air-oil partition coefficient of 2-CP was found equal to 0.136 Pa m^3 mol^{-1}, which is five times lower than the air–water value (0.619 Pam^3 mol^{-1}). The absorbed 2-CP was then subject to enzymatic degradation by cross-linked BRP aggregates (BRP-CLEAs). The degradation step was affected by four parameters (contact time; 2-CP, hydrogen peroxide and enzyme concentrations), which were optimized in order to obtain the highest conversion yield. A maximal conversion yield of 69% and a rate of 1.58 mg L^{-1} min^{-1}were obtained for 100 min duration time when 2-CP and hydrogen peroxide concentrations were respectively 80 mg L^{-1} and 6 mM in the presence of 2.66 UI mL^{-1} BRP-CLEAs. The reusability of BRP-CLEAs in silicone oil was assessed, showing promising results since 59% of their initial efficiency remained after three batches.

1. Introduction

Among the phenolic compounds, chlorinated phenols (CPs) are a major group of pollutants which are used in various industrial processes as pesticides, herbicides, fungicides, disinfectants, antiseptics, wood and glue preservatives, paints, solvents and as intermediates in the production of dyes and pharmaceuticals (Nicolella et al., 2009; Olaniran and Igbinosa, 2011). They penetrate the atmosphere through volatilization and combustion of coal or wood (Olaniran and Igbinosa, 2011). The evaporation of chlorophenols may occur also from shallow surface waters, when ambient temperature is above 20 °C. Among chlorinated phenols, mono and di-Chlorophenols are the most volatile derivatives.

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2-chlorophenol (2-CP) belongs to the 19 possible congeners of chlorophenols; it is the most volatile, characterized by a vapour pressure of 0.99 mmHg and could be found frequently in municipal waste incinerators (Olaniran and Igbinosa, 2011; Szatkowski and Dybala-Defratyka, 2013). Physical technologies, such as activated carbon adsorption, are only partial up-stream technologies and could not be considered as efficient methods for CP removal (Li et al., 2011). Advanced oxidation processes (AOP) are among the most used methods for chlorophenols elimination (Pera-Titus et al., 2004; Sung and Huang, 2007). Despite their efficiency, AOPs could be a source of undesirable by-products. Atmospheric pollution by CP could be also treated by biofiltration (Nicolella et al., 2009). However, and even if microbial degradation of chlorophenols was reported by several authors as an efficient cost effective method (Field and Sierra-Alvarez, 2008), chlorophenols at high concentration could be inhibitory for a wide range of microorganisms (Marsolek et al., 2006). During the last decade, more attention has been paid to the use of enzymes as potential catalysts for the degradation of recalcitrant compounds including phenolic compounds (Kirk et al., 2002).

As catalysts in aqueous phase, enzymes like peroxidases have been used since decades in emerging process of wastewater charged in phenolic compounds (Bansal and Kanwar, 2013; Bayramoğlu and Arıca, 2008; Nazari et al., 2007). Enzymatic treatment using peroxidase and hydrogen peroxide as a co-substrate was proposed in the early 1980s as an alternative treatment, which is highly selective and efficient for removing phenols from aqueous solutions (Klibanov et al., 1980). Enzymatic polymerization offers the advantages of low process energy requirements as well as low solubility of the polymerized product.

The use of organic solvents as reaction media can also greatly expand the repertoire of enzyme-catalysed transformations. In order to overcome the denaturing effect of organic solvents on enzymes, methods such as enzyme cross-linking were developed (Sheldon et al., 2006). A number of potential applications of enzymes, which are either impossible or marginal in water, due for instance to limited solubility of substrate or reaction shift due to the presence of water, become quite feasible and commercially attractive in other solvents (Illanes et al., 2012; Klibanov, 2001). Hence, hydrophobic volatile atmospheric pollutants could be transferred in an organic phase to undergo a rapid, efficient enzymatic treatment under mild conditions. Since chlorophenols presents a limited solubility in water, silicone oil (Rhodorsil® 47V20) was used as a solvent for their absorption. Indeed, the effectiveness of silicone oil as an alternative absorbent for VOCs was reported in recent studies by several authors (Darracq et al., 2010, 2012; Dumont et al., 2006, 2010).

In the current research, a new combined method was conducted at laboratory-scale for the degradation of 2-CP from air streams by absorption into silicone oil followed by enzyme degradation by cross-linked *Brassica rapa* peroxidase aggregates (BRP-CLEAs). Effects of parameters, like substrate, enzyme concentrations and contact time on process efficiency were also evaluated.

2. Materials and methods

2.1. Reagents, silicone oil and peroxidase

The substrates, 2-CP (99.9%), hydrogen peroxide (30%, v:v), guaiacol (98%) and the reagents, glutaraldehyde (25% solution in water), hexane (99.9%), were all of analytical grade and obtained from Fluka and Sigma–Aldrich (Saint Quentin Fallavier, France).

The polydimethylsiloxane or Silicone oil (Rhodorsil® 47V20) was purchased from the Rhodia Company (Boulogne-Billancourt, France). It is characterized by a molecular weight ranging between 2800 and 3200 g mol^{-1}, a viscosity and density of 20 mPa s and 950 kg m^{-3}respectively at 25 °C and a dielectric constant of 2.68 (Bluestar silicones, 2012).

Peroxidase was extracted from *B. rapa* turnip bought from local market of Medea city, Algeria.

2.2. Procedure for BRP-CLEAs preparation

The cross-linked enzyme aggregates were prepared using the standard method described by Sheldon, (Sheldon, 2011) and has already been optimized in a previous work (Tandjaoui et al., 2015). Crude peroxidase was extracted from fresh turnips of about 13–15 cm using an automatic juice extractor. The juice obtained was filtered by four layers of cheesecloth. The filtrate was stored at 4 °C after the determination of its residual activity. To prepare BRP-CLEAs, three volumes of ice-cold acetone was added to 12 mL of crude peroxidase extract diluted with phosphate buffer solution (10 mM, pH = 7). The mixture was then cross-linked using glutaraldehyde solution (25% in water) at a volume concentration of 2%, and after 3 h of magnetic agitation at 300 rpm, the mixture was centrifuged and the BRP-CLEAs formed were washed thoroughly with distilled water and stored in phosphate buffer (50 mM, pH = 7) at 4 °C.

2.3. Activity assay for peroxidase

Activity of cross-linked *B. rapa* peroxidase was measured by the guaiacol colourimetric assay (Egley et al., 1983; Singh et al., 2012). The reaction solution containing 3.9 mL of 50 mM sodium phosphate buffer pH = 7.0 and 4.05 μL of guaiacol (98%) was mixed with 2 mg of CLEAs or 90 μL of crude peroxidase. 5 μL of hydrogen peroxide (0.8 M) was then added quickly to initiate the reaction. The colour development was monitored at 470 nm using Shimadzu UV–VIS spectrophotometer (model UV mini-1240 with a molar extinction coefficient value of 4279 M^{-1} cm^{-1}) (Marne-la-Vallée, France). One unit (1.0 U) of peroxidase activity was defined as the amount of enzyme protein that catalyses the oxidation of 1.0 μmol of hydrogen peroxide per min at 25 °C and pH = 7.

2.4. Analytical method

The 2-CP concentrations in the gas phase and in silicone oil were determined using gas chromatography (GC- thermo Focus) equipped with aflame ionisation detector (FID), using an RTx^{-1}column (15 m × 0.32 mm) with 0.25 μm thickness of film. The chromatographic operating conditions are as follows: high-purity nitrogen was used as the carrier gas at a flow rate of 21 ml min^{-1}. Detector temperature was 202 °C. Injector temperatures were 140 °C for the gas phase and 220 °C in the case of silicone oil, while oven temperatures were maintained at 120 °C and 140 °C for the gas phase and silicone oil respectively.

2.5. Partition coefficient determination

In order to evaluate the capacity of silicone oil to absorb 2-CP, liquid/air partition coefficients of 2-CP in pure water and silicone oil (Rhodorsil® 47V20) were determined at 25 °C using a static method (Darracq et al., 2010, 2012; Dumont et al., 2010). For this purpose, 5 mL of silicone oil (47V20) or pure water were introduced in glass vials of 22 mL, closed with PTFE-coated silicone rubber septa (PerkinElmer, France) and sealed with aluminium caps. A precise volume of 2-CP was injected in the vials using a 100 μL microsyringe. The vials were placed in a swivel support in a laboratory thermostatic oven at 25 °C and kept under agitation for 72 h. Once the equilibrium between the two phases was reached, a sample