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Single step peroxidase extraction and oxidation of highly concentrated ethanol and phenol aqueous solutions using supercritical carbon dioxide

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ABSTRACT

Extraction of peroxidase from raw vegetables using supercritical carbon dioxide ($scCO_2$) and its simultaneous application for oxidation of organics in wastewater in the presence of hydrogen peroxide has been demonstrated. Ethanol and phenol were used as the model organic contaminants and potato and cabbage were employed as the sources of peroxidase. The extractions were carried out at 10 MPa for 2 h over a temperature range of 25 °C to 60 °C. Oxidation of ethanol increased by 330% when the treatment was carried out in the $scCO_2$ medium compared to conventional aqueous medium when cabbage was employed, while use of potato as the enzyme source resulted in an increase of 85%. In the case of phenol, $scCO_2$ exhibited a huge increase of 400% in presence of cabbage and 140% in presence of potato. Comparison of vegetable extracts with commercial horseradish peroxidase indicated the feasibility, importance and advantage of employing vegetables as a peroxidase source. This work presents the simultaneous extraction of enzymes and its application for oxidation of organics and opens up new opportunities for recovery of resources from unwanted/discarded plant materials and their use for environmental remediation in a single step.

1. Introduction

Peroxidases, typically found in a variety of fruits and vegetables, are heme proteins that are employed for the oxidation of a wide variety of organic and inorganic compounds [1–3]. Its conjugation with and catalytic decomposition of H_2O_2 is well-known [4–11]. Categorized as an oxidoreductase, peroxidase has been extensively used as a vital component of reagents for clinical diagnoses and many laboratory experiments [12,13]. It also has critical physiological roles in indole-3-acetic acid metabolism, lignification, cross-linking of cell wall polymers and resistance to infection [14]. Novel applications of peroxidase include treatment of wastewater containing phenolic compounds and removal of peroxide from industrial effluents and foodstuffs to increase their shelf life [2,15–22].

The low solubility of peroxidase in water has always presented a serious obstacle [23,24] for various applications and has stimulated the search for non-aqueous solvents in order to increase the solubility of the hydrophobic enzymes [25,26]. Additionally, structural rigidity of the peroxidase source (i.e. vegetables and fruits) renders the aqueous solution an ineffective medium to extract all peroxidase from the source. Hence, developing a right combination of temperature, pressure and medium that can yield higher peroxidase extraction is important.

Supercritical carbon dioxide ($scCO_2$) extraction has been well-received by fabric [27], food, bio and pharmaceutical industries, for example, in coffee decaffeination, extraction of drugs and hops, fatty acid refining and production of herbal products [28–32]. Moreover, Yu et al. showed the extraction of pesticides from agro wastewater which later were degraded using Fenton's reagent [33]. The relatively low critical temperature of CO_2 at 31.1 °C enables the extraction of thermally sensitive compounds such as enzymes, which would otherwise pose problems when traditional methods that require elevated temperatures are employed [29]. Many researchers reported the benefits of $scCO_2$ in presence of co-solvent for enzyme stability and solubility [34–36]. $ScCO_2$ rapidly pen-

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etrates and exits solid matrices, resulting in swift and efficient extractions compared with the use of organic solvents with higher viscosities [37–43].

Cano and Hernandez showed that enzyme activity increased until pressure of 350 MPa at room temperature (20 °C) [44]. Fricks et al. investigated the residual activity level of radish peroxidase after treatment using high pressure carbon dioxide in the supercritical region. Peroxidase activity was found to increase after treatment, with the maximum residual activity of around 215% attained at the experimental condition of 30 °C, 7 MPa for 1 h [45]. Comparable results were obtained when treatment was done at a higher pressure of 14.2 MPa (viz., a higher density) for 6 h which could be a more practical duration for actual enzymatic reactions [45]. These prior works show the importance of employing high-pressure environment in order to attain higher activity of peroxidase enzymes.

Currently, enzymes are employed either by immobilizing on costly substrates or by direct application; both routes render enzyme application a costly process for oxidation [46,47]. To overcome this deterrent, extraction of enzymes from waste bio-material such as discarded vegetables and plant materials or fruit peels is desirable. However, the low extraction efficiency of peroxidase from vegetables using conventional methods such as use of buffers makes the overall process impractical [46]. In this work, simultaneous extraction of peroxidase from plant matter and its application to remediate simulated wastewater streams via $scCO_2$ has been reported.

In this context, the objective of this work is to investigate the oxidation of ethanol and phenol in the presence of potato and cabbage which are employed as cheap and easily available sources of peroxidase. Ethanol and phenol are employed as the model compounds since they are fairly common organic contaminants in waste water. They have significantly different molecular structures, stability and therefore different extents of ease for oxidation. Ethanol represents a simple straight chain molecule which is easy to oxidize whereas phenol is an aromatic molecule and therefore difficult to oxidize. High concentrations (> 500 ppm) of phenol and ethanol in waters are extremely difficult and challenging to treat by using biological methods [11,48]. By deploying $scCO_2$ as the medium for extraction of the peroxidase, the simulated contaminants fortuitously played the role of co-solvent which enhanced the enzyme solubility significantly. Cabbage yielded higher oxidation compared to potato; the greater extent of oxidation of the organics using cabbage peroxidase compared to peroxidase from potato is due to different activities of peroxidase extracted from different sources. The work also presents the importance of $scCO_2$ as a medium of extraction of peroxidases from vegetables.

2. Materials, experimental procedure and mechanism

Ethanol (Fisher Scientific, 99.99% purity), phenol crystals (Alfa Aesar, 99%), hydrogen peroxide (Merck, 30% w/v), horseradish peroxidase (140U/mg, Sigma- Aldrich- Fluka analytical), acetonitrile (Tedia, >99.9% purity), purified carbon dioxide (Soxal, 99.8% purity with siphon), potato (Nadine potato (Solanum tuberosum), Sumich Australian potato), cabbage (white cabbage (Brassica oleracea var. capitata), Fairprice Beijing cabbage) were used as received.

2.1. Conventional oxidation

The solution (1 mol of C_2H_5OH to 3.7 mol of H_2O_2 or 1 mol of C_6H_5OH to 28 mol of H_2O_2) was placed in a 100 mL round-bottomed flask and brought to the desired reaction temperature while being stirred at 350 rpm (Fig. 1). These ratios are adopted as per the results reported by Yu et al. [49]. The enzyme source (5% potato or cabbage (w/v)) was added to the flask and the contents held at these conditions for 2 h (The highest oxidation of ethanol was achieved at 2 h and therefore all the experiments were performed for the same duration; Results of oxidation of ethanol and phenol at various temperature and duration are presented in supporting information). The flask was then cooled to room temperature using an ice pack and the contents were analyzed by GC-TCD and UV–vis spectroscopy. The self- oxidation of ethanol/phenol was also tested at various temperatures in the absence of H_2O_2. Adsorption of ethanol/phenol while using catalyst was also taken into account by carrying a control run in the absence of H_2O_2.

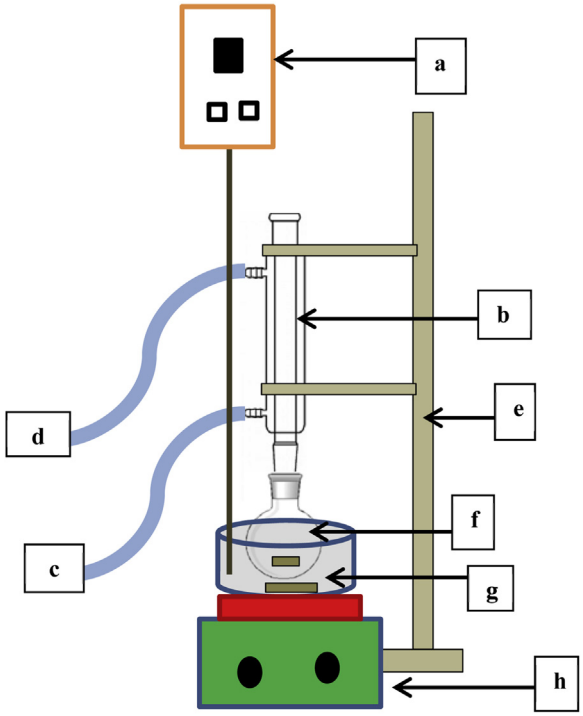

Fig. 1. Typical setup employed for conventional oxidation of ethanol and phenol. a) Temperature controller, b) condenser, c) condenser inlet: water from tap, d) condenser outlet: water to drainage, e) retort stand with clamps, f) round-bottomed flask with reactants and magnetic stirrer, g) silicone oil bath with magnetic pellet, h) heater.

2.2. Oxidation in $scCO_2$ medium

10 mL of reactant solution was loaded into the SS316 reactor (height- 10 cm and radius- 1.27 cm) (Fig. 2) with the desired amount of catalyst (5% potato or cabbage (w/v) and 0.1% pure HRP (w/v)). The reactor was purged with carbon dioxide for a predetermined time to displace air and then pressurized to 10 MPa at 25 °C, 40 °C or 60 °C for 2 h. Three different temperatures were employed in order to achieve different phases of CO_2; 25 °C corresponds to subcritical phase whereas 40 °C and 60 °C both are supercritical phase but with different properties. The reactor was depressurized subsequently while the two traps at the outlet ensured capture of volatile organics. While analyzing the final concentration, the amount present in traps was also taken into account. Self-oxidation of ethanol/phenol has been tested at various temperatures in absence of H_2O_2. Again, adsorption while using catalyst has been taken into account.

2.3. Analysis

Phenol samples were analyzed using UV–vis spectrometer (Shimadzu-3600) at 270 nm. The ethanol samples were analyzed using a Hewlett Packard 6890 G1530A Gas Chromatograph (GC).