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Use of the Electrically Driven Emulsion-Phase Contactor for a Biphasic Liquid–Liquid Enzyme System

The Oxidation of *p*-Cresol by Aqueous Phase-Horseradish Peroxidase† Scientific Note

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INTRODUCTION

One approach to the operation of bioprocessing systems within nonaqueous environments would require that the biocatalyst be chemically (*1*) or genetically (*2*) modified so it can be used directly in the organic phase. An alternative approach to this type of bioprocessing option would require the development of reaction systems that would provide effective interfacial contact between the biocatalyst, contained within an aqueous phase, and the organic phase containing the substrate. A biphasic liquid–liquid (BLL) bioreactor that provides for intimate liquid–liquid contact would be the most probable approach for this application. Dispersed-phase reaction systems have been investigated for many years for solvent

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extraction and other nonbiological applications. However, the production and control of two-phase dispersions are still primarily an empirical art with limited understanding of the fundamental behavior of the process. With addition of effects of potentially toxic organics and interfacial influences on biocatalysts, design of such systems becomes an even more formidable task.

For the BLL reactions considered in this work, the overall effectiveness of the system will depend on both compatibility of the biocatalyst with the chemical species present and intrinsic reaction and interfacial transport phenomena typically involved with liquid–liquid operations. The biocatalyst will almost exclusively reside in the aqueous phase or at the interface so that, potentially, four mechanisms of substrate and product transport become important:

1. Diffusion through the outer organic liquid film to the interface;
2. Adsorption and desorption processes at the interface;
3. Diffusion through an inner aqueous film; and
4. Diffusion through the bulk aqueous phase (*3*).

Once the substrate enters the biocatalyst phase, mass transfer and reaction occur simultaneously. The nature of the coupling of the reaction and transport mechanisms depends on the relative rates of the two processes.

The focus of this article is to investigate the removal and oxidation of *p*-cresol dissolved in toluene by aqueous-phase horseradish peroxidase. Contacting of the liquid–liquid biphasic enzyme system is carried out in an advanced solvent extraction contacting device, the electrically driven emulsion-phase contactor (EPC).

MATERIALS AND METHODS

Biphasic Enzyme System

An oxidative model system has been developed to study the reaction and interfacial transport phenomena of BLL systems. Horseradish peroxidase (HRP) (Sigma Chemical Co., St. Louis, MO) has been shown to catalyze the oxidation of a variety of substrates, especially phenols (*4*). The mechanisms of these types of reactions have been fairly well documented in the literature, and it has been determined that the enzyme accepts the two oxidizing equivalents of hydrogen peroxide and then transfers them to the hydrogen donor molecule (e.g., phenol) in separate one-step reactions (*5*). *P*-cresol has been chosen as the secondary donor substrate. The enzymatic oxidation of *p*-cresol generates phenoxy radicals, which react with other *p*-cresol molecules, forming water-insoluble polyaromatic products that precipitate out of aqueous solution, but may be quite soluble in the bulk organic phase. The immiscible bulk phase used in this study was toluene.

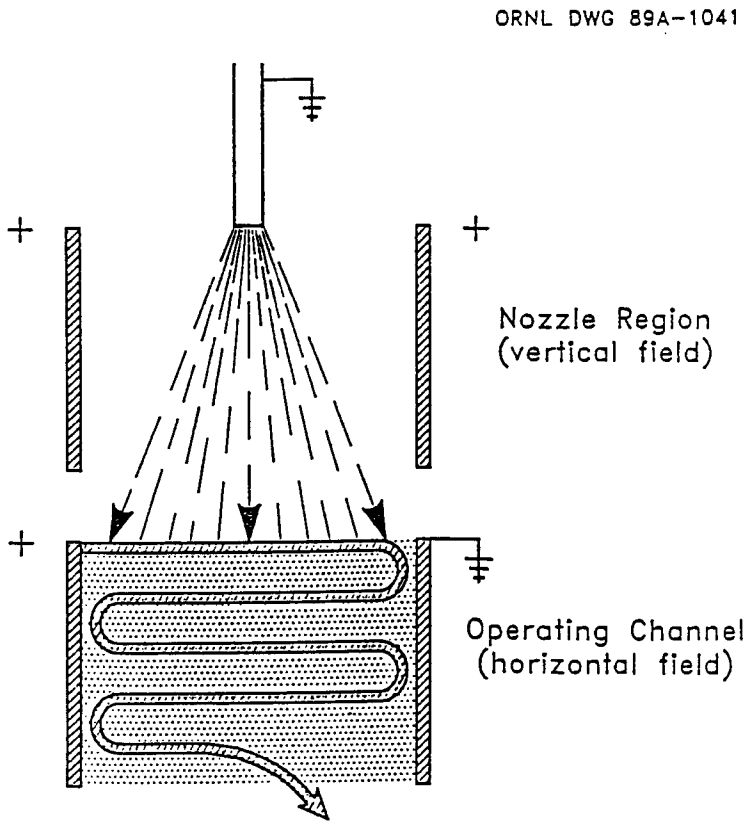

Fig. 1. The two-zoned approach to the emulsion-phase contactor. The upper zone disperses the aqueous phase directly out of the grounded nozzle using a charged electrode array, whereas the lower zone or operating channel uses a horizontal field to promote continuous coalescence and redispersion.

The aqueous phase used during experimentation contained 2 m*M* hydrogen peroxide, $1.21 \times 10^{-8}M$ (0.1 purpurogallin U/mL) HRP and was held at pH 9.0 by using a 0.1*M* borate buffer. The organic phase was 100 ppm ($9.4 \times 10^{-4}M$) *p*-cresol in toluene. *P*-cresol concentration was determined by gas chromatography (GC) using a J&W Scientific DB-5 column with a flame ionization detector. Samples from the toluene phase were directly injected into the GC, whereas *p*-cresol from aqueous samples were extracted by methylene chloride and injected into the GC.

Emulsion-Phase Contactor

Figure 1 is a schematic diagram depicting the two-zoned approach of the EPC. At the top of the contactor, a grounded nozzle is surrounded by a charged electrode array. This causes the aqueous drops to disperse into the organic phase in the general downward direction. As the dispersed aqueous phase reaches the lower region or operating channel, the droplets are subjected to an electric field that is more horizontal in nature. The

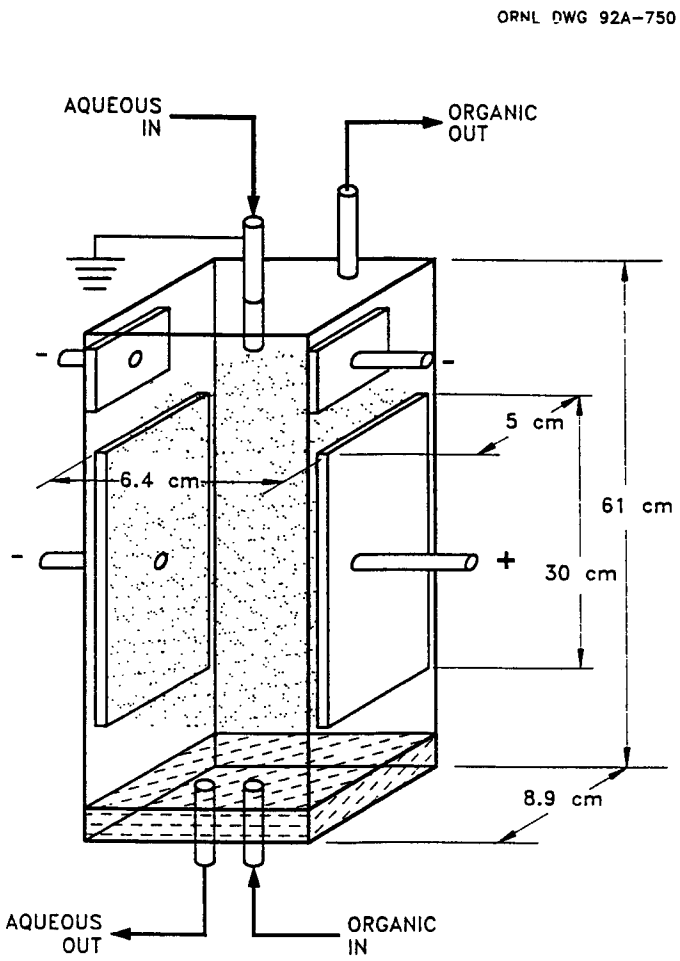

Fig. 2. Schematic diagram of EPC unit used in this study.

droplets continually redisperse and coalesce as they fall through the organic phase. The nozzle region serves to provide primarily an initial reduction in droplet size for entry into the operating channel where the majority of dispersed-phase holdup occurs. The high-intensity electric field creates aqueous droplets that are in the 5–50 μm range, and thus provides a vast amount of interfacial surface area for mass transport and reaction.

Figure 2 is a schematic diagram of the EPC used in this study. The vessel was machined out of Teflon™ to minimize wetting by aqueous solutions. External dimensions were 10 × 10 cm with a height of 61 cm. This device had a 6.4-cm electrode spacing and a 30-cm electrode length. The distance between the front and rear walls and the electrodes was 1.9 cm. Electrodes were stainless steel. The front and rear plates were clear Lexan™ with a clear Teflon™ FEP film spacer (0.05-cm thick) that allowed visual inspection of the flow patterns, and minimized wetting and charge

transport by the aqueous phase. A peristaltic pump (Masterflex) was used to meter the aqueous feed, whereas a gear pump (Cole Parmer Micropump) was used for organic flow. Further description of the apparatus and the high-voltage power supply are given in a previous publication (*6*).

Experimental Procedure

Aqueous enzyme solution and hydrogen peroxide solutions were delivered separately in a 1:1 ratio and mixed in a tee just before entry into the nozzle. The combined aqueous phase was pumped at flow rates from 2 to 5 mL/min to the top of the EPC through a grounded stainless-steel nozzle that was immersed in the bulk organic phase. The bulk phase was delivered separately to the bottom of the EPC at flow rates between 25 and 40 mL/min to yield aqueous/organic flow ratios of 1/20, 1/10, and 1/5.

A high-voltage pulsed electric field was applied to the electrode surrounding the nozzle and to the parallel plates in the operating channel of the EPC. Characteristic peak voltages were −27 kV in the nozzle region and ±27 kV in the operating channel. The pulse frequency was 414 Hz with a secondary 2.5 Hz modulation in the operating channel that yielded a 69% duty cycle. This field imposes intense stresses on the aqueous-phase fluid exiting the nozzle and disperses the aqueous-phase into micron-sized droplets that are then continually coalesced and redispersed as they fall against the upward countercurrent flow of the organic phase. At the bottom of the vessel, a final coalescence takes place, and the aqueous-phase is collected in a puddle that is continuously removed from the vessel. The organic phase is continuously removed through the top of the vessel through an overflow pipe. The EPC total volume is 2.2 L, so the organic phase residence time decreases from approx 1.5 to 0.9 h as the flow rate increases from 25 to 40 mL/min, whereas the aqueous phase is in the vessel for approx 5–10 min. The aqueous phase samples were quenched by addition to 0.1*N* HCl to stop any further enzymatic reaction outside the EPC.

RESULTS AND DISCUSSION

The system ran smoothly with continuous countercurrent flow of both phases and only intermittent electrical arcing primarily in the nozzle region. For the 1/20 phase ratio, electric dispersion of the aqueous phase was complete throughout the contactor. For the experiments with the 1/10 and 1/5 phase ratios, a vigorous dispersion was only observed in the lower third of the operating channel. This was believed to be the result of the increased aqueous phase flow through the system. Evidence for the reaction of *p*-cresol was present in both liquid phases for the cases in which enzyme was present. In the organic phase, several unidentified GC peaks appeared, whereas in the aqueous phase, a white precipitate was formed.

Table 1
Concentration of *p*-cresol in the Exiting Organic and Aqueous Phases at Various Aqueous/Organic Phase Flow Ratios

Aqueous/organic flow ratio	*P*-cresol concentration in aqueous phase, ppm	*P*-cresol concentration in organic phase, ppm	*P*-cresol expected in aqueous phase, ppm	Comments
1/20 (no enzyme)	11	100	N/A	*
1/20 (enzyme)	0	100	N/A	* *p*-cresol in aqueous phase apparently reacted
1/10 (no enzyme)	6	100	N/A	*
1/10 (enzyme)	3	95	50	94% of removed *p*-cresol reacted
1/5 (no enzyme)	2	100	N/A	*
1/5 (enzyme)	4	92	40	90% of removed *p*-cresol reacted

*Not enough removal from organic phase to measure.

Table 1 contains results for the steady-state exiting concentrations of *p*-cresol in both phases for aqueous/organic phase ratios of 1/20, 1/10, and 1/5. In each case where no enzyme was present, there was no evidence of a change in *p*-cresol concentration in the organic phase. This is to be expected since the distribution coefficient of *p*-cresol was measured to be 0.2 (A/O), thus indicating a preference for the toluene. Given the phase ratios used, one would expect minimal removal of *p*-cresol for mass transfer without enzyme reaction. The aqueous-phase concentrations for the no-enzyme runs also indicate that only minimal transfer of *p*-cresol had occurred.

For the experiments where HRP was included in the aqueous phase, there was evidence of significant removal of *p*-cresol from the toluene for the 1/10 and 1/5 phase ratios. The aqueous-phase *p*-cresol concentrations are small for all three phase ratios, indicating that enzymatic conversion had occurred in the aqueous phase. For example, if no enzymatic reaction were to occur, the amount of *p*-cresol removal from the organic phase would all reside in the aqueous phase. The 1/10 and 1/5 phase ratio experiments would have *p*-cresol concentrations of 50 ppm instead of 3 ppm, and 40 ppm instead of 4 ppm, respectively. This suggests that 90% or more of the *p*-cresol removed from the toluene in these three runs was converted to oxidized products. Based on organic flow rate and volume of active portion of the EPC, the 1/10 and 1/5 runs have both demonstrated *p*-cresol volumetric removal rates of 11 mg *p*-cresol/L/h. It was also evident that significant enzyme activity was retained on exit from the EPC,

since aqueous-phase samples that were allowed to sit for several hours without quenching by 0.1*N* HCl consistently showed no remaining *p*-cresol.

These preliminary experiments serve to provide a proof of principle for the use of the EPC in BLL enzyme systems. Apparently, the enzyme remains active during the electrified dispersion process that is used to contact the two liquid phases, and the enzymatic reaction process serves to enhance removal greatly. Further studies will be carried out in which extraction/reaction efficiencies will be measured as a function of aqueous/organic phase ratio and temperature in order to obtain more complete removal of the *p*-cresol. In addition, it will be necessary to develop more intricate analytical techniques to allow accurate measurement of both substrate and reaction products.

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