

# Comparison of Batch-Stirred and Electro-Spray Reactors for Biodesulfurization of Dibenzothiophene in Crude Oil and Hydrocarbon Feedstocks\*

ERIC N. KAUFMAN\*\*, JAMES B. HARKINS,  
AND ABHIJEET P. BOROLE

*Bioprocessing Research and Development Center, Chemical Technology  
Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6226*

Received September 16, 1997; Accepted February 23, 1998

## ABSTRACT

Biological removal of organic sulfur from petroleum feedstocks offers an attractive alternative to conventional thermochemical treatment, because of the mild operating conditions afforded by the biocatalyst. In order for biodesulfurization to realize commercial success, reactors must be designed that allow for sufficient liquid-liquid and gas-liquid mass transfer, while simultaneously reducing operating costs. Electro-spray bioreactors were investigated for use as desulfurization reactors because of their reported operational cost savings relative to mechanically agitated reactors. Unlike batch-stirred reactors, which mix the biocatalyst-containing aqueous phase with the organic feedstock by imparting momentum to the entire bulk solution, electro-spray reactors have the potential for tremendous cost savings, creating an emulsion <5  $\mu\text{m}$  in diameter, at a cost of only 3 W/L. Power law relationships indicate that mechanically stirred reactors would require 100–1000-fold more energy to create such a fine emulsion, but these relationships generally do not

\*The submitted manuscript has been authored by a contractor of the U.S. Government under contract DE-AC05-96OR22464. Accordingly, the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U.S. Government purposes.

\*\*Author to whom all correspondence and reprint requests should be addressed. E-mail: [ekn@ornl.gov](mailto:ekn@ornl.gov)

account for the effect of endogenously produced surfactant in the system. Here, the rates dibenzothiophene (DBT) oxidation to 2-hydroxybiphenyl (2-HBP) in hexadecane, by *Rhodococcus* sp IGTS8 are compared in the two reactor systems. Desulfurization rates ranged from 1.0 to 5.0 mg 2-HBP/(dry g cells · h), independent of the reactor employed. The batch-stirred reactor was capable of forming a very fine emulsion in the presence of the biocatalyst IGTS8, similar to that formed in the emulsion phase contactor (EPC™), presumably because the biocatalyst produces its own surfactant. Although EPC did not prove to be advantageous for the IGTS8 desulfurization system, it may prove advantageous for systems that do not produce surface-active bioagents, in addition to being mass-transport limited.

**Index Entries:** Oil desulfurization; *Rhodococcus*; electrostatic spraying; dibenzothiophene; biodesulfurization.

## INTRODUCTION

Biological refining of fossil fuel feedstocks offers an attractive alternative to conventional thermochemical treatment, because of the mild operating conditions and greater reaction specificity afforded by the nature of biocatalysis. Efforts in microbial screening and development have identified microorganisms capable of petroleum desulfurization (*see*, for example, ref. 1–12), denitrification (6), demetalization (6), cracking (6,13), and dewaxing. Further investigation and manipulation of enzymatic pathways responsible for these reactions (14–18) has led to processes that are approaching commercial application, particularly in the area of biological desulfurization (7,19). Biological desulfurization of petroleum may occur either oxidatively (*see*, for example, refs. 3,5,8,9,12,18,20–22) or reductively (2,23–25). In the oxidative approach, organic sulfur is converted to sulfate, and may be removed in process water. This route is attractive because it would not require further processing of the sulfur, and may be amenable for use at the wellhead, where process water may then be reinjected. In the reductive desulfurization scheme, organic sulfur is converted into hydrogen sulfide, which may then be catalytically converted into elemental sulfur; this is an approach of utility at the refinery. A sampling of desulfurization rates achieved with oxidative and reductive microorganisms has been summarized in ref. 26. Regardless of the mode of biodesulfurization, key factors affecting the economic viability of such processes are biocatalyst activity and cost, differential in product selling price, sale or disposal of co-products or wastes from the treatment process, and the capital and operating costs of unit operations in the treatment scheme.

The selection of the petroleum feedstock in biodesulfurization will play a large role in the overall economic viability of the process. Biodesulfurization may be utilized as a pretreatment to crude oil before entering pipelines, may be applied as an alternative to hydrotreating the crude at the refinery, or may be applied in the polishing of refinery products such as diesel or gasoline. The particular application will determine the extent of desulfurization necessary, and hence the treatment cost per barrel. At the wellhead, a biodesulfurization unit may be used to treat marginally sour crudes (0.6–0.7% S) converting them to sweet crudes (<0.5% S), and claiming the price differential in sweet sour crude in segregated pipeline systems (currently, the premium for sweet crude is ~\$1/bbl). For this application, the extent of desired desulfurization is quite low, and this may serve as an attractive initial niche for biodesulfurization. When utilized for refinery applications, the biodesulfurization process must compete with conventional hydrotreating. Here, the economic viability of biorefining will be based on its competitiveness relative to the catalyst replacement, hydrogen, and octane penalty costs associated with hydrotreating.

There is significant progress in the engineering and chemical modification of enzymes, so that they may function in purely organic solutions (27–29), but inherent to all of the current bioprocessing of fossil feedstocks schemes is the need to contact a biocatalyst-containing aqueous phase with an immiscible or partially miscible organic substrate. Factors such as liquid–liquid and gas–liquid mass transport, amenability for continuous operation and high throughput, capital and operating costs, as well as ability for biocatalyst recovery and emulsion breaking, are significant issues in the selection of a reactor for aqueous–organic contacting. Traditionally, impeller-based stirred reactors are utilized for such mixing, because of their ease of operation and wide acceptance in the chemical and biological processing industries. Such mechanically stirred reactors contact the aqueous and organic phases by imparting energy to the entire bulk solution, i.e., the impeller must move the contents of the reactor. Energy input in the stirred reactor is a function of the phase ratio, oil viscosity, density, reactor size, impeller speed, and so on (30). Typically, impeller-based reactors are capable of achieving water or oil droplet sizes of 100–300  $\mu\text{m}$  in diameter when surfactants are not present (26), and require on the order of 1–6 W/L to do so, based upon empirical correlation's (30). It is estimated that if impeller-based systems were capable of producing 5- $\mu\text{m}$  droplets, it would require ~25 kW/L (31) if surfactants are not present. Furthermore, no capacity exists for biocatalyst separation or emulsion breakage within the reactor.

Alternative processing schemes (19) propose the use of motionless mixers, in which the two phases are pumped over a reversing helical coil, which creates turbulent eddies. Although this method reduces the number

of moving parts in the reactor, it does not reduce power requirements, since costs are transferred from the impeller to the pumps required to move the liquids past the coil. Liquid velocities greater than 4 m/s are required to form emulsions ( $\sim 10\ \mu\text{m}$ ), and one cannot form emulsions in coil tubes greater than 3 mm in diameter (32). For tubes greater than 10 cm in diameter, one cannot form droplets smaller than 1 mm. Like the stirred reactor, no capacity exists for emulsion-breaking within the motionless mixer.

Recent advances in the area of contactors for solvent extraction have led to the development of electrically driven emulsion-phase contactors (EPC<sup>TM</sup>) for efficient contact of immiscible phases (33–38). In this concept, the differing electrical conductivity between the aqueous and organic phases causes electrical forces to be focused at the liquid–liquid interface, creating tremendous shear force (*see*, for example, ref. 39). This shear causes the conductive phase to be dispersed (5  $\mu\text{m}$  droplet size; ref. 31) into the nonconductive phase, but does so with decreased energy requirements relative to mechanical agitators because energy is imparted only at the liquid–liquid interface, and not the entire bulk solution. Electrostatic crude oil desalters have been operated for several years (40). More recently, devices based on the EPC have been used commercially to water-wash methyl tert-butyl ether feedstocks (with greater than 10-fold reduction in stage height (41), and for organic extraction from aqueous analytical samples (42). Energy consumption on a volumetric basis has been measured to be 2.4 W/L for a 30% tributyl phosphate/70% dodecane/distilled water system, which is four orders of magnitude less than mechanical systems creating as fine an emulsion (31,37).

The configuration of the EPC developed at the Oak Ridge National Laboratory is shown in Fig. 1, in which the contactor serves to disperse a liquid with a greater density than the continuous phase. The reactor employs two different types of electrode regions in order to increase liquid throughput. The first, termed the nozzle region, provides a high-capacity droplet dispersion by providing an electric field with a significant vertical component. This vertical field creates the dispersion at the nozzle entrance and accelerates it into the continuous phase. A second region termed the operating channel employs parallel plates carrying a modulated DC offset with high voltage spikes. This signal creates an oscillating horizontal electrical field which controls the residence time of the dispersed phase and serves to continuously coalesce and redisperse the droplets as they progress in a serpentine manner through the reactor. At the base of the reactor, an electrical field exists between the electrified central plate and the grounded aqueous phase, which accelerates the aqueous droplets to the organic–aqueous interface. In this manner, droplet coalescence and hence separation on the interface is enhanced. The EPC creates droplets of

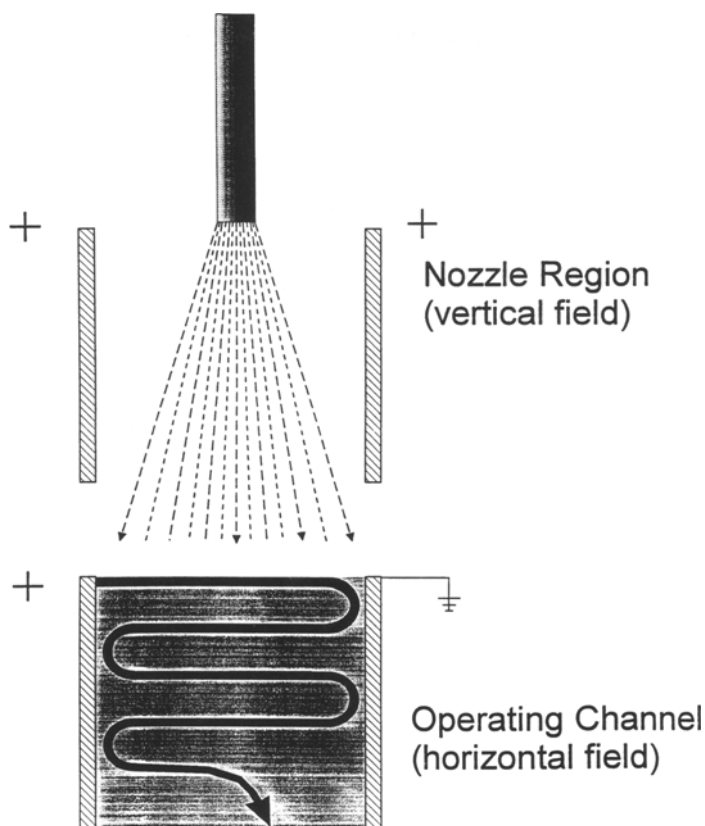
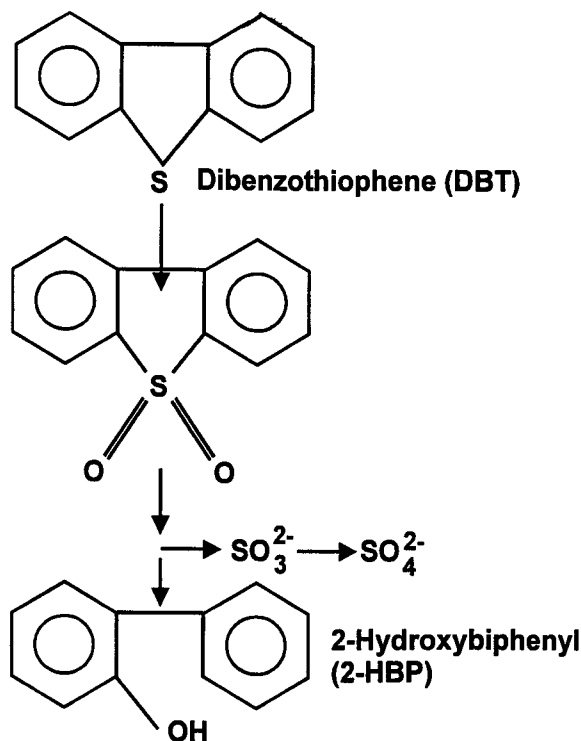


Fig. 1. Dual electrode region concept for the emulsion-phase contactor. The nozzle region disperses the aqueous phase into the organic, and the operating channel serves to control droplet residence time within the reactor. Adapted with permission from ref. 37.

water containing biocatalyst  $\sim 5 \mu\text{m}$  in diameter within an organic phase, and does so with a power requirement of  $3 \text{ W/L}$  (26).

With the success the EPC has exhibited in the area of solvent extraction, it was proposed that it could be an efficient reactor system for aqueous-oil contacting in biorefining (43). The authors' previous work (26) characterized the emulsion quality and power requirements of the EPC, and demonstrated that there was no detrimental effect on the cells caused by the electric fields. This article compares the performance of the EPC to that of a batch-stirred reactor (BSR), investigates the required level of biocatalyst activity before the surface area afforded by the EPC becomes a factor in reactor performance, and characterizes the emulsion formed by both reactors in the presence of bacteria.



### ***Rhodococcus* sp. strain IGTS8 Pathway**

Fig. 2. Biochemical pathway showing the sequence of DBT oxidation by IGTS8.

## **MATERIALS AND METHODS**

### **Biocatalyst and Solvent Systems**

The oxidation of dibenzothiophene (DBT) in hexadecane was studied to investigate reactor design and performance in an easily tractable chemical system. *Rhodococcus* sp wild strain IGTS8 (ATCC 53968) was provided by Energy BioSystems (Woodlands, TX) and served as the biocatalyst. The sequence of DBT oxidation by IGTS8 is shown in Fig. 2. (20), and detailed enzymatic steps in the pathway are discussed in ref. 18. Cells were supplied as a frozen paste, and had a cell dry wt of 0.28 g/g of original frozen material. The aqueous phase in all experiments consisted of 0.156 M, pH 7.5, potassium phosphate buffer. DBT (Aldrich, St. Louis, MO, D3,220-2), dissolved in *n*-hexadecane (Aldrich, H670-3), served as the organic phase, with typical initial DBT concentrations being 0.6 wt%.

## Analytical

Liquid samples collected from the reactors were centrifuged at 12,500g for 5 min, to separate the aqueous phase and cell debris. DBT and 2-hydroxybiphenyl (2-HBP) concentrations in *n*-hexadecane were measured by gas chromatography, using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. A 1- $\mu$ L sample was injected onto a 15-m DB-1 column (J&W Scientific, cat. no. 125-1012), which was used with a helium carrier flow of 10 mL/min. The temperature program was 150°C for 1 min, followed by an initial ramp rate of 5°C/min, up to 200°C, and a final ramp rate of 25°C/min, to a final temperature of 280°C. The column was calibrated with DBT and 2-HBP (Aldrich, no. 24,021-4) standards. The injector was operated at 250°C, and the detector was operated at 300°C. In the experiments reported here, DBT and 2-HBP concentrations in the aqueous phase were below levels of detection (6 ppm), hence, only concentrations in the organic phase are reported.

## Batch-Stirred Reactor Experiments

Experiments conducted in BSRs typically utilized 50 g frozen *Rhodococcus* sp wild-type strain IGTS8 (ATCC 53968) cell paste, which were brought up to 750 mL with 0.156 M (pH 7.5) phosphate buffer (1X cell density), and added to 250 mL 0.6 wt% DBT in *n*-hexadecane. The reactor vessel was a 1-L VirTis Omni-Culture fermentor (VirTis, Gardiner, NY, model 178657), utilizing a six-bladed Rushton-type impeller with two baffles. The impeller was mounted on the shaft, 1 cm from the aerator and 5 cm from the bottom of the vessel. The reactor was kept at 30°C, agitated at 800 RPM, and aerated with either room air or pure oxygen at a rate of either 0.2 or 1.0 SLPM. Specific aeration procedures are noted in the text. To collect samples, agitation and aeration were ceased for 5 min, to allow the aqueous and organic phases to separate. A 1.5-mL sample from the top of the organic phase was then taken for analysis.

Mass-transport experiments were conducted in the BSR to determine if the desulfurization process was liquid–liquid (l–l) or gas–liquid (g–l) mass-transfer-limited. The mass-transport experiments were conducted as above, except that aeration was performed using 6-mm stainless-steel tubing, rather than the aerator supplied by the manufacturer. Unlike the previous experiments, the mass-transport experiments were organic continuous while 250 mL aqueous phase was dispersed into 750 mL hexadecane containing 0.6 wt% DBT. Five batch-stirred reactors were run simultaneously, with different quantities of *Rhodococcus* sp wild-type strain IGTS8 (ATCC 53968) cell paste added to each reactor. Using a 250-mL basis for the aqueous phase, the quantities of frozen biocatalyst added

to the 0.156 M (pH 7.5) phosphate buffer in each reactor were 8.3, 16.7, 83.3, 166.7, and 250 g, respectively. The cell density corresponding to 16.7 g frozen biocatalyst in 250 mL aqueous phase was considered as 1X cell concentration. Thus, the cell density was varied from 0.5X to 15X in this experiment. To collect samples, agitation and aeration were stopped for 5 min, to allow the aqueous and organic phases to separate. A 1.5-mL sample was taken from the top of the organic phase in each reactor every hour for 10 h, and again at 24 h into the experiment.

Gas-liquid mass-transport experiments were conducted using air and oxygen at 0.2 and 1.0 SLPM. In order to determine whether or not an oxygen mass-transport limitation was a factor in the experiments using different cell densities, two batch-stirred reactors were run at 800 RPM, 30°C, containing 750 mL hexadecane with 0.6 wt% DBT, and 166.7 g biocatalyst (10X cell density) in 250 mL phosphate buffer. The aeration rate was 1.0 SLPM of room air for the control condition, and 1.0 SLPM pure oxygen in the experimental condition. Samples were collected every hour for 8 h, using the procedure described above.

## EPC Experiments

The design and operation of the EPC utilized in these experiments are adaptations of those described by Scott et al. (37). A schematic of EPC operation is shown in Fig. 3. The Teflon body of the EPC measured 10 × 10 × 61 cm. The front and rear plates were made of clear Lexan, allowing for visual inspection of reactor operation. A thin sheet of Teflon was placed between the body and the front and rear plates, to prevent wetting and current leakage to the Lexan. Three stainless steel electrodes, placed parallel to each other, measured 30 × 6 cm. The center electrode was charged; the two outer electrodes were grounded. This adaptation to the original EPC design was found to minimize biomass fouling of the reactor. The center electrode was connected to the high-voltage electric supply through a supporting steel rod, to avoid disturbance of electrostatic spraying in the nozzle region. High-voltage (up to 40 kV) was generated using a pulsed DC power supply and automobile ignition parts. A power supply (Hewlett Packard 6653A, Avondale, PA) and two sweep/function generators (BK Precision 3030, Chicago, IL) were used to produce the signal, which was then passed through an ignition coil (Mallory Promaster 29901, Carson City, NV), to step up the signal. In order to prevent discharge of the electrodes through the circuit, a high-voltage diode (Collmer Semiconductor CS4107X30, Dallas, TX) was placed between the coil and the EPC. From the diode, the positively charged terminal was connected to the rings surrounding the capillary tubes, and the negative lead was attached to the center electrode. The charged rings created an initial dis-



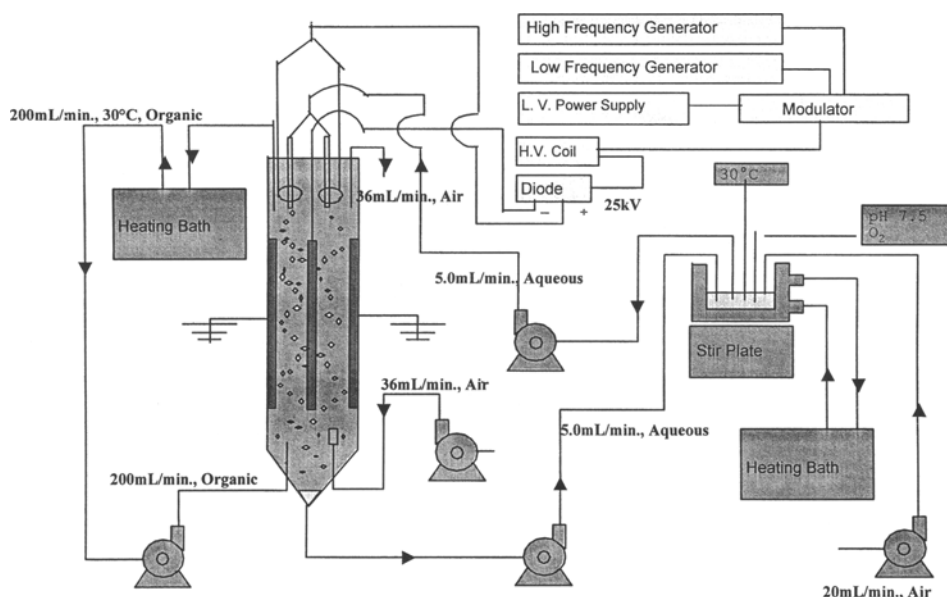


Fig. 3. Schematic of the emulsion-phase contactor and ancillary equipment.

persion of biocatalyst as the aqueous phase emerged from the capillary tubes. The parallel electrodes lower in the reactor enhanced droplet dispersion as the more dense aqueous phase descended to the bottom of the reactor.

In EPC experiments, the organic liquid served as the continuous phase into which an aqueous biocatalyst was dispersed. The organic phase consisted of 2400 mL *n*-hexadecane containing 0.6 wt% dibenzothiophene. The temperature of the organic phase was controlled at 30°C by pumping the liquid from the top of the EPC, through a stainless-steel coil submerged in a heated bath, and then returning the hexadecane to the bottom of the EPC. Typically, biocatalyst (26.7 g of frozen cell paste was brought to a volume of 100 mL with potassium phosphate buffer) was recirculated through the reactor at 5.0 mL/min, using a peristaltic pump. The cell density, with respect to the amount of aqueous phase used in these EPC experiments, corresponded to a 4X concentration. A higher cell density was used in the EPC compared to the BSR experiments, because of the lower aqueous:organic phase ratio in the EPC, and to enable DBT conversion significant enough to be observed by the analytical procedure employed. Aqueous phase containing the biocatalyst was sprayed into the reactor at the nozzle region, was continuously coalesced and redispersed in the operating region, and coalesced at the base of the EPC. To better aerate,

sample, and control the aqueous phase, it was circulated from the reactor base to an external reservoir at a rate of 5.0 mL/min. The external container allowed for temperature control, pH and O<sub>2</sub> measurement, agitation, and aeration of the biocatalyst. The liquid was then returned to the top of the reactor through two 1.6-mm ad, 1-mm id capillary tubes (U-140, Upchurch Scientific, Oak Harbor, WA), where it was again sprayed into the hexadecane. A water bath controlled the temperature of the jacketed aqueous reservoir at 30°C. The pH was monitored throughout the experiment, and remained in the pH range of 7.0–7.5. Agitation of the aqueous reservoir from a magnetic stirrer, and room air aeration through a diffuser at 20 mL/min, permitted more favorable conditions for the biocatalyst than did the long residence time at the bottom of the EPC, a location that lacked both aeration and agitation. In order to help alleviate possible oxygen deficiency inside the reactor, another diffuser was introduced 3 cm from the bottom of the EPC at airflow rate of 36 mL/min. Samples from the EPC were drawn hourly for 12 h through a 2 m × 3.175 mm Teflon tube placed 10 cm through the top of the reactor against a sidewall. Using a 30 mL glass-bodied syringe, the tubing was flushed with liquid from the EPC 3× before a 1.5 mL sample was drawn. Samples were centrifuged as described above. Because of the small amount of 2-HBP production in the EPC, and hence greater associated error in GC analysis, samples were run in triplicate.

In experiments to determine possible mass-transport limitations in the EPC, 66.7 g of frozen cell paste was brought to 100 mL with potassium phosphate buffer (10X cell density), and used as the aqueous phase. In order to evaluate if oxygen mass transfer was a limiting factor, the aqueous reservoir used for recirculating the aqueous phase was sparged with pure oxygen at a flow rate of 50 mL/min. This experiment was also conducted at a 10X cell density.

## RESULTS AND DISCUSSION

### 2-HBP Production in EPC and BSR

Desulfurization activity of the *Rhodococcus* sp in both reactors was typically between 1 and 5 mg 2-HBP produced/dry g of biocatalyst/h. The amount of 2-HBP released into the hexadecane was between 55 and 72% of the amount of DBT removed. Nonspecific DBT removal, e.g., by mere adsorption onto the cell surface, was negligible, because DBT loss during a control run in EPC reactor with no aeration was insignificant. Rates of 2-HBP production in the two reactor systems were within experimental variance, and no appreciable difference in desulfurization rates was seen between the two reactors. Note that in the experiments reported here, the

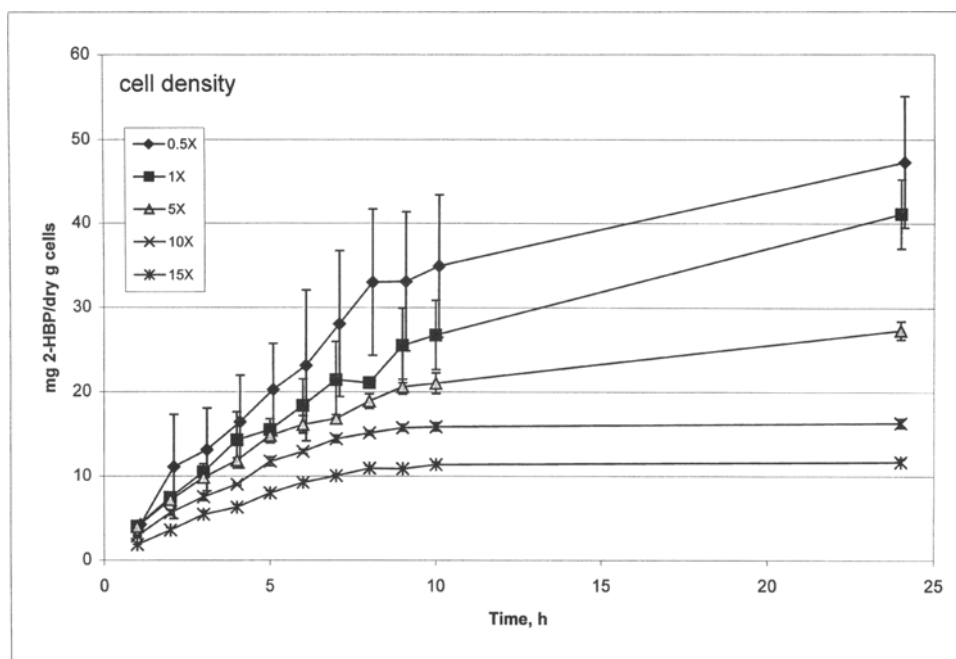


Fig. 4. Effect of cell density on 2-HBP production rate. The 0.5–15X refers to cell density and 1X corresponds to 16.7 g cell paste in 250 mL aqueous phase. As observed from this plot, mass transfer may be a limiting factor in the DBT desulfurization process.

only available carbon and energy source for the biocatalyst other than what may be carried over in the frozen cell paste, was hexadecane and DBT. Other studies (outlined in ref. 26) have utilized additional external carbon and energy sources, and have reported higher activities with *Rhodococcus* sp. Because of the high surface area reported in the EPC (26), higher rates were expected in the EPC; however, similar performance was observed in both reactors. Experiments were conducted with higher cell densities, to determine at what point the BSR becomes mass-transfer-limited and the high surface area afforded by the EPC would become beneficial.

### Mass-Transport Limitations

Results of the DBT desulfurization experiments conducted at varying cell densities in BSRs are given in Fig. 4. The rate of desulfurization, when normalized with respect to cell mass, was found to decrease with increasing cell density, indicating that mass-transfer resistance was the controlling process in desulfurization. A number of different approaches may be utilized in assessing these data, to determine when the system becomes

Table 1  
Results of Analysis of Variance (ANOVA) and *t*-Test (Assuming Unequal Variance) Comparing Desulfurization Rates Over a Range of Cell Densities.<sup>a</sup>

Time, h	ANOVA (p)	p (0.5 and 1)	p (1 and 5)	p (5 and 10)	p (0.5 and 5)
1	3.62E-10	0.2170	0.7420	0.0010	0.0739
2	4.53E-02	0.2150	0.0953	0.0177	0.1820
3	2.60E-03	0.0787	0.3380	0.0066	0.0600
4	5.40E-03	0.4706	0.3340	0.0006	0.0950
5	5.30E-03	0.3250	0.4600	0.0146	0.2190
6	3.89E-05	0.7440	0.3580	0.0370	0.1310
7	3.00E-03	0.0750	0.0384	0.0025	0.0390
8	2.00E-04	0.0328	0.0510	0.0190	0.0180
9	6.00E-05	0.0951	0.1984	0.0031	0.0110
10	1.18E-02	0.5820	0.1458	0.0066	0.1920
24	9.00E-07	0.1343	0.0301	0.0005	0.0110

<sup>a</sup> The second column gives p values for an analysis of variance over the complete range of data. The third through sixth columns gives p values for *t*-tests comparing two experiments at a time. Numbers in parentheses indicate the cell densities compared. The p values comparing the data at 5X and 10X cell densities are all below 0.05, showing a significant difference between the two sets. This suggests that the desulfurization process may be mass-transfer limited at 10X cell density with statistical certainty.

mass-transport limited. When analyzing the data time-point by time-point, as is demonstrated in Table 1 using the analysis of variance (ANOVA) test and the *t*-test, a significant difference in the rates of HBP production was observed between 5X and 10X cell density, based on a 95% confidence interval. If the slopes of the rate curves in Fig. 4 are compared, a significant difference is seen, even between the 0.5X and 1X data sets. By either method of analysis, it is suggested that the system is definitely mass-transport-limited at 10X cell density, at which further experiments were conducted. The mass-transfer limitation may be caused by gas-liquid or liquid-liquid mass-transport resistance.

The results of experiments conducted in the BSR at 10X cell density, to evaluate possible gas-liquid mass-transfer limitations, are given in Fig. 5. Increasing the rate of air supply, or increasing the oxygen tension in the reactor through the use of pure oxygen rather than air, was not seen to affect HBP production. Pure oxygen has been shown to be toxic to some bacterial cells, but because desulfurization rates were invariant with respect to both the rate of room air supply and oxygen tension, oxygen toxicity was not a factor in these experiments. Further, measurement of dissolved oxygen in the aqueous phase indicated that oxygen concentrations were >60% of saturation under all conditions, indicating that oxygen mass

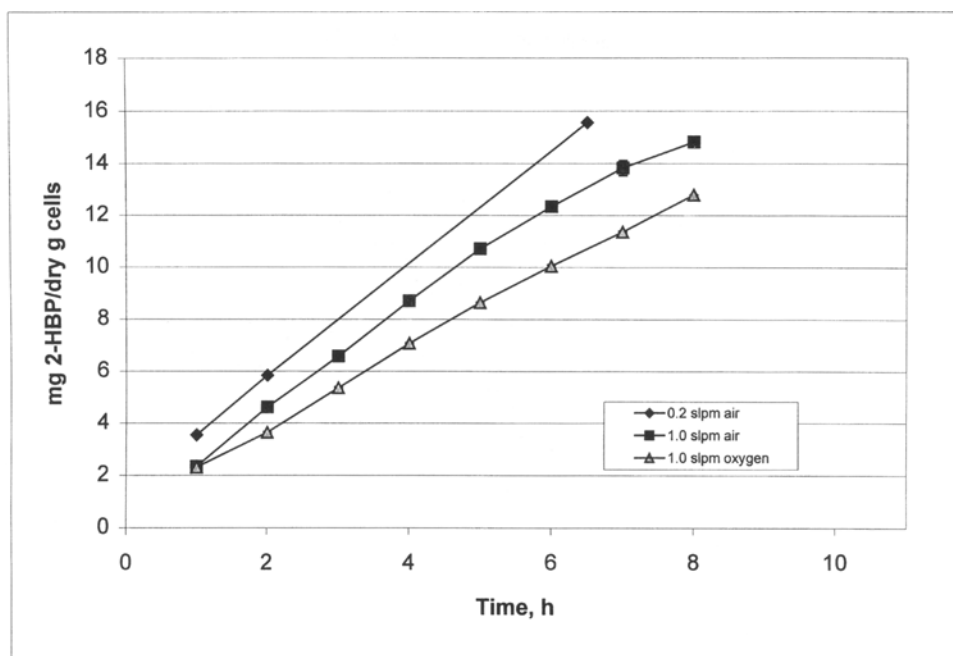


Fig. 5. Effect of aeration rate and oxygen tension on 2-HBP production in BSR. The experiment was conducted by dispersing 250 mL aqueous phase containing 166.7 g cell paste (10X cell density) in 750 mL hexadecane containing 0.6 wt% DBT.

transfer was not the limiting factor in the desulfurization of DBT, even at 10X cell density. This suggests that the system may be limited by liquid-liquid mass transfer. Since the EPC reportedly provides larger liquid-liquid interfacial area, the BSR was compared with the EPC for desulfurization activity at equal cell density.

### EPC vs BSR at 10X Cell Density

Comparison of the EPC and BSR at 10X cell density is given in Fig. 6. As shown, no difference was observed in the desulfurization rates between the two reactors. For this reason, the emulsion quality was evaluated to determine if indeed EPC provides a larger surface area for reaction under the present conditions.

### Emulsion Characterization

To determine whether the EPC offers larger surface area than BSR, samples were collected from the reactors and observed under a microscope using a  $\times 100$  oil-emersion objective. Because of the opaqueness ren-

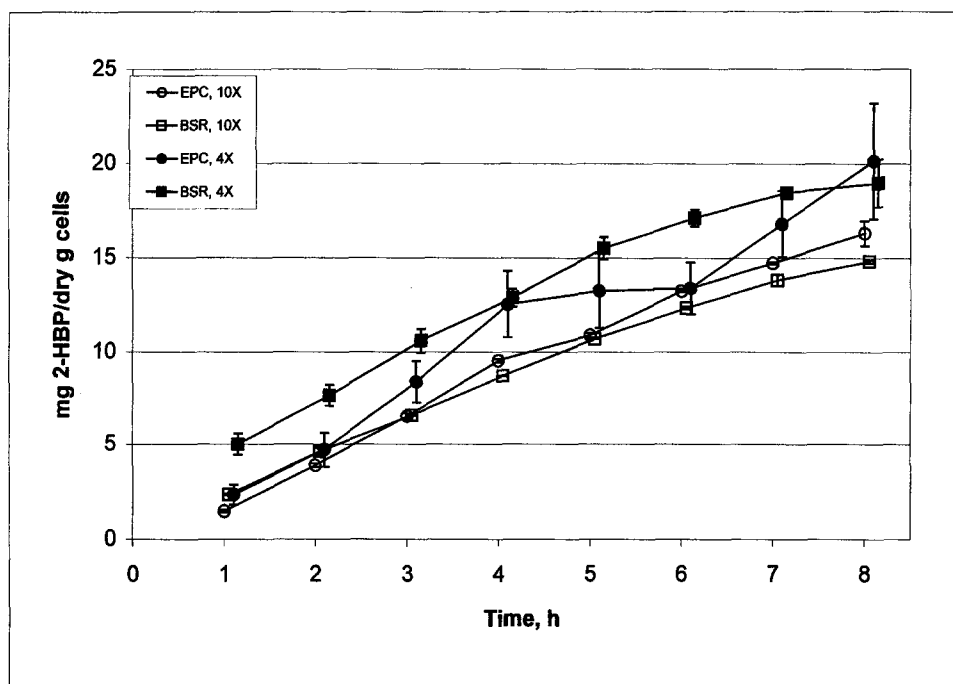


Fig. 6. Comparison of 2-HBP production rate in EPC and BSR. The BSR experiment was conducted by contacting 250 mL aqueous phase containing 166.7 g cell paste (10X cell density) in 750 mL hexadecane containing 0.6 wt % DBT. In the EPC experiment, 107 mL aqueous phase containing 66.7 g cell paste (10X) was contacted with 2400 mL organic phase.

dered by high cell densities employed, observations could not be made *in situ* during reactor operation. Samples collected during the run were immediately mounted on a slide and observed under a microscope. In addition to sampling conducted during a run, samples were also collected at the end of a run from a cuff layer formed after gravity settling of the aqueous and organic phases. The cuff layer (which was previously characterized as being 92.7% hexadecane) was observed to contain a major portion of the biocatalyst. In addition, a significant amount of the biocatalyst was extracted, and existed in the organic phase. Microscopic examination of samples from the cuff layer of the BSR showed a very fine emulsion, with droplet sizes ranging from 1 to 10  $\mu\text{m}$ . A similar cuff layer was also formed during EPC experiments. Average droplet size for EPC and BSR samples collected at 4 h during normal operation were  $2.54 \pm 2.40 \mu\text{m}$  and  $3.08 \pm 1.78 \mu\text{m}$ , respectively ( $n > 300$ ). Figure 7 shows a micrograph of the emulsion obtained in an EPC. Thus, a very fine emulsion is formed in the EPC as well as the BSR, and it appears that, for this reason, an augmentation in desulfurization rate is not seen in the EPC relative to the BSR.

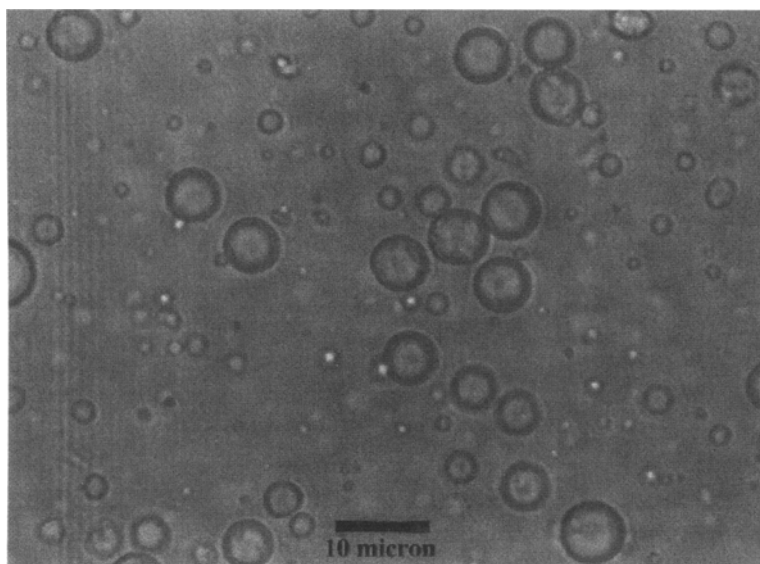


Fig. 7. Micrograph of an EPC sample collected at 4 h after beginning of reactor operation. The sample was collected from the central region between the electrodes, using a Teflon tubing connected to a syringe and observed under a microscope, as described earlier. The average droplet size was measured to be  $2.54 \pm 2.40 \mu\text{m}$  ( $n = 300$ ).

## MATERIALS AND METHODS

### EPC Experiments

Formation of such an emulsion in the BSR may be presumed, because of production of biosurfactants by the biocatalyst IGTS8. Other *Rhodococcus* sp have been previously reported to produce glycolipids in the presence of hexadecane, as well as some crude oils (44). Earlier characterization of droplet size in a BSR (26) was performed, either with no biocatalyst present or with an extremely small amount, to allow *in situ* observation of the droplets in the reactor. The authors' previous work revealed a decrease in droplet size from 210 to 118  $\mu\text{m}$  when adding just 5 g of cells in 750 mL aqueous phase. Thus, the emulsion characterization reported here (with 67 g of cells in 250 mL aqueous phase) is in no way contradictory to previous reports.

In total, the experiments presented in this work demonstrate that the biodesulfurization process is mass-transport-limited, either at the organic-aqueous interface or within the aqueous phase. Preliminary experiments varying the concentration of DBT 10-fold did not affect the rate of desulfurization, indicating that the aqueous-side liquid mass transfer may be controlling. This is consistent with the decreasing rate of desul-

furization with increasing cell density. Given the minimal solubility of DBT in the aqueous phase, the steady-state DBT concentration is believed to be negligible, and it may be speculated that the process is limited by the rate of surface renewal as new cells communicate with the organic-aqueous interface to acquire the DBT substrate.

## CONCLUSIONS

The performance of EPC was similar to BSR for desulfurization of a model system containing DBT in hexadecane treated with biocatalyst *Rhodococcus rhodochrous* IGTS8. The system was not limited by gas-liquid oxygen mass transfer at high cell densities (10X). The equal desulfurization rates in two reactors were the results of almost equal interfacial area and the high degree of cell extraction into the organic phase. Higher interfacial area than normally expected in mechanically stirred reactors were realized in BSR, presumably because of formation of surfactants by the biocatalyst present. Although EPC did not prove to be advantageous for the IGTS8 desulfurization system in terms of rates of desulfurization, it may prove advantageous for electrostatic emulsion breaking, in reducing power requirements for mixing, and for creating large amounts of surface area for systems that do not produce surface-active bioagents.

## ACKNOWLEDGMENTS

This work was supported by the Office of Oil and Gas Processing, U.S. Department of Energy, under contract DE-ACO5-96OR22464, with Lockheed Martin Energy Research Corp. The authors acknowledge the material contributions of Energy BioSystems Corp., and the technical assistance of Punjai Selvaraj and Costas Tsouris at the Oak Ridge National Laboratory.

## REFERENCES

1. Constanti, M., Giral, J., and Bordons, A. (1994), *World J. Microbiol. Biotechnol.* **10**, 510-516.
2. Finnerty, W. R. (1993), *Fuel* **72**, 1631-1634.
3. Kayser, K. J., Bielaga-Jones, B. A., Jackowski, K., Odusan, O., and Kilbane, J. J. (1993), *J. Gen. Microbiol.* **139**, 3123-3129.
4. Kim, T. S., Kim, H. Y., and Kim, B. H. (1990), *Biotechnol. Lett.* **12**, 757-760.
5. Lee, M. K., Senius, J. D., and Grossman, M. J. (1995), *Appl. Environ. Microbiol.* **61**, 4362-4366.
6. Lin, M. S., Premuzic, E. T., Yablon, J. H., and Zhou, W. M., (1996), *Appl. Biochem. Biotechnol.* **57/58**, 659-664.



7. Monticello, D. J. (1994), *Hydrocarbon Processing* **February**, 39–45.
8. Ohshiro, T., Hine, Y., and Izumi, Y. (1994), *FEMS Microbiol. Lett.* **118**, 341–344.
9. Ohshiro, T., Hirata, T., and Izumi, Y. (1995), *Appl. Microbiol. Biotechnol.* **44**, 249–252.
10. Shennan, J. L. (1996), *J. Chem. Tech. Biotechnol.* **67**, 109–123.
11. Sorokin, D. Y. (1993), *Microbiology (NY)*. **62**, 575–581.
12. Wang, P. and Krawiec, S. (1994), *Arch. Microbiol.* **161**, 266–271.
13. Premuzic, E. T., Lin, M. S., Jin, J. Z., Manowitz, B., and Racaniello, L. (1993). in *Biohydrometallurgical Technologies* Torma, A. E., Apel, M. L., and Briarley, C. L. eds. Minerals, Metals, and Materials Society, Warrendale, PA, pp. 401–413.
14. Denome, S. A., Oldfield, C., Nash, L. J., and Young, K. D. (1994), *J. Bacteriol.* **176**, 6707–6716.
15. Kim, T. S., Kim, H. Y., and Kim, B. H. (1990), *Biotechnol. Lett.* **12**, 757–760.
16. Oshiro, T., Kanbayashi, Y., Hine, Y., and Izumi, Y. (1995), *Biosci. Biotech. Biochem.* **59**, 1349–1351.
17. Vazquez-Duhalt, R., Westlake, D. W. S., and Fedorak, P. M. (1993), *Enzyme Microb. Technol.* **15**, 494–499.
18. Gray, K. A., Pogrebinsky, O. S., Mrachko, G. T., Xi, L., Monticello, D. J., and Squires, C. (1996), *Nature Biotechnol.* **14**, 1705–1709.
19. Dounias, G. A. and Stavropoulos, K. D. (1995), *Economic Feasibility of Biochemically Upgrading Heavy Crudes*. Brookhaven National Laboratory, Upton, NY.
20. Kilbane, J. J. and Bielaga, B. A. (1990), *Chemtech*. **December**, 747–751.
21. Olson, E. S., Stanley, D. C., and Gallagher, J. R. (1993), *Energy Fuels* **7**, 159–164.
22. Omori, T., Saiki, Y., Kasuga, K., and Kodama, T. (1995), *Biosci. Biotech. Biochem.* **59**, 1195–1198.
23. Kim, H. Y., Kim, T. S., and Kim, B. H. (1990), *Biotechnol. Lett.* **12**, 761–764.
24. Lizama, H. M., Wilkins, L. A., and Scott, T. C. (1995), *Biotechnol. Lett.* **17**, 113–116.
25. Kim, B. H., Kim, H. Y., Kim, T. S., and Park, D. H. (1995), *Fuel Processing Technol.* **43**, 87–94.
26. Kaufman, E. N., Harkins, J. B., Rodriguez, M., Selvaraj, P. T. Tsouris, C., and Murphy, S. E. (1997), *Fuel Processing Technol.* **52**, 127–144.
27. Almarsson, O., and Klibanov, A. M. (1996), *Biotechnol. Bioeng.* **49**, 87–92.
28. Scott, C. D., Scott, T. C., Blanch, H. W., Klibanov, A. M., and Russel, A. J. (1995), *Bioprocessing in Nonaqueous Media Critical Needs and Opportunities*. Oak Ridge National Laboratory, Oak Ridge, TN.
29. Woodward, C. A. and Kaufman, E. N. (1996), *Biotechnol. Bioeng.* **52**, 423–428.
30. Perry, R. H., Green, D. W., and Maloney, J. O., eds (1984), *Perry's Chemical Engineering Handbook*, 6th ed. 1984, McGraw-Hill, New York, p. 2336.
31. Scott, T. C. and Sisson, W. G. (1988), *Separation Sci. Technol.* **23**, 1541–1550.
32. Chemineer (1988), *Kenics Static Mixers KTEK Series*. North Andover, MA, p. 32.
33. Byers, C. H. and Ammi, A. (1995), *Chem. Eng. Prog.* **91**, 63.
34. Kowalski, W. and Ziolkowski, Z. (1981), *Int. Chem. Eng.* **21**, 323.
35. Martin, L., Vignet, P., Fombarlet, C., and Lancelot, F. (1983), *Separation Sci. Technol.* **18**, 1455.
36. Scott, T. C. and Wham, R. M. (1989), *Ind. Eng. Chem. Res.* **28**, 94.
37. Scott, T. C., DePaoli, D. W., and Sisson, W. G. (1994), *Ind. Eng. Chem. Res.* **33**, 1237–1244.
38. Thorton, J. D. (1968), *Rev. Pure Appl. Chem.* **18**, 197.
39. Ptasinski, K. J. and Kerkhof, P. J. (1992), *Separation Sci. Technol.* **27**, 995.
40. Warren, K. W., and Prestridge, F. L. (1988), in *National Petroleum Refiners Association Annual Meeting*. National Petroleum Refiners Association, San Antonio, TX, Report No. AM-88-78.

41. Schwartz, E., Rock, K., Byeseda, J. and Pehler, R. (1992), in *First Separations Division Topical Conference on Separations Technologies: New Developments and Opportunities*. American Institute of Chemical Engineers, New York, p. 236.
42. Wood, T. S., Cernohlavek, L. G., Grant, D. T., Kelly, K. P., Lochhaas, P. D., Maynard, A. E., et al. (1992), *First Separations Division Topical Conference on Separations Technologies: New Developments and Opportunities*, American Institute of Chemical Engineers, New York, p. 231.
43. Lizama, H. M., Scott, T. C., and Scott, C. D. (1995), *Apparatus and Method for the Desulfurization of Petroleum by Bacteria*. US Patent 5,458,752.
44. Finnerty, W. R., and Singer, M. E. (1984), *Dev. Microbiol.* **25**, 31–46.