2001

Two-phase partitioning bioreactors in fermentation technology

Janusz J. Malinowski

Available at: https://works.bepress.com/janusz_malinowski/9/
Research review paper

Two-phase partitioning bioreactors in fermentation technology

Janusz J. Malinowski

Polish Academy of Sciences, Institute of Chemical Engineering, ul. Baltycka 5, 44-100 Gliwice, Poland

Abstract

The two-phase partitioning bioreactor concept appears to have a great potential in enhancing the productivity of many bioprocesses. The proper selection of an organic solvent is the key to successful application of this approach in industrial practice. The integration of fermentation and a primary product separation step has a positive impact on the productivity of many fermentation processes. The controlled substrate delivery from the organic to the aqueous phase opens a new area of application of this strategy to biodegradation of xenobiotics. In this review, the most recent advances in the application of two-liquid phase partitioning bioreactors for product or substrate partitioning are discussed. Modeling and performance optimization studies related to those bioreactor systems are also reviewed. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Extractive fermentation; Liquid partitioning; Liquid extraction; Downstream separation; Xenobiotics biodegradation

1. Introduction

Fermentation processes are hampered by a variety of problems originating from the accumulation of products in the bioreactor. Integration of fermentation and a primary product separation step can accelerate the product formation, improve the product yield, and facilitate downstream processing. Among the different approaches to integrating reaction and product recovery steps in a biological system, the liquid–liquid extractive bioconversion process seems to have the greatest potential. From the early eighties, this processing strategy has been extensively exploited, mainly for recovery of low-molecular weight volatile products (ethanol and acetone–butanol) and organic acids (acetic, lactic, propionic, and butyric). The reviews by Daugulis (1994, 1997), van der Wielen and
Luyben (1992), Freeman et al. (1993), and Schügerl (2000) summarize much of the research on the extractive fermentation carried out to the mid-nineties.

Product removal can be performed within the bioreactor (in situ) or externally (ex situ), in a separate extraction column through which a fraction of the fermentation broth or cell-free medium is circulated. The study of Eiteman and Gainer (1989) on the extraction of 2,3-butanediol produced by Klebseilla oxytoca suggests that in the bioreactor combined with the external extraction column, the toxicity of the solvent towards cells is reduced to a large extent. So far, a majority of works in this field has been done for extractive fermentation with in situ product removal (e.g. see Daugulis et al., 1994; Gyamerah and Glover, 1996; Jones et al., 1993; Weilnhammer and Blass, 1994).

The concept of the two-phase partitioning bioreactor can be applied to controlled delivery of a toxic substrate dissolved in an organic phase to a cell-containing aqueous phase. This approach has been shown to be effective in the biodegradation of a xenobiotic like phenol (Collins and Daugulis, 1996, 1997a,b) or pentachlorophenol (Munro and Daugulis, 1996). As found by Munro and Daugulis (1996), the solvent’s high capacity for the toxic substrate and distribution coefficient providing subinhibitory aqueous substrate level is of primary concern. It is evident that biocompatibility and nonbiodegradability of the solvent are also essential features.

In this review, the most recent developments in two-phase partitioning bioreactors applied, either for the product or substrate partitioning, will be discussed. The focus is on cell, rather than enzyme, bioreactors because the concept of this type of reactor has been exploited more extensively for such systems. I will emphasize the use of immiscible organic solvents with aqueous systems, which allow molecules to be partitioned between two phases, excluding the two polymers aqueous systems discussed recently by Sinha et al. (2000). The liquid–liquid partitioning methods, including the various integrated production and recovery processes, for the separation of antibiotics and other secondary metabolites from fermentation broths, has been evaluated by Schügerl (1994, 2000) and Gu (2000).

2. Solvent selection considerations

The selection of a suitable extractant is of crucial importance for the development of an effective extractive fermentation process. The biocompatibility of the organic solvent is one of the most important characteristics for this processing strategy. It is widely accepted that the solvent-tolerance of microorganism can be, to some extent, correlated with the log \( P \) (the octanol/water partition coefficient) parameter (Bruce and Daugulis, 1991; Inoue and Horikoshi, 1991; Salter and Kell, 1995). It has been demonstrated experimentally that, in many situations, an appropriate mixture of biocompatible and toxic solvents yields an extractant with improved characteristics that is still biocompatible (Bruce and Daugulis, 1991).

A systematic approach to solvent screening presented by Kollerup and Daugulis (1986) made use of the calculation of multicomponent liquid–liquid equilibria by
UNIFAC and UNIQUAC models. The method has been successfully applied for solvent selection for liquid extraction in different situations, including extractive fermentation (Daugulis et al., 1994; Malinowski, 1999, 2000; Malinowski and Daugulis, 1993, 1994). As regards the separation of aliphatic alcohols or carboxylic acids, the reviews by Kertes and King (1986, 1987) additionally give an insight into an extraction chemistry of those chemicals.

Since the success in extractive fermentation depends exclusively on the properties of the extracting solvent, new ideas on how to overcome the toxicity of solvents possessing good extractive characteristics are welcome. Kapucu and Mehmetoğlu (1998) reported on the extractive ethanol fermentation by yeast cells immobilized in sodium alginate gel and decanol as a solvent. The special immobilization procedure was used to reduce the toxic effect of extractant on the yeast cells. They observed that the addition of sunflower oil and/or Al2O3 to the immobilization medium prevented cells from detrimental effect of the solvent. The best results have been obtained if 30% of sunflower oil and 5% of Al2O3 were added. In fact, this approach is similar to that used by Honda et al. (1986), who immobilized yeast cells within alginate gel with castor oil as a protector to prevent the toxic effect of o-isopropylphenol and o-tert-butylphenol. Kapucu and Mehmetoğlu (1998) did not investigate the reuse of immobilized beads and the regeneration of decanol-saturated beads with oil. However, in light of the results presented by Honda et al. (1986), the reusability of gel beads should not be a problem in this case.

It is also the case in developing a viable extractive fermentation system for organic acids production. Besides good extractive characteristics in terms of high distribution ratio and high capacity for the solute, the solvent has to work well in the range of pH in which only the undissociated form of acid is present (only undissociated acid form is extracted). Vandač et al. (1997) have recently reported the results of the systematic screening of extractants for the possible use in the production of butyric acid. Oleyl alcohol and Hostarex A327 (the equimolar mixture of tri-n-octyl and tri-n-decyl amines) in oleyl alcohol (20% w/w) appeared to be nontoxic to butyric acid-producing bacteria. However, due to the larger value of the distribution coefficient for the mixture of oleyl alcohol with Hostarex A327 (3.0) than pure oleyl alcohol (1.0), the former system can be recommended.

An interesting article (Marták et al., 1997) has recently focused on the toxicity of organic solvents used in the extractive fermentation of lactic acid by Rhizopus arrhizus. The fungal lactic acid fermentation has an advantage of giving only the l-form of the acid compared to a racemic mixture obtained in most bacterial fermentations. To date, most data concerning biocompatibility of extractant used in lactic acid fermentations have been obtained from observations of bacteria. Yet, as pointed out by Roffler et al. (1991), the solvent biocompatibility depends on whether yeast or bacteria are used in the process. Generalizations can hardly be done at this stage. The presented toxicity data show that solvents containing tertiary amine, Hostarex A327, or trioctylamine and secondary amine with isotridecanol (the best from the tested alcohols) as a modifier and trihexylphosphate, are suitable for in situ extraction. Oleyl alcohol, despite its biocompatibility, cannot be applied as a modifier since it is utilized by microorganisms.
3. Product partitioning

The main interest in refining the existing fermentation technology, especially at the downstream processing side, stems from potential large-scale biofuel production using renewable resources. The extractive fermentation approach has advantages over conventional fermentation techniques mainly due to the lower product recovery costs and reduced effluent treatment costs as a result of the use of a more concentrated feedstock (Daugulis et al., 1994). Extractive ethanol fermentation is among the most mature product partitioning technologies and the information of its precommercial stage of development has been made public already in the early nineties (Anonymous, 1993).

Gyamerah and Glover (1996) reported on ethanol fermentation by immobilized yeast cells with ex situ liquid extraction. This process configuration was selected to avoid the emulsion formation observed when an extracting solvent, n-dodecanol, was applied in situ. The authors confirmed the ability of this technique to use very concentrated feedstocks (up to 45.8% w/w) without by-products inhibition even when the glycerol concentration increased by a factor of six if the glucose feed concentration was increased from 10 to 45.8% w/w. An important advantage of this process from both economical and environmental aspects, was the reduction of aqueous effluent stream from the typical 12.5 m$^3$/m$^3$ of anhydrous ethanol produced, generated by a traditional cane molasses distillery, to 2.8 m$^3$ in this process.

The simultaneous saccharification and extractive fermentation process described by Moritz and Duff (1996) is an interesting example of the application of this approach to ethanol production from cellulosic substrates. The paper industry produces huge quantities of cellulosic waste materials and technologies, which use this feedstock to generate value-added products, are of considerable interest. The authors claim that ethanol productivity in a novel reactor configuration was up to 65% higher than in the conventional simultaneous saccharification and fermentation reactor. The environmental advantages of this processing concept stem from the reduction of waste material to be landfilled.

The acetone–butanol–ethanol (ABE) fermentation is another example of a process amenable to the concept of reduction of end-product inhibition, thus enhancing solvent productivity by in situ product removal. Ishizaki et al. (1999) have recently reported on the application of methylated crude palm olive as extractant in batch ABE fermentation. Applying this extractant, about 47% of the total butanol produced was extracted and butanol production increased from 15.9 kg m$^{-3}$ obtained in the conventional fermentation to 20.9 kg m$^{-3}$ in the extractive mode. Methylated crude palm olive has less favorable extraction characteristics compared to oleyl alcohol widely used on laboratory scale in situ extractive ABE fermentation in the past. However, its low price and availability, especially in the palm oil-producing countries, makes the extractive bioconversion process with this extractant very promising. Moreover, these extractants can be used directly as a fuel in agricultural machineries (Isigigur et al., 1993), thus eliminating the need of the solvent recovery step. Promising results obtained by the authors in the application of palm oil mill effluents as a substrate for ABE fermentation make possible biodiesel production in integrated systems with the aforementioned extraction process. This concept, when efficiently developed, could provide a source of potentially inexpensive liquid fuel in developing countries.
Carboxylic acids are the commodity chemicals with several possible uses in different industries. Currently, most of them are manufactured by chemical synthesis. However, there is increasing interest in the production of these acids by fermentative route utilizing inexpensive and renewable substrates. As in many bioconversion processes, low productivity, low final concentration of products in the broths, and difficult product isolation make such production not competitive with the synthetic route. The effective separation of the inhibition product simultaneously with its enhanced production by the use of the two-phase partitioning bioreactor concept can be one of the possible ways to improve economy of the whole biotechnological process. Marták et al. (1997) and Vandaık et al. (1997) have presented recent results of comprehensive studies of extractant selection strategy for lactic and butyric acids fermentations.

Citric acid is another important organic acid with a wide range of applications. Wieczorek and Brauer (1998a,b) tested a process scheme consisting of the fermentation stage integrated with the product recovery by in situ liquid extraction in a spray column using tridodecylamine in kerosene with octanol as modifier. The continuous fermentation with recirculation of the fermentation broth after acid recovery was characterized by a long-time operation (66 days) with the productivity (up to 55th day) as good as this observed in five consecutive batchwise fermentations. However, the continuous fermentation process resulted in considerable (up to 32%) reduction in the volume of waste; thus, it may have a positive impact on environment protection.

Efforts have also been made towards applying the products partitioning concept to propionic acid fermentation (Gu et al., 1998, 1999; Jin and Yang, 1998). Hollow-fiber membrane extractors were used to prevent direct contact between the organic and the aqueous phases. The solvents used in those studies were ditridecylamine (Jin and Yang, 1998) and trilaurylamine (Gu et al., 1999) diluted in oleyl alcohol.

Application of the extractive mode resulted in a fivefold increase in productivity ($\sim 1$ kg m$^{-3}$ h$^{-1}$) and more than 20% increase in the propionate yield (0.66 kg kg$^{-1}$) in comparison with the conventional batch fermentation (Jin and Yang, 1998). The performance of this process has been demonstrated in the continuous operation for more than 1.5 months. It is evident however, that the use of the immobilized cells (Gu et al., 1999) instead of free cells could be advantageous for large-scale operation. Gu et al. (1999) evaluated the process of 47 million kg annual production scale with a mixer–settler extractor in place of the hollow fiber modules. Their analysis demonstrates that even with favorable assumptions, the process reaches break-even point for the current acid values. When credits are included for acetic acid, the fermentation process reaches marginal profitability. The use of low-cost substrates could improve further the process economy. Additionally, the use as substrate of the waste materials like corn steep liquor, sulfite waste liquor, or whey-based material can subsequently reduce the waste treatment cost.

In Table 1, the performance of two-phase partitioning bioreactors in selected fermentation processes is summarized.
achieved by continuous conversion of the product extracted into organic solvent, to one of its derivatives (Schügerl, 2000). In this light, it seems worth presenting the examples of such process configuration, although extractive biocatalysis is beyond the scope of this review.

An interesting application of the two-phase partitioning bioreactor concept to extractive biocatalysis has been recently presented by Oliveira et al. (1997, 1998, 2001). They used an integrated approach involving extractive alcoholic fermentation coupled with enzymatic esterification of ethanol. Oleic acid was used as extractant and substrate for the esterification reaction catalyzed by a free or immobilized lipase from Rhizomucor miehei. The process feasibility was tested in the fermentation of high concentrated glucose feeds, up to 400 kg m$^{-3}$. The addition of lipase to the extractive fermentation system led to almost complete glucose consumption and lower ethanol concentration in the aqueous phase as a result of its esterification with oleic acid. Similar results were obtained for the system with free and immobilized lipase. The latter can be advantageous due to possibility of repeated use of the biocatalyst in such a form. More data concerning the enzymatic side of this process (not provided by the authors) would be helpful for its technical–economic assessment for the production of esters using the high-glucose concentration substrates.

Kiss et al. (1999) recently tested a similar approach using coimmobilized yeast cells and lipase. In this work, the fermentation culture media including glucose were inside the gel beads only. These beads were suspended in oleic acid that functioned in the same way as in

<table>
<thead>
<tr>
<th>Process</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol from cellulosic substrates (Moritz and Duff, 1996)</td>
<td>simultaneous saccharification and extraction in fed-batch mode; 48% higher productivity than in conventional process</td>
</tr>
<tr>
<td>Propionic acid from lactose (Jin and Yang, 1998)</td>
<td>membrane extractor used; productivity, final product concentration, product purity—1 g L$^{-1}$ h$^{-1}$, 75 g L$^{-1}$, 90%, respectively, comparing to 0.2 g L$^{-1}$ h$^{-1}$, 18.5 g L$^{-1}$, 71%, obtained in conventional batch fermentation</td>
</tr>
<tr>
<td>Propionic acid from glucose (Gu et al., 1999)</td>
<td>continuous process with immobilized cells; propionic acid yield was doubled in extractive mode (0.43 g g$^{-1}$); productivity 0.46 g L$^{-1}$ h$^{-1}$ comparing to 0.32–0.42 g L$^{-1}$ h$^{-1}$ in nonextractive mode</td>
</tr>
<tr>
<td>ABE (Ishizaki et al., 1999)</td>
<td>butanol overall productivity: 0.55 g L$^{-1}$ h$^{-1}$; butanol increase by 32% from 15.9 g L$^{-1}$ (conventional) to 20.9 g L$^{-1}$ (extractive); yield increases from 38% to 40%</td>
</tr>
<tr>
<td>Citric acid (Wieczorek and Brauer, 1998a,b)</td>
<td>continuous fermentation with recirculation of the broth after product extraction; mass of citric acid produced over 65 days of operation comparable to that obtained in five consecutive batchwise fermentation, considerable reduction in the waste stream</td>
</tr>
</tbody>
</table>
the work of Oliveira et al. (1997, 1998). The inhibitory effect of ethanol was reduced by its esterification by lipase to ethyl oleate. According to the presented results, the maximum ethanol production, 50 kg m\(^{-3}\), can be achieved for glucose concentration of 100 kg m\(^{-3}\) and lipase content of 4 U mL\(^{-1}\). However, the data are too scarce to speculate on the possibility to apply this process beyond the laboratory scale.

4. Substrate partitioning

The concept of two-phase partitioning bioreactor can be also applied to the controlled delivery of toxic substrates. In such a two-phase aqueous–organic system, the substrate is solubilized in the immiscible organic phase and allowed to transfer into the organic phase. The microorganisms degrade or transform the substrate at the aqueous/organic interface and/or in the aqueous phase. Thus, the substrate concentration in the biotic phase can be maintained below the inhibitory level. The partition process itself is controlled to some extent by the metabolic activity of microorganisms. The system is well suited for biodegradation of hazardous pollutants.

Gardin et al. (1999) have recently demonstrated its application to biodegradation of a mixture (70/30% wt./wt.) of xylene isomers and butyl acetate originated from the paint industry. When present in low concentrations, below 0.05% wt./vol., they could be degraded. The microbial consortium, composed of yeasts and bacteria, completely degraded 10 kg m\(^{-3}\) of the mixture in 96 h. Silicon oil was selected as an organic phase reservoir for the toxic substrate. The biodegradation process resulted in the production of acidic compounds and consequently in the fall of the pH value from 7.0 to 2.5–3.0. The experiments proved that periodical adjustments of pH to 6.0 led to a better degradation efficiency. The specific degradation rates were 14, 53, and 65 mg L\(^{-1}\) h\(^{-1}\) for \(m\)-xylene, butyl acetate, and \(p\)-xylene, respectively. The global rate of the xylene isomers and butyl acetate degradation reached 63 mg L\(^{-1}\) h\(^{-1}\). The authors claimed that the rates obtained were much larger than those reported for the one-phase systems with very low concentration of xenobiotics.

A two-phase bioreactor system has been thoroughly examined for the bioremediation of benzene, toluene, and \(p\)-xylene by Collins and Daugulis (1999a,b,c). An industrial grade of oleyl alcohol, Aldol 85NF (Sherex Chemical, Dublin, OH) was used throughout the experiments. This solvent has been shown previously to be effective in the extractive ethanol fermentation (Malinowski and Daugulis, 1993; Daugulis et al., 1994). The microorganism used was *Pseudomonas* sp. ATCC 55595, which is able to degrade these xenobiotics simultaneously. Moreover, benzene degradation by this strain appeared to be induced by the presence of toluene while \(p\)-xylene was codegraded with toluene. Thus, the higher concentration of toluene than of other compounds was required in the bioreactor system. The 2-L volume bioreactor employed a 1-L cell containing aqueous phase, and a 0.5-L organic phase, which partitioned xenobiotics into the aqueous phase. In the experiments, the organic phase was loaded with 10.15 g toluene, and either 2.0 g benzene or 2.1 g \(p\)-xylene. The simultaneous fermentation of toluene and benzene resulted in degradation rates of 67 and 24 mg L\(^{-1}\) h\(^{-1}\) for the compounds involved, respectively. In the case of the simultaneous
degradation of toluene and \( p \)-xylene, the rates of, respectively, 66 and 18 mg L\(^{-1}\) h\(^{-1}\), were achieved. Further improvement of the degradation rates for all three xenobiotics was achieved through the application of a sequential feeding strategy: toluene was first added to the organic phase whereas benzene or \( p \)-xylene was added only when half of the initial toluene had been consumed. The observed degradation rates for toluene and benzene were 79 and 56 mg L\(^{-1}\) h\(^{-1}\), respectively. In the toluene/\( p \)-xylene sequential biodegradation, the degradation rates were 74 and 25 mg L\(^{-1}\) h\(^{-1}\), respectively. However, the microbial activity was oxygen-limited during the rapid growth phase in these conditions. The aeration/agitation conditions were not too aggressive and the system operated as two distinct phases. The oxygen limitation was not observed in the completely dispersed system as in the work of Gardin et al. (1999).

Examination of potentials of the two-phase partitioning reactor scheme to mineralize simultaneously the mixture of the three aforementioned xenobiotics was a natural extension of the two-xenobiotic degradation study described above. The results of such a study have been reported recently (Collins and Daugulis, 1999c). The bioreactor system was able to biodegrade a mixture of 2 g of benzene, 10.15 g of toluene, and 2.1 g of \( p \)-xylene within 144 h. The supply of enriched air eliminated the oxygen limitation observed previously and reduced the fermentation time to 108 h. The two-phase partitioning bioreactor system used in this process required minimal operational control of pH and temperature. The delivery of substrates to the microbes containing an aqueous phase was controlled by the metabolic activity of bacteria. Finally, this concept proved viable in the laboratory-scale bioremediation of the contaminated soil. In this experiment, the organic solvent was used to recover the ‘spilled’ xenobiotics from the moist sand contaminated with 1.75 mg of benzene, 8.89 mg of toluene, and 1.84 mg of \( p \)-xylene per gram of soil. The Aldol loaded with toxic compounds was then transferred to the two-phase bioreactor where the biodegradation took place. After complete degradation, the organic phase would be reused to recover contaminants from the sand once again and the biodegradation procedure was repeated. The total recovery of each compound was about 99% both times. Ultimately, the 2-L bioreactor system degraded 4 g of benzene, 20.2 g of toluene, and 4.2 g of \( p \)-xylene within 144 h. The results obtained represent an unprecedented level of biodegradation of this toxic mixture.

In Table 2, the performance of the two-phase partitioning bioreactors compared with the conventional systems used for biodegradation of toxic organic compounds is presented.

5. Modeling and optimization

Modeling and optimization issues were addressed in a few recent publications (Arnold et al., 1996; Cruickshank et al., 2000a,b; Sajc and Vunjak-Novakovic, 2000). The reports deal mostly with conventional stirred tank reactors operated continuously, fed-batch, or batch, except for the study of Sajc and Vunjak-Novakovic (2000) on the extractive bioconversion in an external-loop airlift bioreactor.

Arnold et al. (1996) proposed a dynamic model for a continuous extractive ethanol fermentation process, which can be used for both the process simulation and determination of optimal operating procedures. The changeover from the conventional fermentation to the
extractive mode, and the initial stage of the latter process are of particular interest to optimization, due to the most pronounced system dynamics occurring at that time. The optimal profiles of the feed substrate concentration, the solvent and feed flow dilution rates have been identified using a Dynamic Programming method. These optimum profiles can be used as setpoints for feedback controllers and for the process control design.

A similar methodology has been used recently for the modeling and optimization of two-phase partitioning bioreactors for the biodegradation of phenol (Cruickshank et al., 2000a,b). Fed-batch and continuous modes of operation have been analyzed. The first study was aimed at the determination of the most favorable fed-batch feeding strategies for consuming the maximum amount of xenobiotics in the shortest period of time. The model predictions were in good agreement with the available experimental data (Collins and Daugulis, 1997a). The feeding strategy optimization have predicted optimal performance of the bioreactor by using a

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Performance of TPPB</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>batch mode: 80 mg L(^{-1}) h(^{-1})</td>
<td>batch mode: 37.5 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1996)</td>
<td>(Fujita et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>fed-batch with sequential feeding: 167 mg L(^{-1}) h(^{-1})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1997a)</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>batch mode: 79 mg L(^{-1}) h(^{-1})</td>
<td>batch mode: 2 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1999a)</td>
<td>(Alvarez and Vogel, 1991)</td>
</tr>
<tr>
<td>Toluene and benzene</td>
<td>batch mode: toluene: 67 mg L(^{-1}) h(^{-1})</td>
<td>batch mode: toluene and benzene: 2 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>benzene: 24 mg L(^{-1}) h(^{-1})</td>
<td>(Alvarez and Vogel, 1991)</td>
</tr>
<tr>
<td></td>
<td>fed-batch with sequential feeding: toluene: 79 mg L(^{-1}) h(^{-1})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>benzene: 56 mg L(^{-1}) h(^{-1})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1999b)</td>
<td></td>
</tr>
<tr>
<td>Toluene and (p)-xylene</td>
<td>batch mode: toluene: 66 mg L(^{-1}) h(^{-1})</td>
<td>batch mode:</td>
</tr>
<tr>
<td></td>
<td>(p)-xylene: 18 mg L(^{-1}) h(^{-1})</td>
<td>toluene: 1 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>fed-batch with sequential feeding: toluene: 74 mg L(^{-1}) h(^{-1})</td>
<td>(p)-xylene: 0.5 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(p)-xylene: 25 mg L(^{-1}) h(^{-1})</td>
<td>(Alvarez and Vogel, 1991)</td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1999b)</td>
<td></td>
</tr>
<tr>
<td>Toluene, benzene, and (p)-xylene</td>
<td>batch mode, oxygen supplemented: toluene: 135 mg L(^{-1}) h(^{-1})</td>
<td>batch mode:</td>
</tr>
<tr>
<td></td>
<td>benzene: 42 mg L(^{-1}) h(^{-1})</td>
<td>toluene: 0.5 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(p)-xylene: 35 mg L(^{-1}) h(^{-1})</td>
<td>benzene: 0.4 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(Collins and Daugulis, 1999c)</td>
<td>(p)-xylene: 0.1 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td>(o)-Xylene, (m)-xylene, and butyl acetate</td>
<td>batch mode: (o)-xylene: 65 mg L(^{-1}) h(^{-1})</td>
<td>batch mode: butyl acetate: 0.04 mg L(^{-1}) h(^{-1})</td>
</tr>
<tr>
<td></td>
<td>(m)-xylene: 14 mg L(^{-1}) h(^{-1})</td>
<td>(Alvarez and Vogel, 1991)</td>
</tr>
<tr>
<td></td>
<td>butyl acetate: 53 mg L(^{-1}) h(^{-1})</td>
<td>(Vandenbergh, 1988)</td>
</tr>
<tr>
<td></td>
<td>(Gardin et al., 1999)</td>
<td></td>
</tr>
</tbody>
</table>
5-h feeding interval with a smooth ramp-up in the concentration of phenol added to the system followed by constant addition to the organic phase. The authors claim that such feeding policy should provide substantial improvements in the amount of phenol degraded compared to the non-optimized heuristic approach. In the second report, the possibility of phenol degradation in a continuous mode was explored. A mechanistic model was proposed and the preliminary investigations using both steady-state and dynamic simulations were performed. To date, the continuous operation of such a bioreactor has not yet been examined experimentally. However, the examination of results led to guidelines for a continuous operation of this system in the future.

Sajc and Vunjak-Novakovic (2000) developed a simple mathematical model of co-current liquid–liquid extraction in the riser of an external-loop airlift bioreactor. This bioreactor system, integrating in a single-unit biosynthesis and separation, was used for the production of anthraquinones by immobilized plant cells of *Frangula alnus*. The effect of flow conditions, solvent properties, solvent droplet size, and contactor length on the product extraction efficiency and product concentration profiles in the continuous aqueous phase and dispersed organic phase (silicon oil and n-hexadecane) are studied.

Mass transfer between aqueous and organic phases is an important parameter in extractive fermentation. A comprehensive overview of the mechanisms of mass transfer during extraction process can be found in the book by Schügerl (1994). One of the well-recognized rationale of application of two-phase partitioning bioreactors in integrated production and separation is the expected enhancement of mass transfer rates (Sajc et al., 2000). Besides, such bioreactor concept when applied to biotreatment has the potential of controlling the mass transfer rates and the aqueous phase concentration of toxic substrates.

Considering the extraction process with biological systems, it is known that the rate of mass transfer is strongly influenced by the presence of microbial cells, cell debris, proteins, lipids, and other active components. The simple mechanisms involve blockage of the interfacial area available for mass transfer due to cell’s absorption at the liquid–liquid interface, increase of the interfacial rigidity through absorption of interfacially active components of the fermentation broth, etc. Crabbe et al. (1986) noted the substantial reduction in the rate of ethanol extraction from the fermentation broth compared with the pure system due to the absorption of yeast cells at the liquid–liquid interface. Similarly, Eldridge et al. (1989) attributed a considerable decrease in mass transfer efficiency to the reduction of internal circulation in droplets due to the effect of surface-active agents in the broth. Srivastava et al. (2000) have reported recently on the application of colloidal liquid apheres to enhance mass transfer in bioextraction processes.

Increase of the interfacial area available for mass transfer is usually achieved by dispersion of one phase into the other one. Moreover, the proper choice of extraction equipment can result in a sufficient degree of mixing and turbulence in dispersion to free the liquid–liquid interface. Thus, the mass transfer performance of the whole or clarified broth could be similar (Liddell, 1994). General guidelines for extraction equipment selection have been given by Godfrey and Slater (1994), and for biological systems, by Schügerl (1994) and Liddell (1994).

So far, the CSTR reactors with completely mixed aqueous and organic phases have been used mostly in laboratory-scale extractive fermentation studies limiting the maximum removal
efficiency to one theoretical stage. Collins and Daugulis (1997b, 1999a,b) operated a system without intermixing the phases but oxygen limitation was observed in this case. The operation of the two-phase bioreactor with the continuous solvent phase could prevent the formation of emulsion, which makes phase separation more difficult. However, the application of rigorous solvent selection strategy can minimize the emulsion formation problem (Daugulis, 1997).

Information concerning mass transfer optimization and modeling in biological systems with emphasis on the extractive fermentation application are scarce. Eldridge et al. (1989) showed that conventional mass transfer models could be applied in these cases. Nevertheless, it seems that the understanding of physical and biochemical properties of such systems influencing mass transfer performance is still insufficient to rely on this approach beyond large laboratory-scale experiments.

6. Concluding remarks

Recent reports on the application of the two-phase partitioning bioreactor concept in bioprocessing have been reviewed. Its earliest use involved the in situ removal of inhibitory products, mainly ethanol, acetone, and butanol, organic acids. In the light of results obtained, it can be seen that some incremental improvements have been made in this area in recent years. Scarce information indicates a possible use of this bioreactor concept in ethanol production from renewable resources (Anonymous, 1993). However, only reports from the large laboratory-scale experimental studies are available.

A new, potentially attractive application of two-phase partitioning bioreactors is in environmental biotechnology. This bioreactor configuration has been shown to be effective in a treatment of highly concentrated xenobiotics such as phenol, benzene, toluene, p-xylene, which was successfully demonstrated by laboratory-scale experiments of bioremediation of soil contaminated with these compounds. This may open new opportunities for application of two-phase partitioning bioreactors.

Acknowledgments

The help of Prof. A.B. Jarzebski (Silesian Technical University, Gliwice, Poland) in the preparation of this manuscript is gratefully acknowledged. The author thanks Prof. A.J. Daugulis (Queen’s University, Kingston, Canada) for stimulating discussions and introduction to the subject.

References


