ARTICLE

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Bioproduction of the Aroma Compound 2-Phenylethanol in a Solid–Liquid Two-Phase Partitioning Bioreactor System by *Kluyveromyces marxianus*

Fang Gao, Andrew J. Daugulis

Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6; telephone: 613-533-2784; fax: 613-533-6637; e-mail: andrew.daugulis@chee.queensu.ca

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ABSTRACT: The rose-like aroma compound 2-phenylethanol (2-PE) is an important fragrance and flavor ingredient. Several yeast strains are able to convert L-phenylalanine (L-phe) to 2-PE among which *Kluyveromyces marxianus* has shown promising results. The limitation of this process is the low product concentration and productivity primarily due to end product inhibition. This study explored the possibility and benefits of using a solid–liquid Two-Phase Partition Bioreactor (TPPB) system as an in situ product removal technique. The system applies polymer beads as the sequestering immiscible phase to partition 2-PE and reduce the aqueous 2-PE concentration to non-inhibitory levels. Among six polymers screened for extracting 2-PE, Hytrel® 8206 performed best with a partition coefficient of 79. The desired product stored in the polymer was ultimately extracted using methanol. A 3 L working volume solid–liquid batch mode TPPB using 500 g Hytrel® as the sequestering phase generated a final overall 2-PE concentration of 13.7 g/L, the highest reported in the current literature. This was based on a polymer phase concentration of 88.74 g/L and aqueous phase concentration of 1.2 g/L. Even better results were achieved via contact with more polymers (approximately 900 g) with the aqueous phase applying a semi-continuous reactor configuration. In this system, a final 2-PE concentration (overall) of 20.4 g/L was achieved with 1.4 g/L in the aqueous and 97 g/L in the polymer phase. The overall productivities of these two reactor systems were 0.38 and 0.43 g/L h, respectively. This is the first report in the literature of the use of a polymer sequestering phase to enhance the bioproduction of 2-PE, and exceeds the performance of two-liquid phase systems in terms of productivity as well as ease of operation (no emulsions) and ultimate product recovery.

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Correspondence to: A.J. Daugulis

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Introduction

2-Phenylethanol (2-PE) is an important fragrance and flavor ingredient in the cosmetic and food industry, mainly because of its popular rose-like smell. Most of the 2-PE currently in use is produced chemically (Etschmann et al., 2002). The major problem in its chemical synthesis is the costly product purification process. Additionally, the solvents and by-products can impart off-odors in the final product and therefore influence the grade of 2-PE (Etschmann et al., 2002). In the last two decades, consumers have tended to favor "natural" ingredients in products and this behavior has driven up the market demand for naturally produced 2-PE. Because products derived from bioprocesses starting with natural substrates are in principle defined as "natural" if they have already been identified in plants or other natural sources (Schrader et al., 2004), an option of obtaining natural 2-PE is to use microbial biocatalysts.

Yeasts have shown great ability to produce higher alcohols and esters as aromas (Etschmann et al., 2002). When provided with L-phenylalanine as the precursor, several food grade yeasts are able to convert it to 2-PE via the Ehrlich pathway (Albertazzi et al., 1994). The yeast strains *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* have shown promising results in producing 2-PE (Etschmann et al., 2003; Fabre et al., 1998; Stark et al., 2002). Even with the efforts to optimize medium and choose the best-producing microorganisms, this biological system is still limited by low 2-PE concentration and low productivity primarily due to end product inhibition. 2-PE at even low

concentrations can be used as a biocide because it is toxic to yeast cultures once it exceeds a critical inhibitory level. Fabre et al. (1998) reported that 2.0 g/L of 2-PE could cause complete growth inhibition for *K. marxianus*. Several in situ product removal (ISPR) approaches for reducing product inhibition have been attempted. Such a technique allows the aqueous phase 2-PE to be removed as it forms in situ by an extractant. Liquid–liquid solvent extraction is the most commonly studied ISPR technique for 2-PE biotransformation. Solvents such as oleyl alcohol (Etschmann et al., 2003), oleic acid (Stark et al., 2002), and polypropylene glycol 1200 (PPG 1200) (Etschmann and Schrader, 2006) have been tested and were able to enhance the system performance. Etschmann and Schrader (2006) achieved the highest 2-PE concentration (10.2 g/L overall) reported by using *K. marxianus* CBS 600. Other ISPR techniques such as microcapsulation (Serp et al., 2003; Stark et al., 2003); and organophilic pervaporation (Etschmann et al., 2005) have also been reported.

There are several drawbacks of the liquid–liquid two-phase system. First, organic solvents, especially those with high carbon content, are hard to evaporate and remove. Therefore, their residual odor could affect the quality of the final product. Second, organic solvents may exhibit cytotoxicity and affect cell viability (Bruce and Daugulis, 1991). Third, although PPG 1200 showed the highest partition coefficient in extracting 2-PE from the aqueous phase, it formed emulsions with the aqueous phase upon stirring (Etschmann and Schrader, 2006), causing problems for OD measurements and separation of the two phases. Compared with two liquid phase systems, the solid–liquid Two-Phase Partitioning Bioreactor (TPPB) is a new technique for ISPR. There have been several successful demonstrations in the environmental biotechnology field (Littlejohns and Daugulis, 2008; Prpich and Daugulis, 2006; Rehmann and Daugulis, 2007), and two biotransformation applications have also shown promising results (Morrish and Daugulis, 2008; Prpich and Daugulis, 2007). In such a system, polymers (usually in the form of polymer beads) act as the sequestering phase to absorb and store the desired product, not only targeting the product inhibition problem, but also acting as the initial concentrating step in downstream processing. Polymers have several advantages over organic solvents (Prpich and Daugulis, 2004; Rehmann et al., 2007) in that they are generally biocompatible, non-biodegradable, non-volatile, inexpensive, and for this particular application, polymers will not compromise the product quality by affecting its organoleptic property.

In this article, a solid–liquid TPPB system has been investigated for the production of 2-PE by *K. marxianus* CBS 600. Several polymers were screened to select the best sequestering phase. The polymer that showed the best results was then used in a TPPB system. A single-phase biotransformation, biotransformations with different amounts of polymer loading, and a semi-continuous solid–liquid reactor configuration were studied. The reactor performances were quantified with regard to the final product concentration, productivity, and yields; reactor operability and the recovery of the desired product from the polymer were also assessed.

Materials and Methods

Chemicals and Polymers

$MgSO_4$, K_2HPO_4, citric acid and glucose, and the solvents 1-dodecene and 1-dodecane were purchased from Fisher Scientific (Oakville, ON, Canada). L-phenylalanine, 2-PE, 2-phenylethylacetate (2-PEA) and polypropylene glycol 1000 (PPG 1000) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Yeast nitrogen base (YNB) without amino acids and ammonium sulfate was purchased from BD Difco™ (Oakville, ON, Canada). Polymers with the trade name: Hytrel® 8206, Nylon® 6,6, Elvax® were kindly donated by DuPont Canada. Other polymers used for screening purpose were Desmopan®, kindly donated by Bayer Canada (Toronto, ON, Canada); Kraton®, kindly donated by Kraton (Houston, TX), and high density polyethylene, purchased from Sigma-Aldrich (Oakville, ON, Canada).

Medium Formulation and Culture Preparation

The yeast strain *K. marxianus* CBS 600 was obtained from the American Type Culture Collection (ATCC 200965, Manassas, VA). The medium formulation was adapted from Etschmann et al. (2004) and Wittmann et al. (2002). Defined media were used throughout the inoculum preparation and bioreactor runs. For preparing inocula, the following medium formulation was used (g/L): L-phe 7, glucose 30, $MgSO_4$ 0.5, citric acid 10.3, K_2HPO_4 27.1, YNB without amino acid and ammonium sulfate 6.8. For bioreactor medium formulation, glucose and L-phe concentrations were increased to 70 and 26 g/L, respectively for TPPB systems, and only glucose concentration was increased to 70 g/L for the single-phase system.

Seven flasks each containing 50 mL medium were inoculated with 20 μL cells. One flask was used for tracking cell OD. Once it reached 1.2 (approximately after 24 h of incubation), the remaining six flasks were added to the bioreactor. All operations were conducted aseptically.

Analytics

Cell Measurement

A cell dry weight versus optical density (OD) calibration curve was used. Samples taken from the reactor were measured immediately for their OD at 600 nm (Biochrom Ultraspec, Edmonton, AB, Canada).

Concentration Measurements

High performance liquid chromatography (HPLC) was used to quantify 2-PE, 2-PEA, and L-phe. After measuring cell OD, the mixture was centrifuged at 3,500 rpm for 15 min at

4°C. The supernatant was then passed through a 0.45-μm syringe filter (Waters, Mississauga, ON, Canada) prior to the injection into the HPLC (Waters 2478). Twenty microliters of sample was injected and passed through a RP-C18 column (Spherisorb ODS-2 column), and the compounds were detected by a Waters UV–Visible Detector at 258 nm. Thirty-five percent sterile water and 65% methanol was pumped isocratically through a Waters 515 pump at 1 mL/min. The column was kept at room temperature. Ethanol concentration was tracked by an enzymatic kit purchased from R-Biopharm (Marshall, MI). Glucose concentration was measured by a DNS assay adapted from Miller (1959).

Polymer Partition Coefficient

Six 20 mL scintillation vials were used to test partition coefficient. One vial containing 10 mL of 4 g/L 2-PE solution was used as the control with no polymer, and the other five vials contained 2-PE solution with concentrations (g/L) 2, 3, 4, 5, and 6. Equal amount of polymers (approximately 0.33 g) were added to each of the five vials, and the vials were put onto a shaker for mixing overnight. After equilibrium was established, the aqueous phase 2-PE concentration was analyzed by HPLC. The amount left in the polymer phase was back calculated using mass balance. The control showed no detectable change of the 2-PE concentration. The slope of the straight line of polymer phase concentrations versus aqueous phase concentrations of 2-PE was the partition coefficient of that polymer towards 2-PE. The same tests were performed with other molecules such as 2-PEA and L-phe. Similar method was applied to test partition coefficient using organic solvents.

Batch Reactor Operation

A 5 L bioreactor (3 L medium) equipped with pH, temperature, and dissolved oxygen control was used for all reactor runs (New Brunswick Scientific, Edison, NJ, BioFlo III). The pH was controlled automatically by adding 6 M KOH. The aeration rate was controlled at 1 vvm (3 L/min) and the agitation was set to 600 rpm for single phase and 700 rpm for TPPB runs. The temperature was controlled at 35°C. Dissolved oxygen was monitored to ensure the reactor was non-oxygen limited. Two trials of solid–liquid TPPB were performed with 100 g of Hytrel® and 500 g of Hytrel®. The glucose concentration was tracked online after hour 18 to closely monitor the depletion of the carbon source. Saturated glucose solutions were added at various time points during the bioreactor runs, and the glucose concentration was maintained in this fashion between 10 and 30 g/L after the initial rapid consumption.

Semi-Continuous Reactor Operation

A glass column (0.5 L; length: 30 cm; diameter: 4.6 cm) filled with polymer beads was connected to the reactor via sterile tubing and a peristaltic pump. The contact between the aqueous phase and the polymer phase was achieved by circulating the broth (containing cells) through the external column packed with polymer beads at a rate of 40 mL/min. The polymer beads were around 2 mm in diameter, therefore, the column was not tightly packed due to the void space between polymer beads. Therefore, blockage caused by cells did not occur in the column. The assembly and preparation of this reactor was adapted from Morrish and Daugulis (2008). Three glass columns were used each containing approximately 300 g of polymer beads. Other reactor conditions were kept the same as the batch TPPB operations. Besides glucose addition, substrate L-phe was also added at time 29 h.

Product Recovery From Polymer

An extraction procedure with methanol was performed after the polymers were recovered from the reactor. In a 20 mL vial, 10 mL of methanol was added together with approximately 1 g of polymer randomly selected from the recovered polymers. The mixture was put onto a shaker to equilibrate overnight. After 24 h, the methanol was decanted completely to a new vial. Ten milliliters of fresh methanol was then added to the vial that contained the polymers for a second round of extraction overnight. The procedure was repeated several times until there was no detectable 2-PE in methanol. The total amount of 2-PE stored in the polymer was calculated by adding the amount of 2-PE extracted into methanol from all rounds of methanol extractions.

Results and Discussion

Polymer Screening

Initial efforts were made to find a polymer that had the highest partition coefficient towards 2-PE. Two important functional groups in the 2-PE chemical structure are the aromatic ring and the hydroxyl group. Therefore, polymers that can attract 2-PE through π interaction and/or through hydrogen bonding may be considered as promising absorbants. Previously, PPG 1200, Oleic acid, and Oleyl alcohol were used and showed good results. Generally speaking, these solvents were polar solvents with no aromatic functionality.

A similar trend was found in the polymer partition coefficient tests shown in Figure 1. Hytrel® 8206 possessed the highest affinity toward 2-PE among all polymers tested. Hytrel® is a block copolymer of polybutylene ester and polyether (Prpich and Daugulis, 2004). Both ester and ether linkages open the possibility for hydrogen bonding, a strong intermolecular force, between the polymer and the hydroxyl

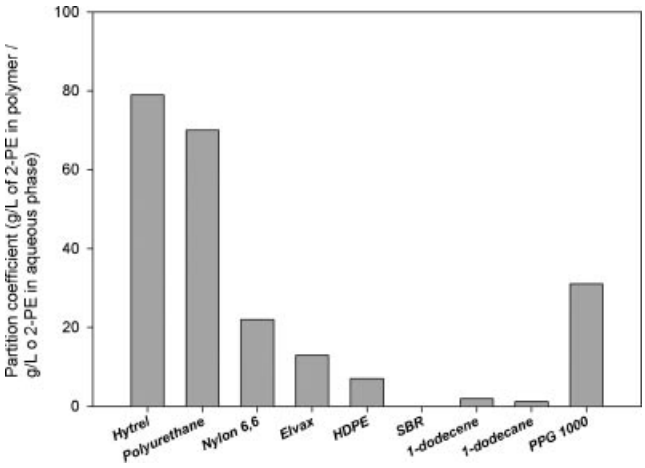

Figure 1. Partition coefficient screening of all polymers and organic solvents tested. The number was obtained based on the linear relationship (slope) between the aqueous phase 2-PE concentration and polymer phase 2-PE concentration. The former one was from direct measurement while the latter one was calculated by mass balance.

group in 2-PE. By the same mechanism, polyurethane also showed good results in attracting 2-PE. Nylon® 6,6 also had hydrogen-bonding potential due to its polyamide linkage in the structure; however, it appeared to have a lower partition coefficient. The reason for this is that Nylon 6,6 can form intramolecular hydrogen bonding inside the polymer chains, and it reduces the sites available for bonding with 2-PE. This phenomenon is also reflected in its high degree of crystallinity because the intramolecular hydrogen bonding holds and arranges the chains in a regular and ordered way. A high degree of crystallinity is undesirable when polymers are used in a TPPB system. In order to absorb the target compound, not only attraction between the polymers and 2-PE has to occur, but also the polymer needs to possess large free volume to accommodate the target compound. This requires the polymer to have a large portion of its structure in an amorphous condition instead of being crystalline. As a result, Nylon® 6,6 was not as good a candidate as Hytrel®. Elvax®, a type of ethylene-vinyl acetate, had modest affinity to 2-PE because the acetate group can hydrogen bond with 2-PE. However, due to the presence of a large portion of ethylene making the polymer chains packed in an ordered pattern, its structure has a high degree of crystallinity. HDPE, showed even lower affinity, and the highly aromatic polymer styrene butadiene rubber (SBR) showed no measurable affinity. This observation led to the hypothesis that the primary uptake mechanism of 2-PE by polymer beads is through hydrogen bonding interaction instead of aromatic π interaction. Similar results can also be found when organic solvents were tested. The most polar solvent PPG 1000 had a partition coefficient of 31 while the non-polar solvents 1-dodecene and 1-dodecane showed almost no affinity for 2-PE.

The best polymer Hytrel® 8206 was then tested for its affinity towards L-phe and 2-PEA. L-phe was mainly in its dissociated form ($pK_a = 2.59$) in the medium (pH = 5). The tests showed no measurable uptake of L-phe by Hytrel®. Hytrel® was also tested for its affinity towards 2-PEA, a valuable fragrance by-product in this biotransformation. The partition coefficient was found to be 50. Hytrel® is an FDA compliant polymer for use in the food and fragrance industry (personal communication, DuPont Canada). It is odorless and its properties were not affected by autoclaving at 121°C. Hytrel® was also determined to be non-bioavailable and biocompatible, and was thus a good choice for this biotransformation.

Batch Reactor Runs With a Single Phase and a Solid–Liquid TPPB System

Prior to solid–liquid TPPB operation, a single-phase run with only aqueous medium was performed to provide a benchmark. The time course graph of cell growth and product accumulation is illustrated by Figure 2. The arrows represent the time points when glucose was added (same for Figs. 3, 4, and 6). A final cell density of 5.8 g/L was obtained at the end of 42.5 h with 1.4 g/L of 2-PE in the aqueous broth. The results were similar but with slightly lower numbers than Wittmann's results which found 7 g/L of biomass and 1.83 g/L of 2-PE in a single-phase bioreactor (Wittmann et al., 2002). However, although the two studies both used *K. marxianus*, the specific strains were different. Using the same strain of *K. marxianus* CBS 600, Etschmann et al. (2003) had accumulated 0.89 g/L of 2-PE in a single-phase reactor with non-optimal medium and carbon source. Even though the reported toxic threshold of 2-PE for *K. marxianus* was 2 g/L, it is difficult to reach that concentration in a reasonable time period. By the end of

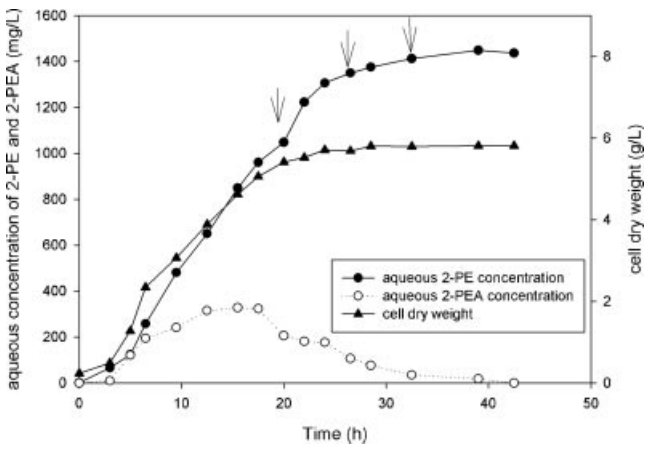

Figure 2. Aqueous phase 2-PE and 2-PEA concentration and cell density as a function of time in a single-phase reactor. The time points marked with arrows indicate the addition of 30 mL of saturated glucose solution (600 g/L).

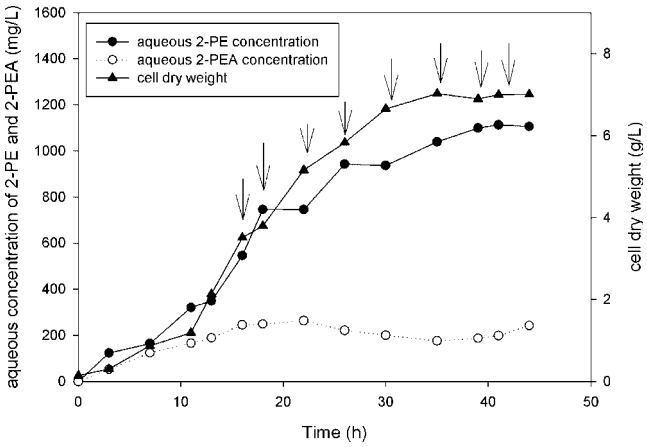

Figure 3. Aqueous phase 2-PE and 2-PEA concentration and cell density as a function of time in a TPPB batch system in which 100 g of Hytrel® polymer was added as the sequestering phase. The time points marked with arrows indicate the addition of 60 mL of saturated glucose solution (600 g/L).

the biotransformation, there was 14 g/L of glucose and 4.2 g/L of L-phe left which indicated sufficient carbon and nitrogen source, and the termination of the biotransformation was due to product inhibition. To ensure this was the case, at the end of the run, 50 mL of broth was taken from the reactor followed by the addition of 10 g of Hytrel® beads, and was put into an incubator. After 6 h, the OD increased indicating the resumption of cell growth. Furthermore, this test indicated that during a short period of time, the damage to the cells caused by 2-PE toxicity was not permanent, and cell growth can be restored.

Two batch trials of the solid–liquid TPPB with Hytrel® polymer beads (100, 500 g) were performed and the results

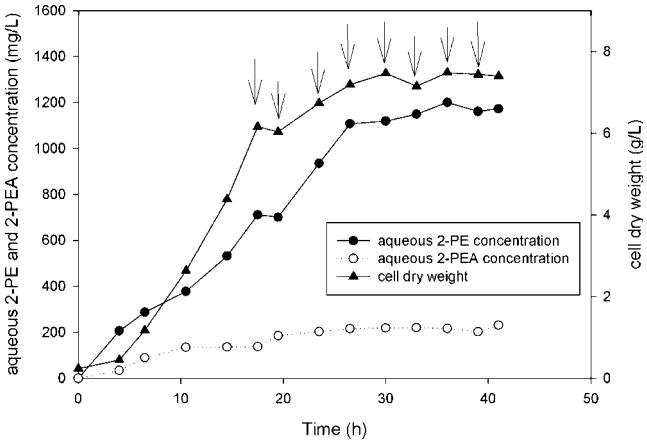

Figure 4. Aqueous phase 2-PE and 2-PEA concentration and cell density as a function of time in a TPPB batch system in which 500 g of Hytrel® polymer was added as the sequestering phase. The time points marked with arrows indicate the addition of 60 mL of saturated glucose solution (600 g/L).

are shown in Figures 3 and 4, respectively. The amount of 2-PE in the polymer beads was measured via the desorption test in methanol. After the third round of extraction, there was no detectable 2-PE in the methanol. The 1st wash was able to extract 80% of the total 2-PE stored in the polymer beads. A final partition coefficient was calculated and the results agreed well with the partition coefficient tests obtained earlier. It is obvious that by adding the selected polymer as the sequestering phase, nearly all the important measurements of reactor performance improved significantly (Table I). The total 2-PE concentration in the TPPB system was 2.6 times (100 g Hytrel® run) and 9 times (500 g Hytrel® run) that of the single phase and 3 g/L (500 g Hytrel® run) more than the highest reported 2-PE production using *K. marxianus* (Etschmann and Schrader, 2006). The overall productivity using the solid–liquid TPPB system was two times (100 g Hytrel® run) and six times (500 g Hytrel® run) that of its single-phase counterpart. The polymer appeared to have no affinity for L-phe and glucose, therefore the substrates were delivered to the cells in the same manner as the single-phase system. The fact that aqueous 2-PE accumulation curve was not affected by adding the sequestering phase showed that the two-phase system was kinetically limited instead of being mass transfer limited, which means that the diffusion and absorption of 2-PE by Hytrel® beads were fast enough to accommodate 2-PE production by the cells. One additional observation is that in the single-phase system, the 2-PEA concentration reached 0 at the end of the fermentation while in the TPPB system did not. The single-phase 2-PEA profile agreed with Wittmann's results when they analyzed the metabolic physiology of *K. marxianus* (Wittmann et al., 2002). However in the TPPB system, a portion of 2-PEA produced at the beginning of the fermentation was likely stored in the polymer. When the aqueous 2-PEA concentration started to decrease, 2-PEA stored in the polymer diffused out of the polymer driven by the increased concentration gradient between the two phases, and a new equilibrium was established. Therefore, the 2-PEA concentration in the aqueous phase never reached 0. This is also consistent with Etschmann's observation (Etschmann and Schrader, 2006).

The starting glucose concentrations were around 70 g/L for all reactor runs to provide enough carbon source. Initially it was consumed rapidly and at around 20 h, its concentration dropped below 10 g/L. Glucose was then fed to the reactor at the time points marked by arrows in Figures 3 and 4, and by applying this feeding strategy, the glucose level was maintained between 10 and 30 g/L after initial consumption. The ethanol concentration was kept below 10 g/L for all reactor runs (data not shown). Ethanol was produced at a faster rate initially at higher glucose concentration, and as the glucose was utilized by cells, the production of ethanol slowed and stopped. As glucose approached depletion, the ethanol concentration dropped because cells used ethanol as a carbon source, consistent with Wittmann's finding (Wittmann et al., 2002). The

Table I. Comparison of reactor performance of the biotransformation process carried out in a single-phase system, TPPB batch system, and TPPB semi-continuous system.

	Single phase	100 g Hytrel	500 g Hytrel	External column
Final CDW (g/L)	5.81	7.01	7.40	7.90
[Aqueous 2-PE] (g/L)	1.45	1.12	1.20	1.40
[Polymer 2-PE] (g/L)	N/A	80.45	88.74	97.00 (average)
[Polymer 2-PEA] (g/L)	N/A	12.10	11.50	N/A
[2-PE] overall (g/L)	1.45	3.82	13.70	20.38
Partition coefficient	N/A	71.19	73.95	79.23 (average)
Overall productivity (g/L h)	0.05	0.10	0.38	0.43
Y2-PE/L-phe (mol/mol)	0.60	0.55	0.93	0.91

[] represents concentrations.

The overall 2-PE concentration was calculated based on the total amount of 2-PE in the aqueous phase and the polymer phase divided by the total reactor volume which include both the aqueous medium and the polymer beads.

The concentration of 2-PEA in the polymer phase was calculated based on the partition coefficient test and the aqueous phase 2-PEA concentration at the end of the reactor run.

primary substrate L-phe was also provided as the sole nitrogen source for the cells. Shown in Figure 5, its consumption was significantly enhanced by the presence of Hytrel®. This is expected since the increase in cell density required more nitrogen, and more importantly, the sequestration of the product by the polymer drove the biotransformation towards the formation of more 2-PE and therefore the consumption of L-phe was increased.

The reactor operation was trouble-free for all cases. Since solid polymer beads were used the potential problems of forming emulsions upon stirring were not encountered. The presence of 500 g of Hytrel® beads in 3 L of liquid volume did not cause any mechanical problems associated with stirring, aeration, sampling, and operation in general. The oxygen transfer was sufficient under 700 rpm agitation and 1 vvm aeration. With more vigorous aeration, major product loss might be expected due to the high volatility of this aroma compound. A condenser was attached to the bioreactor and at the exhaust end of the condenser, there was slight rose-like smell even under 1 vvm aeration rate indicating minor product loss via evaporation.

Methanol was chosen because of its complete miscibility with 2-PE and therefore high extraction capability. Also, the separation of methanol from 2-PE can be achieved by distillation given the large difference in boiling point of the two compounds. However, a less toxic solvent such as ethanol, which is also miscible with 2-PE, could be a better choice, and its potential should be further explored.

Semi-Continuous Solid–Liquid TPPB Reaction System

It can be seen from the batch mode runs that by adding more polymer beads, the reactor performance was greatly enhanced. However, there exists a maximum practical amount of beads that can be added, limited by the physical

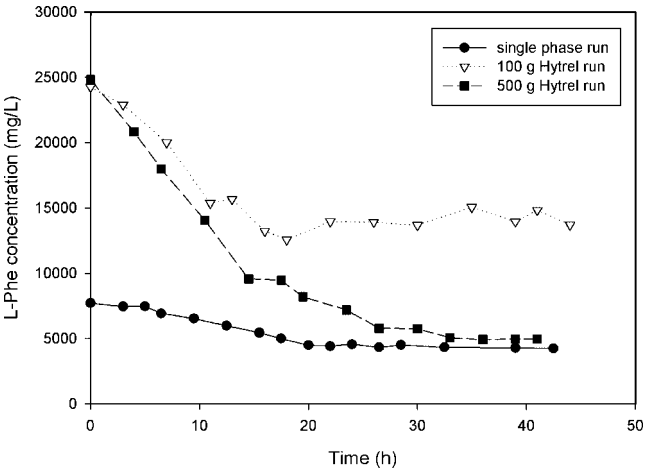

Figure 5. Substrate L-phe consumption curve as a function of time for three different reactor runs.

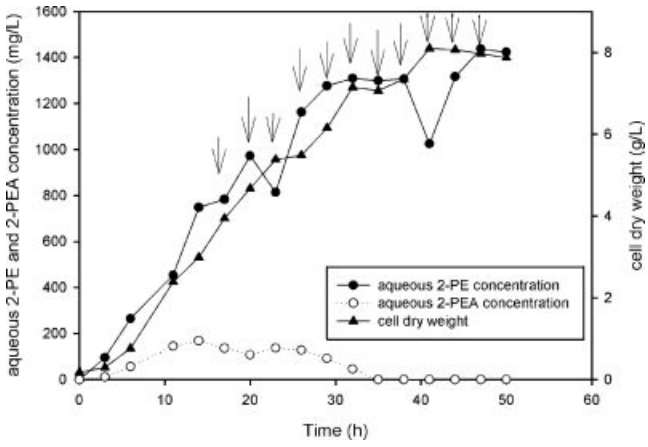

Figure 6. Aqueous phase 2-PE and 2-PEA concentration and cell density as a function of time in a TPPB semi-continuous system with approximately 900 g of Hytrel® polymer. The time points marked with arrows indicate the addition of 60 mL of saturated glucose solution (600 g/L).

operation of the reactor. The compromise between adequate bead loading and good operability will limit the reactor performance. However, this problem can be solved by the use of an external column loaded with polymer beads through which the bioreactor contents are circulated. Once the beads are fully loaded, the column can be taken offline and a new column with fresh beads can be attached. This configuration not only makes it possible to bring large amount of polymer into contact with the aqueous broth, but also makes the process semi-continuous. Two column changes were made at hour 20 and hour 38. The decision about when to change columns was based on previous experience and online tracking of 2-PE concentration. The cell viability can be greatly reduced when the 2-PE concentration reached 0.89 g/L (Etschmann et al., 2003), and in order to preserve reasonable cell viability, the first column change was made when 2-PE concentration was just below 1 g/L. The new column contained fresh beads; therefore, the concentration gradient at the time of column change was high. As the biotransformation progressed, the driving force decreased until the beads were saturated and no absorption would occur. This change was monitored by tracking 2-PE concentration in the aqueous phase and once it plateaued, it was time for the second column change. The progress is shown in Figure 6. As shown, after the second column change, the 2-PE concentration was brought down to 1 g/L from 1.3 g/L. The cells kept producing 2-PE and kept growing due to the less toxic level of 2-PE.

The overall reactor performance of all bioreactor runs is summarized by Table I. The polymer phase 2-PE concentration was calculated based on the average of all three columns used. Since more beads (over 900 g) were brought into contact with the aqueous broth, the overall 2-PE concentration and overall productivity were greatly improved compared with the 500 g Hytrel® run. It is worth noting that the 2-PE/L-phe yield was also increased when comparing the TPPB system with the single-phase system. It has been reported that about 65.2% of the L-phe consumed is transformed to 2-PE in a single-phase setting (Wittmann et al., 2002). Although the reason for the increase in yield (2-PE/L-Phe) to 93% by adding the sequestering phase (500 and 900 g Hytrel®) is unclear, our initial hypothesis is that the higher starting L-phe concentration in the TPPB system favored the Ehrlich pathway to make 2-PE over the Cinnamate pathway. Etschmann's group also showed that by supplying L-phe initially at high concentration, the biotransformation achieved better results (Etschmann and Schrader, 2006).

This investigation showed that by selecting an appropriate polymer, a solid–liquid TPPB system can greatly enhance the biotransformation process and achieve high final 2-PE concentration and high productivity. Hytrel® showed a high partition coefficient towards 2-PE, however, the issue of selectivity has not been addressed yet. The methanol extraction showed that although 2-PE is the most dominant component in the polymer, small quantities of other by-products from the biotransformation were also extracted. Further polymer screening may identify an even more effective sequestering phase than Hytrel®. Alternatively, the use of mixtures of polymers may provide the ability to preferentially absorb mixtures of molecular species from fermentation media. Nonetheless, this solid–liquid TPPB system showed promising results in removing a toxic product as an ISPR technique and as an initial concentrating step for downstream processing. Efforts are currently underway to explore the application of solid–liquid TPPBs for producing other flavor and fragrance compounds.

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