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Optimization and Scale-Up of a Lipase-Catalyzed Enzymatic Resolution of an Indole Ester Intermediate for a Prostaglandin D_2 (DP) Receptor Antagonist Targeting Allergic Rhinitis

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Abstract:

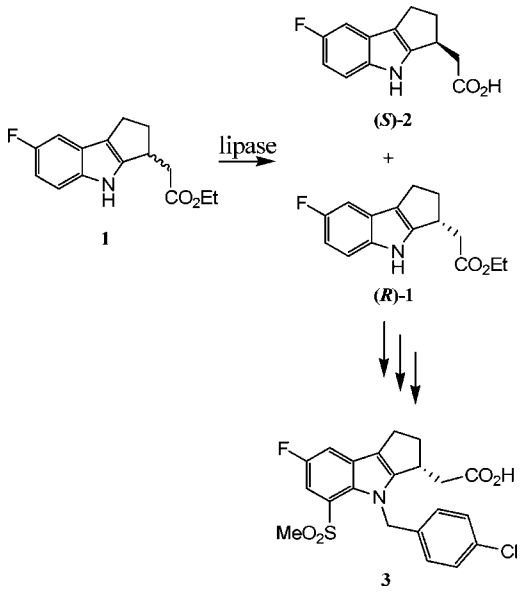
***Pseudomonas fluorescens* lipase was found to catalyze the asymmetric hydrolysis of racemic indole-ethyl ester 1.[1] The lipase converted the undesired *(S)* enantiomer to its corresponding acid, while leaving the desired *(R)* enantiomer untouched, yielding optically enhanced *(R)*-ester with an ee of 95%[1] at 48% conversion. The original process conditions for this resolution contained a second-phase immiscible organic solvent to solubilize the substrate, and required 128 g/L enzyme for a 100 g/L substrate charge (Shafiee, A.; Upadhyay, V.; Corley, E.; Biba, M.; Zhao, D.; Marcoux, J.-F.; Campos, K.; Journet, M.; King, A.; Larsen, R.; Grabowski, E.; Volante, R.; Tillyer, R. *Tetrahedron Asymmetry* 2005, *16*, 3094). A study of substrate solubility and phase-partitioning behavior in a wide range of miscible and immiscible solvent concentrations demonstrated that the presence of the immiscible organic solvent caused the reaction to reach a mass transfer limited regime at conversions >40%. Process optimization led to the elimination of the second-phase immiscible organic solvent, a greater than 4-fold reduction in enzyme charge, and an increased product ee of >99%. The optimized process has been demonstrated at scale on two 40-kg batches and has resulted in product ester ee of 99.75% at 50% conversion.**

1. Introduction

The resolution of esters via selective hydrolysis catalyzed by lipases has been demonstrated as a practical way to arrive at a number of optically enhanced pharmaceutical intermediates.[2−6] Enzymatic routes to chiral amino acids,[2] *(S)*-naproxen and *(S)*-ibuprofen[4] are just a few examples.

The asymmetric hydrolysis of racemic indole-ethyl ester **1** using *Pseudomonas fluorescens* lipase was shown to be an efficient way to afford optically pure indole-ethyl ester ***(R)*-1**[1] as shown in Scheme 1. This intermediate ***(R)*-1** was used to produce chiral compound **3**, a prostaglandin DP receptor antagonist targeted against allergic rhinitis.[7]

Scheme 1. **Enzymatic indole-ethyl ester resolution to form intermediate for compound 3**



The baseline process called for 100 g/L substrate ester to be charged to a solution of 70% buffer, 20% heptane, and 10% DMF (by volume). To this was added a total of 128 g/L enzyme in four equal shots throughout the reaction age. This enzyme-charging strategy was required to push the reaction to completion (toward a maximum theoretical yield of 50%) as the reaction progress slowed considerably above 40% conversion.

Because of the low solubility of many esters in aqueous hydrolytic reaction systems, the substrate is typically dissolved in an immiscible organic solvent, such as heptane used in the original baseline process conditions. The thought behind this type of reaction system is that an immiscible organic solvent will have minimal contact with the enzyme (protecting enzyme stability) and will improve biocatalyst performance by providing an interface for interfacial activation via a reservoir of substrate in an organic solvent second phase.[8]

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The purpose of this work was to optimize the reaction system and reduce the enzyme charge primarily by studying the substrate solubility and phase-partitioning behavior in a variety of miscible and immiscible organic solvent systems. The addition of an appropriate organic solvent to an aqueous reaction mixture can increase the solubility of the substrate in the reaction system and alleviate mass transfer issues.[9] This technique has been shown to increase the rate of reaction of enzymatic resolutions.[10,11]

2. Materials and Methods

2.1. Enzymes. This work was conducted with Amano AK-AF lipase from *P. fluorescens*. A comprehensive screen of enzymes was conducted, and this lipase was found to be the most selective.[1]

2.2. Preparation of Indole-Ethyl Ester. The preparation of the indole-ethyl ester **1** substrate for the enzymatic resolution is described previously by Journet et al.[12]

2.3. Biotransformation Reactions. *2.3.1. Small-Scale (5 mL) Reactions.* Small-scale reactions were run at 5-mL scale in scintillation vials. The vials were placed in an Infors AG Multitron temperature controlled shaker at 900 rpm. All reactions were run in potassium phosphate buffer, and pH was adjusted manually as needed with a 3.8 N NaOH solution. Because pH could not be controlled continuously, these small-scale reactions were used to determine initial rate data and end points of reactions only.

2.3.2. Medium-Scale (200 mL) pH-Controlled Reactions. When precise pH control was needed, reactions were run in the Infors AG Sixfors reaction unit. Six reactions can be run simultaneously with separate set points for agitation, temperature, and pH. Reactions were run at 200-mL scale with agitation set at 400 rpm. The Sixfors use a Rushton-type impeller for stirring. pH was controlled automatically using a 3.8 N NaOH solution.

2.3.3. Front Run (1 L Reaction). A front run was conducted at 1-L scale in a Braun reaction vessel. The temperature was set to 28 °C with agitation at 300 rpm. The pH was controlled at 8.0 using a manual pH control method, which consisted of adding 5 mL shots of 3.8 N NaOH solution to the reaction vessel whenever the pH dropped to 7.8. The NaOH shot raised the pH of the reaction from 7.8 to 8.2.

2.3.4. GMP Pilot-Plant Run (400 L Reaction). The pilot=plant runs were conducted in a 750-L baffled reaction vessel equipped with jacketed temperature control and a retreat curve impeller. The temperature was controlled at 26 ± 2 °C, and agitation was set to 100 rpm. The agitation was set such that the reaction mixture was visibly well mixed, while minimizing air entrainment at the surface of the liquid. Two batches were run, each at 400-L scale. pH control was implemented using the method of shot-wise base addition previously described in section 2.3.3, using 2-L shots of 3.8 N NaOH for the 400-L scale.

2.4. Analytical Methods. *2.4.1. HPLC Analysis.* High-pressure liquid chromatography (HPLC) was used to determine the enantiomeric excess (ee) of the desired unconverted *R* enantiomer of compound **1**. A Chiralcel OJ-RH (4.6 mm × 150 mm, 5 μm) chiral column was used. The mobile phase was methanol containing 0.1% TFA with a flow rate of 1.5 mL/min. Temperature was set to 25 °C, and the detection wavelength was 215 nm. The undesired *S*-acid eluted at 2.5 min, and the desired *R*-ester eluted at 3.6 min.

2.4.2. Colorimetric Lipase Activity Assay. A colorimetric lipase activity assay was used to quickly determine enzyme activity. The activity assay was used to map the inactivation of the lipase in the reaction mixture over time. The surrogate substrate used for this assay was *p*-nitrophenyl butyrate (*p*-NPB). When the lipase reacts with *p*-NPB, *p*-nitrophenol (*p*-NP) is released. *p*-NP is yellow in color and could easily be quantified by spectrophotometer.

To test the enzyme activity, samples were diluted to an enzyme concentration of 1 g/L. Potassium phosphate monobasic buffer (1.95 mL of 50 mM) at pH 7.2 and 50 μL of a solution containing 9 mM *p*-NPB in acetonitrile were added to a cuvette. The cuvette was placed in the spectrophotometer, and 100 μL of the enzyme solution was added. The absorbance at a wavelength of 410 nm was then recorded every 30 s for 2 min. A linear plot was generated, the slope of which was directly proportional to the enzyme activity.

2.5. Enzyme Deactivation/Inhibition Studies. *2.5.1. Effect of Organic Solvent on Enzyme Deactivation.* Reactions were run at 5-mL scale using the procedure outlined in section 2.3.1. The following four mixtures were made (all percentages are by volume):

(1) 4.50 mL of 10% DMF/20% heptane/70% buffer (200 mM potassium phosphate at pH 7.5)

(2) 3.60 mL of 10% DMF/90% buffer (200 mM potassium phosphate at pH 7.5)

(3) 4.05 mL of 20% heptane/80% buffer (200 mM potassium phosphate at pH 7.5)

(4) 3.15 mL of buffer (200 mM potassium phosphate at pH 7.5)

To each 5-mL solution, 160 mg (32 g/L) of *P. fluorescens* lipase was added. The solutions were then placed in a temperature controlled shaker (800 rpm) at 28 °C for 48 h. After the 48 h, 0.9 mL of heptane was added to solution 2, 0.45 mL DMF was added to solution 3, and 0.9 mL heptane and 0.45 mL DMF were added to solution 4. The solvent addition brought each solution to the baseline process conditions of 10% DMF/20% heptane/70% buffer. Next, 0.5 g (100 g/L) of indole-ethyl ester **1** substrate was charged to each solution. Conversion was monitored, and the initial rate of reaction was determined.

2.5.2. Enzyme Product Inhibition Study. Reactions were run at 5-mL scale using the procedure outlined in section 2.3.1. *P. fluorescens* lipase (160 mg (32 g/L)) was added to each of two 4.5 mL solutions of 10% DMF/20% heptane/70% buffer (200 mM potassium phosphate at pH 7.5). Next,

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Table 1. **Substrate solubility and phase partitioning study concentrations**

solution	DMF/heptane	% DMF	% heptane	% buffer	solution	DMF/heptane	% DMF	% heptane	% buffer
1	0:1	0	100	0	16	1:1	15	15	70
2	0:1	0	80	20	17	1:1	10	10	80
3	0:1	0	50	50	18	2:1	60	30	10
4	0:1	0	30	70	19	2:1	53	27	20
5	0:1	0	20	80	20	2:1	40	20	40
6	1:2	31	61	8	21	2:1	20	10	70
7	1:2	30	60	10	22	2:1	13	7	80
8	1:2	27	53	20	23	1:0	90	0	10
9	1:2	20	40	40	24	1:0	80	0	20
10	1:2	10	20	70	25	1:0	75	0	25
11	1:2	7	13	80	26	1:0	70	0	30
12	1:1	45	45	10	27	1:0	60	0	40
13	1:1	43	43	14	28	1:0	40	0	60
14	1:1	40	40	20	29	1:0	30	0	70
15	1:1	30	30	40	30	1:0	20	0	80

Table 2. **DMF/heptane ratio optimization concentrations**

solution	% DMF	% heptane	% buffer
1	0	30	70
2	10	20	70
3	15	15	70
4	30	0	70

both solutions were charged with 0.5 g of indole-ethyl ester **1** substrate. The reactions were run for 28 h, and conversion was monitored. At 28 h, one of the reactions was charged with another 0.5 g of substrate, and conversion was monitored for an additional 32 h (60 h total).

2.6. Substrate Solubility and Phase Partitioning. Solutions of 4.5 mL were made that contained varying concentrations of each reaction solvent (DMF, heptane, and 200 mM potassium phosphate at pH 7.5). Organic solvent and buffer concentrations were studied over a wide range, 0−100 vol %, and the ratios of DMF to heptane studied were the following: 0:1, 1:2, 1:1, 2:1, 1:0. Table 1 lists each set of conditions studied.

To each 4.5-mL solution was added 0.5 g (100 g/L) of indole-ethyl ester. The solutions were well mixed and then allowed to settle for 2 h. The aqueous and organic phases were sampled and assayed for ester concentration. Many of the solutions had a third oil phase that was also sampled from and assayed for ester concentration. The volume of each phase (aqueous, organic, and oil) was then measured.

2.7. Reaction Optimization. *2.7.1. Organic Solvent Concentration Optimization.* Reactions of 200 mL were run using the procedure outline in section 2.3.2. All reactions were run with 32 g/L enzyme and 100 g/L indole-ethyl ester at 28 °C. The first set of reactions was run with a fixed buffer (200 mM potassium phosphate at pH 7.5) concentration of 70% and a variable DMF/heptane (immiscible/miscible) solvent ratio. The DMF and heptane concentrations studied are shown in Table 2.

The reactions were run for 48 h until conversion stopped. Final conversion was then measured.

A second set of reactions was run using only DMF as an organic solvent. Reactions were run with DMF concentrations ranging from 10 to 45%. Conversion was monitored for the first 2 h.

2.7.2. Temperature Optimization (Enzyme Activity and Stability vs Temperature). Solutions of 5 mLwere made that contained 75% 200 mM potassium phosphate buffer at pH 7.5, 25% DMF, and 32 g/L enzyme. The solutions were temperature controlled and shaken as described in section 2.3.1. The solutions were incubated at a range of temperatures from 15 to 45 °C. Samples were taken at time increments of 0, 1, 2, 4, 8, 24, and 48 h. Remaining enzyme activity of each sample was determined using the activity assay described in section 2.4.2. From these data, a profile of enzyme activity and stability vs temperature was generated.

Reactions of 400 mL were run according to the procedure outlined in section 2.3.2. Reactions were run at 24, 28, 32, and 37 °C under the following conditions: 75% 200 mM potassium phosphate buffer at pH 7.5, 25% DMF and 32 g/L enzyme. Reactions were sampled for conversion and product ee over 50 h.

2.7.3. pH Optimization and Control Strategy. Reactions of 200 mL were run using the procedure outline in section 2.3.2. Reactions were set up with 75% 200 mM potassium phosphate buffer, 25% DMF, 32 g/L enzyme, and 100 g/L indole-ethyl ester at 28 °C. Potassium phosphate buffer (200 mM) was found to be optimal for this reaction system with regards to enzyme activity and the buffering capacity needed to allow for pH control. Four reactions were set up at the following pH values: 7.0, 7.5, 8.0, 8.5. Reaction progress was monitored over 60 h.

A pH control strategy was developed for manual pH adjustment throughout the reaction age, as the reaction pH drops as a result of acid **2** formation. The control strategy used fixed shots of 3.8 N NaOH to keep the pH in a suitable range for enzyme activity.

3. Results and Discussion

3.1. Baseline Process Description. The baseline process conditions were as follows: 100 g/L substrate ester and 128 g/L enzyme in a solution of 70% buffer, 20% heptane, and 10% DMF (by volume) at 25 °C and pH 7.5. The 128 g/L enzyme was charged to the reaction in four 32 g/L shots throughout the reaction age. Four enzyme shots were required to push the reaction toward completion. Adding the enzyme

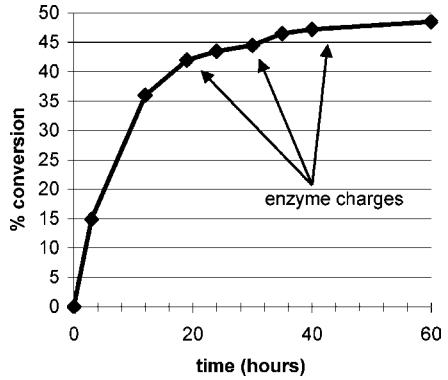

Figure 1. **Reaction profile of baseline process.**

Table 3. **Results of reaction component enzyme incubation study**

reaction no.	components incubated	rate (g/L−h)	conversion (%)	ester ee
1	DMF/heptane/buffer	6.0	44.9	80.3
2	DMF/buffer	6.2	44.8	80.0
3	heptane/buffer	6.3	44.5	79.0
4	buffer	6.0	45.4	80.9

in shots was found to be more effective than increasing the enzyme charge 4-fold at the start of the reaction. A typical profile of the reaction is shown in Figure 1. The multiple enzyme charges pushed the reaction to 48% conversion with a desired *(R)*-ester ee of 95%. The key to reducing the enzyme charge was determining why the reaction rate slowed above 40% conversion and required additional enzyme charges.

3.2. Enzyme Deactivation/inhibition Studies. *3.2.1. Reaction Component Enzyme Deactivation Study.* A study was conducted to determine if there was enzyme deactivation due to any of the components in the reaction system. The enzyme was incubated with varying components of the reaction system (DMF, hetpane, and buffer) for 48 h. After the incubation period, each system was brought to baseline conditions by adding the omitted reaction components. All of these reactions proceeded to ∼45% conversion with an ee of ∼80% for the desired (*R*)-ester, which was typical for a reaction containing only the initial 32 g/L enzyme charge and no additional enzyme shots. Table 3 shows the experimentally determined initial rate, final conversion, and ester ee for each incubation experiment. These data demonstrated that there was no significant enzyme deactivation due to reaction components over the time period studied.

3.2.2. Enzyme Product Inhibition Study. Product inhibition was investigated by running two identical reactions at baseline conditions with only the initial 32 g/L enzyme charge. Both reactions proceeded to 45% conversion (Figure 2) in 24 h. At 28 h, an additional 100 g/L ester substrate charge was added to one of the reactions. Over the course of the next 32 h, the reaction receiving the substrate charge proceeded to 44% conversion, demonstrating both that the enzyme was still active and that the product does not inhibit the enzyme at the concentrations studied.

3.3 Substrate Solubility and Phase Partitioning. Ruling out enzyme deactivation due to reaction components and

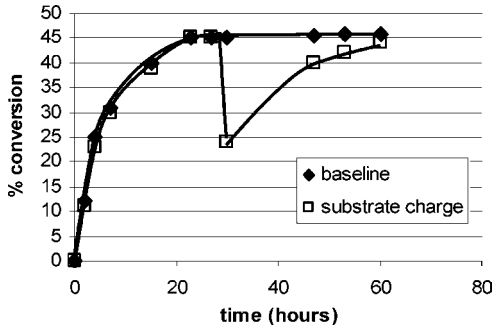

Figure 2. **Substrate-charging study.**

inhibition due to product formation, our attention focused on substrate mass transfer limitations as a possible cause for the decrease in reaction rate above 40% conversion.

A series of solutions containing between 0 and 80% buffer were made with DMF and heptane organic solvents. The ratios of DMF/heptane organic solvent concentrations studied were 0:1, 1:2, 1:1, 2:1, 1:0. In this way, a wide experimental space of miscible and immiscible solvent concentrations was analyzed. To each solution was added 100 g/L of indole-ethyl ester. In most cases, at least two and sometimes three phases were observed. The three phases consisted of an aqueous phase (consisting mostly of buffer), an organic phase (consisting mostly of heptane), and an oil phase (consisting mostly of indole-ethyl ester). Each phase of the mixture was sampled and assayed for ester concentration. The volume of each phase was then measured, and the percentage of substrate partitioned in each phase was calculated. The percentages of substrate partitioned in the aqueous, organic, and oil phases are shown in Figure 3a,b, and c, respectively. The arrow on the *y* axis points in the direction of increasing hetpane/DMF (immiscible/miscible) organic solvent ratio.

Increasing the amount of the water-miscible solvent DMF in the solution (decreasing the heptane/DMF ratio and operating at low buffer concentrations) partitions more substrate into the aqueous phase (Figure 3a). Increasing the amount of the water-immiscible solvent heptane in the solution (increasing the heptane/DMF ratio) partitions more substrate into the organic phase (Figure 3b). At buffer concentrations >40%, most of the ester substrate partitioned to an oil phase (Figure 3c) separate from the aqueous (Figure 3a) and organic phases (Figure 3b). The two most important trends observed were the following: most of the substrate partitioned to an oil phase and an organic heptane phase at buffer concentrations >50%, and increasing the immiscible-to-miscible solvent (heptane/DMF) ratio shifts more substrate out of the oil phase into the organic heptane phase.

Lipases display much higher catalytic activity at a hydrophobic/hydrophilic interface than in solution.[13] This phenomenon of interfacial activation has been shown to occur because of the displacement of a protein loop lid that exposes that enzyme catalytic site when it comes in contact with a hydrophobic surface.[14−16] The observation that the majority of the ester partitioned to an oil phase at buffer concentrations

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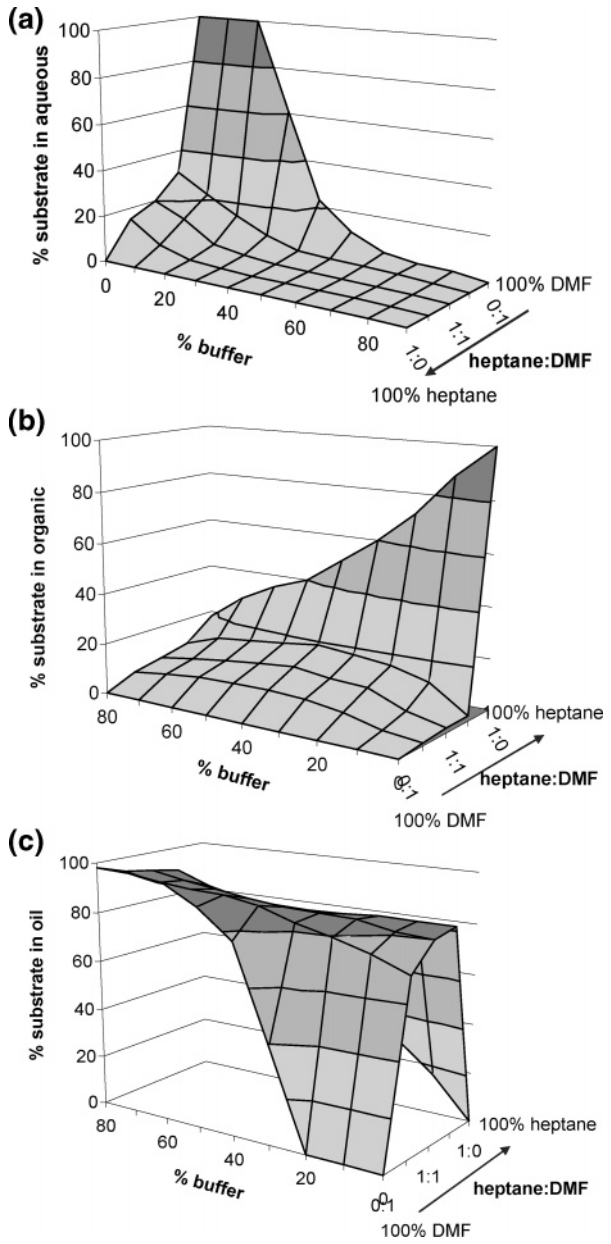

Figure 3. **(a) Percent of substrate ester in aqueous phase as a function of buffer concentration and immiscible-to-miscible solvent ratio. (b) Percent of substrate ester in organic (heptane) phase as a function of buffer concentration and immiscible-to-miscible solvent ratio. (c) Percent of substrate ester in oil phase as a function of buffer concentration and immiscible-to-miscible solvent ratio**

>50% led to the notion that heptane may not be needed in this reaction system to provide an interface for lipase interfacial activation, as an oil/water interface already existed in most of the solutions, including those that did not contain any heptane. The substrate partitioned in the organic (heptane) phase may also have slow mass transfer and, therefore, may be limiting the rate of conversion of the ester in the organic phase.

3.4. Reaction Optimization. *3.4.1. Organic Solvent Concentration Optimization.* On the basis of the information obtained from the substrate solubility and phase-partitioning studies, an attempt was made to eliminate heptane from the reaction system. To show the effect of the immiscible solvent

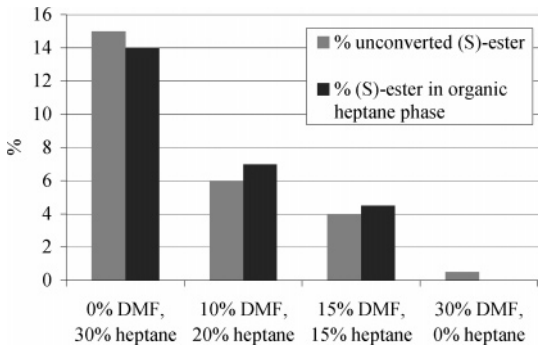

Figure 4. **Amount of unconverted (*S*)-ester vs (*S*)-ester partitioned in the organic heptane phase.**

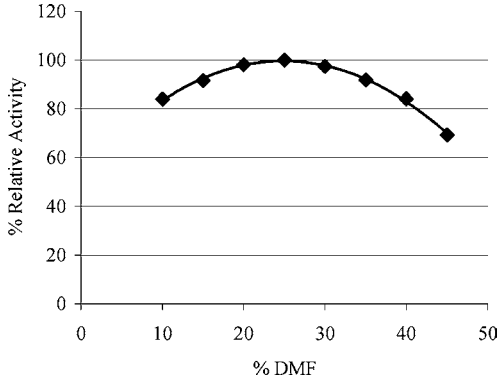

Figure 5. **Enzyme activity vs DMF solvent concentration.**

heptane on the reaction system, reactions were run with a buffer concentration of 70% and varying DMF/heptane ratios of 0:1, 1:2, 1:1, and 1:0. The reactions were run until conversion appeared to stop (48 h). The amount of (*S*)-ester in the organic heptane phase at the start of the reaction (determined by the solubility and phase-partitioning studies) was then compared to the amount of unconverted (*S*)-ester in each reaction system after 48 h. Figure 4 shows that the amount of (*S*)-ester in the organic heptane phase corresponds well with the amount of unconverted material after 48 h, suggesting that the substrate partitioned in the organic heptane phase has very slow mass transport and is limiting the end of reaction rate. Running reactions without heptane enabled conversion to proceed to near completion. On the basis of these data, experiments using DMF as the only organic solvent were undertaken.

Next, the DMF concentration was optimized. Reactions were run with DMF concentrations ranging from 10 to 45%. Conversion was monitored over an initial reaction period of 2 h. Enzyme activity, normalized to the maximum rate observed, is shown in Figure 5. Visually, increasing levels of DMF in the reaction system aided dispersion of the oil phase by making it more fluid. We hypothesize that the DMF partitions to the oil phase, thereby reducing its surface tension and viscosity. With no DMF, the oil phase was so viscous that it would not mix in the reaction vessel. Increasing the DMF concentration increased the dispersion of the oil phase, thereby enabling a faster reaction rate by providing more

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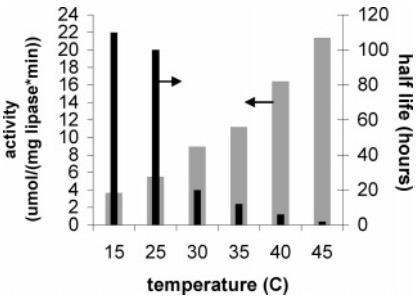

Figure 6. **Enzyme activity and stability (half-life) vs temperature.**

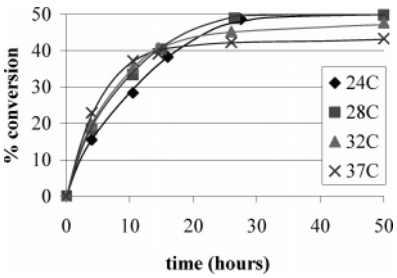

Figure 7. **Temperature study reaction profiles.**

surface area for enzyme interfacial activation and better mass transfer. However, the benefit of increased DMF concentration is balanced by the tendency of organic solvents to deactivate enzymes. DMF at 24% was found to be the optimal DMF concentration for this reaction system.

3.4.2. Temperature Optimization (Enzyme Activity and Stability vs Temperature). To optimize the temperature, a balance must be reached between enzyme activity and thermal deactivation of the enzyme. Enzyme activity in a 75% buffer/25% DMF solution was monitored over 48 h at various temperatures ranging from 15 to 45 °C. Plots of enzyme half-life vs activity were obtained from these data and are shown in Figure 6. The enzyme shows a steep drop in half-life (stability) at temperatures ≥30 °C.

Reactions of 200 mL were run at the following temperatures 24, 28, 32, and 37 °C. The results are shown in Figure 7. The reactions run above 30 °C did not proceed to completion (50% conversion) due to thermal inactivation of the enzyme as suggested by the activity and stability data. The optimum temperature for this reaction was found to be 28 °C. For pilot-plant operations the spec was set at 26 °C (±2 °C). This was done to ensure that the temperature did not reach 30 °C at which point extreme thermal instability is observed.

3.4.3. pH Optimization and Control Strategy. pH optimization studies were conducted with pH control at the 200-mL scale. Figure 8 shows the results of these reactions. The optimal pH range for this process was found to be pH 7.5−8.5.

Controlling the reaction pH at scale was done manually with a shotwise addition of 3.8 N NaOH that would keep the reaction mixture in the pH range of 7.8−8.2. When the pH of the reaction system reached 7.8, a shot of 3.8 N NaOH (equal to 5 mL of 3.8 N NaOH per 1 L of batch) was added to the reaction. A 1-L front run conducted with this manual

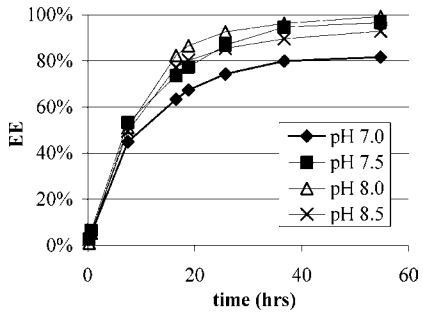

Figure 8. **pH study reaction profiles.**

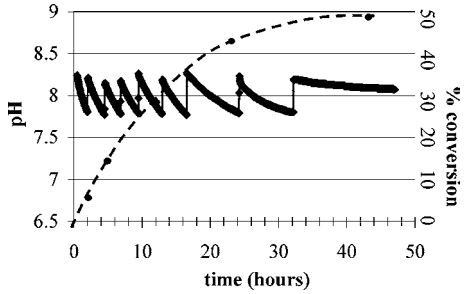

Figure 9. **pH and reaction profile for shot-wise pH control strategy.**

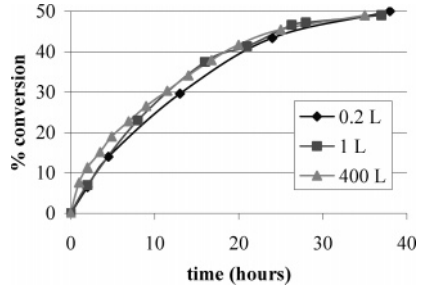

Figure 10. **Reaction profiles comparing a range of reaction scales.**

pH control strategy is shown in Figure 9. Eight shots of base were needed throughout the course of the reaction.

3.5. Front Run and GMP Pilot-Plant Run. A 1-L front run and two 400-L pilot-plant batches were run according to the procedures outlined in sections 2.3.3 and 2.3.4, respectively. The reactions were run at 100 g/L indole-ethyl ester and 32 g/L enzyme concentrations in 200 mM potassium phosphate buffer with 25% v/v THF at pH 8.0 and 28 °C. Figure 10 shows the reaction profiles of the 1-L front run and the 400-L pilot-plant batch compared to a 200-mL reaction under the same conditions. All reactions proceeded to completion within 40 h, and the process was shown to be reproducible at a wide range of scales from 20 to 40 kg.

4. Conclusions

The enzymatic resolution of the indole-ethyl ester intermediate was successfully optimized with the following conditions: 100 g/L indole ester substrate, 32 g/L *P. fluorescens* lipase, 25% v/v DMF, 200 mM potassium phosphate buffer, 28 °C, pH 8.0. An analysis of the substrate solubility and phase partitioning in the reaction system along with an understanding of lipase interfacial activation led to the elimination of the immiscible organic solvent heptane

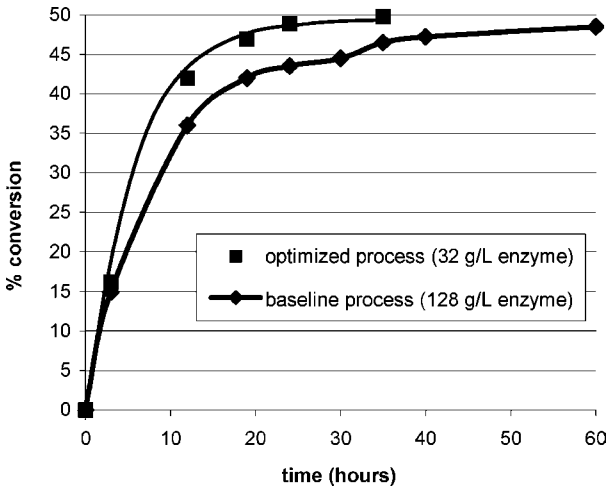

Figure 11. **Optimized vs baseline process reaction profiles.**

from the reaction mixture. Mass transfer (and therefore reaction rate) was optimized by tuning the fluidity of the substrate oil phase and its dispersion in the aqueous reaction system by adjusting the miscible organic solvent DMF concentration. Finally, enzyme stability and activity were determined for a wide range of temperatures, and reaction temperature and pH were optimized.

This work led to a 4-fold decrease in enzyme charge along with an increase in product ee from 95% to >99% and a 2-fold reduction in reaction time (Figure 11). This decrease in enzyme usage enabled a cost-effective process for large-scale production of the desired chiral intermediate. A shot-wise pH control strategy was developed for use in the pilot plant, and reactions were successfully run at 400-L scale, yielding the desired product ester with an ee of 99.73% at 50% conversion (theoretical maximum).

These findings demonstrate that a complete analysis and understanding of the reaction system was essential to the process development and scale-up of this lipase-catalyzed ester resolution. The simple addition of a second-phase immiscible organic solvent (heptane) to solubilize the substrate was detrimental to the reaction system, as it put the reaction in a mass transfer limited regime that negatively affected productivity. Combining the substrate's natural hydrophobicity with the inclusion of a miscible organic solvent (DMF) enabled a suitable interface to exist for interfacial activation of the enzyme, while improving the mass transfer of the substrate, thereby increasing productivity.

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