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Scale-Up of a Chiral Resolution Using Cross-Linked Enzyme Crystals

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Abstract:

The scale-up of a biocatalytic process using a cross-linked enzyme crystal (ChiroCLEC-PC, Altus Biologics Inc., Cambridge, MA) for the resolution of a racemic mixture (*R*/*S*-*sec*-phenethyl acetate to *R*-*sec*-phenethyl alcohol) is reported. Reaction parameters were initially investigated at the 20-mL and 1-L scale to determine optimum reactor conditions for operation at the 100-L scale. At this scale, reproducibility trials were conducted with a single charge of 50 g of ChiroCLEC-PC for the entire nine trials. Results demonstrated that, although there was a steady decrease in reaction rate over the trials (primarily as a result of sub-optimal CLEC catalyst recovery between each trial) all nine reactions proceeded to >48% conversion, with ee_p's of 99.9% and ee_s's of 91−95% recorded. In total, 270 kg of racemate was converted into *R*-phenethyl alcohol and *S*-phenethyl acetate during the reproducibility trials using a standard stirred tank design with pH control. With further work to improve CLEC catalyst recovery between runs, the technology is suitable for industrial exploitation to compete favourably alongside alternative chiral technologies.

Introduction

In a recent article, Persidis[1] predicted that by the year 2000 total pharmaceutical sales will exceed $US 300 billion. About $US 100 billion of these sales will be chiral therapeutics. The annual growth rate for sales of single enantiomer chiral pharmaceuticals has been steady at over 20% for several years. Recently, the U.S. Food and Drug Administration (FDA) has increased the necessity for investigating the chirality of drug candidates by requesting evaluation of the chiral forms of all potential new drugs where technically possible.[2,3] The reason for the upsurge in interest in chirality is that the distinct chiral forms of a drug can have different reactivities and pharmokinetic properties. These events have contributed to a resurgence of interest in developing chiral technologies that should prove to be a lucrative area of biopharmaceutical research and commercialisation.

Stinson,[3] in a report on chiral drugs, stated that among the ways to produce enantiomeric drugs, resolution of racemic mixtures often is more economical than asymmetric syntheses. When companies do choose enantioselective preparations, they have frequently chosen enzyme-catalysed reactions over organic synthetic reactions. Lalonde[4] summarised the main advantages in using enzymes: they are highly regio/stereoselective, they are efficient, they work under ambient conditions at neutral pH, they are environmentally benign, a broad range of reactions are possible, and they are ideal "green" catalysts, producing less waste and consuming less energy.

However, on the downside, enzyme-catalysed reactions can exhibit slow reaction rate, generally occur in higher dilution than organic syntheses, are subject to inhibition, and can be unstable owing to narrow operating conditions, their downstream processing can be complex, and the bulk supply is currently limited to hydrolases. These disadvantages, along with the generally held view by chemists and chemical engineers that enzymes are delicate, probably account for the reason enzymes have yet to make ground in the chemical process industries.

One technology to emerge which addresses the disadvantageous properties of enzymes is the use of cross-linked enzyme crystals (CLEC, a registered trademark of Altus Biologics Inc.).[5] CLEC catalysts are reported to comprehensively address all the cited limitations of biocatalysts by combining the desirable features of a pure catalyst with those of immobilised biocatalysts in a stable form that is amenable to large-scale production.[6] The basis of CLEC technology lies in the crystallisation of the protein catalyst and then cross-linking of these highly pure microcrystals with a bifunctional reagent such as glutaraldehyde. CLEC catalysts are reported to maintain their structure and hence remain active in environments that can be detrimental to enzyme function, including multiple reaction cycles, prolonged exposure to high temperatures, near-anhydrous organic solvents, and aqueous−organic solvent mixtures.

This paper describes the scale-up of a biocatalytic process using a CLEC catalyst for the resolution of a racemic mixture and assesses whether such a technology is applicable for use in the pharmaceutical/chemical process industries to compete alongside alternative chiral technologies. It is an attempt to show organic chemists and chemical engineers in the specialised organics industry that biocatalysis can fit well into overall chemical processes that convert material inputs into products.

In this work, we follow a structured approach to biocatalytic process design as proposed by Woodley and Lilly[7] in order to select the correct process on the basis of fundamental

(1) Persidis, A. *Nature Biotechnol.* **1997**, *15*, 594−595.
(2) Caldwell, J. *Chem. Ind.* **1995**, 6 March, 176−179.
(3) Stinson, S. C. *Chem. Eng. News* **1995**, *73* (Oct 9), 44−74.
(4) Lalonde, J. *Chem. Eng.* **1997**, *9*, 108−112.
(5) Margolin, A. L. *Trends Biotechnol.* **1996**, *14*, 223−230.
(6) Lalonde, J. *Manuf. Chem.* **1996**, *4*, 19−22.
(7) Woodley, J. M.; Lilly, M. D. *Chem. Eng. (Rugby, England)* **1996**, *611*, 28−30.

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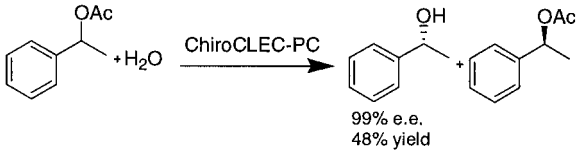

Figure 1. **Hydrolysis of racemic *sec*-phenethyl acetate by ChiroCLEC-PC.**

characterisation of the reactants, products, reactions, and biocatalyst. The biocatalytic process chosen is the chiral resolution of *R-sec*-phenethyl alcohol from racemic *R*/*S-sec*-phenethyl acetate using ChiroCLEC-PC (Figure 1).

Materials and Methods

Biocatalyst. ChiroCLEC-PC (a lipase from *Pseudomonas cepacia*), sourced from Altus Biologics Inc. (Cambridge, MA), was used for the work. The CLEC catalyst is supplied in slurry form in a 10 mM Tris, 10 mM $CaCl_2$, pH 6.0 buffer.

Inherent Activity Assay. CLEC esterase activity (units per mg of ChiroCLEC-PC) was measured as the rate of hydrolysis of a 6% (v/v) glycerol triacetate (BDH no. 28456 4H, >99% by GLC) solution, using a Mettler DC40 RC Memo Titrator. One unit is the activity of ChiroCLEC-PC that hydrolyses 1 μmol of glycerol triacetate per minute, at pH 6.0 and 25 °C.

CLEC Characterisation: Stability Trials. To 1.5-mL plastic Eppendorf tubes were added 475 μL of a buffer (pH 3.0 (10 mM citric acid), 4.0, 4.5, 5.0, 5.5 (10 mM sodium acetate), 6.0, 7.0, and 8.0 (10 mM phosphate)) and 25 μL of ChiroCLEC-PC (90.9 g L^{-1}), and these solutions were incubated at 30, 40, 50, or 70 °C. Ten-microliter samples were taken at intervals for inherent activity determination.

Solvent Effects. Two milliliters of solvent (methanol, ethanol, acetone, isopropyl alcohol, *sec*-phenethyl acetate, and water (control)) and 1 mL of ChiroCLEC-PC catalyst (10 g L^{-1}) were put into a sealed glass test tube for 1 h with regular vortexing. The solvent/CLEC catalyst mixtures were filtered (2.5 cm diameter, No. 1 Whatman filter paper) under vacuum, and the CLEC cake was washed with reverse osmosis (RO) water (10 mL) followed by 10 mM phosphate buffer, pH 6.0 (10 mL). *Note:* Phenethyl acetate-suspended ChiroCLEC-PC was washed only with 10 mM phosphate buffer, pH 6.0 (30 mL). The washed CLEC cake was resuspended (10 mM phosphate buffer, pH 6.0) to 1 mL for activity determination.

Two-Liquid-Phase Reaction—20-mL Scale. Fourteen milliliters of 0.2 M phosphate buffer, pH 6.0, and 6 mL of *R*/*S-sec*-phenethyl acetate were agitated in a 50-mL glass, jacketed vessel (attached to a circulating waterbath at 30 °C) for 1 h, and then 1 g L^{-1} (aqueous basis) ChiroCLEC-PC was added. The reaction was allowed to run for 24 h, with samples being taken periodically to monitor the reaction progress.

Experimental Design—1-L Scale. A series of experiments were planned using a factorial design to allow us to determine the effects of all the major parameters while reducing the number of experiments required. Parameters varied were stirrer speed, impeller type, and CLEC loading. A total of 21 experiments were required, including five duplicates. The reactor conditions for these experiments are shown in Table 1.

Table 1. **Reactor conditions for 1-L scale runs**

run	agitation (rpm)	impeller type	[ChiroCLEC-PC] in reactor (g L^{-1})	phase ratio (initial)
1	700	rushton	1	0.1
2	500	rushton	1	0.4
3	700	marine	1	0.1
4	500	marine	0.5	0.1
5	500	marine	1	0.1
6	500	rushton	1	0.1
7	700	rushton	0.5	0.1
8	700	rushton	1	0.4
9	700	marine	0.5	0.4
10	700	rushton	0.5	0.1
11	500	rushton	0.5	0.1
12	700	rushton	1	0.1
13	500	marine	0.5	0.1
14	700	marine	0.5	0.1
15	700	marine	1	0.1
16	700	marine	1	0.4
17	500	rushton	0.5	0.4
18	500	marine	1	0.4
19	500	rushton	1	0.4
20	500	marine	0.5	0.4
21	700	rushton	0.5	0.4

Two-Liquid-Phase Reaction—1-L Scale. These reactions were carried out in 2-L glass bioreactors (New Brunswick Multigen System), with a working volume of 1 L. Agitation was achieved by a magnetically coupled agitator with either a single rushton turbine or a marine impeller. pH was controlled to 6.0 by the addition of 10 M NaOH. The agitation rate was set to at least 500 rpm in order to ensure complete homogeneity of the phases. The aqueous (RO water) and organic (*sec*-phenethyl acetate) phases were added to the reactor and allowed to equilibrate (under agitation) for 30 min at 30 °C. ChiroCLEC-PC (between 0.25 and 1 g L^{-1}, reactor basis) was added to the reactor. The weight of NaOH added was monitored over a 24-h period. One-milliliter samples were taken at 0, 1, 2, 3, 4, 6, 8, and 24 h for GC analysis.

Conditions for all the 1-L experiments were planned, and results were analysed using statistical software, EChip v6.01 from Echip Inc. The design was a simple linear screen, with the analysis concentrating on interaction effects.

CLEC Recovery—1-L Scale. Reactor contents were filtered through No. 42 Whatman filter paper in a Buchner funnel. The CLEC catalyst was rinsed using the desired solvent solution (generally 70% IPA in RO water). This wash was repeated as required and was followed by one or more washes using a 10 mM phosphate buffer, pH 6.0. The CLEC catalyst was removed from the filter and resuspended in 5 mL of 10 mM phosphate buffer, pH 6.0 for storage. The ChiroCLEC-PC was tested for activity using the inherent activity assay.

Two-Liquid-Phase Reaction—100-L Scale. These reactions were carried out in a 250-L SS316 bioreactor (IRL in-house design), with a working volume of 100 L. Agitation was achieved by a bottom entry agitator with a single 45° pitched six-bladed impeller. pH was controlled to 6.0 by

the addition of 18 M NaOH. The agitation rate was 200 rpm in order to ensure complete homogeneity of the phases. The aqueous (RO water) and organic (*sec*-phenethyl acetate) phases were added to the reactor with a phase ratio of 0.3 and allowed to equilibrate (under agitation) for 30 min at 30 °C. ChiroCLEC-PC (initially at 0.5 g L^{-1}, reactor basis) was added to the reactor. The weight of NaOH added was monitored over the course of the reaction, and samples were taken at intervals for GC analysis.

pH Control. The reaction produces acetic acid, which required neutralisation. This was done using ~18 M NaOH dosed in via a peristaltic pump that was controlled using a software package (FixDMACS, Intellution Software) and a PLC (Modicon Compact). For the first hour of each run, pH deadband was set at ±1 pH, dropping to ±0.2 pH for the next phase, with a final reduction to ±0.1 pH for the final 30−60 min of each run.

Data Logging. Throughout the 100-L reactions, temperature, pH, and mass of NaOH consumed were monitored using the data logging capabilities of the reactor control system (FixDMACS SCADA software). Data were logged directly to the hard disk of the machine before being transferred to a personal computer for analysis and manipulation.

Sampling from a Two-Liquid-Phase Reaction—1-L and 100-L Scale. Samples of ~1.5 mL were placed in 1.5-mL glass reaction vials with conical bases and sealed with a PTFE lined lid. These were centrifuged (at room temperature) at 4000 rpm for 5 min. Three phases separated: organic, aqueous, and solid CLEC catalyst (at low catalyst concentrations, the crystals were frequently found adhered to the liquid−liquid interface). A 100-μL sample of the liquid was taken (with an aqueous:organic ratio equivalent to the phase ratio in the vessel). This was extracted twice with 1 mL of butyl acetate, using octanol internal standard, and was analysed by chiral GC.

Chiral GC. A J&W 30-m × 0.25-mm × 0.25-μm CyclodexB column was used. Hydrogen carrier was at 85 kPa, split 50 mL/min, purge 2.5 mL/min. Sample was 10 mg/mL in methanol. Manual injection of 0.1 μL was done. Temperature program was as follows: initial, 80 °C; gradient rate, 1 °C/min; final temperature, 99 °C.

Monitoring Reaction. Reaction progress was monitored by viewing the mass of NaOH consumed. As the reaction neared completion, consumption of NaOH (used to neutralise the acetic acid produced in the reaction) dropped to very low levels. The reaction was considered complete after a period of 60 min with no addition of NaOH. Samples were taken at intervals to allow us to compare the theoretical conversion as calculated from NaOH consumption with actual conversion at a later date.

Catalyst Recovery—100-L Scale. Reactor contents were filtered through a 27-μm filter cloth (27-μm nylon monomesh, Filter Corp., Auckland, New Zealand) (runs 1−3) or a 5-μm filter cloth (heavy duty nylon, code BCNY-HD005-5, NZ Filter Specialists Ltd., Auckland, New Zealand) (runs 4−9). The CLEC catalyst was rinsed by resuspending the catalyst in 10 L of RO water for 10−30 min, followed by filtration. This was repeated four times and was followed by a wash using 1 L of 10 mM phosphate buffer, pH 6.0. The CLEC catalyst was removed from the filter and resuspended in 500 mL of 10 mM phosphate buffer, pH 6.0, for storage. The ChiroCLEC-PC was tested for activity using the inherent activity assay. The CLEC catalyst recovered from each run was used as the catalyst in the subsequent run. A small sample of the recovered CLEC catalyst from each run was taken and dried for 24 h at 80 °C. The dry weight of this sample was used to estimate the total dry mass of ChiroCLEC-PC present in the subsequent run.

Experimental Rationale

Solvent Effects. Initial work concentrated on determining the effects of solvents and the substrate on the activity of the ChiroCLEC-PC. Methods for use of CLEC catalysts recommend that the catalyst be washed in a suitable solvent to improve the catalyst life. To this end, a number of solvents selected from those used in similar systems in the literature were examined for possible detrimental effects on the catalyst activity.

Reaction Characteristics. ChiroCLEC-PC (Altus Biologics Inc) is used to preferentially hydrolyze the *R*-phenethyl acetate to the *R*-phenethyl alcohol. There is interest in this fine bulk chemical for synthesis, and it is presently produced via asymmetric reduction of acetophenone.

At the small scale, CLEC was found to confer the greatest stability and activity for this reaction at pH 6.0 and 30 °C (results not shown). These conditions were used for all experiments referred to here.

Phenethyl acetate is poorly water soluble (approximately 1.5 mM), and the product phenethyl alcohol partitions between the organic (phenethyl acetate) and aqueous phases in the ratio 9:1. Interestingly, phenethyl acetate and phenethyl alcohols have specific densities of 1.028 and 1.02, respectively, and there is high interfacial tension between phenethyl acetate and the aqueous phase. Therefore, to achieve a high substrate concentration in the reactor, the phenethyl acetate will be present as a discrete second liquid phase.

While the ChiroCLEC-PC appeared to act as a classic lipase, with the CLEC catalyst adhering to the interface, it is unlikely that the reaction was occurring at the interface on a molecular level. Lalonde et al.[8] showed that there is no interfacial activation with ChiroCLEC-CR by studying systems with and without an interface and observing that the reaction rate in biphasic systems was not appreciably different from the reaction rate in single-phase systems. It is thought that this is because the lipase is held rigidly in the CLEC structure and is not able to align itself with the interface.

While the reaction may not be occurring at the interface on the molecular level, because of the propensity of the ChiroCLEC-PC to physically locate itself at the interface and in order to maximize mass transfer between the phases, experimental conditions were chosen that facilitated high interfacial area.

(8) Lalonde, J.; et al. *J. Am. Chem. Soc.* **1995**, *117*, 6845−6852.

Acetic acid is a byproduct of this reaction, and therefore pH has to be controlled to maintain optimum activity of the ChiroCLEC-PC. A stirred tank reactor was selected for this reaction as it provides a high interfacial area between the liquid phases and also allows easy control of pH. It is worth noting that the stirred tank reactor is the type of reactor most commonly found in process plants and a type of equipment that most engineers are familiar with.

A reaction with a phase ratio (volume fraction organic) of 0.3 will produce approximately 1 mol L^{-1} of acetic acid. Because of this, it was impractical to buffer the system (aqueous phase), and instead alkali addition was used as a means to control the pH. This was also an easy and accurate method to follow the rate of reaction. The disadvantage of this method is that, on a large scale, large quantities of alkali are required. Therefore, a high molarity sodium hydroxide (18 M) was used to minimise the increase in volume of the reactor contents.

Reactor Operation. By maximizing the liquid−liquid interfacial area, the reaction rate is unlikely to be affected by diffusion rate limitations. The agitation rate, the phase ratio, and the impeller type (type of mixing) will affect the interfacial area. Increasing the liquid−liquid interface by increasing the agitation rate and using a higher shear impeller type will increase the shear, a situation that is detrimental to traditional biocatalysts (above a certain level).

To operate an efficient process, one needs to maximize the product produced per unit time and biocatalyst mass. For an optimal system, the catalyst concentration needs to be just sufficient to complete the reaction in the required time frame. Use of more catalyst than the minimum would be economically undesirable, while the desired time frame will be highly dependent on the cost structure of the facilities being used.

These four reaction variables—impeller type, phase ratio, biocatalyst concentration, and agitation rate—were identified as parameters to be further investigated. A full factorial experimental design was carried out to investigate the effect of these parameters and their interactions on reaction performance.

Results

Effect of Solvents on Catalyst Activity. The effect of mixing the ChiroCLEC-PC in various organic solvents was quite severe even after 1 h (Figure 2). All the common solvents, which were originally intended to be used for washing the CLEC catalyst between runs, decreased the inherent activity by >50%. However, the drop in activity in the presence of the reaction substrate, phenethyl acetate, was within experimental error of the tests.

Two-Liquid-Phase Reaction Trial−20-mL Scale. Figure 3 shows the reaction profile of a ChiroCLEC-PC resolution of racemic *sec*-phenethyl acetate on the 20-mL scale. Chiral GC results showed that *S*-phenethyl acetate was unaffected by ChiroCLEC-PC activity and no *S*-phenethyl alcohol was produced.

Two-Phase Reaction−1-L Scale. The results from the 21 small-scale (1 L) reactions are shown in Table 2, while the qualitative effects of the reactor parameters are as follows:

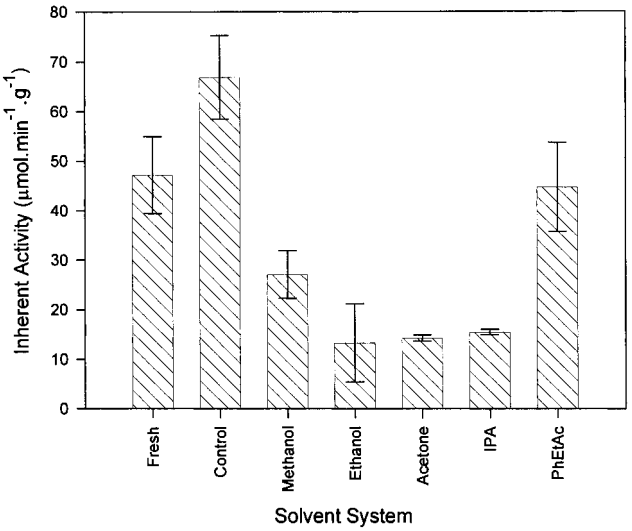

Figure 2. **Effects on ChiroCLEC-PC activity after mixing for 1 h in various solvents.**

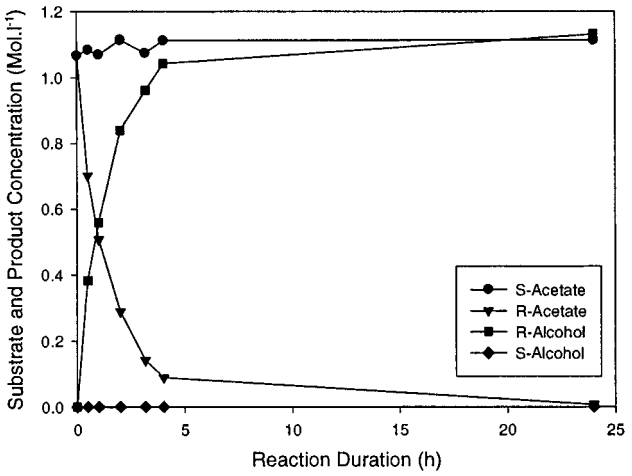

Figure 3. **Enantiomeric analysis of a 20-mL scale ChiroCLEC-PC-catalysed *R*/*S*-*sec*-phenethyl acetate hydrolysis reaction.**

(i) CLEC concentration has a positive effect on reaction rate and a negative effect on specific initial activity (both at the 5% level).

(ii) Phase ratio has a positive effect on reaction duration (at the 1% level).

(iii) [rpm × phase ratio] and impeller type both have a positive effect on the initial activity.

The qualitative effects were determined by multivariate analysis using a statistical software package. The most significant effect was the effect of phase ratio on the time taken for completion of the reaction. This was expected, as the greater the amount of substrate, the longer it will take to completely react at any given rate. The ChiroCLEC-PC concentration has a negative effect on the specific initial activity (Figure 4) but a positive effect on the overall reaction rate. The initial activity was significantly affected by the agitation rate and phase ratio interaction (Figure 5), and higher reaction rates were also found with the rushton impeller, a higher shear impeller than the marine impeller. It appears that the CLEC catalyst is not detrimentally affected by high shear in the reactor, with the rpm and impeller type having no statistically significant effects on the inherent

Table 2. **Results from 1-L scale experiments**

run	initial activity (mmol min^{-1}) NaOH basis	specific activity (mmol min^{-1} g^{-1}) NaOH basis	time for 48% conversion (h)	*E* at end of reaction	rate to 48% (mmol/h)
1	11.7	11.67	1.00	1256	621.2
2	6.6	6.59	24.00	2001	103.5
3	10.1	10.12	1.15	2227	540.2
4	5.5	11.00	2.62	1821	237.1
5	7.9	7.91	4.35	1072	142.8
6	18.0	18.00	1.23	1635	505.0
7	14.2	28.36	2.08	993	298.7
8	12.7	12.67	5.46	11541	455.1
9	14.5	29.00	18.33	5476	135.6
10	4.0	8.00	3.35	1695	185.4
11	10.7	21.34	1.69	1532	367.6
12	16.0	16.00	1.46	2095	425.5
13	4.5	9.00	2.58	9717	240.8
14	7.5	15.00	2.54	9348	244.6
15	6.5	6.55	1.65	976	376.5
16	9.0	9.00	7.46	1906	333.1
17	4.2	8.40	23.62	2389	105.2
18	7.7	7.67	6.81	4105	364.9
19	8.6	8.62	7.48	4371	332.2
20	5.3	10.60	7.68	2063	323.5
21	6.2	12.46	18.69	3334	132.9

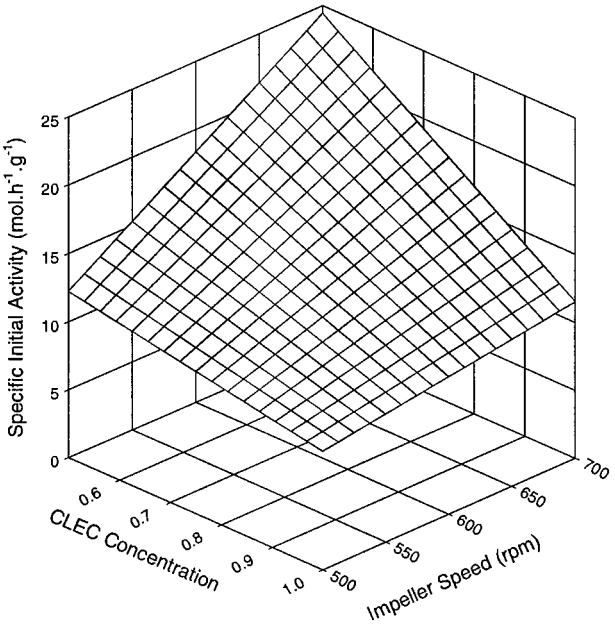

Figure 4. **Specific initial activity as a function of CLEC catalyst concentration and impeller speed.**

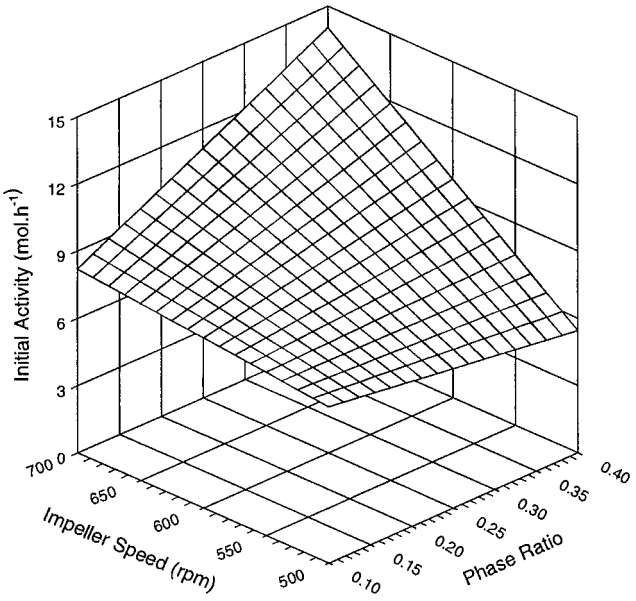

Figure 5. **Initial activity as a function of phase ratio and impeller speed.**

activity of the ChiroCLEC-PC after the completion of the trial. This confirms the results presented by Lalonde et al.[9]

Although there were no unexpected results, this exercise highlighted the relevant significance of parameters in this system and their interactions. The findings from this work were used to define the reactor conditions for scaling-up to the 100-L scale.

100-L Experimental Conditions. Phase ratio was decided as 0.3 mainly for economic reasons. Any phase ratio between 0.1 and 0.4 could be chosen, depending on the required economics. A catalyst concentration of 0.5 g L^{-1} was chosen in conjunction with the phase ratio to fit the economic constraints. Agitation at 200 rpm on the 100-L scale produced a high level of agitation in the reactor, arguably even greater than that used in the 1-L runs. The 45° pitched blade impeller was the closest in type to the rushton impeller that was available for the 100-L reactor. The temperature of 30 °C and pH 6.0 were chosen from the optima for CLEC activity and stability as determined at the 1-mL scale (results not shown).

Pilot Plant Trials and Reaction Reproducibility. The identification of the significant reactor parameters allowed this reaction to be scaled-up for the pilot plant (100-L working volume). The reaction parameters were chosen to ensure that the process took approximately 18 h, whilst

(9) Lalonde, J.; et al. *Proceedings of Industrial Biocatalysis: InBio 96*; Spring Innovations Ltd.: Bramhall, England, 1996.

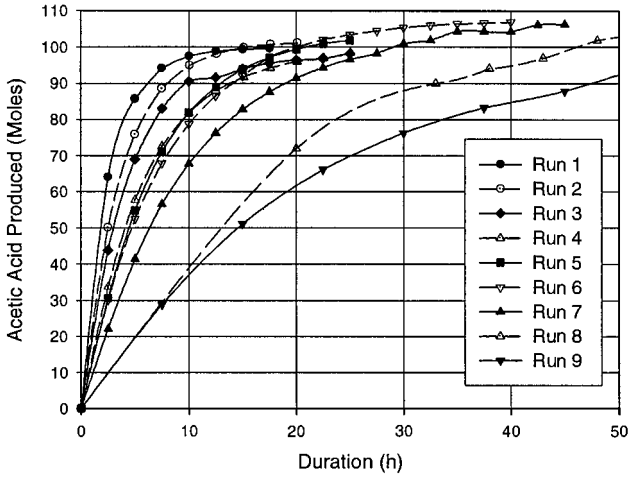

Figure 6. **100-L reproducibility trials—hydrolysis of *sec*-phenethyl acetate using ChiroCLEC-PC.**

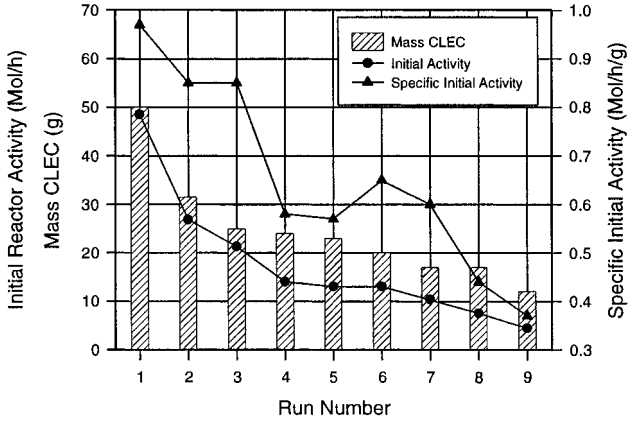

Figure 7. **100-L reproducibility trials—drop in initial activity and CLEC catalyst.**

maintaining productivity (maximizing product formed per unit time and per unit mass of catalyst). The reactor conditions are outlined in the Materials and Methods Section.

During run 1, the reaction reached 48.8% conversion in 18 h, with enantiomeric excess of the product and substrate of 99.9% and 94.8%, respectively. The initial activity of the reaction was 48.5 mol h^{-1}, and the specific initial activity was 16.2 mmol min^{-1} g^{-1}.

Run 1 demonstrated that the reaction could run efficiently on the pilot scale with high enantioselectivity. Runs 2−9 were conducted in a manner simulating a production process with recovery of the ChiroCLEC-PC for use in the next identical production run. No fresh top-up of catalyst was performed, so that the effects of processing (such as catalyst recovery and effects due to repeated contact with solvent) on the reaction rate and enantioselectivity could be assessed.

The ChiroCLEC-PC from these pilot-scale runs was recovered using filtration. After recovery, the CLEC catalyst was resuspended in 10 mM phosphate buffer, with 1 mM $CaCl_2$ at pH 6.0, to a total volume of 500 mL. A 1-mL homogeneous sample was taken to determine the CLEC catalyst dry weight. This process was repeated for eight reactor trials, and at no time was any fresh catalyst added.

Figures 6 and 7 respectively are graphical representations of the reaction profiles and trend in the drop of specific initial

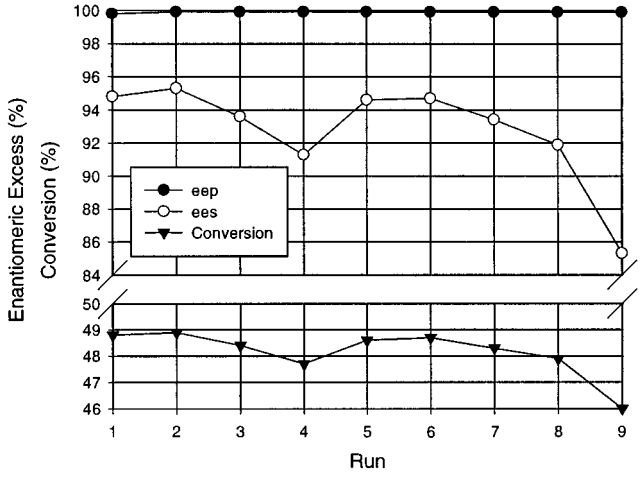

Figure 8. **100-L reproducibility trials—enantiomeric excess and conversion.**

activities as the runs proceed to nine. It can be clearly observed that there is a steady decrease in reaction rate over the nine runs (Figure 7). This decrease in activity by a factor of approximately 10 appears to be due to a physical loss of catalyst (from 50 to 12.5 g) and a drop in catalyst activity (as indicated by the specific initial activity) by a factor of 2.5 (from 0.96 to 0.37 mol h^{-1} g^{-1}).

The extent of the loss of CLEC catalyst during the recovery process was not realised until after run 3 was completed due to delays in obtaining the dry weights of samples. Thereafter, the original 27-μm filter was used in conjunction with a similar 5-μm cloth.

While the majority of the catalyst loss occurred during the first three runs, some loss was still evident after run 4. It was initially thought that the high agitation rate of 200 rpm in the reactor may have broken up the ChiroCLEC-PC into smaller particles which were then lost during filtration, but literature indicates that this is unlikely to have been the case.[10] Subsequent contact with Altus revealed that the batch of ChiroCLEC-PC used had an average particle size of 20-μm and a standard deviation of 6.67 μm.[11] This indicates that it is certainly possible that the smaller particles were lost during the recovery process in the first three runs. The combination of reducing the pore size of the filter cloth and the fact that the majority of the CLEC catalyst remaining would tend to be at the larger end of the size distribution would lead to the observed trend of sharply decreasing mass of catalyst over the first few runs, followed by a sustained but lower loss over the remaining runs. It is apparent that the pore size of the filter cloth was still too coarse to retain all the catalyst. In future work, it was decided to use an inert material added with the CLEC catalyst to improve both the filtration rate and the overall recovery of the catalyst.

Even after nine runs with only a quarter of the original CLEC catalyst present (38 g lost), the reaction proceeded to completion (albeit for a longer duration as the runs stacked up), with no adverse effect on the quality of the reaction, as shown in Figure 8.

(10) Lalonde, J.; et al. *Proceedings of Chiral USA '97 USA*; Boston, MA, May 1997; Spring Innovations Ltd.: 1997.
(11) Baust-Timpson, M., Altus Biologics. Personal communication, 1998.

Since this experimental work was completed, improvements have been made in the recovery and reuse of CLEC catalysts. With ChiroCLEC-PA, recovery and reuse has been proven for 500 cycles without any measurable material or activity loss.[10]

Enantiomeric excess of the product is 99.9%, and that of the substrate is between 91 and 95% (for runs 1−8). All reactions (apart from run 9) were allowed to run to approximately 48% conversion. Enantioselectivity (*E* values) were greater than 10^4 for all reaction runs.

Discussion

The key issues to consider with regard to the suitability of this technology for industrial-scale application are, in our view, the following:

(1) What is the cause of the decline in activity as runs proceed through the cycles and can it be overcome?

(2) Is the quality of the reaction affected by the recycling of the catalyst?

(3) Given the ChiroCLEC-PC charge, is the yield of enantiomers acceptable and can they be manufactured for an acceptable cost?

At this juncture, only some of these questions can be answered.

(1) Decline in Catalyst Activity. Run 1 demonstrated 48.8% conversion in 18 h with an initial specific activity of approximately 1 mol h^{-1} g^{-1} ChiroCLEC-PC. By run 9, this had decreased to 0.37 mol h^{-1} g^{-1}, or 38% residual activity. Over these runs, it was observed that 75% of the CLEC catalyst was lost. It is a reasonable assumption that this loss was mainly of the smaller CLEC particles rather than the larger particles breaking through the filter. These smaller particles probably exhibit higher specific activity owing to a higher surface area-to-volume ratio.

Further repeat runs are required which retain 100% ChiroCLEC-PC recovery between cycles. A stirred tank design incorporating a 0.2-μm flat sheet filter at the outlet would be an ideal CLEC reactor. The inclusion of an inert filter aid would be necessary to facilitate filtration of the products from the reactor at the completion of each batch. The CLEC catalyst would remain in the reactor amongst the inert filter aid cake caught on the 0.2-μm filter ready, after washing, for the next substrate charge. In this manner, no handling of small CLEC catalyst quantities is required between runs, minimising catalyst losses.

Until CLEC catalyst losses are minimised by this (or other) means, the effect of other processing activities and conditions on the activity of the CLEC catalyst cannot be adequately addressed. Figure 2 demonstrates that pure phenethyl acetate had no effect on the ChiroCLEC-PC activity after 1 h. This needs to be repeated for a much longer duration, and it also cannot be discounted that the phenethyl alcohol product is affecting ChiroCLEC-PC activity.

(2) Quality of Reaction. Even though there was a 62% loss in specific activity and a 75% physical loss in catalyst mass, the quality of the reaction was unaffected, with enantiomeric excess for product of >99.9% recorded for all nine runs. Enantiomeric excess for substrate is lower, at 91−95%, as runs were stopped before reaction completion owing to time constraints.

(3) Yield and Cost. Fifty grams of ChiroCLEC-PC was used to convert 270 kg of racemate over the nine runs. Without catalyst loss, it is estimated that this could be further improved to at least 20 cycles. Undoubtedly, there is a limit to the number of cycles one could subject ChiroCLEC-PC to under these operating conditions.

If 20 cycles were possible, each with a 30-kg racemate charge, then this would produce 223 kg of chiral *R-sec*-phenethyl alcohol. Fifty grams of ChiroCLEC-PC costs $US 5000 at $100 g^{-1} (Altus Biologics, personal communication). This ChiroCLEC-PC kinetic resolution process is relatively simple to operate and would not require a large expense in capital equipment. The other major operating costs would be the substrate cost (600 kg × $US 15 kg^{-1} = $US 9000) and utility costs during distillation to separate the *S*-acetate from the *R*-alcohol. Depending on the production scale and market demand, the value of the product should be between $US 100 and 1000 kg^{-1}, which would indicate that this process is economically feasible.

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