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Kinetic resolution of 1-phenylethanol in the spinning mesh disc reactor: Investigating the reactor performance using immobilised lipase catalyst

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Abstract

The spinning mesh disc reactor (SMDR) is an innovative catalytic rotating reactor to aid process intensification. In this study, the application of the SMDR has been demonstrated for the enzymatic kinetic resolution of racemic 1-phenyethanol using amano lipase immobilised on wool as a catalyst. Physical characterisation of wool was carried out to confirm the presence of lipase. The reaction was tested for a range of solvents and temperatures for both free and immobilized lipase and the optimised reaction conditions were employed in the SMDR for different flowrates and spinning speeds. The SMDR showed better reaction efficiency compared to the batch reactor: the feed throughput was scaled-up from 10 ml to 250 ml and the productivity increased from 7.05 g l^{-1} h^{-1} in batch to 10.92 g l^{-1} h^{-1} in the SMDR. An increase in catalyst loading was achieved by adding more lipase cloths and the reaction rate increased from 0.16 mmol min^{-1} (one cloth) to 0.28 mmol min^{-1} (three cloths). These results show the first demonstration of novel reactor design for scale-up of enzymatic kinetic resolution using an inexpensive lipase. The SMDR thus shows potential for scale-up and continuous processing for versatile applications in the fine chemicals and pharmaceutical industry.

Keywords: Spinning mesh disc reactor; lipase immobilisation; kinetic resolution; intensified enzyme catalysed reaction; reaction scale-up

1. Introduction

There is a demand for enantiopure compounds for bulk manufacture of pharmaceuticals and agrochemicals [1]. Biotransformation of racemates into enantiomers using enzymes is an attractive option owing to the high regio, enantioselectivity and a safer alternative to chemical synthesis [2, 3]. The difference in the reaction rates of the enantiomers with the enzyme (chiral catalyst) results in the resolution of the racemates. One such important reaction is the resolution of 1-phenylethanol via acylation using a suitable acyl donor and catalysed by lipase, as the chiral derivatives of phenylethanol are often used as starting materials in the pharmaceutical and natural products industries [4-9]. Non-amano lipases been studied extensively for this reaction as they show high selectivity and product yield [6, 8-11]. However, they are very expensive and not economical for process scale-up. An alternative is to use inexpensive amano lipases for kinetic resolution, although in comparison they have shown maximum conversions of approximately 30% and 97% enantioselectivity [5]. Other challenges with this reaction are prolonged reaction times, low recoverability of expensive enzymes, solvent compatibility and process scale-up [11, 12].

The spinning disc reactor (SDR) is a promising process intensification technology in which the centrifugal force associated with the spinning disc causes the liquid feed impinged on the centre to spread out into a thin film with high surface shear [13, 14]. This is responsible for rapid mixing, accompanied by short residence times for reactions and improving the overall heat and mass transfer [15, 16]. The application of a SDR has been well reported in literature for a wide range of chemical processes, such as precipitation, polymerisation, photocatalytic reactions, production of pharmaceuticals and synthesis of nanoparticles [15, 17-21]. The spinning mesh disc reactor (SMDR) is a novel reactor built on a similar concept to the SDR, but additionally houses a cloth with an immobilised catalyst attached on the disc surface allowing the centrifugal force of the spinning disc to create a thin film over and within the cloth. This improves mixing and mass transfer within the film, accelerating the reaction rate, as well as protecting the catalyst from the shear forces associated with the spinning disc [22]. The SMDR is characterised by two key design parameters, the spinning speed and the feed flow rate. Optimisation of these parameters are achieved based on the effect of the shear forces on the catalyst stability and the optimal flowrate is determined based on the catalyst activity and concentration.

Textile based supports have diverse structural and mechanical properties and are cheap [23]. Wool has shown effective support properties for both biological and non-biological catalysts as it is rich in surface functional groups, allowing the catalysts to be firmly bound onto the surface, offers a high surface area and a sustainable alternative to synthetic supports [24-26]. Wool has been demonstrated as a catalyst support in the SMDR so far for two reaction systems, enzymatic hydrolysis of tributyrin (water-enzyme) [22, 27] and copper catalysed Henry reaction (organic solvent-metal catalyst) [25]. Intensification of enzymatic hydrolysis of tributyrin using immobilized lipase was achieved successfully in the SMDR and both the rate and the final conversion increased compared to a traditional batch reactor. Liquid flow characterisation studies indicated that the flow patterns in the SMDR was analogous to a well-mixed reactor in contrast to the plug flow behaviour of the SDR [27]. The catalyst loading was easily increased by increasing the number of cloths with the immobilised lipase on the disc. We also showed that the SMDR is suitable for traditional organic synthesis, as demonstrated by the Henry reaction catalysed by copper triflate immobilised on wool, achieving an increase in both final conversion and rate [25]. An additional advantage with the SMDR is the use of immobilized catalysts which enables easy catalyst separation, recovery and reuse. One of the aims of this research is therefore, for the first time, to achieve process intensification of enzyme catalysed synthesis in organic solvents. This will also further demonstrate the versatility of the SMDR. Also, to the best of the author's knowledge, enzyme catalysed organic synthesis in other process intensified reactor, like microreactors have been investigated on a small scale (~ 10 ml) and the present study demonstrates the application of the reaction on a larger scale [28].

The aim of the present work is thus to: (i) demonstrate the potential of the SMDR for kinetic resolution of 1-phenylethanol using amano lipase immobilised on wool as a catalyst, (ii) optimise the reaction conditions in the SMDR based on flow rate and spinning speed. Physical characterisation will be carried out to verify immobilisation of lipase on wool. Reactions in batch will be carried out using a range of different solvents and temperatures to determine the optimum condition for use in the SMDR for both free and immobilised lipase.

2. Materials and Methods

2.1 Materials

Unbleached wool (1.5 mm thickness, cream colour) was obtained from Urbanara (Berlin, Germany). All chemicals, lipase from *pseudomonas florescence* (PF) and solvents were purchased from Sigma Aldrich and used as received unless specified. All solutions were prepared using deionised water (Elga).

2.2 Lipase immobilisation on wool

A detailed immobilisation procedure has been published elsewhere [29]. In summary, woollen cloth was cut into circular pieces (12 cm diameter). The cloth was pre-treated using a solution containing hydrogen peroxide and sodium silicate in a pH 9 carbonate buffer for 70 minutes. Surface modification of the pre-treated cloths was carried out by dipping the cloths in 2% PEI (polyethylene imine) solution at pH 8 for 2 hours at room temperature. The cloths were thereafter rinsed with deionised water and soaked in 2 gL^{-1} lipase solution in pH 6 phosphate buffer for 24 hours. The lipase immobilised cloths were then crosslinked using 0.5% glutaraldehyde in pH 6 phosphate buffer for 10 minutes and rinsed with deionised water to remove excess enzyme and crosslinking solution. The lipase activity was measured using the tributyrin emulsion method [22] and was found to be 248 Ug^{-1} of cloth, where one enzyme unit (U) is defined as the amount of lipase that catalyses the release of 1 µmol of butyric acid per minute.

2.3 Material characterisation of wool

Lipase wool was characterised by Fourier transform infrared spectroscopy (FTIR) using a Perkin-Elmer-100 FTIR spectrometer. Plain and lipase wool samples were scanned between the wavelengths 4000 to 500 cm^{-1} without any further sample preparation. The background scan was carried out without any sample before the samples of interest were scanned. The surface morphology and elemental composition studies of plain and lipase wool were done using SEM (JEOL SEM6480LV) and EDX (Oxford INCA X-Act SDD) respectively. Individual fibres of plain wool and lipase wool were stuck on a double sided carbon tape and coated with gold to improve the imaging quality for SEM. Cross section of wool fibres were used for EDX analysis. Individual wool fibres were stuck on a double sided tape and embedded in Epotin Z resin, mixed with the corresponding hardener. The samples were cured for 12 hours in vacuum and polished with a diamond polisher (Buehler

Ecomet 250 Pro) and finally washed with soap and ethanol. The resin surface was coated with chromium (5 nm) before the analysis. The surface composition of plain and lipase wool was analysed by X-ray photoelectron spectroscopy (XPS) using a Thermo k-alpha[+] system at the XPS facility at Cardiff University. Fibres of plain wool and lipase wool were stuck on a double sided tape of 1cm^2 and used for analysis.

2.3 Kinetic resolution of 1-phenylethanol in batch

In a typical batch reaction, 1-phenylethanol (0.5 mmol), vinyl acetate (2.5 mmol) and the desired amount of catalyst were added to a suitable solvent (3 ml) and stirred (Fig 1). The reactions were carried out in a reaction carousel for 24 hours. Control experiments were carried out without the catalyst under the same conditions and no reaction occurred. Samples were drawn periodically and the reaction conversion was monitored using gas chromatography (Varian CP-3800, CP-Sil-8 CB column). Enantiomeric excess was measured using HPLC.
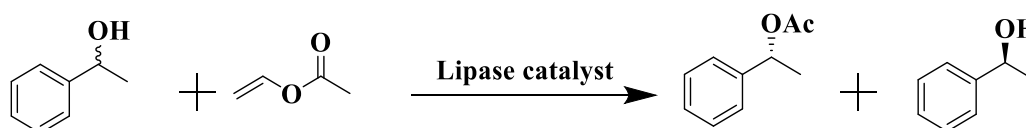

Fig 1: Kinetic resolution of 1-phenylethanol with vinyl acetate as the acyl donor, catalysed by lipase.

2.4 Kinetic resolution of 1-phenylethanol in the SMDR

As shown in Fig 2, the SMDR consists of an overhead liquid feeding system, a disc connected to an overhead stirrer and a funnel like vessel to channel the spin-off feed from the edge of the disc to the reactant storage vessel. The cloth was placed on a glass disc and connected to a rotor (Heidolph RZR 2021), operating at variable speeds. Fresh lipase cloths were used for each study. Further details about the reactor set-up can be found in our previous publication [25]. 1-phenylethanol (0.5 mmol), vinyl acetate (2.5 mmol) were dissolved in 250 ml of solvent and the reaction was caried out at 25°C for 5 hours. Samples were drawn every 5 minutes for the first 30 minutes and once every hour thereafter. The samples were analysed using GC as above and the sample at the end of the reaction was also analysed using NMR (300 MHz Bruker Spectrometer). Analysis of ^{1}H NMR integral of 1-phenylethanol at 4.72 ppm to the integral of 1-phenylethyl acetate at 5.79 ppm was used to calculate the conversion. The reactor performance was characterised based on the average shear on the surface of the disc ($\bar{S}$) given by the following equation:

$$\overline{S} = \frac{1}{R}\int_0^R S dr = \frac{3}{4}\left(\frac{3QR\omega^4}{2\pi v^2}\right)^{1/3}$$

where, S = surface shear (s^{-1}); Q = volumetric flow rate (m^3 s^{-1}); R = radius of the disc (m); ω = angular velocity (rad s^{-1}); v = kinematic viscosity (m^2 s^{-1}).

An increase in the spinning speed results in a proportional increase of the average surface shear and thus an increase in the conversion is expected. However, research has shown that enzyme leaching from the woollen cloth occurs over a certain value of average surface shear known as the critical surface shear, which is 9500 s^{-1} [22].
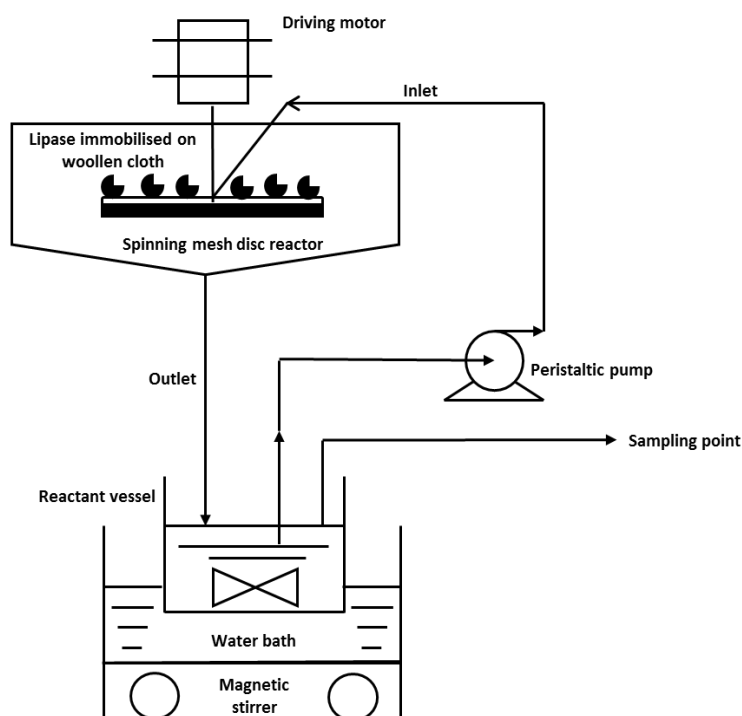

Fig 2. Schematic representation of the SMDR with the lipase cloth placed on the disc.

2.5 High performance liquid chromatography (HPLC)

The enantiomeric excess (ee) was determined by HPLC (Agilent Technologies 1260 Infinity-II, Chiracell OD-H column with Hexane/IPA (90/10) at 1 ml min^{-1} and 254 nm wavelength using a UV detector). The 'ee' of the reaction was calculated using the following equation:

$$ee = \frac{(S - R)}{(S + R)}$$

2.6 Kinetic studies

The ping-pong bi-bi kinetic model has been used successfully to describe the kinetics of the resolution reaction [30]. The enzyme first forms a complex with the acyl donor (acyl-enzyme), conforming to the ordered bi-bi mechanism. The complex then undergoes an isomerisation reaction to form the reaction intermediate, followed by the release of the alcohol product. The second substrate, 1-phenylethanol binds to the acyl-enzyme complex, undergoes an isomerisation reaction resulting in the formation of an ester-enzyme complex, followed by the release of the acetate product and the free enzyme. Furthermore, the substrates and the products are considered as competitive inhibitors. The rate expression is given by:

$$v = \frac{(v_{max}/K_m)S}{1 + (S/K_m) + (S_o - S)/K_i}$$

where, v is the reaction rate, v_{max} is the maximum rate of the reaction, K_m and K_i are the kinetic constants, S is the final concentration of the substrate and S_o is the initial substrate concentration. The initial rate was calculated from the concentration-time profile for the reaction carried out at different temperatures and compared with the theoretical rate values obtained from the model.

3. Results and Discussion

3.1 Characterisation of lipase immobilised wool

FTIR, SEM, EDX and XPS was used to show that lipase was successfully immobilized on wool. The functional groups present in wool were determined by analysis by FTIR spectroscopy (see supplementary material A). Figure 3 shows the SEM images of plain and lipase immobilized wool. As seen in fig 3 (a-c), the morphology of plain wool is characterised by a uniform arrangement of cuticle cells on the surface of the wool fibres (diameter 2.5 µm). Changes in morphology of wool fibres were observed at almost every stage of immobilisation (presented in supplementary material B). Fig 3(d-f) shows loss of surface smoothness after lipase immobilisation which may be due to a coating of lipase on the wool surface. Fig 3(g-h) shows the elemental ratios from the cross section EDX analysis from different stages of lipase immobilisation at the surface and fibre core. As lipase immobilisation occurs mainly on the surface of the wool, changes in the composition near the surface is more significant compared to the fibre core. Further, wool and lipase are mainly made of amino acids (containing carbon, nitrogen, oxygen and sulphur) and the reagents used for

immobilisation have similar elemental composition. Thus, it would be expected to see a continuous increase in the carbon ratio throughout the course of immobilisation. Although it can be seen that this trend is not consistent throughout indicating possible interaction of the reagents with the surface functional groups containing sulphur and nitrogen resulting in decrease in carbon ratios at different stages of immobilisation.
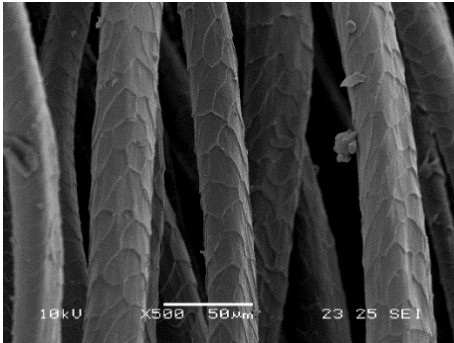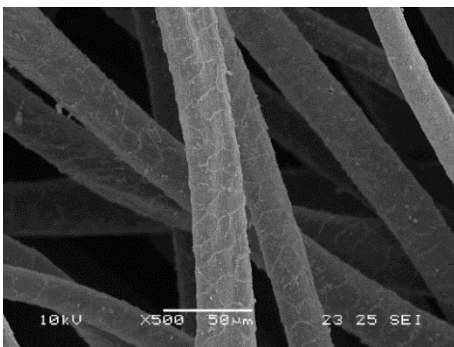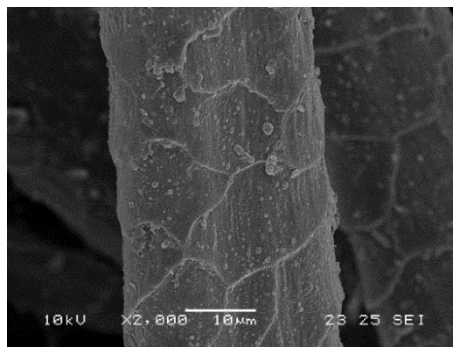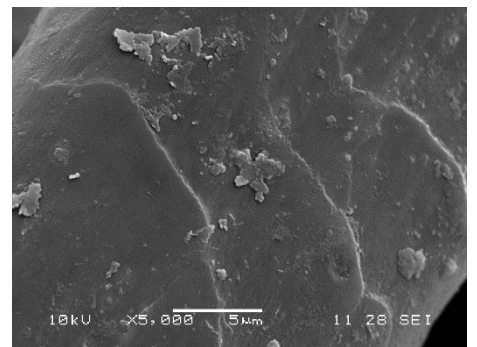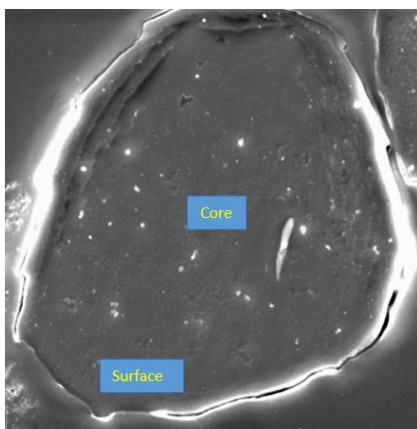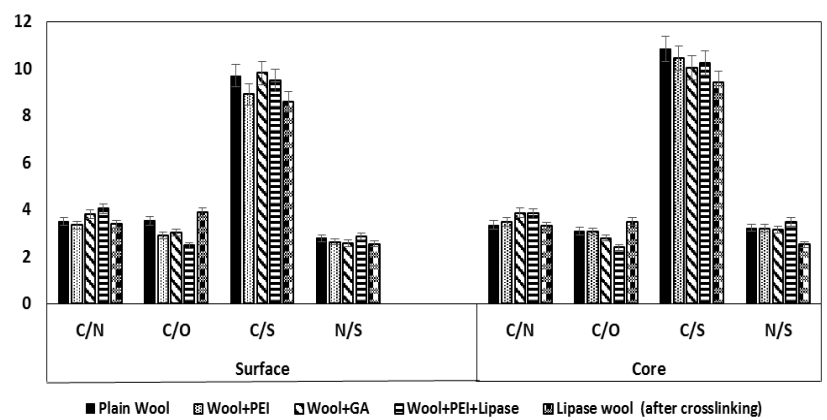

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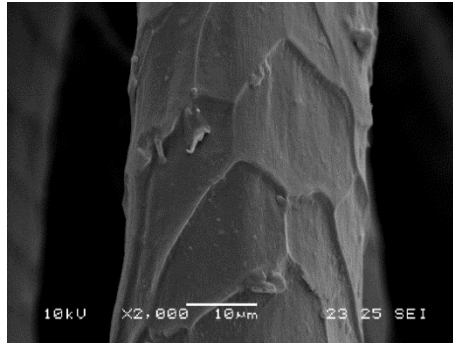
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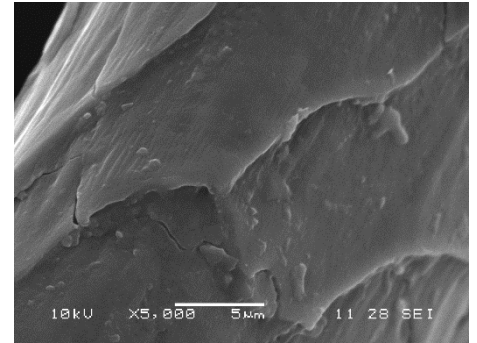
(c)

(d)

(e)

(f)

(g)

(h)

Fig 3. SEM images of (a-c) plain wool, (d-f) lipase wool and (g) SEM image of wool fibre cross section (h) Elemental ratios from the EDX analysis during different stages of lipase immobilisation

The survey spectrum for plain wool obtained from XPS analysis indicated the presence of carbon (78%), oxygen (12%), nitrogen (7%) and sulphur (2%) (see supplementary material C). In difference, the survey spectrum of lipase wool showed the presence of phosphorous (2%) in further indicates successful lipase immobilisation on wool as only lipase of all the immobilization chemicals contain phosphorous. The elemental scans (Figure 5) show a slight shift in the binding energy for O1s and S2p for lipase wool indicating a possible interaction of the immobilisation media and the surface functional groups present in wool.
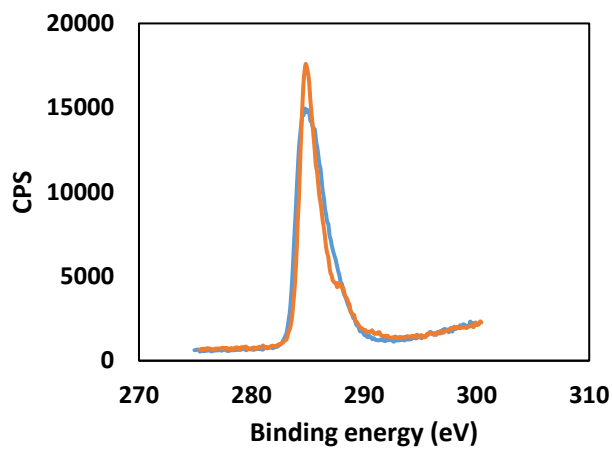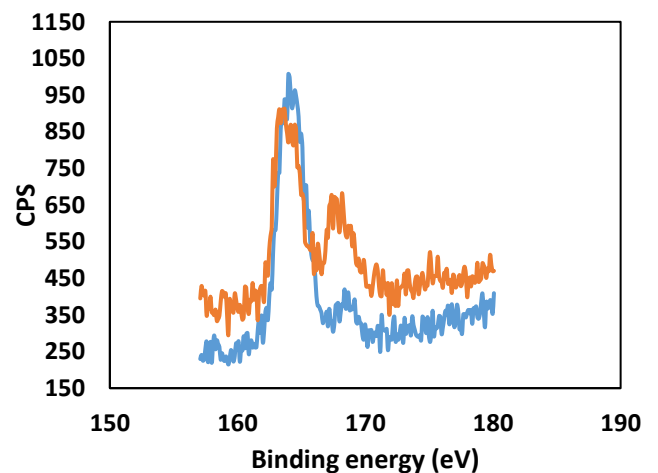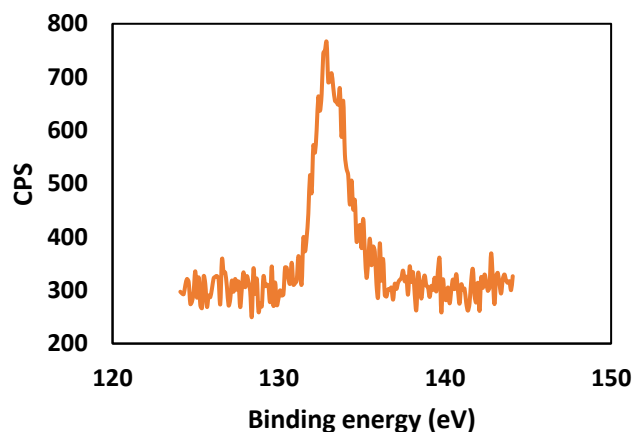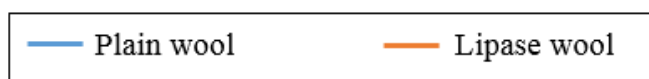

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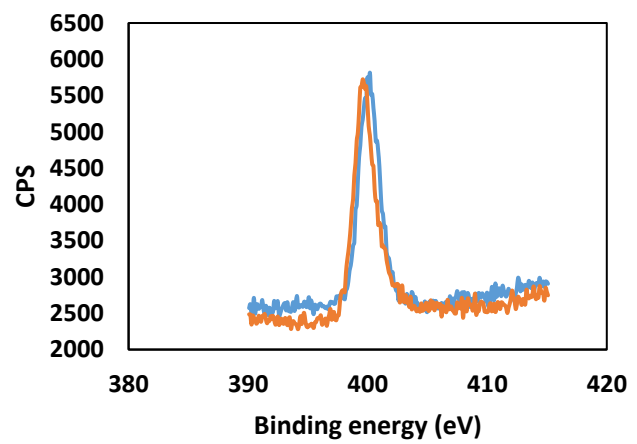
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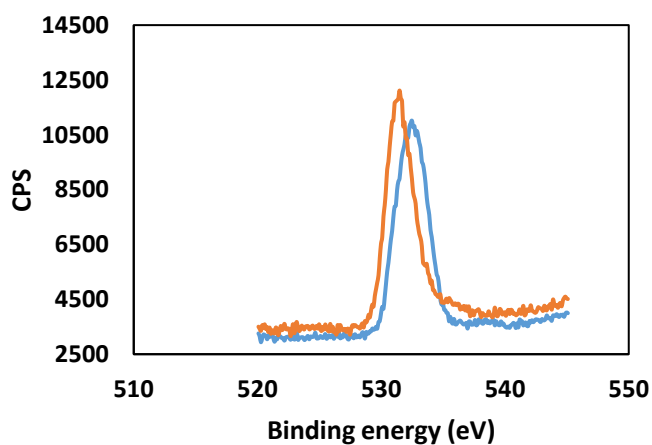
(c)

(d)

(e)

Figure 4: Elemental scans of plain wool and lipase wool. (a) C1s, (b) N1s, (c) O1s, (d) S2p and (e) P2p

3.2 Effect of different solvents on catalyst performance and enantioselectivity in batch

The choice of organic solvent for enzymatic resolution plays an important role as it affects the activity and the stability of the enzyme but also the substrate characteristics. Further, it has been reported that the enantioselectivity of the enzyme is significantly affected by the reaction solvent as there can be changes in the structural conformation of the enzyme and the nature of the enzyme-substrate intermediate compound [31].

A range of solvents, both polar and non-polar, were investigated and their effect of conversion and enantioselectivity are presented in Table 1. It shows that in a non-polar solvent like toluene, free lipase exhibited good reaction conversion and enantioselectivity but only little conversion was observed with the immobilised lipase. Aromatic solvents like toluene show poor wettability of wool resulting in a reduced contact between the catalysts present on and within the woollen cloth. Hence, a lower reaction conversion was observed with immobilised lipase [32]. Table 1 further shows that there is no activity using polar organic solvents like ethanol and DMSO. Research has previously shown that they offer least diffusion resistance through wool fibres but have been reported to cause an activity loss of enzymes by denaturing them, resulting in no conversion and enantioselectivity for the reaction [32, 33]. As toluene demonstrated the highest conversion for free lipase and ethanol is known to have better penetration through the wool fibres, a combination of ethanol and toluene in equal volume proportions was also examined as a reaction solvent.

However, as per Table 1, there was no change in the conversion indicating that the enzyme was denatured in the presence of ethanol. Moderate conversion and enantioselectivity was observed using DCM for both free and immobilised lipase. For ethyl acetate, similar conversions to toluene was observed using free lipase, but higher conversion for the immobilized lipase was achieved. Though there is only a marginal difference in the enantioselectivity for ethyl acetate and DCM, the former was chosen as the solvent for the reaction as it has a higher boiling point than DCM, minimising solvent losses and is also a more environment friendly solvent. Thus, all further experiments were carried out using ethyl acetate.

The results from the present study for both free and immobilised lipase are in line with that reported by de Souza et.al [5], using free lipase from PF for the same reaction system. As mentioned in the earlier sections, kinetic resolution of racemic alcohols have been mainly carried out using lipases from *Candida* species as employed in Novozyme 435 and other non-amano lipases due to their higher enantioselectivity. Amano lipase from PF was chosen for the present study as it was readily available, inexpensive and has been successfully immobilised on wool in the past [29]. In the present study, it is also a trade-off between solvent compatibility with lipase immobilised on wool and obtaining measurable conversions for the reaction to demonstrate the potential of the SMDR for an enzyme catalysed reaction, as seen in later sections. Also, other lipases have not been screened for this reaction as it out of scope for the present study as the main focus is to demonstrate reaction scale-up using an inexpensive lipase.

Table 1: Effect of solvent on reaction conversion using free and immobilised lipase in batch reactions after 24 hours

Solvent	% Conversion with free lipase[a]	%ee$_s$[b]	% Conversion with immobilised lipase[a]	%ee$_s$[b]
Toluene	49	91	3	<1
Ethanol	<1	-	0	-
Ethanol+Toluene	<1	-	0	-
DMSO	-	-	-	-
DCM	27	93	10	20
Ethyl acetate	48	92	30	23

[a]Conversion was determined by ^{1}H NMR analysis. The maximum conversion possible is 50% as the starting material was racemic.

[b]The enantiomeric excess was determined by chiral HPLC.

3.3 Catalyst efficiency at different temperatures in batch

To determine the best operating temperature for the catalytic activity of the lipase, the reaction in ethyl acetate was monitored between 25°C to 45°C. Maximum reaction conversion was achieved at room temperature for both free and immobilised lipase, and the conversion decreased with an increase in temperature (Fig 5). Our previous reports on hydrolysis of tributyrin using immobilised lipase have shown an increase in the activity of the enzyme up to 55°C demonstrating the superior heat resistance of the lipase [29]. However, it was a non-enantioselective reaction and carried out in an aqueous medium. Traditionally, amano lipases (like *pseudomonas fluorescens* used in the present study) have shown maximum activity at room temperature for enantiomeric reactions as they are sensitive to the thermal effects of conventional heating process in an organic medium [6, 30, 34, 35]. They are also susceptible to temperature induced racemisation at higher temperatures resulting in reduced conversion and enantioselectivity [33]. It is natural to expect immobilised lipase to yield better conversions at higher temperatures as they are known to be more stable to thermal effects. In the present case however, in addition to temperature effect, reduction in conversion using lipase immobilised on wool may also be due to the alteration of the conformation of the activity centre of the enzyme during the immobilisation process [34].
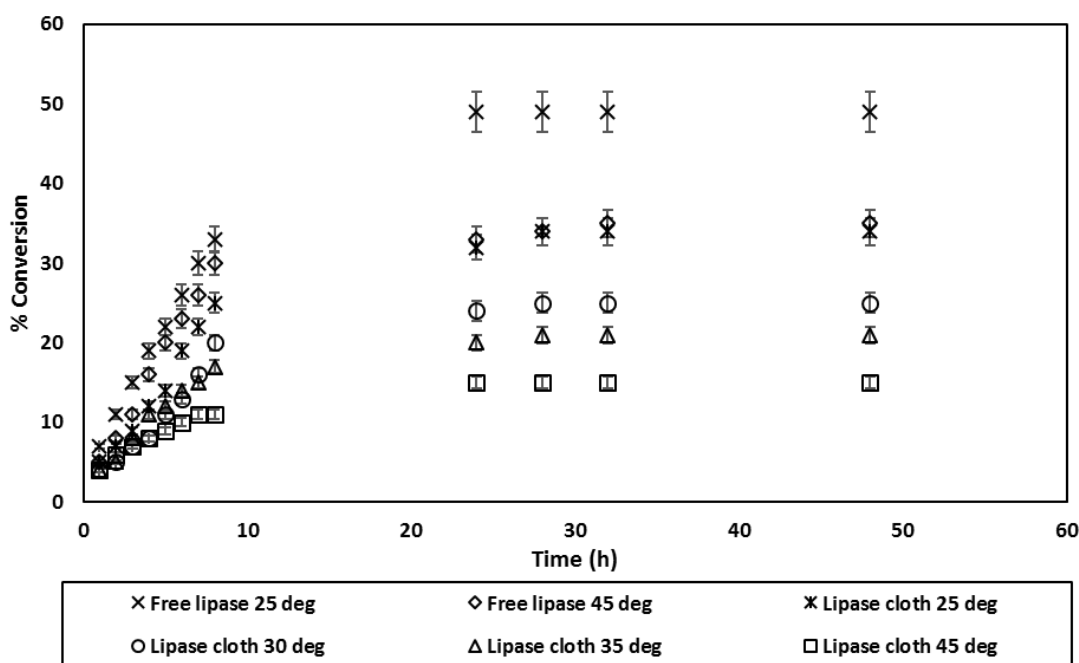

Fig 5: Effect of temperature on reaction conversion using free and immobilised lipase in batch

3.4 Kinetic resolution in the SMDR using free and immobilized enzymes

The SMDR was used for reaction scale-up and the reaction conversion was compared with that obtained in batch after 5h for 250 mL. Fig 6(a) shows that the reaction conversion increased for both free and immobilised lipase in the SMDR compared to the reaction in batch. Previous research [22] has shown that, in addition to overcoming mass transfer resistances, the cloth and disc geometry creates a higher interfacial surface area on the disc for the enzyme-substrate throughout the reaction and discrete residence time of the substrate on the spinning disc result in the enhanced reaction conversion using immobilised lipase in the SMDR. Hence, the lipase immobilised cloth was used for further reaction optimisation. The volumetric feed scale-up of the SMDR was tested by running a 1L feed of the same concentration through the SMDR using similar disc size and reaction conditions. Although the conversion decreased by 20% compared to the 0.25 L feed, it was still higher than that achieved in batch using free lipase. Changing reaction volumes in the batch reactor has an effect on mixing and turbulence, in turn affecting the reaction rate. In the SMDR, the reaction volume only increases the volume of the substrate as the reaction does not occur in the bulk of the reactant vessel. The productivity in the SMDR using lipase cloth (10.92 g l^{-1} h^{-1}) was 35% higher than that obtained in batch (7.05 g l^{-1} h^{-1}). This is also higher than the productivity of 2.2 g l^{-1} h^{-1} achieved by Hartmeier et.al [36] using immobilised lipase in a fixed bed reactor.

Figure 6(b) shows that with the lipase immobilised cloth in the SMDR, reaction conversion increased with increase in the spinning speed of the disc, and a maximum conversion of 31% was achieved at 450 RPM in 5 hours, which is comparable with that in batch at the end of 24 hours. The centrifugal force associated with the spinning disc allows for an even spread of liquid film on and within the woollen cloth immobilised with lipase, resulting in better mixing within the film accompanied by reduced resistance to mass transfer and increase in conversion, in agreement with our previous results for enzyme hydrolysis [22]. It should be noted that although mass transfer resistance was successfully overcome in the SMDR, the lower overall conversion for the reaction is due to (i) the solvent compatibility with the immobilised lipase woollen cloth, (ii) probable loss of chiral active centres of the lipase as a result of its immobilisation on wool. As can be seen from table 2, the reaction conversion from the present study for both free and immobilised lipase in the SMDR are higher compared to other reports using lipase from *pseudomonas*.sp for the same reaction. Also, the reported scale for this reaction

to date is between 5 to 50 ml, whereas we have scaled it up to 250 ml without a loss in reaction efficiency. This indicates the potential of the SMDR for handling larger throughput compared to a batch reactor, and hence its applicability for industrial scale application of the reaction system.

Figure 6(c) show a small 2% increase in conversion with an increase in flowrate from 3 ml/s to 5 ml/s. This marginal increase in conversion has also previously been confirmed in the SMDR and is because an increase in flowrate results in a higher contact frequency between the feed and the immobilised lipase, although it also result in a lower mean residence time between the cloth and the feed (i.e. a lower contact time per pass). The reaction rate also increased with an increase in the spinning speed comparable with the rate of the batch reaction, and a good agreement was found between the experimental and model values (Fig 6(d)). As can be seen from figure 6(e), similar reaction rates were obtained for the reaction in batch using free lipase and the SMDR.

Table 2: Comparison between different amano lipases for kinetic resolution of (rac)1-phenyl ethanol

Enzyme description	Reaction time (h)	Reaction volume (ml)	%Conversion	Reference
Free PFL	12	4	28	[37]
PFL immobilised on PPU	12	4	17	[37]
Pseudomonas immobilised on diatomite	2	30	18	[11]
Amano AK-homogeneous	2	1.5	7	[5]
Lipase from PF-homogeneous batch	24	10	48	Present study
Lipase from PF – Immobilised batch	24	10	30	Present study
Lipase from PF-homogeneous in SMDR	5	250	26	Present study
Lipase from PF – Immobilised in SMDR	5	250	31	Present study
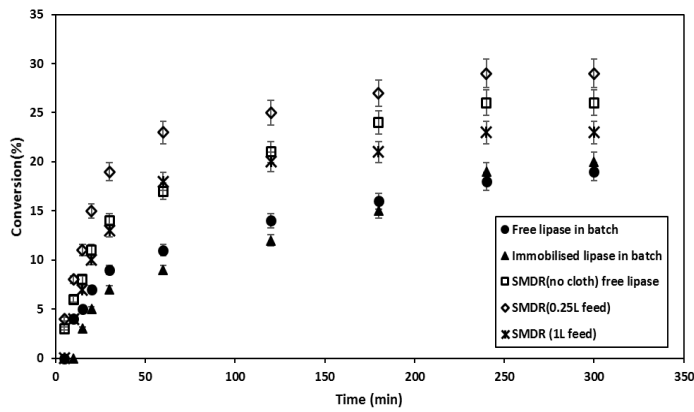
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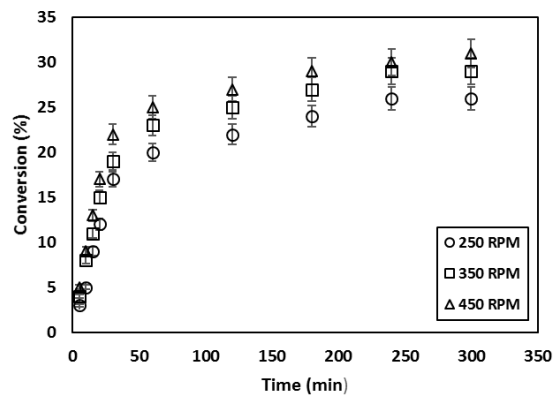
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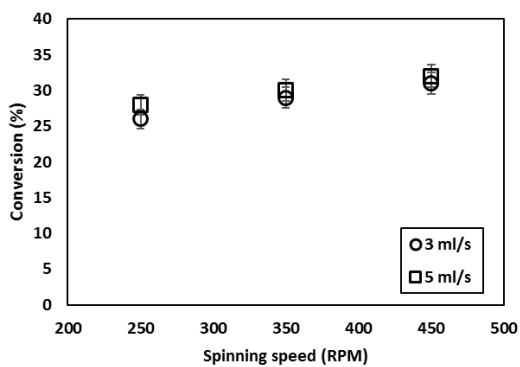
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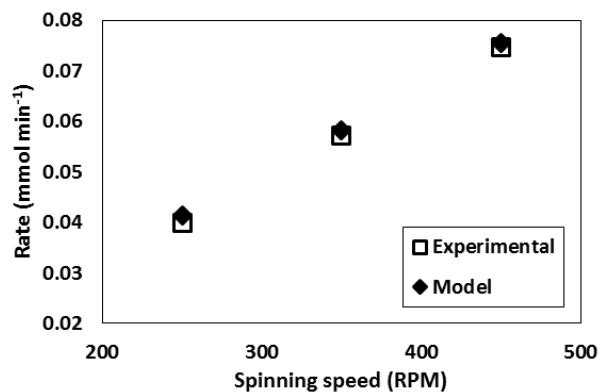
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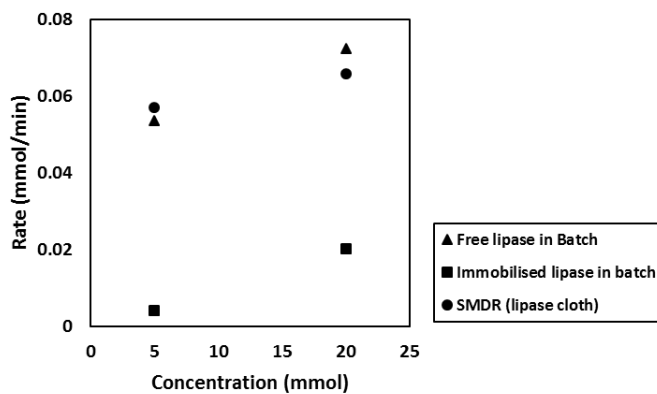
(e)

Fig 6: (a) Comparison of reaction in batch and SMDR with free and immobilised lipase at spinning speed of 350 RPM and 3ml s^{-1} flowrate, (b) Effect of spinning speed on reaction conversion at flowrate of 3ml s^{-1}, (c) Effect of flowrate on reaction conversion at different spinning speeds and (d) Experimental and model results for reaction rate and (e) Initial reaction rates in SMDR and batch reactor

3.5 Effect of multiple cloths in the SMDR

The SMDR follows a concept of 'numbering up', which means that the overall catalyst loading of the system can be increased by adding more lipase cloths on the disc. From Fig 7(a & b), it was observed that the addition of cloths resulted in an increase in the initial reaction rate accompanied by a small increase in reaction conversion. As the cloth number increases from, there is an increase in the cloth volume and enzyme loading. The increase in the surface area of the cloths is not significant compared to the increase in the cloth volume which further proves that the reaction is not solely catalysed by the enzymes bound on the outer surface but also occurs within the cloths. Also, the reaction proceeds at a faster rate for similar conversion from one cloth (0.16 mmol min^{-1}) to three cloths (0.28 mmol min^{-1}) as more number of active sites are available for a higher enzyme-substrate ratio and for a given residence time. Visual studies from the previous reports have indicated that although the centrifugal force causes the reaction liquid to flow tangentially across the disc, gravitation forces acting on the cloth stack promotes complete penetration of the reaction liquid through the cloth stack [12]. Additionally, multiple cloths on the disc surface causes an increase in the mean residence time ensuring a longer contact time between the immobilised enzymes and the substrate. The presence of multiple cloths also causes the flow patterns to change compared to a single cloth SMDR as more flow channels are present within the fibre matrix, leading to increased mixing on and within the cloth stack [27]. The flow pattern in a conventional SDR is similar to plug flow, whereas with the addition of a cloth, the SMDR is deviates from the plug flow behaviour. Addition of more clothes further reduces the number of tanks (N) in series with a wider distribution of the residence time indicating a well-mixed behaviour of the reactor. This is beneficial in the SMDR as it facilitates better contact between the substrate and the enzymes.
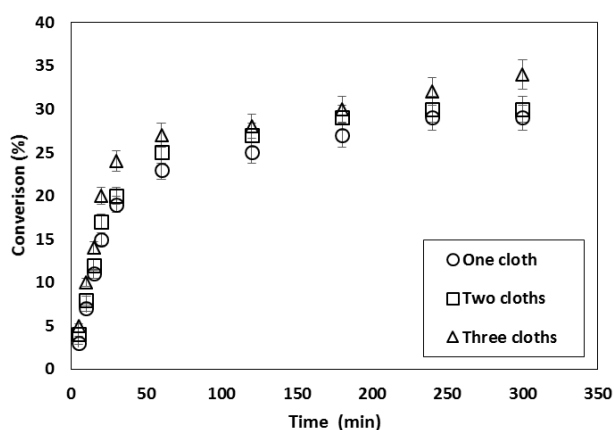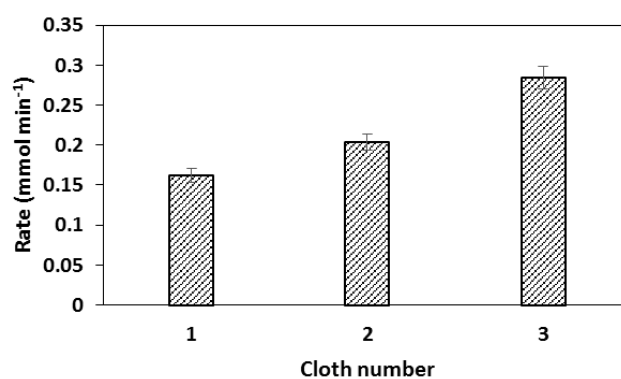
(a)   (b)

Fig 7: (a) The effect of number of cloths on conversion in the SMDR and (b) effect of cloth number on the initial reaction rate at spinning speed of 350 RPM and flowrate of 3 ml s^{-1}

3.6 Re-usability of lipase cloth in the SMDR

Figure 8(a) demonstrates re-usability of the lipase cloths for up to 3 cycles, retaining 83% of the original activity when used in the SMDR. The loss in the activity can be due to two reasons: (1) Detachment of enzymes from the cloth support; (2) Enzyme deactivation caused by the reaction environment (substrate/intermediate/product). Since the reactor was operated below the critical shear stress (9500 s^{-1} as tested with ethyl acetate), only the loosely bound enzymes may have been detached from the support causing a loss in the activity.

Figure 8(b) shows a decrease in the concentration of phosphorous at the surface, further indicating the possibility of loosely bound enzymes leaching from the surface of wool. The resolution reaction of 1-phenylethanol is being carried out in an organic solvent medium with vinyl acetate as an acyl donor, resulting in the formation of a vinyl alcohol intermediate, followed by the product 1-phenylethyl acetate. The reaction media is characterised by the presence of alcohol functional groups, which has shown to cause enzyme deactivation (see 3.2), and can be one of the reasons for activity loss of the lipase cloth on re-use. Han et.al [37] have reported that the formation of acetaldehyde during the resolution reaction can inhibit the enzyme activity and selectivity, which may also be the reason for reduced activity on repeated use of lipase cloth in the present study. Other reports [10, 11] for this reaction have shown that there was little or no loss in conversion upon re-using the enzyme and this may be due to the nature of lipase/ the immobilisation technique and lower agitation speeds (50 to 200 RPM) used for the reaction.
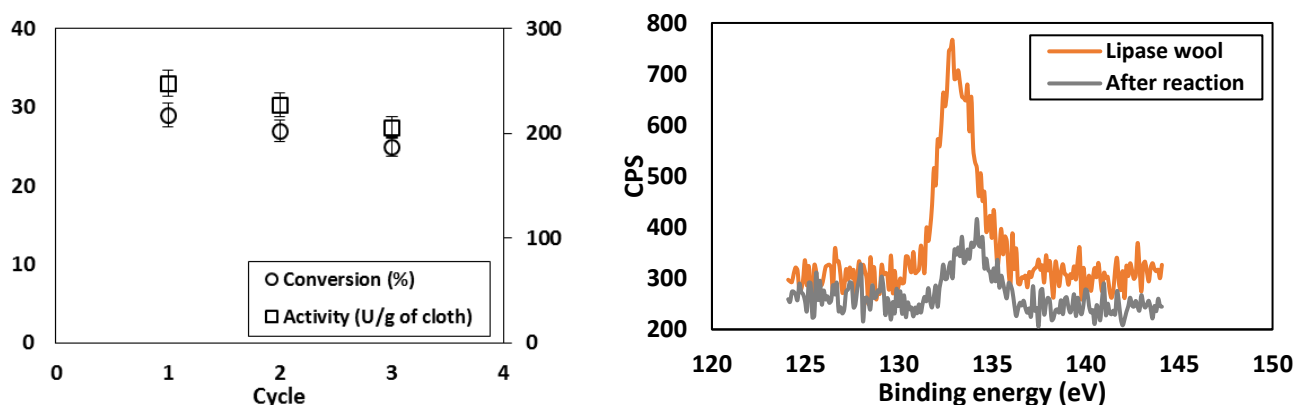
Fig 8: (a) Re-usability and activity of lipase cloth for multiple cycles in the SMDR at spinning speed of 350 RPM and flowrate of 3 ml s^{-1} and (b) P2p scan of used lipase wool

4. Conclusion

In this study, amano lipase from *pseudomonas fluorescence* was immobilised on wool and was used as a catalyst for the kinetic resolution of 1-phenylethanol. Lipase was immobilised on wool using a simple protocol and the immobilisation was verified via characterisation techniques like SEM, EDX and XPS. The effect of different solvents and temperatures were investigated on the catalyst efficiency and enantioselectivity in batch to establish optimum reaction conditions. Maximum reaction conversion was observed at 25°C in ethyl acetate and the conversion decreased with an increase in temperature for both free and immobilised lipase in batch. The reaction conversion increased with increasing spinning speed and flowrate in the SMDR due to higher mass transfer as a result of better mixing on the surface of the disc and high surface shear. The production output in the SMDR using immobilised lipase was 35% higher than batch and other conventional reactors under similar reaction conditions and the reactor successfully handled higher feed throughput. Further, the catalyst loading in the reactor was increased by simply adding more cloths on the surface of the disc and the reaction rate doubled from one cloth to three cloths. The lipase cloth was re-used for up to three cycles and 83% of the original activity was retained, demonstrating the robustness of this catalyst for organic reactions. The kinetics of the reaction in both batch and SMDR conformed well to the Ping Pong bi-bi mechanism. The overall conversion for this reaction system is comparable to that reported in literature for amano lipase catalyst and reaction intensification and scale-up was achieved in the SMDR compared to the batch reactor in the present study. This indicates potential of the reactor to carry out enzyme catalysed reactions as demonstrated through the kinetic resolution of 1-phenylethanol. The results from the study also highlight the merits of the SMDR as a process intensification technology as the reactor can support a wide range of mesh supports and catalysts, and scaled-up by adding more mesh cloths immobilised with the catalyst. Future work is required to further optimise the reaction to achieve higher conversion through altering the support and catalyst for better compatibility with polar solvents.

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