Citation for published version:
Feng, X, Patterson, DA, Balaban, M & Emanuelsson, E 2014, 'Increasing reaction rate and conversion in the spinning cloth disc reactor: Investigating the effect of using multiple enzyme immobilized cloths', Chemical Engineering Journal, vol. 255, pp. 356-364. https://doi.org/10.1016/j.cej.2014.06.049

DOI:
10.1016/j.cej.2014.06.049

Publication date:
2014

Document Version
Early version, also known as pre-print

Increasing reaction rate and conversion in the Spinning Cloth Disc Reactor: Investigating the effect of using multiple enzyme immobilized cloths

Xudong Feng[1,2], Darrell Alec Patterson[3], Murat Balaban[2]

and Emma Anna Carolina Emanuelsson[3]*

[1]School of Life Science, Beijing Institute of Technology, 100081 Beijing, PR China

[2] Department of Chemical and Materials Engineering, University of Auckland,

Private Bag 92019, Auckland Mail Centre, Auckland, 1142, New Zealand.

[3]Department of Chemical Engineering and Centre for Sustainable Chemical Technologies, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom.

*Corresponding author at:

Department of Chemical Engineering and Centre for Sustainable Chemical Technologies, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom.

Tel: 0044 1225 385312,Fax: 0044 1225 385713, Email: eaep20@bath.ac.uk

Abstract

The spinning cloth disc reactor (SCDR) is a novel mesh supported enzyme rotating reactor system for process intensification. In this study, to increase the enzyme loading in the SCDR, a new reactor operational mode was designed by increasing the number of cloths used in the SCDR to form a multi-cloth stack on the spinning disc. To test its effectiveness, the influence of the number of cloths in the SCDR on reaction conversion and rate was investigated. The flow within the multi-cloth stack was characterized by residence time distribution (RTD) analysis and an imaging of the flow and dye penetration in the SCDR.

For different tributyrin substrate concentrations (10-40 g L^{-1}), the reaction rate and conversion increased when the number of cloths was increased from one to two, indicating that the enzyme loading in the SCDR can be easily tailored to the desired reaction system by simply changing the number of immobilized enzyme cloths. The mean residence time increased with an increase in the number of cloths at different spinning speeds and flow rates, due to flow existing inside the volume of the multi-cloth stack. The number of tanks-in-series (N) decreased as the increase of cloth number on the spinning disc, indicating that more cloths caused larger deviation from plug flow. The visual study showed that the multi-cloth stack would not essentially change the flow types in the SCDR, and the fluid could penetrate through the three layers of multi-cloth at both low (100 rpm) and high (400 rpm) spinning speeds.

Keywords: spinning cloth disc reactor; lipase immobilization; residence time distribution; visualization; multi-cloth stack.

1. Introduction

The spinning disc reactor (SDR) is a process intensification technology, which utilizes centrifugal forces to produce a thin and highly sheared film on the spinning disc surface, resulting in rapid mixing and short residence time. Research has shown that the heat and mass transfer in the SDR can be significantly enhanced due to the fluid dynamics within the films [1-4]. The SDR has been applied in several chemical reactions such as polymerization [5], photocatalysis [6, 7], transesterification [8] and nanoparticle preparation [9-12]. Recently, the SDR concept has been introduced to enzymatic reactions by using a novel mesh supported enzyme rotating reactor system: the spinning cloth disc reactor (SCDR) [13-15]. As shown in Fig. 1, similar to the SDR configuration, the SCDR is also driven by the centrifugal forces on the spinning disc, however, this disc has immobilized enzymes on a woolen cloth resting on it. Therefore, the thin film is expected to be produced both on top of and within the cloth, where mass transfer enhancement and rapid mixing can be achieved.

The SCDR, like the conventional SDR, is scaled up through the microreactor concept of 'numbering-up' rather than traditional scale-up. This means that feasibility proven at the small scale in these reactors (such as in this work) can be almost directly translated into an industrially feasible system. The SCDR has been successfully applied to oil hydrolysis reactions, primarily to tributyrin emulsion hydrolysis. The results have shown higher reaction rates and conversions in comparison to the equivalent reaction system in a conventional batch stirred tank reactor (BSTR), for example, under comparable conditions (i.e. the same reaction conditions and the same enzyme to substrate ratio), the conversion of tributyrin hydrolysis increased by 18.1% and 13.5% in 4 h for substrate concentration of 10 g L^{-1} and 40 g L^{-1} respectively, indicating process intensification has been achieved. This reaction rate and conversion enhancement is considered to take place through a combination of enhanced mass transfer and mixing, increased interfacial surface area, the protection of the enzymes by woolen cloth from deactivation, and the increased residence time of the substrate on the disc due to the liquid holdup within the cloth [13]. Besides, the immobilized enzyme in the SCDR had good reusability maintaining 80% of its original activity after 15 consecutive runs. The enzyme leakage from the cloth support was very slight when the SCDR was operated under surface shear of 9500 s^{-1}: only accounting for 0.32% of total immobilized enzyme amount on the cloth [13]. The thermal stability of immobilized lipase was significantly improved compared to its free form. The thermal deactivation rate of immobilized lipase was found to follow the Arrhenius law with the thermal deactivation energy of 199 kJ mol^{-1} [14].

The flow characteristics in the SCDR was also investigated by using residence time distribution (RTD) analysis and visual dye staining of the cloths with immobilized enzyme [15]. The results indicated that the flow pattern in the SCDR was essentially well-mixed – a vast contrast to the plug flow behavior found in the conventional SDRs. This indicates that the SCDR is a different class of rotating process intensification reactor from the traditional SDR – a new reactor class the authors have classified as the Spinning Mesh Disc Reactor (SMDR). The flow patterns and regimes in the SCDR were also classified at different spinning speeds and flow rates, with two flow regimes observed in the visual study within the spinning cloth: radial finger-like flow and concentric flow [15].

The previous research has also shown that the liquid in the SCDR can penetrate through the cloth and there is immobilized enzyme inside the cloth, thus allowing the enzyme catalyzed reaction to occur inside as well as on the outside of the woolen cloth [13, 16]. This allows the SCDR to utilize all the available surface area of the woolen cloth that has been occupied by the immobilized enzymes for reactions. Currently only one woolen cloth has been characterized in the SCDR, however this has also limited the enzyme loading in the SCDRs to the maximum amount that can be immobilized on one woolen cloth. In order to increase the enzyme loading in the SCDR, the simplest method would be to increase the number (or thickness) of cloths used in the reactor to form a multi-cloth stack on the spinning disc. This is desirable, since a higher catalyst loading brings more catalytic sites being available and therefore should result in faster reaction rates and a higher volumetric efficiency in the SCDR, as long as there are no negative consequences of additional cloth layers present in the SCDR (which could include: poor penetration of the liquid throughout the multi-cloth stack, poor mass transfer and mixing within the cloth stack). Therefore, to test this hypothesis, in this study the effect of the number of cloths in a stack in the SCDR on reaction conversion was investigated using tributyrin emulsion hydrolysis as a model reaction. The flow within the multi-cloth was characterized by conventional RTD analysis and image study [15].

2. Materials and methods

2.1. Materials

Unbleached organic woolen cloth (color: natural cream, thickness: 1.5 mm) was purchased from Treliske (Otago, New Zealand). Amano lipase derived from *Pseudomonas fluorescens*, tritonX-100, tributyrin (98%), sodium bicarbonate, sodium carbonate and polyethyleneimine (PEI) were obtained from Sigma-Aldrich (New Zealand). Hydrogen peroxide 30% was obtained from Scharlau (Thermofisher, New Zealand). Glutaraldehyde (GA) 25%, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium chloride and hydrochloric acid were purchased from Unilab (ECP, New Zealand). Water color dyes (Reeves, UK) were obtained from a local market. All solutions were prepared using deionized water (produced from Milli-Q Gradient A10 made by Millipore).

2.2. Preparation of immobilized lipase on woolen cloth

The main immobilization procedure has been described in our recent publications [14, 16]. The woolen cloth was cut into circular pieces with diameter of 250 mm. The natural woolen cloth was first pretreated with a solution containing 30 mL L^{-1} hydrogen peroxide (30%) and 2 g L^{-1} sodium silicate at pH 9 (0.1 M Na_2CO_3, $NaHCO_3$ buffer) at 55 °C for 70 min. The pretreated woolen cloth was then soaked in 500 mL 2% PEI solution at pH 8 for 2 h at room temperature and rinsed with deionized water. The cloth was then dipped in 1 L 2 mg mL^{-1} lipase solution (0.1 M Na_2HPO_4, NaH_2PO_4 buffer, pH 6) for 24 h, followed by immersion in 500 mL 0.5% (w/v) GA solution (0.1 M Na_2HPO_4, NaH_2PO_4 buffer, pH 6) for 10 min to achieve crosslinking. The cloth was finally washed with deionized water until no free enzyme was detected in the washed solution. The enzyme loading was 46.8 mg per gram of dry cloth and activity was 178.3 U per gram of dry cloth determined by using the tributyrin emulsion hydrolysis method [16].

2.3. Equipment

As shown in Fig. 1a, the SCDR consists of a liquid feeding system, an overhead stirrer connected to a disc, a vessel for catching, containing and funneling liquid from the disc, and a reactant solution storage vessel. The spinning disc for supporting the cloth in this SCDR was a Perspex disc, 250 mm in diameter, which was driven by a variable speed motor (Glas-Gol, US). The multi-cloth (up to 4 cloths) with immobilized lipase was rested (with no means of fastening) on the disc as shown in Fig. 1b. Further details can be found in our previous publication [13].

The tributyrin emulsion was made by adding tributyrin and triton X-100 to phosphate buffer (0.1 M, pH 7) with a final concentration range of 10-40 g L^{-1} and 3.5-14 g L^{-1}, respectively. The mixture was then emulsified with a motor homogenizer (IKA T25 digital, Japan) at 12,000 rpm for 5 min. The reaction was carried out at 45 °C for 4 h. During the hydrolysis, sodium hydroxide was added into the reactant vessel with a pH stat to keep a constant pH and the data was recorded continuously with a PC. Reaction conversion was calculated through the amount of sodium hydroxide consumed during the reaction according to Eq. 1:

$$Conversion(\%) = \frac{moles\ of\ free\ butyric\ acid}{moles\ of\ original\ esters\ in\ tributyrin} \times 100\% \qquad (1)$$

2.4 RTD study

The schematic diagram of the equipment for the RTD study is shown in Fig. 1a. The cloths with immobilized lipase prepared as described in Section 2.2 were used for the RTD study. The experimental procedure for this was the same as that used in characterizing the flow properties in the SCDR with a single cloth [15]. A solution of 0.5 M KCl was used as the tracer and deionized water was used as the test fluid. The tracer conductivity and concentration showed a good linear relationship in a concentration range between 0.005 and 0.5 M, so the RTD can be directly related to the conductivity of the tracer. A conductivity probe (Mettler Toledo, Switzerland) was placed in a small vessel which collected the outlet solution continuously and the data was logged to a PC every second via LabX direct software (Mettler Toledo, Switzerland).

The deionized water was first fed to the reactor at the desired spinning speed and flow rate until the conductivity in the outlet was stable and close to that of deionized water. Then, 1 mL of KCl was injected quickly to the center of the spinning disc with a syringe. The concentration change in the outlet was measured as a function of time.

The RTD data was analyzed according to references [17, 18]. For the pulse injection of tracer, the distribution density function of the residence time $E(t)$ was obtained from the following equation:

$$E(t)=\frac{c(t)}{\int_0^t c(t)dt}=\frac{c(t_i)}{\sum_0^{t_{\max}} c(t_i)\Delta t_i} \quad (2)$$

The mean residence time was calculated as follows:

$$\bar{t}=\frac{\int_0^\infty tE(t)dt}{\int_0^\infty E(t)dt}=\int_0^\infty tE(t)dt=\frac{\sum_0^{t_{\max}} t_i c(t_i)\Delta t_i}{\sum_0^{t_{\max}} c(t_i)\Delta t_i} \quad (3)$$

The variance of RTD, σ_t^2, stands for the discrete level and was obtained from Eq. 4:

$$\sigma_t^2=\frac{\int_0^\infty \left(t-\bar{t}\right)^2 E(t)dt}{\int_0^\infty E(t)dt}=\int_0^\infty t^2E(t)dt-\bar{t}^2=\frac{\sum_0^{t_{\max}} t_i^2 c(t_i)\Delta t_i}{\sum_0^{t_{\max}} c(t_i)\Delta t_i}-\bar{t}^2 \quad (4)$$

The dimensionless forms of σ_t^2 can be derived from Eq. 5:

$$\sigma_\theta^2=\frac{\sigma_t^2}{\bar{t}^2} \quad (5)$$

The number of tanks-in-series (*N*) has been widely used to describe the flow pattern in a quantitative way. *N* was calculated as follows:

$$N=\frac{1}{\sigma_\theta^2} \quad (6)$$

As can be seen from Eq. 6, *N* is in reverse relation with σ_θ^2, so a high *N* is characteristic of the ideal plug flow (N>50 is usually considered to be a small deviation from plug flow [18]).

2.5 Visual study

A dye staining technique was used to study the flow characteristics on and within the spinning multi-cloth. The equipment setup for visual study is shown in Fig. 1a, and the feed is a dye solution. A blue water color was selected as the tracer. For each run, deionized water was first fed to the spinning multi-cloth at the desired spinning speed and flow rate until the cloth was fully saturated. The feed was then changed to the dye solution. As soon as the dye came out at the center of the cloth, images were taken until the cloth was completely covered with the dye.

3. Results and discussion

3.1. Preparation of multiple lipase immobilized cloths with similar enzyme activity

Ten new cloths with immobilized lipase were prepared for the multi-cloth SCDR study. Each of the ten new cloths was placed in the SCDR using deionized water as feed and spun at 350 rpm for 2 h to wash off the free lipase. After that, the activity of each cloth was tested in the SCDR for 1 h with 10 g L^{-1} tributyrin emulsion as feed. The reaction time course is shown in Fig. 2. The conversion among the ten different new cloths is similar (conversion ranges from 48.4 % to 51.6 %) and the relative standard deviation among them was 2.4 %. This shows that the immobilization technique developed gives a repeatable and consistent immobilization onto the woolen cloths, which further indicates that this protocol is a significant step toward enabling the use of wool as a cheap, renewable and effective lipase support material [16].

Although the cloths can provide similar enzyme activity, there are still some small differences in activity, so the ten cloths were used equally across the experiments and divided into four groups for testing the differences among one cloth, two cloths, three cloths and four cloths in the SCDR. To keep the activity balanced between each group, the cloths with relatively high and low activity were divided into the same group. By doing this, the spread of activity between the groups (determined as relative standard deviation in terms of conversion) for the four groups is reduced to 0.76 %, which is acceptable within the experimental uncertainty present in the data.

3.2. Comparison of usage of one and two cloths at different substrate concentrations

As can be seen from Fig. 3, at all the different concentrations studied, tributyrin showed a higher conversion with two cloths, which further proves that the reaction also happens inside the cloth, since the cloth volume (enzyme loading) increases with the number of cloths present. Since the outer surface area of cloth present does not increase significantly with two cloths, this result means that it is unlikely that it is only the outer surface bound enzymes that are catalyzing the reaction. In addition, different concentrations showed different magnitudes of increases in conversion: only a slight increase in conversion was observed for tributyrin concentration of 10 g L^{-1} and 20 g L^{-1} (1.5 % and 2.3 % respectively), whilst a larger increase in conversion was observed for tributyrin concentration of 30 g L^{-1} and 40 g L^{-1} (5.7 % and 5.3 % respectively). For lower substrate concentrations, the reaction goes to completion faster (since the catalyst-substrate ratio is higher) even with one cloth, indicating that the required number of active sites (lipase) available for the reaction time is most likely already present, and so an additional cloth (and therefore additional enzymes) would not further increase the reaction rate or conversion. However, for reactions with a higher substrate concentration, there is a lower catalyst-substrate ratio, and it is likely that there is insufficient enzymatic catalyst available for the amount of substrate present in the residence time on the cloth, resulting in only a partial conversion of the substrate, so an increase in enzyme loading (via an increase in the number of cloths) results in a higher reaction rate and conversion.

Overall, these results confirm the hypothesis that increasing the number of lipase immobilized cloths in the SCDR is an effective way of increasing enzyme loading.

3.3. Optimization of number of cloths in the SCDR

As stated in Section 3.2, a significant increase in tributyrin conversion was achieved by increasing the cloth number from one to two at the higher substrate concentration of 40 g L^{-1}.

To find the optimal number of cloths for this substrate concentration (since increasing the enzyme loading should in theory increase reaction rate and overall conversion in the time period studied, up to the point at which all enzyme active sites are occupied for the residence time available), three and four cloths in the SCDR were further investigated in this section.

As can be seen from Fig. 4, as expected, the tributyrin conversion increased with an increase in the number of cloths in the SCDR. As the number of cloths increased from two to three, the conversion of the 40 g L^{-1} tributyrin increased from 55.4 % to 67.2 % after 4 h, which was close to the completion of reaction, since the conversion becomes very slow after 66.7 % conversion due to 1,3 specific property of the lipase used in this study [19]. Considering the relative standard deviation was 1.3 % and 0.98 % for 2 cloths and 3 cloths respectively, this increase in conversion caused by using more cloths (and therefore more enzyme) was significant and again indicates that there is most likely good wetting and penetration of the reaction liquid throughout the cloth stack (this will be further studied in Section 3.4 and 3.5). As the number of cloths in the SCDR stack further increased to four, the conversion was similar to that of three cloths, and only a slight increase of 2 % conversion was obtained. However, as can be seen from Fig. 5, the reaction rate in the first 30 min of four cloths (4.0 mM min^{-1}) was higher than that of three cloths (3.0 mM min^{-1}). Therefore, for 40 g L^{-1} tributyrin, three cloths could completely hydrolyze all the substrate within 4 h, and further increase in cloth number would not significantly increase the conversion, but the reaction would proceed much faster with higher reaction rate, due to the more available active sites for the residence time provided by the higher catalyst-substrate ratio with more immobilized enzyme cloths. These results indicate that the enzyme loading in the SCDR can be simply adjusted by changing the cloth number on the spinning disc.

3.4. RTD analysis of the effect of multiple lipase immobilized cloths

The above results indicate that an increase in the number of cloths can increase the enzyme loading in the SCDR, thus producing higher reaction rates and conversions, up to the point at which no further rate increase is possible at the substrate concentration being used. To determine if this limitation is a result of a limit on substrate to enzymes/active sites needed in the residence time available, or is related also (or perhaps even primarily) to the flow behavior within the cloth, an analysis of the RTD and a visual characterization of the liquid flow within and on top of the cloths was performed. The results from the RTD analysis will be presented first.

As can be seen from Fig. 6, the mean residence time (from center of disc to the reactor outlet) increased with an increase in the number of cloths. As expected (based on our previous work [15]), this increase in mean residence time was also controlled by spinning speed and flow rate: at a low spinning speed and flow rate, the increase in mean residence time with number of cloths was significant for all of the conditions tested (for example, the mean residence time is 56.2 s, 65.2 s, 77.5 s at disc speed of 50 rpm and flow rate of 2 mL s^{-1} for one cloth, two cloths and three cloths respectively); however, as the increase of spinning speed and flow rate, the increase in mean residence time with increasing numbers of cloth in the SCDR became insignificant (for example, the mean residence time is 15.2 s, 17.8 s, 18.4 s at disc speed of 500 rpm and flow rate of 8 mL s^{-1} for one cloth, two cloths and three cloths, respectively). These results can be explained in terms of the increased liquid trapped hold-up volume (considered as a resistance to flow) created by increasing numbers of cloths in the SCDR versus the increased centrifugal force on the fluid at increased spinning speeds and the amount of volume that can be trapped within the cloth being exceeded with increased flow rates. For a greater number of cloths, the trapped volume of liquid will increase under the same conditions, since there is a greater volume and therefore greater resistance to flow over the cloth covered, increasing the residence time of the disc as a result. At higher spinning speeds, the increased centrifugal force on the fluid will act to decrease the amount of liquid hold-up (essentially compressing the volume and forcing liquid out of the cloths and off the disc at a faster rate than at lower spinning speeds). At higher flow rates, the residence time decreases for the same number of cloths, since the increased flow rate is pushing liquid through the cloth discs at a faster rate (as expected). Therefore, as the spinning speed and flow rate increases, the effect of the trapped volume of liquid provided by multi-cloth becomes less significant compared to the higher centrifugal force, thus leading to the similar mean residence time for different cloth numbers.

There is one exception to this however - at flow rate of 2 mL s^{-1}, the residence time of two cloths and three cloths at 150 rpm was longer than that at 50 rpm, which was abnormal because in theory, no matter how many cloths were on the disc, the mean residence time should decrease with an increase in disc speed and flow rate. One explanation is that at disc speed of 50 rpm, the centrifugal force was too low to facilitate the liquid going through all the cloths, which means the liquid only passes through part of the volume of the cloths. This is an exception, since as the disc speed increased to 150 rpm, there was sufficient centrifugal force to force the liquid through more of the volume of the cloths, therefore increasing the mean residence time.

Therefore, there are two reasons for the higher reaction rates and conversion when increasing numbers of lipase immobilized cloths used in the SCDR: the increased catalyst loading (and therefore active site availability) and the increased mean residence time of multiple cloths on the disc.

To determine the effect of increasing the number of cloths on the reactor flow behavior, the number of tanks-in-series was determined for the RTD data, as presented in Fig. 7. As previously noted, a large number of tanks-in-series (*N*) means that the flow pattern is close to plug flow (N>50 is usually considered a small deviation from plug flow) and the opposite indicates a more well-mixed (CSTR) behavior.

As can be seen from Fig. 7, for the SCDR with one cloth, two cloths and three cloths, N generally increased with the increase in spinning speed and flow rate, implying that a more narrow distribution of residence time was obtained, which brings the reactor performance closer to plug flow. However, the overall flow pattern is always closer to a well mixed reactor than a plug flow reactor, which is in stark contrast to conventional SDRs. This is also consistent with the previous RTD study with one cloth in the SCDR [15] . N decreased with the increase in the number of cloths in the SCDR, indicating the reactor becomes better mixed with a greater number of cloths. For example, N decreased from 10 to 6 as the cloth number increased from one to three at spinning speed of 500 rpm and flow rate of 8mL s^{-1}. This is expected since the application of one cloth makes the SCDR deviate away from the typical plug flow compared to conventional SDRs [15], so adding more cloths should make this phenomena more pronounced. The mechanism would include the change in flow pattern caused by the resistance to flow that fiber mesh would create (which would increase mixing, sieving and back-mixing) and the fact that more cloths result in more multi-flow channels within the SCDR, thus leading to a wider distribution of residence time. In conventional SDRs, increased mixing is considered as a disadvantage, however in the SCDR it is an advantage, since it facilitates more contact between the substrate and enzyme.

3.5. Visual analysis of flow types and flow penetration in a multi-cloth SCDR

The multi-cloth with three layers in the SCDR was run with a blue dye in the water feed to allow the flow types and flow penetration to be visualized. As can be seen from Fig. 8 and Fig. 9, the two flow types determined for the single cloth SDR are present in the multi-cloth SCDR: radial finger-like flow and concentric flow [15]. However, the variation profile of flow type with spinning speed and flow rate was different: for example, concentric flow was observed for the multi-cloth SCDR at spinning speed of 400 rpm and flow rate of 8 mL s^{-1} but radial finger-like flow was observed for single cloth SCDR under the same conditions. This might be due to the higher inertial forces (caused by the natural liquid flow paths changes within the cloth) present in the three multi-cloth stack, allowing the concentric flow to form at a lower spinning speed in the three multi-cloth SCDR [15]. This indicates that the use of multiple cloths would not essentially change the flow types, but would instead change the flow types present at different spinning speeds and flow rates in the SCDR. Therefore to fully characterize the multi-cloth SCDR, these should therefore be mapped out for all the different numbers of cloth stacks (as was done for the single cloth SCDR in ref. [15]). However, these results will not be presented here.

One of the key problems that could occur with a multi-cloth SCDR also needs to be tested – potentially poor penetration of the liquid throughout the multi-cloth stack. Fig. 8 and Fig. 9 show that this is not the case: the dye can penetrate through the three layers at both low (100 rpm) and high (400 rpm) spinning speeds. Generally, the dye did not spread synchronously on all the three layers: it spread very fast on the first (top) layer and slowly on the third layer. This is due to gradual penetration of the dye through the cloth stack. It was faster for the dye to reach the bottom layer at a low spinning speed: cloth layer 3 was completely covered with dye after 5 min with a spinning speed of 100 rpm, but was only partially covered with dye even after 10 min at spinning speed of 400 rpm.

These results can be reconciled in terms of the forces acting on the flow over and through the multi-cloth layers: gravity force and centrifugal force. The gravity force favors the dye to go through the multiple layers since it acts to push fluid down through the cloth and therefore is a force for fluid penetration through the cloth stacks. The centrifugal force prevents the fluid from penetrating through the cloth and facilitates it to exit tangentially from the cloth stack instead. At low spinning speeds, the magnitude of the gravity force is greater compared to the centrifugal force, so the dye reaches the bottom layer faster. With an increase in spinning speed, the magnitude of the centrifugal forces becomes more significant with most of the dye forced to move towards to the edge of the cloth and thus less dye penetrating through the multiple layers. However, in both cases the dye did eventually penetrate through to the bottom cloth layer, indicating that at steady state operation (if the reactor was to be run continuously), penetration of the fluid and surface coverage of the reactant onto all the available immobilized enzyme is not an issue.

Overall, by combining the RTD and visual analysis of the flow properties, it can be concluded that complete wetting does occur in the multi-cloth SCDR and therefore the increased reaction rate and conversions observed with greater number of immobilized cloths is caused primarily by two factors: increased enzyme loading and increased residence time. These results indicate that there is a further factor that allows easy control and tunable operation of the SCDR: the number of cloths. Through this, the enzyme loading can be easily varied and tailored to the reaction system desired.

4. Conclusions

In this study, a multi-cloth stacked SCDR (using one to four cloths) was investigated using tributyrin emulsion hydrolysis as a model reaction. The flow characteristics within the multi-cloth stack were characterized by RTD and visual/image analysis. Initial experiments showed that increasing the number of cloths in the SCDR could increase reaction rate and conversion: for different tributyrin concentrations (10, 20, 30, 40 g L^{-1}), the reaction rate and conversion increased when the number of cloths was increased from one to two. The reaction rate and conversion were then optimized: for the higher concentration of 40 g L^{-1} tributyrin, the use of a three-cloth stack was sufficient to completely hydrolyze all the tributyrin substrate. A further increase in number of cloths in the stack would not result in an increase in overall conversion. However, reaction rate was affected by increasing number of cloths: the reaction rate kept increasing from 1.9 to 4.0 mM min^{-1} as the number of cloths in the stack increased from one to four.

The increased reaction rate and conversion observed with greater number of immobilized cloths was determined to be caused primarily by two factors: increased enzyme loading and increased residence time. RTD studies showed that the mean residence time increased with the increase of number of cloths at different spinning speeds and flow rates, due to flow existing inside the volume of multiple cloths. The number of tanks-in-series (N) decreased as the increase of the number of cloths in the SCDR. This indicates that more cloths can increase the well-mixed behavior of the SCDR (decreasing the plug flow behavior), since more cloths result in more multiple flow channels within the cloths thus leading to a wider distribution of residence time. A visual study tracking a dye staining of the cloths over time showed that the multi-cloth stack would not essentially change the flow types already observed for a single cloth SCDR. Importantly it showed that the fluid could penetrate through the three layers of multi-cloth stack at both low (100 rpm) and high (400 rpm) spinning speeds, indicating that complete wetting did occur in the multi-cloth SCDR. Therefore, hydrodynamics in the multi-cloth stack SCDR is not a problem.

Overall, these results indicate that the enzyme loading in the SCDR can be easily varied and tailored to the desired reaction system by simply changing the immobilized enzyme cloths number, which allows easy control and tunable operation of the SCDR. Considering the merit of reaction intensification of SCDR, the results in this study further indicate that the SCDR is a superior and flexible new process intensification technology for enzyme catalyzed reactions.

Future work will now determine if these benefits can be achieved in a range of different enzyme catalyzed reactions.

Acknowledgements

The authors acknowledge Laura Liang, Peter Buchanan, Raymond Hoffmann, Cecilia Lourdes, Jessie Matthew, Frank Wu and Allan Clendinning for their technical help in this work.

References

[1] R.J.J. Jachuck, C. Ramshaw, Process intensification: Heat transfer characteristics of tailored rotating surfaces, Heat. Recov. Syst CHP., 14 (1994) 475-491.

[2] K.V.K. Boodhoo, R.J. Jachuck, Process intensification: Spinning disc reactor for condensation polymerisation, Green Chem., 2 (2000) 235-244.

[3] I. Boiarkina, S. Norris, D.A. Patterson, Investigation into the effect of flow structure on the photocatalytic degradation of methylene blue and dehydroabietic acid in a spinning disc reactor, Chem. Eng. J., 222 (2013) 159-171.

[4] F. Visscher, J. van der Schaaf, M.H.J.M. de Croon, J.C. Schouten, Liquid–liquid mass transfer in a rotor–stator spinning disc reactor, Chem. Eng. J., 185–186 (2012) 267-273.

[5] K.V.K. Boodhoo, R.J. Jachuck, Process intensification: Spinning disk reactor for styrene polymerisation, Appl. Therm. Eng., 20 (2000) 1127-1146.

[6] I. Boiarkina, S. Pedron, D.A. Patterson, An experimental and modelling investigation of the effect of the flow regime on the photocatalytic degradation of methylene blue on a thin film coated ultraviolet irradiated spinning disc reactor, Appl. Catal. B-environ, 110 (2011) 14-24.

[7] I. Boiarkina, S. Norris, D.A. Patterson, The case for the photocatalytic spinning disc reactor as a process intensification technology: Comparison to an annular reactor for the degradation of methylene blue, Chem. Eng. J., 225 (2013) 752-765.

[8] Z. Qiu, J. Petera, L.R. Weatherley, Biodiesel synthesis in an intensified spinning disk reactor, Chem. Eng. J., 210 (2012) 597-609.

[9] C.Y. Tai, Y.H. Wang, H.S. Liu, A green process for preparing silver nanoparticles using spinning disk reactor, AIChE J., 54 (2008) 445-452.

[10] H.S. Liu, Y.H. Wang, C.C. Li, C.Y. Tai, Characterization of agi nanoparticles synthesized in a spinning disk reactor, Chem. Eng. J., 183 (2012) 466-472.

[11] H.S. Liu, K.A. Chen, C.Y. Tai, Droplet stability and product quality in the higee-assisted microemulsion process for preparing caco3 particles, Chem. Eng. J., 197 (2012) 101-109.

[12] B.C.Y. Chan, X.L. Wang, L.K.W. Lam, J.M. Gordon, D. Feuermann, C.L. Raston, H.T. Chua, Light-driven high-temperature continuous-flow synthesis of tio2 nano-anatase, Chem. Eng. J., 211 (2012) 195-199.

[13] X. Feng, D.A. Patterson, M. Balaban, G. Fauconnier, E.A.C. Emanuelsson, The spinning cloth disc reactor for immobilized enzymes: A new process intensification technology for enzymatic reactions, Chem. Eng. J., 221 (2013) 407-417.

[14] X. Feng, D.A. Patterson, M. Balaban, E.A.C. Emanuelsson, Characterization of tributyrin hydrolysis by immobilized lipase on woolen cloth using conventional batch and novel spinning cloth disc reactors, Chem. Eng. Res. Des., 91 (2013) 1684-1692.

[15] X. Feng, D.A. Patterson, M. Balaban, E.A.C. Emanuelsson, Characterization of liquid flow in the spinning cloth disc reactor: Residence time distribution, visual study and modeling, Chem. Eng. J., 235 (2014) 356-367.

[16] X. Feng, D.A. Patterson, M. Balaban, E.A.C. Emanuelsson, Enabling the utilization of wool as an enzyme support: Enhancing the activity and stability of lipase immobilized onto woolen cloth, Colloid Surf. B-Biointerfaces, 102 (2013) 526-533.

[17] O. Levenspiel, Chemical reaction engineering, 3rd ed., Wiley, New York, 1999.

[18] S. Mohammadi, K.V.K. Boodhoo, Online conductivity measurement of residence time distribution of thin film flow in the spinning disc reactor, Chem. Eng. J., 207–208 (2012) 885-894.

[19] Y. Kojima, M. Yokoe, T. Mase, Purification and characterization of an alkaline lipase from pseudomonas fluorescens ak 102 Biosci. Biotechnol. Biochem., 58 (1994) 1564-1568.

Figure legends

Figure 1(a) Schematic diagram of the multi-cloth in the SCDR setup for RTD analysis and visual study. (b) Top view of a two-cloth stack on the disc, each cloth containing immobilized enzymes. (c) Top view of a three-cloth stack on the disc, each cloth containing immobilized enzymes.

Figure 2 Time course of tributyrin hydrolysis with the ten newly prepared lipase immobilized cloths for this study. Reaction temperature: 45 °C, reaction time: 1 h, flow rate: 5 mL s^{-1}, spinning speed: 350 rpm.

Figure 3 Comparison of one and two cloths in the SCDR for tributyrin conversion at different substrate concentrations. Each experiment was repeated three times and error bars are the mean± one standard deviation.

Figure 4 The effect of using multi-cloth stack (one to four cloths) in the SCDR on conversion over time with tributyrin concentration of 40 g L^{-1}. Each experiment was repeated three times and error bars are the mean± one standard deviation.

Figure 5 The effect of number of cloths in the SCDR on the overall reaction rate at tributyrin concentration of 40 g L^{-1}. Each experiment was repeated for three times and error bars are the mean± one standard deviation.

Figure 6 Results from the RTD analysis of multi-cloth stacks in the SCDR: the mean residence time of different multi-cloth stacks in the SCDR at different disc speeds and flow rates. Each experiment was repeated three times and the error bars are the mean ± one standard deviation.

Figure 7 Results from the RTD analysis of multi-cloth stacks in the SCDR: the number of tanks-in-series (N) of different multi-cloth stacks at different spinning speeds and flow rates. Each experiment was repeated three times and the error bars are the mean ± one standard deviation.

Figure 8 Images of the different layers in the multi-cloth stack with dye at flow rate of 8 mL s^{-1} and spinning speed of 400 rpm.

Figure 9 Images of the different layers in the multi-cloth stack with dye at flow rate of 8 mL s^{-1} and spinning speed of 100 rpm.

Figure 1(a) Schematic diagram of the multi-cloth in the SCDR setup for RTD analysis and visual study. (b) Top view of a two-cloth stack on the disc, each cloth containing immobilized enzymes. (c) Top view of a three-cloth stack on the disc, each cloth containing immobilized enzymes.

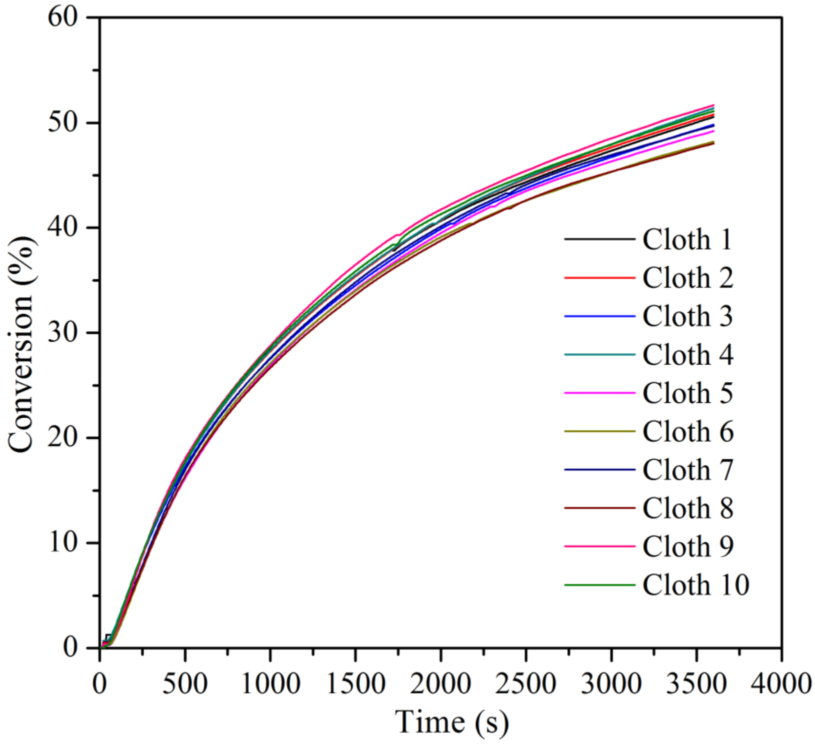

Figure 2 Time course of tributyrin hydrolysis with the ten newly prepared lipase immobilized cloths for this study. Reaction temperature: 45 °C, reaction time: 1 h, flow rate: 5 mL s^{-1}, spinning speed: 350 rpm.

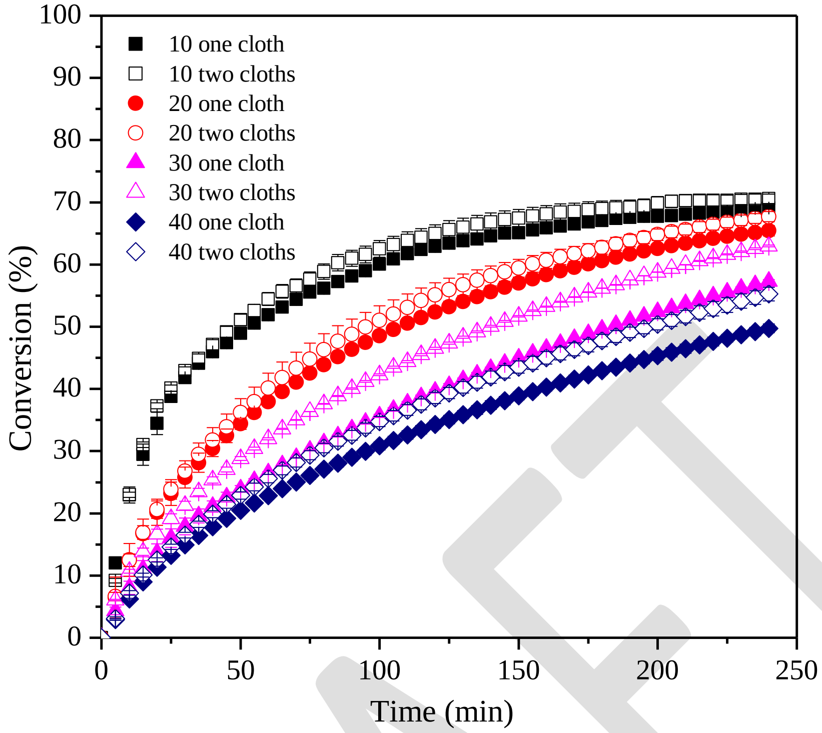

Figure 3 Comparison of one and two cloths in the SCDR for tributyrin conversion at different substrate concentrations. Each experiment was repeated three times and error bars are the mean ± one standard deviation.

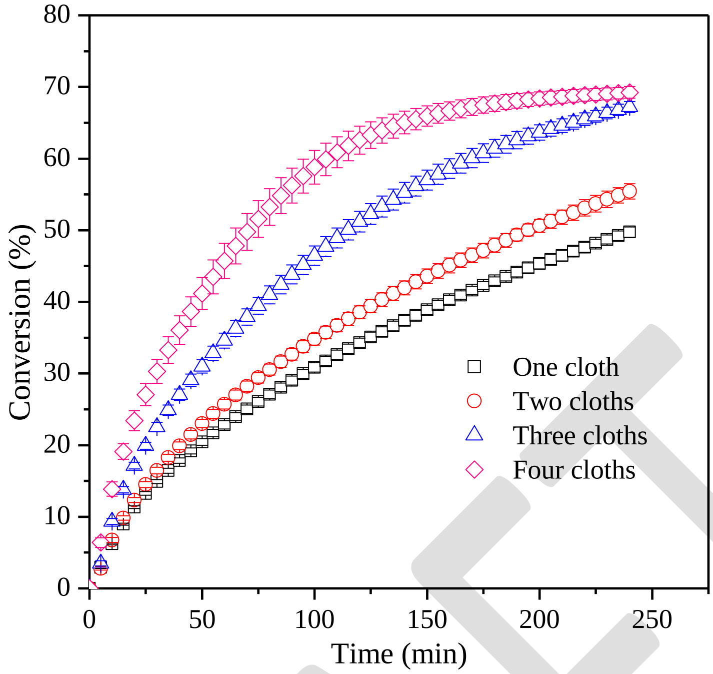

Figure 4 The effect of using multi-cloth stack (one to four cloths) in the SCDR on conversion over time with tributyrin concentration of 40 g L^{-1}. Each experiment was repeated three times and error bars are the mean ± one standard deviation.

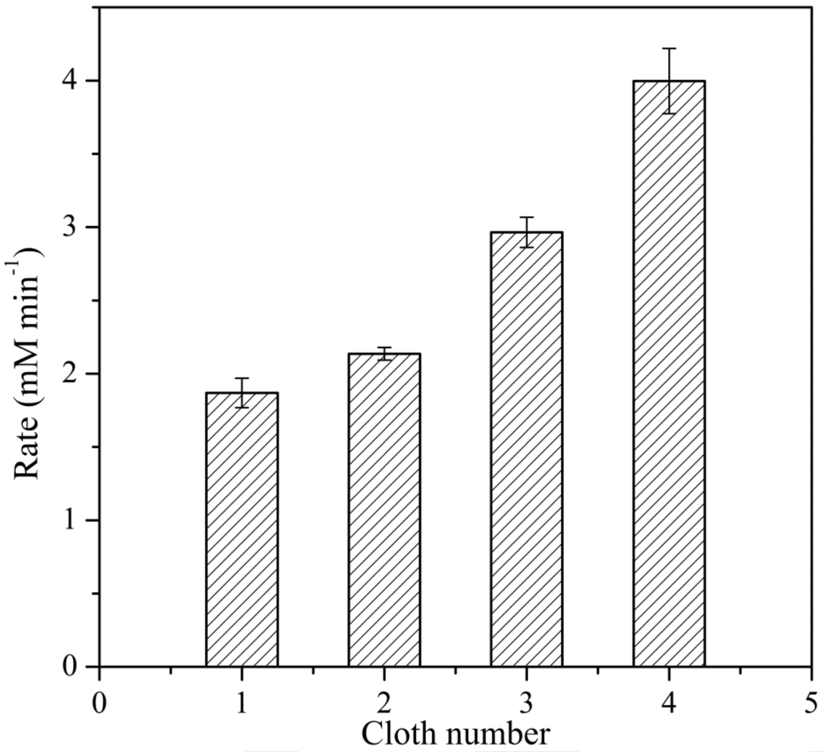

Figure 5 The effect of number of cloths in the SCDR on the overall reaction rate with tributyrin concentration of 40 g L^{-1}. Each experiment was repeated for three times and error bars are the mean ± one standard deviation.

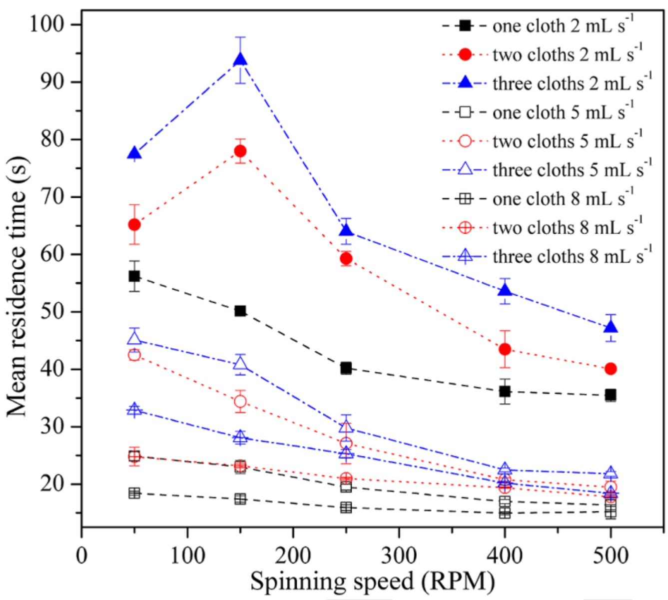

Figure 6 Results from the RTD analysis of multi-cloth stacks in the SCDR: the mean residence time of different multi-cloth stacks in the SCDR at different disc speeds and flow rates. Each experiment was repeated three times and the error bars are the mean ± one standard deviation.

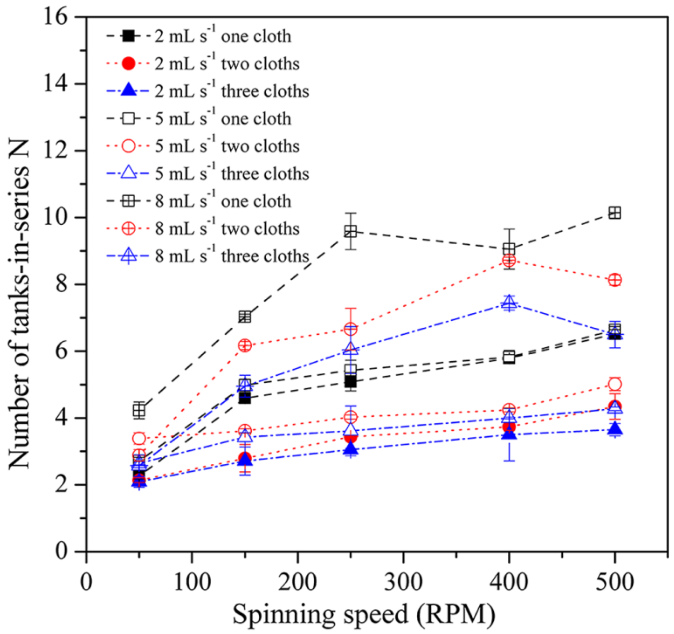

Figure 7 Results from the RTD analysis of multi-cloth stacks in the SCDR: the number of tanks-in-series N of different multi-cloth stacks at different spinning speeds and flow rates. Each experiment was repeated three times and the error bars are the mean ± one standard deviation.

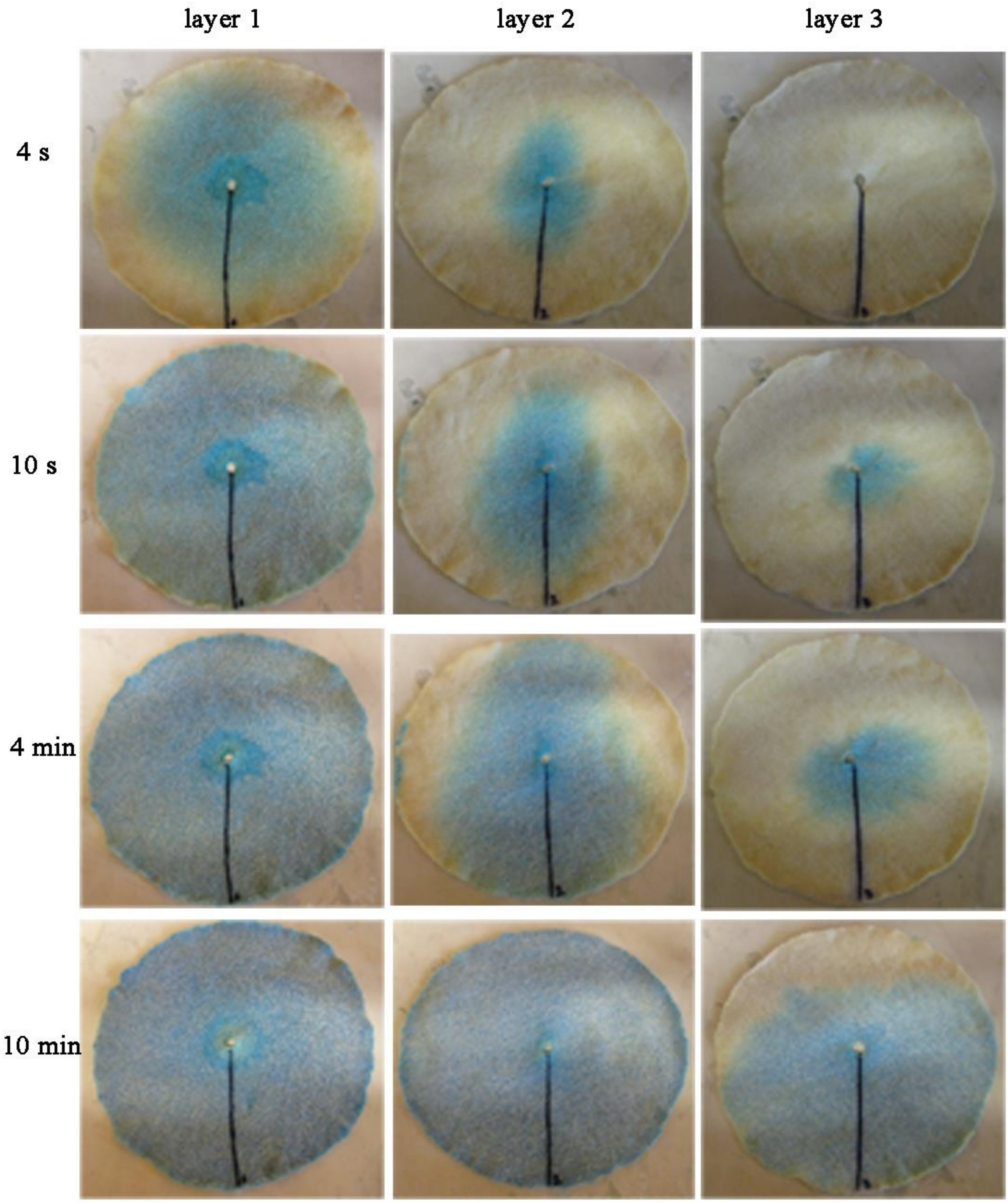

Figure 8 Images of the different layers in the multi-cloth stack with dye at flow rate of 8 mL s^{-1} and spinning speed of 400 rpm.

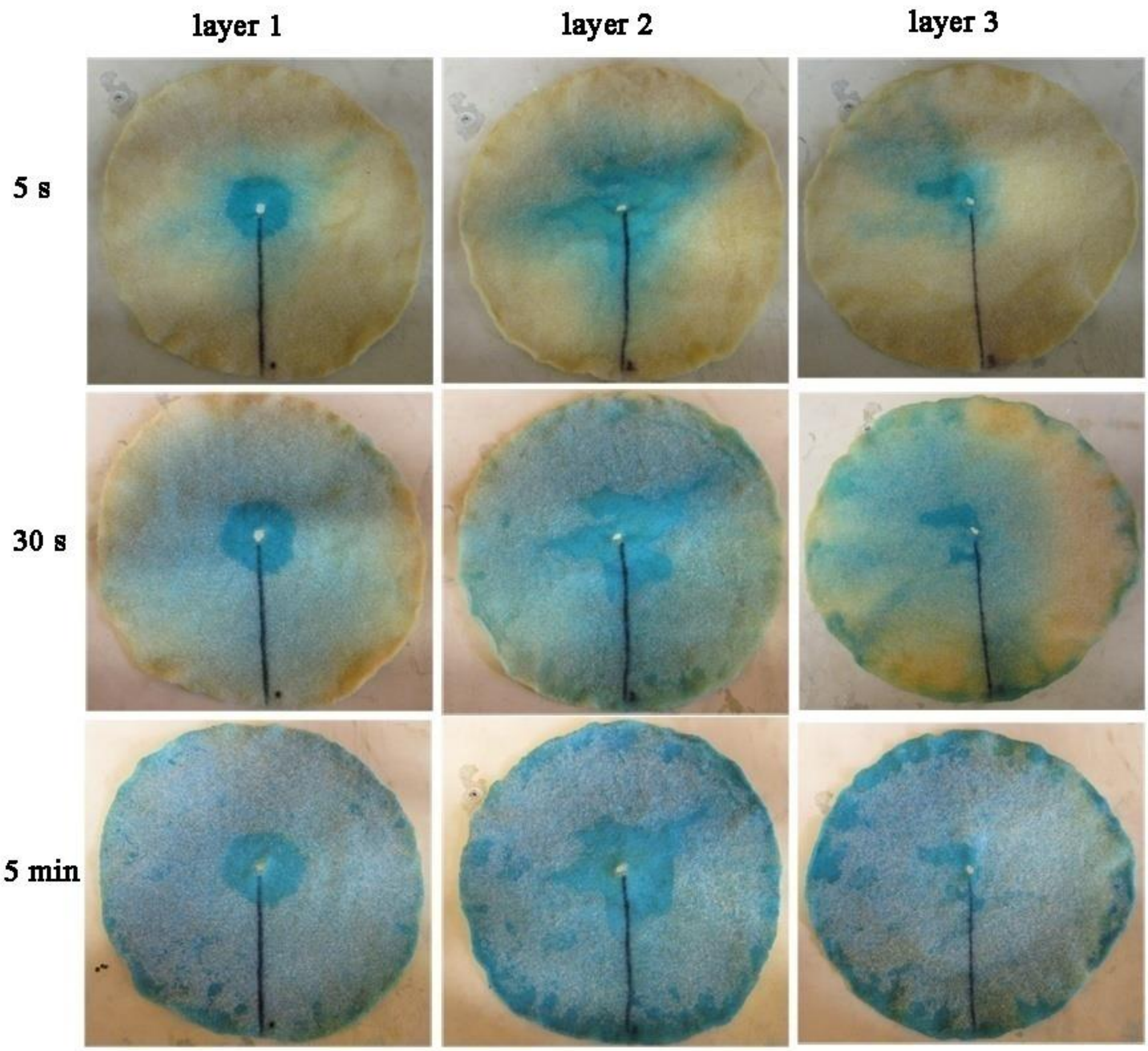

Figure 9 Images of the different layes in the multi-cloth stack with dye at flow rate of 8 mL s^{-1} and spinning speed of 100 rpm.