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The spinning cloth disc reactor for immobilized enzymes: A new process intensification technology for enzymatic reactions

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HIGHLIGHTS

- A novel rotating enzyme reactor was developed: spinning cloth disc reactor (SCDR).
- Process intensification occurred in SCDR: conversion and rate were largely improvfed.
- Below an average surface shear of 9500 s^{-1} enzyme loss in SCDR was very slight.
- Immobilized enzyme retained 80% initial activity after 15 long term reuses in SCDR.
- A Ping Pong Bi Bi kinetic model fitted well with the experimental data.

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ABSTRACT

The spinning cloth disc reactor (SCDR) is an innovative enzyme reaction intensification technology. Based on spinning disc technology, the SCDR uses centrifugal forces to allow an even spread of a thin film across a spinning horizontal disc which holds a cloth with immobilized enzyme. This geometry promotes accelerated reactions due to high mass transfer rates and rapid mixing. Here, the SCDR has been benchmarked against a conventional batch stirred tank reactor (BSTR) using tributyrin emulsion hydrolysis as a model reaction and lipase immobilized on woolen cloth as the biocatalyst. Reaction intensification has been shown to occur: the conversion in the SCDR was significantly higher than that in a conventional BSTR under comparable conditions. Spinning speed and flow rate control reaction rate and conversion: conversion increased nearly 7% on average as the flow rate rose from 2 to 5 mL s^{-1} and the highest conversion (72.1%) occurred at 400 rpm. A Ping Pong Bi Bi kinetic model fitted reaction progress data well. The immobilized lipase showed excellent stability to repeat reactions in the SCDR: 80% of the original activity was retained after 15 consecutive runs. The robustness of the SCDR to industrially relevant feeds was also demonstrated through successful hydrolysis of different vegetable oils at reaction rates 5 times higher than other reactors in the literature. Overall, the above results indicate that the SCDR is an innovative, superior and robust technology for enhancing enzyme reactions, taking enzyme reactors beyond the current state-of-the-art. This concept can readily be extended to other enzyme-catalyzed reactions, where enhanced mass transfer and enzyme stability is needed.

1. Introduction

Enzymatic hydrolysis of triglycerides into acids by lipase is an environmentally sustainable alternative to chemical hydrolysis and can be used in many important industrial applications (such as in the fat and oleochemical industry, the dairy industry, and wastewater treatment), due to the potential energy savings and alleviation of thermal deactivation of unsaturated fatty acids through lowering the reaction temperature [1,2]. One significant characteristic of lipase in this reaction is its activation at the oil–water interface; therefore such hydrolysis reactions catalyzed by lipase are more effective in oil/water emulsions [3]. In practical applications, immobilized lipase is more favorable due to the prominent advantages over its free form: enhanced stability, ease of enzyme recovery and reuse, simplified product separation. Thus, immobilized enzyme reactors have been widely studied for industrial processes [4] . Batch stirred tank reactors (BSTRs) are the most

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commonly used reactors for enzymatic processes, however this type of reactor suffer from a series of disadvantages, including the fact that active enzymes (in their free form) are complicated to recover and reuse and have low productivities [5]. Furthermore, mass transfer can be limiting, with only increased stirring speed as the means of reducing the mass transfer resistances inherent in any enzyme immobilization and support used. Consequently, a range of enzyme reactors have been proposed for lipase catalyzed reactions to overcome such disadvantages. For example:

- Packed bed reactors (PBRs), which offer great advantages for immobilized enzymes, such as high efficiency, low cost and ease of construction. PBRs with immobilized lipase have been used for rice bran oil hydrolysis [6,7]. However, the main drawbacks of PBRs are the associated large pressure drops (if the packing is too small) as well as potential bypassing and channeling if the catalyst is improperly packed [8]. To produce a low pressure drop, large particles are required; however this decreases the amount of enzyme per volume in the reactor (decreasing overall reactor efficiency).
- Fluidized bed reactors (FBRs) have been reported for oil hydrolysis with immobilized lipase [9]. One of the potential advantages of FBRs are that small particles can be used (since pressure drop is unaffected), however large particles are usually required anyway due to the low density difference between fluid and particles used in immobilized enzyme FBR systems, and the high viscosity of the fluids usually used. This again decreases the amount of enzyme per volume in the reactor, decreasing overall reactor efficiency. Moreover, significant channeling and bypassing of the particles as well as significant particle agitation (and therefore the potential for enzyme loss and deactivation) also make FBRs complicated to operate for enzymatic reactions.
- Enzyme membrane reactors (EMRs) have become increasingly popular due to their integration of enzyme catalyzed conversion and product separation into a single process. EMRs have been widely used with immobilized lipase for triglyceride hydrolysis [10–12]. However, EMRs typically undergo a decrease in reaction rate and yield during operation caused by loss of catalyst and mass transfer efficiency (including the effects of membrane fouling), limiting their industrial application.

Based on this, it is clear that a robust and stable enzyme reactor that maintains a stable immobilized enzyme at a high amount of enzyme per volume in the reactor combined with intensified mass transfer is very desirable. Therefore, this study investigates the application of process intensification to immobilized enzyme reactors, applying the concept of the spinning disc reactor (SDR) for the first time to immobilized enzyme systems.

Process intensification has been recognized as a promising development path for the chemical process industry, aiming to improve production efficiency, lower cost, enhance safety and reduce environmental pollution [13,14]. The SDR is one such technology, which consists of a rotating disc with a jet of liquid impinging onto the center of its top surface. The centrifugal force of the spinning disc forces this liquid to form a thin (100–200 μm) and highly sheared film on top of the rotating surface. Research has shown that the heat and mass transfer in such device can be significantly enhanced by the fluid dynamics within these films [15–17]. In addition, the SDR also has several benefits over conventional reactors, such as the rapid mixing in the liquid film and short liquid residence times [18]. Due to these factors, the SDR has been used to enhance reaction rates in a range of chemical reactions including: condensation polymerizations [19], nanoparticle preparation [20–23], biodiesel synthesis [24], pharmaceutical manufacture [25], and thin film photocatalysis [26]. Despite the wide range of reactions that SDRs have been applied to, to authors' best knowledge, the SDR concept has not yet been applied to enzyme reactions nor to catalyst systems immobilized within a three dimensional mesh, such as a fibrous cloth. Recently, a simple and effective protocol has been developed by the authors to immobilize lipase on woolen cloth with high enzyme load, activity and good reusability [27]. The protocol consists of four main steps: (1) bleaching of the wool surface to release viable functional groups for immobilization, (2) modification of the bleached wool surface with polyethyleneimine, (3) adsorption of lipase onto the polyethyleneimine modified wool by electrostatic interaction, and (4) cross-linking with glutaraldehyde to stabilize the immobilized lipase. Confocal laser scanning microscopy was used to confirm that immobilization occurred both on the outer surface and within the volume of cloth. This provides a large outer and inner fiber surface area facilitating contact and reaction of substrates with the immobilized enzymes [27]. This immobilized lipase on wool therefore has great potential as a matrix for immobilized enzymes in a spinning disc reactor system.

Therefore, through combining the SDR concept with this superior woolen cloth enzyme immobilization, this paper presents both a new method of process intensification in enzymatic reactions as well as a novel type of rotating reactor system: the spinning cloth disc reactor (SCDR). Based on the principles of the SDR, the SCDR also uses centrifugal forces to allow a spread of a thin film across a spinning horizontal disc; however this disc has a cloth with immobilized enzyme. The SCDR therefore potentially produces a thin liquid film flow both on top of and through the cloth. The cloth surface is key to increasing the potential of immobilized enzymes in a variety of reactions, since it should produce accelerated reaction rates due to high mass transfer rates and rapid mixing on top and within the cloth, with the cloth potentially helping protect the enzyme from excessive hydrodynamic forces, as well as providing an additional structure that can promote mixing and turbulence at the appropriate spinning speeds and feed flow rates. As such, the purpose of this research is to prove the viability of the SCDR concept and characterize its performance, using immobilized lipase onto woolen cloth with tributyrin emulsion hydrolysis as the model reaction. The SCDR will be benchmarked against a conventional BSTR run under equivalent conditions to determine if enzyme process intensification is achieved (and therefore if the SCDR is a worthwhile technology to pursue).

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*, polyethyleneimine (PEI; branched, average MW of 10,000), tributyrin (98%), tritonX-100, coomassie brilliant blue G 250, sodium bicarbonate and sodium carbonate were obtained from Sigma–Aldrich (New Zealand). Glutaraldehyde (GA) 25% (w/v), sodium dihydrogen phosphate, disodium hydrogen phosphate and hydrochloric acid were purchased from Unilab (ECP, New Zealand). Hydrogen peroxide 30% (v/v) was obtained from Scharlau (Thermofisher, New Zealand). Bovine serum albumin was obtained from Gibcobrl (Life Technologies, New Zealand). Canola oil, olive oil, soybean oil and sunflower oil were obtained from the local market. The phosphate buffer (pH 6 and pH 7) used in this study was composed of 0.1 M sodium dihydrogen phosphate and disodium hydrogen phosphate. All solutions were prepared using deionized water (produced from Milli-Q Gradient A10 made by Millipore).