Journal of Molecular Catalysis B: Enzymatic 119 (2015) 48–53

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Modelling of immobilised enzyme biocatalytic membrane reactor performance

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ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 15 May 2015
Accepted 27 May 2015
Available online 1 June 2015

Keywords:
Immobilised enzyme
Biocatalytic membrane reactor
Mathematical modelling
Bioprocess

ABSTRACT

Immobilised enzyme-catalysed conversions frequently provide specific advantages of selectivity over chemical conversions and further, facilitate continuous operation through biocatalyst retention and reuse. This study focuses on the development and modelling of an enzyme-catalysed continuous immobilised enzyme biocatalytic membrane reactor (BMR). The conversion of the amidase-catalysed lactamide to lactic acid process was used as an industrially representative system with which to evaluate the process performance of the BMR.

The model was developed from unsteady state differential mass balances incorporating a second order enzyme decay. This model was validated from empirically determined conversions in dual experiments using 80 and 40 mM amide substrate, 6.4 and 20.1 mg immobilised amidase and a flow rate of 0.0005 and 0.0001 L/min respectively.

Model predictions over a range of amidase amounts and stabilities, flow rates and initial amide concentrations quantified the direction and extent of the influence of these parameters on the maximum conversions attainable, consequently identifying the critical parameter ranges defining optimal BMR performance. Although the model has been developed and validated for the prediction of BMR performance of the specific lactamide-lactic acid system, it nevertheless has broad applicability for and relevance to broad-based prediction of the performance of immobilised enzyme BMR processes in general, irrespective of the specific enzyme or substrate moieties.

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1. Introduction

Biocatalytic processes frequently provide specific advantages of regio-, chemo- and enantio-selectivities over chemical conversions [1,2]. Moreover, biocatalytic processes using isolated enzymes rather than whole cells may significantly reduce the complexity of downstream processing [3]. Retention and reuse of the isolated enzymes by immobilisation in or on a matrix provide further benefit by facilitating continuous operation strategies. Membrane immobilisation has been specifically highlighted as a route to maximise process performance [4]. Membrane immobilisation techniques include ultrafiltration membranes where the enzyme is immobilised in or on the pores while the product, with a lower molecular weight relative to the enzyme, passes freely through [5]. Ultrafiltration membranes have been used successfully to retain isolated enzymes such as fumarase [3], polyphenol oxidase [6] and lipase [5]. These membranes are typically configured in flat or hollow fibre geometries in the construction of biocatalytic membrane reactors (BMRs), where substrate is fed to either the shell or lumen side, with products removed from lumen or shell side respectively.

In this study, the amidase-catalysed conversion of lactamide to lactic acid was examined and modelled in a BMR. Although soluble [7,8] and cross-linked [9] amidase-catalysed conversions have been reported, ultrafiltration matrices have not yet been evaluated as potential immobilisation matrices. The amidase-catalysed production of lactic acid provided a model system to evaluate the process performance of an enzyme-catalysed BMR process in general and an industrially relevant process in particular. Lactic acid has various applications in the food, pharmaceutical, leather and textile industries. The world market for lactic acid is 350,000 tonnes of lactic acid and this is growing by 15% annually [10]. Food-related applications account for 85% of the commercial product – as an acidulant, pH-buffering agent, flavouring agent, and bacterial inhibitor [11]. Its production by a biological-based process is economically important since this allows its being marketed as a “natural” product.

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http://dx.doi.org/10.1016/j.molcatb.2015.05.015
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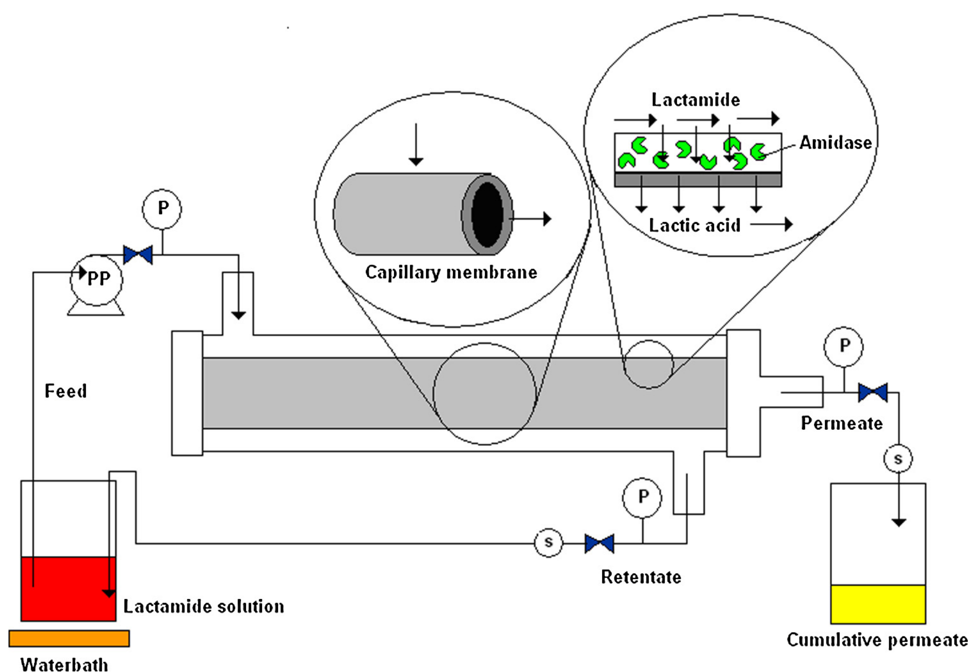

Fig. 1. Schematic diagram of the membrane immobilised amidase bioreactor system (PP = peristaltic pump, P = pressure gauge, s = sample point).

A major focus of this work was the development of a mathematical model which describes the kinetic performance of an amidase-catalysed biotransformation in a BMR system and subsequent prediction of conversion performance facilitated by the model. The model was developed from unsteady state differential mass balances incorporating enzyme deactivation. Model accuracy was validated by comparison with experimentally determined substrate conversions under different operating conditions. The model successfully predicted the kinetics in terms of conversion over wide ranges of enzyme amounts, stability, initial substrate concentration and volumetric flow rate. Importantly, the model is not constrained to the amidase system and has broad-based value in application to BMR immobilised enzyme systems in general.

2. Materials and methods

2.1. The BMR system

The BMR comprised a borosilicate glass tubular shell (Glass-chem, South Africa) with a ceramic capillary membrane insert (Tami-Industries, France) (Fig. 1). The active membrane layer (molecular weight cut off = 8 kDa) was positioned on the lumen side. Amidase (molecular weight = 35 kDa), produced from *Geobacillus pallidus* RAPc8, cloned and over-expressed in *Escherichia coli* BL21 pNH 223 pLysS [12], was absorbed into the pores on the shell side. The substrate, DL-lactamide (Sigma Aldrich), 97% purity, was fed through the shell side by means of a peristaltic pump (Vera Manostat, Sigma Aldrich). The reaction took place in the pores of the membrane, where the enzyme was immobilised, with the reaction path from the shell side to the lumen side of the BMR.

Since a major aim was to determine a fundamental kinetic relationship, it was necessary to use a single membrane to ensure that the pressure drop was uniform over the entire surface and to avoid secondary effects of the specific system geometry. An understanding of this relationship has the advantage that it can then be used to predict behaviour in more complex systems, such as increased membrane surface area.

2.2. Enzyme immobilisation

Amidase was immobilised by circulating 300 mL chilled enzyme solution through the shell side of the BMR at 200 mL/min and a transmembrane pressure (TMP) of 0.5 bar (gauge) for 2 h. The system was washed with distilled water until no enzyme protein could be detected in either the retentate or permeate streams. The amount of enzyme protein immobilised was determined from a mass balance of the residual protein in the circulating solution and the protein in the retentate and permeate washings. Effective retention of protein was confirmed since no losses occurred in the permeate or retentate streams during operation for over 20 h after immobilisation procedures in similar experiments with different feed enzyme protein concentrations (0.021 mg/L and 1.07 mg/L).

2.3. Enzyme and product analyses

Enzyme activity was determined by quantifying the amount of ammonia released during the amidase-catalysed conversion of lactamide to lactic acid, using the phenol-hypochlorite ammonia detection method [13,14]. Enzyme activity was expressed in units where 1 unit was defined as the amount of amidase which catalysed the release of 1 μmol of ammonia (equal to 1 μmol lactic acid) per minute at standard assay conditions ($T = 50\,^{\circ}$C, pH = 8.0 and lactamide concentration = 80 mM). Specific enzyme activity was expressed in units per mg of enzyme protein. Enzyme protein was measured by the Bradford Coomassie Brilliant Blue dyebinding assay and the micro Bradford Coomassie Brilliant Blue dyebinding assay (Bradford Reagent, Product information, Sigma Aldrich) for protein concentrations in the ranges 50–1000 μg/mL and 1–20 μg/mL respectively. The lactic acid product was quantified according to the amount of ammonia released (as above) since equimolar quantities of lactic acid and ammonia are formed from one mole of lactamide.

3. Results and discussion

3.1. Development of the BMR system for amide conversion

The BMR system was established for the conversion of lactamide to lactic acid using immobilised amidase as described (Section 2.1).

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