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Scaling Up Biocatalysis Reactions in Flow Reactors

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ABSTRACT: Biocatalytic oxidase processes can benefit from improved mixing and mass transfer, as can all multiphase processes. Flow reactors are established for their mixing capabilities, but there are practical challenges in terms of slurry and gas/liquid handling. The oxidase of the DL-amino acid was studied and scaled up in flow in a Coflore system and compared to batch processing conditions. The improved mass transfer under flow conditions resulted in a reduction in reaction time, enzyme consumption, and pressure drop.

INTRODUCTION

Unnatural amino acids (UAA) are extremely relevant to pharmaceutical drug substance development, especially for peptidomimetic pharmaceuticals. Many UAAs have been very effective in peptide based drugs used as HIV inhibitors, since they introduce resistance to proteolytic degradation in the bloodstream, thereby extending drug half-life and improving pharmacokinetic properties. Following the success of HIV protease inhibitors, many new pharmaceuticals in the area of virology and oncology now incorporate UAAs (examples are atazanavir, nafarelin and amoxicillin). Chiral amines represent a substantial class of chiral pharmaceutical intermediates for which there are no general, scalable, and economical biological methods of synthesis. Resolution methods employing lipase enzymes have been used to prepare a limited range of chiral amines.[1] Resolution approaches are widely used for the production of chiral chemicals at manufacturing scale despite 50% maximum single pass yield due to the simplicity of the approach. Resolution of racemic amino acids or amines using amine or amino acid oxidases represents a simple method for production of chiral amines and amino acids with high optical purity. In this example the two products of the resolution (an amino acid and a ketoacid) may be separated very easily. Where the process economics demand, amino acid oxidases have been used in conjunction with metal catalysed reduction to allow deracemisation of amines and amino acids in high optical purity and yield.[2] The optimisation of such processes begins with the optimisation of the enzymatic oxidation half reaction (effectively the kinetic resolution of the racemic acid).

Biochemist is a project cofunded by the UK Technology Strategy Board. The aim is to develop continuous manufacturing solutions for enzyme catalysed reactions. The partners in this project are Ingenza, CTech Innovation, and AM Technology.

Many of the opportunities for process intensification (PI) have already been identified by analysis of existing commercial manufacturing routes.[3] In a study[4] of 47 commercial chemical manufacturing processes, only three were continuous, whereas the remainder were batch or fed batch. About half of the processes surveyed could be accelerated by removing the mass or heat transfer limitations. Over 60% of processes involved the feed of a solid material, and over 40% involved the feed of a solid that is essentially insoluble in the solvent used. Thus, there is a clear need for PI equipment capable of handling solids, from feedstock handling, through reaction, to product recovery and drying.

Although many continuous-flow processes have already been established in synthetic chemistry,[5] the use of flow reactors in bioapplications is still limited, with only a handful reported in the literature.[6] Long residence times and multiphase media are common features in bioprocesses, and traditional tubular flow reactors have proved poorly suited to this type of operation.

Larger scale flow reactors fall into two categories depending on whether they use static or dynamic mixing. Statically mixed systems rely on turbulent flow and/or baffles to promote bending and folding of the fluid. For these systems, both mixing and orderly (plug) flow (i.e., product leaving the reaction channel in the same order that it enters it) rely on high fluid velocities through the channel, and this implies long narrow tubes. Such systems are vulnerable to suboptimal mixing, blockage, and phase separation. Dynamically mixed flow reactors employ mechanical stirrers. These provide very high mixing efficiencies independent of fluid velocity. By decoupling the relationship between fluid velocity and performance, low aspect ratio (tube length/tube diameter) reactor tubes can be used. This approach has inherent advantages over statically mixed systems for processes with long reaction times, demanding mixing conditions or the presence of solids. In this study, a Coflore Agitated Tube Reactor (ATR) was used. This is a dynamically mixed reactor which relies on loose agitator elements and mechanical shaking of the reactor body for mixing. This approach overcomes the problems of traditional rotating mixers in tubes (shaft flexing, mechanical seals, baffles, and centrifugal separation). In previous studies, Coflore reactors have demonstrated low pressure drop,[7] high mixing efficiency with long reaction times,[8] and the ability to handle slurries and gas/liquid mixtures.[9,10]

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EXPERIMENTAL SECTION

The DL-amino acid mixture is resolved biocatalytically by selective oxidation of the D-amino acid flavin adenine dinucleotide (FAD) as a redox cofactor, giving a mixture of L-amino acid and the α-ketoacid using wild-type D-amino acid oxidase (Figure 1). The biocatalyst is produced by fermentation

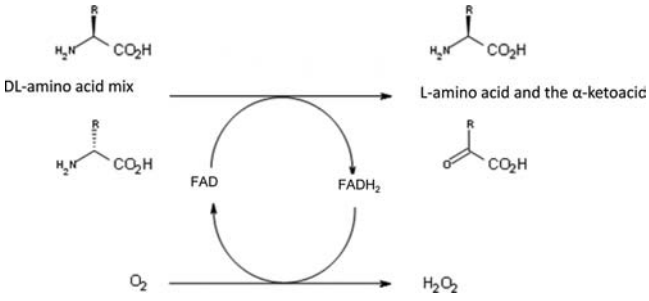

Figure 1. Biocatalytic resolution of DL-alanine.

of *Pichia pastoris* expressing the DAAO enzyme. The whole cells from the fermentation are freeze-dried and added to the biotransformation vessel. Oxygen is required as cosubstrate and is added to the reaction via a sparged gas inlet (ground glass fritted glass tube). Initially, the reactions are usually run under oxygen limited conditions due to the gas–liquid mass transfer constraints of the vessels used. The reactions are monitored by HPLC and are deemed to be complete when the enantiomeric excess (ee) of the L-amino acid is >99%.

The requirement for the cosubstrate oxygen in the bioprocess introduces additional parameters which, using conventional stirred tank reactor vessels, increases the complexity and time to develop and optimize the process toward each target. In particular, the gas–liquid mass-transfer characteristics must be well understood to ensure optimal enzyme usage and minimization of plant time. Additionally, a byproduct of the reaction is hydrogen peroxide, which can affect the reaction by (a) causing decomposition of the biocatalyst and, therefore, reducing the rate of reaction and b) reacting with the keto-acid product, giving rise to the C-1 carboxylic acid (decarboxylation, e.g. pyruvic acid to acetic acid).

Reactions were carried out at a concentration of 1 mol of alanine (89.09 g) per litre of water. Some initial tests were carried out in batch mode to obtain kinetic data to highlight the links between reaction rate and mixing intensity. Different batch vessel sizes, agitation speeds, and enzyme load were tested. The type of stirrer was a 4 cm impeller in all cases.

The process material in this study contains gas (oxygen), liquid (alanine solution), and solids (enzyme immobilized within whole cells). It also requires efficient mixing for reaction times lasting many hours. A Coflore ATR flow reactor was used for the flow experiments. This is a tubular reactor with free moving agitators. These agitators generate strong radial mixing when the reaction tubes are subjected to lateral shaking (Figure 2). The Coflore ATR has good slurry handling capabilities and can maintain orderly flow (plug flow) and good mixing for reaction times ranging from a few seconds to many hours.

When working in continuous processing mode, the alanine and enzyme suspension was prepared and kept stirred in the absence of oxygen. The slurry dispersion was pumped into the Coflore ATR system using a peristaltic pump (Watson Marlow), while adjusting the flow rate to achieve different

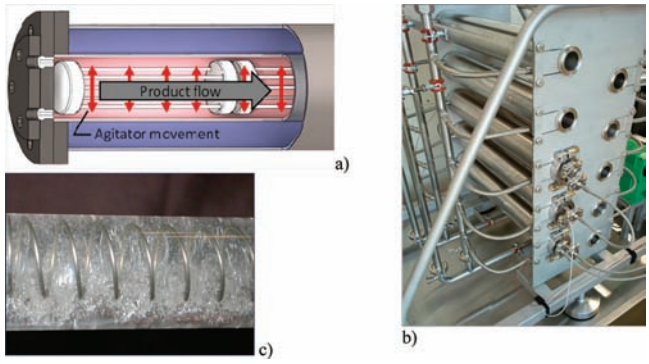

Figure 2. Coflore ATR: (a) section of a tube with agitator; (b) assembly; (c) gas/liquid mixing.

residence times for a given reactor volume. The oxygen was injected via a second inlet point directly in the Coflore ATR tube (or at multiple stages depending on the scale, if more than one tube was used) (Figure 3). When operating in continuous mode, the oxygen flow per unit volume was maintained at the same rate as that used in batch mode. The mixing intensity directly depends on the shaking frequency, and for the results shown, this was kept constant at 120 cycles per minute for all cases, equivalent to 2 Hz.

RESULTS AND DISCUSSION

Subject to the presence of adequate enzyme loading, the reaction rate for this reaction is primarily constrained by the uptake of oxygen, and therefore, good mixing is required for fast reaction times. In Figure 4, the agitator speed is varied in a 1 L batch vessel. The results show a substantial increase in reaction rate as the agitator speed is increased. After about 5 h, however, the reaction rate falls to a lower and approximately constant rate. The reaction rate during the initial 5 h varies from 13 to 3.6% per hour depending on stirrer speed. After 5 h, this falls to 2.3–2.6% per hour and is believed to be due to depletion of enzyme. When this reaction was performed in a smaller 250 mL batch reactor, however, a higher reaction rate of 21% per hour was achieved for a comparable agitator speed (Figure 5). When scaled up to 4 L capacity, the reaction rate fell to 4.5% per hour. These results demonstrate the practical problems of scaling up gas/liquid reactions in batch reactors. As the physical size of the reactor increases, the problems of maintaining a uniform gas/liquid dispersion and efficient distribution of mixing energy increase. As a result, batch equipment becomes disproportionately large as throughput is increased. This not only impacts on capital and operating cost but also has serious implications for enzyme consumption (since the enzyme has a short service life). The problem of catalyst life versus reaction time can be managed by using multiple small batch reactors. This, however, compounds the problems of capital and operating cost.

The scale constraints of batch reactors can be addressed by using dynamically mixed flow reactors. The Coflore reactor belongs to this category but, unlike conventional stirred systems, it uses transverse mixing by lateral movement of the agitators. This has the advantage of being self-baffling and eliminates the problems of phase separation due to centrifugal action (of rotating stirrers). Even at the 1 L scale (Figure 6), the reaction rate in a 1 L Coflore tubular reactor is three times faster than a 1 L batch reactor. When the Coflore reactor is scaled up to 10 L (Figure 7), the reaction rate remains

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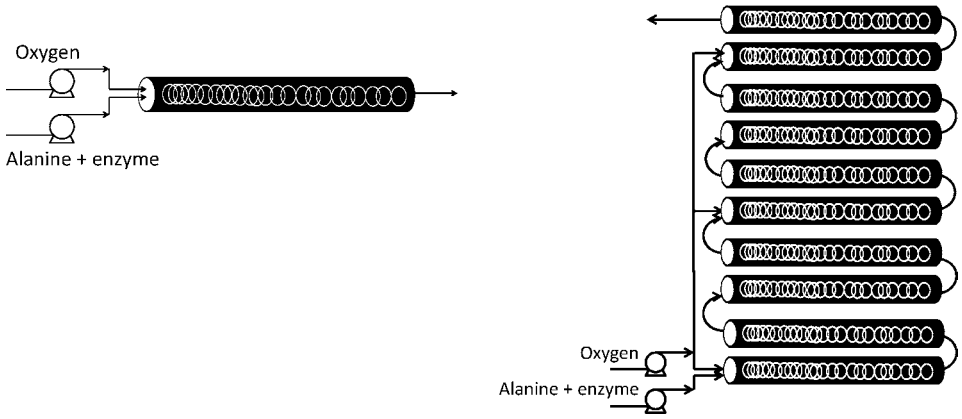

Figure 3. Experimental setup.

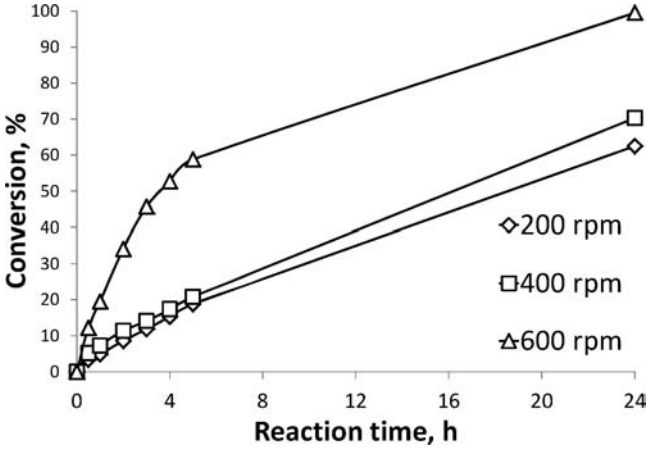

Figure 4. Effect of stirrer speed in a 1 L batch reactor. Enzyme load = 7 g/L, oxygen flow = 0.625 L/min.

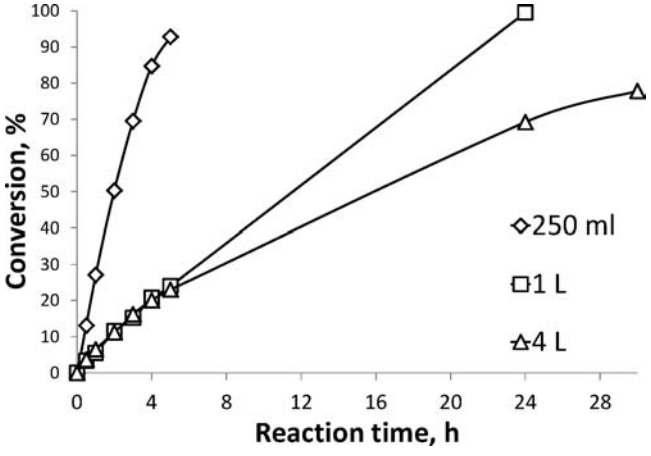

Figure 5. Effect of vessel size. Enzyme load = 21 g/L, stirrer speed = 400 rpm, oxygen flow = 0.25 (L/min)/L_{vessel}.

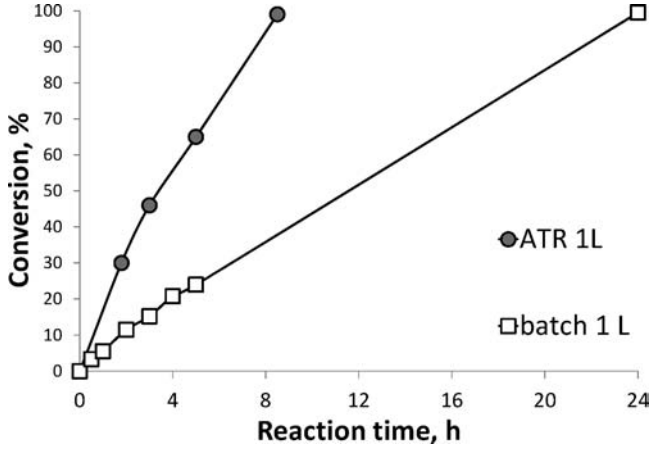

Figure 6. Effect of continuous operation. Enzyme load = 21 g/L, batch stirrer speed = 400 rpm, ATR agitation frequency = 2 Hz, oxygen flow = 0.25 L/min.

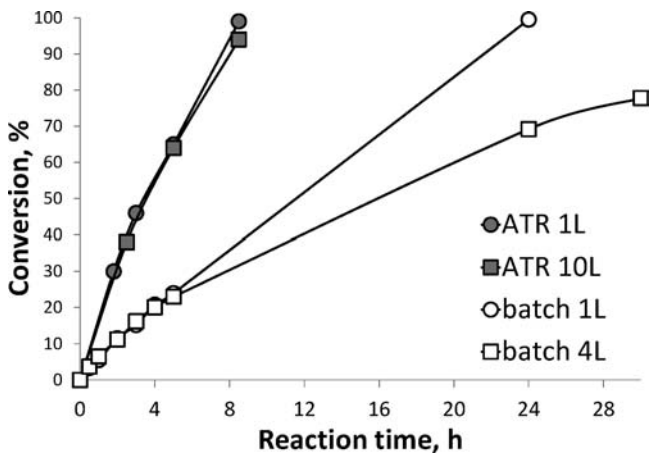

Figure 7. Effect of scale up in continuous and batch. Enzyme loading = 21 g/L, batch stirrer speed = 400 rpm, ATR agitation frequency = 2 Hz, oxygen flow = 0.25 (L/min)/L_{vessel} for batches and ATR 1 L. For ATR 10 L, oxygen flow = 0.75 L/min.

substantially unchanged. It is also worth noting that the 10 L Coflore reactor used 70% less oxygen than the 1 L system due to experimental constraints. This highlights the fact that, in batch, oxygen is used in large excess and wasted.

Scaling up a batch reactor alters the vessel diameter, height, and agitator speed (relative to the process fluid). This has a marked impact on mixing performance and gas/liquid distribution. The Coflore ATR reactor, in contrast, is scaled up by increasing tube length, which leaves mixing and gas/liquid distribution unchanged.

The lengths of the Coflore tubes were 0.7 and 7 m for the 1- and 10-L systems, respectively (4.2 cm in diameter for all cases). Table 1 illustrates the very low linear velocities employed. Compared to a statically mixed flow reactor, this

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Table 1. Comparison between Batch and Continuous at Different Scales

performance parameter	1-L batch	4-L batch	1-L ATR	10- L ATR
velocity (m/s)	N/A	N/A	0.00002	0.0002
pressure drop (mm water gauge)	N/A	N/A	<1[a]	<1[a]
reaction rate at 50% conversion (%/h)	4	2.4	9.8	10.3
conversion at 4 h (%)	20.8	20.1	55.7	54.6
conversion at 8 h (%)	35.9	30.4	94.1	89.7
oxygen consumption ((L/h)/L)	0.25	0.25	0.25	0.075

[a]By calculation.

represents a substantial (orders of magnitude) reduction in required overall tube length. Table 1 also shows very low pressure drops through the reactor, which is a product of low linear velocities and large tube diameters. In conventional statically mixed flow reactors, mixing performance is dependent on minimum fluid velocities, and therefore, overall pressure drop is directly proportional to reaction time. For long reaction times, this can present formidable pump cost/selection problems. In this case, the pressure drop through the Coflore reactor was sufficiently low to dispense with the use of pumps and employ gravity transfer (although pumps were used in this case for metering purposes).

Apart from gases and liquids, this process also contained solids in the form of live cells and organic debris. Materials of this type have a tendency to accumulate and block in statically mixed flow reactors. No blockage problems were encountered. This can be attributed to the high mixing efficiency and large tube diameters in the Coflore reactor.

CONCLUSIONS

The rate of reaction in this oxidation process is primarily determined by the rate of oxygen uptake. This in turn is dependent on mixing efficiency and uniformity of gas/liquid distribution. This makes it a difficult process to scale up in batch reactors. By employing a dynamically mixed Coflore flow reactor, it was demonstrated that transverse mixing was not only substantially more effective than traditional rotational mixing but that throughput can be increased by an order of magnitude with negligible impact on reaction rate. Despite the presence of live cells and organic debris, no problems of blockage were encountered with the Coflore ATR unit. The commercial implications of this system are reduced cost of capital equipment, lower operating costs, and reduced catalyst consumption, due to faster throughput, for manufacturing processes.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Breuer, M.; et al. *Angew. Chem., Int. Ed.* **2004**, *43* (7), 788.
(2) Carr, R.; et al. *Angew. Chem., Int. Ed.* **2003**, *42* (39), 4807.
(3) Birse, D. *EPIC Symp. Ser.* **2011**, *157*, 53.
(4) Atherton, J. H.; Double, J. M.; Gourlay, B. *Survey of Process Intensification Equipment Requirements in the Fine Chemicals and Pharmaceuticals Sector*; 2007.
(5) Ley, S. V.; Saaby, S.; Tranmer, G. K. *Chem. Commun.* **2006**, 2566.
(6) Coughlin, R. W.; et al. *Biotechnol. Bioeng.* **2004**, *17* (4), 515.
(7) Reay, D.; Ramshaw, C.; Harvey, A. *Process Intensification*; Butterworth-Heinemann: 2008; p 157.
(8) Delogu, P.; Gasparini, G. *Spec. Chem.* 2010, May, 34.
(9) Browne, D. L.; et al. *Org. Process Res. Dev.* **2011**, *15* (3), 693.
(10) Gómez-Quero, S.; et al. *Chem. Eng. J.* **2011**, *166* (3), 1044.