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In the Lab

Bioreaction Engineering for the Implementation of Biocatalysis in Industry

Johnson Matthey Technology Review features new laboratory research

John M. Woodley is a Professor of Chemical Engineering at the Department of Chemical and Biochemical Engineering at the Technical University of Denmark (DTU). Originally from the UK, his research focuses on the relatively new field of bioreaction engineering, using chemical engineering to design and implement the next generation of chemical processes with enzymatic and microbial catalysts. In 2014 he was a Gambrinus Forum Lecturer at Technical University of Dortmund, Germany, and in 2016 he was the elected Chair of the Gordon Research Conferences 2016 Meeting on Biocatalysis held at the University of New England, USA. He has published over 170 peer-reviewed papers and is a Fellow of the Institution of Chemical Engineers and the Royal Academy of Engineering in the UK.

About the Researcher

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About the Research

The area of enzymatic catalysis for organic synthesis (commonly termed biocatalysis) has grown enormously in recent years, fuelled in particular by developments in protein engineering which allow the modification of specific enzyme properties. Protein engineering involves swapping of amino acid residues, and can result in enzymes with enhanced properties such as activity (reaction rate) as well as stability. The attractive features of enzyme-catalysed reactions include superb selectivity under mild reaction conditions (ambient temperature, atmospheric pressure and neutral pH) and today many biocatalytic reactions are used by organic chemists to assist in laboratory-scale syntheses. Of these reactions, around 150 or so have been scaled up and operate in industry, mostly in the pharmaceutical sector, with particular application in the synthesis of optically-pure chiral compounds. The biocatalysts used can vary in format and include the use of isolated soluble enzymes, enzymes immobilised on an inert support and enzymes encompassed within permeabilised microbial cells in a non-growing state.

Despite the strong rationale for using biocatalytic processes on a large scale, the requirements of industrial processes are far from those usually found in nature. For example the most valuable industrial products are made from substrates which are rarely found in nature and therefore frequently have not been seen by an enzyme. Likewise the reactor operating conditions, often involving high substrate and product

concentrations, are far from those occurring in the natural environment of an enzyme. With very few exceptions therefore it is essential to undergo an engineering programme to alter both enzyme (activity and stability under particular conditions) and process (feeding substrate, immobilising the enzyme) in such a way as to apply it effectively at a larger scale.

This integration of protein and process engineering is exemplified very well in the case of transferase reactions, where a functional moiety is transferred from a co-substrate (donor) molecule to a substrate (acceptor) molecule, yielding a new product and co-product. **Figure 1** schematically illustrates such a reaction. In these reactions, in order to shift equilibrium towards the product in situations where the reaction is not thermodynamically favourable it is necessary to either use an excess of co-substrate to 'push' the equilibrium to the product, or to 'pull' the reaction by removing either the product as it is formed (*in situ* product removal (ISPR)) or the co-product as it is formed (*in situ* co-product removal (IScPR)), or combinations of all three methods. Each method presents challenges where protein engineering can also help.

Another excellent example of utilising protein engineering with process engineering is ISPR itself. Schematic representations of the four modes of ISPR/IScPR operations are shown in **Figure 2**. Analysis indicates that selectivity of the separation is paramount, but so too is the need for a sufficient driving force to remove the product fast enough. Here protein engineering can assist in producing a workable solution along with a technology driven approach.

Challenges such as these have provided the motivation for research in the Bioreaction Engineering Group based in the Department of Chemical and

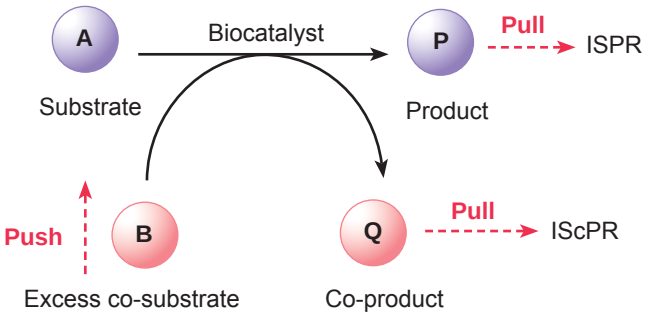

Fig. 1. Schematic representation of transferase reaction. Co-substrate (B) donates a functional moiety to substrate (A), yielding product (P) and co-product (Q). (© John Wiley & Sons, Inc)

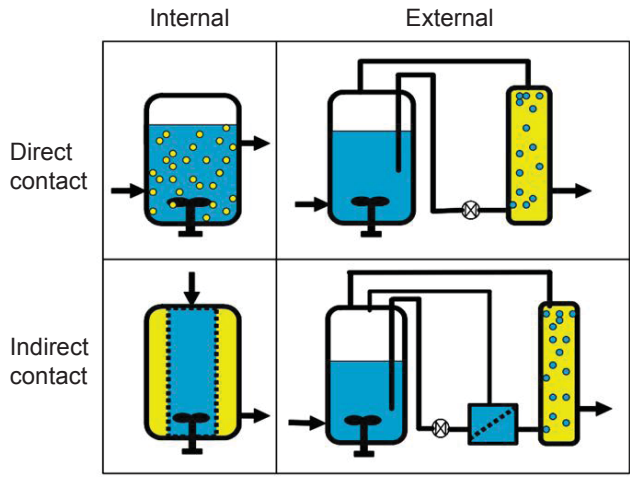

Fig. 2. Schematic flowsheets showing internal and external modes of ISPR operation with direct and indirect biocatalyst contact. (© John Wiley & Sons, Inc)

Biochemical Engineering at DTU. The emphasis of the research is at the interface of biotechnology and chemical engineering. The DTU group is one of only a handful worldwide that have such a focus and it aims to target solutions using both protein and process engineering. The work is necessarily interdisciplinary and the group has academic collaborators around the world: Bernhard Hauer, University of Stuttgart, Germany; Nicholas Turner, University of Manchester, UK; Dick B. Janssen, University of Groningen, The Netherlands; Marco Fraaije, University of Groningen, The Netherlands; Volker Sieber, Technical University Munich, Germany; and Masanao Imai, Nihon University, Japan. Likewise validation and testing of new process concepts necessitates industrial collaboration: BASF, Germany; Dr Reddys Laboratories Ltd, India and UK; Johnson Matthey Plc, UK; B.R.A.I.N. Biotechnology Research and Information Network AG, Germany; Prozomix Ltd, UK; DSM, The Netherlands; and Novozymes, Denmark.

The current work of the group is in five main directions:

1. **Multi-step biocatalysis, enzymatic cascades and systems biocatalysis:** Increasingly multiple enzymes are being linked together in synthetic sequences and modelling this, with the intention of reaction and reactor design is an important objective of the group
2. **Biocatalysis in flow:** The shift towards flow chemistry is particularly valuable for enzyme reactions, linked with neighboring operations such as product recovery, other enzyme steps

or chemical catalysis. A particular interest is multi-phase flow reactor systems

3. **Thermodynamic and kinetic measurements:** The interest here is in establishing rapid and automated methods for collecting data to assist in enzyme, reaction and reactor design
4. **Oxygen supply methods:** Oxidases and oxygenases are particularly valuable enzymes for synthesis and the group are interested in understanding the best way to supply oxygen, model this and build suitable scale-down equipment to understand the implications of different supply methods
5. **Techno-economic evaluation of new bioprocesses:** Use of costing metrics to evaluate new bioprocesses, and benchmark against pre-defined targets as a means of identifying the required enzyme and process improvements.

Current enzyme systems under investigation include galactose oxidase, Baeyer-Villiger monooxygenase, glucose oxidase, cytochrome P450, ω-transaminase, amine dehydrogenase, alcohol dehydrogenase and nicotinamide adenine dinucleotide (phosphate) hydrogen (NAD(P)H) oxidase.

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