Ensuring the Sustainability of Biocatalysis

Woodley, John M.

Published in:
ChemSusChem

Link to article, DOI:
10.1002/cssc.202102683

Publication date:
2022

Document Version
Peer reviewed version

ChemSusChem

Chemistry–Sustainability–Energy–Materials

Chemistry Europe

European Chemical Societies Publishing

Accepted Article

Title: Ensuring the Sustainability of Biocatalysis

Authors: John M. Woodley

To be cited as: *ChemSusChem* **2022**, e202102683

Link to VoR: https://doi.org/10.1002/cssc.202102683

WILEY-VCH

10.1002/cssc.202102683

WILEY-VCH

CONCEPT

Ensuring the Sustainability of Biocatalysis

John M Woodley*[a]

[a] Professor John M Woodley
Department of Chemical and Biochemical Engineering
Technical University of Denmark
2800, Kgs Lyngby, Denmark
E-mail: jw@kt.dtu.dk

Abstract: Biocatalysis offers many attractive features for the synthetic chemist. In many cases, the high selectivity and ability to tailor specific enzyme features via protein engineering already make it the catalyst of choice. From the perspective of sustainability, several features such as catalysis under mild conditions and use of a renewable and biodegradable catalyst also look attractive. Nevertheless, to be sustainable at a larger scale it will be essential to develop processes operating at far higher concentrations of product, and which make better use of the enzyme via improved stability. In this concept, I will argue that a particular emphasis on these specific metrics is of particular importance for the future implementation of biocatalysis in industry, at a level which fulfills its true potential.

Introduction

Biocatalysis offers enormous potential for the catalysis of reactions comprising new sustainable routes for the synthesis and production of many chemicals of industrial interest.[1] To support this claim, the features of enzymatic reactions frequently cited in the scientific literature are that reactions are highly selective under mild conditions and that protein engineering offers the ability to tailor enzyme properties to the particular needs of the chemist or process engineer, responsible for implementation.[2] Protein engineering offers the possibility to engineer enzymes for new-to-nature reactions as well as new-to-nature conditions.[3] Many industrial examples of biocatalysis, especially in the pharmaceutical sector, illustrate well both these features.[4] Moreover, as catalysts, enzymes are both renewable, as well as biodegradable. Furthermore, in recent years cascade biocatalysis, where the product of one reaction serves as the substrate for the subsequent one has also been implemented, enabling the creation of de novo pathways and in many cases shortening alternative syntheses.[5] All of these features mean that such catalysts suit the obvious requirements for sustainable processing, and it is therefore often concluded that biocatalysis is a sustainable process technology.[6] Whilst in many cases laboratory syntheses using enzymes do indeed meet the requirements of green and sustainable syntheses, as so often the devil is in the detail and some additional features also need to be addressed. Amongst the additional features are the need for high concentrations of product and effective use of both the reactor and catalyst. Indeed, addressing these additional requirements also helps to enable the translation of laboratory syntheses into industrial processes, which in turn, should enable the production of low-priced products by biocatalysis. Such low-priced products today represent the greatest challenge for a sustainable chemical industry and therefore are the very ones that can benefit most from biocatalytic methods.[7]

Table 1 lists some of the key requirements for sustainable industrial catalysts. A simple comparison is made between metal-based catalysis (often lacking the required reaction selectivity), fermentation (often lacking the required reaction rate) and biocatalysis (often lacking the required reaction longevity).

Table 1. Key Requirements of Sustainable Industrial Catalysts.

Requirement	Metal-based catalysis	Fermentation with growing cells	Biocatalysis with resting cells	Biocatalysis with isolated enzyme
High selectivity	■	■	■	■
Operation under mild conditions	■	■	■	■
Tunable properties	■	■	■	■
Renewable Catalyst	■	■	■	■
Degradable catalyst	■	■	■	■
Operation at high product concentration	■	■	■	■
High catalyst stability	■	■	■	■
Catalytic rate	■	■	■	■

■ Required performance in the majority of cases; ■ Performance requires improvement in the majority of cases; ■ Performance does not meet the requirement.

Both fermentation and biocatalysis offer attractive routes. However, fermentation suffers from a lack of selectivity as well as speed. The rate of such syntheses being entirely dependent upon the cell concentration, which itself needs to be built-up in the early stages of conversion of the substrate. Metabolic engineering tools can focus the pathway on the product of interest, thereby increasing selectivity[8], but improving rate of reaction is more challenging. In recent years' the development of two-stage fermentation (first stage, cell growth and second stage, conversion) has been shown to enhance reactor productivity[9], but for low-priced products where the volumes of product to be produced are very large, this remains a challenge. There are of course notable exceptions, but in the main fermentation does not convert substrate into product fast enough. On the other hand, biocatalysis suffers from higher catalyst costs linked also to lower stability when using enzymes out of the cell. This trade-off is schematically shown in Figure 1.

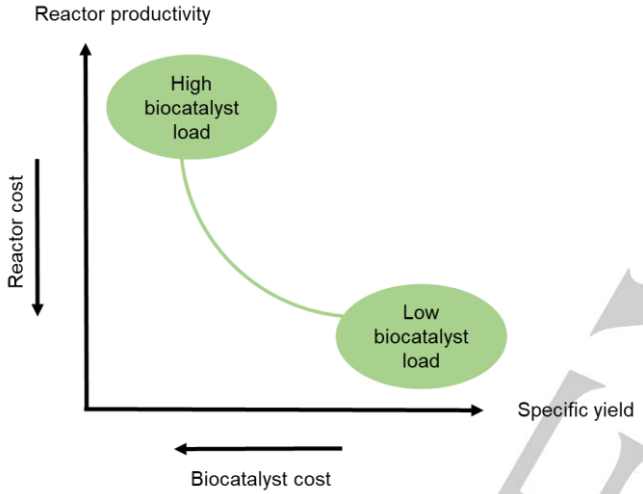


Figure 1. Trade-off of reactor productivity against specific yield.

If this trade-off is not addressed then despite all the enormous progress in developing new synthetic pathways using biocatalysis, industrial implementation will not fulfil its full potential. In this concept I will argue that an important focus for biocatalysis in order to ensure scalable sustainable processes for the future should be on improving both the concentration of product obtained and the stability of enzymes under industrially relevant conditions.

Increasing the product concentration

It is clear that the yield of any catalytic reaction is of importance in order that a sufficient amount of substrate is converted into product. Dependent upon the value difference between substrate and product this can determine the economic potential of the process. In many biocatalytic processes very high yield can be obtained where the thermodynamics are favorable, using to great effect the high selectivity of enzymes. In contrast to fermentation, a single pathway is highlighted in biocatalysis, resulting in by-products alone which are part of the reaction chemistry, rather than side-products through unwanted degradation or other unwanted enzyme activities. In cases where the thermodynamic equilibrium is unfavorable then it is essential to push or pull the equilibrium using Le Chatelier's principle with an excess of a second substrate, removal of product or by-product (either using in situ product removal or alternatively a second enzyme reaction).[10] Such principles have been well studied in the last decade. However, aside from adequate conversion, of equal importance is the need for an adequate concentration of product entering the downstream process. A low concentration implies a large amount of solvent (usually water) which then needs to be removed from the reaction mixture. The cost of removing the water needs to be considered due to the particularly high heat of evaporation of water.[11] Another observation is that the E-factor (mass waste / mass product) is far higher in processes which have a low product concentration.[12] Hence, reactions need to be developed to operate at higher product concentrations in order to provide a reasonable feed to the downstream process. For low-priced products (< 5 USD / kg) values over 100 g/L are required and for high-priced products (> 100 USD / kg) values between 10 and 50 g/L are required, depending on the final purity required and the difficulty of the downstream operations. A few recent excellent examples illustrate the possibilities well[13], but in many cases the concentrations are too low. Again, this emphasizes the importance of upstream and downstream process scientists working closely together. In many cases, enzymes cannot convert high concentrations of substrate due to inhibitory effects. Hence, feeding or supply from a two-liquid phase system (for poorly water-soluble substrates) are required methods to ensure the concentration in the vicinity of the enzyme is low enough to maintain sufficient activity, while supplying a sufficient pool of substrate to give a high final product concentration.[14] Likewise, the product may also inhibit the enzyme and here in situ product removal or else combining the next enzyme in a synthetic route can be beneficial. Prior to scale-up these issues should be addressed.

Improving enzyme stability

Aside from the need to ensure the reaction yield and product concentration are adequate, the two other metrics of importance to ensure scalability are the activity and stability of the enzyme.[15] In short, this means ensuring a minimal amount of enzyme is used to make a certain amount of product. It is often expressed as the catalytic turnover or specific yield (mass product / mass enzyme). Both the rate of the enzyme catalysed reaction (activity), but also the maintenance of this rate (stability) are important here.[16] The specific activity (rate / mass enzyme) can be enhanced through protein engineering. Together with the loading of enzyme in the reactor (mass enzyme / volume reactor) this will determine the reactor productivity (mass product / volume reactor / time). In many reactors, relatively high concentrations of enzyme can be used, especially if they are soluble and not immobilized. In this way, the reaction rate can always be improved. Interestingly, a recent review highlighted the excellent and scalable values of reactor productivity (also termed space-time yield by chemical engineers) achievable in many biocatalytic reactions today.[17] Many of these values are an order-of-magnitude or more above those achievable in fermentation. High reactor productivity results in smaller reactors to achieve a given annual productivity. For low-priced products with a high annual

productivity (> 100,000 tons / year) this becomes essential if it is to be competitive with existing catalytic routes. However adding more enzyme also implies an extra cost unless the stability of the enzyme can be enhanced, such that it can be used for a long time (potentially in multiple batches). In other words, there is a trade-off between reactor productivity and specific yield. This drives a less researched field which is the study of enzyme stability, not under natural conditions, but rather under those conditions found in large-scale reactors.

Classical enzymology describes enzyme stability in terms of the time-dependent effects of temperature and pH. However, in synthetic reactions the presence of substrate and product (at high concentrations) over prolonged periods of time may also have an effect on the retention of catalytic activity.[18] In the laboratory, more enzyme can be added if stability proves inadequate, but at a large scale, unless the enzyme can be stabilized by methods such as immobilization[19] , the addition of enzyme becomes costly and is reflected in the lowering of specific yield (mass product / mass enzyme). Of still greater interest scientifically is to understand what happens to enzymes in industrial reactors (whether immobilized or freely soluble in solution). Table 2 lists some of the conditions present in an industrial reactor, including the presence of agitation, but also in many cases with gas-liquid interface.

Table 2. Examples of typical conditions in an industrial biocatalytic reactor.

Conditions	Rationale linked to intensification	Rationale linked to scale-up	Effect on biocatalyst activity	Effect on biocatalysts stability
High substrate concentration	■		■	■
High product concentration	■		■	■
Concentration gradients		■	■	■
pH gradients		■	■	■
Gas-liquid interface	■			■
Liquid-liquid interface	■			■

Since the 1970s it was established that the primary cause of enzyme damage in agitated tanks is the entrainment of air from the surface, rather than shear.[20] The argument being that the size of an enzyme molecule is well beneath the Kolmogorov scale of mixing. Nevertheless, there remain many questions about the exact mechanism of stability loss. Of greater complexity, but no less important is the effect of deliberately added gas-liquid interface, present in most biocatalytic oxidations. Oxidoreductases are a hugely important class of enzymes for the synthetic chemist and in these cases supplying oxygen (even from air) is required on a continuous basis (usually via bubbling) in order to replenish the very low concentration of oxygen transferable to the aqueous phase where the enzyme catalyses the reaction.[21] However, the presence of significant gas-liquid interface can lead to multi-meric enzymes losing their quaternary structure and unfolding.[22] The amount of interface appears to be of great importance, but here too there are many questions that remain to be answered.[23] An interesting alternative to supplying oxygen from gas is to consider the use of unspecific peroxygenases, where hydrogen peroxide (a liquid) is supplied.[24] While this overcomes the gas-liquid interface it also introduces another challenge only seen at larger scale. Because hydrogen peroxide is harmful to the enzyme, to add it all at once in a batch risks losing activity. Hence, it needs feeding, either intermittently or continuously. As the scale of a reactor is increased so the mixing time increases (inversely proportional to the speed of agitation). This means that at a large scale the feeding of hydrogen peroxide will result in 'hot spots' of high hydrogen peroxide concentration, and other 'cold spots' of low concentration. The study of such effects is also of importance to help understand pH control via acid or alkali addition, as well as feeding of other liquid substrates which would otherwise prove toxic or inhibitory at the required concentrations to give a sustainable (and low E-factor) process. The study of such on-classical enzymology is complicated and it is maybe correct to argue that such studies are best carried out by bioprocess engineers. Indeed it makes sense for upstream scientists in organic chemistry / biocatalysis groups to focus on reaction discovery and characterization, which they do very well. However the key point to be made here is to ensure awareness of the importance of enzyme stability. Beyond that, it may well be that in some cases collaboration efforts are required together with bioprocess engineers to experimentally assess enzyme stability.

Future outlook

It is evident that improving the enzyme selectivity and activity under given conditions can be very well served through the various protein engineering tools available today, provided a suitable assay can be developed.[25] In principle, enzymes can also be made increasingly tolerant to higher and higher concentrations of product, although this is a harder challenge. Such approaches can be complemented through process engineering methods (e.g. feeding substrate and in situ product removal). Other challenges may require additional process engineering tools such new agitator or reactor design to minimize direct exposure to gas-liquid interface. Nevertheless, in all cases an essential dialogue is required between protein engineers and process engineers.[26] Protein engineering can solve many problems, but the number of traits to be enhanced simultaneously mean that process engineering is an essential complement. This not only requires a common language, but also a set of common goals. Those goals are today often described in terms of activity under given conditions, but require a shift in the future to encompass other metrics such as productivity and specific yield. By ensuring such a dialogue with a common focus it will in stages become possible to ensure that biocatalysis is not only attractive to implement for economic reasons, but in the longer-term for sustainability reasons as well.

Acknowledgements

The author has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement No 101021024).

Keywords: Biocatalysis • Scale-up • Product concentration • Productivity • Enzyme stability

[1] a) V.S. Ferreira-Leitao, M.C. Cammarota, E.C.G. Aguieiras, L.R. Vaconcelos de Sá, R. Fernandez-Lafuente, D.M. Guimaraes Freire, *Catalysts* **2017**, *7*, 7010009; b) R.A. Sheldon, J.M. Woodley, *Chem. Rev.* **2018**, *118*, 801-834; c) R.A. Sheldon, D. Brady, *Chem. Comm.* **2018**, *54*, 6088-6104.

[2] a) U.T. Bornscheuer, G.W. Huisman, R.J. Kazlauskas, S. Lutz, J.C. Moore, K. Robins, *Nature* **2012**, *485*, 185-194; b) J. Lalonde, *Curr. Opin. Biotechnol.* **2016**, *42*, 152-158.

[3] a) F.H. Arnold, *Angew. Chem. Int. Ed*. **2018**, *57*, 4143-4148; b) K. Chen, F.H. Arnold, *Nature Catal.* **2020**, *3*, 203-213.

[4] a) C.K. Savile, J.M. Janey, E.C. Mundorff, J.C. Moore, S. Tam, W.R. Jarvis, J.C. Colbeck, A. Krebber, F.J. Fleitz, J. Brands, P.N. Devine, G.W. Huisman, G.J. Hughes, *Science* **2010**, *329*, 305-309; b) P.N. Devine, R.M. Howard, R. Kumar, M.P. Thompson, M.D. Truppo, N.J. Turner, *Nature Rev. Chem.* **2018**, *2*, 409-421; c) J.P. Adams, M.J.B. Brown, A. Diaz-Rodriguez, R.C. Lloyd, G.-D. Roiban, *Adv. Synth. Catal.* **2019**, *361*, 2421-2432; d) A. Bornadel, S. Bisagni, A. Pushpanath, S.L. Montgomery, N.J. Turner, B. Dominguez, *Org. Process Res. Dev.* **2019**, *23*, 1262-1268.

[5] a) P.A. Santacoloma, G. Sin, K.V. Gernaey, J.M. Woodley, *Org. Process Res. Dev.* **2011**, *15*, 203-212; b) J.M. Sperl, V. Sieber, *ACS Catal.* **2018**, *8*, 2385-2396; c) S. Gandomkar, A. Zadlo-Dobrowoska, W. Kroutil, *ChemCatChem* **2019**, *11*, 225-243; d) M.A. Huffman, A. Fryszkowska, O. Alvizo, M. Borra.Garske, K.R. Campos, K.A. Canada, O.N. Devine, D. Duan, J.H, Forstater, S.T. Grosser, H.M. Halsey, G.J. Hughes, J. Jo, L.A. Joyce, J.N. Kolev, J. Liang, K.M. Maloney, B.F. Mann, N.M. Marshall, M. McLauglin, J.C. Moore, G.S. Murphy, C.C. Nawrat, J. Nazor, S. Novick, N.R. Patel, A. Rodriguez-Granillo, S.A. Robaire, E.C. Shere, M.D. Truppo, A.M. Whittaker, D. Verma, L. Xiao, Y. Xu, H. Yang, *Science* **2019**, *366*, 1255-1259; e) J. Nazor, J. Liu, G. Huisman, *Curr. Opin. Biotechnol.* **2021**, *69*, 182-190.

[6] a) J.M. Woodley, *Trends Biotechnol.* **2008**, *26*, 321-327; b) J.M. Woodley, *Curr. Opin. Green. Sustain. Chem.* **2020**, *21*, 22-26; c) A. Aguilar, T. Twardowski, R. Wohlgemuth, *Biotechnol. J.* **2019**, *14*, 1800638.

[7] a) P.N.R Vennestrøm, C.H. Christensen, S. Pedersen, J.-D. Grunwaldt, J.M. Woodley, *ChemCatChem* **2010**, *2*, 249-258; b) J.M. Woodley, M. Breuer, D. Mink, *Chem. Eng. Res. Dev.* **2013**, *91*, 2029-2036; c) H. Arastoopour, *Chem. Eng. Sci.* **2019**, *203*, 247-258.

[8] a) M.C. Bassalo, R. Liu, R.T. Gill, *Curr. Opin. Biotechnol.* **2016**, *39*, 126-133; b) Y. Liu, J. Nielsen, *Curr. Opin. Biotechnol.* **2019**, *60*, 188-197.

[9] a) J.M. Burg, C.B. Cooper, Z. Ye, B.R. Reed, E.A. Moreb, M.D. Lynch, *Curr. Opin. Chem. Eng.* **2016**, *14*, 121-136; b) S. Klamt, R, Mahadevan, O. Hädicke, *Biotechnol. J.* **2018**, *13*, 1700539.

[10] a) R. Abu, J.M. Woodley, *ChemCatChem* **2015**, *7*, 3094-3105; b) D. Hülsewede, L.-E. Meyer, J. von Langermann, *Chem. Eur. J.* **2019**, *25*, 4871-4884.

[11] P. Dominguez de Maria, *Curr. Opin. Green Sus. Chem*. **2021**, *31*, 100514.

[12] a) Y. Ni, D. Holtmann, F. Hollmann, *ChemCatChem* **2014**, *6*, 930-943; b) R.A. Sheldon, *Green Chem.* **2017**, *19*, 18-43.

[13] a) A. Hinzmann, S. Glinski, M. Worm, H. Gröger, *J. Org. Chem.* **2019**, *84*, 4867-4872; b) Z. Yang, H. Fu, W. Ye, Y. Xie, Q. Liu, H. Wang, D. Wei, *Catal. Sci. Technol*. **2020**, *10*, 70-78; c) Z. Yang, H. Fu, W. Ye, Y. Xie, Q. Liu, H. Wang, D. Wei, *Org. Process Res. Dev*. **2020**, *24*, 1068-1076; d) Z. Cui, Y. Mao, Y. Zhao, M. Zheng, Z. Wang, H. Ma, T. Chen, *Catal. Sci. Technol.* **2020**, *10*, 7734-7744.

[14] a) P. Tufvesson, J. Lima-Ramos, N. Al-Haque, K.V. Gernaey, J.M. Woodley, *Org. Process Res. Dev.* **2013**, *17*, 1233-1238; b) D. Leis, B. Lauß, R. Macher-Ambrosch, A. Pfennig, B. Nidetzky, R. Kratzer, *J. Biotechnol.* **2017**, *257*, 110-117; c) L.-E. Meyer, J. von Langermann, U. Kragl, *Biophysical Rev.* **2018**, *10*, 901-910; d) M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends Biotechnol.* **2019**, *37*, 943-959.

[15] J.-P. Lange, *Nature Catal.* **2021**, *4*, 186-192.

[16] M.T. Reetz, *Chem. Rec.* **2016**, *16*, 2449-2459.

[17] S. Wu, R. Snajdrova, J.C. Moore, K. Baldenius, U.T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 88-119.

[18] A.S. Bommarius, *Annu. Rev. Chem. Biomol. Eng.* **2015**, *6*, 319-345.

[19] a) A. Basso, S. Serban, *Molecular Catal*. **2019**, *479*, 35-54; b) J. Boudrant, J.M. Woodley, R. Fernandez-Lafuente, *Process Biochem*. **2020**, *90*, 66-80; D.-M. Liu, C. Dong, *Process Biochem*. **2020**, *92*, 464-475; R.A. Sheldon, A. Basso, D. Brady, *Chem. Soc. Rev*. **2021**, *50*, 5850-5862.

[20] C.R. Thomas, G. Geer, *Biotechnol. Lett.* **2011**, *33*, 443-456.

[21] A. Toftgaard Pedersen, W.R. Birmingham, G. Rehn, S.J. Charnock, N.J. Turner, J.M. Woodley, *Org. Process Res. Dev.* **2015**, *19*, 1580-1589.

[22] a) A.S. Bommarius, A. Karau, *Biotechnol. Prog.* **2005**, *21*, 1663-1672; b) M. Dias Gomes, R. Moiseyenko, A. Baum, J.M. Jørgensen, J.M. Woodley, *Biotechnol. Prog.* **2019**, *35*, e2878; c) S.R. Anderson, B.R. Bommarius, J.M. Woodley, A.S. Bommarius, *Chem. Eng. J.* **2021**, *417*, 127909.

[23] G. Dalkas, S.R. Euston, *Curr. Opin. Coll. Interf. Sci.* **2019**, *41*, 1-10.

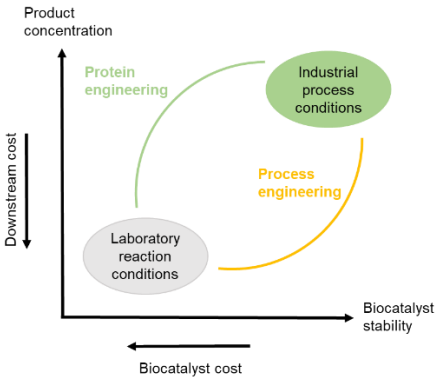
[24] Y. Wang, D. Lan, R. Durrani, F. Hollmann, *Curr. Opin. Chem. Biol.* **2017**, *37*, 1-9.

[25] B. Hauer, *ACS Catal.* **2020**, *10*, 8418-8427.

[26] J.M. Woodley, *Curr. Opin. Chem. Biol.* **2013**, *17*, 310-316.

10.1002/cssc.202102683

Entry for the Table of Contents



Enzyme-based biocatalysis offers selective catalysis under mild conditions with a renewable, biodegradable and tunable catalyst. A close dialogue between protein engineers and process engineers will be essential to ensure the sustainability of scalable biocatalytic processes, operating at high product concentrations (to reduce E-factor) and high enzyme stability (to reduce costs).