



HAL
open science

Wall-immobilized biocatalyst vs. packed bed in miniaturized continuous reactors: performances and scale-up

Maïté Michaud, Guillaume Nonglaton, Zoé Anxionnaz-minvielle

► To cite this version:

Maïté Michaud, Guillaume Nonglaton, Zoé Anxionnaz-minvielle. Wall-immobilized biocatalyst vs. packed bed in miniaturized continuous reactors: performances and scale-up. *ChemBioChem*, 2024, 25 (11), pp.e20240008. 10.1002/cbic.202400086 . cea-04770888

HAL Id: cea-04770888

<https://cea.hal.science/cea-04770888v1>

Submitted on 7 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

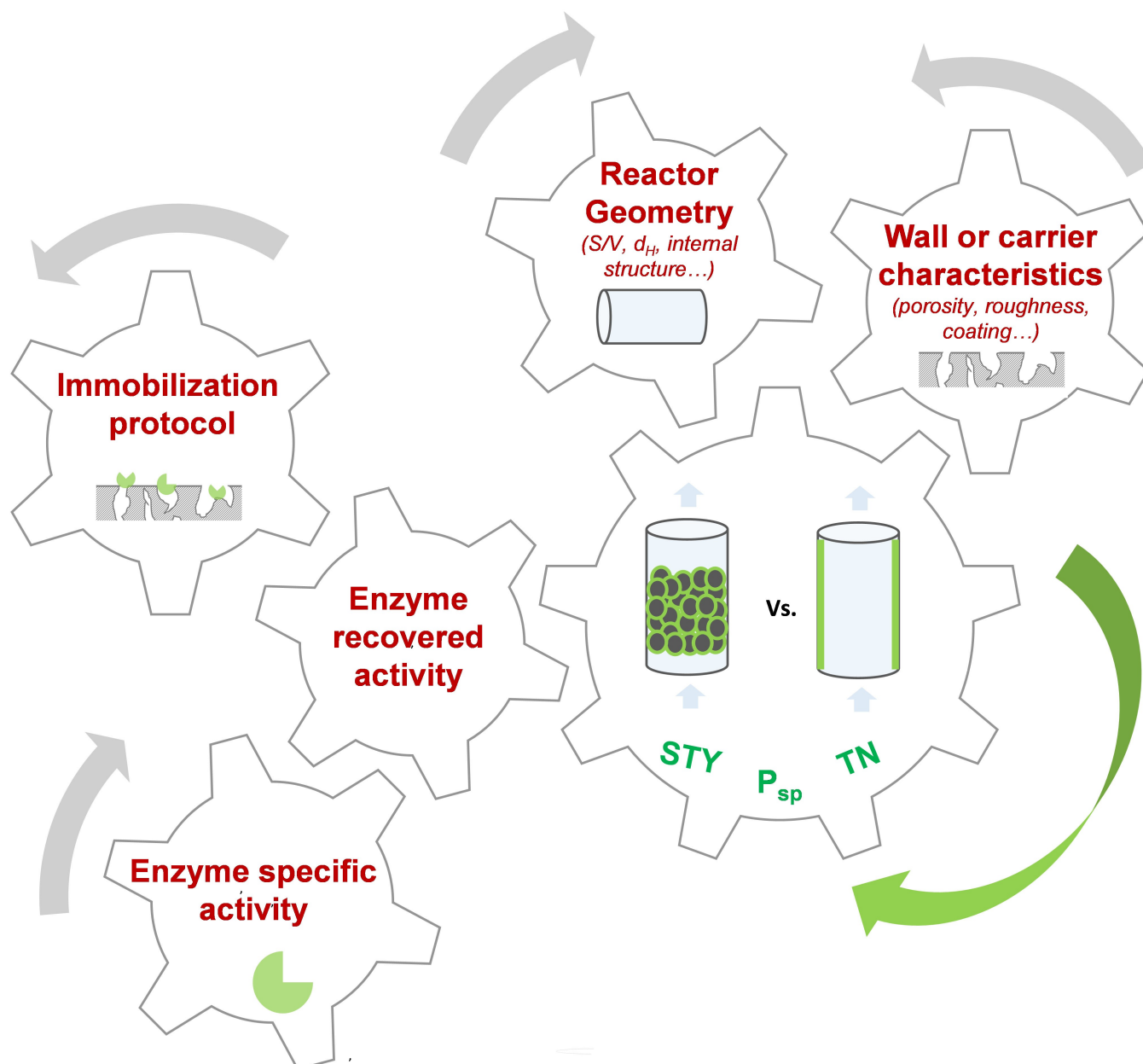
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Wall-Immobilized Biocatalyst vs. Packed Bed in Miniaturized Continuous Reactors: Performances and Scale-Up

Maité Michaud,^{*[b]} Guillaume Nonglaton,^[c] and Zoé Anxionnaz-Minvielle^{*[a]}



Sustainable biocatalysis syntheses have gained considerable popularity over the years. However, further optimizations – notably to reduce costs – are required if the methods are to be successfully deployed in a range of areas. As part of this drive, various enzyme immobilization strategies have been studied, alongside process intensification from batch to continuous production. The flow bioreactor portfolio mainly ranges between packed bed reactors and wall-immobilized enzyme miniaturized reactors. Because of their simplicity, packed bed reactors are the most frequently encountered at lab-scale. However, at industrial scale, the growing pressure drop induced by the increase in equipment size hampers their implementa-

tion for some applications. Wall-immobilized miniaturized reactors require less pumping power, but a new problem arises due to their reduced enzyme-loading capacity. This review starts with a presentation of the current technology portfolio and a reminder of the metrics to be applied with flow bioreactors. Then, a benchmarking of the most recent relevant works is presented. The scale-up perspectives of the various options are presented in detail, highlighting key features of industrial requirements. One of the main objectives of this review is to clarify the strategies on which future study should center to maximize the performance of wall-immobilized enzyme reactors.

1. Introduction

With a broad selectivity, enzymes or whole cells are very attractive catalyst tools for chemical synthesis in particular for valuable chiral compounds.^[1,2] As an example, lipase-mediated production of the herbicide Dimethenamide-P by BASF allows the formation of a single enantiomer instead of the racemic mixture produced by a chemical process.^[3] In addition to the improved selectivity of this reaction, the other major breakthrough with biocatalysis is in environmental sustainability compared to classical chemical synthesis, with mild operating conditions (e.g. aqueous media, low temperature and atmospheric pressure). For example, 90% of nicotinic acid, an essential nutrient for humans, is produced at industrial scale through the oxidation of 5-ethyl-2-methylpyridine in harsh operating conditions. The reaction takes place in a highly corrosive environment at 190–270 °C, 2–8 Mpa, with nitric acid in large excess; nitrous oxide is produced as a side-product.^[4] When the metal catalyst is replaced by its biological counterpart, nitrilase, the reaction proceeds at ambient temperature and pressure, there is no need for large quantities of acid, and no greenhouse gases are produced.^[5] Moreover, the ecological burden of the industrial wastes, particularly by-products generated in the food industry, can be sustainably transformed via enzymatic reactions into high-value products such biofuels,

or biomaterials.^[6] Thus, the direct consequences in replacing metallo/organocatalysts with their biological counterparts are reduced energy demands and waste production, along with diminished consumption of toxic and chemical solvents.^[7] Along with the growing interest in biocatalysts, advances in protein and metabolic engineering have expanded the operational and stability windows for enzymes – broadening substrate scope and useable concentrations, as well as extending working temperature and pH ranges, and increasing activity. As a result, the number and variety of enzymes is continuously expanding. Nevertheless, three main catalyst classes attract the most intense study: lipases, ketoreductases and transaminases.^[8] At industrial scale, biosynthetic applications are so far limited to the pharmaceutical, fine chemical and food industries.^[9] To make this green tool more cost-effective for use in other industrial applications, alternatives to the traditional batch-wise production process must be developed to reduce both equipment size and operation time. The pharmaceutical industry have issued requests for further research to develop flow reactors to allow changes in the preferred technology.^[10,11] Indeed, the widespread large and costly batch reactors used in the pharmaceutical industry are not well adapted for newly developed products. For clinical assays, production is only required at a rate of 3 to 15 kg/day.^[12,13] Continuous operations make manufacturing processes more flexible and responsive to fluctuations in demand. They also improve processes in terms of safety (e.g. reduced equipment size and handling) and performances (e.g. increased yield, purity, and productivity). These claims are supported by a demonstration of the capacity to synthesize prexasertib monolactate monohydrate at kilogram-scale in a continuous system.^[12] Another example at pilot-scale, with an end-to-end continuous production of active pharmaceutical ingredient (API) decreased capital investment by 90% and reduced operation costs by 33.6%.^[14] As part of this shift in focus, the biocatalyst recycling is a critical to be addressed. Indeed, despite a decline in purchase costs for biocatalysts, their re-use is still indispensable to make continuous operations profitable, especially for large-scale manufacturing processes.^[15] In this context, immobilization strategies play an important role, making it possible to combine biocatalysis with flow chemistry. Interest in these strategies is underlined by the growing number of articles and reviews related to this topic (Figure 1). To be noted that in some cases, the economic

[a] Dr. Z. Anxionnaz-Minvielle
Univ. Grenoble Alpes, CEA, LITEN, DTCH, Laboratoire Composants et Systèmes Thermiques (LCST)
F-38000, Grenoble, France
E-mail: zoe.minvielle@cea.fr

[b] Dr. M. Michaud
Univ. Grenoble Alpes, CEA, LITEN, DTCH, Laboratoire Composants et Systèmes Thermiques (LCST)
F-38000, Grenoble, France
E-mail: maite.michaud@cea.fr

[c] Dr. G. Nonglaton
Univ. Grenoble Alpes, CEA, LETI, DTIS, Plateforme de Recherche Intégration, fonctionnalisation de Surfaces et Microfabrication (PRISM)
F-38000 Grenoble, France

© 2024 The Authors. ChemBioChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

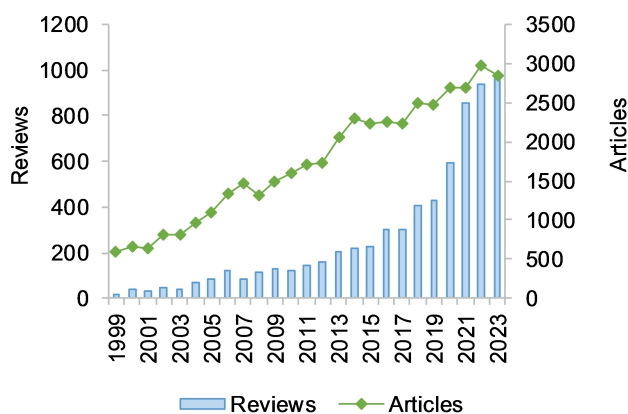


Figure 1. Number of articles and reviews per years on enzyme immobilization (Source: Scopus with query: "enzyme immobiliz*" OR "biocatalyst immobiliz*" – Update: February 2024)

viability of enzyme immobilization is not always compensated by its benefits, in particular for enzymes with overly high or overly low turnover frequencies (i.e. TOF in min^{-1}) and excessively short lifetimes.^[16,17] Consequently, to reduce investments costs, industrial processes tend to favour simple immobilization protocols, with few steps and cheap supports in order to reduce the investments costs. As an example, D-glucose/xylose isomerase has been immobilized on an inexpensive inorganic carrier, bentonite clay, for the manufacture of high-fructose corn syrup.^[9] Cost considerations should therefore be taken into account in future process developments and immobilization strategies, but beyond this basic requirement,

immobilized enzymes present considerable interest and seem to hold potential compared to free-enzyme (Figure 1).

Some recent reviews have examined the combination of biocatalysis with continuous processes from various perspectives (Table 1).

Basso and Serban listed biosynthetic examples at industrial scale with their associated processes. They concluded that batch processes remain the reference, except in the food and chemical industries.^[9] At laboratory scale, examples of processes in continuous mode can be found in literature reviews with an updated list reported by Tamborini and co-workers in 2018^[23] or also by Britton *et al.* in 2018.^[24] In their review, Zhu *et al.* focused specifically on immobilized enzyme reactors (IMERs) using microfluidic devices (μ -IMERs) and Žnidaršič-Plazl in 2019, emphasised the microflow systems benefits as a cheap solution for ultrahigh-throughput screening to collect datas at various operating conditions.^[21,22] Indeed, microscale have found particular favour for flow biocatalysis as a result of their well-known intensification capacity, thanks to their higher surface-to-volume ratio (S/V). However, for high productivity, scaling-up a process based on microdevices requires costly pumping technologies and reactor manufacturing and as a result interest has returned to mesoreactors.^[23] By combining channel sizing-up and numbering-up strategies, miniaturized equipments from laboratory to production scale are commercially available.^[25] The most-well known are the Advanced Flow Reactor (i.e. AFR reactors including LF, G1, G2, G3, G4 and G5) from Corning and ART®, Lonza®, and Miprowa® systems from Ehrfeld Mikrotechnik. Also, with the increased interest in integrated continuous manufacturing in the pharmaceutical field, automated micro



Dr. Zoé Minville-Anxionnaz is a senior expert and research engineer at French Alternative and Atomic Energy commission. She received her Engineer, M.Sc. and Ph.D. degrees from the University of Toulouse (National Polytechnic Institute) in chemical and process engineering. She has been working for 15 years in the field of heat exchanger fouling and multi-functional heat exchangers (heat exchanger/reactor) for process intensification. She has also been in charge of several PhD students and post-doctoral students. She has been involved in 23 industrial or academic research projects, 2 of them being European projects, and was project leader for 17 of them.



Dr. Maïté Michaud is currently working as a post-doctoral research associate at French Alternative and Atomic Energy commission. She obtained her Engineer M.Sc. in chemical and process engineering from University of Lorraine (ENSIC, Nancy). After finishing her Ph.D. at the University of Lyon in 2020, she further completed her experience in process intensification on reactive systems with post-doctoral experiences from 2020 to 2024. She is particularly interested in the development of green processes. Her research includes the use of immobilized enzymes in flow reactors.



Dr. Guillaume Nonglaton, earned a M.Sc. in fine chemistry (2001) and a Ph.D. in organic synthesis (2005) from the University of Nantes. His research focused on innovative supports for biological analysis. He worked on surface chemistry as an invited researcher at the University of Florida and later conducted postdoctoral research at Uppsala University, specializing in peptide immobilization for bio-analytical microchips. In 2007, he joined CEA-Leti as a senior scientist in surface chemistry. Appointed CEA expert in 2013, he currently serves as senior expert, leading surface functionalization projects, managing a team, and contributing to numerous national, European, and industrial projects.

Table 1. Selected relevant reviews in-flow biocatalysis.	
Authors (date)	Title
Bolivar et al. (2022) ^[16]	Is enzyme immobilization a mature discipline? Some critical considerations to capitalize on the benefits of immobilization
Žnidaršič-Plazl (2021) ^[18]	Biocatalytic process intensification <i>via</i> efficient biocatalyst immobilization, miniaturization, and process integration
Bolivar et al. (2020) ^[19]	Characterization and evaluation of immobilized enzymes for applications in flow reactors
Romero-Fernández (2020) ^[20]	Protein immobilization technology for flow biocatalysis
Žnidaršič-Plazl (2019) ^[21]	The promises and the challenges of biotransformations in Microflow
Basso and Serban (2019) ^[9]	Industrial applications of immobilized enzymes – A review
Zhu et al. (2019) ^[22]	Microfluidic immobilized enzyme reactors for continuous biocatalysis
Tamborini et al. (2018) ^[23]	Flow Bioreactors as Complementary Tools for Biocatalytic Process Intensification
Britton et al. (2018) ^[24]	Continuous flow biocatalysis

flow systems via computational tools and continuous monitoring have been successfully applied to multiple API fabrications.^[26] But, the integration of biocatalysis into flow systems at production scale, also depends on biocatalyst immobilization performances for long-term re-use.^[27] Some recent reviews highlight progress in the field of enzyme immobilization.^[18,20] Despite all the accumulated data published by the scientific community, the potential of biocatalyst has yet to be fully exploited and enzyme immobilization is far from mature as explained in the review of Bolivar *et al.*^[16] Fair quantitative comparison of the various solutions is made difficult by the diversity of enzymes available, the variety of immobilization protocols, types of support, and technology configurations, but also by the lack of uniform metrics. In an attempt to remedy this situation, in 2020, Bolivar *et al.* gave a clear list of metrics that should be reported when it comes to in-flow biocatalysis.^[19] Finally, the end use of the biocatalysts is not usually considered in early developments. This is one of the main reasons explaining the gap between academia and industry.^[16] Reactor engineering should be considered in the early stages of development with appropriate technology choice and design to facilitate immobilization protocol transposition. In complement to these latest reviews of in-flow biocatalysis, the goal of the present article is to provide a more extensive overview of the potential of wall-immobilized enzyme miniaturized reactor compared to packed bed reactors, particularly in terms of scale-up considerations. Both micro- and milli-size IMERs have been analysed to assess their performance. We selected some relevant studies and, when possible, homogenized the experimental data in standard metrics. We start this review by summarizing the technology portfolio. Then, we discuss scale-up considerations, presenting the relative advantages and disadvantages of micro- and meso-scale reactors.

Then, we explain the quantitative comparison strategy, and finally we compare some of the most recent advances to the two most common process configurations.

2. Immobilized Enzyme Reactors: A Portfolio Of Options

One of the first conventional reactor to be proposed is the packed bed reactor either randomly filled with small packed objects (Figure 2a-1) or loaded with a monolith type material (Figure 2a-2). In the biocatalysis field, the PBR acronym (i.e. packed bed reactor) usually referred to the first category with typically commercial nanoporous microspheres (i.e. size of 50–200 μm).^[18] Initially, this robust and versatile technology was very popular as high enzyme loads are easily achievable thanks to the nanopores.^[23] Many recent studies reveal this technology to also be the most prevalent in a milli-scale format for proof of concept studies of in-flow biocatalysis combined with newly developed immobilization forms.^[5,28–30]

Nevertheless, this simple technology has some well-known technical limitations: (i) excessive backpressures due to limited bed porosity (i.e. at best 0.45 with perfectly spherical particles^[31]) which considerably hamper scale-up attempts,^[32] (ii) poor mixing control leading to back mixing and the creation of preferred paths (i.e. generating dead zones) along the length of

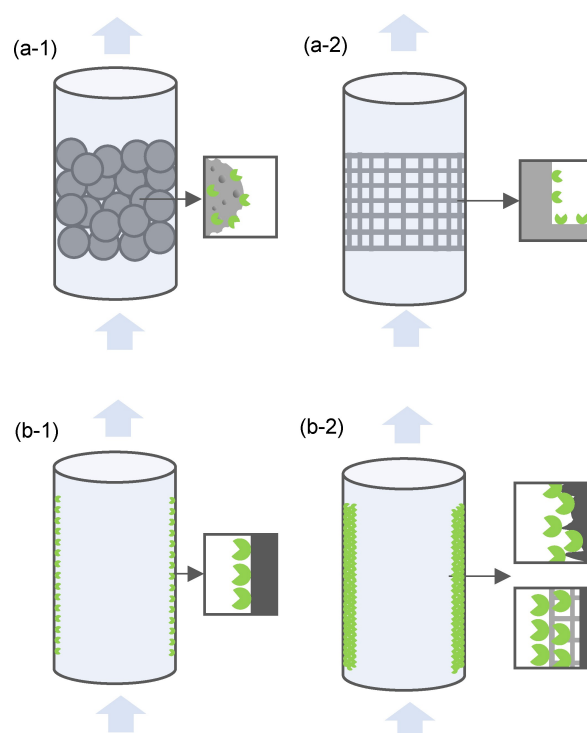


Figure 2. Conventional technologies in flow biocatalysis: (a) Packed bed reactor with (a-1) bulk packing, usually nanoporous microspheres or with (a-2) monolith packing (i.e. ceramic mainly silica material, metallic, or polymer like foam, hydrogel); (b) Coated-surface reactors with (b-1) monolayer attachment or (b-2) multilayer attachment with rough surfaces or with an additional porous layer linked to the reactors surface;

the column at meso and macro-scale, (iii) and limitations on diffusion in particle pores.^[33] Alternative packings have been studied to mitigate each of the cited drawbacks in turn, but at the expense of the high specific area. Thus, hollow microsphere carriers have been promoted as a means to reduce the backpressure effect by increasing the proportion of void.^[34,35] To suppress pore diffusion limitations, non-porous nanoparticles have been proposed^[29] but this strategy is incompatible with scale-up due to even greater pressure drops. Another typical process configuration using beads is the fluidized bed reactor, which is mostly applied at industrial scale when dealing with highly viscous systems.^[9] In this case, the liquid flows from the bottom side to the top of the column to induce a bead suspension. Consequently, the amount of enzyme concentration is reduced compared to the packed bed reactors, usually in the range of 3 to 10% w/v of immobilized enzyme per reaction volume, leading to an increase in the size equipment, and operating costs.^[9] Monolithic reactors are filled with a single piece of structured material.^[36,37] Monolith packing is a good alternative, minimizing the limitations of PBR especially in terms of backpressure build-up, with porosity that can exceed 0.9.^[23,38] Many reports have been published on ceramic materials, particularly silica monoliths as silica surfaces and particles have been investigated for use in combination with enzymes, facilitating transposition of the immobilization protocol from one support to another.^[39,40] An alternative to inorganic materials is organic monolith carriers (i.e. polymers) such as polyurethane foam for example.^[41,42] These solutions have been proposed in particular in the wastewater treatment field.^[43,44] Alternatively, polymers in the form of hydrogels have gained popularity over the years and remain relevant.^[41,45,46] This specific class of polymer exhibits a swelling capacity resulting from a high affinity for water. In general, the aqueous environment generated by these superabsorber-gel matrices provides conditions preserving high enzyme activity.^[47] However, despite widely reported analytical systems, industrial examples of monolith bio-reactors remain rare, notably because of the complex and costly in situ fabrication processes involved which could make them less attractive than other carriers.^[33,48] Indeed, monolith columns are mainly used in the bio-industry for purification and downstream operations. Several products, commercialised under the trademark convective interaction media®, CIM®, are available on the market.^[49,49] Nevertheless, recently, interest in monolithic reactors with more intricate geometries has been growing thanks to the emergence of additive manufacturing in chemical engineering.^[40,45,48,50] The questions surrounding their economic viability and industrial scalability appear to have been reopened thanks to the emergence of 3D-printed reactors, with claims that they are more flexible, cost-effective, and easier to manufacture.^[23,51,52]

In the wall immobilized enzyme miniaturized reactors, enzymes are immobilized directly on the reactor surface (Figure 2b-1). The simplest miniaturized reactor is a tubular-based reactor, usually with a capillary tube (internal diameter smaller than 1 mm) or with an array of capillaries to increase the overall reactor volume.^[53] Microcapillary reactors have been applied to biochemical reactions for a controllable reaction environment

with improved heat and mass transfer efficiencies.^[54] With chip-based devices, more complex and flexible reactor designs can be manufactured. Features and specific structures inside the reactor (e.g. meandering channels^[55-57] or pillars^[58]) can be used to optimize the mixing conditions and/or to increase the surface-to-volume ratio.^[59] Indeed, compared to batch processes or large-size continuous reactors, the increase of the surface-to-volume ratio (about 10 to 50 mm⁻¹ for micro-structured reactors^[60]), provides higher level of enzyme concentrations, resulting in a faster bio-kinetics. But geometrical adjustments are not the only way to increase the quantity of biocatalyst that can be immobilized. To obtain a highly active wall, most case studies indicate that biocatalyst should be attached in multiple layers (Figure 2b-2), even with micro-size reactors. This strategy is even more important when working with enzyme that have a low activity (e.g. ≈ 5 U/mg_{enz}).^[60] To increase the effective surface area, the reactor walls can be modified by etching with a corrosive chemical solution.^[50] However, the most widely use strategy, is to coat the reactor surface with a thin nanoporous layer after the manufacturing step^[61-67]. Depending on the nature of the surface to be modified, the surface coating protocol is adapted. To comply with bio/chemical and stability requirements, reactors are made of (i) silica or polymeric materials (mainly polydimethylsiloxane, PDMS and polymethylmethacrylate, PMMA) or (ii) alloy and stainless steel materials. Among non-metallic reactors, silica polymer coating materials have been extensively studied with various synthesis routes (e.g. sol-gel, nanospring, etc).^[65,66] In this category, hydrogel is also another interesting type of polymer coating, as previously mentioned, because of its swelling capacity which creates favourable environmental conditions for sustained enzyme activity.^[61] With metallic reactors, enzymes are immobilized on surfaces modified by washcoat treatment.^[62-64] Other techniques like sol-gel deposition, electrophoretic deposition, chemical or physical vapor deposition would be suitable for similar surface modification.^[68] With washcoating, the metallic surface is calcinated before exposure to a polymer/metallic powder slurry.^[69,70] After drying and a final calcination step, the resulting thin nanoporous layer is chemically functionalized in preparation for enzyme immobilization. This final step it the same for metallic and non-metallic reactors. The difference relative to monolithic reactors is that with surface-coated reactors, the volume of the polymeric layer is relatively limited compared to the channel volume, as in a monolithic reactor, the polymer structure occupies the entire reactor body.

In addition to these conventional technologies (Figure 2), examples of membrane processes with immobilized enzymes on the surface or/and in the pores have been presented in the literature. The examples found mainly have disc shaped reactors, macroporous materials are selected for their non-limiting diffusion rate for this process configuration,^[71,72] but recent reports have also presented nanofibrous membranes as offering an higher enzyme load capacity.^[73] However, one of the major issues with these systems is the energy required to ensure sufficient backpressure for the fluid to flow through the membrane. As a result, they have similar drawbacks to the PBR. Moreover, membranes tend to have a lower enzyme-loading

capacity due to a reduced surface area.^[33] Also, an alternative widespread immobilization strategy is the ex-situ immobilization method, with the formation of cross-linked enzymes aggregates, CLEAs.^[74] These ex novo solids are advantageously independent of the reactor design and can subsequently be placed into the reactor.^[75] However, the major challenge reported with self-assembling of enzymes is the poor mechanical resistance which makes them difficult to be used in industrial processes.^[16] To improve the stability, they can be entrapped in solids with better mechanical feature,^[76] self-assembled with an hydrogel polymer^[77] or combined with magnetic nanoparticles to facilitated the handling.^[78,79] Finally, innovative process configurations involving the biocatalyst in motion have emerged over the years. Indeed, some specific reaction media may justify the use of compartmented phases in segmented flow. For example, when the water-solubility of organic substrates and products is too low, biocatalytic reactions must be performed in an organic phase. However, due to enzyme deactivation by the organic phase, a two-phase liquid system is required, where the enzyme is in the aqueous phase, and the organic phase carries the substrate and collects the product. Additional surfactant may be introduced to prevent the biocatalyst from direct contact with the organic solvent at the droplet interface^[80] and biocatalyst immobilization can also limit such biocatalyst deactivation.^[81] However, post-separation of the two phases can be difficult, making downstream operations challenging, and the biocatalyst recovery and recyclability has not been proven yet.^[32] Microfluidic oscillation reactors (μ MORE) with magnetic nanoparticles (MNPs) are another novel trend emerging in the scientific research community, not only with enzymes. With magnetic particles, the handling and separation of immobilized enzymes after deactivation is facilitated by the use magnets or a magnetic field. Several points to address are reported using static beads and/or magnetic particle layers such as reactor plugging^[82,83] and non-uniform flow velocity.^[84] An interesting alternative is the combination of magnetic mixing and retention with magnetic bead movement. The needs for an easy and adjustable controlled magnetic field, that does not generate heat or cause particles to agglomerate are the new challenges to be tackled with these fluidized magnetic beads.^[82,85,86] Indeed, the heat generation can have a negative side effect on the biocatalyst thermal stability as reported by Robotjazi *et al* with immobilized cells.^[87] However, contradictory effect of the external magnetic field on *S. cerevisiae* growth have also been reported according to an analytical review from Hristov and Perez.^[88] Thus, magnetic field assisted bioreactor required deeper investigations of the magnetic effect on the biocatalyst activity and stability.^[89] Finally, the large surface-to-volume ratio of nanomaterial has been also investigated with other type of support like metal-organic frameworks (MOFs), covalent-organic frameworks (COFs) or silica nanoparticles for example. The increase popularity of MOFs is justified by their versatility and tuneable structure design.^[16] But so far, these investigations have mainly focused on the preparation and characterization steps.^[90] As they are still at an early stage of development, all

these new processes are beyond the scope of the quantitative analysis.

To be noted that sub-optimal performances encountered with batch process can be assigned to product and/or substrate inhibitions at elevated concentrations.^[91] Flow reactors overcome this challenge, with a fine control over reaction conditions close to the enzyme location and with a constant removal of the product.^[92] This challenge may also justify some specific technical choices like the use of membranes when enzymatic products can be continuously removed from the reaction mixture based on size-exclusion separation, reducing product inhibition effects and the formation of side-product (i.e. peptide and oligosaccharide production).^[93,94] Optimal operating conditions and appropriate reactor design, integrating kinetic parameters, can be computationally guided to avoid time-consuming and expensive experiments. Several examples of model-based design optimization methods have been published.^[95–98]

3. Process Scale-Up: Considerations

At lab-scale, a huge proportion of bioreactors are based on microscale processes, such as chip or microcapillary systems, with channel widths of less than 500 μ m.^[23,99] This scale of reactor presents advantages in terms of increased surface-to-volume ratios and intensified mass and heat transfers.^[55] These reactors can be scaled-up by increasing the characteristic dimensions of the channel, by adding more reactors, or by adding internal structures within the unit (known as numbering up, parallelization or scale-out). When multiple micro-device systems are connected, the inherent benefits of the microscale format - mixing and heat efficiency - are maintained, but outlay is increased due to the need for multiple pumping and heating devices as well as the cost of the manufacturing the multiple reactors.^[26,100] To limit the expense, micro-size reactors can be gathered into interconnected reactor stacks. However, it is difficult to ensure an equal flow distribution upon scale-up.^[23] Indeed, a recent study of microreactors in heterogeneous catalysis, demonstrated the need for optimization to avoid significant performance degradation when microreactors are stacked to increase production scale.^[101] Productivity of a microreactor at lab scale is usually in the range of g/h or g/min.^[102] To meet industrial needs (i.e. kg/h or more), thousands of microreactor units would have to be used.^[103] This mismatch in the throughput between lab scale and production site results in a challenging scale-up factor of 100–1000.^[25] For industrial scale applications, a combination of both strategies, sizing up and numbering-up, appears to be the current best option.^[104] Corning developed its reactor portfolio from lab-scale (LF reactor of 0.6 mL, productivity of 5 t/y) to production scale (G5 reactor, productivity of 10 000 t/y) in five gradual sizing-up and internal numbering-up steps, achieving a total scale-factor of 400.^[25] No degradation of the reactor performances in term of mass transfer efficiency have been reported with the 2.75-fold increase in channel height between the LF system (height 400 μ m) and the G1 system (height 1.1 mm) for liquid-liquid

systems,^[105] nor with an emulsion system considering the 1.4-fold increase in channel height between G1 and G2.^[106] With Miprowa® technology, Ehrfeld Mikrotechnik has reduced the scale-up process to 2 steps; a 2-fold increase in the milli-size channel cross-sectional area of the laboratory reactor and parallelization of the channels.^[107] Thus, to guarantee the scale-up robustness, with a reduced number of steps, channel size at lab and production-scale should not more than 2 to 3-fold apart.

In the biocatalysis domain, microfluidic devices have been extensively used as wall-immobilized enzyme miniaturized reactors but they have potential beyond this process configuration.^[55,60–66] For example, miniaturized packed bed (μ -PBR) with a single, double or triple particle layer is a related option that intensifies the volumetric productivity of PBR while mitigating the backpressure issue thanks to the reduced device size.^[108] For production objectives measured in g/h, millireactors (e.g. structure dimensions above 1 mm according to DIN EN ISO 10991) seem more appropriate. However, with this size of reactor, the technological configuration mainly reported is the PBR. Indeed, very few publications describe wall-immobilized enzyme miniaturized reactors with channel dimensions exceeding 1 mm.^[56] Especially, with heterogeneous catalysis not only the mixing efficiency must be considered with the increase in the channel dimension but also the influence of the reduced surface-to-ratio on the amount of biocatalyst that can be immobilized on the reactor walls. Indeed, geometrical surface-to-volume ratio is greatly affected by the reactor characteristic size. For microfluidic devices, they are in the range of 10 to 50 mm⁻¹^[60] whereas for milli-sized reactors they are around 1 to 5 mm⁻¹.^[56,107,109] For example, from G2 to G3 reactor (i.e. scale-up factor of 4), the decrease in the heat transfer efficiency due to a lower surface-to-volume ratio has been balanced with a material of higher conductivity.^[25] Thus, to facilitate scale-up of miniaturized reactor with wall-immobilized enzymes, additional work at lab-scale should be investigated with milli-sized channel close to those that would be implemented at production scale.

For the transfer from lab to industrial scale, the main consideration when it comes to preferred technology is cost, taking in initial investment, as well as operational and maintenance expenses. When the volume of production required is relatively low (e.g. for high-value products), stirred batch reactors tend to be preferred due to their simplicity and reduced cost compared to PBR.^[110] Nevertheless, examples of implementation of continuous processing are growing due to evolving research priorities in the pharmaceutical industry.^[10] In contrast, in the food and chemistry industries, where long-term productivity is the key criterion, PBR tend to be favoured.^[9]

4. Metrics and Standardization: Facilitating Cross-Study Comparisons

Despite the tremendous increase in the number of reviews of biocatalysis, comparative studies are difficult, partially due to a

lack of standardization in reported data.^[15] A methodology with a list of the relevant metrics was recently published by Bolivar and López-Gallego to clarify this point^[19] (Figure 3). They identified two categories of metrics: those representing the activity considering enzyme load, recovered activity (η in %) and space-time-yield (STY in mg/mL/h); and those analysing long-term stability based on specific productivity (P_{sp} in mg/mg_{enz}/h) or accumulated productivity, referred to as turnover number (TN in mg_{product}/mg_{enz}). STY scales with both the amount of enzyme immobilized and the recovered activity but the “investment cost” is not clearly identifiable. For that parameter, TN is used as it gives an overview of the immobilized enzyme’s durability. Therefore, stability metrics are normalized for time based on the enzyme’s operational longevity.

These seemingly simple metrics actually depend on several parameters with multiple and sometimes contrary influences (Figure 3). For example, as illustrated by Valikhani *et al.*^[66] and Bolivar *et al.*^[55] studies, a selected immobilization strategy to increase the enzyme loading (i.e. from 6^[55] to 140 mg_{enz}/m²^[66]) can negatively affect the recovered activity (i.e. from 70^[55] to 20%^[66]). Thus, this given parameter has, in these reported studies, a contradictory effect on the STY, explaining the relatively small gain of 1.4-fold.^[66] Among these parameters, independent adjustable parameters for biocatalytic performance optimization can be classed in two categories: enzymology parameters – enzyme specific activity (A_{sp} U/mg_{enz}) and immobilization protocol – and engineering parameters – reactor geometry and support characteristics. Enzyme specific activity can be tuned using protein engineering to achieve higher activity, enhanced robustness over time, and/or a broader substrate scope. For flow processes, the liquid flowrate is not considered an independent parameter for optimization due to its opposing effects on mixing conditions and biocatalytic conversion. With increased throughput, the mixing conditions are improved to some extent (provided there is no enzyme deterioration due to overly harsh flow conditions), but the reduction in residence time leads to a lower conversion level. Consequently, the liquid flowrate is adjusted depending on the industrial requirement in terms of productivity and conversion level. Figure 3 gives a first indication of the complexity of a strict comparison between studies. Studies often present STY and TN as measures of process performance, but a thorough analysis of the operating conditions must be performed to allow comparison with data from other studies. For example, under specific biocatalytic conditions (enzyme type, substrate concentration, temperature), STY must always be considered alongside the degree of substrate conversion. Indeed, as the net-yield increases, STY tends to decrease. This detail can be omitted only when using the maximum space-time-yield, STY_{max} obtained at the beginning of the enzymatic reaction (i.e. at very low conversion levels). This STY_{max} is an interesting intensification criterion as it assesses the maximum productivity obtainable per volume of reactor. Comparisons of TN values are even more complex due to their dependence on STY and on the choice of criteria to stop experiments. For example, Menegatti *et al.* may have underestimated the TN value (at 15 mg/mg) because no significant drop in the enzyme activity

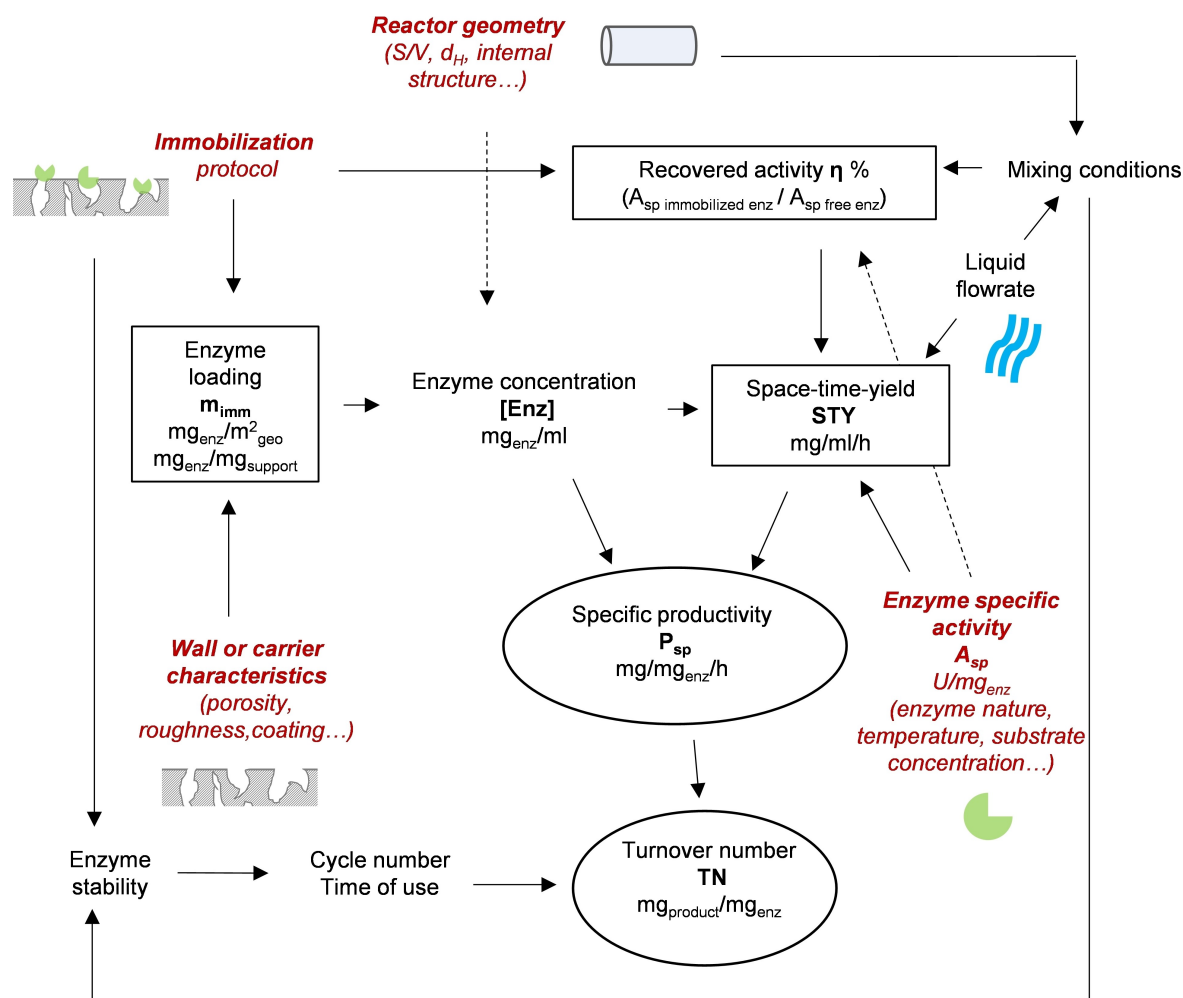


Figure 3. Biocatalytic metrics with square for those representing the “activity” and oval for those representing the “stability” which are time-averaged based on the enzyme operational time. The red colour and italic topography indicate the independent adjustable parameters to be optimized.

have been obtained after 7 days of continuous use, resulting in a stable conversion level at 100%.^[61] On the contrary, Stojković *et al.* reported a conversion drop from 40% to only 4% which could have contributed to an over estimation of the TN at 7100 mg/mg.^[57] To give an idea of the order of magnitude, at the industrial scale, production is typically stopped when the remaining enzyme activity is in between 50 and 10% of the initial activity measured, which constitutes a relatively wide operating window.^[9] To meet the industrial requirements, the TN must reach values up to 10000 g of product per g of immobilized enzyme for commodities and 100 g/g for high-value products.^[9]

5. Quantitative Analysis: Selected Examples

The enzymes selected for this review were not restricted to a certain category, consequently a large range of specific activities are covered: 0.32 to 800 U/mg_{enz} (Table 2). Two examples with immobilized cells are also proposed as this configuration is becoming more popular in the biocatalysis field

to reduce enzyme costs and create a more stable environment. The enzymatic activity has a significant impact on the kinetics of biocatalysis, and thus on the resulting STY, productivity and TN. However, strict quantitative comparisons between studies can be difficult, as explained above (Section 3). Our goal is to map the metrics of both wall-immobilized enzyme reactors and PBRs. Unfortunately, despite the recommendations of Bolívar and López-Gallego,^[19] the results of some recent studies are not expressed in standard metrics. As a result, some of them had to be excluded from our analysis because of insufficiently comparable experimental data.^[28,30,40]

Regarding the selected bioreactors (Table 2), the geometry of wall-immobilized enzyme reactors falls into three categories: meandering channel, parallel plates, and multiple parallel channels (array of capillaries or straight channels). Most wall-immobilized enzyme reactors are at microscale, whereas PBR studies encompass both micro- and milli-scale reactors. Very few reports of successful bioreactor scale-up were found, although Bajic *et al.* reported a μ -PBR scale-up that included an overview of how geometric parameters influenced reactor performance.^[108] For higher productivity without biocatalytic

Table 2. Some examples of experimental results selected from the literature on biocatalysis flow processes.

Substrate (concentration)	Enzyme (A_{sp})	Product	Reactor (reactor volume – channel dimension ^[a])
Racemic mixture of methylbenzylamine (5 mM ^[56] , 40 mM ^[108] , 100 mM ^[112])	ω -transaminase “ATA117”, R selective (A_{sp} not given) ^[56] ω -transaminase “AsR- ω TA”, R selective (≈ 15 U/mg _{enz}) ^[112] ω -transaminase “ATA-wt”, S selective (5 U/mg _{enz}) ^[108]	Acetophenone + other chiral compounds (S or R)	Single meandering channel (800 μ L–1.5 mm) ^[56] PBR with nano-porous microsize bead (157 μ L) ^[112] PBR with one layer of millisize bead (7 μ L or 1.2 mL) ^[108]
Sucrose (50 mM)	Sucrose Phosphorylase “Z_BISpase” (77 U/mg _{enz})	α -D-glucose 1-phosphate	Single meandering channel (9 μ L–92 μ m) ^[55] 2 parallel rectangular plates (83 μ L–225 μ m) ^[60] , ^[66]
Sucrose (800 mM) Glycerol (200 mM)	Engineered sucrose Phosphorylase Zbasic2_LMSPase (5 U/mg _{enz})	Glycosyl glycerol	Multiple parallel straight channels (25 μ L–300 μ m) ^[64]
Lactose (100 mM)	β -glycosidase “CelB” (800 U/mg _{enz})	Glucose	Multiple parallel straight channels (25 μ L–300 μ m) ^[62]
Sucrose (50 mM)	Saccharomyces cerevisiae invertase (A_{sp} not given)	Glucose / fructose	Array of capillaries (350 μ L–300 μ m) ^[54]
Fumaric acid (5 mM ^[61] and 20 mM ^[57])	Permeabilized Saccharomyces cerevisiae cell (16 U/g _{enz} , based on dry cell ^[61] , not given in [57])	L-malic acid	Single meandering channel (25 μ L–250 μ m) ^[57] 2 parallel rectangular plates (350 μ L–300 μ m) ^[61]
Benzaldehyde (30 mM)	Engineered amine dehydrogenase “LEAmDH-v1” and formate dehydrogenase “Cb-FDH” (A_{sp} not given)	Benzylamine	Hydrogel bed with 4 parallel channels (6 mL ^[b]) ^[45]
DMB-SMMP (1 mM) MJA (2 mM)	Engineered Acyltransferase “LovD-BuCH2” (A_{sp} 0.41 U/mg _{enz})	Simvastatin	PBR with nano-porous microsize beads (6 mL ^[c]) ^[30]
3-cyanopyridine (10 mM)	Commerciale nitrilase (A_{sp} 0.8 U/mg _{enz})	Nicotinic acid	PBR with nano-porous microsize beads (500 μ L) ^[5]
Acetophenone (5 mM)	Alcohol dehydrogenase «GcAPRD» (A_{sp} 2.8 U/mg _{enz})	(S)-1-phenylethanol	Single meandering channel (600 μ L–1.5 mm) ^[109]
8-hydroxyquinoline “8-HQ” (50 μ M)	Permeabilized human liver microsomes “b-HLM” containing UDP-glucuronosyltransferase “UGT” (A_{sp} 0.005 U/mg _{enz})	8-hydroxyquinoline glucuronide	Pilar structure (18.3 μ L – \approx 200 μ m) ^[58]
5-Methoxytryptamine (500 mM)	Acetyltransferase “MsACT” (A_{sp} not given)	Melatonin	PBR with nano-porous microsize bead (1.2 mL) ^[113]

[a] For PBR, the reactor volume refers to the void volume (i.e. where the liquid flows) [b] Hydrogel porosity is not given, the reactor volume refers to the column volume. [c] Bed porosity is not given; the reactor volume refers to the column volume as used in the article calculation

performances decrease, the chamber width increase should be privileged. The chamber length extend is limited because of the backpressure effect and the chamber depth because of the preferred paths formation. With wall-immobilized enzymes, no geometrical optimization for scale-up purposes has been proposed so far. According to their conclusions, for higher productivity without decreasing biocatalytic performance, scaling should focus on increasing chamber width. Any extension of chamber length should be limited because of the backpressure effect. Similarly, increases in chamber depth lead to the formation of preferred paths. With wall-immobilized enzymes, no geometrical optimization for scale-up purposes has been proposed so far.

5.1. Activity Analysis

The activity metrics of the selected studies are given in Table 3 and Table 4. To some extent, the reactor geometry can affect

the bioreactor's productivity when using wall-immobilized enzymes. For example, STY can be improved 2-fold with a higher S/V ratio by changing from a rectangular to a parabolic channel cross-section.^[55,60] But compared to the surface condition, the influence of reactor geometry on enzyme performances seems to be rather limited.

When a nanoporous layer is added to the reactor surface, the enzyme load is usually in the range of 91–170 mg_{enz}/m² or more,^[62,64,66] without such a layer, it is 2 to 9 mg_{enz}/m².^[54,55,60] Generally, due to limited diffusion, the deeper layers of the immobilized enzymes are less accessible, leading to lower recovered activity, η (down to 20%^[66] instead of 80%^[60]). However, the reactor productivity increases considerably since the STY_{max} is greatly improved, as shown by an example where the addition of a nanospring layer on a given reactor resulted in a 19-fold increase,^{[60][66]} With the hydrogel study, a massive difference in cell loading was achieved because of the difference in immobilization scenario: the cells were entrapped during hydrogel formation.^[61] The greater thickness (200 μ m)

Table 3. Metrics to analyse the bio-activity performances of wall immobilized reactors.

[Ref]	Support characteristics	m_{imm} ($\text{mg}_{\text{enz}}/\text{m}^2$)	S/V^a (mm^{-1})	[Enz] ^b ($\text{mg}_{\text{enz}}/\text{ml}$)	η (%)	A_{sp} ($\text{U}/\text{mg}_{\text{enz}}$)	STY_{max} ($\text{mg}/\text{ml}/\text{h}$)
Native or functionalized surface reactors (polymeric film of few nanometers)	[55] Borosilicate surface	6	43	0.3	70	77	216
	[60] Smooth borosilicate surface	2	9	0.02	80	77	16
	[54] Glass smooth surface	9	9	0.2	–	–	–
	[57] PFA smooth surface	357 (dry cells)	16	6 (dry cells)	–	–	6
	[58] OSTE surface (thiol-enes)	33 (cells)	33	1.2	70	0.005 (cells)	0.1
	[109] Polypropylene 3D-printed reactor coated with polymer films made of polydopamine and polyethylenimine	133	3	0.7	86 ^c	2.8	–
Coated surface reactors (added layers of several micrometers)	[56] Nylon 3D-printed reactor	71	3	0.2	–	–	–
	[66] Borosilicate surface coated with silica nanospring ($\approx 70 \mu\text{m}$)	140	9	1.3	20	77	296
	[64] Stainless steel surface coated with nano-porous aluminium oxide layer ($\approx 20 \mu\text{m}$)	> 170	14	> 2.3	–	5	191
	[62]	91	14	1.2	50	800	–
	[61] PMMA surface coated with hydrogel layer ($200 \mu\text{m}$)	33000 (dry cells)	6	189 (dry cell)	65– 72 ^c	0.016 (dry cells)	3

[a] Calculated from the reactor geometry [b] Calculated based on the total amount of immobilized enzyme and not only the active enzyme [c] Measured from batch assay and not from in-flow experiments

Table 4. Metrics to analyse the bio-activity performances of packed bed reactors.

[Ref]	Support characteristics	m_{imm} ($\text{mg}_{\text{enz}}/\text{mg}$)	[Enz] ^a (mg/ml)	η (%)	A_{sp} ($\text{U}/\text{mg}_{\text{enz}}$)	STY_{max} ($\text{mg}/\text{ml}/\text{h}$)
[108]	One layer of milli-size particles (reactor volume of $7 \mu\text{L}$)	0.05	≈ 130	$< 10^b$	5	277
	One layer of milli-size particles (reactor volume of 1.3 mL)		≈ 60			–
[45]	Hydrogel bed	–	5	–	–	–
[30]	Nano-porous glass μ -bead	$3.4 \cdot 10^{-4}$	0.2	83	0.41	–
[5]	Nano-porous PMMA μ -bead	$3 \cdot 10^{-3}$	3	23	0.8	6
[112]	Nano-porous glass μ -bead with a functionalized polymer (Fe^{3+})	0.15	≈ 190	≈ 40	≈ 15	–
[113]	Nano-porous glyoxyl agarose bead	$1 \cdot 10^{-3}$	≈ 3	73 ^b	–	–

[a] Calculated based on the total amount of immobilized enzyme and not only the active enzyme [b] Measured from batch assay and not from in-flow experiments

Despite all the attempts to increase the wall-immobilized enzyme load, the resulting enzyme concentrations – 0.02–2.3 mg/mL – do not reach those obtained with PBR: around 60–130 mg/mL. As a result, STY_{max} is more likely to be higher with PBR and must be balanced against the increase in backpressure due to void reduction.

also contributed to the tremendous increase in cell load. However, the resultant drawback with such a thick polymer layer is limited mass-transfer, as mentioned by the authors. As a consequence, the actual η was lower than 10%. The mass-transfer issue combined with low specific activity when cell mass is taken as a reference, resulted in a low STY_{max} compared to other studies. Further improvements in the hydrogel thickness should increase η .

The 3D-manufacturing also appear to provide promising results, with enzyme loads close to those obtained with coating techniques (i.e. 71–133 $\text{mg}_{\text{enz}}/\text{m}^2$ ^[56,109]). However, so far this manufacturing technique has mostly been applied for tailor-

made carrier supports to be placed inside the reactor, for example in packed bed or monolith reactors.^[40,48,111]

Finally, geometric influences are not considered significant in the studies cited when using wall-immobilized enzymes. However, in milli-scale reactors, this conclusion might have to be reconsidered. Indeed, the geometry of milli-scale reactors is expected to have a greater influence on mixing than observed in microfluidic devices. For example, Bajic *et al.* studied a micro-size PBR scale-up, they proposed the inclusion of a triangular-pillar section at the entrance and outlet of the reactor to ensure homogeneous fluid distribution despite the increased chamber length.^[108] They also emphasized the importance of maintaining good mixing conditions when channel height is increased from

one layer of beads to two or three layers. In these conditions, the biocatalytic performances were reduced with multiple layers due to imperfect mixing conditions.

5.2. Stability Analysis

The stability metrics reported in the selected studies are collected in Table 5.

The P_{sp} decreases as the enzyme load increases, primarily due to a reduction in activity efficiency. This relation has been demonstrated by Valikhani et al. studies where P_{sp} has been reduced from 464 mg/mg_{enz}/h^[55] to 126 mg/mg_{enz}/h^[66] with a 3.5 fold drop in activity efficiency. In Table 5, this observation is further supported by reports of PBR with P_{sp} below 1 mg/mg_{enz}/h_r.^[45,108] This result can be explained by very low activity efficiency (<10%) combined with poor mixing efficiency in PBR. Finally, to be noted that small P_{sp} can also be assigned in tests with the lowest substrate concentrations at the reactor inlet^[56,58,109] or with the lowest enzyme specific activities (in particular for whole-cells studies.^[30,57,58,61]

A lack of standardization in long-term study protocols is observed based on the conversion levels selected. Thus, some authors select the "optimum" STY with a conversion level starting at 50%, but change the end point (e.g. to 26% or 42%).^[55,66] Other authors consider total substrate conversion^[30,56,61,112,113] but – almost certainly due to time

constraints – stop their experiments before the enzyme's maximum capacity is reached, leading to an underestimated TN. From a stability point of view, provided the hydrogel composition is appropriately adapted, hydrogel seems to be interesting compared to surface-immobilized cells, with no fluctuation in the conversion level observed over 7 days.^[61] However, TN – at 15 mg/mg_{enz} – was undoubtedly underestimated in this study, and longer experiments would be required to complete the stability analysis.

5.3. Stability and Analysis Metrics Interaction

Since TN cannot be directly compared due to significant process time as mentioned in 5.2 Section (i.e. with various reaction conversions), an overall mapping has been performed as proposed by Bolivar and López-Gallego,^[19] with the natural logarithmic representation of TN as a function of STY in Figure 4.

This mapping gives a similar trend to that reported by Bolivar and López-Gallego based on their selection of studies.^[19] Consequently, with improvements in STY, TN is more likely to be increased. We also noticed that values for PBR tend to be lower than those obtained for wall-coated reactors. This may be explained by lower P_{sp} values (Table 5). For this type of reactor, the high enzyme concentration and low recovered activity must

Table 5. Metrics of the bioreactor longevity.

[Ref]	P_{sp} ^[a] (mg/mg _{enz} /h)	STY ^[a] (mg/ml/h)	TN ^[a] (mg/mg _{enz})	Number of cycle (time)	X variation from t_0 to t_f
Native or fonctionalized surface reactors					
[55]	464	119	≈ 11 000	800 (24 h)	50–26%
[60]	–	–	–	–	–
[54]	879	69	≈ 630 000	900 (30 days)	100–90%
[57]	0.5 (dry cells)	3	≈ 7100	20 (4 days)	40–4%
[58]	0.1 (cells)	0.1	≈ 20	250 (15 h)	50–20%
[109]	1.7	1	≈ 200	200 (5 days)	90–40%
[56]	4	1	≈ 360	100 (4 days)	100–97%
Coated surface reactors					
[66]	126	152	≈ 3500	840 (29 h)	50–42%
[64]	–	99	–	900 (16 days)	23–11%
[62] ^[b]	323	400	≈ 32 000	1900 (4 days)	70–60%
[61] ^[c]	0.003 (dry cells)	1	≈ 15	200 (7 days)	100%
Packed bed reactors					
[108]	0.33	18	≈ 170	1200 (21 days)	≈ 100–80%
[45]	0.07	0.3	8	150 (5 days)	90–25%
[30]	17	5	35	20 (4 h)	≈ 100–90%
[5]	1.1	3	500	2000 (30 days)	85–40%
[112]	3.5	331	330	13 000 (96 h)	100%
[113]	126	1280	2500	580 (24 h)	92%

[a] When not directly given by the authors, the calculation was approximated considering the mean value of the conversion level. This mean value was applied to the entire experiment duration. [b] The real volumetric activity was reduced at 180 U/mL for the long-term experiment leading to η at 18% and not 50% as compared to table 3. [c] P_{sp} and TN are calculated based on the mass of dry cells.

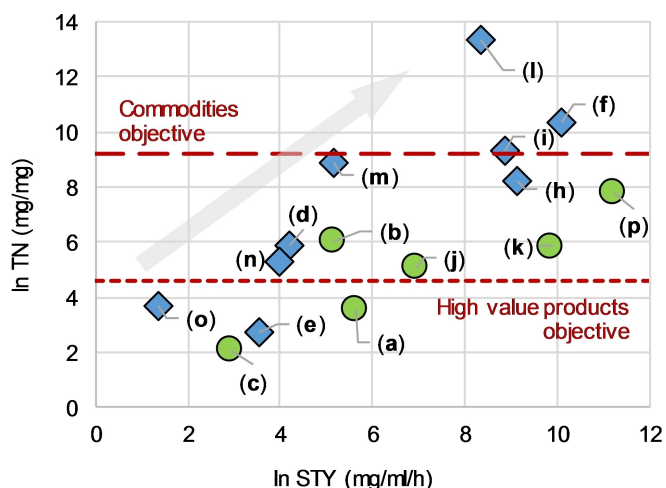


Figure 4. Logarithmic representation of TN as a function of STY with the most recent and relevant studies selected in this review. Green circles represent the packed bed reactors and blue diamonds the wall immobilized enzyme reactors. References: (a) García-Marquina *et al.*, 2022; (b) Teepakorn *et al.*, 2021; (c) Croci *et al.*, 2022; (d) Peris *et al.*, 2017; (e) Menegatti and Žnidaršič-Plazl, 2019; (f) Thomsen and Nidetzky, 2009; (g) Bolivar *et al.*, 2017; (h) Valikhani *et al.*, 2017b; (i) Valikhani *et al.*, 2017; (j) Bajić *et al.*, 2017; (k) Böhmer *et al.*, 2019; (l) Carvalho *et al.*, 2017; (m) Stojković and Žnidaršič-Plazl, 2012; (n) T.sriwong and Matsuda, 2022; (o) Kiiski *et al.*, 2021; (p) Contente *et al.*, 2019

be weighed against the highly stable performance, which increases the TN.

In Table 5, the most promising operating STY values seem to be linked to highly active enzymes.^[55,62] This interaction is confirmed with an almost linear tendency relation between the natural logarithm of STY as a function of the Asp logarithm (Figure 5-a).

The growing interest in micro-sized bioreactor is mainly justified by better mixing performances and higher S/V ratio which would improve STY.^[22] A recent study took miniaturization even further with a nanoscale reactor, but the choice of

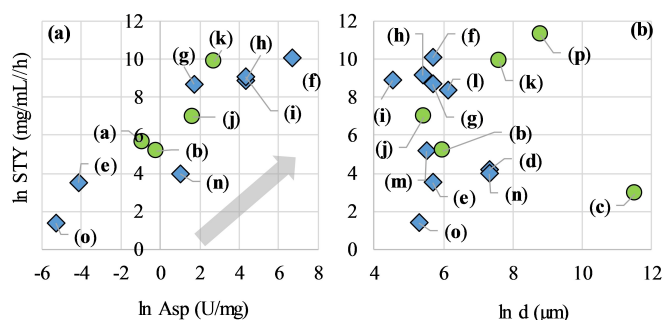


Figure 5. Logarithmic representation of STY as a function of Asp (a) and the channel height/diameter or column diameter (b). Green circles represent the PBR and monolith reactors and blue diamonds the wall immobilized enzyme reactors. References: (a) García-Marquina *et al.*, 2022; (b) Teepakorn *et al.*, 2021; (c) Croci *et al.*, 2022; (d) Peris *et al.*, 2017; (e) Menegatti and Žnidaršič-Plazl, 2019; (f) Thomsen and Nidetzky, 2009; (g) Bolivar *et al.*, 2017; (h) Valikhani *et al.*, 2017b; (i) Valikhani *et al.*, 2017; (j) Bajić *et al.*, 2017; (k) Böhmer *et al.*, 2019; (l) Carvalho *et al.*, 2017; (m) Stojković and Žnidaršič-Plazl, 2012; (n) T.sriwong and Matsuda, 2022; (o) Kiiski *et al.*, 2021; (p) Contente *et al.*, 2019

such scale reduction was justified by the application. Indeed, analytical systems do not require high throughput.^[114] In addition, the benefit of reducing reactor channel size on STY is not clearly observable from the selected results (Figure 5-b). Either the difference is not significant or the influence of enzymatic activity prevails over other parameters. To truly compare two distinct technologies or reactor sizes, it would be necessary to have the same enzyme immobilized using the same protocol.

6. Summary and Outlook

Due to environmental concerns, research in the biocatalysis field is expanding in multiple directions: (i) novel engineered enzymes, (ii) multiple enzyme immobilization protocols and carrier types, and (iii) technological solutions for continuous biocatalytic production. The two most widely studied configurations at lab-scale are: packed bed reactors (PBR), with enzymes immobilized on beads or on a monolithic support; and wall-immobilized enzyme miniaturized reactors.

Continuous biosynthesis is already implemented through the use of bulk beads in some applications in the food industry. At lab-scale, monolithic reactors have been extensively studied because they minimize backpressure effects. Even though industrial applications of monolithic columns in downstream operations with multiple convective interaction media are commercially available, biocatalytic applications at production scale remain rare. This is mainly justified in the literature by complex and costly fabrication processes. However, the economic viability of an enzyme immobilization strategy is complex and multi-factorial.^[16] Thus, the cost of the support may not be the only reason for the lack of industrial examples of monolithic bioreactor applications.

Wall-immobilized enzyme reactors are another solution that reduces backpressure effects, while also providing improved mixing efficiency. These types of flow reactor would be more attractive for the production of high-value products.

Proof of concept of biocatalytic flow are not at a first try. Consequently, a certain number of studies has been published, but the articles do not always report sufficient data, or the most relevant data to allow critical examination. Biocatalysis flow reactors should be compared based on activity and stability aspects in line with the standard metrics reported in the literature. These powerful tools are relatively simple to calculate and can be used as the basis for a more complex interpretation due to the influence of multiple factors. In addition, the enzyme portfolio often makes fair comparison difficult. Thus, these metrics should be considered with care and analysed from a very general point of view when comparing distinct studies.

From the data-mapping presented here, that higher enzyme concentrations can be reached with PBR than with wall-immobilized enzyme miniaturized reactors. However, since the specific productivity is generally lower, sometimes below $1 \text{ mg} \cdot \text{mg}_{\text{enz}}^{-1} \cdot \text{h}^{-1}$, the advantage is counterbalanced by the need for highly stable enzymes. With wall-immobilized enzyme miniaturized reactors, microfluidic devices combined with coat-

ing techniques to add a nanoporous layer have been the most widely studied solutions to date, with many iterations to maximize performance. Promising examples have been reported in the literature, with highly active enzymes with turnovers close to industrial requirements for commodities production. As demonstrated from the data-mapping, enzymatic activity is also a key factor contributing to reactor space-time efficiency. The relationship between space-time efficiency and turnover revealed by Bolivar and López-Gallego^[19] seems to be also confirmed in the studies analysed here, even though some of them report an underestimated turnover due to overly short experiments and/or low substrate levels.

For up-scaling purposes, both increased reactor characteristic length and numbering up must be considered. Thus, despite its positive effect on space-time efficiency, micrometer reactor scaling should not be systematic. So far, critical comparisons between microfluidic devices and mesoreactor performances is rather difficult as: (i) very few studies report on milli-scale reactors, (ii) the influence of channel size on reactor performance cannot be clearly determined from a global point of view since the influence of enzymatic activity outweighs other potential parameters. More scale-up studies on meso-scale reactors, with channel characteristic dimensions exceeding 1 mm will now need to be performed. Ideally, the characteristic channel size should not be more than 2 to 3 times that used at industrial scale to avoid creating a scale-up factor that is too big and too challenging. Optimization of the geometry to preserve intensified mixing conditions should also be investigated along with strategies to overcome the decrease in surface-to-volume ratio, for example through efficient coating and immobilization techniques that would allow higher recovered activities.

Acknowledgements

The authors acknowledge CEA/LITEN – CARNOT project “Milli-Biocat” for funding the activities presented in this paper. The authors acknowledge the Agence Nationale de la Recherche for partial financial support through the LabEx ARCANÉ program (ANR-11-LABX-0003-01) and the Graduate School of Chemistry, Biology and Health of University Grenoble Alpes CBH-EUR-GS (ANR-17-EURE-0003).

Conflict of Interests

The authors declare no commercial or financial conflict of interest.

Keywords: Biosynthesis · enzyme catalysis · flow biocatalysis · process intensification · reactor design

[1] S. Salama, H. S. Mostafa, S. Husseiny, M. Sebak, *Chem. Biodiversity* **2024**, *21*, e202301205.

[2] S. Pandya, A. Gupte, *Microbiol. Biotechnol. Lett.* **2023**, *51*, 333–352.

- [3] F. Balkenhohl, K. Ditrach, C. Nübling, *Racemate separation of primary and secondary heteroatom-substituted amine by enzyme-catalysed acylation*, **1996**, WO1996023894 A1.
- [4] D. Lisicki, K. Nowak, B. Orlińska, *Materials* **2022**, *15*, 765.
- [5] C. Teepakorn, P. Zajkoska, G. Cwicklinski, V. De Berardinis, A. Zaparucha, G. Nonglaton, Z. Anxionnaz-Minvielle, *Biotechnol. J.* **2021**, *16*, 2100010.
- [6] M. Bilal, H. M. N. Iqbal, *Food Res. Int.* **2019**, *123*, 226–240.
- [7] G. Rossino, M. S. Robescu, E. Licastro, C. Tedesco, I. Martello, L. Maffei, G. Vincenti, T. Bavaro, S. Collina, *Chirality* **2022**, *34*, 1403–1418.
- [8] R. M. Phelan, M. J. Abrahamson, J. T. C. Brown, R. K. Zhang, C. R. I. Zwick, *Org. Process Res. Dev.* **2022**, *26*, 1944–1959.
- [9] A. Basso, S. Serban, *J. Mol. Catal.* **2019**, *479*, 110607.
- [10] C. Jiménez-González, P. Poechlauer, Q. B. Broxterman, B.-S. Yang, D. am Ende, J. Baird, C. Bertsch, R. E. Hannah, P. Dell’Orco, H. Noorman, S. Yee, R. Reintjens, A. Wells, V. Massonneau, J. Manley, *Org. Process Res. Dev.* **2011**, *15*, 900–911.
- [11] M. M. Nasr, M. Krumme, Y. Matsuda, B. L. Trout, C. Badman, S. Mascia, C. L. Cooney, K. D. Jensen, A. Florence, C. Johnston, K. Konstantinov, S. L. Lee, *J. Pharm. Sci.* **2017**, *106*, 3199–3206.
- [12] K. P. Cole, J. M. Groh, M. D. Johnson, C. L. Burcham, B. M. Campbell, W. D. Diseroad, M. R. Heller, J. R. Howell, N. J. Kallman, T. M. Koenig, S. A. May, R. D. Miller, D. Mitchell, D. P. Myers, S. S. Myers, J. L. Phillips, C. S. Polster, T. D. White, J. Cashman, D. Hurley, R. Moylan, P. Sheehan, R. D. Spencer, K. Desmond, P. Desmond, O. Gowran, *Science* **2017**, *356*, 1144–1150.
- [13] A. Adamo, R. L. Beingessner, M. Behnam, J. Chen, T. F. Jamison, K. F. Jensen, J.-C. M. Monbaliu, A. S. Myerson, E. M. Revalor, D. R. Snead, T. Stelzer, N. Weeranoppanant, S. Y. Wong, P. Zhang, *Science* **2016**, *352*, 61–67.
- [14] C. J. Testa, C. Hu, K. Shvedova, W. Wu, R. Sayin, F. Casati, B. S. Halkude, P. Hermant, D. E. Shen, A. Ramnath, Q. Su, S. C. Born, B. Takizawa, S. Chattopadhyay, T. F. O’Connor, X. Yang, S. Ramanujam, S. Mascia, *Org. Process Res. Dev.* **2020**, *24*, 2874–2889.
- [15] M. P. Thompson, I. Peñafiel, S. C. Cosgrove, N. J. Turner, *Org. Process Res. Dev.* **2019**, *23*, 9–18.
- [16] J. Bolivar, J. Woodley, R. Fernandez-Lafuente, *Chem. Soc. Rev.* **2022**, *51*, DOI 10.1039/D2CS00083K.
- [17] M. Janssen, C. Müller, D. Vogt, *Green Chem.* **2011**, *13*, 2247–2257.
- [18] P. Znidaršič-Plazl, *Curr. Opin. Green Sustain. Chem.* **2021**, *32*, 100546.
- [19] J. M. Bolivar, F. López-Gallego, *Curr. Opin. Green Sustain. Chem.* **2020**, *25*, 100349.
- [20] M. Romero-Fernández, F. Paradisi, *Curr. Opin. Chem. Biol.* **2020**, *55*, 1–8.
- [21] P. Znidaršič-Plazl, *Biotechnol. J.* **2019**, *14*, 1800580.
- [22] Y. Zhu, Q. Chen, L. Shao, Y. Jia, X. Zhang, *React. Chem. Eng.* **2019**, *5*, 9–32.
- [23] L. Tamborini, P. Fernandes, F. Paradisi, F. Molinari, *Trends Biotechnol.* **2018**, *36*, 73–88.
- [24] J. Britton, S. Majumdar, G. A. Weiss, *Chem. Soc. Rev.* **2018**, *47*, 5891–5918.
- [25] Z. Dong, Z. Wen, F. Zhao, S. Kuhn, T. Noël, *Chem. Eng. Sci. X* **2021**, *10*, 100097.
- [26] K. F. Jensen, *AIChE J.* **2017**, *63*, 858–869.
- [27] P. Znidaršič-Plazl, *J. Flow Chem.* **2017**, *7*, 111–117.
- [28] C. Alvarez-Gonzalez, V. E. Santos, M. Ladero, J. M. Bolivar, *Catalysts* **2022**, *12*, 80.
- [29] H. B. S. Bento, C. E. R. Reis, P. A. Pinto, D. V. Cortez, R. N. Vilas Bôas, T. A. Costa-Silva, A. K. F. Carvalho, H. F. de Castro, *Catal. Lett.* **2022**, *152*, 2434–2444.
- [30] G. García-Marquina, J. Langer, M. Sánchez-Costa, G. Jiménez-Osés, F. López-Gallego, *ACS Sustainable Chem. Eng.* **2022**, *10*, 9899–9910.
- [31] A. Jungbauer, R. Hahn, *J. Chromatogr. A* **2008**, *1184*, 62–79.
- [32] N. Adebar, H. Gröger, *Eur. J. Org. Chem.* **2020**, *2020*, 6062–6067.
- [33] Y. Mao, R. Fan, R. Li, X. Ye, U. Kulozik, *Electrophoresis* **2021**, *42*, 2599–2614.
- [34] M. Oláh, S. Suba, Z. Boros, P. Kovács, M. Gosselin, C. Gaudreault, G. Hornyánszky, *Period. Polytech. Chem. Eng.* **2018**, *62*, 519–532.
- [35] W. Snoch, M. Tataruch, O. Zastawny, E. Cichoń, M. Gosselin, H. Cabana, M. Guzik, *Bioorg. Chem.* **2019**, *93*, 102813.
- [36] V. Balakotaiah, Z. Sun, R. Ratnakar, *Rev. Chem. Eng.* **2021**, *38*, DOI 10.1515/revce-2020-0113.
- [37] A. Podgornik, A. Savnik, J. Jančar, N. L. Krajnc, *J. Chromatogr. A* **2014**, *1333*, 9–17.
- [38] P. Krajnc, N. Leber, D. Štefanec, S. Kontrec, A. Podgornik, *J. Chromatogr. A* **2005**, *1065*, 69–73.

- [39] N. Ghéczy, W. Xu, K. Szymańska, A. B. Jarzębski, P. Walde, *ACS Omega* **2022**, *7*, 26610–26631.
- [40] A. Valotta, M. C. Maier, S. Soritz, M. Pauritsch, M. Koenig, D. Brouczek, M. Schwentenwein, H. Gruber-Woelfler, *J. Flow Chem.* **2021**, *11*, 675–689.
- [41] M. Michaud, F. Bornette, E. Rautu, S. H. More, M. Leonardo Martinez Mendez, L. Jiery, D. Edouard, *Chem. Eng. J.* **2023**, *452*, 138604.
- [42] G. Nicoletti, E. P. Cipolatti, A. Valério, N. G. Carbonera, N. S. Soares, E. Theilacker, J. L. Ninow, D. de Oliveira, *Bioprocess Biosyst. Eng.* **2015**, *38*, 1739–1748.
- [43] L. Majul, S. Wirth, L. Levin, *Environ. Technol.* **2022**, *43*, 684–695.
- [44] W. Xu, M. Luo, L. Xinyue, Z. Ye, T. Jeong, *Water* **2022**, *14*, 2595.
- [45] F. Croci, J. Vilim, T. Adamopoulos, V. Tseliou, P. J. Schoenmakers, T. Knaus, F. G. Mutti, *ChemBioChem* n.d., n/a, e202200549.
- [46] Q. Yang, Y. Yan, X. Yang, G. Liao, D. Wang, H. Xia, *Chem. Eng. J.* **2019**, *372*, 946–955.
- [47] J. Meyer, L.-E. Meyer, S. Kara, *Eng. Life Sci.* **2022**, *22*, 165–177.
- [48] B. Schmieg, M. Nguyen, M. Franzreb, *Front. Bioeng. Biotechnol.* **2020**, *8*.
- [49] A. Podgornik, A. Strancar, in *Biotechnology Annual Review*, Elsevier, **2005**, pp. 281–333.
- [50] J. Ye, T. Chu, J. Chu, B. Gao, B. He, *ACS Sustainable Chem. Eng.* **2019**, *7*, 18048–18054.
- [51] Y. Shao, Z. Liao, B. Gao, B. He, *ACS Omega* **2022**, *7*, 11530–11543.
- [52] T. Pose-Boirazian, J. Martínez-Costas, G. Eibes, *Macromol. Biosci.* **2022**, *22*, 2200110.
- [53] P. L. Suryawanshi, S. P. Gumpfekar, B. A. Bhanvase, S. H. Sonawane, M. S. Pimplapure, *Chem. Eng. Sci.* **2018**, *189*, 431–448.
- [54] F. Carvalho, M. P. C. Marques, P. Fernandes, *Catalysts* **2017**, *7*, 42.
- [55] D. Valikhani, J. M. Bolivar, M. Pfeiffer, B. Nidetzky, *ChemCatChem* **2017**, *9*, 161–166.
- [56] E. Peris, O. Okafor, E. Kulcinskaja, R. Goodridge, S. V. Luis, E. Garcia-Verdugo, E. O'Reilly, V. Sans, *Green Chem.* **2017**, *19*, 5345–5349.
- [57] G. Stojković, P. Žnidaršič-Plazl, *Process Biochem.* **2012**, *47*, 1102–1107.
- [58] I. Kiiski, E. Ollikainen, S. Artes, P. Järvinen, V. Jokinen, T. Sikanen, *Eur. J. Pharm. Sci.* **2021**, *158*, 105677.
- [59] M. Viefhues, S. Sun, D. Valikhani, B. Nidetzky, E. Vrouwe, T. Mayr, J. Bolivar, *J. Micromech. Microeng.* **2017**, *27*, 065012.
- [60] J. M. Bolivar, D. Valikhani, B. Nidetzky, *Biotechnol. J.* **2019**, *14*, 1800244.
- [61] T. Menegatti, P. Žnidaršič-Plazl, *Micromachines* **2019**, *10*, 867.
- [62] M. S. Thomsen, B. Nidetzky, *Biotechnol. J.* **2009**, *4*, 98–107.
- [63] A. Schwarz, M. S. Thomsen, B. Nidetzky, *Biotechnol. Bioeng.* **2009**, *103*, 865–872.
- [64] J. M. Bolivar, C. Luley-Goedl, E. Leitner, T. Sawangwan, B. Nidetzky, *J. Biotechnol.* **2017**, *257*, 131–138.
- [65] K. F. Schilke, K. L. Wilson, T. Cantrell, G. Corti, D. N. McIlroy, C. Kelly, *Biotechnol. Prog.* **2010**, *26*, 1597–1605.
- [66] D. Valikhani, J. M. Bolivar, M. Viefhues, D. N. McIlroy, E. X. Vrouwe, B. Nidetzky, *ACS Appl. Mater. Interfaces* **2017**, *9*, 34641–34649.
- [67] S. Matsuura, R. Ishii, T. Itoh, S. Hamakawa, T. Tsunoda, T. Hanaoka, F. Mizukami, *Chem. Eng. J.* **2011**, *167*, 744–749.
- [68] L. He, Y. Fan, L. Luo, J. Bellettre, J. Yue, *Chem. Eng. J.* **2020**, *380*, 122424.
- [69] R. Zapf, G. Kolb, H. Pennemann, V. Hessel, *Chem. Eng. Technol.* **2006**, *29*, 1509–1512.
- [70] G. Germani, A. Stefanescu, Y. Schuurman, A. C. van Veen, *Chem. Eng. Sci.* **2007**, *62*, 5084–5091.
- [71] I. Pottratz, I. Müller, C. Hamel, *Catalysts* **2022**, *12*, 7.
- [72] H. Rasouli, I. Iliuta, F. Bougie, A. Garnier, M. C. Iliuta, *Chem. Eng. J.* **2021**, *421*, 129587.
- [73] D. Zhao, M. Louise Leth, M. Abou Hachem, I. Aziz, N. Jančić, T. Luxbacher, C. Hélix-Nielsen, W. Zhang, *Chem. Eng. J.* **2023**, *451*, 138902.
- [74] L. Cao, F. van Rantwijk, R. A. Sheldon, *Org. Lett.* **2000**, *2*, 1361–1364.
- [75] M. Bajić, S. Khiawjan, S. T. Hilton, G. J. Lye, M. P. C. Marques, N. Szita, *Biochem. Eng. J.* **2024**, *205*, 109260.
- [76] J. Cui, S. Jia, L. Liang, Y. Zhao, Y. Feng, *Sci. Rep.* **2015**, *5*, 14203.
- [77] P. Bitterwolf, S. Gallus, T. Peschke, E. Mittmann, C. Oelschlaeger, N. Willenbacher, K. S. Rabe, C. M. Niemeyer, *Chem. Sci.* **2019**, *10*, 9752–9757.
- [78] T. Peschke, M. Skoupi, T. Burgahn, S. Gallus, I. Ahmed, K. S. Rabe, C. M. Niemeyer, *ACS Catal.* **2017**, *7*, 7866–7872.
- [79] D. Zheng, S. Wang, S. Qiu, J. Lin, X. Diao, *J. Biotechnol.* **2018**, *281*, 123–129.
- [80] R. Karande, A. Schmid, K. Buehler, *Langmuir* **2010**, *26*, 9152–9159.
- [81] P. V. Iyer, L. Ananthanarayan, *Process Biochem.* **2008**, *43*, 1019–1032.
- [82] D. Jussen, H. Soltner, B. Stute, W. Wiechert, E. von Lieres, M. Pohl, *J. Biotechnol.* **2016**, *231*, 174–182.
- [83] Y. Li, X. Xu, B. Yan, C. Deng, W. Yu, P. Yang, X. Zhang, *J. Proteome Res.* **2007**, *6*, 2367–2375.
- [84] F. M. W. G. Silva, A. O. Imarah, O. Takács, L. Tuba, L. Poppe, *Catalysts* **2023**, *13*, 384.
- [85] A. Šalić, K. Pindrić, G. H. Podrepšek, N. Novosel, M. Leitgeb, B. Zelić, *J. Flow Chem.* **2016**, *6*, 27–32.
- [86] E. Gkantzou, M. Patila, H. Stamatis, *Catalysts* **2018**, *8*, 282.
- [87] S. M. Robotajzi, S. A. Shojaosadati, R. Khalilzadeh, E. V. Farahani, N. Balochi, *Bioresour. Technol.* **2012**, *104*, 6–11.
- [88] J. Hristov, V. Perez, *Int Rev Chem Eng* **2011**, *3*.
- [89] Z. Al-Qodah, M. Al-Shannag, M. Al-Busoul, I. Penchev, H. Al-Ahmadi, K. Alqdah, *Rev. Chem. Eng.* **2017**.
- [90] Y. Wu, W. Xu, L. Jiao, W. Gu, D. Du, L. Hu, Y. Lin, C. Zhu, *Chem. Soc. Rev.* **2022**, *51*, 6948–6964.
- [91] S. C. Cosgrove, A. P. Matthey, *Chem. Eur. J.* **2022**, *28*, e202103607.
- [92] F. Paradisi, L. Poppe, in *10 Contin.-Flow Biocatal. Enzym. Cells*, De Gruyter, **2021**, pp. 277–312.
- [93] J. Luo, S. Song, H. Zhang, H. Zhang, J. Zhang, Y. Wan, *Eng. Life Sci.* **2020**, *20*, 441–450.
- [94] P. Andrić, A. S. Meyer, P. A. Jensen, K. Dam-Johansen, *Biotechnol. Adv.* **2010**, *28*, 407–425.
- [95] V. Venezia, V. Califano, G. Pota, A. Costantini, G. Landi, A. Di Benedetto, *Micromachines* **2020**, *11*, 790.
- [96] T. Menegatti, I. Plazl, P. Žnidaršič-Plazl, *Chem. Eng. J.* **2024**, *483*, 149317.
- [97] F. Strniša, M. Bajić, P. Panjan, I. Plazl, A. M. Sesay, P. Žnidaršič-Plazl, *Chem. Eng. J.* **2018**, *350*, 541–550.
- [98] A. Amani, E. Jalilnejad, *Biochem. Eng. J.* **2017**, *122*, 47–59.
- [99] Y. Asanomi, H. Yamaguchi, M. Miyazaki, H. Maeda, *Molecules* **2011**, *16*, 6041–6059.
- [100] A. Prasnikar, T. Urbic, I. Plazl, *Chim. Oggi-Chem. Today* **2017**, *35*, 12–14.
- [101] K. Fu, X. Wang, P. Li, C. Cao, W. Du, *AIChE J.* **2023**, *69*, e18190.
- [102] L. Vaccaro, *Sustainable Flow Chemistry: Methods and Applications*, John Wiley & Sons, **2017**.
- [103] L. Hajba, A. Guttman, *J. Flow Chem.* **2016**, *6*, 8–12.
- [104] S. G. Newman, K. F. Jensen, *Green Chem.* **2013**, *15*, 1456–1472.
- [105] A. Woitalka, S. Kuhn, K. F. Jensen, *Chem. Eng. Sci.* **2014**, *116*, 1–8.
- [106] D. Lavric, P. Woehl, *Chem. Today* **2009**, *27*, 45–48.
- [107] F. Herbstritt, J. Heck, in *Compact Heat Exchangers: Designs, Materials and Applications*, **2017**, pp. 144–159.
- [108] M. Bajić, I. Plazl, R. Stloukal, P. Žnidaršič-Plazl, *Process Biochem.* **2017**, *52*, 63–72.
- [109] K. T. Sriwong, T. Matsuda, *React. Chem. Eng.* **2022**, *7*, 1053–1060.
- [110] P. Poechlauer, J. Manley, R. Broxterman, B. Gregertsen, M. Ridemark, *Org. Process Res. Dev.* **2012**, *16*, 1586–1590.
- [111] L. Wenger, C. P. Radtke, J. Göpper, M. Wörner, J. Hubbuch, *Front. Bioeng. Biotechnol.* **2020**, *8*, 713.
- [112] W. Böhmer, T. Knaus, A. Volkov, T. K. Slot, N. R. Shiju, K. Engelmarm Casimjee, F. G. Mutti, *J. Biotechnol.* **2019**, *291*, 52–60.
- [113] M. L. Contente, S. Farris, L. Tamborini, F. Molinari, F. Paradisi, *Green Chem.* **2019**, *21*, 3263–3266.
- [114] K. Yamamoto, K. Morikawa, H. Imanaka, K. Imamura, T. Kitamori, *Analyst* **2020**, *145*, 5801–5807.

Manuscript received: January 30, 2024
Revised manuscript received: April 12, 2024
Accepted manuscript online: April 15, 2024
Version of record online: May 10, 2024