Journal of Flow Chemistry (2024) 14:211–218
https://doi.org/10.1007/s41981-023-00283-z

PERSPECTIVES

Perspectives on flow biocatalysis: the engine propelling enzymatic reactions

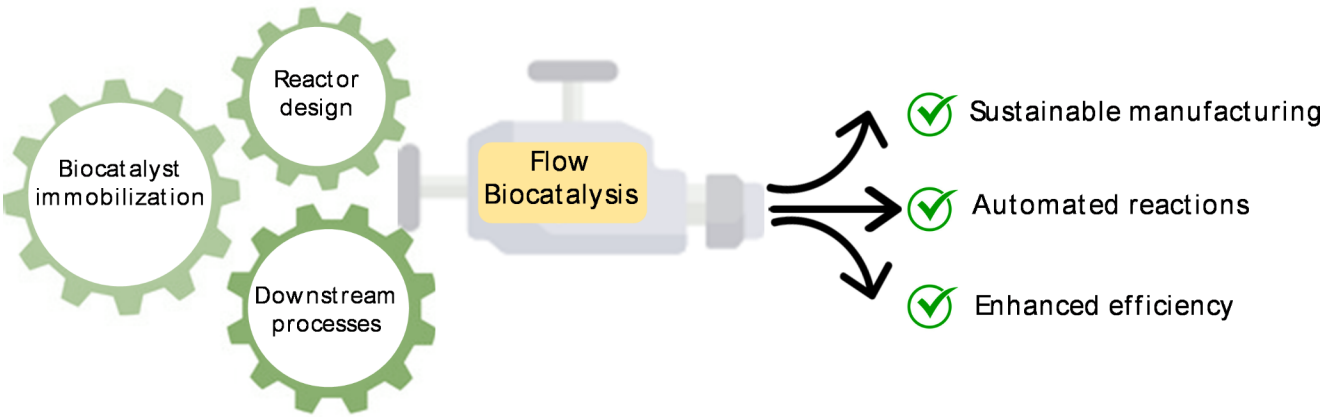
Ana I. Benítez-Mateos[1] · **Francesca Paradisi**[1]

Received: 31 August 2023 / Accepted: 5 October 2023 / Published online: 23 October 2023

Abstract

Flow biocatalysis has emerged as an empowering tool to boost the potential of enzymatic reactions towards more automatized, sustainable, and generally efficient synthetic processes. In the last fifteen years, the increasing number of biocatalytic transformations carried out in continuous flow exemplified the benefits that this technology can bring to incorporate biocatalysis into industrial operations. This perspective aims to capture in a nutshell the available methodologies for flow biocatalysis as well as to discuss the current limitations and the future directions in this field.

Graphical abstract



Article highlights

- Integration of enzymatic reactions into flow reactors is an exponentially growing technology.
- Flow biocatalysis can produce valuable molecules for food, cosmetics, and pharmaceuticals in a more eco-friendly manner.
- An interdisciplinary effort is needed to overcome the current challenges for industrial flow biocatalysis.

Keywords Flow chemistry · Biocatalysis · Biotransformations · Chemoenzymatic reactions · Enzyme immobilization · Process intensification

Introduction

Nowadays, enzymes are broadly used as highly selective and greener biocatalysts with respect to other traditional chemical catalysts that can be toxic and/or result into more waste production [1, 2]. However, the full implementation of biocatalytic reactions on large scale is still a challenging

✉ Ana I. Benítez-Mateos
ana.benitez@unibe.ch

✉ Francesca Paradisi
francesca.paradisi@unibe.ch

[1] Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland

task [3]. Enzymes may suffer from (i) low stability under harsh conditions such as presence of organic co-solvents, extreme temperatures (i.e. mesophilic enzymes operate at 20–45ºC), or extreme pH (i.e. highest activity of most enzymes remains between pH 6 and 8), (ii) substrate/product inhibition, (iii) dependency on costly cofactors (i.e. price for NADPH can be > 1,000 €/g), [4] and (iv) low productivity rate, among others. Several technologies have emerged with the aim to alleviate those drawbacks and bring biocatalysis closer to industrial set-ups [3]. A clear step to boost the potential of biocatalysis is the incorporation of enzymatic reactions into continuous flow reactors, which is generally known as *Flow Biocatalysis* [5]. The transition from batch to flow offers numerous advantages. For instance, flow set ups offer a higher surface-volume ratio; increased automation of the systems, reducing the risks associated with hazardous intermediates; better experimental reproducibility, lower production of waste; etc. Specifically, flow biocatalysis offers the great advantage to be able to continuously synthesize a product of interest with high control of the reaction parameters (i.e. substrate concentration, reaction rate) and a straightforward coupling of downstream processes (i.e. product purification, reuse of co-solvents). Such a set-up is highly desired to develop more cost-effective processes for chemical manufacturing, while contributing to greener synthetic routes.

The rise of flow biocatalysis is easily recognizable when looking at the steady increase of scientific articles, conference presentations, and lectures over the last years. In fact, scientific publications containing the keyword '*Flow Biocatalysis'* started to increase intensely in 2008 according to the database *Scopus* (Fig. 1). Just in the last five years almost 400 publications came out, including entire reviews focusing on this topic [5–13]. Flow biocatalytic approaches are also gaining attention in industry, especially in pharmaceutical synthesis [14–17]. Yet, while enzymatic reactions in continuous flow are far from being extensively exploited in industrial processes, the growing interest in biocatalytic transformations is clear, as it has been recently shown by the survey carried out by Gallou, Gröger, and Lipshutz to different chemical industries around the world [18]. Herein, we will discuss the current and future directions for flow biocatalysis and its role in the leverage of enzymatic transformations towards more efficient and sustainable chemistry.

Current landscape of enzymatic reactions in continuous flow

Enzyme immobilization

Enzyme immobilization as the linkage of enzyme molecules to a solid support is typically associated with the integration of biocatalytic reactions into flow systems [19]. Not only, enzyme immobilization can stabilize the protein structure under the required operational conditions, but also facilitates the reusability of the biocatalyst through easy separation of the heterogenous biocatalyst (enzyme-support entity) from the reaction medium that continuously flows through the reactor. This is one of the key concepts that enhances the sustainability and efficiency of biocatalytic reactions in flow. Notwithstanding, the impact of the costs and waste production on to the final productivity must be carefully evaluated when using immobilized enzymes. In order to evaluate the suitability of a heterogeneous biocatalyst for its implementation into a flow reactor, Bolivar and López-Gallego have summarized the most fundamental aspects that should be considered [20]. Surface-protein engineering, material engineering, and reactor design are the three pillars which must be optimized to achieve a balance between the activity and stability of the biocatalyst. Therefore, the

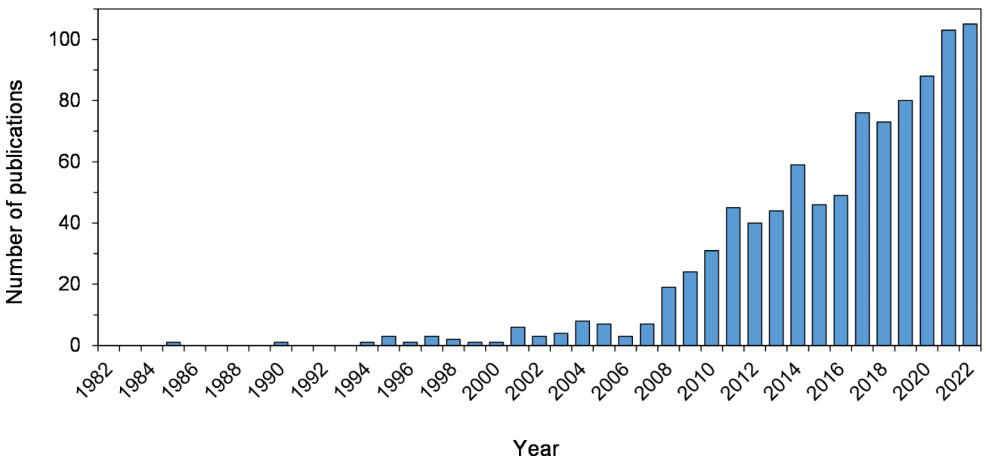


Fig. 1 Scientific publications including the keywords '*Flow biocatalysis'* reported yearly during the last four decades. The database *Scopus* has been used as data source

field of flow biocatalysis advances parallelly to the field of enzyme immobilization.

The immobilization of enzymes on solid supports has indeed come a long way during the last 50 years. The first examples of biocatalyst immobilization featured the adsorption of only one enzyme, typically a hydrolase, to a solid particle [21]. Nowadays, the co-immobilization of several enzymes working synergistically on an enzymatic cascade is possible, even tuning each enzyme immobilization through a different binding chemistry [22–24]. Furthermore, the reversible co-immobilization of cofactors needed for the biocatalytic reactions have been achieved, allowing the development of self-sufficient heterogenous biocatalysts that contribute to reduce the costs of the synthetic processes [25]. Regarding the support onto which the enzyme is attached, different techniques are available: anchoring to microparticles, entrapment into hydrogels, or carrier-free immobilization (such as crosslinking of enzymes) [19, 26]. Noteworthy, the immobilization of biocatalysts from whole cells or cell extracts can be also performed through all those techniques [27–29].

In the industrial landscape, the increasing interest on stabilization of enzymes via immobilization has encouraged the establishment of companies specifically focused on the preparation of immobilized biocatalysts [30].

Reactor design

Transferring enzymatic reactions from batch to flow mode requires the adaptation of the reactor as well. Several types of reactors which were already used in flow chemistry have been adopted for the integration of enzymatic reactions in continuous flow (Fig. 2). The most user-friendly configuration are likely continuous stirred tank reactors (CSTRs) as their set-up is very similar to a stirred batch reactor [31–33]. The operation of CSTRs in continuous mode involves an inlet to feed the substrate solution to the reactor vessel and the outlet line to recover the product within the reaction mixture. This is a good option for newcomers to the field of flow biocatalysis, but the use of immobilized enzymes in CSTRs must be carefully considered because the mechanical stirring can damage the heterogeneous biocatalysts by shredding the support. As an alternative, membrane reactors (MRs) offer the opportunity to work with free biocatalysts due to the use of a membrane that can separate the biocatalyst from the product molecules which are much smaller [34], although MR can be also operated with immobilized biocatalysts.

The most promising and widely used reactors for flow biocatalysts at present are packed-bed reactors (PBRs) [22, 35–38]. These reactors are column-shaped and contain the heterogenous biocatalyst occupying around half of the reactor volume. Due to the high surface/volume ratio, the reactants flowing through the PBR can be transformed to the corresponding products accomplishing high conversion rates. Unfortunately, PBRs also suffer limitations such as the inability to process solid reactants or slurries.

In order to screen and optimize the reaction conditions, microreactors are an attractive solution that permit to perform different reactions simultaneously while reducing the reagent consumption and waste generation [39, 40]. The microfluidic devices also allow the study of immobilized enzymes as those are usually attached to the reactor wall. The last trend on (micro)reactor design for flow biocatalysis are 3D-printed reactors which allow the tailor-made design of the reactor in consideration of the reaction challenges [41, 42].

Downstream processing

One of the biggest advantages of performing enzymatic reactions under flow conditions is the *in-line* coupling of downstream processing. After the enzymatic reaction is completed, the separation of the product from the unreacted substrates, by-products, or co-solvents used during the reaction requires special attention. In-line downstream processing contributes to reducing the waste production as well as simplifying the multi-step synthetic reactions. Recently, Kara and co-workers have reviewed the main downstream techniques used in flow biocatalysis [43]:

- Liquid-liquid extraction is a simple strategy in which the different physico-chemical properties of the product

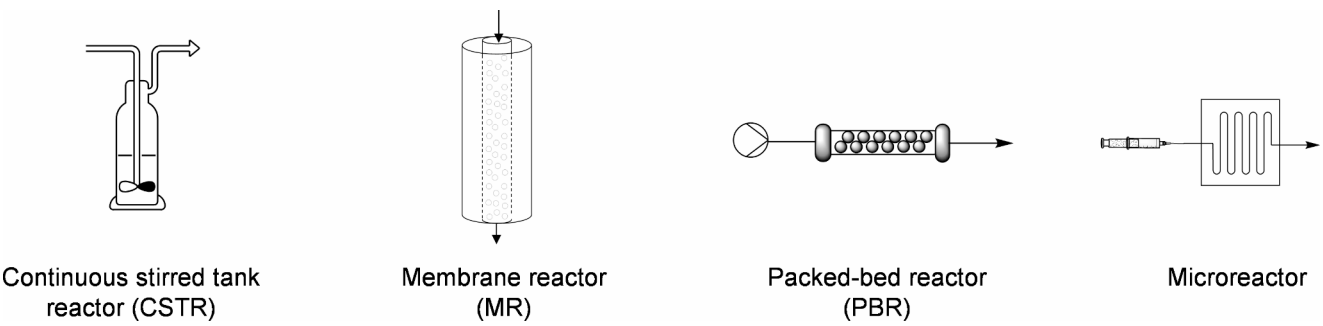


Fig. 2 Schematic representation of different types of flow reactors

are harnessed to enable its solubility into a target solvent, thus promoting its purification. This strategy also allows for the separation of unreacted starting materials or cofactors that are water-soluble and can be recycled into the flow system (Fig. 3A) [44–48].

- Scavenger resins are widely employed to purify a target molecule in a liquid-solid extraction. The process known as "catch and release" strategy allows for the separation of the product (or other target molecules) by attachment to the resin, and then the purified molecule is released into a new liquid phase (Fig. 3B) [35–37, 49]. Typically, sorbent materials which are commercially available are packed into a column that is connected downstream to the flow bioreactor. In addition, novel scavenger materials (i.e. lignin) from natural sources have been successfully applied [50].
- Precipitation and filtration are often employed for an easy product recovery (Fig. 3C) [38]. Different methods are available for the crystallization of the product, not only to allow its downstream purification but also to shift the reaction equilibrium when the crystallization occurs in situ [51].

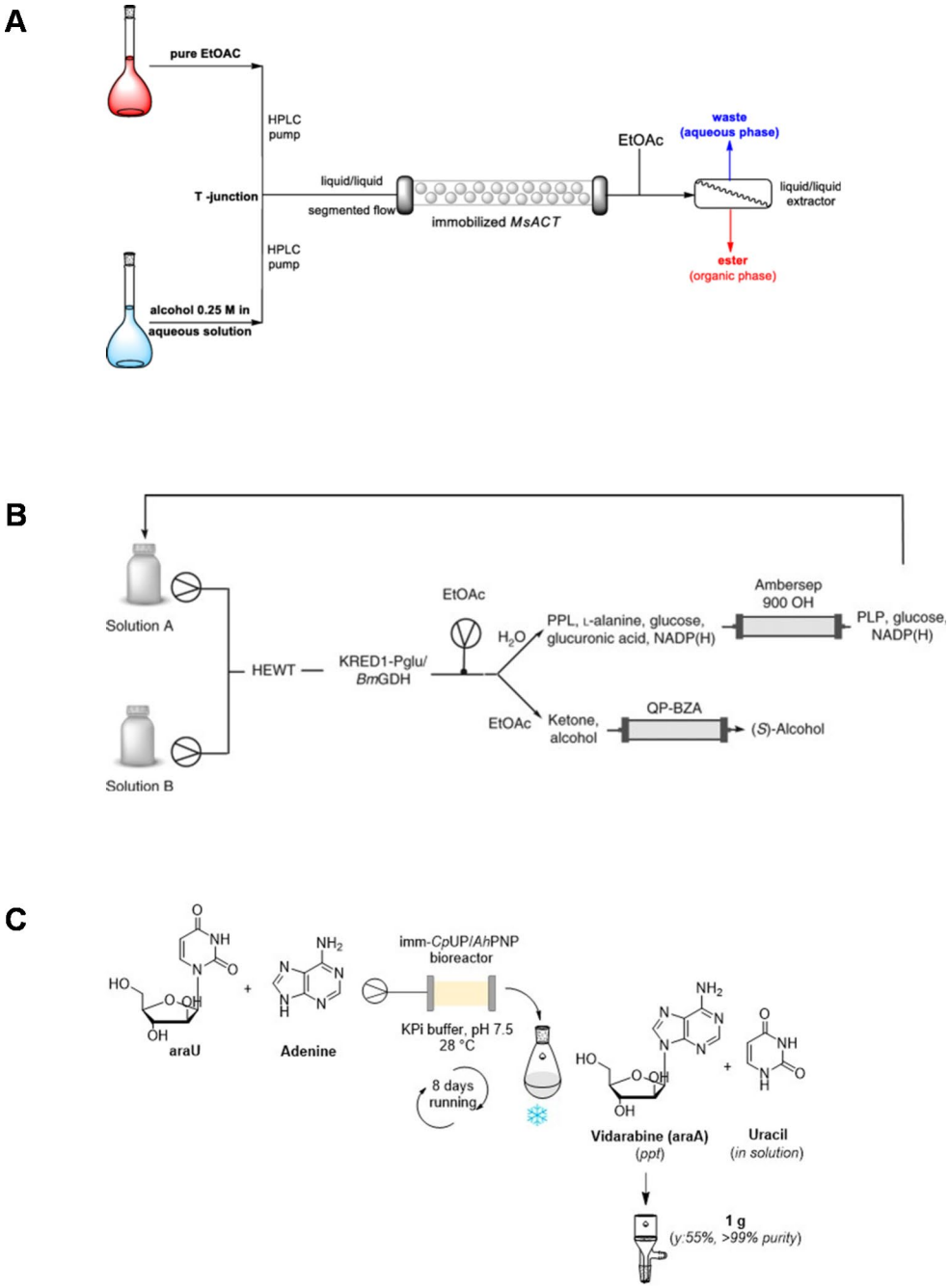


Fig. 3 Flow biocatalytic reactors coupled to in-line downstream processes. (A) liquid-liquid-extraction was used to separate the product (ester) in the organic phase [45]. (B) liquid-liquid extraction allowed a first separation of the co-substrates and side product from the intermediate and product. In a second step, a scavenger column was used to isolate the final product (*S*-alcohol). Moreover, another scavenger column was employed for the separation and recycling of the co-substrates by "catch-and-release" strategies. For example, glucuronic acid was retained onto Ambersep 900 OH which is a strongly basic resin, and could be released by the addition of an acidic solution [35]. (C) Product precipitation was achieved by cooling down the solution exiting the flow stream. Then, in a separate step, filtration and drying were carried out to isolate the final product (vidarabine) [38]. Figure 3A and 3B are reproduced from ref. [45] and ref. [35] with permissions of Springer Nature

Noteworthy, in-line analysis by UV or NMR among others can be also coupled downstream the continuous flow reactor [32].

New trends in flow biocatalysis

Multi-enzyme cascades

The current accessibility to numerous and diverse biocatalysts has certainly unveiled the potential of multi-enzyme reactions [52]. In this regard, flow biocatalysis facilitates the integration of different enzymatic steps into a cascade. On one hand, the compartmentalization of each biocatalytic step into single reactors allows to operate each enzyme at its optimal conditions (temperature, pH, co-solvents) [53]. On the other hand, the integration of different biocatalysts into the same flow reactor, either by co-immobilization of enzymes on the same support [25, 54, 55] or by mixing of different immobilized biocatalysts [35, 56, 57], has proven to favor the reaction rate compared to batch reactions. Certainly, what makes flow biocatalysis truly valuable for enzymatic cascades is the elimination of issues related to product inhibition, as the flow itself continuously removes the newly formed product. Despite all the above-mentioned benefits, the intensification of a multi-enzymatic process in flow is still a very complex task that requires optimization efforts for each individual enzyme/reaction [16, 58, 59]. For this reason, the integration of multi-enzyme cascades into industrial set-ups has been barely explored to date, although this field is advancing steadily.

Chemoenzymatic approaches

The combination of chemical and enzymatic steps in synthetic procedures is a very appealing strategy to introduce (flow) biocatalysis at large scale [58]. The integration of telescoped chemoenzymatic approaches in flow systems facilitates the separation of chemo- and biocatalysts, allowing them to perform at their best reaction conditions that might be difficult to achieve, if at all possible, when performed in batch mode (for instance, enzyme incompatibility with a chemical reaction that happens at 150ºC). In-line purification/separation methods are also a great advantage of flow systems to promote the compatibility of chemo- and biocatalytic reactions, as precise control over the product of one catalytic step can be executed before carrying out the next one. As result, reduced reactions times and more reproducible yields can be obtained. Hence, chemo-enzymatic flow reactions are a very active research area in both academia and industry as it can be appreciated in the recent literature [48, 60–64].

Photobiocatalysis in flow

One of the most recent additions to the field of flow biocatalysis are photobiocatalytic reactions [65]. Light can be harnessed either (i) to excite the substrate before the reaction proceeds, or (ii) to bring the biocatalyst to an excited state in which it is active [66, 67]. In photobiocatalytic reactions, the use of whole-cell biocatalyst is more common than free enzymes due to the instability of the latter. Indeed, this is an important parameter when integrating photobiocatalysis in flow reactors. New types of reactors have been designed to adapt photobiocatalytic reactions to flow set-ups, for instance, to avoid the decrease on enzyme stability due to exposure to high temperatures while ensuring the right light penetration into the photobioreactor [68]. Overall, photobiocatalysis has brought new-to-nature reactivities as well as an alternative manner to improve the sustainability of biotransformations (i.e. light-dependent cofactor regeneration). Now, the biggest challenge to overcome is the obtainment of industrially-relevant processes since both the techniques have limitations in terms of instruments/reactors.

Bioinformatics and machine learning

In recent years, bioinformatic tools have played a key role in the development of enzymatic processes, from the optimization of the catalytic activity of the enzyme to the reaction process [69–72]. Now, artificial intelligence and machine learning strategies are entering the field of continuous flow biocatalysis aiming at further automation of continuous processes [73–75]. Nevertheless, not all reactions can be operated in continuous flow and databases are still limited. But the potential of computational tools on the development of more efficient biocatalysts, reaction optimization, and reaction screening can be revolutionary in the near future by reducing the experimental time required.

Conclusions

In this perspective, we have highlighted the main research streams within the field of flow biocatalysis, focusing on the biocatalyst, the reactor, and the downstream processes. The advantages of this key enabling technology have been discussed and summarized in Table 1. Yet, Flow Biocatalysis is a field in development that present certain challenges, but it is rapidly growing to meet the request of process intensification at industrial scale.

Table 1 Summary of the benefits that flow biocatalysis technology has brought to enzymatic reactions, and the current challenges that still must be overcome to completely leverage its potential

Benefits of Flow Biocatalysis	Current challenges
Fine control over reaction conditions	Poor knowledge transfer from academia to industry
In-line product separation and analysis	Lack of expertise on enzyme/cell immobilization
Automation	Need for cofactor-regeneration
Enhanced efficiency	Necessity for regulatory guidance in industrial processes
Reduced waste generation	Low productivity for some applications

It is worth mentioning that the integration of enzymatic reactions into continuous flow processes is not a one-step procedure but rather a multidisciplinary effort. Therefore, enzymologists, material scientists, and process engineers work in synchrony to develop the most optimal flow biocatalytic processes. Nevertheless, not all the enzymatic reactions can be intensified in continuous flow, and batch mode may offer more advantages in certain scenarios. New technologies such as photocatalysis or computational studies are entering this field and providing valuable tools to tackle the current limitations as well as expanding the opportunities to apply flow biocatalysis. We envision an even more increasing number of works integrating this technology in the next years. Specially, (chemo)enzymatic reactions in continuous flow are expected to be applied in the chemical and pharmaceutical industries.

Acknowledgements The authors acknowledge the SNSF for financial support (grant 200021_192274, F.P.).

Funding Open access funding provided by University of Bern

Declarations

Competing interests the authors declare the absence of financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

References

1. Sheldon RA, Brady D, Bode ML (2020) The Hitchhiker's guide to biocatalysis: recent advances in the use of enzymes in organic synthesis. Chem Sci 11:2587–2605. https://doi.org/10.1039/c9sc05746c
2. Winkler CK, Schrittwieser JH, Kroutil W (2021) Power of biocatalysis for organic synthesis. ACS Cent Sci 7:55–71. https://doi.org/10.1021/acscentsci.0c01496
3. Woodley JM (2019) Accelerating the implementation of biocatalysis in industry. Appl Microbiol Biotechnol 103:4733–4739. https://doi.org/10.1007/s00253-019-09796-x
4. https://www.sigmaaldrich.com/DE/de/product/mm/481973 (visited on 26.09.2023)
5. Benítez-Mateos AI, Contente ML, Roura Padrosa D, Paradisi F (2021) Flow biocatalysis 101: design, development and applications. React Chem Eng 6:599–611. https://doi.org/10.1039/d0re00483a
6. De Santis P, Meyer LE, Kara S (2020) The rise of continuous flow biocatalysis-fundamentals, very recent developments and future perspectives. React Chem Eng 5:2155–2184. https://doi.org/10.1039/d0re00335b
7. Tamborini L, Fernandes P, Paradisi F, Molinari F (2018) Flow bioreactors as complementary tools for biocatalytic process intensification. Trends Biotechnol 36:73–88. https://doi.org/10.1016/j.tibtech.2017.09.005
8. Britton J, Majumdar S, Weiss GA (2018) Continuous flow biocatalysis. Chem Soc Rev 47:5891–5918. https://doi.org/10.1039/c7cs00906b
9. Crotti M, Robescu MS, Bolivar JM et al (2023) What's new in flow biocatalysis ? A snapshot of 2020–2022. Front Catal 1–10. https://doi.org/10.3389/fctls.2023.1154452
10. Thompson MP, Peñafiel I, Cosgrove SC, Turner NJ (2019) Biocatalysis using immobilized enzymes in continuous Flow for the synthesis of fine chemicals. Org Process Res Dev 23:9–18
11. Adebar N, Nastke A, Gröger H (2021) Concepts for flow chemistry with whole-cell biocatalysts. React Chem Eng 6:977–988. https://doi.org/10.1039/d0re00331j
12. Cosgrove SC, Mattey AP (2022) Reaching New Biocatalytic Reactivity using continuous Flow reactors. Chem - A Eur J 28. https://doi.org/10.1002/chem.202103607
13. Žnidaršič-Plazl P (2019) The promises and the Challenges of Biotransformations in Microflow. Biotechnol J 14. https://doi.org/10.1002/biot.201800580
14. Roura Padrosa D, Lehmann H, Snajdrova R, Paradisi F (2023) Sustainable synthesis of L-phenylalanine derivatives in continuous flow by immobilized phenylalanine ammonia lyase. Front Catal 3:1–9. https://doi.org/10.3389/fctls.2023.1147205
15. Heckmann CM, Dominguez B, Paradisi F (2021) Enantio-Complementary Continuous-Flow synthesis of 2-Aminobutane using covalently immobilized Transaminases. ACS Sustain Chem Eng 9:4122–4129. https://doi.org/10.1021/acssuschemeng.0c09075
16. Leemans Martin L, Peschke T, Venturoni F, Mostarda S (2020) Pharmaceutical Industry Perspectives on Flow Chemo- and biocatalysis. Curr Opin Green Sustain Chem 25:100350. https://doi.org/10.1016/j.cogsc.2020.04.011
17. Benítez-Mateos AI, San Sebastian E, Ríos-Lombardía N et al (2017) Asymmetric reduction of Prochiral Ketones by using self-sufficient heterogeneous biocatalysts based on NADPH-Dependent ketoreductases. Chem - A Eur J 23. https://doi.org/10.1002/chem.201703475
18. Gallou F, Gröger H, Lipshutz BH (2023) Status check: biocatalysis; it's use with and without chemocatalysis. How does the fine chemicals industry view this area? Green Chem. https://doi.org/10.1039/d3gc01931d

19. Romero-Fernández M, Paradisi F (2020) Protein immobilization technology for flow biocatalysis. Curr Opin Chem Biol 55:1–8. https://doi.org/10.1016/j.cbpa.2019.11.008
20. Bolivar JM, López-Gallego F (2020) Characterization and evaluation of immobilized enzymes for applications in flow reactors. Curr Opin Green Sustain Chem 25:100349
21. Ballesteros A, Guisan JM (1980) Preparation of immobilized sepharose-micrococcal nuclease derivatives: activity and stability. 4:245–252
22. Romero-Fernandez M, Paradisi F (2021) Biocatalytic access to betazole using a one-pot multienzymatic system in continuous flow. Green Chem 23:4594–4603. https://doi.org/10.1039/d1gc01095f
23. Velasco-Lozano S, Santiago-Arcos J, Grazia Rubanu M, López-Gallego F (2022) Cell-free biosynthesis of ω-Hydroxy acids boosted by a synergistic combination of Alcohol Dehydrogenases. Chemsuschem 15. https://doi.org/10.1002/cssc.202200397
24. You C, Myung S, Zhang YHP (2012) Facilitated substrate channeling in a self-assembled trifunctional enzyme complex. Angew Chem Int Ed 51:8787–8790. https://doi.org/10.1002/anie.201202441
25. Velasco-Lozano S, Benítez-Mateos AI, López-Gallego F (2017) Co-immobilized phosphorylated cofactors and enzymes as self-sufficient heterogeneous biocatalysts for chemical processes. Angew Chem Int Ed 56:771–775. https://doi.org/10.1002/anie.201609758
26. Ölçücü G, Krauss U, Jaeger KE, Pietruszka J (2023) Carrier-free enzyme Immobilizates for Flow Chemistry. Chem-Ing-Tech 95:531–542. https://doi.org/10.1002/cite.202200167
27. Paradisi F Poppe L 10 Continuous-flow biocatalysis with enzymes and cells
28. Guisan JM, Bolivar JM, López-Gallego F, Rocha-Martín J (2020) Immobilization of enzymes and cells. Springer US
29. Pinto A, Contente ML, Tamborini L (2020) Advances on whole-cell biocatalysis in flow. Curr Opin Green Sustain Chem 25:100343. https://doi.org/10.1016/j.cogsc.2020.04.004
30. www.inseit. ch; www.zymtronix.com; www.cleatechnologies.com; www.novozymes.com; www.inofea.com
31. Hülsewede D, Temmel E, Kumm P, Von Langermann J (2020) Concept study for an integrated reactor-crystallizer process for the continuous biocatalytic synthesis of (S)-1-(3-methoxyphenyl)ethylamine. Crystals 10:1–13. https://doi.org/10.3390/cryst10050345
32. Claaßen C, Mack K, Rother D (2020) Benchtop NMR for online reaction monitoring of the Biocatalytic synthesis of aromatic amino alcohols. ChemCatChem 12:1190–1199. https://doi.org/10.1002/cctc.201901910
33. Lindeque RM, Woodley JM (2022) Modeling and experimental validation of continuous Biocatalytic Oxidation in two continuous stirred Tank Reactors in Series. Org Process Res Dev 26:2030–2037. https://doi.org/10.1021/acs.oprd.1c00429
34. Pottratz I, Müller I, Hamel C (2022) Potential and scale-up of pore-through-flow membrane reactors for the production of prebiotic galacto- oligosaccharides with immobilized β-galactosidase. Catalysts 12. https://doi.org/10.3390/catal12010007
35. Contente ML, Paradisi F (2018) Self-sustaining closed-loop multienzyme-mediated conversion of amines into alcohols in continuous reactions. Nat Catal 1:452–459. https://doi.org/10.1038/s41929-018-0082-9
36. Roura Padrosa D, Benítez-Mateos AI, Calvey L, Paradisi F (2020) Cell-free biocatalytic syntheses of l -pipecolic acid: a dual strategy approach and process intensification in flow. Green Chem 22:5310–5316. https://doi.org/10.1039/d0gc01817a
37. Benítez-Mateos AI, Paradisi F (2022) Sustainable Flow-Synthesis of (Bulky) Nucleoside drugs by a Novel and highly stable Nucleoside phosphorylase immobilized on Reusable supports. Chemsuschem 15:1–9. https://doi.org/10.1002/cssc.202102030
38. Tamborini L, Previtali C, Annunziata F et al (2020) An enzymatic flow-based preparative route to vidarabine. Molecules 25:1223. https://doi.org/10.3390/molecules25051223
39. Žnidaršič-Plazl P (2021) Biocatalytic process intensification via efficient biocatalyst immobilization, miniaturization, and process integration. Curr Opin Green Sustain Chem 32. https://doi.org/10.1016/j.cogsc.2021.100546
40. Zhu Y, Chen Q, Shao L et al (2020) Microfluidic immobilized enzyme reactors for continuous biocatalysis. React Chem Eng 5:9–32. https://doi.org/10.1039/c9re00217k
41. Maier M, Radtke CP, Hubbuch J et al (2018) On-Demand production of Flow-Reactor Cartridges by 3D Printing of Thermostable enzymes. Angew Chem Int Ed 57:5539–5543. https://doi.org/10.1002/anie.201711072
42. Peng M, Mittmann E, Wenger L et al (2019) 3D-Printed Phenacrylate Decarboxylase Flow reactors for the Chemoenzymatic synthesis of 4-Hydroxystilbene. Chem - A Eur J 25:15998–16001. https://doi.org/10.1002/chem.201904206
43. Meyer LE, Hobisch M, Kara S (2022) Process intensification in continuous flow biocatalysis by up and downstream processing strategies. Curr Opin Biotechnol 78:102835. https://doi.org/10.1016/j.copbio.2022.102835
44. Baumer B, Classen T, Pohl M, Pietruszka J (2020) Efficient Nicotinamide Adenine Dinucleotide phosphate [NADP(H)] recycling in Closed-Loop continuous Flow Biocatalysis. Adv Synth Catal 362:2894–2901. https://doi.org/10.1002/adsc.202000058
45. Contente ML, Tamborini L, Molinari F, Paradisi F (2020) Aromas flow: eco-friendly, continuous, and scalable preparation of flavour esters. J Flow Chem 10:235–240. https://doi.org/10.1007/s41981-019-00063-8
46. Colacicco A, Catinella G, Pinna C et al (2023) Flow bioprocessing of citrus glycosides for high-value aglycone preparation. Catal Sci Technol 4348–4352. https://doi.org/10.1039/d3cy00603d
47. Contente ML, Farris S, Tamborini L et al (2019) Flow-based enzymatic synthesis of melatonin and other high value tryptamine derivatives: a five-minute intensified process. Green Chem. https://doi.org/10.1039/C9GC01374A
48. Cosgrove SC, Miller GJ, Bornadel A, Dominguez B (2023) Realizing the continuous Chemoenzymatic synthesis of Anilines using an immobilized nitroreductase. ACS Sustain Chem Eng 11:8556–8561. https://doi.org/10.1021/acssuschemeng.3c01204
49. Meyer LE, Plasch K, Kragl U, Von Langermann J (2018) Adsorbent-based Downstream-Processing of the decarboxylase-based synthesis of 2,6-Dihydroxy-4-methylbenzoic acid. Org Process Res Dev 22:963–970. https://doi.org/10.1021/acs.oprd.8b00104
50. Benítez-Mateos AI, Bertella S, Behaghel de Bueren J et al (2021) Dual valorization of Lignin as a versatile and renewable matrix for enzyme immobilization and (Flow) Bioprocess Engineering. Chemsuschem 14:3198–3207. https://doi.org/10.1002/cssc.202100926
51. Hülsewede D, Meyer LE, von Langermann J (2019) Application of in situ product crystallization and related techniques in biocatalytic processes. Chem - A Eur J 25:4871–4884. https://doi.org/10.1002/chem.201804970
52. Benítez-Mateos AI, Roura Padrosa D, Paradisi F (2022) Multistep enzyme cascades as a route towards green and sustainable pharmaceutical syntheses. Nat Chem 14:489–499. https://doi.org/10.1038/s41557-022-00931-2
53. Hartley CJ, Williams CC, Scoble JA et al (2019) Engineered enzymes that retain and regenerate their cofactors enable continuous-flow biocatalysis. Nat Catal 2:1006–1015. https://doi.org/10.1038/s41929-019-0353-0
54. Romero-Fernandez M, Heckmann CM, Paradisi F (2022) Biocatalytic production of a Nylon 6 precursor from caprolactone

in continuous flow. Chemsuschem 202200811:1–10. https://doi.org/10.1002/cssc.202200811
55. Liu H, Tegl G, Nidetzky B (2021) Glycosyltransferase co-immobilization for natural product glycosylation: Cascade Biosynthesis of the C -Glucoside nothofagin with efficient reuse of enzymes. Curr Opin Green Sustain Chem 25:2157–2169. https://doi.org/10.1002/adsc.202001549
56. Mangas-sanchez J, Sharma M, Cosgrove SC et al (2020) Asymmetric synthesis of primary amines catalyzed by thermotolerant fungal reductive aminases †. Chem Sci 11:5052–5057. https://doi.org/10.1039/d0sc02253e
57. Shortall K, Arshi S, Bendl S et al (2023) Coupled immobilized bi-enzymatic flow reactor employing cofactor regeneration of NAD + using a thermophilic aldehyde dehydrogenase and lactate. Green Chem 25:4553–4564. https://doi.org/10.1039/d3gc01536j
58. Hu Y, Chen J, Wang Y et al (2022) Biocatalysts used for multi-step reactions in continuous flow. Chem Eng J 437:135400. https://doi.org/10.1016/j.cej.2022.135400
59. Clayton AD, Labes R, Blacker AJ (2020) Combination of chemocatalysis and biocatalysis in flow. Curr Opin Green Sustain Chem 26:100378. https://doi.org/10.1016/j.cogsc.2020.100378
60. Gianolio S, Roura Padrosa D, Paradisi F (2022) Combined chemoenzymatic strategy for sustainable continuous synthesis of the natural product hordenine. Green Chem 24:8434–8440. https://doi.org/10.1039/d2gc02767d
61. Ho CH, Yi J, Wang X (2019) Biocatalytic continuous Manufacturing of Diabetes Drug: plantwide process modeling, optimization, and Environmental and Economic Analysis. ACS Sustain Chem Eng 7:1038–1051. https://doi.org/10.1021/acssuschemeng.8b04673
62. Schober L, Tonin F, Hanefeld U, Gröger H (2022) Combination of Asymmetric Organo- and Biocatalysis in Flow processes and comparison with their analogous batch Syntheses. Eur J Biochem e202101035. https://doi.org/10.1002/ejoc.202101035
63. Farkas E, Oláh M, Földi A et al (2018) Chemoenzymatic dynamic kinetic resolution of Amines in fully Continuous-Flow Mode. Org Lett 20:8052–8056. https://doi.org/10.1021/acs.orglett.8b03676
64. Annunziata F, Contente ML, Betti D et al (2020) Efficient chemoenzymatic Flow Synthesis of High Value Amides and Esters. Catalysts 10:939
65. Chanquia SN, Valotta A, Gruber-woel H, Kara S (2022) Photobiocatalysis in Continuous Flow. 1:1–15. https://doi.org/10.3389/fctls.2021.816538
66. Schmermund L, Jurkas V, Feyza FO et al (2019) Photo-Biocatalysis: Biotransformations in the Presence of Light. https://doi.org/10.1021/acscatal.9b00656
67. Emmanuel MA, Bender SG, Bilodeau C et al (2023) Photobiocatalytic Strategies for Organic Synthesis. https://doi.org/10.1021/acs.chemrev.2c00767
68. Huck WTS, Winkler CK, Kroutil W et al (2022) Strategies for transferring photobiocatalysis to continuous Flow exemplified by photodecarboxylation of fatty acids ˇ. ACS Catal. https://doi.org/10.1021/acscatal.2c04444
69. Li G, Dong Y, Reetz MT (2019) Can Machine Learning revolutionize Directed evolution of selective enzymes ? https://doi.org/10.1002/adsc.201900149
70. Roura Padrosa D, Marchini V, Paradisi F (2021) CapiPy: python-based GUI-application to assist in protein immobilization. Bioinformatics 37:2761–2762. https://doi.org/10.1093/bioinformatics/btab030
71. Casadevall G, Duran C, Osuna S (2023) AlphaFold2 and deep learning for elucidating enzyme conformational flexibility and its Application for Design. https://doi.org/10.1021/jacsau.3c00188
72. Sampaio PS, Fernandes P (2023) Machine Learning: A Suitable Method for Biocatalysis. 1–26
73. Plehiers PP, Coley CW, Gao H et al (2020) Artificial Intelligence for computer-aided synthesis in Flow: analysis and selection of reaction components. Front Chem Eng 2. https://doi.org/10.3389/fceng.2020.00005
74. Lashkaripour A, Rodriguez C, Mehdipour N et al (2021) Machine learning enables design automation of microfluidic flow-focusing droplet generation. Nat Commun 12. https://doi.org/10.1038/s41467-020-20284-z
75. Vasina M, Vanacek P, Hon J et al (2022) Advanced database mining of efficient haloalkane dehalogenases by sequence and structure bioinformatics and microfluidics. Chem Catal 2:2704–2725. https://doi.org/10.1016/j.checat.2022.09.011