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Opinion

Flow fermentation: microsystems for whole-cell bioproduction processes

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Industrial biotechnology utilizes whole cells for the production of value-added goods in large-scale bioreactors. The miniaturization of bioreactors has greatly contributed to the understanding and optimization of bioprocesses. However, microsystems for the production of value-added goods have thus far only been established in chemistry and biocatalysis/biotransformation but are rarely applied for whole-cell bioprocesses. Here, we discuss the fundamental and translational aspects of how microsystems could be used as production units for future whole-cell bioproduction processes. The characteristics and resulting advantages of microsystems are introduced and current production approaches are highlighted. Finally, we provide perspectives on establishing future whole-cell bioproduction processes at the microscale, here introduced as flow fermentation. Flow fermentation potentially enables entirely new bioprocesses and application fields.

Highlights

Microsystems offer many possibilities for the cultivation of cells and production of value-added goods due to their characteristics and resulting advantages (e.g., small size, fast mass transfer, precisely controllable environmental conditions).

Proof-of-concept microsystems have been developed for whole-cell bioproduction processes.

There are numerous whole-cell bioproduction processes that could benefit from the use of microsystems, introduced here as the emerging field of 'flow fermentation'. Application fields include small product quantities, decentralized bioprocesses, toxicity and inhibition, surface-to-volume ratio, mass transfer, and spatiotemporal arrangement.

The field of flow fermentation faces various technical challenges that need to be solved, including cell retention and nutrient supply, material and manufacturing methods, downstream processing, and scalability.

Industrial bioprocesses

The utilization of **whole cells** (see Glossary), whether prokaryotic or eukaryotic, for the production of **value-added goods** has an extremely wide range of applications. What began with the production of beer and wine has evolved over several thousand years into a multibillion-dollar industry [1,2]. Applications range from the production of biopharmaceuticals to food and food supplements, fine and bulk chemicals, biogas, and bioethanol [3,4]. Actual production typically occurs in industrial-scale bioreactors ranging from 5000 to >500 000 liters [5,6]. The development of these industrial processes is a time- and cost-intensive workflow [7]. Starting with a product idea, a host organism and substrate are selected and then optimized in small-scale cultivation devices (e.g., microtiter plates, shake flasks). Promising candidates are then transferred to laboratory-scale bioreactors for process optimization and then scaled up to pilot and industrial scales, each with unique scale-related challenges [5]. Despite advanced process development strategies (e.g., strain engineering, high-throughput screening, **scale-down** studies), the probability of successful **scale-up** remains quite low [8]. As a result, society misses out on numerous products and industry misses out on potential revenues. **Microsystems** enable alternative pathways and possibilities for the development and realization of novel bioprocesses by means of the unique laws governing the microscale. In this opinion, we first introduce microsystems as production platforms for **whole-cell bioprocesses**, their advantages, and their current applications. Finally, we discuss whole-cell bioproduction processes that will significantly benefit from realization in microsystems and highlight the most important challenges that need to be overcome.

Microsystems for the production of value-added goods: state of the art

Compared with traditionally used reactors and scales, microsystems offer completely novel characteristics and advantages (Box 1). Here, microsystems are seen as reaction units that contain microstructures or have volumes in or below the milliliter dimension. These systems are already being used for chemical production ('**flow chemistry**') [9,10] and **biocatalysis** ('**flow biocatalysis**')

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Box 1. Characteristics and advantages of microsystems for (bio)chemical reactions

Fundamentally, microsystems are characterized by their small size, with dimensions in the nano- to millimeter range corresponding to small volumes from femto- to milliliters [73,74]. Working on a small scale allows the systems to be made portable as well as parallelized due to the small space requirement (Figure I) [75]. Working with small volumes allows cost savings through relatively low reagent consumption and, at the same time, very precise liquid handling (Figure I). In addition, small volumes are beneficial from an ecological and hazard potential perspective because they consume lower quantities of chemicals or hazardous materials and minimize their release in the event of accidents. This effectively increases operational safety by reducing direct impacts [56]. Another key advantage is the high surface-to-volume ratio in these systems, which is the basis for very fast heat and mass transfer (Figure I) [75]. This makes it possible to perform reactions under highly defined conditions. Interfacial effects and interactions between phase boundaries and cell/surface interactions play a dominant role. These properties not only provide the basis for optimal control of process variables but also lay the foundation for completely new reaction principles and their technical application. Moreover, very low Reynolds numbers result in **laminar flow** conditions rather than turbulence, which is the dominant flow regime in larger bioreactor systems [16]. As a result, the environmental conditions can be kept constant and can be controlled precisely with high **spatio-temporal** resolution [75,76].

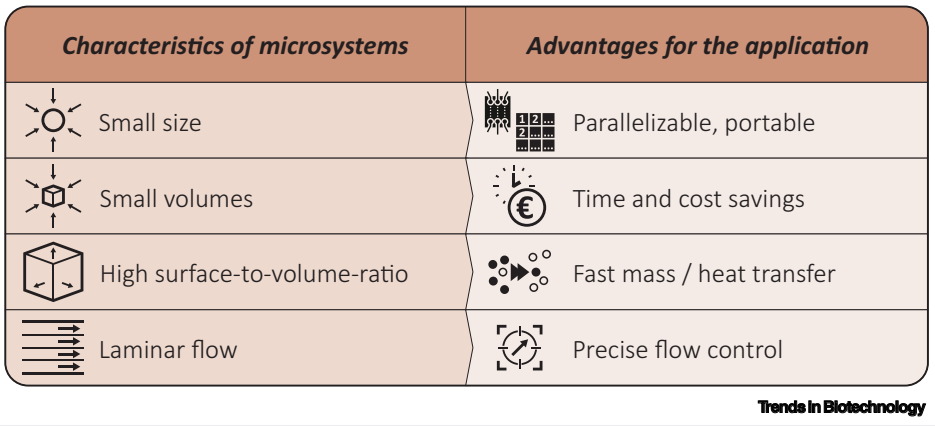

Figure I. Characteristics of microsystems and their resulting advantages for potential applications in bioproduction.

[11,12] (Box 2). Compared with conventional bioprocesses (liter-scale or greater) operated in batch, fed-batch, continuous, or perfusion mode, microsystems enable fast mass transfer through specific surface areas between 5000 and 50 000 m^2 m^{-3} [13], low mean residence times of the fluid [14,15], and precisely controllable, homogeneous environmental conditions because of small Reynolds numbers [16].

Microsystems for whole-cell bioprocesses

Compared with flow biocatalysis, microsystems in whole-cell bioprocesses have so far mostly included the fields of analytics and high-throughput screening of potential biocatalysts and were used to understand and optimize large-scale bioprocesses (Box 2). By contrast, microsystem-based whole-cell bioproduction processes are still in their infancy. Several proof-of-concept studies with cells as products as well as producers have been established (Figure 1A).

Microsystems for whole-cell bioprocesses: cells as products

Microsystems have been used for applications in red biotechnology, where the production of various types of cells for **cellular therapies** has been shown, including the production of chimeric antigen receptor T cells [17]. Sin and coworkers utilized a commercially available 2-ml microbioreactor (Mobius Breez, Millipore Sigma) to set up a whole production process within a microfluidic chip and were able to yield a sufficient amount of cells needed for clinical doses (>200 million cells) [17]. Fitzgerald and coworkers produced mesenchymal stromal cells in compliance with current good

Glossary

Biocatalysis: use of biological systems or their components, primarily enzymes, to catalyze chemical reactions.
Bioconversion: synthesis of a product from basic nutrients by whole cells. The desired product is in this case produced via the cellular metabolism encompassing a multitude of enzymes.
Biotransformation: conversion of a preformed substrate into a product involving only one or a few enzymatic steps.
Cellular therapies: medical transfer of autologous or allogeneic cells into a patient for therapeutic purposes.
Flow biocatalysis: a combination of flow chemistry and biocatalysis; use of biocatalysts (mostly enzymes) to perform chemical reactions in a continuous flow inside microreactors.
Flow chemistry: chemical reactions performed in a continuous flow inside microreactors.
Flow fermentation: bioproduction processes performed in microsystems with whole, viable eukaryotic or prokaryotic cells used as biocatalysts. In contrast to flow biocatalysis, the growth of the cells is addressed within the microsystem and as a crucial first part of the process. In the second part, value-added goods are produced by either biotransformation or bioconversion.
Laminar flow: fluid movement in smooth layers without turbulence.
Microsystems: reaction units that contain microstructures or have volumes in or below the milliliter dimension.
Modularization: concept of dividing systems into separate, interchangeable functional units or modules.
Numbering-up: increasing the number of modularly applicable systems. The reactor dimensions do not change.
Scale-down: approach that mimics representative large-scale bioreactor conditions in laboratory-scale bioreactors.
Scale-up: increasing the scale of biomanufacturing processes.
Spatio-temporal: having both spatial and temporal properties.
Value-added goods: metabolites, chemicals, and cells as products of bioprocesses.
Whole cell: eukaryotic or prokaryotic cell with intact cell membrane containing

Box 2. History of microsystems for (bio)production

The applications of microsystems can historically be split into two fields: microsystems for the production of value-added goods and microsystems for the analysis of catalysts, where the latter is highlighted only from the whole-cell perspective.

The first patents for microsystems that were used for production are associated with flow chemistry and were filed between 1980 and 1990 (Figure II) [77]. Since then, production with flow chemistry has established itself in the chemical and pharmaceutical industries [9,78–82]. One property of microsystems that, among others, is particularly exploited in this field is the rapid mass and heat transfer resulting from their small size. This leads to increased safety and the possibility of performing reactions that are considered too dangerous in larger systems due to their hazardous potential [83,84]. Examples include the industrial production of highly explosive nitroglycerin for medical purposes or the application of highly exothermic and very rapid reactions such as the Grignard reaction [85–87]. For deeper insight, the field has already been reviewed elsewhere [9,10,88,89]. Originating from flow chemistry, in the early 2000s/2010s, the field of flow biocatalysis started to develop and has gained increasing interest (Figure II). Flow biocatalysis utilizes microsystems, among other advantages, due to improved mass transfer, the possibility of cascade reactions, intensified processes, and the ability to perform multiphase reactions [90]. Examples include the production of melatonin [91], L-phenylalanine derivatives [92], vidarabine [93], and (*S*)-piperazine-2-carboxylic acid [94]. Further examples have been extensively reviewed by others [11–13,95]. In recent years, the first proof-of-concept studies for whole-cell bioproduction have been developed (see main text), marking the beginning of the flow fermentation field (Figure II).

The use of microsystems for the analysis of whole cells began to arise in the early 2000s with microfluidic high-throughput screening of cell libraries (Figure II) [96]. Microtiter plate-based systems and microfluidic bioreactors were applied to screen for strains and bioprocess parameters [97,98]. Other examples include the screening of cells in microfluidic droplet systems [99]. Since the beginning of the 2010s, single-cell cultivation in combination with live-cell imaging has made it possible to trap and cultivate cells while analyzing selected environmental process parameters [14,100]. At the same time, single-cell omics has emerged, where various omics (e.g., transcriptomics, metabolomics) techniques have been developed and applied to investigate the behavior of small cell clusters and even single cells [101,102]. In recent years, both single-cell cultivation and single-cell omics have been increasingly applied to bioprocess-relevant questions [76]. However, the full potential of microsystems for the analysis of catalysts has not yet been fully exploited.

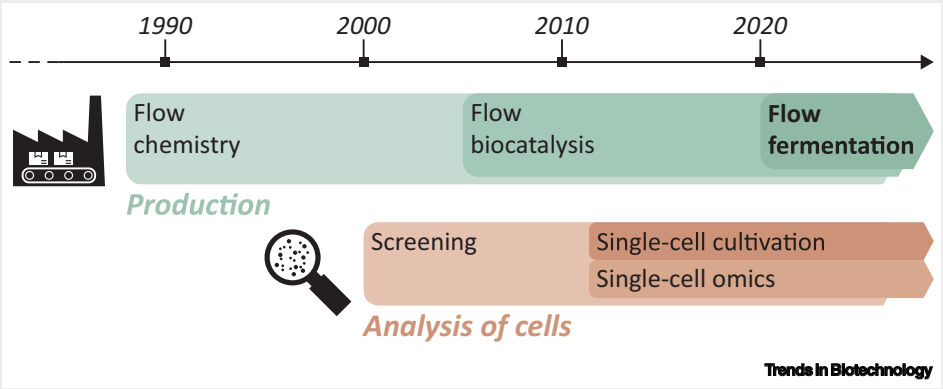

Figure II. History of microsystems for production purposes and their applications for the analysis of whole cells.

all organelles and cellular machinery that can be used as biocatalyst.

Whole-cell bioprocesses: processes that utilize viable eukaryotic or prokaryotic cells as biocatalysts for the production of value-added goods through bioconversion or biotransformation.

manufacturing practices [18]. A commercially available small-scale bioreactor (NANT 001 bioreactor, VivaBioCell) was used to perform the autologous production process. The production of the cells itself was successful, and the process was also shown to be economically advantageous by a cost-effectiveness analysis due to its highly automated mode of operation [18]. By developing an automated expansion protocol with a novel cytokine cocktail, Jones and colleagues produced T cell-depleted hematopoietic stem and progenitor cells (Figure 1B, Product type) [19]. By using a 124-ml hollow fiber bioreactor (Quantum System, Terumo Blood and Cell Technologies), the power of perfusion in the form of a constant supply of fresh medium and removal of waste was harnessed (Figure 1B, Setup). The developed protocol was shown to be effective, as the number of cells increased 100-fold over 8 days, as experimentally shown in triplicate (Figure 1B, Results) [19]. The production of human pluripotent stem cells was shown by Cohen and colleagues [20]. Here, a hybrid form of microsystem and large-scale was applied, as 3D

(A)

Product type	Setup	Operation mode	Biocatalyst	Product	Study
Cells as product	3D micro-compartments in alginate beads	Batch	Human pluripotent stem cells	Human pluripotent stem cells	Cohen *et al.* 2023
	Cell culture flask (NANT 001 bioreactor, VivaBioCell)	Batch	Mesenchymal stromal cells	Mesenchymal stromal cells	Fitzgerald *et al.* 2022
	Microreactor (Mobius Breez, MilliporeSigma)	Continuous	Chimeric antigen receptor T cells	Chimeric antigen receptor T cells	Sin *et al.* 2023
	Hollow-fiber bioreactor (Quantum system's dynamic perfusion bioreactor)	Continuous	T cell-depleted hematopoietic stem and progenitor cells	T cell-depleted hematopoietic stem and progenitor cells	Jones *et al.* 2022
Cells as producer	PDMS-glass based microreactor	Batch (repetitive)	CHO-ahIFN-α2b adherent cell line	Anti-hIFN-a2b recombinant scFv-Fc monoclonal antibody	Bourguignon *et al.* 2022
	Capillary biofilm microreactor	Continuous	*Synechocystis sp. PCC 6803*	Oxidation of cyclohexane to cyclohexanol	Hoschek *et al.* 2019
	PDMS-glass based microreactor	Continuous	*Escherichia coli*	4-vinylphenol	Lemke *et al.* 2024
	PDMS-glass based microreactor	Continuous	*Escherichia coli*	Reduction of 5-nitrononane-2,8-dione	Peschke *et al.* 2019
	Hydrogel microreactor	Continuous	*Saccharomyces cerevisiae*	L-malic acid	Menegatti *et al.* 2019
	Column bioreactor packed with alginate beads	Continuous	*Escherichia coli*	γ-amino butyric acid	Ham *et al.* 2023
	Column bioreactor packed with alginate beads	Continuous	*Rhodotorula rubra*	Reduction of β-ketonitriles	Annunziata *et al.* 2022
	3D-printed scaffold-perfusion bioreactor system	Continuous	Mesenchymal stem cells	Extracellular vesicles	Kronstadt *et al.* 2023
	Hydrogel microreactor	Continuous	*Escherichia coli*	L-erythrulose	Bajić *et al.* 2024

(B)

Product type	Setup	Results	Study
Cells as product Stem and progenitor cells	Hollow-fiber bioreactor 200 µm Intracapillary Extracapillary	Total Cells at Harvest Cell Seed Run 1 Run2 Run 3 Quantum CES Runs	Jones *et al.* 2022
Cells as producer Recombinant monoclonal antibodies	PDMS-glass based microreactor Cystern 100 mm	anti-hIFN-α2b (ug/mL) F-12/DMEM (50/50) FBS 10% F-12/DMEM (50/50) FBS 0.5% Time (days)	Bourguignon *et al.* 2022

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microcompartments were engineered in alginate capsules and used to scale-up the production of stem cells to the industrial scale. By doing so, the authors were able to achieve an amplification of 277 in 6.5 days [20].

Microsystems for whole-cell bioprocesses: cells as producers

Proof-of-concept studies on the production of chemicals and metabolites have been performed with a variety of cell types as producers. The production of recombinant antibodies in Chinese hamster ovary (CHO) cells was shown by Bourguignon and coworkers (Figure 1B, Product type) [21]. The cells were cultivated in a polydimethylsiloxane (PDMS)-glass-based microfluidic bioreactor with cistern-like structures (Figure 1B, Setup). Here, after the growth phase (days 0–7), the medium was switched, initiating the production phase (day 7). Antibody production was sustained until day 18 at concentrations ranging from 32 to 57 µg/ml (Figure 1B, Results) [21]. Kronstadt and coworkers produced extracellular vesicles with mesenchymal stem cells [22]. For that, a 3D-printed scaffold-perfusion bioreactor was created. Compared with conventional flask cell culture, an up to 80-fold increase in yield was achieved [22]. In contrast to the two mentioned examples from red biotechnology, there are several examples that address the application of white biotechnology. Bühler and colleagues oxidized cyclohexane to cyclohexanol with *Synechocystis* sp. [23,24]. These authors co-cultivated O_2-producing *Synechocystis* with O_2-respiring *Pseudomonas* to form mixed-species biofilms in capillary bioreactors and achieved high cell densities with a cyclohexane conversion of >98% [23]. Lemke and colleagues converted 4-hydroxycinnamic acid to 4-vinylphenol in *Escherichia coli* biofilms [25]. A PDMS-glass-based microreactor with pillars pointing in the opposite direction to the flow to perform flow-induced deposition was developed. With the microreactor, space–time yields of 8000 g l^{-1} day^{-1} were reached for >50 h [25]. Another biofilm application was shown by Peschke and coworkers for 5-nitrononane-2,8-dione reduction with *E. coli* biofilms [26]. The cells were cultivated in a meandering channel structure in a PDMS-glass-based microfluidic chip system. They were able to reach a space–time yield of 4.6 g l^{-1} day^{-1} [26]. Menegatti and colleagues produced L-malic acid with *Saccharomyces cerevisiae* [27]. In the two-plate microreactor used, the cells were immobilized in a copolymeric hydrogel. A space–time yield of 69.6 g l^{-1} day^{-1} was obtained [27]. Ham and colleagues produced γ-amino butyric acid with *E. coli* [28]. A column bioreactor was packed with alginate beads to immobilize the cells. After 96 h, a total of 165 g of γ-amino butyric acid was produced [28]. Annunziata and coworkers demonstrated the reduction of β-ketonitriles by *Rhodotorula rubra* [29]. Here, a column bioreactor packed with alginate beads was also utilized and a conversion of >95% could be reached [29]. Bajić and coworkers reported the synthesis of L-erythrulose by *E. coli* [30]. They developed a hydrogel microreactor for this purpose. With its cartridge-like insertion of the reaction chamber or its lid, the microreactor is designed to be configured for whole cells and enzymes, allowing a wide range of reactions. A volumetric productivity of 380.5 ± 138.5 g l^{-1} day^{-1} was achieved [30]. Furthermore, there are initial approaches for microsystems for the potential microbial utilization of alternative substrates, such as those derived from lignocellulose [31].

In summary, most of the demonstrated studies reported remain at the proof-of-concept level and do not systematically utilize the advantages of the microscale (Box 1), thus leaving the potential underexploited. The implementation of model bioprocesses in microsystems for whole-cell bioproduction processes needs to transcend trial-and-error approaches and advance toward more rational designs that exploit microscale advantages and fulfill process requirements.

Figure 1. Overview of proof-of-concept studies of microsystems for whole-cell bioproduction processes. (A) Proof-of-concept studies categorized into the underlying product type with cells as products and cells as producers. (B) Example studies from each category shown with their product type, the approach chosen for the setup, and representative results. Reprinted, with permission, from [19,21]. See [17–23,25–30]. Abbreviations: CES, cell expansion system; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; PDMS, polydimethylsiloxane.

Potential applications of microsystems for whole-cell bioproduction

The characteristics and advantages of microsystems shown in Box 1 offer completely new opportunities for bioprocess engineering. Microsystems could thus be particularly complementary in areas where conventional systems have physical and/or engineering constraints restricting their range of application. However, they should not be seen as a substitute for conventional scales but rather as a complement that creates new technological possibilities [32]. This marks the beginning of the new field of **flow fermentation**, which, analogous to flow biocatalysis, exploits the advantages of microscale laws to enable and increase the bioproductivity of selected (niche) applications. In contrast to flow biocatalysis, flow fermentation refers to bioproduction processes in which whole, viable eukaryotic or prokaryotic cells are used as biocatalysts to produce value-added goods, such as the cells themselves or chemicals/metabolites through **biotransformation** or **bioconversion**. However, flow fermentation needs to ensure optimal growth of the biocatalyst first; in a second phase, growth and production need to be managed and balanced (Figure 2A).

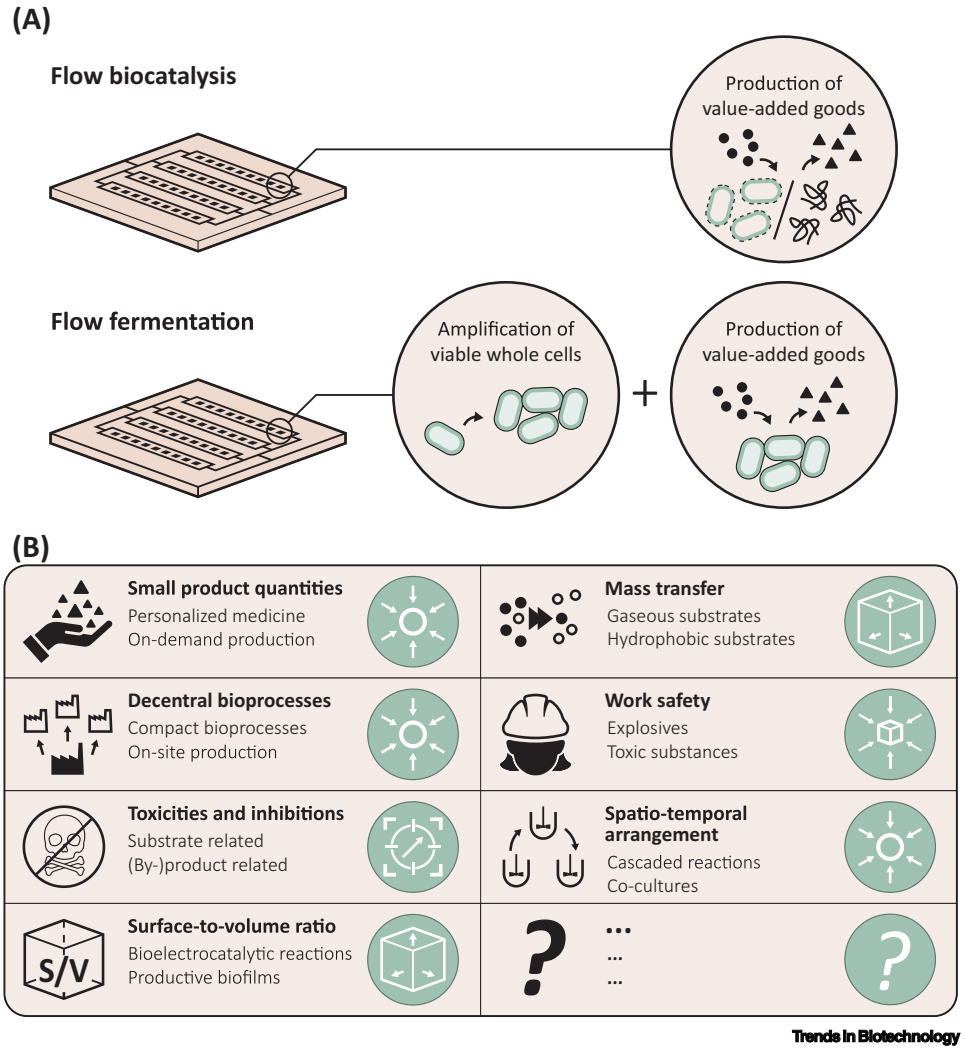

Figure 2. Overview of flow fermentation and its potential application fields. (A) Schematic drawing of flow biocatalysis and flow fermentation highlighting the differences between the two concepts. (B) Overview of potential application fields of flow fermentation benefiting from the advantages of working at the microscale. Green icons indicate the main characteristics or advantages of microsystems exploited in related areas. For additional explanations, see Figure I in Box 1.

Ideally, these systems are operated in a continuous mode in analogy to plug-flow tubular reactors (e.g., tubes, capillary systems) used in flow biocatalysis. Potential application fields can be clustered into the following seven groups (Figure 2B). Several applications are associated with more than one field but are, for simplicity, only listed in one of the groups.

Small product quantities

Due to their small size, microsystems meet the requirements for small product quantities and on-demand production at the point of use (Box 1). This qualifies flow fermentation for the manufacture of products required only in small quantities and at widely dispersed points of use. These include, for example, products for the medical sector, such as orphan drugs, cells for early phases of clinical trials, stem cell therapies, and personalized medicine in general [33]. In this context, the ability of microsystems to ensure a high degree of parallelization is an additional asset, for example, to provide parallel cell production for personalized therapies at the same time.

Decentral bioprocesses

The small size of microsystems makes flow fermentation compact and creates opportunities for decentralized on-site and on-demand bioprocesses [34]. The most prominent field is the production of pharmaceutical biomolecules [35,36], but application fields also include the food, agriculture, and material sectors [34]. Furthermore, flow fermentation could become key for life-supporting systems in space, where the compactness of processes plays a critical role [37].

Inhibition and toxicity

A class of potential applications can be summarized under substrate/(by)product-inhibited bioprocesses. The advantages exploited here are the continuous operation of flow fermentation, which results in permanent perfusion of the microsystem and precisely controllable concentration profiles. Thus, bioprocesses based on cytotoxic substrates could be realized by continuously exposing the cells to low concentrations only. For example, the cytotoxic one-carbon (C1) feedstock formaldehyde could be utilized as a substrate, which has great potential, especially with regard to a greener future, as it can be sustainably produced from CO_2 and renewable energy [38–40]. In addition, operation in perfusion leads to the permanent removal of potentially inhibitory or toxic (by) products. Therefore, flow fermentation could improve the production of antibiotics, antimicrobial peptides [41], or recombinant immunotoxins used as cytostatics [42]. An additional area of application is bioprocesses in which product synthesis occurs only under very specific environmental conditions, as with secondary metabolites; for example, microbial siderophore production [43,44]. Thus, precise control of the environmental conditions is mandatory, as enabled by flow fermentation. In addition, flow fermentation could be beneficial for bioprocesses that are currently conducted in conventional reactors with a second organic phase for *in situ* extraction. One such example is the biotransformation of styrene to the corresponding epoxide by *E. coli* or *Pseudomonas taiwanensis*. Both molecules are toxic to cells at a concentration of approximately 1 mM, requiring a second organic phase to provide styrene and to remove the product from the bulk water. Using a biocatalytic biofilm, styrene diffused through a silica membrane was converted to styrene oxide, and the product was subsequently extracted from the bulk substrate, thereby maintaining high catalytic activity in a continuous system [45]. Bubble or slug flow microreactors allow precise reaction kinetic optimization while forming and maintaining biofilms [46].

Surface area and mass transfer

Large-scale bioreactors are characterized by the fact that the energy-intensive gas input and mixing of the system are never homogeneous, leading to limited mass transfer and emerging heterogeneities. This can negatively affect the growth, production, and predictability of the process or prevent the process from being realizable at all [47]. Flow fermentation enables very fast mass

transfer due to very high surface-to-volume ratios. It is therefore particularly interesting for processes based on gaseous or hydrophobic substrates (two-phase systems). Furthermore, the very high surface-to-volume ratio could be exploited for processes that require large surface areas [48]. These include bioelectrocatalytic or biofilm-related processes. In bioelectrocatalytic bioprocesses, large surface areas are crucial because the process productivity is closely correlated with the electrochemically active surface area needed for the exchange of electrons with whole cells [49,50]. In biofilm-based production processes, surfaces are required for organisms to form biofilms. A large surface area is therefore associated with a high catalytic biomass [51]. Likewise, bioprocesses with phototrophic organisms would benefit from such large surfaces, as areas of light exposure could be ensured [23,52].

Work safety

Similar to flow chemistry, microsystems could be used to increase occupational safety and thus implement bioprocesses, which represent a major security risk in large-scale reactors. For example, nonideal gas mixtures are used in large-scale bioprocesses to ensure occupational safety. By contrast, at a small scale, gas mixtures that have an ideal stoichiometry to support whole-cell bioproduction could be used, as at any point in time the absolute amount of gas exposed is small, significantly reducing the hazard potential. The use of oxyhydrogen to cultivate *Cupriavidus necator* for the production of diverse high-value terpenes [53–55], bioplastics, or biomass (single-cell protein) is such a challenge. The same applies to the use of highly toxic and biohazardous substances, since a lower input quantity is also associated with a low occupational risk [56]. Flow fermentation could thus expand the range of substances that can be used in bioproduction, reducing risks for operators and the environment alike.

Spatial arrangement

The use of microsystems enables an optimized spatial arrangement with extremely short distances between the individual compartments. This would allow the cascading of process steps, which often fails in large-scale systems due to the apparent distances and the resulting limitations. In flow fermentation, a precise flow could be set between the individual steps to accurately adjust the respective concentrations. The cascading of process steps is of high interest in microbial co-cultures where different whole cells share catalytic tasks, converting, for example, molecules one after the other, and each whole cell ideally requiring different cultivation conditions [57]. Compared with monocultures, co-cultures are very versatile and can also handle sophisticated synthesis, which grants them a diverse substrate and product spectrum [58,59].

Challenges

The use of microsystems as a production platform for flow fermentation is still in its infancy. Alongside the opportunities they offer (Figure 2 and Box 1), there are also technical hurdles and challenges to overcome. Here, different technical challenges arise, such as finding the right trade-off between cell retention and flow profile (Figure 3). Compared with flow biocatalysis, flow fermentation requires the growth and maintenance of viable cells before and during production. Therefore, a suitable cell retention concept and process modes must be established to ensure optimal interplay between cell growth and production. Initial approaches include the encapsulation of cells in beads [20] or thin films [27], membrane-based methods [19,60], or surface immobilization (e.g., in the form of biofilms) [26]. Emerging technologies that are also beneficial include microencapsulation [61,62], microcarriers [63], and microstructured surfaces [64]. A potential process mode blueprint could be a retentostat [65], where cells are kept at zero growth after biomass formation, as well as process modes with controlled continuous release of excess cells. The process parameters, including flow rates and an adequate gaseous supply, must be optimized to obtain optimal production environments. Furthermore, especially for continuous production,

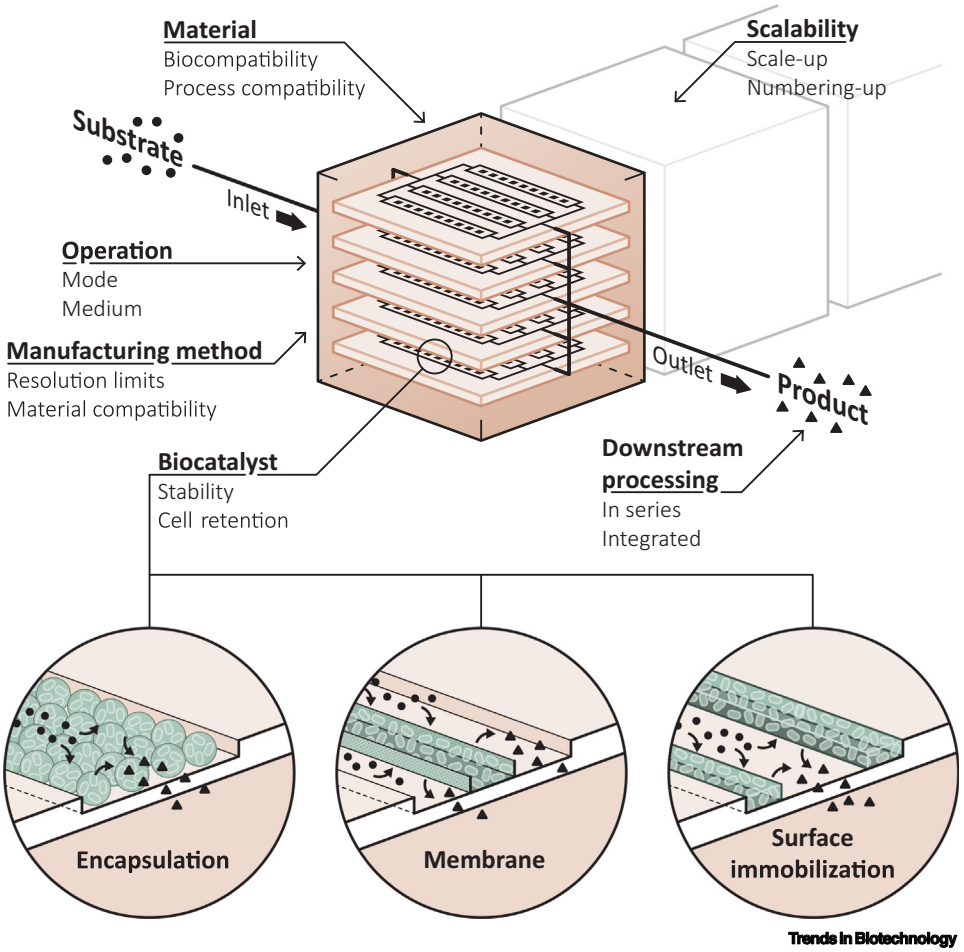

Figure 3. Overview of the technical challenges for the development and establishment of microsystems as a production platform for flow fermentation.

much more research needs to be conducted to stabilize cellular integrity and prevent drift of the production host [66]. Here, efforts in synthetic and molecular biology will accelerate the development of robust and stable production hosts [67].

An additional challenge is the choice of material and the manufacturing method. Furthermore, biocompatibility and compatibility with the process – for instance, solvent stability and gas permeability – are important points to consider regarding the material. Materials used in flow biocatalysis include polymers such as PDMS, polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), polymethyl methacrylate (PMMA),and perfluoroalkoxy (PFA), metals (e.g., aluminum, stainless steel), glass, and ceramics [11,68]. Microsystems for whole cells were manufactured using various polymers, such as polyvinyl chloride (PVC), PDMS, and acrylates, while borosilicate glass was also used. The choice of material goes hand in hand with the choice of fabrication method. There is a wide range of options available, such as photo- and soft lithography, 3D printing, and milling, which must be weighed primarily regarding the resolution limits and materials that can be implemented.

Further challenges include the miniaturization of the periphery, including analytical methods, monitoring, and downstream processes for product purification, which can be either integrated

with or performed in series with the microsystem. Flow fermentation could benefit, for example, from the integration of sensors [69], miniaturized unit operations such as cell separation [70], or product purification [71].

The key challenge of flow fermentation is the transfer of production units from the laboratory to industry. Here, the transfer will be easy for applications in red biotechnology since **numbering-up** (sometimes also called scaling out) is easy to perform with microsystems [72]. Scale-related difficulties, as in conventional systems, are eliminated because the reactor dimensions do not change [32]. However, this strategy is associated with high costs (individual periphery) and is thus economical only for expensive products. By comparison, the transfer of flow fermentation to industrial use in white biotechnology will be challenging. A trade-off between scale-up and numbering-up is needed here to reduce cost but maintain the advantageous production conditions offered by the microscale. In this context, **modularization** and standardization of reactions units could be concepts to consider.

Concluding remarks

Does flow fermentation and the associated use of microsystems for whole cells have a *raison d'être* for bioproduction processes? As discussed, the advantages of microsystems lie in their distinct microscale properties and thereby offer opportunities where conventional systems reach their limits. Flow fermentation could thus expand the portfolio of bioprocesses, and its greatest potential lies in the possibility of highly personalized production near the point of use due to its small size and ability to create optimal conditions within the systems through precise environmental control. The path to realization of these processes involves technical hurdles (see Outstanding questions) for which individual solutions have already been proposed. This lays the foundation to establish the field of flow fermentation and thus addresses emerging questions and challenges in the field (see Outstanding questions).

Acknowledgments

Graphics and illustrations were created in collaboration with Ruben Epping. We would like to thank our numerous discussion partners in the bioprocess engineering and bioprocess microfluidics research community for the enriching scientific exchange. Additionally, we would like to thank both anonymous reviewers for their valuable discussion points that significantly improved the manuscript.

Declaration of interests

The authors declare no competing interests.

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Outstanding questions

What are the genuine reactions/bioprocesses that benefit from microsystems and are thus ideal for flow fermentation?

Will flow fermentation benefit and learn from already established microsystems in the field of flow chemistry and flow biocatalysis?

How should the microsystems be designed (reactor format) and operated (continuous or perfusion mode) to enable optimal flow fermentation?

What are the actual volumes and characteristic dimensions of reaction units needed for flow fermentation? Where do the frontiers between meso-, milli-, and microreactors lie and how can they be reconciled with the trade-off between numbering-up and scale-up in flow fermentation?

How can whole-cell growth and production be balanced to perform optimal flow fermentation?

Should the biocatalyst (enzyme or whole cell) adapt to the state of the art of reactor engineering or should the reactor engineering adapt to the requirements of the biocatalyst (enzyme or whole cell)?

How can benchmarking between flow fermentation and different traditional reactor types, scales, and process modes be performed?

How can we number up to realize the appropriate production scale to obtain sufficiently large quantities of product in an economic manner?

Can modularization and standardization of reaction units help to accelerate the field of flow fermentation?

How do you convince the conservative field of whole-cell bioprocess engineering that microsystems can be not only an analytical tool but also a production system?

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