Review

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Microscale technology and biocatalytic processes: opportunities and challenges for synthesis

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Despite the expanding presence of microscale technology in chemical synthesis and energy production as well as in biomedical devices and analytical and diagnostic tools, its potential in biocatalytic processes for pharmaceutical and fine chemicals, as well as related industries, has not yet been fully exploited. The aim of this review is to shed light on the strategic advantages of this promising technology for the development and realization of biocatalytic processes and subsequent product recovery steps, demonstrated with examples from the literature. Constraints, opportunities, and the future outlook for the implementation of these key green engineering methods and the role of supporting tools such as mathematical models to establish sustainable production processes are discussed.

Microfluidic devices for biocatalytic processes

Increasing research efforts and the growing industrial interest in using microscale technology for continuous-flow organic synthesis and for various continuous-flow separations [1–6] shed new light on basic process development and sets a new paradigm for chemical production [7]. Progress in the fundamentals of biocatalysis and its applications in various industrial sectors over the past 50 years [8–10] have already established biocatalysis as a key technology for sustainable chemistry in numerous manufacturing processes for pharmaceuticals, flavors, fragrances, vitamins, and chemicals [11]. The quest for high-performance manufacturing technology places the combination of biocatalysis and microscale technology as a key green engineering method for process development and production [12–14]. The strategic advantages of microscale technology are reviewed with regard to minimizing the gap between the laboratory and the full-size manufacturing scale enabling sustainable manufacturing of high added-value bioproducts based on biocatalytic processes.

Strategic advantages of microscale technology for manufacturing

Small reactor size (at least one dimension below 1 mm, typically in the range 50–500 μm) and high surface-to-volume ratio (typically 10.000–50.000 m^2/m^3) together with a continuous operation mode resulting in, for example, improved mixing and energy efficiency, heat management, scalability, and safety and lower waste production, are strategic advantages of microfluidic systems over large-scale reactors [1–7].

Safety, health, and environmental advantages

Point-of-use generation of toxic, explosive, and hazardous chemicals *in situ* within integrated microfluidic systems improves safety, health, and environment issues and allows multiple reactions and separations. For example, a robust

Glossary

Flow chemistry: continuous reactions conducted in microreactors combined with other microscale unit operations such as mixing, heating/cooling, phase separation, concentration, and extraction.
***In situ* product removal (ISPR):** a strategy aimed at the removal of an inhibitory product from the reaction mixture as soon as the product is formed, providing immediate relief of the inhibitory action.
Lab on a chip: a device that integrates one or several laboratory functions on a single chip, while transporting and manipulating microliter amounts of fluids.
Microfluidics: a research field that develops methods and devices to control, manipulate, and analyze flows on a nano- to microliter scale.
Microreactor: a reactor with characteristic dimensions from submillimeter to submicrometer whose operation depends on precisely controlled design features.
Microscale technology: technology based on processes and unit operations within microstructured devices.
Monolithic microreactors: microscale reactors using organic polymers and silica-based monoliths with porous rod structures providing very efficient mass transfer due to convective flow, giving better accessibility to the biocatalyst active site, low back pressure, stability in most solvents, versatility of functional groups available for surface chemistry, and the possibility of preparing reactors in any size and shape.
Multiscale modeling: computational methodology ranging from the molecular level to the continuum level.
Process window: a collection of (biocatalytic) process parameter values that allow the reaching of a specific target, usually expressed in terms of productivity, product quality (purity), or economic process viability.

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microfluidic set-up for the *in situ* generation of hazardous ethyl diazoacetate used a dual-channel membrane enabling liquid–liquid separation and a capillary microreactor to perform cascade reactions in a sequential and continuous manner yielding negligible toxic waste [15]. The environmental impact could be significantly improved by using microfluidic devices offering better selectivity and yield and fewer byproducts. The type of manufacturing technology employed contributes to the lower amount of waste produced per amount of product for a petrochemical compared with products in the pharmaceutical/fine chemical industries [16]. The dimension-dependent benefits apply equally well to bioprocesses (e.g., enzymatic cyanohydrin synthesis) where smaller reaction volumes translate into smaller amounts of hydrogen cyanide (HCN) and hence improved safety with fewer risks [17].

High surface-to-volume ratio

One of the most prominent benefits of microscale technology, compared with conventional platforms, is the dramatic increase in heat and mass transfer due to the higher surface-to-volume ratio of microreactors and the short diffusion paths [1–7]. For example, it is advantageous for catalytic reactions occurring on the inner surfaces of microchannel and monolithic reactors with immobilized biocatalysts as well as for packed-bed microreactors offering high catalyst load. Several approaches for surface modification enabling efficient enzyme and cell immobilization are known [18–21]. Further increases in the available surface for biocatalyst immobilization are obtained by the implementation of nanostructured materials such as nanosprings [22,23].

Product removal/product isolation

The high surface-to-volume ratio of microscale reactors is of extreme value for integrated product removal (ISPR) (see Glossary) using either membranes [24] or two-liquid flow for *in situ* extraction [25–27], which among other factors enable improved catalytic efficiency overcoming thermodynamic limitations, enhanced product purity and prevention of catalyst poisoning, and deactivation by removing undesirable byproducts from the reaction zone. Membranes are integrated within microchannels either to achieve product separation or as a catalyst support. They could be made from various materials including zeolites, carbon nanofibers, metals, nylon, polytetrafluoroethylene (PTFE), ceramics, or thin layers of ionic liquids acting as selective media for separation [24].

Continuous processing at smaller scales

The introduction of miniaturized versions of tubular reactors, traditionally used for continuous processing in the petrochemical industry and for heterogeneous catalysis, into organic synthesis has established flow chemistry, opening new approaches and bridging the efforts of engineers and chemists toward sustainable production technology [5]. Continuous processing and bioprocesses have been recognized as the most important key green engineering research areas by pharmaceutical and fine chemicals manufacturers alike [13,28]. Continuous operations have major benefits in reduced costs, equipment size, energy consumption, solvent utilization, and waste. Furthermore, high product quality can be maintained in highly automated production plants incorporating control loops. Numerous applications such as 24/7 manufacturing, multistep synthesis, and repetitive or routine steps are possible [29]. Entirely new pathways can be developed using new processing steps available to continuous manufacturing, as recently demonstrated for the production of the pharmaceutical Aliskiren from advanced intermediates [30]. The easy scaling of reaction conditions in microfluidic systems (Figure 1) is advantageous for the whole process chain [31]. Continuously operated biocatalytic reactions in microreactors, especially reactions at phase boundaries, have been shown to be superior to batch reactors [17–21,25–27].

Mobile process plants

The size reduction to microscale devices opens new perspectives for on-demand production of high-energy or reactive reagents instead of their challenging storage and transport. The possibility of transporting the microreactor itself to the place where the starting material is available or where the product is required is an additional degree of freedom that is a great strategic advantage (Figure 1) and can be tailored to new application areas [7].

Better spatial and temporal control

Among the most distinct strategic advantages of microreactors is their spatial and temporal reaction control. For example, the reaction time can be adjusted by altering the flow rate of the reagents added (e.g., in the lipase-catalyzed ring-opening polymerization of ε-caprolactone to polycaprolactone [32]) or the reaction channel length [33]. Reaction time control is advantageous for short-lived reactive species, which can be generated and transported to the place of the next reaction before decomposition occurs.

Improved transport in multiphasic systems

The application of microstructured mixing devices, using active and passive mixing principles, have led to great improvements for miscible liquids and represent a strategic advantage over mixing in batch reactions. Mixing multiphase systems like immiscible liquids becomes even more important, because if one reactant in one phase needs to react with another reactant in a second phase, the flow dynamics and the promotion of phase contacting and mixing are critical parameters for the subsequent reaction [2]. Better control of fluid flow, short diffusion paths, and high interfacial areas have driven the application of microstructured devices for biocatalytic processing in multiphase systems. Enzymatic microscale reactions in two-liquid-phase systems, often used for poorly water-soluble compounds, to increase biocatalyst stability and/or the yield of thermodynamically limited reactions by *in situ* product extraction, has been reviewed [26,27]. The use of multiphase systems in enzymatic microreactors shows benefits for bioprocess intensification (Table 1).

Energy efficiency

The microstructured reactor consumes two to three times less mechanical energy than the equivalent fixed-bed reactor, achieving equal productivities for a heterogeneously

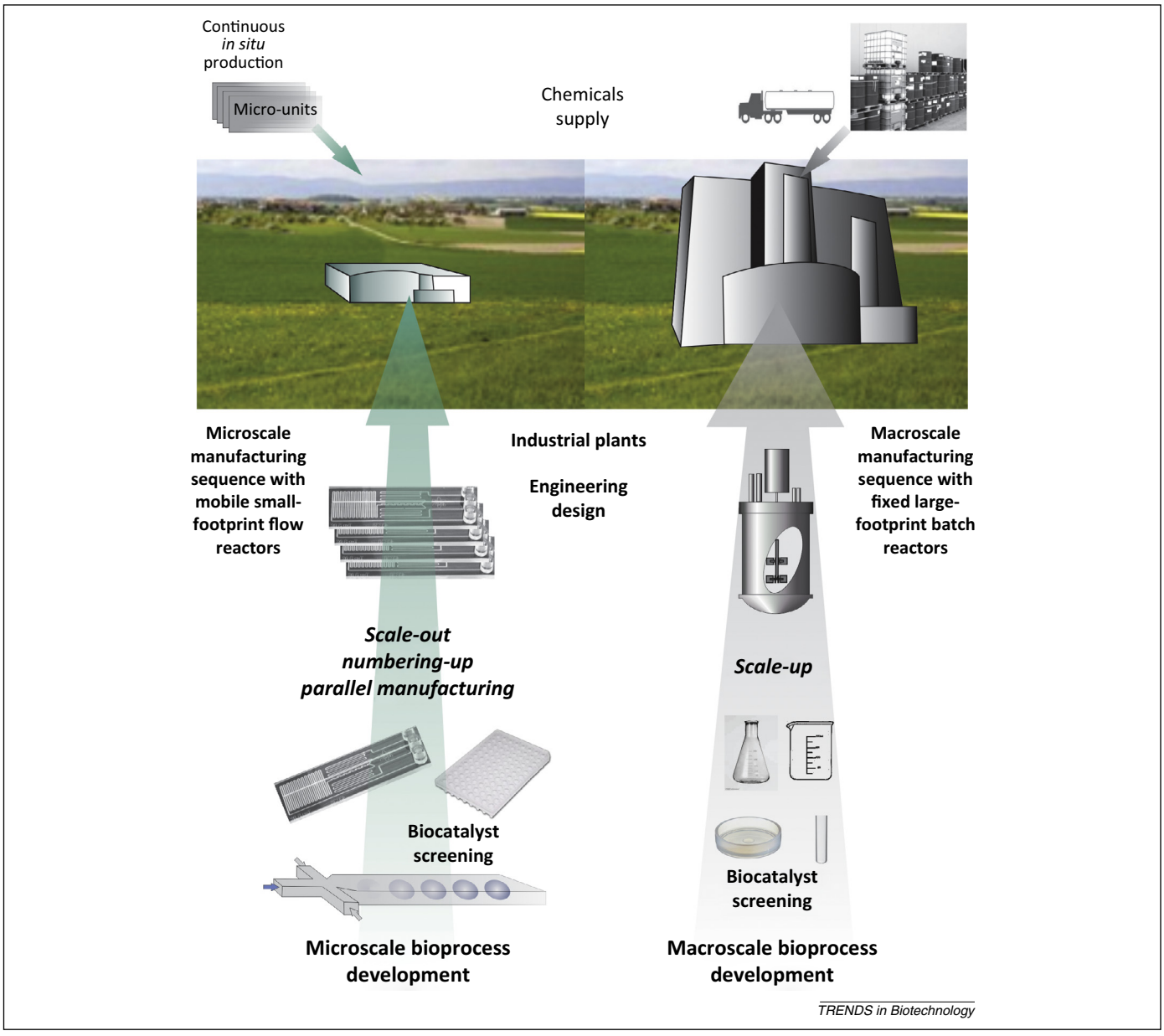

Figure 1. Architecture and scaling of batch and microflow manufacturing modes. The small footprint of mobile flow reactors and the different supply chain logistics in a microscale manufacturing sequence compared with the large footprint of fixed batch reactors is illustrated for an industrial plant, but this strategic advantage extends far beyond industrial synthesis to various areas of decentralized just-in-time manufacturing.

catalyzed gas-phase reaction [34]. Lower power consumption for mixing multiphase systems is evident from comparing microdroplet/microemulsion formation by pumping two phases within microfluidic systems of specific geometry and energy-consuming stirring in large-scale reactors. The continuous-flow synthesis of a key steroid intermediate for an endothelin receptor antagonist in a microreactor by the Barton reaction using a 15-W black-light source illustrates efficient energy use in photomicroreaction systems [35].

Plug-and-play construction of process configuration

The ease with which configuration changes can be made at a microscale is often perceived as an additional advantage compared with large-scale manufacturing equipment. Efficient lipase-catalyzed synthesis of isoamyl acetate in a two-liquid-phase system enabling the reuse of solvent with the dissolved enzyme was developed using commercially available microfluidic units. A miniaturized process system comprising an X-junction glass microfluidic chip provided droplet flow with a very large interfacial area for the reaction and simultaneous product separation in a silanized polymeric microchannel and a membrane-based phase microseparator [36]. The fully integrated continuous two-step synthesis of Aliskiren at a nominal throughput of 41 g/h illustrates a process configuration comprising tubular reactors and membrane-based separators that were scaled up from microfluidic designs [30].

Faster process development

A broader range of reaction conditions can be explored in the development of a process with a given amount of starting material because experiments at a microscale can be performed with reduced reagent consumption.

Table 1. Multiphase enzymatic reactions in microfluidic devices[a]

Multiphase system and fluid flow	Solvent	Microreactor characteristics	Biocatalyst	Reaction	Comparison of a continuous process in a microreactor with a batch process in a conventional laboratory-scale reactor	Refs
Liquid/liquid two-phase system	Citric acid aqueous buffer/ MTBE	Glass microchannel with Y-shaped inlet and outlet; pillar structure in the middle of the microchannel	Crude cell lysate with (*S*)-selective hydroxynitrile lyase from *Hevea brasiliensis*	C–C bond formation for the synthesis of various enantiopure cyanohydrins	Faster and cheaper catalyst screening: two process parameters estimated in 4 h with only 150 μl of crude cell lysate	[17]
	Phosphate aqueous buffer/ isooctane	PC channels with hydrophobic coating; tapered inlet channel, electrostatic phase separation; recirculation of substrate solution	Pentaerythritol tetranitrate reductase + glucose 6-phosphate dehydrogenase	Stereoselective reduction of α/β-activated alkenes + NADPH regeneration	Up to 2.5 higher productivity; 10 times higher surface-to-volume ratio	[89]
	[bmpyr][dca]/ *n*-heptane	Glass X-shaped micromixer; silanized PFA tube; integrated membrane microseparator	*Candida antarctica* lipase-B aqueous solution	Esterification of isoamyl alcohol and acetic anhydride	Volumetric productivity of 29.8 g/(m^3.s), the highest reported so far for this reaction	[36]
	Glycine-pyrophosphate aqueous buffer/ *n*-hexane	Glass microchannels with Y-shaped inlets and outlets of various geometries; one of these equipped with swirl micromixer	Alcohol dehydrogenase from *Saccharomyces cerevisiae*	Oxidation of hexanol to hexanal	30-fold higher maximum reaction rate	[49]
	Aqueous Bis–Tris buffer/ hexadecane	Glass T-shaped micromixer, PTFE tubes of various diameters and lengths	TADH from *Thermus* sp. + FDH from *Candida boidinii*	Reduction of 1-heptaldehyde to 1-heptanol + NADH regeneration	Volumetric productivity of up to 48 mmol/(l.h); eliminated emulsion formation: reduced downstream costs	[90]
	Aqueous phase with cells/ acrylonitrile	Stainless steel membrane dispersion microreactor	*Rhodococcus ruber* resting cells containing nitrile hydratase	Hydration of acrylonitrile to acrylamide	Microscale reactor: up to 45.8 wt% acrylamide in 35 min; conventional stirred-tank reactor: 39.5 wt% in 245 min; better temperature control at microscale	[91]
Gas/liquid two-phase system	Oxygen/aqueous buffer	Falling film microreactor	Glucose oxidase	Oxidation of β-D-glucose to gluconic acid	Microscale reactor: up to 50% final conversion in 25 s; conventional bubble column: 27% final conversion; much higher interfacial area at microscale	[92]

[a]Abbreviations: MTBE, methyl tert-butyl ether; PC: polycarbonate; [bmpyr][dca], 1-butyl-3-methylpyridinium dicyanamide; PFA, perfluoroalkoxy; TADH, thermophilic alcohol dehydrogenase; FDH, formate dehydrogenase.

A highly sensitive and high-throughput screening system for new enzymes was demonstrated in microfluidic droplet compartments using only picoliter amounts of cell lysate. A microfluidic platform for directed enzyme evolution in which water-in-oil droplet compartments serve to miniaturize cell lysate assays by a million-fold was reported by Kintses and coworkers [37]. Automated on-line determination of reaction rate parameters can provide reaction kinetics faster and more efficiently [38]. Enzyme inhibition can be measured rapidly with a uniform substrate concentration, an inhibitor concentration gradient using a single microchannel, and a single initial inhibitor concentration [39]. Fast estimation of the immobilized enzyme activity has also been achieved in a segmented-flow reactor [40]. Process conditions like pH, substrate inlet concentration and flow rate, cell permeabilization conditions, and catalyst stability were evaluated, and optimized, within a very short time and under more controlled conditions when polymeric microchannels were used for L-malic acid production by permeabilized yeast cells compared with their batch counterparts [41].

Expanded biocatalytic process windows

The benefit of microreactors for increased process performance by applying novel process windows such as high temperatures, high pressures, and high concentrations

Box 1. Expanded biocatalytic process windows

Reaction boundaries like reaction rate, toxicity, catalyst inactivation over time (total turnover number), and downstream processing using a window of operation for whole-cell biocatalysis were introduced by Woodley and Titchener-Hooker [93]. Reaction rate acceleration due to the reduction in diffusion path and increased mass transfer in two-phase systems within microscale reactors is the most suitable driving force for bioprocess intensification.

Considering toxicity and biocatalyst deactivation, various studies have proved the benefit of using microscale technology. Continuous microprocessing with multi-input addition of toxic substrates was suggested to prevent catalyst inactivation [94]. Almost 2 months of production at maximal volumetric productivity was enabled by the continuous feed of a toxic substrate and *in situ* product extraction over a silicone tube that prevented the inhibition of *Pseudomonas* sp. cells performing asymmetric epoxidation of styrene [95]. The use of a flow reactor enabled higher quantities of oxidant to be employed for lipase-mediated oxidation of alkenes over prolonged periods of time without deactivation of the biocatalyst [62]. Very short reaction times for acrylamide synthesis in microreactors also greatly weakened the inhibition of *Rhodococcus ruber* cells by acrylonitrile and acrylamide. Furthermore, the temperature of this exothermic reaction could be much more efficiently controlled compared with traditional stirring in a conventional reactor leading to additional lowering of biocatalyst deactivation and prevention of byproduct formation [91].

Another critical parameter for enzyme-catalyzed reactions is leaching of enzymes from solid beads, contaminating products and reducing biocatalyst (immobilized enzyme) lifetime. A study of ring-opening polymerization of ε-caprolactone to polycaprolactone using a packed-bed microreactor with *Candida antarctica* lipase-B in the form of Novozym 435 indicated that enzyme leaching from porous beads was lower in microreactors compared with batch reactors [32].

The last boundary of the bioprocess window of operation (Figure I) is the efficiency of downstream processing. Typically very low bioproduct concentrations represent a huge challenge resulting in up to 90% of production costs needed for product isolation. Studies considering liquid–liquid extraction of bioproducts within microfluidic systems clearly confirm the benefit of taking advantage of the high surface-to-volume area and short diffusion paths in either microflow systems with parallel flow enabling phase separation at the exit [25] or droplet or segmented flow, where further phase separation is needed [36].

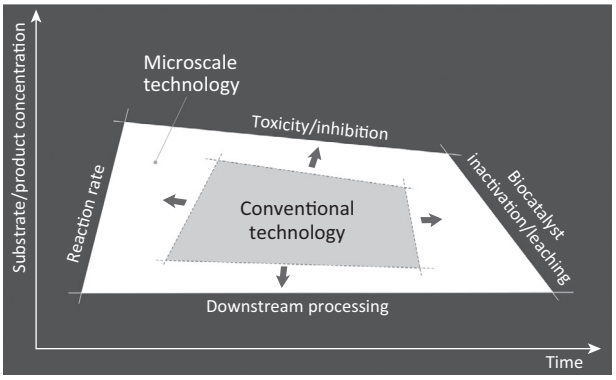

Figure I. Microscale technologies offer improvements in reaction rate and downstream processing efficiency as well as an increased possibility of preventing the toxic effects of substrate/product on cells and biocatalyst deactivation and/or leaching compared with conventional technology, primarily due to increased mass transfer, higher surface-to-volume ratio, and better process control. Based on Woodley and Titchener-Hooker's illustration of the process window for whole cells [93].

demonstrated for various types of chemical reaction [7] provides new opportunities for biocatalytic reactions. High pressure is a reaction parameter with huge potential for enzyme catalysis and has already been highlighted [42]. Dissolving carbon dioxide at 280 bar in the lipase-B-catalyzed solvent-free synthesis of polyglycerol-3-laurate resulted in a fourfold reaction rate increase [43]. Limitations of bioprocess operation windows related to reaction rate, substrate or product toxicity/inhibition, biocatalyst stability and/or leaching from the reactor, and downstream processing could be overcome by using microscale devices (Box 1).

Microreactor technology for manufacturing of biosynthetic products

The availability of technologies for building microstructured devices in a controlled and repeatable manner enables the design of microreactors from various materials using a large number of strategies for integrating and releasing reagents [44]. Furthermore, in-line process analysis and control and biocatalyst configuration, as well as integration with downstream processing and possibilities for scale-out, are important considerations in the manufacturing of microfluidic systems [45].

Microreactor manufacturing and materials

Materials used in microreactor manufacturing have to be accepted and compatible with industrial production requirements, reagents, solvents, and processes. A wide range of reaction conditions and single and multiphase reactions are possible with widely applicable, cost- and resource-efficient manufacturing techniques in modular microreactors made of suitable materials [46]. Microreactors can be adapted to bioprocess needs, as demonstrated with the multi-input reactor in the transketolase-catalyzed synthesis of L-erythrulose [22]. That there are only two patents for microreactors designed for biocatalytic reactions indicates the development opportunities [47]. Biocatalytic processes in microfluidic systems encompass a broad range of materials and systems, ranging from simple capillaries and tubing to custom-made microchannels, monoliths, and microstructured reactors with inserted nanomaterial (Table 2).

Enzymatic microreactors can be classified with respect to shape, materials used, and interface preparation or with respect to flow characteristics. Y-shaped, Ψ-shaped, T-shaped, X-shaped, or tapered are the most common microchannel shapes, made mainly from glass, silicon, ceramic, stainless steel, or polymer materials. Immobilized enzyme processing and interface preparation in microreactors are emerging technologies implemented to increase the surface area as a major factor in enzymatic microreactors. Structures can be etched from, cast into, deposited onto, or grown directly from microchannel surfaces [46]. Wall-coated supports and especially nanosprings or other nanostructured materials are recognized as another efficient method to increase surface area and enzyme loading in microreactors [22,23,48]. Some of the most common types of enzymatic microreactor already demonstrate the specific features of

Table 2. Biocatalyst configurations used in microfluidic devices[a]

Biocatalyst configuration	Microreactor characteristics	Biocatalyst and immobilization technique	Reaction	Comparison of a continuous process in a microreactor with a batch process in a conventional laboratory-scale reactor	Refs
Dissolved enzyme in a homogeneous system	Glass microreactor with Y-shaped inlet and outlet	LACCASE from *Trametes* sp.	Oxidation of catechol	18- to 167-fold higher oxidation rates	[50]
	Glass microreactors with two inlets and one outlet; one microreactor with teardrop micromixer structures	Benzoylformate decarboxylase from *Pseudomonas putida*	(S)-2-Hydroxypropiophenone synthesis from benzoylformate and acetaldehyde	Up to 72-fold higher volumetric productivities than in ultrafiltration membrane reactor	[51]
	PMMA microreactor with a filtration unit based on PDMS gasket and regenerated cellulose filter	TK (wild type and mutant)	L-Erythrulose synthesis from hydroxypyruvate (HPA) and glycolaldehyde; (3S)-1,3-dihydroxypentan-2-one synthesis from HPA and propanal	Complete substrate-to-product conversion, complete enzyme retention and product separation; the reaction rate is comparable to batch reactors, facilitating high-throughput evaluation of process conditions and rapid process development	[52]
	PMMA and PDMS-based membrane microreactor	Pectin lyase	Pectin depolymerization	Faster and cheaper process development, better understanding of the enzyme-catalyzed reaction dynamics; similar kinetic profiles in micro- and laboratory-scale operations	[53]
Dissolved enzyme in a two-phase system	See Table 1				
Biocatalyst immobilized on the inner surface of the microchannel	Perfluoroalkoxy microchannel with silanized inner surface	*Saccharomyces cerevisiae* cells; covalent binding with glutaraldehyde as a crosslinker	L-Malic acid synthesis from fumaric acid	Four times higher specific volumetric productivity than in the bench-scale membrane reactor; attractive alternative for process development studies or for synthesis	[41]
	Glass microchannel with Y-shaped inlet and outlet with silanized inner surface	Fumarase from porcine heart; covalent binding with glutaraldehyde as a crosslinker	L-Malic acid synthesis from fumaric acid	Volumetric productivity in a microreactor up to 2.57 mmol/(l.min) with 80% conversion; in a laboratory-scale membrane reactor, up to 0.81 mmol/(l.min) productivity with 68% conversion; known reaction area enables accurate process description	[54]
	Fused silica capillary with NTA surface derivatization	His-tagged TK and TA immobilized on functionalized surface	TK–TA coupled reaction for synthesis of 2-amino-1,3,4-butanetriol from lithium hydroxypyruvate, glycolaldehyde and (S)-α-methylbenzylamine	Development of a continuous-flow immobilized enzyme microreactor as a tool for fast and inexpensive *in vitro* evaluation of multienzyme pathways	[55]
	Not yet tested in a microreactor; tested on a glass support	Immobilization via positively charged miniprotein Z_{basic2}	–	Potential for reversible immobilization	[21,56]
	Capillary column with highly cationic chemically modified bovine serum albumin (cBSA-147)	Planktonic bacterium *Escherichia coli* BL21 star (DE3); electrostatically mediated immobilization	Enantioselective reduction of ethyl acetoacetate to R-(−)ethyl hydroxybutyrate	30% higher productivity than the conventional batch system	[57]
Biocatalyst immobilized on the inner surface of the monoliths	Disks from microcellular polymers with various pore structures	*Pseudomonas syringae*	Phenol degradation	At least 20-fold higher volumetric productivity than the packed-bed reactor; monolayered biofilm	[59]
	Macroporous monolithic minidisk	A complex of extracellular chitinolytic enzymes isolated from *Clostridium paraputrificum*	Chitin hydrolysis	Fast and efficient immobilization and evaluation of a multienzyme system	[60]
	Monolithic silica rods exhibiting very open and uniform 3D hierarchical pore structure	Invertase; covalent immobilization using glutaraldehyde crosslinking	Sucrose hydrolysis	Over 1000 times faster hydrolysis; notably larger enzyme affinity to the substrate	[61]

Table 2 (*Continued*)

Biocatalyst configuration	Microreactor characteristics	Biocatalyst and immobilization technique	Reaction	Comparison of a continuous process in a microreactor with a batch process in a conventional laboratory-scale reactor	Refs
Biocatalyst in the packed-bed microreactor	Aluminum microchannel covered with Kapton film using a thermally cured epoxy	Novozym 435	Ring-opening polymerization of ε-caprolactone	At least one order of magnitude higher apparent reaction rate; extended biocatalyst stability	[32]
	Borosilicate glass capillary	Novozym 435	Chemoenzymatic oxidation of aromatic and aliphatic alkenes	Up to 381-fold reduction in reaction time; possibility of using higher quantities of H_2O_2 over prolonged periods of time	[62]
	Stainless steel tubular mesoscale reactor	Various lipases; immobilization on beads of various materials	Kinetic resolution of racemic secondary alcohols and cycloalkanols	Similar enantiomer selectivities but higher productivities compared with batch production of various esters: e.g., 8.3 vs 7.0 mmol/(g.min) at 51–52% conversion, 5.6 vs 3.9 mmol/(g.min) at 24–29% conversion, 6.1 vs 4.9 mmol/(g.min) at 53–54% conversion	[63,64]
	Layers of olefin-based film between two PMMA plates	Novozym 435	Transesterification of 1-butanol and vinyl butyrate and esterification of isoamyl alcohol and acetic anhydride	Isoamyl acetate: volumetric productivity 20 mmol/l.min in a microreactor, 9.1 mmol/l.min in a batch reactor; butyl butyrate: five times higher volumetric productivity in a microreactor	[65,66]
	Stainless steel tubular mesoscale reactor	*E. coli* cells containing overexpressed ω-TA and PLP immobilized on methacrylate polymeric resin beads	Asymmetric amination of various non-natural ketones to chiral amines	High throughput (30–60 min), clean production, high enzyme stability (the packed-bed reactor can be continuously operated for 1−10 days), and excellent mass recovery	[67]
	FEP tube with a microfilter assembly at the end	His-tagged TK and TA immobilized on Ni-NTA agarose beads	TK–TA coupled reaction for synthesis of 2-amino-1,3,4-butanetriol from lithium hydroxypyruvate, glycolaldehyde, and (S)-α-methylbenzylamine	Short process time (83% conversion in 20 min); fast and inexpensive immobilization and biocatalyst stability evaluation	[68]
Biocatalyst immobilized on nanostructures	Microchannel with inserted mat of silicon dioxide nanosprings functionalized with thiol groups	β-Galactosidase; disulfide linkages between thiolated nanosprings and the modified enzyme	Hydrolysis of o-nitrophenyl β-D-galactosylpyranoside	Microreactor: 37% conversion in 14 s, batch: 10% conversion in 10 min; high solvent-accessible surface area with good permeability and mechanical stability	[22]
	Plates or discs with silicon dioxide nanosprings functionalized with thiol or epoxy groups	Threonine aldolase; linkages between thiolated nanosprings and the modified enzyme	Cleavage of L-threonine to glycine and acetaldehyde	Larger specific surface area and enzyme availability; a new flow reactor concept with tight packing of stacked nanospring disks	[23]
Biocatalyst immobilized on magnetic particles	Magnetic field-assisted PTFE tubular microreactor with silanized magnetic nanoparticles	Alcohol dehydrogenase; immobilization on nanoparticles via glutaraldehyde crosslinking	NADH oxidation	Very simple technique for biocatalyst localization; two times higher volumetric productivity	[69]

[a]Abbreviations: Novozym 435, *Candida antarctica* lipase B immobilized within acrylic beads; TK, transketolase; TA, transaminase; PLP, pyridoxal 5′-phosphate; FEP, fluorinated ethylene propylene; NTA, nitriloacetic acid.

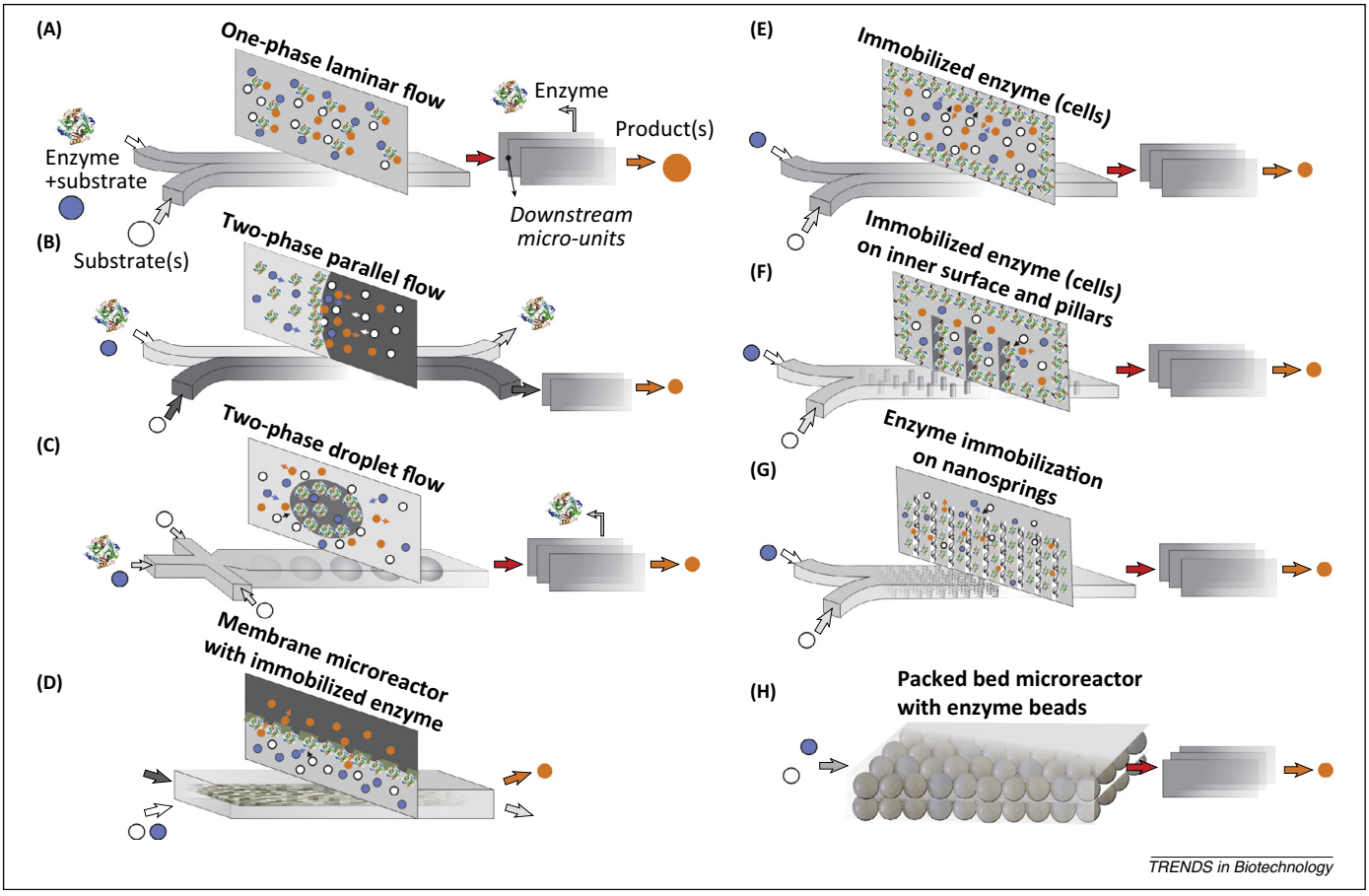

Figure 2. Schematic representation of enzymatic microreactors: **(A)** free enzyme in a two-phase parallel flow; **(B)** free enzyme in a droplet flow; **(C)** free enzyme in a homogeneous system; **(D)** membrane microreactor with enzyme immobilized on the membrane; **(E)** immobilized biocatalyst on the inner surface of a microchannel; **(F)** immobilized biocatalyst on the inner surface and pillars; **(G)** nanospring supports to increase surface area and biocatalyst loading in the microchannel; and **(H)** packed-bed microreactor with beads containing biocatalyst.

microfluidics for biocatalytic processes in homogeneous, heterogeneous, and multiphase systems (Table 2 and Figure 2).

Biocatalyst configuration

Biocatalysts in microreactors can be used in soluble form or flow freely through the reactors in the form of channels [25,49–51] or with integrated membranes enabling enzyme separation and recycling [52,53]. Biocatalyst immobilization, which may also improve stability and simplify product separation [20,21], can be achieved on the inner surface of the microchannels [21,41,54–58], in microscale reactors with monolithic structures [59–61] or nanostructured material within the microspace [22,23], entrapped in the bulk space within porous structures [32,62–68] or with the assistance of a magnetic field [69] (Table 2). For example, a comparison of various immobilization methods and supports for threonine aldolases showed a sufficient loading performance under best performance assumptions only when high-value products like pharmaceuticals were considered [23]. Bioprocess intensification and development using either free or immobilized biocatalysts in enzymatic microreactors with non-aqueous media also shows benefits (Table 3).

Process analytical and control technology

Process analytical technology (PAT) may be efficiently implemented in microreactors due to the high degree of automation and process controllability achievable in small-scale continuous equipment [70]. The use of sensors to analyze critical parameters and incorporation of the corresponding actuators to exploit the results of the analytical devices are important for general process control. Dissolved oxygen concentrations were monitored on line in a microreactor at various flow rates and microchannel dimensions in the glucose oxidase-catalyzed oxidation of D-glucose [71]. Accurate 2D oxygen imaging was achieved with an oxygen-sensitive platinum–porphyrin complex in the microfluidic system and a color charge-coupled device (CCD) camera, a set up that can be parallelized or applied to resolve oxygen concentrations spatially and temporally inside microfluidic channels [72]. While temperature and pH sensors measure key reaction parameters and are routine in-process analysis techniques suitable for industrial manufacturing, spectroscopic analyses provide more detailed information but are limited to the laboratory scale. IR– and UV–visible light detection linked to flow systems is attractive for on-line monitoring [3]. Classical HPLC, often used in microreactor analysis, is a key component of an automated microfluidic system for on-line optimization [73]. Microreactor technology has been combined with capillary flow NMR spectroscopy, whereby the two reactants, fed separately and mixed effectively in a micromixer into the probe head, pass through a capillary

Table 3. Benefits of implementation of enzymatic microreactors using non-aqueous media for bioprocess intensification and development [27]

Biocatalyst format and reaction medium	*Two-liquid-phase systems containing free biocatalyst*	*Non-aqueous media containing immobilized biocatalyst*
Transport intensification	Reduced diffusion length within microreactors	
	Larger and controlled interfacial area	Efficient contact between liquid phase and enzyme within the carrier enabled by suitable flow regimens
Reaction intensification	Use of non-aqueous solvents suppresses hydrolytic reaction Higher substrate concentrations or use of poorly water-soluble reactants enabled by the use of non-aqueous solvents	
		Increased enzyme loads in microreactors compared with traditional batch reactors More stable enzymes due to lower pressure drop compared with large-scale packed-bed reactors
Process intensification	Enzyme and solvent recycling possible with miniaturized reactor integrated with microseparator *In situ* product extraction Enzyme recycling within closed-loop reactors More straightforward downstream processing due to fewer possibilities for stable emulsion formation	Biocatalyst immobilization offers easier enzyme–product separation Integration of miniaturized reactor and a microseparator possible
Acceleration of bioprocess development	Reduced time consumption for kinetic data evaluation, test of enzyme activity and stability, optimization of process parameters	
Efficient use of resources	Lower material consumption for kinetic data evaluation, test of enzyme activity and stability, optimization of process parameters	

NMR flow cell with a solenoidal radiofrequency coil where the NMR signal is acquired [74].

Process integration

Downstream processing integrated with microscale reactions offers process intensification advantages. For example, an integrated microfluidic system was developed that included the cascade generation, extraction, separation, and reaction of hazardous ethyl diazoacetate with aldehydes to yield 2-keto esters with subsequent gas–liquid separation [15]. Efficient enzyme-catalyzed cholesterol oxidation with *in situ* product removal was achieved by the parallel flow of an organic and an aqueous phase with phase separation at the exit of a Y-shaped microchannel system (Figure 2). Phase separation was achieved by the establishment of a laminar interface between the two parallel flows throughout the channel length at the position of the inlet and outlet junctures of Y-shaped channels resulting from the adjustment of flow rates ratio according to the relative viscosity of the fluids. The microfluidic device, further integrated with a packed-bed reactor containing immobilized catalase, resulted in continuous cholestenone synthesis for >300 h at more than 30% of the initial cholesterol oxidase activity [25]. A packed-bed microbioreactor was also integrated with a miniaturized separator for continuous lipase-catalyzed butyl-butyrate synthesis [65].

Simulations enabling process development

Many biocatalytic processes at the microreactor scale have been theoretically described using validated mathematical models on the macroscopic level. Likewise, on-line validated mathematical models have been developed to depict the governing transport characteristics [71]. Finite difference/volume/element (FD/FV/FE) methods, based on the discretization of the continuous form of the relevant transport equations, are the most common macroscale simulation techniques. However, such techniques based on a continuum approximation may not always be readily adapted to describe transport processes and chemical interactions that occur in processes at the microscale. The macroscopic models are often unable to provide conclusive evidence for a given mechanism in systems with the complexity characterizing almost all biochemical processes. By contrast, atomistic-scale simulation techniques, such as molecular dynamics (MD) and Monte Carlo (MC) simulations, can track the motion of individual molecules and allow precise reconstruction of the molecular architecture and properties. Since the use of these atomistic methods to probe the dynamic behavior at higher scales remains impractical, particle-based mesoscale methods possess the unique ability to model relatively large physical systems and simultaneously effectively capture the essential features of the micro- and nanoscale structure, architecture, and relevant interactions [75]. Due to the fact that microfluidic phenomena arising from microdevices are characterized by a hierarchal multiscale nature with respect to space and time, hierarchical multiscale modeling is a promising potential tool for developing, designing, and optimizing bioprocesses at the microscale. It aims to develop a viable multiscale computational methodology to improve understanding and predictions of complex physicochemical phenomena in spatial and temporal domains ranging from the molecular level to the continuum level [76]. Although the chemical engineering community has traditionally been interested in macroscopic continuum models, micro-, meso-, and macro-scales can be linked and integrated to improve understanding and predictions of complex chemical engineering phenomena. Multiscale modeling may help to design and optimize microsystems technology for biocatalytic processes from a microscopic perspective. Moreover, to move lab-on-a-chip biochemistry to the pilot and production scale, multiscale modeling can be implemented for scale out/scale up or numbering up of optimization design (Figure 3).

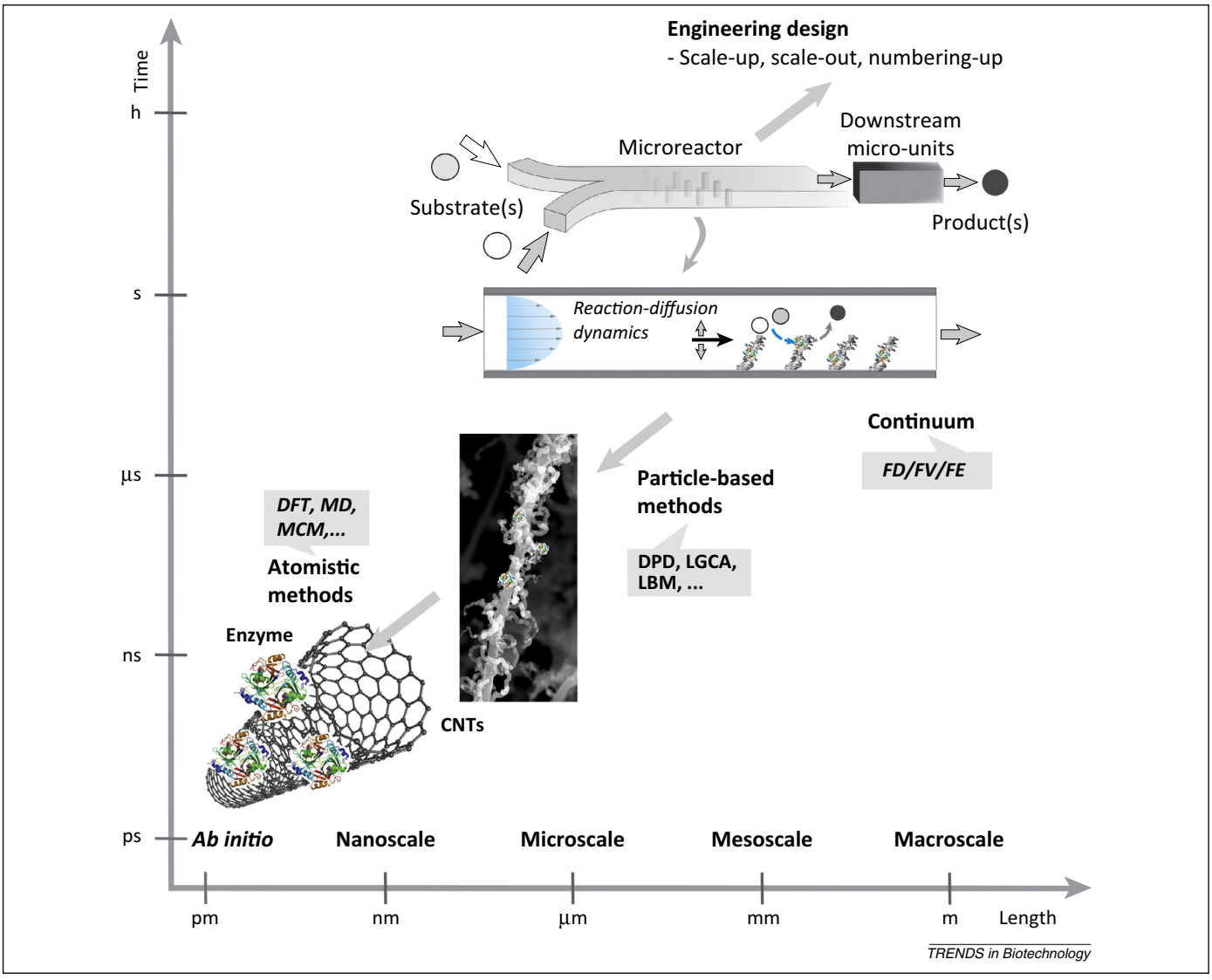

Figure 3. An example of the various time and space scales encountered in hierarchical multiscale modeling. *Ab initio* and atomistic methods model the complete molecular structure: density functional theory (DFT), molecular dynamics (MD), Monte Carlo methods (MCMs). In particle-based methods, particles represent clusters of molecules or fluid pockets: dissipative particle dynamics (DPD), lattice gas cellular automata (LGCA), lattice Boltzmann method (LBM). Methods based on discretization of the continuous form of transport equations: finite difference/volume/element (FD/FV/FE) [76].

Challenges in microreactor technology for manufacturing

The move from traditional batch reactors to continuous-flow processes in industrial manufacturing remains slow due to several perceived barriers [16,77,78]. Standardized reactor modules that can be combined in various sequences to test novel biocatalytic processes are crucial for rapid development. The feasibility of a wide area of dimension- and time-dependent biocatalytic reactions can be explored at the microscale. High space–time yields, high selectivity, and complete conversion are key goals in process intensification and resource efficiency. Microreactors are also of interest for rapid process development if PAT matches the reaction timescale [79,80]. Real-time product analysis is key for quality by design (QbD) of the manufacturing process.

Highly innovative and systematic approaches, protocols, tools, and strategies are currently being developed to minimize the gap between research and industry and between the laboratory and full-size manufacturing scale and to define the smooth transfer of a well-developed, safe, scalable, robust, and economic (bio)chemical process to the industrial environment [29,81,82]. Advances in biocatalyst discovery to biocatalytic microprocess design require not only a new level of understanding of reaction mechanisms and transport phenomena at the microscale, but also computational tools and advanced microsensors to optimize and control bioprocesses in working environments from chemical and energy resources to products. It is important to define key performance indicators as development targets early in projects toward economically viable bioprocesses. Few studies consider increasing the volumetric production rate at the microscale, to design equipment for larger-scale production, and to perform holistic process analysis (e.g., for complex biobased molecules whose production in microdevices remains at an early stage [83]). Microreactor-based synthesis of chiral amino alcohols using threonine aldolase and large-volume fine-chemical synthesis (gluconic acid synthesis with glucose oxidase) reveal that enzyme loading and activity are not yet sufficient for large-volume, low-cost chemicals, al-

though they are adequate for high-value products where enzyme costs can be overcome by recycling [23,47,84]. In practice, challenges related to the handling of particles and slurries, often leading to clogging of microscale channels and thereby preventing stable microscale reactor operation, as well as differences in capacities of unit operations, necessitate customized reactor design and make plug-and-play construction difficult to realize in practice [85].

Concluding remarks and future perspectives

The development of integrated reaction–separation systems at the microscale leading to process intensification of biocatalytic processes [36,65], as well as the increased capability of using mathematical models to predict phenomena in microscale devices, are some of the most significant advances in this field. Development of a microfluidic platform for directed evolution, using water-in-oil-droplet compartments for screening [37], plays a significant role in faster process development, while multi-inlet microfluidic reactors for controlled addition of inhibitory/toxic substrates [22] and microfluidic devices with entrapped biocatalysts using a magnetic field [69] indicate new possibilities for the establishment of highly efficient biocatalytic processes with long-term stability. The further expansion of microreactors into process development will proceed from established analytical platforms using both continuous and droplet assays. Reductions in development time and cost can be achieved by activity-based reaction screening as a function of pH, temperature, reaction conditions, and substrate scope or by end point determinations. Multiphase flow conditions, where flow instabilities, fast biocatalyst inactivation, and low conversion rates are often encountered [26,27], provide opportunities for process intensification. Continuous processes will constitute a key research area for the engineering of green and sustainable processes [28]. Microscale technology and flow chemistry is not yet used as much for manufacturing as is batch reactor technology and myths of it not being useful for production persist. Therefore, to be convincing, documenting and reporting the significant advantages for a rapidly growing range of reaction types, in direct comparison with batch reactor technology, is essential. The recent microreactor synthesis of ibuprofen in three sequential reaction steps with each reaction completed within 1 min, leading to an overall reaction time of 3 min and 83% overall yield, demonstrates the capabilities of continuous-flow manufacturing for multistep syntheses using small-footprint reactors [86]. The further development of more precise reaction control in microreactors by suppressed formation of undesired products or byproducts, as well as *in situ* preparation of reactive species only when they are needed, will enable telescoped flow synthesis from simple precursors [87]. The higher efficiency of microreactors over batch systems for biphasic gas/liquid systems offers the merits of more convenient protocols [87,88] and the tools for new synthetic manufacturing routes. Reagents can be prevented from destructive interactions and the formation of undesired byproducts by introducing reagents at specific stages of the microscale system, leading to a broader substrate scope and higher reactivity and yield compared with batch processes [88]. An (R)-selective ω-transaminase with pyridoxal-5′-phosphate immobilized on methacrylate beads in organic solvent enabled the transamination of ketones in a microreactor with a residence time of 30–60 min, >99% enantiomeric excess (ee), and excellent mass recovery, demonstrating a clean production system without additional work up and purification [67]. The current rapid progress in continuous-flow manufacturing and innovative approaches for overcoming engineering and molecular bottlenecks opens a wealth of exciting new opportunities and process windows for future discovery, development, and production.

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