

Review

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Enzymatic microreactors in biocatalysis: history, features, and future perspectives

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Abstract: Microfluidic reaction devices are a very promising technology for chemical and biochemical processes. In microreactors, the micro dimensions, coupled with a high surface area/volume ratio, permit rapid heat exchange and mass transfer, resulting in higher reaction yields and reaction rates than in conventional reactors. Moreover, the lower energy consumption and easier separation of products permit these systems to have a lower environmental impact compared to macro-scale, conventional reactors. Due to these benefits, the use of microreactors is increasing in the biocatalysis field, both by using enzymes in solution and their immobilized counterparts. Following an introduction to the most common applications of microreactors in chemical processes, a broad overview will be given of the latest applications in biocatalytic processes performed in microreactors with free or immobilized enzymes. In particular, attention is given to the nature of the materials used as a support for the enzymes and the strategies employed for their immobilization. Mathematical and engineering aspects concerning fluid dynamics in microreactors were also taken into account as fundamental factors for the optimization of these systems.

Keywords: Microreactor, microfluidic reactor, enzyme, immobilization, biocatalysis

1 Introduction

The first appearance of the word *microreactor* in a scientific publication was most likely in 1951, in a paper by M.A. Mosesman concerning the use of a micro-apparatus

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for the studies of gas-solid reactions by *in situ* X-ray diffraction [1]. Successively, this technology was applied in several kinds of chemical reactions with the aim to improve the reaction rate of specific processes.

As recently highlighted by R. Wohlgenuth et al. [2], the increasing success of microreactor technology is principally due to the relevant advantages of micro-scaled systems vs. large-scale reactors. These advantages include reduced environmental impact, improved heat and mass transfer, ease of product isolation, and energy efficiency. In addition, microreactors offer unique possibilities for the design of innovative industrial processes in conditions not applicable on a large-scale [3] and for their easy transportability to places where reagents are available and/or products are required [2].

A microreactor can be described as a device with narrow channels (typically with an internal diameter less than 1 mm) constructed from stable and inert materials (e.g., glass, silicon, stainless steel, ceramic, or polymers), and equipped with a tunable pumping system, two or three reservoirs for reagents, and a system for the selective collection of the reaction products [4,5]. Fig. 1 shows the basic scheme of a simple microreactor and the external aspect of a typical commercial system.

In general, the choice of reactor material depends on the operating conditions [6,7]. For example, if the reaction mixture is a liquid under 200°C, a glass reactor can be used, but if the reaction operates at high temperature and pressure, reactors fabricated with silicon and steel are required. At even higher temperatures, such as those reached in reforming reactions, a ceramic reactor must be used [7]. Functionalized polymers may also be effective for this purpose [8]. Some examples of microreactors fabricated with different kind of materials are given in Fig. 2.

Although several different commercial or handmade models are available, the reaction volume in a typical microreactor is about 1,000 μL or less, and the reaction time is determined and regulated by the pump rate [9]. In this condition, the flow is laminar with a low Reynolds number (Section 3.2), and the mixing of reagents typically occurs by diffusion [10].

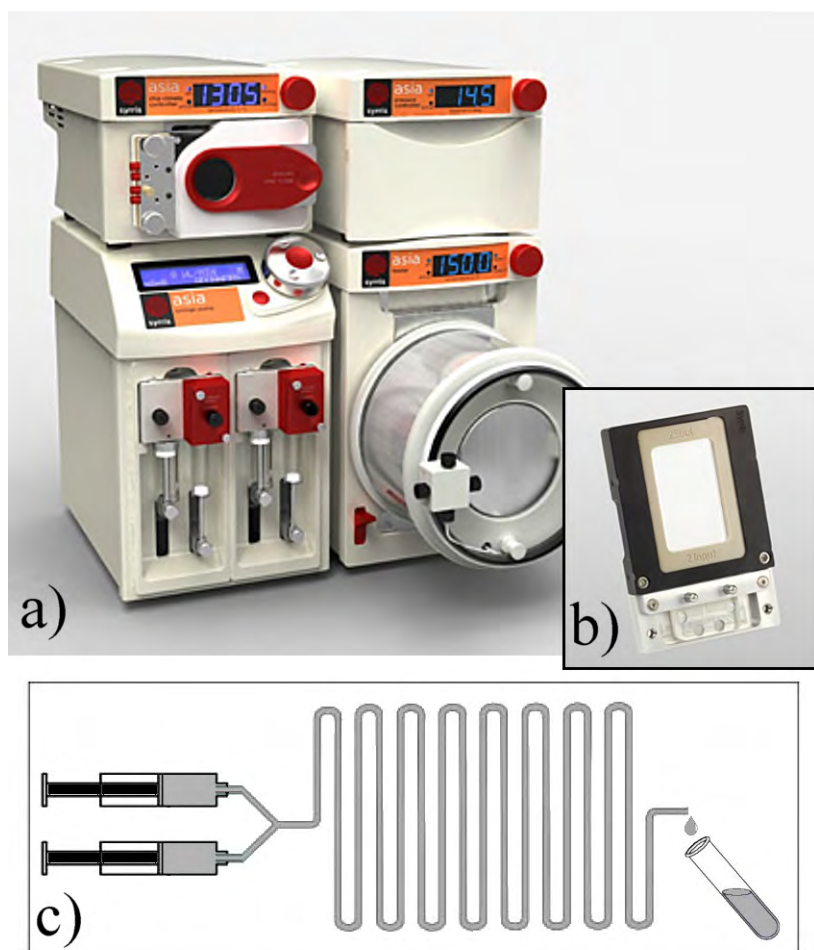


Figure 1: Picture of a commercial microreactor: a) External aspect of Asia120 system by Syrris; b) enlarged view of a glass microreactor; c) Scheme of a microreactor with two input.

Reagent mixing is a crucial phenomenon in a microreactor because it influences the flow profile and the reaction kinetics. Therefore, several different designs of the mixing chamber have been developed, and the corresponding residence time distribution has been analyzed. For example, D. Bošković et al. [11] studied three passive mixing structures with different serpentine designs and their effects on the resulting flow profile and dead volume. On the other hand, the mixing time can be shortened by forcing the collision between reactant fluid streams, by applying shear to the streams and enhancing the number of channel bend, or by dividing the inlet stream in lamellae [12-14]. Furthermore, in some cases, active mixing has been implemented by using electrical and magnetic fluctuant fields or inducing thermal gradients into the microreactor [12,15].

Typically, the reagents are in the liquid phase and their mixing is the result of hydrodynamic pumping by syringe pumps [9], but the apparatus can also be adapted for a

biphasic gas-liquid mixture by introducing an appropriate micromixer and a back pressure control valve (Fig. 3) in order to regulate and maintain the reactor pressure [16,17].

Microreactors are also equipped with a system for temperature control. This is a very important aspect of microreactor design because the typically large surface area/volume ratio allows for rapid heat transfer or removal from the reaction, therefore permitting thermal conditions that are inaccessible with other kind of reactors [5]. Moreover, the enhancement of mass and heat transfer optimize the reaction selectivity [18]. For these reasons, microreactors have proven to be successful in the production of new pharmaceutical compounds [19] or in complex organic synthesis, such as gas-liquid-solid hydrogenations of organic compounds [20]. Finally, studies about micromixing phenomena allow for an understanding of reagent mixing on a large scale [21-23].

Although many papers report in detail the properties of the most recent generation of microreactors and

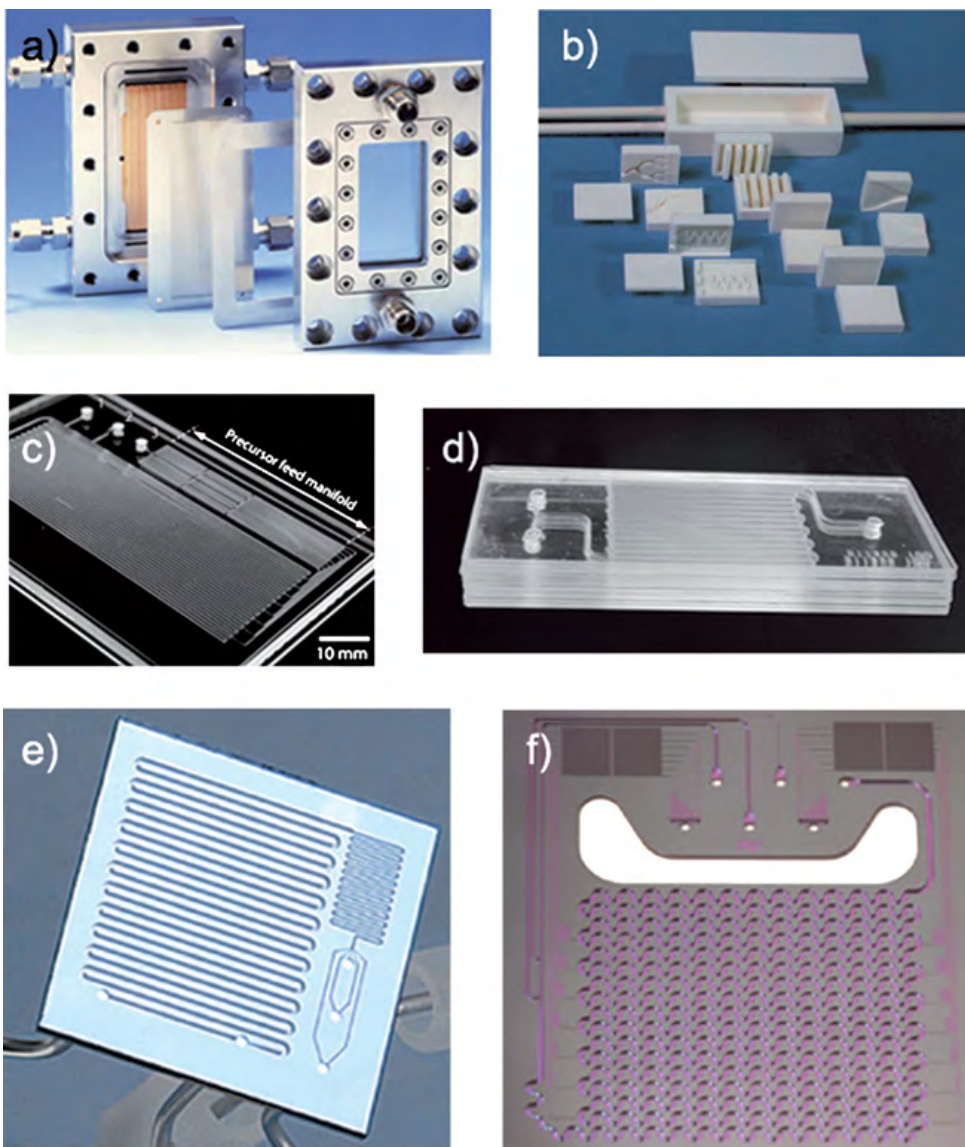


Figure 2: Examples of microreactors fabricated from different materials: a) metal; b) ceramic; c) poly(dimethylsiloxane), d) glass; and e) f) silicon.

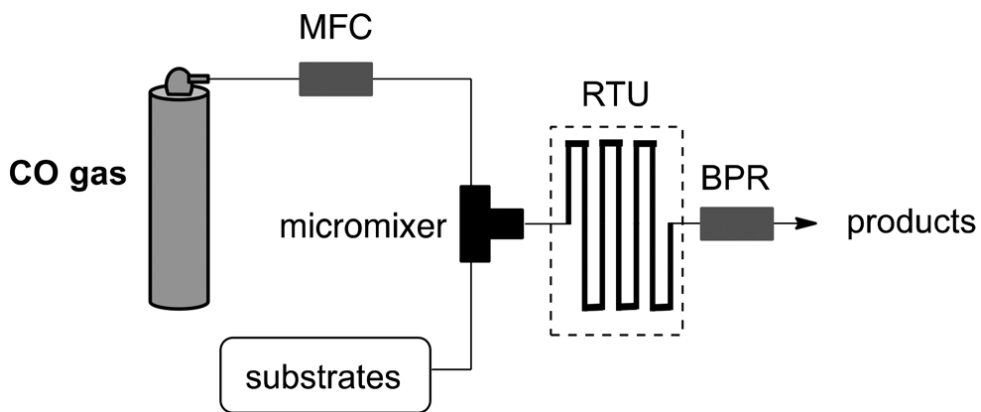


Figure 3: Typical flow reactor system for carbonylation (MFC: mass flow controller; RTU: residence time unit; BPR: back pressure regulator). With permission from reference [17] (license number: 3701431434096).

summarize their applications in various chemical reactions, so far, less attention has been given to corresponding applications in biocatalysis. To address this need, we present an overview of the current leading applications of microreactors in chemistry, with particular focus on biocatalytic applications of microreactors and their specific methodological and technical features.

In contrast to conventional reactors, microreactors display peculiar fluid dynamics. Fast endothermic or exothermic reactions with huge mass and heat transfer is strictly attributed to the microdimensions and design of the reactor. For this reason, this review also provides a general treatment of the physical and mathematical aspects of microreactors and a chemical engineering approach to their fluid dynamics.

2 Microreactors in chemistry

The most significant application of microreactor technology in chemistry is organic or polymer syntheses of a variety of products. For example, microreactors have been used to prepare enantiomerically pure products [24,25], fluoro-organic compounds [26-29], β -peptides [30], tetrahydroisoquinolines [31], and 2-aminothiazoles *via* the

Hantzsch reaction [32]. The Koch-Haaf carboxylation of adamantanol is also described [33].

Furthermore, several polymer chemistry applications are described in the literature, such as the synthesis of linear and branched polymers with different structures, including beads, microcapsules, and fibers [34]. As reviewed by A. Kirschning et al. [35], the use of microreactors in combination with other synthetic techniques, such as microwaves and ionic liquids or other new solvents, permits the development of new synthetic platforms for more efficient and rapid processes (Fig. 4). Two interesting examples are the synthesis in a tubular microreactor of branched polymers [36] and the production of polymer nanoparticles by photopolymerization of methacrylates in a borosilicate microreactor [37]. In the first case, the molecular architecture is controlled by the flow rate, while in the second case, the use of a microreactor leads to a higher reaction yield with a lower irradiation power.

Photochemical applications of continuous-flow microreactors were also recently reviewed by Y. Su et al. [38], taking into account, in particular, the engineering aspects of flow photochemistry, the advantages related to the microreactor technique, and the problems related to scale-up to the industrial level. Technical aspects, such as light sources, reactor materials, and appropriate solvent choice, were also examined and discussed.

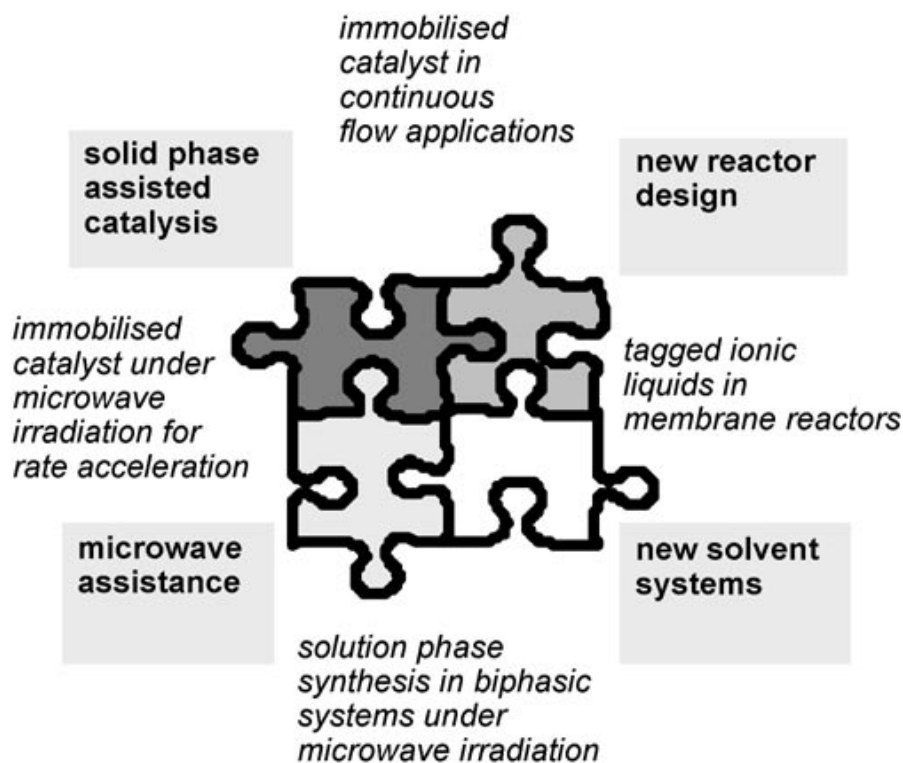


Figure 4: Enabling techniques and selected proposals of possible combinations for developing new synthetic platforms (with special focus on solid-phase assisted catalysis). With permission from reference [35] (license number: 3701440393200).

The final area of application for microreactors in chemistry is energy production. Different strategies have been developed for the synthesis or processing of fuels, where the most notable is the production of molecular hydrogen for fuel cells. Research in this field was extensively reviewed by G. Kolb [39], who focused on the processing of fuels as hydrogen sources for fuel cells and hydrogen storage and methods for the production of methanol, ethanol, and biodiesel.

3 Mathematical and engineering aspects

Microdevices have very different dimensions from conventional equipment. Because the length is on a micrometer scale, the surface term prevails and mass and heat transfer are enhanced. Therefore, the fluid dynamics of microsystems is different from those of industrial- and laboratory-scale equipment, but the same fundamental principles may be applied.

In modeling and simulation in general, a prototype combined with a mathematical model permits a better understanding of a piece of equipment. Therefore, the goal here is to describe, in mathematical terms, a model that may aid in the comprehension of the physical phenomena that occur inside a microreactor. We present a mathematical treatment of homogeneous flow starting from a simple scale analysis, which is sufficient to cover the basic principles of microreactor phenomena. However, more in-depth issues are also described, with examples from the literature.

3.1 Preliminary analysis

Clearly, microreactors have microdimensions. For instance, the volumes of commercial Syrris glass microchips are 62.5, 250, or 1000 μL . The channel depth is from 85 μm to 1,240 μm ; the width, in the reaction section, is about 400 μm ; and the total length is 2 m. In contrast to industrial and laboratory equipment, the channel cross sections are not necessarily circular. Because of the

technology, the channel cross sections can be different shapes, such as rectangular, “oval track” (from oval track racing), and half-oval track (Fig. 5).

The flow rate usually ranges from 1 $\mu\text{L min}^{-1}$ to 10 mL min^{-1} and depends on both the microreactor dimensions and the injection system properties. From the microreactor volume and the flow rate, the residence time can be calculated as a volume-to-flow ratio. The residence time, or time scale, is the fundamental measure for studying kinetics in microreactors [21,40] because they can be considered as flow reactors [41,42].

To study the flow properties, a very simple analysis can be done with dimensionless numbers [12,43,44]. The Reynolds number (Re), as defined by the equation $Re = \rho v d / \mu$, is the relation between the inertial and the viscous forces, where μ is the viscosity in Pa s , ρ the density in kg m^{-3} , v is the velocity based on the actual cross section area of the channel, and d is the diameter of the channel.

In terms of *flow* in a cylindrical channel, laminar flow occurs if Re is less than 2,100. It is necessary to consider the shape of the channel because secondary flow may also occur, for example, in a sinusoidal channel [45] or in a T-shaped microreactor [22]. A low Reynolds number indicates laminar flow, where the viscous forces prevail over inertial forces. In other words, the diffusion prevails over the convection.

Diffusion may be represented by Fick's law, $J_A^* = -D\nabla C_A$, where J_A^* is the molar diffusive flux of the A component, D is the diffusion coefficient or diffusivity, and ∇C_A is the gradient of the molar concentration of A [46]. The coefficient of diffusion has the dimension L^2/time (where L is a characteristic length) and can be viewed in terms of a characteristic dispersion time (t_d), which is the ratio between the square of L and the coefficient of diffusion, $t_d = L^2/D$.

For example, the diffusion coefficient in liquid is about $10^{-5} \text{ cm}^2/\text{s}$, and if a characteristic length of 250 μm is used, the characteristic dispersion time is about 1 minute. However, if a characteristic length of 1 cm is used, t_d will be equal to 100,000 seconds, or approximately 27 hours. Because microreactors are flow reactors, the flow must be considered. The time associated with convection is

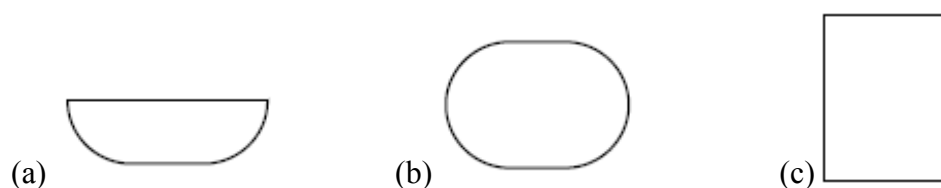


Figure 5: Channel cross section of different Syrris glass microchips (a) 62.5 μL : half oval track; (b) 250 μL : oval track; (c) 1000 μL : rectangular.

the average residence time (t_d), which is calculated by dividing L by the velocity (U). The Fourier number (Fo) is defined as $Fo = t_r/t_{mixing}$, and values that fall within the range $0.1 < Fo < 1.0$ are considered to be good (i.e., there is good mixing for $Fo > 0.1$ and complete mixing for a value of about 1.0) [47]. The Peclet number (Pe) also takes diffusion into account in the relationship: $Pe = u d_H/D$, where u , d_H , and D denote the average velocity, the hydraulic diameter or the transverse diffusion distance (which are assumed to be of the same order of magnitude), and the diffusion coefficient, respectively.

Other dimensionless numbers, like the Bond or Weber numbers, are commonly used in multiphase flows. The Bond number is used to characterize the shape of bubbles and drops, and the Weber number is the ratio of inertia to surface forces. In addition, multiphase microflows may be characterized by the ratio of viscous to surface forces, also known as the capillary number (Ca) [48].

The use of dimensionless numbers in the analysis is sufficient for understanding the fluid dynamics of microdevices. From this analysis, it is possible to identify the best design, or even think about a new design.

3.2 Fluid dynamic characterization

A quantitative study of fluid dynamic behavior may be done through tracer response tests, as first defined by P.V. Danckwerts [49]. Basically, the tracer, a substance that does not interact with the reaction medium, is added at the entrance of the device. At the same time, the concentration of the tracer is monitored at the end of the device. The tracer may be added with three more common types of perturbation: pulse, step, and sinusoidal modification of the tracer feed concentration. The response to the pulse perturbation is the Residence Time Distribution (RTD), which is the distribution of times that each volume element spent in the reactor [50].

The plug flow reactor (PFR) and the batch reactor are the only models in which all the volume elements spend the same time in the reactor. In other reactors, each volume element remains in the reactor for a different period of time, and several distributions of properties can be defined [50].

The tracer perturbation approach may also be applied to microdevices, but a pulse in a microvolume has to be a micropulse and requires special techniques. F. Trachsel and coauthors [51] injected 100 nL of fluorescence-labeled tracer liquid by a piezoelectric actuated injector and monitored the response for the tracer pulse perturbation by means of fluorescence microscopy measurements. In contrast, S. Lohse et al. [52] proposed a non-intrusive

technique involving optical activation of a caged fluorescent dye. Because it is an optical injection, the perturbation can be performed at arbitrary positions in transparent microstructures. Finally, D. Bošković et al. used a spectroscopic technique to measure tracer quantities [11]. In this case, the tracer concentration was calculated by measuring the absorbance simultaneously at the inlet and outlet of the microdevice with two miniature spectrometers.

3.3 Modeling and simulation

Fluid dynamics is the area of physics that covers fundamental principles related to the movement of fluid and its properties. The starting point is the basic equations of transport phenomena (i.e., mass, momentum, and energy balances) [46].

The simplest equation that represents fluid dynamics in tubes is the plug flow (i.e., all elements spend the same time inside the volume),

$$\bar{v}_z \frac{dC_A}{dz} = R_A,$$

where C_A is the molar concentration of species A and \bar{v}_z is the velocity vector along the z-axis (the initial conditions are $z = 0$ and $C_A = C_{A0}$).

It is very common to apply this relationship in chemical reactor engineering to tubular reactors, including the plug flow reactor (PFR). Although the PFR is a very simple model, it can well-represent the experimental data. For example, S.R. Deshmukh et al. compared PFR, continuously stirred tank reactor (CSTR), and computational fluid dynamics (CFD) simulations methods in the Ru/Al₂O₃-catalyzed decomposition of ammonia in an integrated microchemical device [53]. The results show the best approximation of experimental data by the PFR approach. Similarly, G.N. Jovanich et al. studied the dechlorination of *p*-chlorophenol on a Pd/Fe catalyst in a microreactor system, and the ideal plug flow represented the experimental conversions well [54]. On the other hand, V. Burkle-Vitzthum et al. used an annular flow microreactor to study reactions at high temperature (873–1273 K) and very short residence times (10–100 ms), and they found that the fluid dynamics very closely resembled the plug flow reactor [55].

3.3.1 Laminar flow

Microdevices can be seen as microtubes. Assuming a laminar flow for a Newtonian fluid flowing in a cylindrical

tube, the fluid dynamics can be represented by the following equations:

$$\tau_{rz} = \left(\frac{P_0 - P_L}{2L} \right) r \quad (1)$$

$$v_z(r) = \frac{(P_0 - P_L)R^2}{4\mu L} \left[1 - \left(\frac{r}{R} \right)^2 \right] \quad (2)$$

As previously mentioned, microreactors may have other cross-sectional tube shapes. A rectangular cross section is one example, and an approximate solution for velocity is as follows [56,57]:

$$v_z(x, y) = v_{\max} \cdot \left[1 - \left(\frac{x}{a} \right)^s \right] \left[1 - \left(\frac{y}{b} \right)^r \right] \quad (3)$$

In Eq. 3, v_{\max} is the maximum velocity, the width of the channel is equal to $2a$, and the depth of the channel is equal to $2b$, while r and s are parameters that depend on the geometry. A rectangular reactor can be better modeled using the fundamental balances of mass, momentum, and energy. The component of the Cartesian coordinates of the equation of continuity for species A is as follows:

$$\frac{\partial C_A}{\partial t} + v_x \frac{\partial C_A}{\partial x} + v_u \frac{\partial C_A}{\partial y} = D \left(\frac{\partial^2 C_A}{\partial x^2} + \frac{\partial^2 C_A}{\partial y^2} \right) - R_A \quad (4)$$

Where C_A is the concentration of species A, x and y are the Cartesian coordinates, D is the diffusion coefficient, and R_A is the reaction rate. The first term on the left side is the transient term; the second and third are the convective terms of directions x and y , respectively; the term in parentheses on the right-hand side is the diffusional contribution (i.e., the molecular transport); and the last term is the source one. M. Tišma et al. studied the laccase-catalyzed L-DOPA oxidation in a y -shaped continuous flow microreactor [58]. By using this model and the Michaelis-Menten approach to enzyme kinetics, they obtained good agreement between simulations and experimental data.

3.3.2 Non ideal reactor model: Axial dispersion model

The model for a real reactor with one parameter is the axial dispersion model. It considers a plug flow velocity distribution, but some dispersion is added in the direction of the flow. The representative equation is similar to Fick's law:

$$\frac{\partial C_A}{\partial t} + \frac{\partial(C_A v_z)}{\partial z} = D_{axial} \frac{\partial^2 C_A}{\partial z^2} + R_A \quad (5)$$

Although the model appears to only consider deviations by axial mixing, the axial dispersion parameter encompasses non-idealities related to radial mixing, molecular diffusion, and non-ideal velocity profile deviations [59,60].

The axial dispersion model is a good model for low Reynolds numbers [60]. The parameter D_{axial} , which is usually obtained experimentally, may be calculated from molecular diffusion and reactor characteristics from Eq. 6, where d_a is the molecular diffusion of the species A, \bar{v}_z is the average velocity in direction z , and R is the reactor radius.

$$D_{axial} = d_a + \frac{\bar{v}_z^2 R^2}{48 d_a} \quad (6)$$

The model is largely used to represent the RTD curves of tracers (i.e., the fluid dynamics of tracers in reactors). In this case, the tracers should be inert, so the term R_A in Eq. 5 is equal to zero. D. Bošković and S. Loebbecke [61] used an axial dispersed model to fit the measured RTD data. The influence of the ratio of channel depth/channel width in RTD curves and axial dispersion parameters was also studied [62]. S. Schwolow et al. [63] studied the scale-up of a Michael addition reaction in the lab to small-scale commercial production. They used microreactors and the axial dispersion model to evaluate the RTD.

3.4 CFD studies

Computational fluid dynamics (CFD) is a computational tool that solves the balances of mass, momentum, and energy in complex geometries. Mesh generation, turbulence, and system of equations solver are components of CFD. Computational fluid dynamics is a very powerful tool because it can be applied to the study of different phenomena, and the number of papers and patents concerning CFD has significantly grown in recent years [64].

Computational fluid dynamics has been applied to microreactors. For example, Y. Yamaguchi et al. [65] investigated laminar flow in the typical hairpin curves of microreactors, revealing the presence of secondary flow using the software FLUENT; C. Amador et al. used CFD calculations to analyze flow distribution in the mesh microreactor [23]; and X. Li et al. studied the feasibility of a pressure-based recycle flow for the micro-mixing of molecular oxygen in bio-microreactors [66].

A similar approach was used by K.I. Sotowa et al. to evaluate the fluid behavior in two types of deep microchannel reactors and to investigate their performance in the hydrolysis of *o*-nitrophenylgalactopyranoside by β -galactosidase [67]. In addition, W.K. Bodla et al. used CFD analysis to evaluate the biocatalytic transamination for the production of a chiral amine, which revealed that substrate and product diffusion rates were considerably different and depend on the reactor configurations [68].

3.5 Enzyme kinetics

Enzyme kinetics are usually analyzed by starting from a model which takes into account the formation of an enzyme-substrate complex before the formation of the reaction product. Because a differential equation may be used to resolve the rate for each reaction step, a complete analysis of the process results in a system of ordinary differential equations with an initial-value problem; this problem can then be solved by different approaches, such as the Runge-Kutta or Adams-Bashforth-Moulton methods, or Backward Differentiation Formulas (BDF), also known as Gear's method [69].

As in other catalytic processes, the enzyme is not consumed during the reaction. Therefore, the total concentration of the enzyme may be used in the model and if a steady state hypothesis for the enzyme-substrate complex *ES* is assumed, the classic equation proposed by Michaelis-Menten (and later revised by Higgs and Haldane) can be applied, and the enzyme affinity for the substrate can be evaluated by the Michaelis constant (K_M).

Despite the numerous modifications proposed in order to take into account reversible processes or the presence of more than one substrate and inhibitor, this approach it is still largely used to represent enzyme kinetics and is also applied to microreactor systems [58,70,71]. Furthermore, it has been successfully used to compute the steady and laminar mass transfer rates produced by enzymatic reactions [72].

4 Bio-microreactors

One of the advantages of using microreactor technology is the minimum amount of catalyst that is normally required to allow the reaction to occur; this aspect is particularly important when the catalysts are enzymes. Therefore, the possibility of obtaining excellent results using small amounts of enzymes is one of the reasons for the increasing interest in bio-microreactors.

In agreement with the scheme proposed by M. Miyazaki and H. Maeda [73], the fundamental techniques for bio-microreactors can be divided into two main groups: the first includes all continuous flow microreactors with enzymes in solution, and the second pertains to systems containing enzymes immobilized on the microreactor. Because construction features, technical problems, and experimental methods are quite different for the two groups of systems, they are treated separately.

4.1 Bio-microreactors with free enzymes

Some significant examples of bio-microreactors using free enzymes are listed in Table 1. The simpler method consists in continuous flow reactions performed on chip-type commercial microreactors composed of materials like glass or polymers. The reaction begins by introducing both substrate and enzyme solutions from separate inlets, typically by syringe pumps, and proceeds with a continuous flow of reagents for the entire measurement time.

In stopped-flow microreactors, a similar mechanism permits the mixing of reagents, but the reaction rate depends on an applied chemical or physical field, and the flow can be stopped by removing the applied field. An interesting example of this technique is the application of a photothermal laser control system by using an IR diode laser in the near-infrared absorption wavelength of water. In this technique, a direct-radiation heating of the reaction mixture occurred without any effect on the solute's absorption [74,75].

An improvement of the original method is the two-phase flow microchannel system reported by T. Maruyama et al. [76]. In this case, the surface of a glass microchannel was chemically modified with octadecylsilane groups in order to obtain a hydrophobic surface. Then, a two-phase reaction was performed by degrading an isooctane solution of the substrate *p*-chlorophenol by means of laccase dissolved in aqueous buffer. The experimental data show that the glass surface properties and the microchannel dimensions are essential for obtaining a stable two-phase flow. Furthermore, a theoretical model confirms that the substrate is enzymatically degraded at the organic-aqueous interface, and substrate diffusion is the rate-limiting step of the process. This model was an extension of Eq. 4 because the authors considered the mass balance in three dimensions. A similar method was also reported for the synthesis of isoamyl acetate catalyzed by lipase in the organic solvents *n*-hexane and 1-butyl-3-methylpyridinium-dicyanamide [77,78]. Lipase-catalyzed hydrolysis of soybean oil was recently addressed

Table 1: Examples of bio-microreactors with enzymes in solution.

Enzyme(s)	Device material(s)	Results
Alcohol dehydrogenase	Poly(tetrafluoroethylene)	High productivity and easy phase separation are obtained for the enzymatic conversion of 1-heptaldehyde to 1-heptanol in a liquid-liquid phase system [84].
	Glass microchannels	Hexanol oxidation has a 30-fold higher maximum reaction rate in a microreactor than in a cuvette without product inhibition and reverse reaction [85].
Benzoylformate decarboxylase	Glass microchannels	Synthesis of (S)-2-hydroxypropiophenone is studied in three microreactors, obtaining higher productivity compared to an enzyme ultrafiltration membrane reactor (seven-fold) and a batch reactor (five-fold) [86].
β -galactosidase	Poly(methylmethacrylate)	Enzymatic hydrolysis of <i>p</i> -nitrophenyl- β -D-galactopyranoside is five times faster than in batch. The inverse reaction of transglycosylation is also enhanced [87].
Laccase	Glass microchannels	Enzymatic degradation of <i>p</i> -chlorophenol is carried out in a two-phase system with different flow rate and channel shape [76]. In another case, L-DOPA and catechol oxidation are obtained with good reaction rates and high efficiency [88].
Lipase	Glass microchannels	Synthesis of isoamyl acetate is much faster in a two-phases microreactor than in other systems [77, 78].
	Poly(dimethylsiloxane)/plexiglass	Hydrolysis of soybean oil was studied in a continuous microfluidic reaction system. Conversion rate is similar to other published results, but energy consumption is significantly lower [79].
Peroxidase	Glass chip or microchannels	Enzymatic reactions in microchip with ultrafast heating and cooling rates show higher reaction rates and parameter control [74,75,89].
Transketolase	Poly(methylmethacrylate) / poly(dimethylsiloxane)	Modular microfluidic reactors with in-line filtration systems are highly efficient in the synthesis and separation of L-erythrulose [82,83].
Trypsin	Poly(methylmethacrylate)	Hydrolysis of benzoylarginine- <i>p</i> -nitroanilide has reaction rate 20 times greater than in batch [90].

by modifying a microreactor in order to generate a water-oil emulsion in a hydrodynamically controlled slug flow [79]. At the end of the reaction, two microfluidic separators, the first consisting of an array of hydrophobic microchannels and the second using the force of gravity, permit separation of the oil phase from the water solution to recover the products. The results were analyzed using only dimensionless numbers, capillarity, and Reynolds numbers.

The problem of how to separate the reaction mixture components in the final step is an important aspect of microreactor technology. This issue has been resolved in multistep chemical syntheses, where the use of a network of microreactors and separators allows for efficient, continuous synthetic systems [80,81]. The recovery and reuse of enzymes has been achieved using bio-microreactors with immobilized enzymes, a topic discussed later in this paper. Nevertheless, some interesting results were also obtained for continuous flow systems, as in the case of the synthesis of a chiral compound using transketolase [82], where the separation of the enzyme from the other substances was achieved by coupling a tangential flow filtration system to a microfluidic reactor.

Another interesting problem concerning the efficiency

of continuous flow microreactors has been encountered in the transketolase reaction. A lower yield with respect to a typical chemical synthesis was observed with high substrate concentrations, which was attributed to inhibition and denaturation effects induced by the presence of an excess of substrate molecules in the proximity of the enzyme [82]. To overcome this problem, an interesting strategy incorporating a multi-input microfluidic reactor, capable of substrate feeding at multiple points, was designed and successfully applied to the transketolase-catalyzed synthesis of L-erythrulose [83].

4.2 Bio-microreactors with immobilized enzymes

Immobilization of enzymes confers particular advantages in biocatalysis. The possibility of enhancing control of the reaction by separating the catalyst from the reagents and products, preventing contamination of the products by the enzyme, suppressing undesirable side reactions, and reusing the catalyst result in more consistent performance and lower costs in comparison to those previously described. Moreover, miniaturization offers the opportunity to improve

the kinetic performance of enzymes, making these devices particularly attractive for analytical and biotechnological applications [91].

Different kind of materials (e.g., polymers, glass, and silica) have been used as solid supports for enzymes, and the final result depends on the particular material properties, engineering studies, and micro-precision techniques used. All these factors are crucial for improving the system efficiency and contribute to the development of an optimal system, as well as improving the extension of the contact area between enzyme and reagents. In fact, increasing the specific surface of a microreactor is necessary, and techniques such as sol-gel synthesis, electrochemical anodization, zeolite production, membrane deposition, and plasma processes have been used. The ultimate goal is to obtain regular structures without defects in order to improve reaction rates and to avoid the presence of zones that can inhibit the regular flow of substances in the microreactor [92].

In Table 2, examples of bio-microreactors containing immobilized enzymes are summarized. Despite the differences among methods, materials, and enzymes, the most common types of bio-microreactors can be divided in four main categories:

(a) Surface-immobilized enzymes: enzymes are linked to the surface of a pre-constituted microreactor (e.g., by activating the inner channels of a capillary) and exposed to the flow of reagents;

(b) Enzyme activated beads: an appropriate amount of porous beads, previously functionalized with enzymes, are packed together to fill the chamber of a microreactor;

(c) Enzyme-containing monoliths: meso- or macroporous monoliths are coated with a resistant layer or directly prepared in a microchannel and functionalized with enzymes; and

(d) Membranes: enzymes are immobilized onto a selective ultrafiltration membrane.

Table 2: Significant examples of bio-microreactors with immobilized enzyme(s).

Enzyme(s)	Solid support	Methods of enzyme immobilization
Acetylcholinesterase	Graphene oxide/Fe ₃ O ₄ magnetic particles on poly(dimethylsiloxane)	Adsorbed on graphene oxide previously functionalized with Fe ₃ O ₄ by chemical deposition. The system was then packed into a poly(dimethylsiloxane) micro-chip by using an external magnetic field [115].
Alcohol dehydrogenase	Fused-silica capillary	Covalently bound to the inner surface of the capillary by using APTES and GA [130].
Alcohol oxidase	Aminopropyl glass beads	Bound to the beads by reaction with GA and then packed in an acrylic microreactor [109].
Alkaline phosphatase	Polycarbonate and polyethyleneimine	Adsorbed or bound to a polyethyleneimine layer coated on a polycarbonate surface [98].
	Fused-silica capillary	Adsorbed on the capillary wall previously coated with polybrene [128].
Amino acylase	Poly-L-lysine cross-linking on silicon wafer	Cross-linked with poly-L-lysine to generate an enzyme-polymeric membrane on the inner wall of the microchannel [135].
	Poly(glycidyl methacrylate-co-ethylene dimethacrylate)	Covalently bound to the polymer previously formed inside the microreactor channel by photoinitiation [116].
Cytochrome P450 hydroxylase	Ni-NTA agarose beads	Immobilized by reaction with Ni-NTA surface [111].
Fumarase	Glass microtubes	Covalently bound to the inner surface of a microtube by the APTES/GA method [95].
β-Galactosidase	Silica streptavidin-coated microspheres	Conjugated to biotin <i>via</i> amide coupling of aminocaproyl spacer, and then bound to the microbeds [110].
	Glyoxal-agarose on low temperature co-fired ceramics	Directly immobilized on activated glyoxal-agarose gels [112].
	Silica nanosprings	Immobilized by disulfide linkages with SiO ₂ nanosprings, previously activated with APTES, succinimidyl-pyridylidithiopropionate and dithiothreitol [136].
	Multi-walled carbon nanotubes	Coupled to pre-activated nanotubes by reaction with a carbodiimide [137].
β-Glycosidase	Poly(dimethylsiloxane) or γ-Aluminum oxide on stainless steel plate	Bound by the APTES/GA method to stainless steel microstructured plates with different surface layers [104, 138, 139]
Glucose oxidase	Porous silicon	Immobilized by the APTES/GA method on anodized silicon reactors [140, 141].
	Magnetic microparticles	Bound by the APTES/GA method to silica particles previously magnetized by treatment with iron(III) salt [113].

Table 2_{continued}

Enzyme(s)	Solid support	Methods of enzyme immobilization
	Fused-silica capillary	Entrapped in a polyacrylamide-based mixture and bounded to the capillary surface previously activated by γ -methacryl-propyl-trimethoxysilane [119].
	Poly(methyl methacrylate)	Immobilized by GA coupling to polyethyleneimine covered PMMA [101].
Glucose-6-phosphate dehydrogenase	Silica-based hydrogels	Immobilized in a hydrogel obtained by sol-gel method from a mixture containing precursors, APTES and the enzyme [142, 143].
Glutamate dehydrogenase	Fused-silica capillary	Immobilized together with a transaminase by the APTES/GA method [131].
Invertase	Silica monoliths	Immobilized on silica rods previously activated by GA [144].
Laccase	Enzyme-polymeric membrane	Entrapped on the inner surface of microtubes by reaction with poly-L-lysine and cross-linkers [145].
Lactate dehydrogenase	Fused-silica capillary	Bound to the inner surface of electrophoretic capillary by the APTES/GA method [130].
Lipase	Silica micro structured fiber, monolithic network of fused silica capillary, and mesoporous poly(methyl methacrylate)	Immobilized by the APTES/GA method applied to different structures: microstructured fibers [146,147], silica capillaries [148,149], or polymers [150,151].
Organophosphorous hydrolase	Poly(ethylenimine) and poly(styrenesulfonate) on silicon	Immobilized on silicon microchannels by layer-by-layer technique [152].
Peroxidase	Silica streptavidin-coated microspheres	Conjugated to biotin <i>via</i> amide coupling of aminocaproyl spacer, and then bound to the microbeds [110].
	Controlled pore glass	Coupled to alkylamine glass beads by means of GA [96].
	Gold layer on silicon wafers	Covalently immobilized to the surface previously activated by reaction with 11-mercapto undecanoic acid [153].
	Magnetic nanoparticles	Bound by the APTES/GA method to micro and nano-magnetic particles [127].
Transaminase	Silica capillary or agarose beads	Immobilized on capillary glass by the APTES/GA method [131] or on Ni-NTA agarose beds [97,154]
Transketolase	Silica capillary or agarose beds	Covalently bonded to Ni-NTA agarose beds successively packed in glass vials or tubes [97,154,155].
Threonine aldolase	Silica nanosprings and Eupergit CM	Covalently immobilized by different methods [156].
Trypsin	Poly(glycidyl methacrylate-co-ethylene dimethacrylate)	Coupled to the polymer by reaction with GA [125, 157], or by using commercial TPCK-trypsin [100].
	Poly(methyl methacrylate)	Encapsulated in silica gel anchored to the polymer surface as such [99], or modified by zeolite [102].
	Poly-(4-styrenesulfonate)/ β -zeolite multilayer	Introduced in a layer-by-layer multistrata polymer/zeolite structure [103].
	Silica monoliths	Immobilized on silica monoliths by reaction with GA [117,120], or by a thiol-ene strategy [118].
	Controlled pore glass	Covalently immobilized on aminopropylated beads <i>via</i> 1,4-diisothiocyanatobenzene [107].
	Magnetic beads	Bound to magnetic nanoparticles by carbodiimide activation [126].
Tyrosinase	Fused-silica capillary	Immobilized by ionic binding technique with hexadimethrine bromide [129].
Urease	Poly(dimethylsiloxane)	Entrapped in a mono- [105,158,159], or multi-layer [106] polymeric matrix
	Polymer-activated silicon wafer	Bound by a multilayer technique with poly(dimethyldiallyl ammonium chloride) and poly(styrenesulfonate) [106].
	Low temperature co-fired ceramics	Immobilized on commercial glass beads and introduced into the microreactor [108,160].
	Poly(acrylonitrile)	Immobilized on poly(acrylonitrile) supports produced by phase inversion method [161].

In each type of bio-microreactor, enzymes are immobilized onto a solid support by methods such as adsorption, covalent immobilization, or entrapment [93,94]. Some of these procedures are the result of methods previously used in macro-apparatuses, while others have been specifically developed for microstructures and take into account the unique problems related to the small size of these systems.

In the case of surface-immobilized enzymes, each material requires opportune modifications in order to bind a protein. In silica microreactors, the most frequently used method requires activation by (3-aminopropyl) triethoxysilane (APTES) and glutaraldehyde (GA), but analogous reactions with imides or epoxides have also been reported [94-97]. Similar methods were also used to functionalize polycarbonate with a carbodiimide [98] and poly(methyl methacrylate) with several copolymers [99-101].

Poly(methyl methacrylate) was also used as a microchip surface in the immobilization of trypsin after modification with zeolite nanoparticles and sol-gel synthesis [102]. In another case, trypsin was successfully adsorbed onto a poly-(4-styrenesulfonate)/ β -zeolite multilayer [103].

Furthermore, poly(dimethylsiloxane) has been used in a different application, that is, as a material for microreactors. M.S. Thomsen and B. Nidetzky developed a microstructured plate for lactose hydrolysis by immobilizing β -glycosidase via APTES/GA activation [104], while F. Jones et al. affixed polydimethylsiloxane/urease bioplastic on a silicon wafer [105,106]. Bio-microreactors with enzymes immobilized on beads have been prepared by filling the reaction chamber with enzyme-immobilized particles. In this case, various materials were used, such as controlled pore glass [96,107-109], microspheres [110], activated agarose [111,112], and different types of magnetic beads [113-115]. Both covalent immobilization and physical interactions have been used in these systems with good results.

The third type of bio-microreactor concerns inorganic or organic monoliths formed *in situ* from different types of precursors with immobilization strategies similar to those previously described for the production of borosilicate glass [116], silica [117,118], poly(acrylamide) [119] or poly(glycidylmethacrylate-co-ethylenedimethacrylate) [120].

Finally, microreactors that couple enzymatic reactions with membrane separation have been developed. Strictly speaking, in enzymatic membrane microreactors, protein immobilization is not mandatory [86]. In general, however, the immobilization of the enzyme onto the membrane

surface or inside the porous structure is preferred in order to ensure its complete retention and to avoid loss of activity over time [121]. Recently, interesting results were reported for the transesterification of triolein with methanol, catalyzed by lipase in a membrane microreactor with an asymmetric membrane of poly(ethersulfone) [122]. In this example, lipase was immobilized in the sponge layer of the membrane by using an adsorption technique with pressure-driven filtration through the thin layer. The result was a system where the membrane does not act as a selective barrier but rather as a microreactor containing lipase, which enabled the reaction rate to be three times higher than that of native free lipase.

Enzymes play an important role in modern clinical research and diagnosis; therefore, many miniaturized systems have been developed and used in recent years for bioanalytical purposes. The most important example in this field is the use of immobilized enzyme bio-microreactors in capillary electrophoresis [123,124], a well-known analytical technique where an electric field is applied to induce the separation of analytes on the basis of their electrophoretic mobility. Bio-microreactors for capillary electrophoresis are discussed separately in this paper because their preparation requires special approaches. In this case, the main challenge is to optimize the coexistence of the enzymatic reaction with efficient separation of the reaction products.

As recently reviewed by J. Iqbal et al. [123], this problem was solved in two different ways. First, the microreactor and separation capillaries exist as two distinct objects, while in the second, they are united in a single structure. When the two components are separated, both the reaction and separation conditions can be optimized independently, but the correct alignment of the microreactor with the separation capillary is critical. These types of system are mainly used in proteomics research using trypsin or pepsin for protein analysis and peptide mapping [107,125,126] and other appropriate enzymes for nucleic acids studies [123].

Integrated micro bio-reactors were developed by immobilizing the enzyme in the first part of the capillary with the appropriate technique. Despite the increased complexity of construction, this kind of structure is advantageous because it does not have the problems typically associated with the multiple microreactor assembly, such as fluid or electric leakage. These systems have typically been used for kinetic or inhibition studies [127-129] and quantitative determinations of metabolites [119,130,131].

Although different immobilization techniques can lead to good results, some problems remain in

the characterization of the best operative conditions, depending both on the physical effect of mass transfer and the insufficient development of analytical methods [132]. However, new and interesting systems have been proposed for the *in operando* characterization of bio-microreactors, such as opto-chemical sensors for pH and O₂ measurements based on the co-immobilization of luminescent dyes in the microreactor [132], or the so-called “luminescent multiple optical chemical sensors” [133].

Furthermore, from a general perspective, microreactors containing immobilized enzymes can be considered heterogeneous biocatalysts. In agreement with J.M. Bolivar et al. [134], these systems can be studied with convenient techniques (i.e., spectroscopic, opto-chemical, and electrochemical methods) in order to evaluate protein distribution and conformation, and *in operando* stability, activity, and sensing [134].

5 Conclusions

As demonstrated in this review, bio-microreactors represent a very interesting and diverse area of research. They offer many different solutions for chemical and biochemical problems, with the underlying goal to enhance the selectivity and yield of complex reactions.

Nevertheless, as pointed out by J.M. Bolivar and B. Nidetzky, some significant challenges remain [162]. In particular, the development of selective and reversible enzyme immobilization may allow for more versatile systems permitting potential reuse of the unit after the loss of enzymatic activity. Furthermore, improved integration of microreactors with fluid handling and analytical systems could result in fully-automated microreactors. Finally, microreactors could be the solution to the exploitation of the catalytic potential of enzymes in biphasic systems (gas/liquid) and multi-step processes [163].

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References

[1] Mosesman M. A., In situ x-ray diffraction studies of heterogeneous reactions, *J. Am. Chem. Soc.*, 1951, 73, 5635-5639.

- [2] Wohlgenuth R., Plazl I., Žnidaršič-Plazl P., Gernaey K. V., Woodley J. M., *Microscale technology and biocatalytic processes: opportunities and challenges for synthesis*, *Trends Biotechnol.*, 2015, 33, 302-314.
- [3] Hessel V., Kralisch D., Kockmann N., Noël T., Wang Q., *Novel process windows for enabling, accelerating, and uplifting flow chemistry*, *ChemSusChem*, 2013, 6, 746-789.
- [4] Wiles C., Watts P., *Continuous flow reactors, a tool for the modern synthetic chemist*, *Eur. J. Org. Chem.*, 2008, 1655-1671.
- [5] Frost C. G., Mutton L., *Heterogeneous catalytic synthesis using microreactor technology*, *Green Chem.*, 2010, 12, 1687-1703.
- [6] McMullen J. P., Jensen K. F., *Integrated Microreactors for Reaction Automation: New Approaches to Reaction Development*, *Annu. Rev. Anal. Chem.*, 2010, 3, 19-42.
- [7] Brandner J. J., *Microfabrication in metals, ceramics and polymers*, *Russ. J. Gen. Chem.*, 2012, 82, 2025-2033.
- [8] Schönfeld H., Hunger K., Cecilia R., Kunz U., *Enhanced mass transfer using a novel polymer/carrier microreactor*, *Chem. Eng. J.*, 2004, 101, 455-463.
- [9] Watts P., Haswell S. J., *The application of micro reactors for organic synthesis*, *Chem. Soc. Rev.*, 2005, 34, 235-246.
- [10] Sudarsan A. P., Ugaz V. M., *Fluid mixing in planar spiral microchannels*, *Lab. Chip.*, 2006, 6, 74-82.
- [11] Bošković D., Loebbecke S., Gross G. A., Koehler J. M., *Residence Time Distribution Studies in Microfluidic Mixing Structures*, *Chem. Eng. Technol.*, 2011, 34, 361-370.
- [12] Hessel V., Löwe H., Schönfeld F., *Micromixers - A review on passive and active mixing principles*, *Chem. Eng. Sci.*, 2005, 60, 2479-2501.
- [13] Aoki N., Umei R., Yoshida A., Mae K., *Design method for micromixers considering influence of channel confluence and bend on diffusion length*, *Chem. Eng. J.*, 2011, 167, 643-650.
- [14] Marques M. P. C., Fernandes P., *Microfluidic devices: Useful tools for bioprocess intensification*, *Molecules*, 2011, 16, 8368-8401.
- [15] Hartman R. L., McMullen J. P., Jensen K. F., *Deciding Whether To Go with the Flow: Evaluating the Merits of Flow Reactors for Synthesis*, *Angew. Chem. Int. Edit.*, 2011, 50, 7502-7519.
- [16] Fukuyama T., Rahman M. T., Kamata N., Ryu I., *Radical carbonylations using a continuous microflow system*, *Beilstein J. Org. Chem.*, 2009, 5, 34.
- [17] Fukuyama T., Totoki T., Ryu I., *Carbonylation in microflow: Close encounters of CO and reactive species*, *Green Chem.*, 2014, 16, 2042-2050.
- [18] Yoshida J., *Flash chemistry: flow microreactor synthesis based on high-resolution reaction time control*, *Chem. Rec.*, 2010, 10, 332-341.
- [19] Roberge D. M., Ducry L., Bieler N., Cretton P., Zimmermann B., *Microreactor Technology: A Revolution for the Fine Chemical and Pharmaceutical Industries?*, *Chem. Eng. Technol.*, 2005, 28, 318-323.
- [20] Kobayashi J., Mori Y., Okamoto K., Akiyama R., Ueno M., Kitamori T., Kobayashi S., *A Microfluidic Device for Conducting Gas-Liquid-Solid Hydrogenation Reactions*, *Science*, 2004, 304, 1305-1308.
- [21] Aoki N., Hasebe S., Mae K., *Mixing in microreactors: effectiveness of lamination segments as a form of feed on product distribution for multiple reactions*, *Chem. Eng. J.*, 2004, 101, 323-331.

- [22] Bothe D., Stemich C., Warnecke H.-J., Computation of scales and quality of mixing in a T-shaped microreactor, *Comp. Chem. Eng.*, 2008, 32, 108-114.
- [23] Amador C., Wenn D., Shaw J., Gavriilidis A., Angeli P., Design of a mesh microreactor for even flow distribution and narrow residence time distribution, *Chem. Eng. J.*, 2008, 135, Supplement 1, S259-S269.
- [24] Kumaraguru T., Babita P., Sheelu G., Lavanya K., Fadnavis N. W., Synthesis of Enantiomerically Pure 4-Hydroxy-2-cyclopentenones, *Org. Process Res. Dev.*, 2013, 17, 1526-1530.
- [25] Tomida Y., Nagaki A., Yoshida J.-i., Asymmetric Carbolithiation of Conjugated Enynes: A Flow Microreactor Enables the Use of Configurationally Unstable Intermediates before They Epimerize, *J. Am. Chem. Soc.*, 2011, 133, 3744-3747.
- [26] Amii H., Nagaki A., Yoshida J. I., Flow microreactor synthesis in organo-fluorine chemistry, *Beilstein J. Org. Chem.*, 2013, 9, 2793-2802.
- [27] Chambers R. D., Fox M. A., Sandford G., Elemental fluorine. Part 18. Selective direct fluorination of 1,3-ketoesters and 1,3-diketones using gas/liquid microreactor technology, *Lab Chip*, 2005, 5, 1132-1139.
- [28] Chun J. H., Lu S., Lee Y. S., Pike V. W., Fast and high-yield microreactor syntheses of ortho-substituted [(18)F]fluoroarenes from reactions of [(18)F]fluoride ion with diaryliodonium salts, *J Org Chem*, 2010, 75, 3332-3338.
- [29] McMullen J. P., Stone M. T., Buchwald S. L., Jensen K. F., An integrated microreactor system for self-optimization of a Heck reaction: from micro- to mesoscale flow systems, *Angew Chem Int. Ed.*, 2010, 49, 7076-7080.
- [30] Fogel O., Codee J. D., Seebach D., Seeberger P. H., Microreactor synthesis of beta-peptides, *Angew. Chem. Int. Ed.*, 2006, 45, 7000-7003.
- [31] Giovine A., Musio B., Degennaro L., Falcicchio A., Nagaki A., Yoshida J., Luisi R., Synthesis of 1,2,3,4-tetrahydroisoquinolines by microreactor-mediated thermal isomerization of laterally lithiated arylaziridines, *Chem. Eur. J.*, 2013, 19, 1872-1876.
- [32] Garcia-Egido E., Wong S. Y., Warrington B. H., A Hantzsch synthesis of 2-aminothiazoles performed in a heated microreactor system, *Lab Chip*, 2002, 2, 31-33.
- [33] Fukuyama T., Mukai Y., Ryu I., Koch-Haaf reaction of adamantanol in an acid-tolerant hastelloy-made microreactor, *Beilstein J Org Chem*, 2011, 7, 1288-1293.
- [34] Steinbacher J. L., McQuade D. T., Polymer chemistry in flow: New polymers, beads, capsules, and fibers, *J. Polym. Sci. Pol. Chem.*, 2006, 44, 6505-6533.
- [35] Kirschning A., Solodenko W., Mennecke K., Combining enabling techniques in organic synthesis: Continuous flow processes with heterogenized catalysts, *Chem. Eur. J.*, 2006, 12, 5972-5990.
- [36] Bally F., Serra C. A., Brochon C., Hadziioannou G., Synthesis of branched polymers under continuous-flow microprocess: An improvement of the control of macromolecular architectures, *Macromol. Rapid Comm.*, 2011, 32, 1820-1825.
- [37] Lobry E., Jasinski F., Penconi M., Chemtob A., Croutxé-Barghorn C., Oliveros E., Braun A. M., Criqui A., Continuous-flow synthesis of polymer nanoparticles in a microreactor via miniemulsion photopolymerization, *RSC Adv.*, 2014, 4, 43756-43759.
- [38] Su Y., Straathof N. J. W., Hessel V., Noël T., Photochemical transformations accelerated in continuous-flow reactors: Basic concepts and applications, *Chem. Eur. J.*, 2014, 20, 10562-10589.
- [39] Kolb G., Review: Microstructured reactors for distributed and renewable production of fuels and electrical energy, *Chem. Eng. Proc. Process Intensif.*, 2013, 65, 1-44.
- [40] Ajmera S. K., Losey M. W., Jensen K. F., Schmidt M. A., Microfabricated packed-bed reactor for phosgene synthesis, *AIChE J.*, 2001, 47, 1639-1647.
- [41] Knapkiewicz P., Skowerski K., Jaskólska D. E., Barbasiewicz M., Olszewski T. K., Nitration Under Continuous Flow Conditions: Convenient Synthesis of 2-Isopropoxy-5-nitrobenzaldehyde, an Important Building Block in the Preparation of Nitro-Substituted Hoveyda-Grubbs Metathesis Catalyst, *Org. Process Res. Dev.*, 2012, 16, 1430-1435.
- [42] Junior I. I., Flores M. C., Sutili F. K., Leite S. G. F., de M. Miranda L. S., Leal I. C. R., de Souza R. O. M. A., Lipase-Catalyzed Monostearin Synthesis under Continuous Flow Conditions, *Org. Process Res. Dev.*, 2012, 16, 1098-1101.
- [43] Hartman R. L., Jensen K. F., Microchemical systems for continuous-flow synthesis, *Lab Chip*, 2009, 9, 2495-2507.
- [44] Commenge J. M., Falk L., Corriou J. P., Matlosz M., Analysis of microstructured reactor characteristics for process miniaturization and intensification, *Chem. Eng. Technol.*, 2005, 28, 446-458.
- [45] Metwally H. M., Manglik R. M., Enhanced heat transfer due to curvature-induced lateral vortices in laminar flows in sinusoidal corrugated-plate channels, *Int. J. Heat Mass Transfer*, 2004, 47, 2283-2292.
- [46] Bird R. B., Stewart, W.E., Lightfoot, E.N., *Transport Phenomena*, Revised 2nd Edition, 2007, John Wiley & Sons.
- [47] Burns J. R., Ramshaw C., Development of a microreactor for chemical production, *Chem. Eng. Res. Des.*, 1999, 77, 206-211.
- [48] Gunther A., Jensen K. F., Multiphase microfluidics: from flow characteristics to chemical and materials synthesis, *Lab Chip*, 2006, 6, 1487-1503.
- [49] Danckwerts P. V., Continuous flow systems: Distribution of residence times, *Chem. Eng. Sci.*, 1953, 2, 1-13.
- [50] Fogler H. S., *Elements of Chemical Reaction Engineering*. 2005: Prentice Hall.
- [51] Trachsel F., Günther A., Khan S., Jensen K. F., Measurement of residence time distribution in microfluidic systems, *Chem. Eng. Sci.*, 2005, 60, 5729-5737.
- [52] Lohse S., Kohnen B. T., Janasek D., Dittrich P. S., Franzke J., Agar D. W., A novel method for determining residence time distribution in intricately structured microreactors, *Lab Chip*, 2008, 8, 431-438.
- [53] Deshmukh S. R., Mhadeshwar A. B., Vlachos D. G., Microreactor Modeling for Hydrogen Production from Ammonia Decomposition on Ruthenium, *Ind. Eng. Chem. Res.*, 2004, 43, 2986-2999.
- [54] Jovanovic G. N., Žnidaršič Plazl P., Sakrithichai P., Al-Khaldi K., Dechlorination of p-Chlorophenol in a Microreactor with Bimetallic Pd/Fe Catalyst, *Ind. Eng. Chem. Res.*, 2005, 44, 5099-5106.
- [55] Burkle-Vitzthum V., Moulis F., Zhang J., Commenge J.-M., Schaefer E., Marquaire P.-M., Annular flow microreactor: An efficient tool for kinetic studies in gas phase at very short residence times, *Chem. Eng. Res. Des.*, 2015, 94, 611-623.

- [56] Purday H. F. P., An introduction to the mechanics of viscous flow; film lubrication, the flow of heat by conduction and heat transfer by convection, 1949, Dover Publications.
- [57] Hardt S., Microreactors – Modeling and Simulation, in *Ullmann's Encyclopedia of Industrial Chemistry*. 2011, Wiley-VCH Verlag GmbH & Co. KGaA.
- [58] Tišma M., Zelič B., Vasić-Rački D., Žnidaršič-Plazl P., Plazl I., Modelling of laccase-catalyzed l-DOPA oxidation in a microreactor, *Chem. Eng. J.*, 2009, 149, 383-388.
- [59] Levenspiel O., *Chemical Reaction Engineering*, 3rd Edition. 1998: John Wiley & Sons.
- [60] Nauman E. B., *Chemical Reactor Design, Optimization, and Scaleup*, 4th Edition, 2008, John Wiley & Sons.
- [61] Bošković D., Loebbecke S., Modelling of the residence time distribution in micromixers, *Chem. Eng. J.*, 2008, 135, Supplement 1, S138-S146.
- [62] Aubin J., Prat L., Xuereb C., Gourdon C., Effect of microchannel aspect ratio on residence time distributions and the axial dispersion coefficient, *Chem. Eng. Proc. Process Intensif.*, 2009, 48, 554-559.
- [63] Schwolow S., Heikenwälder B., Abahmane L., Kockmann N., Röder T., Kinetic and Scale-up Investigations of a Michael Addition in Microreactors, *Org. Process Res. Dev.*, 2014, 18, 1535-1544.
- [64] Islabão G. I. d., Pinto J. C. C. d. S., Vianna Júnior A. d. S., Technological Trends in CFD Applications, *J. Technol. Manage. Innov.*, 2010, 5, 76-83.
- [65] Yamaguchi Y., Takagi F., Yamashita K., Nakamura H., Maeda H., Sotowa K., Kusakabe K., Yamasaki Y., Morooka S., 3-D simulation and visualization of laminar flow in a microchannel with hair-pin curves, *AIChE J.*, 2004, 50, 1530-1535.
- [66] Li X., van der Steen G., van Dedem G. W. K., van der Wielen L. A. M., van Leeuwen M., van Gulik W. M., Heijnen J. J., Krommenhoek E. E., Gardeniens J. G. E., van den Berg A., Ottens M., Improving mixing in microreactors, *Chem. Eng. Sci.*, 2008, 63, 3036-3046.
- [67] Sotowa K. I., Takagi K., Sugiyama S., Fluid flow behavior and the rate of an enzyme reaction in deep microchannel reactor under high-throughput condition, *Chem. Eng. J.*, 2008, 135, S30-S36.
- [68] Bodla V. K., Seerup R., Krühne U., Woodley J. M., Gernaey K. V., Microreactors and CFD as Tools for Biocatalysis Reactor Design: A case study, *Chem. Eng. Technol.*, 2013, 36, 1017-1026.
- [69] Faires J. D., Burden R. L., *Numerical Methods*. 4th ed. 2012: Brooks Cole, Cengage Learning.
- [70] Song H., Ismagilov R. F., Millisecond Kinetics on a Microfluidic Chip Using Nanoliters of Reagents, *J. Am. Chem. Soc.*, 2003, 125, 14613-14619.
- [71] Baronas R., Ivanauskas F., Kulys J., Mathematical Modeling of Biosensors Based on an Array of Enzyme Microreactors, *Sensors*, 2006, 6, 453-465.
- [72] Pallares J., Ferré J. A., A simple model to predict mass transfer rates and kinetics of biochemical and biomedical Michaelis-Menten surface reactions, *Int. J. Heat Mass Transfer*, 2015, 80, 192-198.
- [73] Miyazaki M., Maeda H., Microchannel enzyme reactors and their applications for processing, *Trends Biotechnol.*, 2006, 24, 463-470.
- [74] Tanaka Y., Slyadnev M. N., Hibara A., Tokeshi M., Kitamori T., Non-contact photothermal control of enzyme reactions on a microchip by using a compact diode laser, *J. Chromatogr. A*, 2000, 894, 45-51.
- [75] Slyadnev M. N., Tanaka Y., Tokeshi M., Kitamori T., Photothermal temperature control of a chemical reaction on a microchip using an infrared diode laser, *Anal. Chem.*, 2001, 73, 4037-4044.
- [76] Maruyama T., Uchida J.-i., Ohkawa T., Futami T., Katayama K., Nishizawa K.-i., Sotowa K.-i., Kubota F., Kamiya N., Goto M., Enzymatic degradation of p-chlorophenol in a two-phase flow microchannel system, *Lab Chip*, 2003, 3, 308-312.
- [77] Žnidaršič-Plazl P., Plazl I., Modelling and experimental studies on lipase-catalyzed isoamyl acetate synthesis in a microreactor, *Process Biochem.*, 2009, 44, 1115-1121.
- [78] Žnidaršič-Plazl P., Pohar A., Plazl I. Theoretical and experimental studies of enzyme-catalyzed isoamyl acetate synthesis with ionic liquid at the microreactor scale. in *Chem. Eng. Trans.*, 2009, 17, 1077-1082.
- [79] Čech J., Schrott W., Slouka Z., Pfištyl M., Brož M., Kuncová G., Šnita D., Enzyme hydrolysis of soybean oil in a slug flow microsystem, *Biochem. Eng. J.*, 2012, 67, 194-202.
- [80] Sahoo H. R., Kralj J. G., Jensen K. F., Multistep continuous-flow microchemical synthesis involving multiple reactions and separations, *Angew. Chem. Int. Edit.*, 2007, 119, 5806-5810.
- [81] Hartman R. L., Naber J. R., Buchwald S. L., Jensen K. F., Multistep Microchemical Synthesis Enabled by Microfluidic Distillation, *Angew. Chem. Int. Edit.*, 2010, 49, 899-903.
- [82] O'Sullivan B., Al-Bahrani H., Lawrence J., Campos M., Cázares A., Baganz F., Wohlgemuth R., Hailes H. C., Szita N., Modular microfluidic reactor and inline filtration system for the biocatalytic synthesis of chiral metabolites, *J. Mol. Catal. B: Enzym.*, 2012, 77, 1-8.
- [83] Lawrence J., O'Sullivan B., Lye G. J., Wohlgemuth R., Szita N., Microfluidic multi-input reactor for biocatalytic synthesis using transketolase, *J. Mol. Catal. B: Enzym.*, 2013, 95, 111-117.
- [84] Karande R., Schmid A., Buehler K., Miniaturizing biocatalysis: Enzyme-catalyzed reactions in an aqueous/organic segmented flow capillary microreactor, *Adv. Synth. Catal.*, 2011, 353, 2511-2521.
- [85] Tušek A., Šalić A., Kurtanjek Ž., Zelič B., Modeling and kinetic parameter estimation of alcohol dehydrogenase-catalyzed hexanol oxidation in a microreactor, *Eng. Life Sci.*, 2012, 12, 49-56.
- [86] Valinger D., Vrsalović Presečki A., Kurtanjek Ž., Pohl M., Findrik Blažević Z., Vasić-Rački D., Continuous enzymatic carbonylation of benzaldehyde and acetaldehyde in an enzyme ultrafiltration membrane reactor and laminar flow microreactors, *J. Mol. Catal. B: Enzym.*, 2014, 102, 132-137.
- [87] Kanno K.-i., Maeda H., Izumo S., Ikuno M., Takeshita K., Tashiro A., Fujii M., Rapid enzymatic transglycosylation and oligosaccharide synthesis in a microchip reactor, *Lab Chip*, 2002, 2, 15-18.
- [88] Tušek A. J., Tišma M., Bregović V., Ptičar A., Kurtanjek Z., Zelič B., Enhancement of phenolic compounds oxidation using laccase from *Trametes versicolor* in a microreactor, *Biotechnol. Bioprocess Eng.*, 2013, 18, 686-696.
- [89] Tanaka Y., Slyadnev M. N., Sato K., Tokeshi M., Kim H. B., Kitamori T., Acceleration of an enzymatic reaction in a microchip, *Anal. Sci.*, 2001, 17, 809-810.

- [90] Miyazaki M., Nakamura H., Maeda H., Improved yield of enzyme reaction in microchannel reactor, *Chem. Lett.*, 2001, 442-443.
- [91] Matosevic S., Szita N., Baganz F., Fundamentals and applications of immobilized microfluidic enzymatic reactors, *J. Chem. Technol. Biotechnol.*, 2011, 86, 325-334.
- [92] Hereijgers J., Desmet G., Breugelmans T., De Malsche W., Strategies to integrate porous layers in microfluidic devices, *Microelectron. Eng.*, 2015, 132, 1-13.
- [93] Asanomi Y., Yamaguchi H., Miyazaki M., Maeda H., Enzyme-immobilized microfluidic process reactors, *Molecules*, 2011, 16, 6041-6059.
- [94] Wong L. S., Khan F., Micklefield J., Selective covalent protein immobilization: Strategies and applications, *Chem. Rev.*, 2009, 109, 4025-4053.
- [95] Stojković G., Plazl I., Žnidaršič-Plazl P., L-Malic acid production within a microreactor with surface immobilised fumarase, *Microfluid. Nanofluid.*, 2011, 10, 627-635.
- [96] Vojinović V., Esteves F. M. F., Cabral J. M. S., Fonseca L. P., Biocatalytic analytical microreactors for glucose, lactate, ethanol, galactose and l-amino acid monitoring in cell culture media, *Anal. Chim. Acta*, 2006, 565, 240-249.
- [97] Matosevic S., Lye G. J., Baganz F., Immobilised enzyme microreactor for screening of multi-step bioconversions: Characterisation of a de novo transketolase- ω -transaminase pathway to synthesise chiral amino alcohols, *J. Biotechnol.*, 2011, 155, 320-329.
- [98] Ogończyk D., Jankowski P., Garstecki P., Functionalization of polycarbonate with proteins; Open-tubular enzymatic microreactors, *Lab Chip*, 2012, 12, 2743-2748.
- [99] Qu H., Wang H., Huang Y., Zhong W., Lu H., Kong J., Yang P., Liu B., Stable microstructured network for protein patterning on a plastic microfluidic channel: Strategy and characterization of on-chip enzyme microreactors, *Anal. Chem.*, 2004, 76, 6426-6433.
- [100] Křenková J., Bilková Z., Foret F., Characterization of a monolithic immobilized trypsin microreactor with on-line coupling to ESI-MS, *J. Sep. Sci.*, 2005, 28, 1675-1684.
- [101] Cerqueira M. R. F., Grasseschi D., Matos R. C., Angnes L., A novel functionalisation process for glucose oxidase immobilisation in poly(methyl methacrylate) microchannels in a flow system for amperometric determinations, *Talanta*, 2014, 126, 20-26.
- [102] Huang Y., Shan W., Liu B., Liu Y., Zhang Y., Zhao Y., Lu H., Tang Y., Yang P., Zeolite nanoparticle modified microchip reactor for efficient protein digestion, *Lab. Chip*, 2006, 6, 534-539.
- [103] Ji J., Zhang Y., Zhou X., Kong J., Tang Y., Liu B., Enhanced protein digestion through the confinement of nanozeolite-assembled microchip reactors, *Anal. Chem.*, 2008, 80, 2457-2463.
- [104] Thomsen M. S., Nidetzky B., Microfluidic reactor for continuous flow biotransformations with immobilized enzymes: The example of lactose hydrolysis by a hyperthermophilic β -glycoside hydrolase, *Eng. Life Sci.*, 2008, 8, 40-48.
- [105] Jones F., Lu Z., Elmore B. B., Development of novel microscale system as immobilized enzyme bioreactor, *Appl. Biochem. Biotechnol. A Enz. Eng. Biotechnol.*, 2002, 98-100, 627-640.
- [106] Jones F., Forrest S., Palmer J., Lu Z., Elmore J., Elmore B. B., Immobilized enzyme studies in a microscale bioreactor, *Appl. Biochem. Biotechnol. A Enz. Eng. Biotechnol.*, 2004, 113, 261-272.
- [107] Dartiguenave C., Hamad H., Waldron K. C., Immobilization of trypsin onto 1,4-diisothiocyanatobenzene-activated porous glass for microreactor-based peptide mapping by capillary electrophoresis: Effect of calcium ions on the immobilization procedure, *Anal. Chim. Acta*, 2010, 663, 198-205.
- [108] Malecha K., Pijanowska D. G., Golonka L. J., Torbicz W., LTCC microreactor for urea determination in biological fluids, *Sens. Actuators B*, 2009, 141, 301-308.
- [109] Alhadeff E. M., Salgado A. M., Cós O., Pereira Jr N., Valero F., Valdman B., Integrated biosensor systems for ethanol analysis, *Appl. Biochem. Biotechnol.*, 2008, 146, 129-136.
- [110] Seong G. H., Heo J., Crooks R. M., Measurement of enzyme kinetics using a continuous-flow microfluidic system, *Anal. Chem.*, 2003, 75, 3161-3167.
- [111] Srinivasan A., Bach H., Sherman D. H., Dordick J. S., Bacterial P450-catalyzed polyketide hydroxylation on a microfluidic platform, *Biotechnol. Bioeng.*, 2004, 88, 528-535.
- [112] Baeza M., Lopez C., Alonso J., Lopez-Santin J., Alvaro G., Ceramic microsystem incorporating a microreactor with immobilized biocatalyst for enzymatic spectrophotometric assays, *Anal. Chem.*, 2010, 82, 1006-1011.
- [113] Nomura A., Shin S., Mehdi O. O., Kauffmann J. M., Preparation, characterization, and application of an enzyme-immobilized magnetic microreactor for flow injection analysis, *Anal. Chem.*, 2004, 76, 5498-5502.
- [114] Li Y., Xu X., Yan B., Deng C., Yu W., Yang P., Zhang X., Microchip reactor packed with metal-ion chelated magnetic silica microspheres for highly efficient proteolysis, *J. Proteome Res.*, 2007, 6, 2367-2375.
- [115] Liang R. P., Wang X. N., Liu C. M., Meng X. Y., Qiu J. D., Construction of graphene oxide magnetic nanocomposites-based on-chip enzymatic microreactor for ultrasensitive pesticide detection, *J. Chromatogr. A*, 2013, 1315, 28-35.
- [116] Ngamsom B., Hickey A. M., Greenway G. M., Littlechild J. A., Watts P., Wiles C., Development of a high throughput screening tool for biotransformations utilising a thermophilic l-aminoacylase enzyme, *J. Mol. Catal. B: Enzym.*, 2010, 63, 81-86.
- [117] Ma J., Liang Z., Qiao X., Deng Q., Tao D., Zhang L., Zhang Y., Organic-inorganic hybrid silica monolith based immobilized trypsin reactor with high enzymatic activity, *Anal. Chem.*, 2008, 80, 2949-2956.
- [118] Chen Y., Wu M., Wang K., Chen B., Yao S., Zou H., Nie L., Vinyl functionalized silica hybrid monolith-based trypsin microreactor for on line digestion and separation via thiol-ene "click" strategy, *J. Chromatogr. A*, 2011, 1218, 7982-7988.
- [119] Mersal G. A. M., Bilitewski U., Development of monolithic enzymatic reactors in glass microchips for the quantitative determination of enzyme substrates using the example of glucose determination via immobilized glucose oxidase, *Electrophoresis*, 2005, 26, 2303-2312.
- [120] Feng S., Ye M., Jiang X., Jin W., Zou H., Coupling the immobilized trypsin microreactor of monolithic capillary with μ RPLC-MS/MS for shotgun proteome analysis, *J. Proteome Res.*, 2006, 5, 422-428.
- [121] Rios G. M., Belleville M. P., Paolucci D., Sanchez J., Progress in enzymatic membrane reactors - A review, *J. Membrane Sci.*, 2004, 242, 189-196.

- [122] Machsun A. L., Gozan M., Nasikin M., Setyahadi S., Yoo Y. J., Membrane microreactor in biocatalytic transesterification of triolein for biodiesel production, *Biotechnol. Bioprocess Eng.*, 2010, 15, 911-916.
- [123] Iqbal J., Iqbal S., Müller C. E., Advances in immobilized enzyme microreactors in capillary electrophoresis, *Analyst*, 2013, 138, 3104-3116.
- [124] Wang X., Li K., Adams E., Schepdael A. V., Recent advances in CE-mediated microanalysis for enzyme study, *Electrophoresis*, 2014, 35, 119-127.
- [125] Schoenherr R. M., Ye M., Vannatta M., Dovichi N. J., CE-microreactor-CE-MS/MS for protein analysis, *Anal. Chem.*, 2007, 79, 2230-2238.
- [126] Li Y., Wojcik R., Dovichi N. J., A replaceable microreactor for on-line protein digestion in a two-dimensional capillary electrophoresis system with tandem mass spectrometry detection, *J. Chromatogr. A*, 2011, 1218, 2007-2011.
- [127] Yu D., Van Antwerpen P., Patris S., Blankert B., Kauffmann J. M., Enzyme immobilized magnetic nanoparticles for in-line capillary electrophoresis and drug biotransformation studies: Application to paracetamol, *Comb. Chem. High T. Scr.*, 2010, 13, 455-460.
- [128] Iqbal J., An enzyme immobilized microassay in capillary electrophoresis for characterization and inhibition studies of alkaline phosphatases, *Anal. Biochem.*, 2011, 414, 226-231.
- [129] Jiang T. F., Liang T. T., Wang Y. H., Zhang W. H., Lv Z. H., Immobilized capillary tyrosinase microreactor for inhibitor screening in natural extracts by capillary electrophoresis, *J. Pharm. Biomed. Anal.*, 2013, 84, 36-40.
- [130] Simonet B. M., Ríos A., Valcárcel M., Analytical potential of enzyme-coated capillary reactors in capillary zone electrophoresis, *Electrophoresis*, 2004, 25, 50-56.
- [131] Yang L., Shi J., Chen C., Wang S., Zhu L., Xie W., Guo L., Dual-enzyme, co-immobilized capillary microreactor combined with substrate recycling for high-sensitive glutamate determination based on CE, *Electrophoresis*, 2009, 30, 3527-3533.
- [132] Bolivar J. M., Consolati T., Mayr T., Nidetzky B., Shine a light on immobilized enzymes: Real-time sensing in solid supported biocatalysts, *Trends Biotechnol.*, 2013, 31, 194-203.
- [133] Stich M. I. J., Fischer L. H., Wolfbeis O. S., Multiple fluorescent chemical sensing and imaging, *Chem. Soc. Rev.*, 2010, 39, 3102-3114.
- [134] Bolivar J. M., Eisl I., Nidetzky B., Advanced characterization of immobilized enzymes as heterogeneous biocatalysts, *Catal. Today*, 2016, 259, Part 1, 66-80.
- [135] Honda T., Miyazaki M., Nakamura H., Maeda H., Facile preparation of an enzyme-immobilized microreactor using a cross-linking enzyme membrane on a microchannel surface, *Adv. Synth. Catal.*, 2006, 348, 2163-2171.
- [136] Schilke K. F., Wilson K. L., Cantrell T., Corti G., McLroy D. N., Kelly C., A novel enzymatic microreactor with *Aspergillus oryzae* β -galactosidase immobilized on silicon dioxide nanosprings, *Biotechnol. Progress*, 2010, 26, 1597-1605.
- [137] Song Y. S., Shin H. Y., Lee J. Y., Park C., Kim S. W., β -Galactosidase-immobilised microreactor fabricated using a novel technique for enzyme immobilisation and its application for continuous synthesis of lactulose, *Food Chem.*, 2012, 133, 611-617.
- [138] Schwarz A., Thomsen M. S., Nidetzky B., Enzymatic synthesis of β -glucosylglycerol using a continuous-flow microreactor containing thermostable β -glycoside hydrolase celB immobilized on coated microchannel walls, *Biotechnol. Bioeng.*, 2009, 103, 865-872.
- [139] Thomsen M. S., Nidetzky B., Coated-wall microreactor for continuous biocatalytic transformations using immobilized enzymes, *Biotechnol. J.*, 2009, 4, 98-107.
- [140] Drott J., Lindström K., Rosengren L., Laurell T., Porous silicon as the carrier matrix in microstructured enzyme reactors yielding high enzyme activities, *J. Micromech. Microeng.*, 1997, 7, 14-23.
- [141] Bengtsson M., Ekström S., Marko-Varga G., Laurell T., Improved performance in silicon enzyme microreactors obtained by homogeneous porous silicon carrier matrix, *Talanta*, 2002, 56, 341-353.
- [142] Cumana S., Simons J., Liese A., Hilterhaus L., Smirnova I., Immobilization of glucose 6-phosphate dehydrogenase in silica-based hydrogels: A comparative study, *J. Mol. Catal. B: Enzym.*, 2013, 85-86, 220-228.
- [143] Cumana S., Ardao I., Zeng A. P., Smirnova I., Glucose-6-phosphate dehydrogenase encapsulated in silica-based hydrogels for operation in a microreactor, *Eng. Life Sci.*, 2014, 14, 170-179.
- [144] Szymańska K., Pudło W., Mrowiec-Białoń J., Czardybon A., Kocurek J., Jarzębski A. B., Immobilization of invertase on silica monoliths with hierarchical pore structure to obtain continuous flow enzymatic microreactors of high performance, *Micropor. Mesopor. Mat.*, 2013, 170, 75-82.
- [145] Lloret L., Eibes G., Moreira M. T., Feijoo G., Lema J. M., Miyazaki M., Improving the catalytic performance of laccase using a novel continuous-flow microreactor, *Chem. Eng. J.*, 2013, 223, 497-506.
- [146] Tuan Anuar S., Villegas C., Mugo S. M., Curtis J. M., The development of flow-through bio-catalyst microreactors from silica micro structured fibers for lipid transformations, *Lipids*, 2011, 46, 545-555.
- [147] Mugo S. M., Ayton K., Lipase immobilized microstructured fiber based flow-through microreactor for facile lipid transformations, *J. Mol. Catal. B: Enzym.*, 2010, 67, 202-207.
- [148] Anuar S. T., Zhao Y. Y., Mugo S. M., Curtis J. M., The development of a capillary microreactor for transesterification reactions using lipase immobilized onto a silica monolith, *J. Mol. Catal. B: Enzym.*, 2013, 92, 62-70.
- [149] Kaneno J., Kohama R., Miyazaki M., Uehara M., Kanno K., Fujii M., Shimizu H., Maeda H., Development of surface modification method and its application for preparation of enzyme-immobilized microreactor, *Kagaku Kogaku Ronbun.*, 2004, 30, 154-158.
- [150] Bhangale A. S., Beers K. L., Gross R. A., Enzyme-catalyzed polymerization of end-functionalized polymers in a microreactor, *Macromolecules*, 2012, 45, 7000-7008.
- [151] Denčić I., De Vaan S., Noël T., Meuldijk J., De Croon M., Hessel V., Lipase-based biocatalytic flow process in a packed-bed microreactor, *Ind. Eng. Chem. Res.*, 2013, 52, 10951-10960.
- [152] Forrest S. R., Elmore B. B., Palmer J. D., Activity and lifetime of organophosphorous hydrolase (OPH) immobilized using layer-by-layer nano self-assembly on silicon microchannels, *Catal. Today*, 2007, 120, 30-34.

- [153] Tudorache M., Mahalu D., Teodorescu C., Stan R., Bala C., Parvulescu V. I., Biocatalytic microreactor incorporating HRP anchored on micro-/nano-lithographic patterns for flow oxidation of phenols, *J. Mol. Catal. B: Enzym.*, 2011, 69, 133-139.
- [154] Abdul Halim A., Szita N., Baganz F., Characterization and multi-step transketolase- ω -transaminase bioconversions in an immobilized enzyme microreactor (IEMR) with packed tube, *J. Biotechnol.*, 2013, 168, 567-575.
- [155] Matosevic S., Lye G. J., Baganz F., Design and characterization of a prototype enzyme microreactor: Quantification of immobilized transketolase kinetics, *Biotechnol. Progress*, 2010, 26, 118-126.
- [156] Fu H., Dencic I., Tibhe J., Sanchez Pedraza C. A., Wang Q., Noel T., Meuldijk J., de Croon M., Hessel V., Weizenmann N., Oeser T., Kinkeade T., Hyatt D., Van Roy S., Dejonghe W., Diels L., Threonine aldolase immobilization on different supports for engineering of productive, cost-efficient enzymatic microreactors, *Chem. Eng. J.*, 2012, 207-208, 564-576.
- [157] Ye M., Hu S., Schoenherr R. M., Dovichi N. J., On-line protein digestion and peptide mapping by capillary electrophoresis with post-column labeling for laser-induced fluorescence detection, *Electrophoresis*, 2004, 25, 1319-1326.
- [158] Elmore B., Besser R., Lu Z., Forrest A., Jiang R., Jones F. Heterogeneous catalysis in a micro scale reactor fabricated from a biologically active polymer. in *Proceedings of SPIE - The International Society for Optical Engineering*. 2001.
- [159] Tang X., Liu S., Wang S., Zhang Q., Cheng Z., Preparation of reversibly immobilized Jack bean urease on microchannel surface and application for enzyme inhibition assay, *Microfluid. Nanofluid.*, 2014, 17, 721-728.
- [160] Malecha K., Pijanowska D., Golonka L., Torbicz W., LTCC enzymatic microreactor, *J. Microelectron. Electron. Packag.*, 2007, 4, 51-56.
- [161] Łukowska E., Pijanowska D., Chwojnowski A., A new polymeric support for enzyme immobilization for bioreactors, *Pol. J. Chem.*, 2008, 82, 1265-1272.
- [162] Bolivar J. M., Nidetzky B., Smart enzyme immobilization in microstructured reactors, *Chim. Oggi*, 2013, 31, 50-54.
- [163] Bolivar J. M., Wiesbauer J., Nidetzky B., Biotransformations in microstructured reactors: more than flowing with the stream?, *Trends Biotechnol.*, 2011, 29, 333-342.