

## Review

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# Multiphase biotransformations in microstructured reactors: opportunities for biocatalytic process intensification and smart flow processing

**Abstract:** Enzymes are gaining increased importance as highly selective catalysts for green chemical synthesis. Multiphase microreaction systems are emerging tools for the development of enzyme-catalysed transformations involving two or more partly immiscible fluids in continuous flow. Mass transfer intensification due to miniaturisation of the flow dimensions and the associated enlargement of the interfacial area presents a powerful approach of effective reaction rate enhancement and thus reactor productivity increase for smart flow bioprocessing. Use of microstructured flow reactors for the study of multiphase (gas-liquid, liquid-liquid) biocatalytic conversions is reviewed. Multiphase flow characterisation based on dimensionless scaling parameters and flow-regime categorisation is presented with emphasis on the different flows applied to experimental studies of enzymatic reactions. Development of instrumented microsystems, flow instabilities, fast inactivation of biocatalysts and low conversion rates are problems often encountered with biotransformation under multiphase flow conditions. Key parameters controlling reaction performance are discussed along with some guidelines for design of scalable multiphase biocatalytic microreactors. Opportunities for biocatalytic process intensification are revealed in examples from fine chemical and materials synthesis.

**Keywords:** biocatalysis; flow synthesis; microchannel; multi-phase flow; process intensification.

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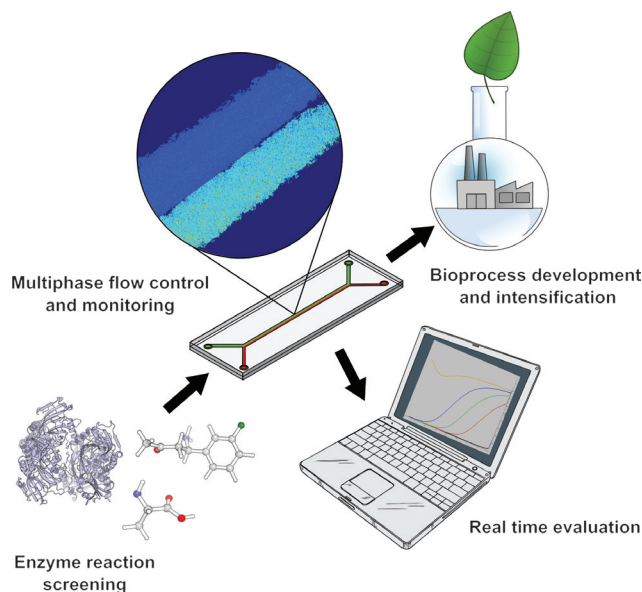
## 1 Introduction

Microstructured reactors are widely recognised as important engineering tools for chemical process research and development [1–8]. They are distinguished from conventional reactor types by the presence of an internal three-dimensional structure, which typically consists of microchannel networks less than one millimetre wide. Generally (not exclusively though), pressure is applied to drive fluid flow through the microchannels, and the typical operation mode of a microstructured reactor is therefore continuous rather than batchwise [9–13]. The flows in microchannels are usually directed, highly symmetric and mostly laminar. Microstructured reactors have been attributed with a great (and diverse) number of advantages over conventional reactors, the most compelling of which are related to intensifications of the mass and heat transfer, having their origin in order-of-magnitude enhancements of surface-to-volume ratio and hence reduced diffusion distances due to geometrical effects [1–8]. To achieve similarly significant reductions in characteristic diffusion distance, in a stirred tank reactor for example, the energy dissipation rate would have to be increased quite substantially through suitable (and often impractical) adjustments of the impeller speed. It has also been shown that by matching microreactor fluidics to the specific requirements of a chemical transformation, a variety of reaction performance parameters (e.g., selectivity) can be improved, not just the effective rate [1, 3, 4, 14]. Accurate intrinsic kinetic data of a chemical transformation, needed during process development at different scales, may be more effectively obtained through experiments in a microstructured reactor than experiments in a shaken microwell or a shaken flask [4, 8].

*Transport intensifications* realised in microstructured reactors potentially translate into significantly reduced material and energy inputs in the process, improved process control and safety and reduced size of apparatus, thereby contributing to implementation of green

chemistry principles at large [9, 15–20]. By application of modularity concept, scale-out of apparatus can occur with high predictability and so the overall development time may be shortened. Development of continuous processes as opposed to processes in batch is currently pursued with high priority in the chemical and pharmaceutical industries, and microreaction technology adopts a leading role in promoting the rethinking of processes from batch to flow [1, 21–29]. Interestingly, the scope of process intensification achievable in microstructured flow reactors has recently been extended to include chemical intensification and process design intensification as two new fields of development [7, 15, 16]. While chemical intensification involves deliberate application of unusually harsh reaction conditions to completely establish new process windows, process design intensification addresses issues such as reduction of process complexity, integration of unit operations and unified utility in modular plants [7, 15, 30–34]. This paper focuses on the application of microstructured flow reactors to a special area of growing importance in chemical process development where biocatalysts (enzymes, non-growing cells) are used to achieve the transformation of interest.

Biocatalysis has become an important enabling technology for synthetic chemistry and chemical process development [35–41]. Enzymes are highly specific catalysts that are usually able to discriminate strictly among different substrate structures (substrate specificity), different reactive groups on the substrate molecule (regioselectivity), and different enantiomeric forms during substrate utilisation and product formation (enantioselectivity) [36, 42]. Moreover, enzymes are usually highly specific for catalysing one particular type of chemical reaction, thereby preventing other (chemically favored) reactions from taking place [36, 42]. Transformations, which are exceedingly difficult to carry out chemically, may thus become possible through the use of biocatalysis. Reduction of carbon-carbon double bonds in  $\alpha$ -unsaturated aldehydes by enone reductases presents an interesting example [43, 44]. In terms of the three fields of process intensification referred to above, the use of enzymes is expected to contribute to chemical process development through chemical intensification by making novel process windows accessible and to process design intensification, by reducing the number of process steps or by improving sustainability metrics, for example [41, 45, 46]. Microreaction technology holds promise to enable successful biocatalytic process development, and its use is being considered in various ways and at different points along the development chain [47–51]. Figure 1 illustrates the application of microstructured reactors for bioprocess development.



**Figure 1** Instrumented multiphase microstructured reactors are powerful tools for biocatalytic process development, providing potential process intensification at different phases of the development chain.

In the field of chemical engineering, flow processes with microstructured reactors are believed to enable shorter development time to production scale. Considering the increasing importance of speed in chemical and pharmaceutical process development, process biocatalysis is confronted with the problem that its development times are usually longer than in other fields of process chemistry [48, 52–54]. Implementation of microreaction technology was therefore thought to support process biocatalysis in responding efficiently to the current needs of accelerated process development and catalyst design [47, 48, 53, 55]. Potential benefits can be categorised broadly according to the development phase in which exploitation takes place. Early on, the use of highly automated and fully instrumented microstructured flow reactors facilitates catalyst and/or reaction screening; evaluation of immobilised catalysts; analysis of intrinsic reaction kinetics to be used later for process optimisation, scale-up and process control; detailed characterization of transport effects on effective kinetics; and determination of enzyme operational stability [47, 49, 56–70]. Opportunities offered by flow microreactors in this phase are different from, and therefore complement the scope of, well-established screening apparatus designed according to the principle of parallelised batch reactors, such as for example, the shaken 96-well plate. At a later stage, microstructured reactors enable flexible production at defined quality and variable scale, thus supporting the product evaluation [24, 25, 47]. Finally, process intensification realised at the

production scale to generate a clear economic benefit justifies plant design, at least partly, based on microreaction technology [9, 15, 16, 47]. A key question therefore concerns the potential for process intensification resulting from the use of microstructured flow reactors in biocatalytic transformations [47, 50]. Rigorous analysis reveals that not all intensification fields described above are equally well applicable [47, 50].

Considering that enzymatic reactions are usually characterised by slow intrinsic kinetics in solution and operate at ambient temperature without generating a large excess of heat, transport intensification will be relevant only in selected cases, typically where contacting or mixing of two or more partially immiscible fluids is a major issue [47]. Homogeneous single-phase enzymatic conversions that are not subject to mixing limitations apparently prevent transport intensification from being carried out in microstructured reactors. However, the poor solubility of organic substrates and products often necessitates biocatalytic reactions to be performed in the presence of a second organic phase next to the aqueous phase [42]. For the most part, therefore, this review will focus on multiphase biotransformations in microreactors, this being a field where the authors recognise the clear potential of the technology to drive development. Because biocatalysts are typically insufficiently stable under very harsh reaction conditions, chemical intensification of enzymatic transformations may often not be possible. However, application of high pressure holds promise to become effectively used, in shifting reaction equilibria for example [71]. Only a few attempts of chemical intensification in biocatalytic conversions have been presented to date [72, 73]. Demonstration of process design intensification provided by flow processes in microstructured reactors will necessitate comprehensive comparison of competing process options, carefully analysing each process in the entirety of steps involved. It is expected that industry will adopt a leading role in such investigations. At this time, however, available evidence appears to be insufficient to comment on the possible role of process design intensification in microstructured reactors for biocatalytic process development. Chemo-enzymatic cascade transformations are often referred to in this context and certainly present an interesting, currently however rather untapped, field of application [74, 75].

In this paper we review multiphase biotransformations in microstructured flow reactors in three parts. Firstly multiphase flow characterisation based on dimensionless scaling parameters and flow-regime categorisation is presented, with emphasis on the different flows applied to experimental studies of enzymatic reactions.

Secondly, the state of the art in the use of microstructured flow reactors for the study of multiphase (gas-liquid, liquid-liquid) biocatalytic conversions is described. Thirdly, major challenges and opportunities for future work in the field are pointed out.

## 2 Multiphase flow characteristics in microstructured reactors

Multiphase flows are generated when two or more partially immiscible fluids are brought into contact. A useful categorisation distinguishes between gas-liquid and liquid-liquid multiphase flows [76, 77]. Heterogeneous catalytic reactions are often encountered in process biocatalysis where immobilised enzymes typically constitute the preferred form of catalyst [36, 78, 79]. Heterogeneously catalysed conversions, therefore, involve gas-liquid-solid or liquid-liquid-solid systems [3, 77]. Multiphase flow systems contain the biocatalyst dissolved in the aqueous liquid phase, adsorbed at the fluids interface, or supported on a solid phase (e.g., microreactor walls; microchannel fixtures; microparticles assembled in microchannel) [47, 49–51, 80]. In liquid-liquid multiphase reactions biocatalytic conversion takes place in the water phase at the solid-liquid interface or occurs directly at the fluids interface [47, 49–51, 80]. In gas-liquid reactions, gaseous substrate usually requires transport into the aqueous liquid phase where it reacts upon contact with soluble or surface-immobilised enzyme [47, 77].

Before discussing the different forms of multiphase flow created in microstructured reactors, it is first of all important to note that the purpose of introducing an immiscible fluid to the main liquid phase is not solely the enhancement of mass transfer across phase boundaries. The performance of single-phase microfluidic systems can be improved when, due to the presence of a second immiscible fluid stream, mixing is increased and Taylor (axial) dispersion is reduced [76]. The rate of mixing and mass transfer limited reactions is enhanced, and the residence time distribution is narrowed due to the reduced dispersion [76]. Moreover, multiphase flows can be applied to prevent one fluid from direct contact with microchannel walls, thereby preventing undesired surface processes from taking place (e.g., enzyme inactivation due to contact with an organic phase; clogging of channels due to deposition of solid material).

Multiphase flows typically exhibit high order between the phases, reflecting the dominance of surface and interface forces on the flow pattern [76, 81].

Multiphase liquid-liquid or gas-liquid flows in microstructured reactors can be distinguished by the principles that are utilised for bringing the phases into contact [77]. Continuous-phase contacting is characterised by non-dispersed phases with large specific interfaces [77]. Dispersed-phase contacting involves dispersion of one phase into the other phase, leading to formation of liquid droplets or gas bubbles [76, 77, 82]. The actual flow pattern is the result of a complex interplay between different forces [76, 83, 84]. There are a variety of possible fluid phase distributions, and the flow may be strongly dynamic and unstable. Interfacial forces shape the often highly regular and well-defined fluid-fluid interfaces and act on microchannel walls. Flow in microchannels is furthermore strongly affected by gravity, by inertia, and also by viscous forces. Multiphase flows through microchannels are considerably different from flows through larger channels or pipes: viscous ( $\sim \mu U/d$ ) and interfacial ( $\sim \sigma/d$ ) forces, both inversely proportional to the hydraulic diameter,  $d$ , are usually more important than inertial ( $\sim \rho U^2$ ) and gravitational ( $\sim \rho gh$ ) forces. Here  $\mu$  is the dynamic viscosity,  $U$  is the characteristic velocity,  $\sigma$  is surface tension,  $\rho$  is fluid density,  $g$  is gravity constant, and  $h$  is height. For microchannels of rectangular geometry with height  $h$  and cross-section  $b$ ,  $d$  is obtained with the relationship  $d = 2bh/(b+2h)$ . Interfacial forces give rise to multiple flow regimes resulting from deformation of the fluid interface and are responsible for flow segmentation into dispersed bubbles or droplets. The multiphase flow properties in microchannels depend on three groups of parameters: channel geometry; physico-chemical properties of the fluids, and flow velocities. These factors can be described by some important dimensionless parameters, related to static (steady state) and dynamic (transient, unsteady) flow conditions.

Under static conditions, the shape of fluid-fluid interfaces is related to capillary pressure. Wetting properties of the fluids affect direct interaction with microchannel walls and can be altered through chemical modification of the wall surface. The bond number ( $Bo$ ) expresses the relative importance of gravitational and interfacial forces.

$$Bo = \frac{\Delta \rho g d^2}{\sigma}$$

$\Delta \rho$  is density difference between the fluids. At microscale ( $d \leq 1$  mm), the Bond number is usually much lower than unity. Fluid velocity ( $U$ ) adds another factor influencing system behavior [50, 81, 83, 84]. The Reynolds number ( $Re$ ) describes the relative importance of inertial forces to viscous forces.

$$Re = \frac{\rho d U}{\mu}$$

As already pointed out above, the flows in microchannels are mostly laminar, related to  $Re < 2100$ . However, microchannel reactors with high mass throughput for production operate in a transitional region between laminar to turbulent flow ( $Re > 4000$ ). The Capillary number ( $Ca$ ) relates viscous force to interfacial tension.

$$Ca = \frac{\mu U}{\sigma}$$

Capillary number together with the ratio of fluid viscosities is key for characterising multiphase flow at microscale. The Weber number ( $We$ ) represents the ratio of inertial and surface tension forces. It is obtained as the product of Reynolds and Capillary number. Values of  $We$  in the range of some hundreds are usually obtained in microreactors. The Weber number is useful for analysing thin flows as well as the formation of droplets and bubbles [50, 81, 83, 84]. Another important dimensionless parameter based on  $Ca$  and  $Re$  is the Ohnesorge number ( $Oh$ ), which expresses the relative importance of viscous forces in relation to inertial and interfacial forces. Fluid density ratio and fluid flow rate ratio are also used for flow characterisation. Experimental techniques applied to flow characterisation are diverse and have been reviewed elsewhere [76, 85].

Multiphase flow patterns can be divided in two major flow categories where the two phases are segregated (segmented flow), or a stable parallel interface is encountered through the microchannel (continuous flow). Segmented flows include slug, bubbly, annular, wavy and droplet flow regimes in dispersed-phase microreactors (Figure 2A) [76, 82, 86]. Continuous flows are obtained by proper selection of individual fluid flows. Continuous flows are stabilised by suitable microchannel wall surface properties, enabling the decoupling of complex multiphase flows into regular single-phase subsystems [76, 84, 87–89]. In this way gas-liquid and liquid-liquid streams can be guided in co- and counter-flowing configurations. In continuous flow two parallel streams are flowing, both phases are continuous, and mass transfer across phase boundaries is due to diffusion only. Even though a relatively low interfacial area is formed under these conditions, continuous flow offers the advantage so that phase separation is readily achieved [1, 89–92]. However, the majority of multiphase flow applications in microstructured reactor exploit segmented flows. It should be noted that flow instabilities present an important problem (but also a design opportunity) of

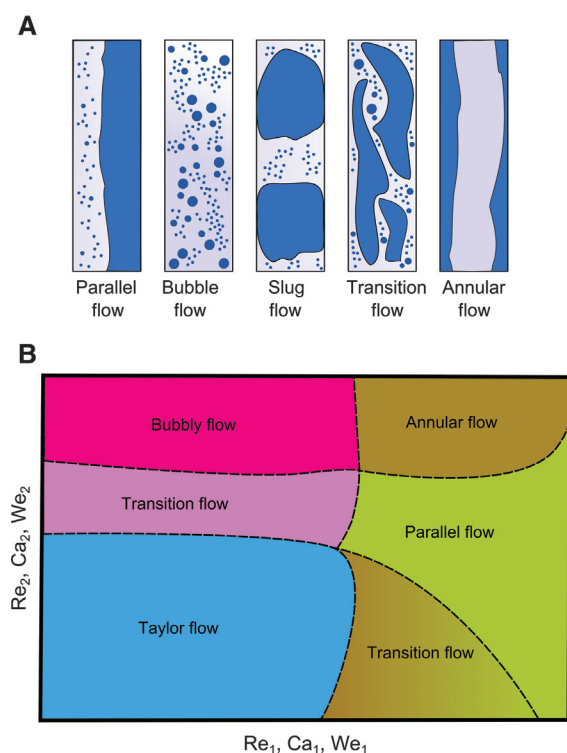


dispersed-phase microreactor operation. The most stable slug (Taylor) flow is therefore often applied. In Taylor flow, series of slugs of one phase are flowing separated by the other phase [76, 82, 86, 93]. Each slug serves as an individual processing sub-volume. Mass transport is due to convection within each slug and diffusion between two adjacent slugs. A relatively high interfacial area is produced in the slug flow regime, and this can be adjusted in a given microreactor by flow rate variation. Phase separation however becomes more difficult [77].

Flow-regime diagrams are used to categorise the types of flow occurring in gas-liquid or liquid-liquid multiphase microreactors (Figure 2B) [76, 82, 94–96]. The diagrams correlate flow rates of the respective two phases. Based on experimental evidence, flow regimes are assigned to certain areas of the diagram. Operating conditions for chemical microprocessing in multiphase systems can be associated with certain flow regimes [76, 82, 94, 95]. More work will be needed to obtain similar flow-regime assignment for different types of biocatalytic conversion.

A variety of microstructured devices are available for the purpose of multiphase contacting [9, 11]. Aside from considering in detail the phases involved, microreactor selection is also dictated by specific requirements of the

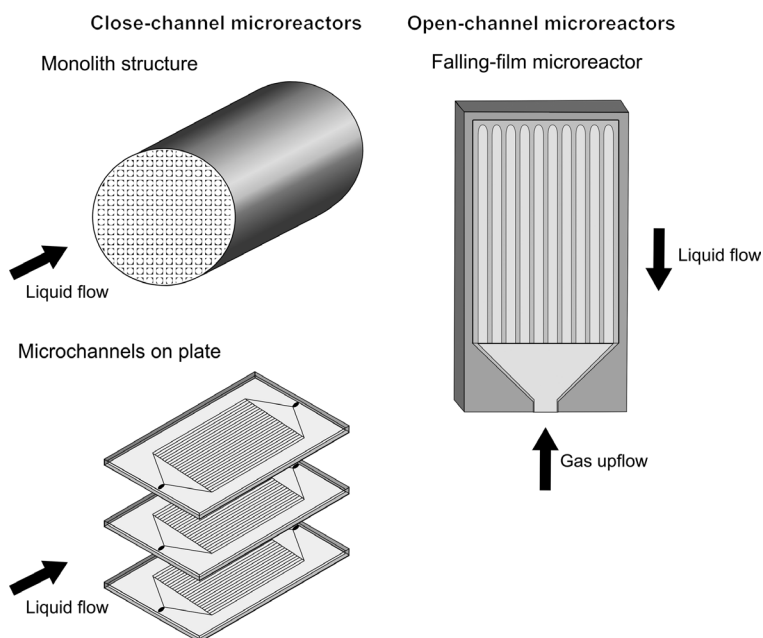
chemical reaction and, in this case, it must also allow for suitable integration of the biocatalyst in soluble or immobilised insoluble form [77]. Phase separation and general work-up considerations are important. Reported multiphase microreactors are classified along the categories mentioned above, enabling dispersed or continuous phase contacting in gas-liquid and liquid-liquid systems [77, 95]. Figure 3 depicts common microstructures used in these reactors. A number of reactors having catalyst deposited as a solid on the internal microstructure surface (micro-channel walls; internal fixtures) have been described [3]. Microchannels reactors fabricated on plate or from capillary tubes and reactors prepared from monolith structures are generally useful for dispersed-phase contacting [9, 11, 77]. Among these, microbubble columns and microbed reactors are special designs. Falling film microreactors are applied to create continuous gas-liquid flows [9, 11, 77]. Microchannel reactors can be further subdivided according to the microstructures used for bringing the phases into contact. We have overlapping-channel and mesh contactors; Y or T shaped contactors; microparticles assembled in microchannels, and multichannel contactors with intermediate redispersion units [50, 77, 84]. Hydrodynamics, mass transfer, pressure drop, and residence time distribution were analysed carefully in different gas-liquid and liquid-liquid microstructured reactors, and the aggregated evidence was presented comprehensively in recent reviews [94, 95]. Not unexpectedly, it is concluded that mass transfer across phase boundaries can be enhanced by up to three magnitude orders in microstructured reactors as compared to conventional chemical reactors. Segmented flows appear to be reasonably well characterised and they are generally quite suitable for flow chemical processing [82, 97, 98].



**Figure 2** Multiphase flow pattern (A) and flow-regime diagram (B) in multiphase microstructured reactors.

### 3 Opportunities for transport intensification in enzymatic reactions in microstructured reactors

Process intensification was originally defined as dramatic ( $\geq 100$ -fold) reduction of the physical size of a reactor while achieving a given production objective. The definition was later extended also to consider improvements of key features of chemical processing such as yield and selectivity [7, 15, 16, 31]. One way of achieving process intensification is transport intensification, which may be realised by matching fluidics of apparatus



**Figure 3** Microstructured elements widely used for phase contacting in microstructured flow reactors.

to the physico-chemical requirements of a reaction. Relevant evidence will be obtained from rigorous comparison of different reactor geometries, and definition of a characteristic time-scale  $\tau_{op}$  for the biocatalytic reaction is useful to perform a meaningful analysis [99]. The process is then characterised by its number of transfer units  $NTU = \tau/\tau_{op}$  where  $\tau = L/U$  is the mean residence time in the reactor, defined by channel length  $L$  and average velocity  $U$  [99]. Biocatalytic reactors operated at identical  $NTU$  show the same chemical efficiency. For homogeneous reactions in which both the substrate and the enzyme are soluble,  $\tau_{op}$  is proportional to the reciprocal catalytic constant of the enzymatic conversion. It is independent of channel diameter and therefore, a global process intensification due to size reduction, i.e., decrease in channel diameter  $d$ , is impossible in this case. For heterogeneously catalysed conversions we can distinguish situations where  $\tau_{op}$  is governed by mass transfer or surface reaction. The operation time-scale is proportional to  $d^2$  for mass transfer-limited processes while it is proportional to  $d$  when the reaction is rate-limiting [99]. Miniaturisation and the accompanying reduction of volume are therefore favorable for reactions catalysed by surface immobilised enzymes. In multiphase reactions where transport across phase boundaries or mixing is rate limiting,  $\tau_{op}$  is likewise dependent on  $d$  and miniaturisation is therefore effective. A useful way of representing the relative importance of reaction and transport effects is through the dimensionless Damköhler number,  $Da$ , which is the reaction rate divided by the transport rate

[50]. The characteristic time of transport  $\tau_{tr}$  is inversely proportional to molecular diffusivity and it scales with diffusion distance according to  $d^2$ . The Peclet number ( $Pe$ ) describes the ratio between mass transport by convection and diffusion.

For miniaturisation to be practical, it is clearly important to consider the pressure drop ( $\Delta P$ ) resulting from reduction of channel dimensions as it increases in proportionality to  $d^4$ - $d^6$ . One can estimate  $\Delta P$  from the relationship  $\Delta P = \lambda_f (L/d) (\rho/2) w^2$  where  $\lambda_f$  is the channel friction factor,  $\rho$  is fluid density, and  $w$  is the mean flow velocity ( $\text{ms}^{-1}$ ) [99]. For laminar flow,  $\lambda_f$  is given by the relationship  $\lambda_f = C_f/Re$  where  $C_f$  is 64 for circular and 56 for rectangular cross sections. However, decrease in  $d$  at constant reactor efficiency does not necessarily imply an increase in  $\Delta P$ . Careful analysis for heterogeneously catalysed gas phase reactions has shown that  $\Delta P$  can be kept constant or even decreased when the number of channels ( $N_c$ ) is increased, while still reducing total reactor volume ( $V$ ) [99]. It has to be kept in mind though, that flow distribution to a large number of microchannels may not be completely uniform and at any rate, it necessitates manifolds that occupy a considerable volume. The reader is referred to useful considerations for the design of microstructured reactors in the literature [9–11]. From the discussion just made it becomes clear that only in selected cases of enzyme catalysed transformations, presumably those involving transport across phase boundaries, will miniaturisation to microscale potentially result in substantial process intensification.

However, the microscale process alternative should always be compared rigorously to the conventional options. One particular advantage of a microstructured reactor could be directly related to the enhancement of a defined specific interfacial area increasing overall performance of reactor [77, 94]. Moreover, due to the characteristic properties of multiphase flow in microchannels where, once bubbles or droplets have been formed, they typically can no longer coalesce, no additional energy is needed for bubble/droplet break-up [97]. This stands in contrast to conventional process equipment where enhancement of chemical conversion per unit volume is usually obtained at the expense of intensified (mechanical) energy dissipation per unit volume. The same mass-transfer behavior can thus be obtained in a multiphase flow microreactor at substantially reduced power input [1, 77, 94, 97]. Interestingly and remarkably, Kreutzer et al. showed that gas-liquid-solid mass transfer in a monolith biocatalytic reactor was even improved with decreased energy input [97]. Using a simple scaling analysis, involving viscous pressure drop, hydrostatic pressure drop, interfacial pressure drop, and penetration theory for mass transfer, it was demonstrated that two-phase laminar bubble-train flow in small channels can exhibit better mass transfer for a given power input than turbulent contactors [82, 97, 98]. It was shown that the resulting flow pattern was applicable to a reaction catalysed at the walls of the capillary channel, and the mass transfer was actually improved by reducing the amount of energy dissipated in the system. In that way, two prime goals of process intensification could be achieved at the same time, namely reduction of the energy requirement and reduction of equipment size.

Finally, the possibility of integrating chemical reaction with product work-up in microreactors is of considerable interest [1]. At microscale, the Bond number usually has a value much lower than unity. Therefore, this implies that methods of separation applied in microstructured devices will not explore gravity and therefore be often fundamentally different from those utilised in conventional apparatus. Continuous separation of two immiscible phases, contacted for reaction either by dispersion or in the form of single-phase subsystems, was achieved at the microscale by a variety of techniques [1] including flow focusing [90], manipulation of surface wetting characteristics [89, 90, 100], and by using capillary forces [91, 92]. In the last case, the immiscible liquids in dispersed flow are separated by flow across a capillary membrane. In other cases, the flow is stabilised in two defined phases by using specific geometries, guides, or surface patterning at the end of microreactor [84, 88].

## 4 Multiphase flow biotransformations in microstructured reactors

Application of biocatalysis to organic chemical synthesis often involves change from purely aqueous systems where enzymes are normally best active and stable, to non-conventional multiphase systems comprising a partly water immiscible fluid [36]. Very few enzymes are active in a low-water environment, and to our knowledge no enzyme has so far been shown to display activity in a completely anhydrous state [42]. True gas-phase reactions as often found in heterogeneous chemical catalysis are therefore not readily accessible to biocatalysis. The non-aqueous fluid used in biocatalysis can be an organic solvent not participating in the reaction or it can be the hydrophobic, hence poorly water-soluble substrate or product of the enzymatic transformation [36]. Multiphase conditions facilitate substrate supply, product removal or both through *in situ* extraction between the aqueous and organic phase. Possible benefits are manifold, but mostly productivity (space-time-yield) enhancement is targeted. There are also few examples of insoluble substrate and/or precipitated product that may be present as solid material during the reaction, but we will not consider these in the following discussion. Solid materials synthesis by polymerisation under flow in biocatalytic microreactors is however an interesting application to be described later [101]. In an alternative biocatalytic application requiring gas-liquid multiphase flow,  $O_2$  is cosubstrate of the enzymatic reaction and must be supplied from the gas phase [44].  $H_2$  dependent biocatalytic reductions have also attracted interest in recent years [102].

Now, mass transport between fluids boundaries in a batch reactor is enhanced by increased energy dissipation due to (intense) mixing, thus reducing the characteristic diffusion distance expressed in the so-called Kolmogorov length scale. In addition to aforementioned energy efficiency considerations for multiphase mixing in microreactors as compared to the conventional stirred vessel, there is a major problem that dynamic mixing of multiphase systems (e.g., agitation, shaking, gasification, recirculation) results in pronounced interfacial inactivation of the biocatalysts used [103–105]. Enforced renewal of interfaces, due to mechanical rupture for example, has a strong promoting effect on protein denaturation [106–109]. Specific interfacial areas are high in microreactors as compared to reactors having conventional geometry [94]. Because phase separation due to bubble coalescence is suppressed in microreactors, there is no need for constant re-dispersion of the phases [97]. Multiphase flow

processes in microstructured structures could therefore lead to enhanced stability of enzymes under operational conditions, enabling high total turnover numbers for the biocatalyst used.

Screening and optimisation studies performed under conditions in which phases are well ordered provide clear information about the characteristic times of mass transfer and enzymatic reaction. Importantly, they also inform about the characteristic time of catalyst inactivation under defined conditions of interfacial contact. Studies of enzyme stabilisation under operational conditions can also be performed. Methods of enzyme immobilisation in microreactors have recently been reviewed [110]. These include assembly of enzyme containing microparticles in microchannels or direct attachment of enzyme on the internal surface of the reactor's microstructure. Liquid-solid mass transport could be an issue in such systems, and multiphase flow operation should be a useful approach to enhance the transport [3, 77].

Table 1 presents a summary of biotransformation studies under liquid-liquid multiphase flow conditions in microstructured reactors. Enzymes, reactor configurations, and multiphase flow conditions used are described. Note that we have not considered studies in miniaturised fixed bed reactors lacking defined microstructure and having internal diameters significantly greater than 1 mm. Research in the field has focused mainly on hydrolases [59, 112, 113, 128], enzymes requiring  $O_2$  as substrate (e.g., oxidases) [114–116], and reductases [117–120]. Goals underlying the use of multiphase microreactors varied among the different studies and included amongst others elimination of transport limitations on  $O_2$  supply, rapid screening of reductases under conditions of nicotinamide coenzyme regeneration, and exploitation of interfacial activation of lipase. Table 1 shows that the microstructured devices used in enzyme studies differed widely in respect to the material applied to microfabrication, however with a clear preference for glass and silica. Also, microstructured elements used varied to include microchannel plates [112–116, 119–122, 128], single-channel capillary tubes [59, 117, 118], and honeycomb monoliths [125, 126]. Multiphase flows included segmented flow, but also continuous co-current flow. Phase contacting was achieved using differently shaped (e.g., Y or  $\Psi$ ) inlets. Using slug flow, mixing in microchannels was reported to have been efficient and large interfacial area was provided for effectively enzyme-catalysed reaction as well as for product extraction [59, 112, 120, 128]. Using parallel flow, the presence of a well-defined interface was advantageous and it made phase separation at reactor outlet easy [113–116, 123, 124]. Stable operation of parallel flow necessitated that fluid velocities

be finely tuned and that small variations in fluid viscosity be considered [113, 114, 116]. Microchannel architecture and wetting properties of the wall surfaces, alternating between hyperhydrophilic on one side and hyperhydrophobic on the other, were important to guide the two parallel phases to the Y-shaped exit, where they were split in a continuous phase separation [114]. In order to achieve phase separation from microreactors operated under slug flow conditions, electrocoalescence or hydrodynamic focusing was applied [119, 120].

Gas-liquid contacting was used in a combined experimental and modeling study of glucose oxidase immobilised in a honeycomb ceramic monolith reactor [125, 126]. Enzymatic conversion of glucose was investigated under variation of reactor channel size and each fluid's flow rate using co-current upflow and downflow operation. Gas-liquid and liquid-solid mass transport rates were determined under these conditions.

Operation of many microstructured reactors involves mixing of two co-currently flowing fluid streams at Y- or T-shaped junctions [63, 111, 129, 130]. Boom and co-workers analysed the effect of mixing efficiency on performance of a single-phase enzymatic microreactor where aqueous solutions of enzyme and substrate are mixed at reactor entry [129]. They showed that at low residence times, biocatalytic conversion was limited by diffusion and the critical residence time for onset of the diffusional limitation increased quadratically with channel width. Guidelines for optimum design of single-phase co-flow microreactors were developed. Even though done in single-phase system, their work is relevant in the context of this article showing the possibility of development of diffusional hindrance to enzymatic conversions in parallel co-flow microreactors. Reaction-diffusion modeling was also applied to oxidation of phenolic compounds by laccase in a continuous liquid-liquid co-flow microreactor [63].

Enzyme-catalysed polymerisation in microreactors presents an innovative application of multiphase flows at the microscale [127]. Authors demonstrated ring-opening polymerisation of  $\epsilon$ -caprolactone to polycaprolactone by immobilised *Candida antarctica* (*Trichosporon oryzae*) lipase B in a single microchannel reactor (width: 2 mm; depth: 1 mm). Immobilised enzyme microparticles were assembled in the microchannel as a micropacked bed.  $\epsilon$ -Caprolactone in dry toluene was fed to the reactor. Enzyme microparticles were the source of water in the system. Reaction in the microreactor proceeded faster and produced polycaprolactone of higher molecular mass than reaction in the batch reactor [127].

With the basic multiphase reaction systems described in Table 1, we now turn to the analysis of (possible) process



**Table 1** Biotransformation studies under multiphase flow conditions in microstructured reactors.

Catalyst	Reactor configuration	Multiphase flow	Multiphase flow comments	Ref
Immobilized CAL-A	Macroporous silica monoliths with controllable porosity, packed within a capillary column	L-L-S, segmented flow, water and <i>n</i> -decane	Segmented flow favoured interfacial activation of the enzyme used	[59]
Soluble CAL-B	Microchannels fabricated on glass plate	L-L, parallel or segmented flow, water and <i>n</i> -decane	Parallel flow/slug flow: For high flow rates, the flow remained parallel. At lower flow rates, the two phases flew in parallel for a few centimeters (from a total length of 1.18 m), then they broke up into droplets	[111]
Soluble CAL-B adsorbed on interface	Two microchannel reactors fabricated on glass plate, a) one outlet, three separate Ψ-shaped inlets; b) Y-shaped inlets and outlets	L-L segmented flow, ionic liquid and <i>n</i> -heptane	With parallel flow, easy separation was possible, but conversion was not detectable. With segmented flow, high reaction rate and yield were obtained.	[112]
Soluble CAL-B adsorbed on interface	Microchannels fabricated on glass plate, Y-shaped inlets and outlets	L-L parallel flow, water and <i>n</i> -hexane	Parallel flow enabled phase separation	[113]
Soluble laccase adsorbed on L-L interface	Microchannel (100 μm width, 25 μm depth) fabricated on glass plate	L-L parallel flow, water and isooctane	Parallel flow was stabilised by wall surface modification	[114]
Soluble cholesterol oxidase	Microchannel fabricated on glass plate, Y-shaped inlet and outlet	L-L parallel flow, water and <i>n</i> -heptane	The organic phase ( <i>n</i> -heptane) acted as a pool for the poorly water-soluble sterol and steroid, whereas the aqueous phase (phosphate buffer) contained the enzyme	[115]
Soluble cholesterol oxidase	Microchannel fabricated on glass plate, Y-shaped inlet and outlet channels	L-L parallel flow, water and <i>n</i> -heptane	As above	[116]
Soluble or immobilized alcohol dehydrogenase	PTFE capillary, T-shaped inlet	L-L segmented flow, water and hexadecane	Segmented flow, influence on enzyme stability was analysed	[117]
Soluble alcohol dehydrogenase	PTFE capillary, T-shaped inlet	L-L segmented flow, water and hexadecane	As above, and reactor performance was analysed	[118]
Soluble pentaerythritol tetranitrate reductase	Microchannels fabricated on polycarbonate sheets	L-L segmented flow, water and isooctane	Phase separation based on electrostatic charges	[119]
Soluble pentaerythritol tetranitrate reductase and thermophilic “ene”-reductase	Microchannels fabricated on polycarbonate sheets	L-L slug flow, water and octanol	Phase separation based on hydrodynamic focusing	[120]
Soluble hydroxynitrile lyase	Microchannels fabricated on borosilicate glass plate	L-L, segmented flow, water and methyl <i>tert</i> -butyl ether	Undefined plug flow was observed inside the microchannel rather than laminar flow, possibly due to detergents or other surfactants present in the cell lysate	[121, 122]
Aminoacylase immobilised as cross-linked enzyme aggregate (CLEA)	CLEA-based enzyme-microreactor consisting in a PTFE tube integrated with a microextractor consisted of glass and silicon plates	L-L parallel flow, water and ethyl acetate	Partial surface modification of microchannels for stable formation of laminar flow	[123]

(Table 1 Continued)

Catalyst	Reactor configuration	Multiphase flow	Multiphase flow comments	Ref
<i>Rhizopus nigricans</i> pellets	Microchannels fabricated on glass plate	L-L parallel flow, water and ethyl acetate	Adjustment of fluid flow rates enabled phase separation after biotransformation performed in conventional bioreactor	[124]
Glucose oxidase immobilised on channel walls	Honeycomb ceramic monolith, different channel sizes	G-L-S, air, water, and catalytic monolith	Slug flow regime for co-current upflow, and liquid annular flow regime for concurrent downflow	[125, 126]
CAL-B immobilised on porous polymethylmethacrylate supports	Microchannels fabricated on aluminium plate	L-L-S, toluene, water, and catalytic particles	Enzyme on solid phase, microparticles provide the water source	[127]

L-L, Liquid-Liquid; G-L-S, Gas-Liquid-Solid; CAL-A, Lipase type A from *Candida antarctica*; CAL-B, Lipase type B from *C. antarctica*; PTFE, polytetrafluoroethylene.

intensification resulting from the use of microstructured reactors.

## 5 Transport intensification for multiphase biotransformations using flow processing in microstructured reactors

In any rigorous evaluation of process intensification due to microscale effects, the reference system used for benchmarking is key [47, 50]. Typically, an agitated tank reactor is selected for that purpose. Even though, in the opinion of the authors, it is not necessary that the reference reactor shows close to ideal mixing behavior because larger tanks are usually far from being ideally mixed, it is still very important that mixing conditions for the reference reactor are both realistic and well characterised. This holds true especially for multiphase systems. Somewhat provocatively stated, the poorer the reference reactor performs, the more likely it is that a microscale process intensification effect will be observed.

Table 2 shows different enzymatic conversions for which the relevant analysis of microscale effects on parameters of reaction efficiency has been performed. Enhancement of (initial) conversion rate and reduction of residence time at equivalent conversion are often applied to measure the degree of intensification. Enzyme requirement for achievement of equivalent conversion at equivalent residence time should also be a useful metric of process intensification. Improvement of product properties would

be another important, alternative parameter of process intensification, as mentioned above for lipase-catalysed ring-opening polymerisation in an enzymatic microreactor [127]. Entries in Table 2 correspond to the microreactor systems described in Table 1. In addition to summarising evidence on process intensification along with some information on the reference reaction, Table 2 also indicates the phase of process development in which microreactor experiments were carried out. Studies of enzymatic reaction kinetics and reaction optimisation clearly predominate. However, there are also examples of successful integration of enzymatic reaction with downstream processing, suggesting that process design intensification resulting from the application of microreaction technology in biocatalytic process development could become possible in the future. Finally, Table 2 shows the analytical tools applied to monitoring of the enzymatic reactions. Widespread use of (slow) off-line analytics is clear indication that development of in-line or at-line monitoring tools presents an important goal of future research in the field of (biocatalytic) microreactors. Interesting recent developments in real-time space-resolved optical sensing in microreactors are noted [47, 131–138]. Methods of *in-situ* analytics in microreactors have been reviewed elsewhere [139]. Flow-through detection systems have great potential to become fully integrated with flow microreactors [119, 140]. Figure 4 illustrates two examples of flow pattern visualisation (A) and space-resolved monitoring of a compound concentration via imaging techniques (B).

Using lipase immobilised on macroporous monolithic support, Haswell and colleagues analysed hydrolysis of nitrophenylbutyrate in capillary microreactor [59]. Water-decane two-phase segmented flow was applied.

Table 2 Bioprocess development and intensification under multiphase flow conditions in microstructured reactors.

Biotransformation	Reference reactor	Bioprocess development	Reaction intensification	Analytics	Ref
Hydrolysis of 4-nitrophenyl butyrate	1 ml stirred reactor (100 rpm) containing 40% (by vol.) organic phase	Stable lipase immobilised, microreactor development Kinetic studies of lipases	Yield was 4-fold higher than in batch reactor, intensification attributed to increase of enzyme interfacial activation. Surface to volume ratio was 8 mm <sup>-1</sup> in the flow system compared to only 0.23 mm <sup>-1</sup> in batch reactor Enzyme stabilisation by immobilisation	UV-Vis spectrophotometry, off-line	[59]
Esterification of propionic acid and 1-butanol	Mixture of 720 µl enzyme aqueous and 720 µl of <i>n</i> -decane mixed by end-over-end incubation at 80 rpm	Kinetic analysis, kinetic parameter determination	No reaction intensification, kinetic parameters at micro and bench scale were similar, enzyme stability was also similar Faster kinetic studies and lower consumption of catalyst and reagent compared to conventional reactor configuration	HPLC-UV, off-line	[128]
Synthesis of isoamyl acetate	Batch esterifications reported in literature	Synthesis development Product removal in organic phase and continuous phase separation Mathematical model of reactor in agreement with experimental data Optimisation of existing bio-chemical process	Up to 35% conversion for 0.5 M acetic acid and isoamyl alcohol concentrations and residence time 36.5 s, at 45°C, superior to those found in the literature Threefold increase in reaction rate of isoamyl acetate synthesis was achieved in a microchannel reactor operated with Taylor flow, as compared to a perfectly mixed batch reactor	HPGC, off-line	[113]
Synthesis of isoamyl acetate	A 4 ml test tube (50% organic phase, by vol.) mixed at 500 or 2000 rpm on a vortex mixer			HPGC, off-line	[112]
Enzymatic degradation of <i>p</i> -chlorophenol	2 ml screw-cap bottle gently shaken or vigorously mixed by magnetic stirring	Oxidation of hydrophobic substrates in two-phase systems Mathematical model of reactor in agreement with experimental data Easy separation of phases at Y-shaped end	Increase of specific interface by two orders of magnitude Enhanced conversion and reaction efficiency (~5-fold) compared to batch reactor. Enzyme was denatured under vigorous stirring in batch reactor.	HPLC-UV, off-line	[114]
Oxidation of cholesterol	250 ml batch reactor (50% organic phase, by vol.) using magnetic stirring at 700 rpm	Integrated bioconversion system, combining cholesterol oxidation with <i>in situ</i> product recovery in microchannel reactors Mathematical model of reactor in agreement with experimental data	About 20-fold decrease in residence time needed to achieve similar conversion of cholesterol (70%)	HPLC-UV, off-line	[115]

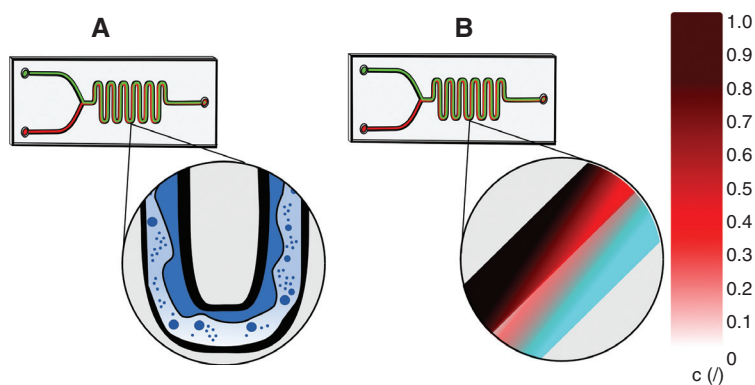
(Table 2 Continued)

Biotransformation	Reference reactor	Bioprocess development	Reaction intensification	Analytics	Ref
Oxidation of cholesterol	300 ml batch reactor with diameter of 6.1 cm, mixing by mechanical stirring (150–500 rpm) through four-pitched-blade turbine with diameter of 4.9 cm, working volume was 50 ml containing 50% organic phase (by vol.) Air flow rate was set to obtain dissolved oxygen concentration in the aqueous phase of 0.24 mmol l <sup>-1</sup> 2 ml packed bed tubular reactor, length was 1.5 cm and outer diameter was 1 cm, filled with 1 g of immobilised cholesterol oxidase in a single organic phase system, oxygen concentration was set at 0.65 mmol l <sup>-1</sup> at inlet	Continuous microproduction platform based on the bio-oxidation of cholesterol performed in microchannel reactors by recycling of enzyme and operational stabilisation due to integration with a packed bed reactor filled with immobilised catalase The applied strategy allowed the maintenance of the catalytic activity of cholesterol oxidase to 30% of the initial activity over 300 h of operation time.	For achieving identical conversions (~70%), a much lower normalised residence time was needed in the microreactor as compared to reference reactors, with intensification factors of 20 and 90 relative to packed bed and stirred tank, respectively	HPLC-UV, off-line	[116]
Enzyme catalyzed redox reaction	Studies of enzyme stabilisation in segmented flow microreactor Influence of enzyme concentration, segment length, fluid flow velocity, and capillary diameter on the stability of the added enzyme			Spectrophotometric determination of enzyme activity GC, off-line	[117]
Reduction of 1-heptaldehyde to 1-heptanol	Studies and evaluation of bioprocess intensification in segmented flow microreactor Important parameters governing the interplay between reaction rates and mass transfer rates were investigated and visualised in an operational window for a systematic optimization of the segmented flow microreactor performance				
Reduction of $\alpha$ , $\beta$ unsaturated activated alkenes	30 ml sealed screw top vials (inner diameter 2 cm) gently shaken at 200 rpm, working volume of 10 ml, 50% organic phase (by vol.), surface to volume ratio was approximately 63 m <sup>-1</sup>	Screening of stereoselective reduction of $\alpha$ , $\beta$ -unsaturated, activated alkenes such as enals, enones, enamides and nitroolefins. Electrostatic phase separation	Enhancement of yield (up to twofold) as compared with reference batch reactor The surface to volume ratio of the organic to aqueous phase in the reactor was determined to be approximately 10 times higher No reaction intensification studied	On-chip spectroscopic detection by UV-vis and IR	[119]
Reduction of 2-cyclohexen-1-one	Not applied	Development of microfluidic electrochemical reactor that can be used to efficiently drive enzyme-catalyzed substrate reductions in the absence of otherwise essential coenzymes and cofactors, system was tested with two different enzymes and applied to different substrates		UV/Vis spectrophotometry, off-line	[120]



(Table 2 Continued)

Biotransformation	Reference reactor	Bioprocess development	Reaction intensification	Analytics	Ref
Synthesis of optically pure cyanohydrins	2 ml vessel with magnetic stirring	Aqueous-organic biphasic system for cyanohydrin synthesis Fast substrate screening under low consumption of reagents Product easily separated from enzyme and obtained as methyl <i>tert</i> -butyl ether solution	No reaction intensification observed The optimal contact between two immiscible phases in a microreactor resulted in enzymatic reactions with a high initial reaction rate and enantioselectivity, comparable to a batchwise process in which optimised conditions were achieved by vigorous stirring (emulsification)	GC and HPLC-UV, off-line	[121, 122]
Deacetylation of <i>N</i> -acetyl D,L-amino acids	Use of L-L flow microextractor for downstream processing Integration of an enzyme immobilised microreactor and the microextractor enabled efficient continuous production of optically pure amino acids.			HPLC UV-Vis, off-line	[123]
Steroid biotransformation Progesterone 11 $\alpha$ -hydroxylation with steroid extraction in a microchannel system Oxidation of glucose	Continuous steroid biotransformation process integrated with product extraction within microchannel system Mathematical model was developed			HPLC UV-Vis, off-line	[124]
	Not applied	Development of continuous oxidation of glucose with immobilised enzyme in segmented flow G-L microreactors Parallelisation using an increase in the numbers of available microchannels led to enhanced conversion	Enhanced mass transfer (4–5 fold) obtained under co-current upflow operation	UV-Vis spectrophotometry, off-line	[125, 126]
Ring-opening polymerization of $\epsilon$ -caprolactone to polycaprolactone	25 ml round-bottom flasks sealed with rubber septum and maintained an argon environment with magnetic stirring	Development of continuous flow microreactor for studying solid-supported enzyme-catalysed polymerisation reactions	Higher conversion, higher conversion rate (10 fold) and higher molecular mass of polymer are obtained in microreactor compared with batch reactor	Raman spectrophotometry, gel permeation chromatography with RI detection, off-line	[127]



**Figure 4** Multiphase flow visualisation (A) and local sensing in microstructured reactors (B).

Compared to the reference reaction carried out using free lipase in (vigorously stirred) batch reactor, reaction at microscale gave fourfold enhanced yield. Peculiarity of lipase structure and function is the so-called interfacial activation. The enzyme undergoes lid-opening conformational change upon contact with liquid-liquid, but also other types of interfaces, which stimulates the activity. Improved conversion rates in the microreactor compared to batch reactor were ascribed to higher degree of interfacial activation at microscale reaction conditions used. However, immobilisation of lipase on solid support can also bring about interfacial activation, making it difficult to compare directly soluble and solid supported insoluble preparations of the enzyme. Immobilisation of the lipase resulted in enhanced stability of the enzyme. The work of Swarts et al. used lipase in a two-phase water and *n*-decane system where esterification of 1-butanol with propionic acid was investigated [128]. The authors demonstrated the possible advantage of kinetic data collection in a microreactor as compared to a bench-scale batch reactor, due to low consumption of reagents and catalyst. Enzyme kinetic parameters obtained from microscale experiments were similar to those obtained from benchscale experiments, probably indicating absence of transport limitations in the different reactors used. Studies of enzymatic cyanohydrin synthesis performed in segmented flow microreactor also showed similar performance (enantioselectivity, initial reaction rate) compared with macroscale, but a substantial decrease of reagent input and fast substrate screening were claimed as the main benefits of performing the reactions at microscale [121, 122]. The soluble enzyme (hydroxynitrilase) was applied directly from cell extract, and it could be used without causing undesirable clogging of microchannels.

Lipase was used in two further studies in which synthesis of isoamylacetate was investigated. The two-phase systems used were water and *n*-hexane with enzyme

dissolved in the aqueous phase [113] or hydrophilic ionic liquid and *n*-heptane containing enzyme adsorbed onto the liquid-liquid interface [112]. Segmented and parallel flows were applied [112, 113]. Detailed mathematical modelling was used to describe experimental data. Microchannel microreactor showed superior performance compared with the well-mixed batch reactor in terms of reaction rate and maximum conversions reached in relation to residence time needed. Product removal into the organic phase and continuous phase separation were successfully accomplished.

Using laccase adsorbed onto the interface of a water-isooctane system operated in parallel flow, Maruyama et al. demonstrated enhanced conversion efficiency for degradation of *p*-chlorophenol in a microchannel microreactor as compared to vigorously mixed batch reactor [114]. Moreover, mixing in the batch reactor caused strong inactivation of the enzyme. Using the microreactor, it was possible to increase the specific water-organic solvent interface by two orders of magnitude as compared to the conventional reactor. Availability of dissolved  $O_2$  may have been a limiting factor for the laccase-catalysed reaction, but the gas-liquid transport was not investigated.

Marques and colleagues performed a detailed study on oxidation of cholesterol in microchannel reactor and compared reaction performance at microscale to reaction performances in stirred batch reactor and continuously operated packed bed reactor [115, 116]. Reaction was carried out in two-phase parallel flow [116]. Residence time required to reach target conversion was decreased about 20-fold compared to a conventional batch reactor. In a later study, authors proceeded by establishing a continuous transformation in a closed system with enzyme recycling [115]. The microstructured reactor was integrated with a packed bed reactor containing immobilised catalase for the decomposition of the hydrogen peroxide formed in the aqueous phase [115]. The microreactor

performance was compared with two realistic bioprocess alternatives, a liquid-liquid phase aerated stirred tank reactor with optimised mixing conditions, and a packed bed reactor containing immobilised cholesterol oxidase in a single organic phase system [115]. Normalised residence time concept was used to account for differences in enzyme concentration applied in the different reactor configurations, and the  $O_2$  concentration was set in the organic phase. The results revealed a ~100-fold decrease in residence time at microscale process operation. Enzyme stability was monitored for 300 h of continuous operation, and only 30% loss of activity was detected. Authors showed that enhanced performance in the microreactor could not be explained by an interfacial area effect, since the stirred tank reactor with small droplet distribution offered a superior specific surface area compared to the microreactor operated with parallel flow. Microscale process intensification requires further study in this particular case. Distances for molecular diffusion may have been shorter in the microreactor [115].

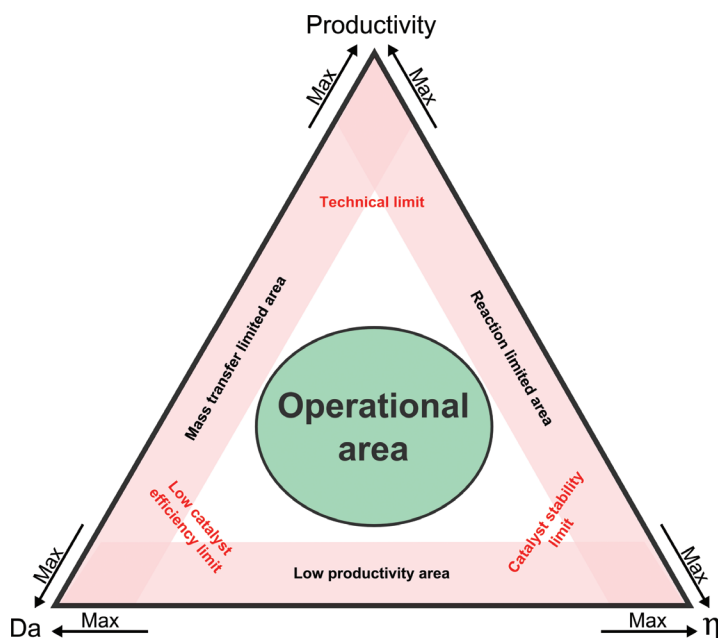
Enzyme-catalysed NADH or NADPH-dependent reductions of diverse functional groups (e.g., carbonyl groups, carbon-carbon double bonds) have been performed in liquid-liquid two-phase flow microreactors, focusing on two important aspects of these biotransformations in particular: nicotinamide coenzyme regeneration, and enzyme instability at liquid-liquid interfaces. Mohr and colleagues developed a continuous flow microstructured reactor, instrumented with on-chip spectroscopic detection capacity, into a powerful platform for substrate screening and reaction analysis [119]. Authors demonstrated practical use of the microreactor and showed that microscale reactions required shorter characteristic times than reactions in conventional two-phase bench reactors. Increase of the specific interfacial area was thought to be responsible for reaction intensification at microscale. In a recent study, a two-phase microstructured electrochemical reactor was developed in which electrochemically active redox mediators were applied to replace nicotinamide coenzymes in enzymatic reactions [120]. Mediator and enzyme were retained in the reactor and continuously reused. Downstream processing was favoured by phase separation at reactor outlet. Operational stability and reaction efficiency (in relation to reaction using NADH) was studied for different combinations of substrate and enzyme. Enzyme stability under conditions of liquid-liquid two-phase segmented flow was analysed by Buehler, Schmid and coworkers [118]. Segment length, fluid velocity, and capillary diameter were investigated as potentially relevant parameters. Addition of surfactant (Tween 20) to the aqueous phase offered a huge stabilisation effect. Enzyme

immobilisation also brought about large stabilisation so that in both cases almost 100% of the initial enzyme activity could be recovered. Prevention of the biocatalyst from coming into direct contact with the liquid-liquid interface was probably essential. The use of surfactants seemed to be more convenient than immobilisation, not only because it enabled greater enzyme activity, higher yield, and reduced complexity, but implementation into the liquid-liquid segment flow reactor was also easier [118].

From the previous discussion it becomes clear that the parallel flow regime has often been preferred for liquid-liquid phase contacting in biotransformation studies, most probably because of its simplicity, the relative ease of achieving controllable conditions, and straightforward phase separation at reactor outlet. Exploitation of segmented two-phase flow in microstructured reactors requires more in-depth analysis. Karande and colleagues have performed an important study of enzyme-catalysed NADH-dependent reductions in a segmented flow capillary microreactor [117]. They examined the influence of capillary diameter, flow velocity, water-organic solvent phase ratio, and enzyme and substrate concentration on process efficiency. Parameters affecting process performance were correlated in a diagram that now defines a window of process operation [117]. The diagram, which is shown in Figure 5, takes into account the Damköhler number, the enzyme effectiveness factor, and process productivity. Specific limitation areas where enzyme inactivation, insufficient productivity, and low enzyme efficiency dominate process performance are recognised. The desirable operational region can be localised in the diagram constituting a useful window of process operation. The work is significant as it provides a generally useful strategy to analyse specific process boundaries of flow processing in microreactors, thus supporting the process design.

Some studies make use of two-phase flow in microchannels to develop innovative procedures of downstream processing [123, 124]. Continuous product removal and easy phase separation have been integrated with biotransformations performed in conventional macro-scale reactors or single-phase microreactors.

Reports about gas-liquid biotransformations in microstructured reactors under well-defined flow conditions are few in number. Two significant papers that have appeared already a long time ago are therefore pointed out where use of glucose oxidase immobilised on honeycomb monolith support is described [125, 126]. Gas-liquid and liquid-solid mass transfer rates were carefully measured at different channel sizes and at different gas and liquid flow rates. Operation in concurrent gas-liquid upflow gave higher mass transfer rates than operation



**Figure 5** Windows of operation analysis for biotransformations in multiphase flow reactors. The diagram was adapted from Karande et al. [117].

in downflow. The effect of the channel size was not very pronounced. Internal numbering-up through multiple channels resulted in enhanced enzymatic conversion of substrate, explicable on account of reduction of internal diffusion resistance of oxygen. Using both experiment and mathematical modelling, it was determined that the effectiveness of the immobilised biocatalyst was 30% of that of the free enzyme. Part of this loss of enzyme effectiveness was proposed to be apparent and result from limitations of the overall conversion rate by mass transfer from the gas-liquid interface to the surface catalyst.

## 6 Conclusions and outlook

The state of the art in multiphase biotransformations in microstructured flow reactors has been reviewed. Current evidence demonstrates high potential of flow processing in microstructured reactors for development of biocatalytic processes that involve contacting between two or more immiscible fluids. It is clear that microreaction technology could contribute to achievement of a major goal in the field of applied biocatalysis, which is “to make available better chemical processes faster”. However, progress in biocatalytic microreactors has been relatively slow. One probable reason is the technical complexity of setting up and operating a microstructured reactor under defined multiphase flow conditions. Another might be that key (“pull”) projects

demonstrating successful biocatalytic process development across scales up to the level of industrial production are not yet available. Reports are therefore mostly from research in an early development phase where characterisation and optimisation of a biotransformation of interest as well as evaluation of process intensification due to microscale effects are of prime interest. Clearly, the microreactor is a great tool for kinetic analysis at defined conditions of mass transport, complementing the scope of other instrumented systems for automated, parallelised experimentation (e.g., 96-well microplates). Moreover, a number of examples demonstrate significant mass transport intensification resulting from performing the biocatalytic reaction in the microreactor as compared to a reference reactor of conventional geometry. However, to date, the observed enhancements in effective reaction rate or in another reaction performance parameter analysed are usually far lower than required by the original definition of process intensification. Systematic studies analysing the basis of transport intensification have already provided useful insight, and rigorous window-of-operation analysis shows clear potential for further intensification. Work in the future will also have to address issues of flow stability in multiphase biocatalytic reactors and consider the available options for scaling out. The need of achieving better integration of microreactors with analytics has been mentioned. Flow processing will require recycling of the biocatalyst used. Enzyme immobilisation and enzyme recovery present possible options. Their integration with existing microreactors



must be demonstrated at a larger scale than has been done so far. *In situ* phase separation for facilitated downstream processing is an interesting example of process design intensification in biocatalytic processes achieved through use of microchannel reactors. Gasparini and colleagues have recently reported on successful scale-up of a gas-liquid multiphase biotransformation (dependent on O<sub>2</sub>) using a flow reactor equipped with non-conventional mixing elements to enhance mass transport [141]. Even though the flow reactor was not microstructured in this case, the study nonetheless demonstrated the high potentiality of smart flow processing for biocatalytic process development. In

a future scenario where applied biocatalysis moves from processing in batch to processing under continuous flow, microstructured reactors are expected to have a key role for development due to the different options for process intensification they can offer.

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