Microfluidic Devices: Useful Tools for Bioprocess Intensification

Marco P.C. Marques 1,2 and Pedro Fernandes 1,2,*

1 Department of Bioengineering, Instituto Superior Técnico (IST), Universidade Técnica de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
2 IBB-Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, IST, Lisboa, Portugal

* Author to whom correspondence should be addressed; E-Mail: pedro.fernandes@ist.utl.pt; Tel.: +351-218419065; Fax: +351-218419065.

Received: 9 August 2011; in revised form: 21 September 2011 / Accepted: 28 September 2011 / Published: 30 September 2011

Abstract: The dawn of the new millennium saw a trend towards the dedicated use of microfluidic devices for process intensification in biotechnology. As the last decade went by, it became evident that this pattern was not a short-lived fad, since the deliverables related to this field of research have been consistently piling-up. The application of process intensification in biotechnology is therefore seemingly catching up with the trend already observed in the chemical engineering area, where the use of microfluidic devices has already been upgraded to production scale. The goal of the present work is therefore to provide an updated overview of the developments centered on the use of microfluidic devices for process intensification in biotechnology. Within such scope, particular focus will be given to different designs, configurations and modes of operation of microreactors, but reference to similar features regarding microfluidic devices in downstream processing will not be overlooked. Engineering considerations and fluid dynamics issues, namely related to the characterization of flow in microchannels, promotion of micromixing and predictive tools, will also be addressed, as well as reflection on the analytics required to take full advantage of the possibilities provided by microfluidic devices in process intensification. Strategies developed to ease the implementation of experimental set-ups anchored in the use of microfluidic devices will be briefly tackled. Finally, realistic considerations on the current advantages and limitation on the use of microfluidic devices for process intensification, as well as prospective near future developments in the field, will be presented.
Keywords: microfluidic devices; bioprocess intensification; modeling and simulation

1. Introduction

Biobased processes have been a mainstay of development for thousands of years, but the current trend towards the use of sustainable production methods has further stressed the role that biotechnology can play in the production of a wide array of products and energy [1,2]. Actually, the need for more cost-effective process, with a lower carbon footprint, and less dependence on fossil fuels spans the chemical, pharmaceutical and bio-based industries [1]. Process intensification has been recognized as a sound approach to comply with such requirements [3]. As defined by Charpentier, this methodology encompasses the replacement of large and expensive equipment/processes with cheaper, smaller, more efficient ones. Preferably, it integrates as many operations as possible into a single one, and has been applied to processes in the chemical, pharmaceutical and bio-based industries [1,2]. Miniaturized devices (viz. microreactors, microseparators, micro heat-exchangers), and concomitantly process design based on their application, are among the tools used to implement process intensification [3,4,6-9]. This is clearly expected, since this trend represents a decrease in size in several orders of magnitude, which results in smaller plants, hence with lower costs in capital and energy, reduced environmental impact, operating in a contained, well controlled and safer environment, where continuous mode of operation is clearly privileged [8,10,11]. Furthermore, process intensification considerably decreases time to market, which is a critical issue in some sectors such as fine chemicals and pharmaceutical industries. Ideally, process intensification allows the direct use of a continuous process developed in lab-scale as the commercial scale process [10]. Since chemical modifications are the core of (bio)chemical processes, miniaturization has focused on microchannel reactors, where at least one dimension is smaller than 1 mm, most likely within tens to hundreds of micrometers, that can be prepared either by microtechnology and precision engineering, or by modification and assembly of microcapillaries. In the former configuration, microchannels are embedded in plates, which can be made of ceramic; glass; polymeric materials (viz. polydimethylsiloxane, PDMS and epoxy-polymer SU-8); stainless steel; and Teflon [12-15]. These materials can actually also be used for the manufacturing of microcapillaries. The selection of the materials used for the fabrication of the microdevices is naturally influenced by the process conditions envisaged, as summarized in Table 1.

The nature of these microreactors enables operation in microfluidic environment, where only microliter volumes of solution are required [12,14,16]. When addressing bioconversions, the present review will thus focus on microchannel reactors. Other type of microreactors, with operating volumes under 1 mL, which emulate typical bench-scale reactors, have been thoroughly reviewed recently [17], and will not be considered here. Besides their application as reactors, microfluidic devices have also been used for downstream steps, namely involving extraction of large biomolecules, as well as for the integration of (bio)conversion and extraction in a single step [16]. As an outcome of the size reduction concomitant to the use of these miniaturized devices, where surface to volume ratios of $5.0 \times 10^4 \text{m}^2\text{m}^{-3}$ are achievable [11,12,18], significant enhancement in heat and mass transfer is observed, due to the
small diffusion path lengths. This allows for nearly gradientless conditions, hence processes take place under a more controlled environment than in conventional set-ups, favoring yield and selectivity [8,19-22]. Therefore, it is not surprising that the environment in microreactors differs from the one found in conventional scale systems, which is reflected by the effect of increased miniaturization of length scale on transport properties and acting forces, as previously reviewed [11,16,23-25]. It has become evident that as a result of downscaling, gravitational and inertial forces tend to lose relevance, whereas viscous and interfacial forces become dominant, while mixing relies mainly on molecular diffusion [26,27]. The small diffusion path has been considered as the major driving force for bioprocess intensification, albeit other process intensification fields/driving forces, such as electric fields, microwaves or pressure can be used. However, although these can be integrated with microreactors for enhancing reaction rates in purely chemical processes, in the case of bioprocesses their integration is more adequate in stages other than the bioconversion/fermentation step [13,28-31].

Table 1. A brief overview of how process parameters may condition the selection of materials for the fabrication of microfluidic devices (adapted from [13,15]).

| Material    | Process variables influencing material selection |
|-------------|--------------------------------------------------|
| Ceramic     | Thermal and chemical endurance, but penalized by significant development costs and by shrinkage after sintering |
| Glass       | Ease of visualization and overall chemical endurance but incompatible with strong aqueous bases |
| Plastic     | Low cost and fast fabrication but incompatible with organic solvents and extreme temperature and pressure |
| Silicone    | Compatible with high temperature and pressure and high-aspect ratio design but incompatible with strong aqueous bases |
| Stainless steel | Compatible with high temperature and pressure but sensitive to corrosive solutions unless expensive metal alloys are used |
| Teflon      | Inertness to several chemicals and extreme resistance against all solvents but relatively unexploited approach |

In the two next sections, aspects related to the hydrodynamics in the microfluidic environment and to the configuration of microchannel devices will be addressed, since these establish some boundaries to the practical use of said devices and contribute to provide an up-to-date insight into the current stage of development within the field of microfluidics. Subsequently, the role of microchannel devices within the scope of bioprocess development will be considered. Such matter will be illustrated with some relevant examples. These will help to establish the potential and limitations of microfluidic devices in different stages and of bioprocess development, either in the transformation or the downstream steps. Microchannel reactors provide suitable platforms for screening of biocatalytic activity at intermediate stages of process development, particularly if integrated with spectroscopic tools enabling in-line analysis. Under well controlled conditions, microfluidic devices can constitute representative scaled-down systems, where sets of realistic operational parameters can be evaluated in short time periods. The use of microchannel reactors in production stage through numbering-up can also be considered, the most straightforward example of said use being for bioconversions involving the use of immobilized enzymes. Yet, the implementation of such process requires a careful evaluation
against the current conventional large scale approaches used, both considering the performance of the reactors as well as cost issues.

2. Hydrodynamics in Microfluidic Environments

Fluid flow is a key issue in microfluidics on which depend the successful applications of both heat and mass transfer relying in three distinct parameters: (i) channel geometry, (ii) properties of the fluids and (iii) flow conditions [32]. Given the small dimensions of the channels involved, the flow regime is typically laminar, despite reports on transitional regimes [32]. This type of flow favors the control and modelling of biochemical reactions while providing high surface area to volume ratio [16].

The establishment of stable flow patterns is very important and is determined by the balance of inertial, viscous and interfacial forces [34,35]. Recently it was described that wetting properties (contact angle), influence two-phase flow patterns in microchannels [36-38]. Depending on the channel geometry and on the flow rates, different flow regimes in co-current can be observed: bubble flow regime, Taylor flow, annular flow and parallel flow [39]. Various reactor configurations have been studied for contacting and mixing fluids, depending on the specific application, including hydrodynamic focusing, manifold splitting and recombination, T- and Y-junctions, chaotic mixing [32]. Factors like physical properties of the dispersion or hydrodynamics of the flow, important for mass transport and reaction applications, must be taken into account.

A combination of dimensionless numbers indicates the importance of forces, energies and time scales present at the microscale [23,32,40] and are briefly described in the following text. The Reynolds number \((Re)\) represents the ratio of inertial to viscous forces and can be defined by:

\[
Re = \frac{\rho L r}{\mu}
\]  

where \(\rho\) is the fluid density \((\text{kg.m}^{-3})\), \(r\) is the characteristic velocity \((\text{m.s}^{-1})\), \(L\) is the hydraulic diameter of the microchannel \((\text{m})\) and \(\mu\) is the dynamic viscosity \((\text{Pa.s})\). The dimensionless number that expresses the ratio of gravitational to surface tension forces is the Bond number \((Bo)\):

\[
Bo = \frac{\Delta \rho g L^2}{\gamma}
\]  

where \(\Delta \rho\) is the density difference \((\text{kg.m}^{-3})\) and \(\gamma\) the interfacial tension between the phase in contact \((\text{N.m}^{-1})\), respectively, and \(g\) is the gravitational acceleration \((\text{m.s}^{-2})\). High Bond number indicates that the system is unaltered by surface tension effects while a low number indicates that surface tensions are dominant.

At this scale, typically both numbers \((Bo\text{ and } Re)\) are small revealing a control of viscous/interfacial forces over inertial/gravity forces. The Capillary number \((Ca)\) expresses the relationship between the two dominating forces (viscous and interfacial) and is defined as:

\[
Ca = \frac{\mu r}{\gamma}
\]  

typically the \(Ca\) is lower than 1, allowing a distinction between two-phase flow patterns and the mechanism of droplet break-up [41,42]. When \(Ca\) values become lower than the critical value \(Ca_{\text{crit}} \approx\)
0.1 to 0.01, the surface tension forces break the liquid filament into droplets, minimizing the interracial area. This phenomenon is called the Rayleigh-Plateau instability [43].

Two other parameters based on the Capillary and Reynolds numbers are the Ohnesorge number \((Oh)\) and the Weber number \((We)\). The Ohnesorge number relates viscous forces to inertial and interfacial tension forces:

\[
Oh = \frac{Ca}{\sqrt{Re}} = \frac{\mu}{\sqrt{\rho CL}}
\]

while the Weber number compares inertial effects to surface tension forces:

\[
We = Re Ca = \frac{\rho L^2 \mu}{\sigma}
\]

Despite of the small paths involved, \(We\) in the range of some hundreds can be obtained in microfluidic devices [44]. Strong surface tensions maintain the droplet as a unique microfluidic entity with a convex interface. If the inertial forces increase, the interface deforms becoming progressively concave and finally disrupting [45,46].

Other dimensionless parameters on fluid properties and operating conditions, include the density ratio \((\alpha)\), viscosity ratio \((\beta)\) and flow rate ratio \((\phi)\):

\[
\alpha = \frac{\rho_c}{\rho_d}
\]

\[
\beta = \frac{\mu_c}{\mu_d}
\]

\[
\phi = \frac{Q}{Q_d}
\]

where \(Q\) is the fluid flow rate \((\mu L/min)\) and \(c\) and \(d\) represent the continuous and dispersed phase, respectively, in the case of non-parallel flow [32].

In biotechnology applications a more complex approach is necessary combining not only physical phenomena related to fluid flow but also bio-chemical reaction data. Within this scope several dimensionless numbers take this into account, particularly the Damköhler number [45]. This number verifies if the overall process is limited by the reaction time or by the transport time of the species involved in the reaction:

\[
Da = \frac{\tau_r}{\tau_t}
\]

where \(\tau_r\) and \(\tau_t\) are the reaction time and the transport time, respectively [47-49]. Within this concept, four Damköhler numbers have been defined [50], the first Damköhler number \((Da_1)\) considers the relative rates of reaction and convective transport, whereas the second Damköhler number \((Da_2)\) considers the relative rates of reaction and diffusion [47,51]. On the whole, it is established that for \(Da\) largely exceeding unity, transport is the rate limiting step of the overall process, whereas for \(Da\) values under 1, the overall process is reaction limited [52]. Swarts and co-workers have evaluated the feasibility of using \(Da_{II}\) while establishing the effect of diffusion on enzyme activity in microchannel
reactors [53]. These authors reported that, under a specific set of conditions, the value calculated for DaII was different according to the equation used, which could limit its applicability [53]. On the other hand, correlated microreactor performance with Da/KM (Michaelis constant), using as case study a microchannel reactor with a porous wall and reactions with kinetics close to first-order type (based on Michaelis–Menten type) with a low reaction rate [51]. The authors were able to establish that Da/KM have a reduced impact since this ratio could not vary much for the reactions close to first-order type used as reference.

The Peclet number, Pe, expresses the rate of forced convection to diffusion [45,54]:

$$\text{Pe} = \frac{Lr}{D}$$

where a Pe value smaller than 1 reflects dominance of diffusion over convection, whereas a Pe larger than 1 suggests that flow mostly depends on the externally applied driving forces [55]. Since the flow velocities in microfluidic environments are typically small, the channel length is often a critical variable in determining Pe [55]. Given the dimensions used in microfluidic devices, diffusion is dominant, thus Pe is typically small [25]. Occasionally long enough channels may result in Pe values larger than 1, meaning that the forced convection exerted by external forces to create a directed flow of the fluid, is dominant [24,55]. Pe can furthermore allow to establish if Taylor dispersion is relevant or not [55]. Taylor dispersion corresponds to an enhancement of the rate of axial dispersion, due to strong density gradients in the radial direction of flow in microchannels, which exceeds what would be expected due to molecular diffusion alone in the absence of flow [56]. Taylor dispersion can be observed when Pe is considerably smaller than the length to width ratio of the microchannel, whereas diffusion mechanisms hardly contribute to dispersion when Pe largely exceeds the length to width ratio of the microchannel [55]. The performance of microreactors has been compared with that of classical plug flow reactors (PFR) and of perfectly mixed continuous stirred tank reactor (CSTR) [57-59]. Although these have been performed using as model systems non-enzymatic reactions, and more specifically systems with pseudo first order and second order kinetics, it has been established that for Pe within 10 and 1, or slightly under, PFR behavior is observed. With a decrease in Pe down to 0.1, a trend towards CSTR is observed, albeit with further decrease of Pe, the performance of the microreactor further shifts from CSTR, and is outperformed by the latter [57-59].

Mixing depends mainly on the molecular diffusion where mixing time can be defined as:

$$t_{\text{mix}} \approx \frac{d^2}{D}$$

where d is the characteristic diffusion path (typically the channel width) and D is the molecular diffusivity (m².s⁻¹). The mixing process by molecular diffusion is slow. A strategy to improve diffusion-induced mixing of reactants is to manipulate the interfacial surface area [60], by using high aspect ratio channels with mixture of different fluid streams accomplished up to tens of seconds, if the channel dimensions are in the hundreds of microns [61,62]. The degree of mixing can actually prove critical regarding product composition for very fast reactions, or when unstable compounds are dealt with [63]. Taking this into account, miniaturized mixers were developed to increase interfacial surface area decreasing consequential the diffusion length [64,65]. The whole concept of micromixing has
actually been thoroughly reviewed recently [40,63,66,67]. Given their small dimensions, and by allowing for a predictable flow pattern, micromixers enable a fast and controllable mixing environment [63]. Besides, micromixers can be used in such a manner that allow for trapping of intermediates (by viz. freeze quenching), and furthermore allow a precise control of reaction conditions by providing a suitable environment for a fast dispersion of added reagents at key time intervals [63]. There exists a great variety of micromixers based on different mixing principles, classified in mainly two basic ways: active and passive micromixers (Figure 1).

In either case, the design aims to decrease the mixing path and enhance the contact surface area [63]. Actually, the range of operation of micromixers in terms of Re and Pe, as well as the mixing efficiency in terms of Re, have been summarized by Kumar and co-workers [40], whereas Lee and co-workers provided thorough data on the performance of micromixers regarding mixing technique, time and length [67]. In active mixing, there is an external energy input are e.g. acoustic, electrical, thermal, pressure disturbance or integrated microvalves/pumps. On the other hand, in passive mixing, there is an induced perturbation on the flow in order to enhance mixing. This is accomplished by e.g., interdigital multi-lamellae arrangements, eddy formation, nozzle injection in flows and collision of jets [62,63,68]. The most common microstructures designs for passive mixing found are zig-zag microchannel, the incorporation of flow obstacle within the channels, T-, ψ- and Y-flow inlet structures and nozzles [23]. The configuration of these some of these inlets is depicted in Figure 2.

The mixing efficiency allowed by active mixers typically exceeds that of passive mixers, but, on the other hand the fabrication of the former is an expensive and complex process, which has furthermore to comprise the integration of external devices (viz. actuators) into the microreactor. In addition, high temperature gradients may occur in some approaches used for active mixing, which may prove deleterious to biological agents. The whole renders active mixers relatively unpopular when microfluidic applications in biotechnology are considered [63], and will therefore be further considered in this work. Again detailed information can be found elsewhere [63,66]. The classical design of passive mixers relies on T- or Y-shaped microchannels [63,66,68]. In the more straightforward design, mixing relies solely on the diffusion of the species at the interface between the two fluids. Hence, the process is slow and long channels are required. In order to overcome such limitation, and thus enhance mixing efficiency, the microchannels where mixing occurs can be narrowed, hence decreasing the diffusion path; obstacles (viz baffles) can be introduced in the channel; the inner surface can be processed in order for the channels to have a rough surface; and operation can be carried out at high Re (viz. over 150) [63,66]. Other designs have been implemented to improve the basic concept, namely [40,63,67]:

a) Multi-lamination, where the inlet stream are divided into several sub-streams, in the form of liquid lamellae, usually within few to several tens of micrometers, which are latter recombined into a laminated stream, the process allowing for enhanced mixing by decreasing diffusion path, while enhancing the contact surface between the two fluids [63,66,69].

b) Hydrodynamic focusing, where three, rather than two inlets are used, resulting in a ψ-shaped format, the inlets being connected to a long microchannel. In this configuration, a solution fed through the middle inlet, flows through the channel, within the outer layer composed by the fluids fed through the side inlets. The flow of the inner fluid is thus constrained, resulting in a
thinner lamination width, the length of which depends on the volumetric flow rate ratio between the inner and outer fluids, a larger difference in flow rate resulting in a thinner width, hence favoring mixing [63,66,70,71].

c) Chaotic micromixers, where the transport of a given molecule occurs in a direction transversal to the direction of flow (chaotic advection). As a result of chaotic advection, diffusion flux across interfaces between fluids increases exponentially and striation concomitantly decreases, hence mixing is favored [63,72,73]. Transversal flow can be generated by inserting obstacles in either in the wall of the microchannel or in the microchannel, but with some exceptions [74], this approach is only effective for producing transversal flow when operating at Re over 100 [63]. Alternatively the use of channels with grooved patterns, viz. staggered herringbones [40,75], has been suggested as a suitable design to promote chaotic advection, as a result of successions of rotational and extensional local flow, at low Re (viz. 1) [63,76,79]. Other approaches for achieving chaotic mixing include serpentine and zigzag flow arrangements [40,67]. In the former configuration, transverse flow in the curved microchannel as an outcome of the consecutive generation of Dean vortices [63,77]. Typically these micromixers are only effective for Re in the range of some hundreds, but improved designs allow effective mixing for low Re [63,78]. In a zig-zag micromixer, transverse flow occurs as a result of recirculation around the turns [79]. Mixing becomes is relatively poor for low Re, and it has been suggested that up to Re around 80, diffusion accounts for mixing [63,67]. The existence of an optimal geometry for this configuration has also been suggested [67].

d) Twisted channels, based on three-dimensional structures of microchannels, with inclined, oblique or wavelike design, where the angle of the bottoms of the channels changes in each subsection. The particular configuration of the microchannel forces the fluid flowing inside to sway around the changeable structures, creating conditions for chaotic mixing to occur, at intermediate Re [67,79,80].

e) Droplet micromixers where the formation of droplets of mixed liquids decreases the path for mixing, as a result of the tree-dimensional internal flow field created by the movement of the droplet, which enables mixing inside the droplet [63,79]. Droplets can be generated as a result of a suitable combination of several factors, namely, significant differences of surface forces (viz. interfacial and viscous) between the fluids, flow in small channels and nature, hydrophobic or hydrophilic of said microchannels, favoring the formation of water-in-oil emulsions or oil-in-water emulsions, respectively. Monodisperse droplets can furthermore be generated, where each of these can be considered an individual reactor, considerably expanding high-throughput capability [63,72,79].

Mixing efficiency can be determined by several flow visualization methods [81] namely visualization experiments (aided by microscopic-, photo-, video- or high-speed camera techniques [33,82,83], reaction experiments (characterization of mixing with a very fast reaction being a more specific the Villernaix–Dushman [84,85]) and concentration profiling (using on- or in-line measurements of optical properties [86]).

This experimental information is of crucial importance since it validates and develops the numerical techniques to represent the system dynamics. The numerical simulations are anchored mainly on
molecular models or on continuum models, depending on the scale of application [87]. The continuum model based on Navier–Stokes equations is used to describe fluid flow when the microchannels are in the range of the micrometers [88-93]. To solve the convection term in the Navier–Stokes equations different discretization methods can be applied such as finite element method, finite difference method, finite volume method, or boundary element method [94]. Moreover, to reduce mathematical efforts, and to incorporate data concerning bio-chemical reactions, mass and heat transfer, Computational Fluid Dynamics (CFD) packages have been developed (e.g., Comsol, Ansys Fluent and Ansys CFX) and applied in microtechnology [36,38,95-100].

**Figure 1.** Selective passive and active micromixer principles [Reprinted from [64]. Copyright (2005) with permission from Elsevier].

**Figure 2.** Different ψ- and Y-flow microchannel inlet geometries in multiphase flows. Arrow = flow direction; α - variable that controls the inlet angle responsible for different flow characteristics. In T-shaped inlet, \( \alpha_1 = \alpha_2 = 90^\circ \).

When the length scale of the microchannel reactors are in the region of the nanometer, molecular models are used and can be classified into deterministic (Molecular dynamics [101-103]) or statistical
approaches (Direct simulation Monte Carlo method [104-107]), and Lattice-Boltzmann method [108-114].

To aid with the choice of the numerical methods between several factors, a dimensionless number can decide this, namely the Knudsen number ($Kn$):

$$Kn = \frac{\lambda}{L}$$

where $L$ represents the physical length path and $\lambda$ the mean free path. At the microscale $Kn$ is lower than 1 while at the nanoscale is larger than 1. Typically, for gases $\lambda$ is roughly $1 \mu m$ while for liquids, it is smaller, in the range of $5-10$ nm [45].

Full description of numerical simulations will not be discussed further here, as this review focuses on giving a general overview on the use of microchannel reactors.

3. Configuration of Microchannel Devices

The more common and straightforward configurations of microchannel devices rely either on the assembly of capillary tubes or in the fabrication of a microchannel network on a chip using high precision micromachining techniques, either bulk or surface techniques. In the former, direct modification of the substrate material (viz. a monocrystalline silicon wafer, with thickness within several tens to hundreds of micrometers) is carried out, whereas the later is based on the deposition of several layers of thin films of different materials, which are ultimately shaped according to a given design [115,116]. Both surface and bulk micromachining involve a sequence of steps, namely: (a) transfer step, encompassing the growth and deposition of materials; (b) additive step, where etching and removal procedures take place; (c) subtractive step, where different structures and substrates are put together; (d) bonding step, where the shape of the structure is transferred from the template into the substrate by e.g. photo- or nano-imprint lithography or hot embossing [116]. Specific techniques for accomplishing the different steps can vary according to the material to be processed [13]. When microchannel networks are considered two major patterns can be distinguished: (a) a continuous flow microfluidic system, where solutions and solvents are fed by syringe pumps into the continuous-flow microfluidic device through tubing connections, and effluent(s) with the intended product(s) collected at the other end of the device; (b) integrated microfluidics, a more complex system, where a microchannel network is integrated with micromechanical valves and control components. Such assembly allows to perform and to automate complex chemical or biological reactions/processes in a single device. Efforts have thus been made aiming to develop such functioning modules that can improve the performance of microfluidic devices [13,115,117,118]. Among those are mixing modules to overcome diffusion limited mixing typical of the turbulence-free microfluidic environment [13,119-121]; pumps enabling the delivery and metering of fluidic components in microchannels [13,122,123]; modules allowing for separation by distillation [124-126], by crystallization [127-129] or by extraction [130,131]. Integration of modules with different functionalities has been used for analytical purposes [132,133], but also for parallel screening of chemical reactions [121,123,134], for the production of nanowires [135], or for the synthesis of radiopharmaceuticals [115,136] and of oligonucleotides [115,137], and for DNA sequencing [138]. Actually, the potential of integrated microfluidic devices in systems biology, namely within the scope of omics processes and de-novo
synthesis, has been reviewed most recently [139]. Given the nature of their configuration, integrated microfluidic devices can comprehend a set of analytics in the form of a suitable microarray. Such linkage allows one to overcome a typical drawback of bioprocess development typically carried out in continuous-flow microfluidic devices, where often a given microdevice has to be sacrificed in order to provide a suitable aliquot for offline analysis [13,140]. Therefore, efforts have been made in order to integrate sensors for the measurement of physical properties, namely temperature and flow rates, and for monitoring. The later strongly anchor in recent developments in fiber optics and flow-cell technology, which ultimately enable the continuous monitoring of streams in microchannels through several methodologies, among them: attenuated total reflectance-infrared (ATR-IR), near-infrared/ultraviolet/visible (NIR/UV/Vis), Raman and X-ray absorption spectroscopy; nuclear magnetic resonance (NMR); laser-induced fluorescence (LIF). The use of chromatographic methods (HPLC, GC) for monitoring has also been referred to, where samples are transferred from the microreactor to the chromatographic apparatus through suitable combination of micro-syringes and valves [13]. Aiming at the high throughput screening of enzyme inhibitors, using as model the protease cathepsin, de Boer and co-workers integrated a chip-based microreactor with HPLC-electrospray ionization mass spectrometry (ESI-MS). The integrated set-up allowed the simultaneous detection of chemical and biological parameters [141]. Recently, Fang and co-workers integrated a continuous-flow capillary-based microreactor with ultra-high-pressure liquid chromatography (UHPLC) for online analysis, as a set-up for the high-throughput screening system for homogeneous catalyst aimed at an intramolecular Friedel-Crafts addition [142]. An integrated microfluidic system coupling the lysis of cell lines of L929 fibroblasts and of A549 epithelial with an optical fiber, fluorescence-based enzyme assay, was designed to quantify the activity of β-glucocerebrosidase activity, the diagnostic marker for Gaucher’s disease. Enzymatic activity is established based on the cleavage of the synthetic β-glucoside, 4-methylumbelliferyl-β-D-glucopyranoside, by monitoring on-line the fluorescence of the released product, 4-methylumbelliferone, as a function of time [143].

4. Application of Microstructured Devices to Bioprocesses: Some Examples

4.1. Biocatalysis: Overall Considerations

The use of microreactors in biocatalysis can prove beneficial in either process development or at production scale, as recently highlighted [12]. In their realistic appraisal, Bolivar and co-workers suggest that, at the current stage of development, and where process development is concerned, namely at the key stage of screening enzymatic activity [144,145], the throughput available from microtiter plates is higher than that from microstructured reactors. The former configuration is furthermore more advanced when automation and integration with in-line analysis is considered, namely with commercially available systems [12,140,146,147]. Significant developments are taking place within the field of optical sensing systems, (such as microresonator sensing systems), with particular focus on the application to microfluidic devices, which are likely to result in the delivery of commercially available microfluidic devices with in-line analysis capability. The whole can lead to commercially available setups that can compete with the microtiter plate configuration [12,148,149]. Evidence for such pattern is considered to be also provided by the recent reviews on the application of microfluidic
lab-on-a-chip platforms [150,151]. While these are developed for diagnostics and specific biochemical assays, the strategies developed for incorporating sensors can be used in the microfluidic platforms targeted for bioprocess development. Again, technological developments are increasing the capability of controlled fluid dispensing within microfluidic devices using suitable functional elements (viz. microvalves and micropumps) and are furthermore allowing the production of such devices heavily integrated with said functional elements [150]. This can be particularly helpful when screening for enzyme activity, and establishing kinetic parameters, designing multi-step bioconversion systems or optimizing bioconversion/fermentation systems is aimed at. An integrated microfluidic reactor harboring a set of functional elements was effectively tested, using a bovine carbonic anhydrase II (bCAII) click chemistry system as proof-of-concept study, where the synthetic activity of the enzyme over acetylenic benzenesulfonamide and a library of 20 complementary azides was screened. A very similar outcome was observed when the results were matched with those obtained in an experimental setup anchored in conventional 96-well microtiter plates [121]. Jambovane and co-authors fabricated a fully integrated microfluidic chip with sample metering, mixing, and incubation functionalities, and tested the feasibility of the device by establishing kinetic parameters, $K_M$ and $k_{cat}$ (turnover number) in a single experiment, and evaluated the effect of inhibitors, phenylethyl $\beta$-D-thio-galactoside and lactose, using as model system the hydrolysis of resorufin-$\beta$-D-galactopyranoside promoted by $\beta$-galactosidase. The authors were also able to report deviations in $K_M$ and $k_{cat}$ under 0.3 and 20.4%, respectively, when comparing on-chip and off-chip runs [152].

A realistic perspective on the feasibility of process intensification during biocatalytic production scale has again been addressed by Bolivar and co-workers [12]. Taking also as reference data and conclusions compiled regarding chemical transformation, the authors esteem that intensification is a logic step at such stage only if the actual chemical transformation is relatively fast but the overall process is hampered by heat or mass transfer condition. Most biotransformations are relatively slow (with typical rates of $k = 0.1–100 \text{ s}^{-1}$), suggesting that mass transfer is not limiting, and besides little heat is released. The authors accordingly suggest that significant process intensification at production scale will only occur when considering enzyme catalyzed reactions involving transport across phase boundaries.

Heijnis and co-workers developed a setup anchored in a Y-shaped commercial microchip to study the cross-linking of $\alpha$-lactalbumin with horseradish peroxidase (HRP) [153]. Hydrogen peroxide, $\alpha$-lactalbumin and HRP were loaded into the microchannel using a syringe pump and the evolution of the reaction was monitored using a UV-detector connected in-line with the microreactor. The authors were able to develop a suitable reaction model. Furthermore, the bioconversion proved reproducible, judging from the size distribution of the reaction products, which suggests that the setup developed can be used as a fast screening method for the cross-linking of proteins promoted by HRP.

### 4.2 Specific Examples

#### 4.2.1. Immobilized Enzyme Microreactors

Microreactors have been used as scale-down system for complex enzymatic synthesis, such as multienzyme catalysis, cascade reactions, rapid characterization of bioconversion processes and design
Molecules 2011, 16 8380

of microfluidic biofuel cells [12,153-156]. The application of microstructured reactors in such bioconversion processes is often associated with the use of immobilized enzymes. Immobilization is typically implemented by coating the wall of the microreactor [157], or by packing the microchannels with either small beads or with a monolith structure. The latter structure comprises open channels, hence providing an alternative to the use of microreactors packed with small beads, where large pressure drop can be eventually associated with continuous operation [12,158,159]. Immobilization of enzymes in the surface of channels overcomes backpressure limitations and provides a large interfacial area per unit volume [160], albeit eventually at the cost of enzyme loading. Some recent illustrative examples of the application of these two different approaches are given in Tables 2 and 3.

A configuration that eludes the two aforementioned approaches, while still involving packing of a microfluidic reactor has been suggested by Schilke and co-workers [161]. It is based in an IR flow cell packed with an enzyme immobilized in silicon dioxide nanosprings in a mat format. Nanosprings are claimed to provide high solvent-accessible surface area, present adequate permeability and mechanical stability, and can be patterned into existing microdevices. The selected enzyme, β-galactosidase, was immobilized in a nanospring mat (2.2 cm² × 60 µm thick) after treating the inorganic support with γ-aminopropyltriethoxysilane, then with N-succinimidyl-3-(2-pyridyl-dithio)-propionate (SPDP), and finally with dithiothreitol, to produce surface thiol groups. Once modified by treatment with SPDT, in order to introduce thiol-reactive pyridyl disulfide groups, the enzyme was covalently bound to the support by a covalent disulfide bond. The nanospring mat biocatalyst was then placed into a 175-µm high microchannel, and used to study the kinetics and steady-state conversion pattern of the hydrolysis of o-nitrophenyl β-D-galactosylpyranoside under different substrate flow rates and concentrations. The authors were able to produce a numerical model to fit the experimental data and to simulate reactor performance. Furthermore, it is suggested that in-situ regeneration by reduction with dithiothreitol followed by incubation with the modified β-galactosidase is possible.

Another alternative configuration for an immobilized enzyme microreactor was developed by Alam and co-workers, combining a microreactor coupled to a cellulose membrane unit, which has been used for the hydrolysis of sugar beet pectin by pectin lyase [162]. In the set-up, which however involves the use of a non-structured reactor, for the latter is of microchemostat-like configuration [12,17,163], process integration is implemented since the membrane unit allows in-situ separation of products from unreacted substrate and enzyme. Comparable results were obtained when the continuous membrane microbioreactor and a lab-scale set-up were matched, illustrating the validity of the approach.

Considering the flexibility of processes anchored in the use of microreactors, along the costs of the microstructured devices, a particular challenging issue lies in the development of immobilization strategies that would allow for full removal of the enzyme (or cell) from the immobilization matrix when the catalytic activity decreases or a new enzyme is to be tested. Without prejudice of the stability of the attachment during operation, removal should be easily performed under elution condition. The successful accomplishment of such goal would allow the reuse of microdevices. Widely applicable strategies for reversible protein binding are therefore been looking for, preferably based on the use of specifically tagged proteins [12].

Microfluidic reactors have also been used in multi-fluid phase systems, namely for processes involving sparingly water soluble molecules. Work on such systems has given valuable insight on the
relevance of several features for the development of an efficient setup, such as nature and ratio of the immiscible phase, or the effect of the chemical composition of the channel surface on phase separation [12,16,154]. Phase separation can be typically achieved taking advantage of small interfacial areas that provide enough capillary pressure to compensate the imposed driving pressure or by modifying the wetting characteristics in order to stabilize the interfaces [177,178]. Since chemically modified surfaces can degrade under process conditions, methodologies for maintaining phase separation by incorporating specifically designed and fabricated materials with different surface properties, viz. hydrophilic glass combined with Teflon, were developed [177,178]. Several applications in biocatalysis involving multi-fluid systems rely on the use of free enzymes, since the latter are preferably retained in one of the phases, a feature that already configures an immobilization pattern, ultimately enabling enzyme recovery and re-use. Some relatively recent representative examples are given in Table 4. Nevertheless coupling enzyme immobilization to two-liquid phase systems has also been implemented. Enzyme immobilization in macroporous silica-monomoliths with controllable porosity, packed within a capillary column, has been coupled to operation in biphasic liquid systems. An example is the hydrolysis of 4-nitrophenyl butyrate in water–decane media with immobilized Candida antarctica lipase A [179]. The kinetic studies performed with showed that $k_{cat}$ was similar to that for free lipase in solution, whereas the apparent $K_M$ for the immobilized enzyme was 12-fold lower than that for the free form. Again, a 96% conversion yield was obtained with the immobilized form, roughly exceeding 4-fold the yield with the free lipase, a feature ascribed to the favorable biphasic system in the continuous flowing micro-reactor system, given the relevant increase in the interfacial activation. Immobilization also enhanced operational stability. Bioconversion systems developed in two-liquid phase systems in microreactor environment are typically performed in co-flow mode.

Given the growing use of microreactors in applied biocatalysis, attempts have been made to establish a rationale for the design of representative experimental setups anchored in said devices. Using as model system the hydrolysis of o-nitrophenyl-β-D-galactopyranoside by β-galactosidase, Swarts and co-authors were able to establish that, for given residence times, diffusion could affect reaction rate [53]. These authors were furthermore able to correlate such critical residence time with operational parameters, namely enzyme concentration, substrate concentration and channel width, therefore contributing for the definition of key issues required for the design of robust and reproducible microreactor system.

Recently, further guidelines have been suggested to optimize the design of co-flow enzyme microreactors [89]. Since mass transfer restrictions can affect reaction rate and productivity, depending on enzyme properties, operation conditions and dimensions of the microreactor, correlations for these later parameters were developed, assuming Michaelis-Menten type kinetics. The authors conclude that effectiveness, defined as the ratio of the observed reaction rate to the reaction rate, is a key parameter for microreactor design, but may not lead to optimized throughput, this depending on the configuration of the microreactor. Actually, the effectiveness decreases as function of the channel width, accordingly smaller microchannels minimize mass transfer restrictions. On the other hand, the throughput of co-flow microreactors displays an optimum as function of the channel width, under mass transfer limiting conditions. Maximum throughput and high effectiveness are therefore considered to establish a window of opportunity for the design of co-flow enzymatic microreactors.
Table 2. Packed-bed type microreactors: some case-studies.

| Enzyme                  | Immobilization method and reactor                                                                 | Comments on the immobilized-based system                                                                                                                                                                                                 | Reference |
|-------------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| (+)-γ-lactamase          | Enzyme cross-linking combined with controlled pore glass (1:1) packed in silica-fritted capillary tubes | Hydrolysis of amides. Evaluation of enzyme stability, activity, kinetics and substrate specificity. High activity retention; similar substrate specificity for most substrates and increased for acrylamide as compared to free form. Enhanced thermal stability, thus allowing extensive screening tasks using a single microreactor. | [164]     |
| Lipase                  | Novozyme® 435 (enzyme adsorbed on crosslinked PMMA resin, Lewatit VP OC 1600) and packed in glass capillary columns | Chemo-enzymatic epoxidation of olefins was proved feasible, with significant reduction in reaction time as compared to operation in standard batch reactor. The potential of this approach as a suitable tool for the study of the reaction was established. | [165,166] |
| Lipase                  | Novozyme® 435 (enzyme adsorbed on crosslinked PMMA resin, Lewatit VP OC 1600) and packed in microchannels milled on aluminum | Polymerization of ε-caprolactone to polycaprolactone. Operation with the microreactor allowed faster polymerization and higher molecular mass of product when compared to operation with batch reactors. Corroborates the potential of these platforms for high throughput screening of enzymes and process conditions. | [167]     |
| L-aminoacylase          | Enzyme immobilization through the reaction of the primary amine groups with epoxy terminal groups on the surface of PGMCED monoliths formed inside the microreactor channels | The authors established the use of the immobilized microreactor as a reliable screening tool for enzyme selectivity, aiming at the production of L-amino acids and their analogues. Additionally, the order of preferred N-protecting group (benzoyl) and the order of preferred N-benzoyl protected amino acids were established. High thermal and operational stability, allowing the use small amounts of organic solvents and temperatures as high as 50ºC for bioconversions where substrate solubility could be a limitation. | [168]     |
| Enzyme System                                                                 | Immobilization Method                                                                 | Description                                                                                                                                                                                                                                                                                                                                 | Reference |
|------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Glucose oxidase (GOD) and choline oxidase (CHO)                              | Each enzyme immobilized in sol-gel monolith, where the precursors were allowed to polymerize in the microchannel of a glass microreactor. The monolith was activated with PEI, and the enzyme immobilized through electrostatic interaction between electronegative enzymes and electropositive PEI polymers | Oxidation of glucose (GOD) and choline (CHO) Hydrogen peroxide formed as product of either reaction was quantified amperometrically using an on-chip electrochemical cell Data for GOD - $k_{cat}$ similar to that for GOD in solution, albeit with $V_{max}$ (maximum reaction rate) 70 fold higher, and $K_M$ 4 fold lower - suggested favorable enzyme concentration in the microenvironment of the monolith and enhanced maintenance of enzyme conformation Data for CHO - $k_{cat}$ and $K_M$ values similar to those for CHO in solution, but a 50 fold increase in $V_{max}$ – corroborate the efficiency of the methodology Regeneration of the microreactor by rinsing with 1 M NaOH solution, making the operation of the microreactor highly flexible | [159]     |
| Horseradish peroxidase (HRP)                                                 | Immobilization onto the surface of gold, with thickness from nano- to micro-scale deposited in a silicon wafers. Binding by adsorption or by covalent interaction with the gold surface | Enzymatic oxidation of phenols The stabilizing effect of immobilization on enzyme activity, the screening capability and the operational stability of the device were established. Regeneration of the microreactor through an electrochemical cleaning procedure, making the operation of the microreactor highly flexible | [169]     |
| Hydroxylaminobenzene mutase and soybean peroxidase                          | Each enzyme entrapped in silica nanoparticles, which were packed in microfluidic chips. | Chemo-enzymatic synthesis of APO, aminophenoxazin-3-one, from nitrobenzene, by connecting in series three individual microreactors, harboring zinc, mutase and peroxidase. The potential of microfluidic reactors for performing chemo-enzymatic multistep reactions was established | [170]     |

PMMA: poly-(methyl methacrylate; PGMCED: poly(glycidylmethacrylate-co-ethylenedimethacrylate); PEI: polyethylenimine
Table 3. Coated-wall type microreactor: some case studies.

| Enzyme                  | Immobilization method and reactor                                                                 | Comments on the immobilized-based system                                                                                                                                                                                                                   | Reference |
|-------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| β-glycosidase           | Covalent binding to the surface-activated walls of the stainless steel microreactor            | The setup was used for the continuous hydrolysis of lactose Conversion yield in excess of 70%, a space time yield 500 mg glucose mL⁻¹ h⁻¹, and a half life of 15 days were observed. Results suggest that the immobilized microreactor is a suitable tool for screening, reaction optimization and preparative synthesis on demand | [171]     |
| β-glycosidase (thermostable) | Covalent binding to the surface-activated walls of the stainless steel microreactor            | Synthesis of β-glucosylglycerol from cellobiose and glycerol Under selected operational conditions, 120 mM of β-glucosylglycerol were obtained from 250 mM cellobiose and 1 M glycerol Conversion behavior similar to that in a batch stirred reactor with soluble enzyme Rapid identification of suitable reaction conditions, corroborating the high-throughput nature of microreactor for process characterization | [172]     |
| Fumarase                | Covalent binding to the inner surface of glass microchannels after silanization with APTES and cross-linking with glutaraldehyde | Hydration of fumaric acid to L-malic acid The immobilized enzyme retained 25% of the activity of the free form, which the authors claimed to exceed previous reported data Selected operational conditions allowed a conversion yield of up to 80%. Development of a predictive 3D model comprising mass transfer and reaction kinetics | [173]     |
| Lipase                  | Adsorption of lipase on mesoporous silica (MPS) thin film deposited on its inner walls of micro-capillary borosilicate tubes | Enantioselective transesterification of vinyl acetate with (±)-1-phenylethanol A 3D cubic structure film allowed a yield of 64%, for an enantioselectivity in excess of 99% in continuous flow experiment The catalytic activity of the immobilized PS exceeded that of the native enzyme High operational stability | [160,174] |
Table 3. Cont.

| Enzyme          | Fluid system and reactor                                                                 | Comments on the micro-scale bioconversion system                                                                                                                                                                                                 | Reference |
|-----------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Lipase          | Covalent binding to the inner surface of silica microstructured optical fiber after silanization with APTES and cross-linking with glutraldehyde. Microreactors were 20 cm long | Synthesis of butyl laurate from n-butanol and lauric acid at 50 °C, with n-hexane and n-heptane as solvents. A 3:1 n-butanol-lauric mole ratio yield of up to 99% in less than 38 s of residence time. Bioconversion pattern was roughly similar with either solvent, but n-heptane may be preferred due to lower toxicity. High operational and storage stability. Full conversion foreseeable in longer microreactors, which could pave the way for scaling production by numbering up. | [175]     |
| Yeast cells     | Covalent binding to the inner surface of microchannels after silanization with APTES and cross-linking with glutraldehyde | Sulfuric acid was shown to be the most effective for surface activation of different materials, namely glass, FEP, PFA, PS and PTFE, prior to silanization with APTES. A cell coverage of about 70% was reported in all materials tested. | [176]     |

APTES: 3-aminopropyl-triethoxysilane; FEP: fluorinated ethylene propylene; PFA: perfluoroalkoxy; PS: polystyrene; PTFE: polytetrafluoroethylene

Table 4. Two-liquid phase bioconversion systems in microreactors: some case studies.

| Enzyme          | Fluid system and reactor                                                                 | Comments on the micro-scale bioconversion system                                                                                                                                                                                                 | Reference |
|-----------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Cholesterol oxidase | Aqueous phase containing enzyme solution; n-heptane phase containing the substrate. These were separately fed through a Y-shaped inflow to the microchannel of the glass microchip reactor. | Oxidation of cholesterol to 4-cholestene-3-one. Selected ratio of the fluid flow rates allowed for phase separation in Y-shaped outflow of the microreactor, enabling *in-situ* recovery of the product (present in the organic phase). Roughly 70% conversion of 0.17 mM cholesterol was obtained for residence times close to 1 minute. Characterization of the bioconversion system through a 3D mathematical model comprising mass transfer, kinetics and velocity profiles. | [93]      |
| Enzyme               | Reaction                                                                 | Details                                                                 | References |
|---------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------|------------|
| Hydroxynitrile      | Enantioselective synthesis of cyanohydrins from aldehydes                | Clogging during addition of the lysates to the microchannels did not 90% and enantioselectivity in excess of 99% were obtained | [180]      |
| lyase               |                                                                          | Results consistent with those from large, batchwise process, validating the microscale approach |            |
|                     | Aqueous phase containing crude enzyme lysates and HCN; organic phase     | These were separately fed through a Y-shaped inflow to the microchannel of the glass microchip reactor. The product was recovered from the single outflow |            |
|                     | containing a selected aldehyde.                                         |                                                                          |            |
|                     |                                                                          | Up to 87% conversion of 0.5 mM of L-DOPA observed at residence times of 100 s | [181]      |
|                     |                                                                          | Increasing the inlet concentration of L-DOPA decreased conversion efficiency, possibly to the low molecular diffusivity of laccase in water. A longer microchannel could overcome this drawback |            |
| Laccase             | Oxidation of L-DOPA                                                     | Characterization of the bioconversion system through a 2D mathematical model considering convection and diffusion, and kinetics |            |
|                     |                                                                          |                                                                          | [181]      |
|                     | Peel phase containing crude enzyme lysates and HCN; organic phase       |                                                                          |            |
|                     |                                                                          |                                                                          | [182]      |
|                     |                                                                          |                                                                          |            |
|                     |                                                                          |                                                                          |            |
| Lipase              | Synthesis of isoamyl acetate from isoamyl alcohol and acetic acid       |                                                                          |            |
|                     |                                                                          |                                                                          | [182]      |
|                     | Feed phase containing isoamyl alcohol and acetic acid; organic phase    |                                                                          |            |
|                     |                                                                          |                                                                          |            |
|                     |                                                                          |                                                                          |            |
|                     |                                                                          |                                                                          |            |
|                     |                                                                          |                                                                          |            |
|                     |                                                                          |                                                                          |            |
Table 4. Cont.

| Lipase | Aqueous phase containing enzyme solution; \( n \)-decane phase containing substrates. These were separately fed through a Y-shaped inflow to the microchannel of the microchip reactor. The product was recovered from the single outflow after centrifugation. | Synthesis of butyl-propionate from the esterification of propionic acid and 1-butanol. The product partitions preferably to the organic phase, while substrates favor the aqueous phase, the whole preventing the reaction to reach equilibrium. A Ping Pong Bi Bi mechanism with alcohol inhibition was developed to describe the reaction. Kinetic parameters and thermal activation and inactivation patterns matched those observed in bench scale run. Validates microfluidic approach for characterization of these systems with evident cost optimization. | [183] |
| Lipase | IL ([bmpyr][dca]) phase, containing lipase, isoamyl alcohol; IL ([bmpyr][dca]) phase containing acetic anhydride; \( n \)-heptane. These were separately fed through a \( \psi \)-shaped inflow to the microchannel of the microchip reactor. | Synthesis of isoamyl acetate from isoamyl alcohol and acetic anhydride. A roughly 3-fold increase in the reaction rate was observed for the synthesis performed in microreactor environment, as compared to that observed in a stirred batch reactor, resulting in better productivity than any reported that far. Results were ascribed to the reaction–diffusion dynamics in the microchannel system, enabling an emulsification that led to a large interfacial area for the reaction and simultaneous product extraction. | [184] |

L-DOPA: 3,4-dihydroxy-L-phenylalanine; IL: ionic liquid; [bmpyr][dca]: 1-butyl-3-methylpyridinium diacyanamide
4.2.2. Downstream Processing

The technological developments that have been taking place at the level of design and fabrication in micro and nanofluidics environments, namely at the level of microfluidic handling liquid, viz. micromixers, micropumps, and microvalves, have led to microfluidic platforms that can comprise a wide set of unit operations. Each unit operation corresponds to a building block of laboratory protocol and encompasses fluid transport, fluid metering, fluid mixing, valving, separation or concentration of molecules or particles [150]. The integration of such unit operation in microfluidic platforms has been gaining relevance at the level of devices for point of care diagnostics and for detection and screening of bacteria and drugs, but application of the concept in further areas is gradually being implemented [150,185]. When bioprocess development is considered, most of the applications encompassing the use of microfluidic devices have been centered in the recovery of macromolecules, through liquid-liquid extraction, but also through chromatography. Applications on extractive two-liquid phase systems in microfluidic environment have focused mainly on the recovery and purification of proteins with therapeutic applications, since the intensification resulting from miniaturization decreases the risks of delays during bioprocess development and product launch [186,187]. Some examples of recent applications are given in Table 5.

Microfluidic chromatography columns have also been developed, aiming at improving the separation of biopharmaceuticals. Shapiro and co-workers used a 1.5 µL volume column packed with different fillings, viz. porous agarose beads, Q Sepharose Fast Flow, and were able to establish breakthrough and elution curves while processing different proteins. The authors were able to establish the reproducibility of the data throughout different scales, up to 30 mL laboratory [188,189].

5. Conclusions

The use of microstructured reactors within the scope of bioprocess intensification has been gaining momentum, namely due to the advantages brought by the enhanced heat and mass transfer, flexibility, ease of operation under controlled hydrodynamic conditions and low requirements of reagents. Technological developments are contributing for the development of microfluidic platforms, which integrate monitoring and fluid handling functionalities that further expand the range of application of microfluidic devices. Still, the contribution of these devices at bioprocess production scale is scarce, and a realistic appraisal on the feasibility of their application within such scope is undergoing. Further expected developments on this field can be related to the development of enzyme/whole cell immobilization strategies that will allow the reuse of the microfluidic platforms and further increase its flexibility as screening and process characterization tools. Furthermore, guidelines for the design of microfluidic devices as representative systems are being developed.

Acknowledgments

The authors would like to thank Fundação para a Ciência e a Tecnologia, Portugal, for financial support through contracts under the program Ciência 2007 awarded to P. Fernandes and for the post-doctoral grant SFRH/BPD/64160/2009 awarded to M.P.C. Marques.
**Table 5.** Application of microfluidic devices in downstream processing: some examples.

| Microfluidic device | Application and comments                                                                                                                                                                                                 | Reference |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Microchip with ψ-shaped inflow and Y-shaped outflow, for independent feeding of three different solutions and recovery of two separate phases | Isolation of fluorescent, genetically tagged proteins from *E. coli* lysates, through extraction in ATPS (PEG/salt)  
Laminar flow and low interfacial tension led to a stable interface along the microchannel. The protein was recovered in the PEG rich phase in one of the outflows; contaminants and interphase were recovered in the second outflow.  
The fluorescent nature of tagged proteins eased the visualization of the extraction process. Roughly 85% of contaminating proteins, unwanted nucleic acids and cell debris, were removed. | [190]     |
| Microchip with ψ-shaped inflow and outflow, for independent feeding of three different solutions and recovery of three separate phases | Purification of bacteriorhodopsin from *Halobacterium salinarium* cells extracts ATPS (PEG/salt) and IL (HHMM/salt) system were compared for protein isolation; cell suspension fed through central inlet, with a three phase flow maintained throughout the microchannel in both ATPS and IL system  
Contaminants were removed to the PEG (or IL) and salt phases.  
The recovery rate of protein was roughly similar for both methods, roughly 90%, with purity of 95%, but IL system proved more sensitive to variations in pH, as reflected by the concomitant decrease in the recovery rate | [191]     |
| Microchip with ψ-shaped inflow and outflow, for independent feeding of three different solutions and recovery of three separate phases | Purification of membrane proteins from crude cell through extraction in ATPS (PEG/detergent)  
Continuous operation in microfluidic environment is claimed to result in increased extraction rate and efficiency when compared to the traditional discontinuous approach | [192]     |
| Microchip with ψ-shaped inflow and outflow, for independent feeding of three different solutions and recovery of three separate phases | Discrimination of live and dead cells from animal cell cultures, through extraction in ATPS (PEG/dextran)  
Optimized flow rates led to stable aqueous two-phase flows along the microchannel  
Live recovered in the PEG phase. The recovery efficiency of live cell was up to 97%, as compared to only 85.5% in the normal macroscale ATPS | [193]     |
| Microchip with ψ-shaped inflow and outflow, for independent feeding of three different solutions and recovery of three separate phases | Use of ATPS (PEG/dextran) for the outflow microchip were used for the separation of leukocyte and erythrocytes from whole blood cells, and for the concentration of leukocytes | [194,195] |
Table 5. Cont.

| Microchip with Y-shaped inflow and outflow, for independent feeding of aqueous and organic phases | Extraction of progesterone and 11α-hydroxyprogesterone from an aqueous phase with ethyl acetate. Model system and integration with a whole cell bioconversion where 11α-hydroxylation is performed by Rhizopus nigricans pellets. Extraction occurred in few seconds and the mathematical model of the extraction developed was shown to correlate with experimental data. Further optimization of the extraction and numbering up of microdevices, is likely to result in a realistic integrated system for the production of 11α-hydroxyprogesterone. | [196,197] |
| --- | --- | --- |
| Microchip with Y-shaped inflow and outflow, for independent feeding of aqueous and organic phases | Enantioselective separation of racemic amino acids. The model systems integrate the enantioselective deacetylation of N-acetyl-D,L-amino acids in aqueous media carried out in a microreactor; the product phase is acidified and fed to the microchip extractor, where the acetyl-D-amino acid is extracted into the organic phase. In most cases, the optical resolution of acetyl-D-amino acid exceeded 98% and the final recovery of an acetyl-D-amino acid from the aqueous phase exceeded 85%. | [198] |

ATPS: aqueous two phase system; HHMM: hexafluorophosphate (1-n-hexyl-3-methylimidazolium); IL: ionic liquid; PEG: polyethylene glycol

Conflict of Interest

The authors have no conflict of interest to declare.
References

1. Wohlgemuth, R. The locks and keys to industrial biotechnology. *New Biotechnol.* **2009**, *25*, 204-213.

2. Clark, J.H.; Deswarte, F.E.I.; Farmer, T.J. The integration of green chemistry into future biorefineries. *Biofuels Bioprod. Bioref.* **2009**, *3*, 72-90.

3. Lutze, P.; Gani, R.; Woodley, J.M. Process intensification: A perspective on process synthesis. *Chem. Eng. Process.* **2010**, *49*, 547-558.

4. Charpentier, J.-C. Process intensification by miniaturization. *Chem. Eng. Technol.* **2005**, *28*, 255-258.

5. Reay, D.; Ramshaw, D.; Harvey, A. *Process Intensification—Engineering for Efficient Sustainability and Flexibility*; Butterworth-Heinemann: Oxford, UK, 2008; pp. 223-322.

6. Ehrfeld, W. Process Intensification Through Microreaction Technology. In *Re-engineering the Chemical Process Plant—Process Intensification*; Stankiewicz, A., Moulijn, J.A., Eds.; Marcel Dekker, Inc.: New York, NY, USA, 2007; pp. 155-175.

7. Hessel, V.; Löb, P.; Löwe, H. Industrial microreactor process development up to production. In *Microreactors in Organic Synthesis and Catalysis*; Wirth, T., Ed.; Wiley-VCH: Weinheim, Germany, 2008; pp. 211-275.

8. Pohar, A.; Plazl, I. Process Intensification through Microreactor Application. *Chem. Biochem. Eng. Q.* **2009**, *23*, 537-544.

9. Titchener-Hooker, N.J.; Dunnill, P.; Hoare, M. Micro biochemical engineering to accelerate the design of industrial-scale downstream processes for biopharmaceutical proteins. *Biotechnol. Bioeng.* **2008**, *100*, 616-624.

10. Stankiewicz, A.; Drinkenburg, A.A.H. Process Intensification: History, Philosophy, Principles. In *Re-engineering the Chemical Process Plant—Process Intensification*; Stankiewicz, A., Moulijn, J.A., Eds.; Marcel Dekker, Inc.: New York, NY, USA, 2007; pp. 1-28.

11. Yang, Y.-Q. Microscale and Nanoscale Process Systems Engineering: Challenge and Progress. *Chin. J. Proc. Eng.* **2008**, *8*, 616-624.

12. Bolivar, J.M.; Wiesbauer, J.; Nidetzky, B. Biotransformations in microstructured reactors: More than flowing with the stream? *Trends Biotechnol.* **2011**, *29*, 333-342 and references therein.

13. McMullen, J.P.; Jensen, K.F. Integrated Microreactors for Reaction Automation: New Approaches to Reaction Development. *Annu. Rev. Anal. Chem.* **2010**, *3*, 19-42 and references therein.

14. Miyazaki, M.; Honda, T.; Yamaguchi, H.; Briones M.P.P.; Maeda, H. Enzymatic processing in microfluidic reactors. *Biotechnol. Gen. Eng. Rev.* **2008**, *25*, 405-428 and references therein

15. Ren, K.; Dai, W.; Zhou, J.; Su, J.; Wu, H. Whole-Teflon microfluidic chips. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8162-8166.

16. Fernandes, P. Miniaturization in biocatalysis. *Int. J. Mol. Sci.* **2010**, *11*, 858-879.

17. Schäpper, D.; Alam, M.N.; Szita, N.; Eliasson Lantz, A.; Gernaey, K.V. Application of microbioreactors in fermentation process development: A review. *Anal. Bioanal. Chem.* **2009**, *395*, 679-695.

18. Borovinskayaa, E.S.; Reshetilovskii, V.P. Microstructural Reactors: Concept, Development and Application. *Russ. J. Appl. Chem.* **2008**, *81*, 2211-2231.
19. Chován, T.; Guttman, A. Microfabricated devices in biotechnology and biochemical Processing. *Trends Biotechnol.* 2002, 20, 116-122.
20. Mills, P.L.; Quiram, D.J.; Ryley, J.F. Microreactor technology and process miniaturization for catalytic reactions—A perspective on recent developments and emerging technologies. *Chem. Eng. Sci.* 2007, 62, 6992-7010.
21. Ehrfeld, W.; Hessel, V.; Löwe, H. *Microreactors: New Technology for Modern Chemistry*; Wiley-VCH: Weinheim, Germany, 2000; pp. 1-14.
22. Stroock, A.D.; Wheeler, T.D.; Kirtland, J. Microfluidic Relief for Transport Limitations. *BioTechniques* 2005, 39, 159-162.
23. Squires, T.M.; Quake, S.R. Microfluidics: Fluid physics at the nanoliter scale. *Rev. Mod. Phys.* 2005, 77, 977-1026.
24. Ong, S.-E.; Zhang, S.; Du, H.; Fu, Y. Fundamental principles and applications of microfluidic systems. *Front. Biosci.* 2008, 13, 2757-2773.
25. Livak-Dahl, E.; Sinn, I.; Burns, M. Microfluidic Chemical Analysis Systems. *Annu. Rev. Chem. Biomol. Eng.* 2011, 2, 325-353
26. Shui, L.; Eijkel, J.C.T.; van den Berg, A. Multiphase flow in microfluidic systems—Control and applications of droplets and interfaces. *Adv. Colloid Interface Sci.* 2007, 133, 35-49 and references therein.
27. Hartman, R.L.; Jensen, K.F. Microchemical systems for continuous-flow synthesis. *Lab Chip* 2009, 9, 2495-2507 and references therein.
28. Agiral, A.; Gardeniers, H.J.G.E. Microreactors with Electrical Fields. *Adv. Chem. Eng.* 2010, 38, 37-102.
29. Cecilia, R.; Kunz, U.; Turek, T. Possibilities of process intensification using microwaves applied to catalytic microreactors. *Chem. Eng. Proc.* 2007, 46, 870-881
30. Akay, G.; Erhan, E.; Keskinler, B. Bioprocess Intensification in Flow-Through Monolithic Microbioreactors with Immobilized Bacteria. *Biotechnol. Bioeng.* 2005, 90,180-190
31. Wu J. Interactions of electrical fields with fluids: laboratory-on-a-chip applications. *IET Nanobiotechnol.* 2008, 2, 14-27.
32. Zhao, C.X.; Middelberg, A.P.J. Two-phase microfluidic flows. *Chem. Eng. Sci.* 2011, 66, 1394-1411.
33. Pohar, A.; Plazl, I. Laminar to Turbulent Transition and Heat Transfer in a Microreactor: Mathematical Modeling and Experiments. *Ind. Eng. Chem. Res.* 2008, 47, 7447-7455.
34. Garstecki, P.; Fuerstman, M.J.; Stone, H.A.; Whitesides, G.M. Formation of droplets and bubbles in a microfluidic T-junction-scaling and mechanism of break-up. *Lab Chip* 2006, 6, 437-446.
35. Akbar, M.K.; Plummer, D.A.; Ghaasaiyaan, S.M. On gas-liquid two-phase flow regimes in microchannels. *Int. J. Multiphase Flow* 2003, 29, 855-865.
36. Gupta, R.; Fletcher, D.F.; Haynes, B.S. On the CFD modelling of Taylor flow in microchannels. *Chem. Eng. Sci.* 2009, 64, 2941-2950.
37. Özkan, F.; Hecht, K.; Pfeiffer, P.; Schubert, K.; Kraushaar-Czarnetzki, B. Influence of the contact angle on two-phase flow in microreactors for nitrobenzene–hydrogen–stainless steel/carbon. *Surf. Interf. Anal.* 2010, 42, 1122-1127.
38. Santos, R.M.; Kawaji, M. Numerical modeling and experimental investigation of gas-liquid slug formation in a microchannel T-junction. *Int. J. Multiphase Flow* 2010, 36, 314-323.
39. Fries, D.M.; Trachsel, F.; von Rohr, P.R. Segmented gas-liquid flow characterization in rectangular microchannels. *Int. J. Multiphase Flow* 2008, 34, 1108-1118.
40. Kumar, V.; Paraschivoiu, M.; Nigam, K.D.P. Single-phase fluid flow and mixing in microchannels. *Chem. Eng. Sci.* 2011, 66, 1329-1373.
41. Kiwi-Minsker, L.; Kashid, M.N. Microstructured Reactors for Multiphase Reactions: State of the Art. *Ind. Eng. Chem. Res.* 2009, 48, 6465-6485.
42. Xu, J.; Li, S.; Tan, J.; Luo, G. Correlations of droplet formation in T-junction microfluidic devices: from squeezing to dripping. *Microfluid. Nanofluid.* 2008, 5, 711-717.
43. Colin, T.; Tancogne, S. Stability of bifluid jets in microchannels. *Eur. J. Mechanics - B/Fluids* 2011, 30, 409-420.
44. Skurtys, O.; Aguiera, J. Applications of Microfluidic Devices in Food Engineering. *Food Biophys.* 2008, 3, 1-15.
45. Berthier, J.; Silberzan, P. *Microfluidics for Biotechnology*, 2nd ed.; Artech House: Norwood, MA, USA, 2009; p. 483.
46. Gupta, A.; Kumar, R. Effect of geometry on droplet formation in the squeezing regime in a microfluidic T-junction. *Microfluid. Nanofluid.* 2010, 8, 799-812.
47. Kockmann, N.; Engler, M.; Woias, P. Theoretische und experimentelle Untersuchungen der Mischvorgänge in T-förmigen Mikroreaktoren - Teil 3: Konvektives Mischen und chemische Reaktionen. *Chem. Ing. Tech.* 2004, 76, 1777-1783.
48. Pompano, R.R.; Li, H.W.; Ismagilov, R.F. Rate of mixing controls rate and outcome of autocatalytic processes: Theory and microfluidic experiments with chemical reactions and blood coagulation. *Biophys. J.* 2008, 95, 1531-1543.
49. Nagatsu, Y.; Kondo, Y.; Kato, Y.; Tada, Y. Miscible viscous fingering involving viscosity increase by a chemical reaction with moderate Damköhler number. *Phys. Fluids.* *Phys. Fluids* 2011, 23, 014109.1-014109.8, doi:10.1063/1.3549844.
50. Kockmann, N. *Transport Phenomena in Micro Process*; Springer: Berlin, Germany, 2008; pp. 225-292.
51. Chen, X.B.; Sui, Y.; Cheng, Y.P.; Lee, H.P.; Yu, P.; Winoto, S.H.; Low, H.T. Mass transport in a microchannel enzyme reactor with a porous wall: Hydrodynamic modeling and applications. *Biochem. Eng. J.* 2010, 52, 227-235.
52. Roy, B.; Das, T.; Maiti, T.K.; Chakraborty, S. Effect of fluidic transport on the reaction kinetics in lectin microarrays. *Anal. Chim. Acta* 2011, 701, 6-14.
53. Swarts, J.W.; Kolfshoten, R.C.; Jansen, M.C.A.A.; Janssen, A.E.M.; Boom, R.M. Effect of diffusion on enzyme activity in a microreactor. *Chem. Eng. J.* 2010, 162, 301-306.
54. Plazl, I.; Lakner, M. Modeling and Finite Difference Numerical Analysis of Reaction-Diffusion Dynamics in a Microreactor. *Acta Chim. Slov.* 2010, 57, 100-109.
55. Kutter, J.P.; Klank, H. Microfluidics—theoretical aspects. In *Microsystem Engineering of Lab-on-a-chip Devices*; Geschke, O., Klank, H., Stankiewicz, P.T., Eds.; John Wiley & Sons: New York, NY, USA, 2004; pp. 13-37.
56. Datta, S.; Ghosal, S. Characterizing Dispersion in Microfluidic Channels. *Lab Chip* 2009, 9, 2537-2550.
57. Ni, Z.; Seebauer, E.G.; Masel, R.I. Effects of Microreactor Geometry on Performance: Differences between Posted Reactors and Channel Reactors. *Ind. Eng. Chem. Res.* 2005, 44, 4267-4271.

58. Jovanovic, G.N.; Znidarsic-Plazl, P.; Sakritti chai, P.; Al-Khaldi, K. Dechlorination of p-Chlorophenol in a Microreactor with Bimetallic Pd/Fe Catalyst. *Ind. Eng. Chem. Res.* 2005, 44, 5099-5106.

59. Plazl, I.; Lakner, M. Modeling and Finite Difference Numerical Analysis of Reaction-Diffusion Dynamics in a Microreactor. *Acta Chim. Slov.* 2010, 57, 100-109.

60. Fries, D.M.; von Rohr, P.R. Liquid mixing in gas-liquid two-phase flow by meandering microchannels. *Chem. Eng. Sci.* 2009, 64, 1326-1335.

61. Watts, P.; Wiles, C. Recent advances in synthetic micro reaction technology. *Chem. Comm.* 2007, 443-467.

62. Aubin, J.; Ferrando, M.; Jiricny, V. Current methods for characterising mixing and flow in microchannels. *Chem. Eng. Sci.* 2010, 65, 2065-2093.

63. Capretto, L.; Cheng, W.; Hill, M.; Zhang, X. Micromixing within Microfluidic Devices. *Top. Curr. Chem.* 2011, 304, 27-68 and references therein.

64. Hessel, V.; Löwe, H.; Schönfeld, F. Micromixers—A review on passive and active mixing principles. *Chem. Eng. Sci.* 2005, 60, 2479-2501.

65. 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.

66. Mansur, E.A.; Ye, M.; Wang, Y.; Dai, Y. A State-of-the-Art Review of Mixing in Microfluidic Mixers Purchase. *Chinese J. Chem. Eng.* 2008, 16, 503-516 and references therein.

67. Lee, C.Y.; Chang, C.L.; Wang, Y.N.; Fu, L.M. Microfluidic mixing: A review. *Int. J. Mol. Sci.* 2011, 12, 3263-3287.

68. Yang, J.-T.; Huang, K.-J.; Lin, Y.-C. Geometric effects on fluid mixing in passive grooved micromixers. *Lab Chip* 2005, 5, 1140-1147.

69. Buchegger, W.; Wagner, C.; Svasek, P.; Lendl, B.; Kraft, M.; Vellekoop, M.J. Fabrication and characterization of a vertical lamination micromixer for mid-IR spectroscopy. *Sensor Actuat. B Chem.* 2011, 159, 336-341.

70. Schneider, T.; Chapman, G.H.; Häfeli, U.O. Effects of chemical and physical parameters in the generation of microspheres by hydrodynamic flow focusing. *Colloid. Surface B* 2011, 87, 361-368.

71. Rhee, M.; Valencia, P.M.; Rodriguez, M.I.; Langer, R.; Farokhzad, O.C.; Karnik, R. Synthesis of size-tunable polymeric nanoparticles enabled by 3D hydrodynamic flow focusing in single-layer microchannels. *Adv. Mater.* 2011, 23, H79-H83.

72. Ottino, J.M.; Wiggins, S. Introduction: mixing in microfluidics. *Phil. Trans. R. Soc. Lond. A* 2004, 362, 923-935.

73. Mouza, A.A.; Patsa, C.-M.; Schönfeld, F. Mixing performance of a chaotic micro-mixer. *Chem. Eng Res. Develop.* 2008, 86, 1128-1134.

74. Bhagat, A.; Peterson, E.; Papautsky, I. A passive planar micromixer with obstructions for mixing at low Reynolds numbers. *J. Micromech. Microeng.* 2007, 17, 1017-1024.

75. Du, Y.; Zhang, Z.; Yim, C.; Lin, M.; Cao, X. A simplified design of the staggered herringbone micromixer for practical applications. *Biomicrofluidics* 2010, 4, 024105, doi:10.1063/1.3427240.
76. Stroock, A.D.; Whitesides, G.M. Controlling flows in microchannels with patterned surface charge and topography. *Acc. Chem. Res.** 2003, 36, 597-604.

77. Howell, P.B., Jr.; Mott, D.R.; Golden, J.P.; Ligler, F.S. Design and evaluation of a Dean vortex-based micromixer. *Lab Chip** 2004, 4, 663-669.

78. Sudarsan, A.; Ugaz, V. Fluid mixing in planar spiral microchannels. *Lab Chip** 2006, 6, 74-82.

79. Nguyen, N.-T.; Wu, Z. Micromixers—A review. *J. Micromech. Microeng.** 2005, 15, R1-R16.

80. Jen, C.P.; Wu, C.Y.; Lin, Y.C.; Wu, C.Y. Design and simulation of the micromixer with chaotic advection in microchannels. *Lab Chip** 2003, 3, 77-81.

81. Hessel, V.; Hardt, S.; Löwe, H.; Schönfeld, F. Laminar mixing in different interdigital micromixers: I. Experimental characterization. *AIChE J.** 2003, 49, 566-577.

82. Lye, G.J.; Ayazi-Shamlou, P.; Baganz, F.; Dalby, P.A.; Woodley, J.M. Accelerated design of bioconversion processes using automated microscale processing techniques. *Trends Biotechnol.** 2003, 21, 29-37.

83. Lindken, R.; Rossi, M.; Grosse, S.; Westerweel, J. Micro-Particle Image Velocimetry ([small micro(PIV): Recent developments, applications, and guidelines. *Lab Chip** 2009, 9, 2551-2567.

84. Falk, L.; Commenge, J.M. Performance comparison of micromixers. *Chem. Eng. Sci.** 2010, 65, 405-411.

85. Commenge, J.-M.; Falk, L. Villernaux-Dushman protocol for experimental characterization of micromixers. *Chem. Eng. Process.** 2011, doi:10.1016/j.cep.2011.06.006.

86. Ghaini, A.; Kashid, M.N.; Agar, D.W. Effective interfacial area for mass transfer in the liquid-liquid slug flow capillary microreactors. *Chem. Eng. Process.** 2010, 49, 358-366.

87. Hu, G.; Li, D. Multiscale phenomena in microfluidics and nanofluidics. *Chem. Eng. Sci.** 2007, 62, 3443-3454.

88. Kieffer, R.; Charcosset, C.; Puel, F.; Mangin, D. Numerical simulation of mass transfer in a liquid-liquid membrane contactor for laminar flow conditions. *Comp. Chem. Eng.** 2008, 32, 1325-1333.

89. Kolfschoten, R.C.; Swarts, J.W.; Janssen, A.E.M.; Boom, R.M. Guidelines for optimal design of coflow enzyme microreactors. *Chem. Eng. J.** 2011, 172, 1072-1077.

90. Kashid, M.N.; Platte, F.; Agar, D.W.; Turek, S. Computational modelling of slug flow in a capillary microreactor. *J. Comp. Appl. Math.** 2007, 203, 487-497.

91. Kashid, M.N.; Agar, D.W.; Turek, S. CFD modelling of mass transfer with and without chemical reaction in the liquid-liquid slug flow microreactor. *Chem. Eng. Sci.** 2007, 62, 5102-5109.

92. Schuster, A.; Sefiane, K.; Ponton, J. Multiphase mass transport in mini/micro-channels microreactor. *Chem. Eng. Res. Des.** 2008, 86, 527-534.

93. Marques, M.P.C.; Fernandes, P.; Cabral, J.M.S.; Znidarsic-Plazl, P.; Plazl, I. On the feasibility of in situ steroid biotransformation and product recovery in microchannels. *Chem. Eng. J.** 2010, 160, 708-714.

94. Quarteroni, A.; Saleri, F.; Veneziani, A. Factorization methods for the numerical approximation of Navier-Stokes equations. *Comp. Meth. Appl. Mechanics Eng.** 2000, 188, 505-526.

95. Balan, C.M.; Broboana, D.; Balan, C. Mixing process of immiscible fluids in microchannels. *Int. J. Heat Fluid Flow** 2010, 31, 1125-1133.
96. Fisher, L.; Ingram-Goble, R.; Wang, H.; Garrison, A.; Peterson, R.B. Development of a Microchannel Based Pasteurizer for Energy Efficient Processing of Liquids. *Appl. Thermal Eng.* 2011, 31, 3604-3614.

97. Liu, M. Computational study of convective-diffusive mixing in a microchannel mixer. *Chem. Eng. Sci.* 2011, 66, 2211-2223.

98. Shao, N.; Salman, W.; Gavriilidis, A.; Angeli, P. CFD simulations of the effect of inlet conditions on Taylor flow formation. *Int. J. Heat Fluid Flow* 2008, 29, 1603-1611.

99. Gupta, R.; Fletcher, D.F.; Haynes, B.S. CFD modelling of flow and heat transfer in the Taylor flow regime. *Chem. Eng. Sci.* 2010, 65, 2094-2107.

100. Uriz, I.; Arzamendi, G.; López, E.; Llorca, J.; Gandía, L.M. Computational fluid dynamics simulation of ethanol steam reforming in catalytic wall microchannels. *Chem. Eng. J.* 2011, 167, 603-609.

101. Crozier, P.S.; Henderson, D.; Rowley, R.L.; Busath, D.D. Model Channel Ion Currents in NaCl-Extended Simple Point Charge Water Solution with Applied-Field Molecular Dynamics. *Biophys. J.* 2001, 81, 3077-3089.

102. Kalra, A.; Garde, S.; Hummer, G. Osmotic water transport through carbon nanotube membranes. *PNAS* 2003, 100, 10175-10180.

103. Yeh, I.-C.; Hummer, G. Nucleic acid transport through carbon nanotube membranes. *PNAS* 2004, 101, 12177-12182.

104. Vlachos, D.G. Stochastic modeling of chemical microreactors with detailed kinetics--induction times and ignitions of H2 in air. *Chem. Eng. Sci.* 1998, 53, 157-168.

105. Xue, H.; Fan, Q.; Shu, C. Prediction of micro-channel flows using direct simulation Monte Carlo. *Prob. Eng. Mech.* 2000, 15, 213-219.

106. Snyder, M.A.; Chatterjee, A.; Vlachos, D.G. Net-event kinetic Monte Carlo for overcoming stiffness in spatially homogeneous and distributed systems. *Computers Chem. Eng.* 2005, 29, 701-712.

107. Wang, M.; Li, Z. Nonideal gas flow and heat transfer in micro- and nanochannels using the direct simulation Monte Carlo method. *Phys. Rev. E* 2003, 68, 046704.

108. Verma, N.; Mewes, D.; Luke, A. Lattice Boltzmann study of velocity, temperature, and concentration in micro-reactors. *Int. J. Heat Mass Transfer* 2010, 53, 3175-3185.

109. Kao, P.H.; Ren, T.F.; Yang, R.J. An investigation into fixed-bed microreactors using lattice Boltzmann method simulations. *Int. J. Heat Mass Transfer* 2007, 50, 4243-4255.

110. Li, C.; Chen, T. Simulation and optimization of chaotic micromixer using lattice Boltzmann method. *Sensors Actuators B Chem.* 2005, 106, 871-877.

111. Wu, L.; Tsutahara, M.; Kim, L.S.; Ha, M. Three-dimensional lattice Boltzmann simulations of droplet formation in a cross-junction microchannel. *Int. J. Multiphase Flow* 2008, 34, 852-864.

112. Vakhansky, A. Numerical analysis of residence time distribution in microchannels. *Chem. Eng. Res. Des.* 2011, 89, 347-351.

113. Derksen, J.J. Scalar mixing with fixed and fluidized particles in micro-reactors. *Chem. Eng. Res. Des.* 2009, 87, 550-556.

114. Parker, J.M.; Jovanovoc, G.N. A Multicomponent Lattice Boltzmann Model for Multiphase Convection, Diffusion, and Reaction in Two Dimensions. *Chem. Biochem. Eng. Q.* 2009, 23, 399-409.
115. Lin, W.Y.; Wang, Y.; Wang, S.; Tseng, H.R. Integrated Microfluidic Reactors. Nano Today. 2009, 4, 470-481.
116. Pisani, M.B.; Tadigadapa, S.A. Microfabrication Techniques for Microfluidic Devices. In Methods in Bioengineering—Biomicrofabrication and Biomicrofluidics; Zahn, J.D., Ed.; Artech House: Boston, MA, USA, 2010; pp. 1-30.
117. Ismagilov, R.F. Integrated Microfluidic Systems. Angew. Chem. Int. Ed. 2003, 42, 4130-4132.
118. Melin, J.; Quake, S.R. Microfluidic large-scale integration: the evolution of design rules for biological automation. Annu. Rev. Biophys. Biomol. Struct. 2007, 36, 213-231.
119. Stroock, A.D.; Dertinger, S.K.W.; Ajdari, A.; Mezic, I.; Stone, H.A.; Whitesides, G.M. Chaotic Mixer for Microchannels. Science 2002, 295, 647-651.
120. Lee, C.C.; Sui, G.; Elizarov, A.; Shu, C.Y.J.; Shin, Y.S.; Dooley, A.N.; Huang, J.; Daridon, A.; Wyatt, P.; Stout, D.; Kolb, H.C.; Witte, O.N.; Satyamurthy, N.; Heath, J.R.; Phelps, M.E.; Quake, S.R.; Tseng, H.R. Multistep Synthesis of a Radiolabeled Imaging Probe Using Integrated Microfluidics. Science 2006, 118, 5402-5407.
121. Wang, J.; Sui, G.; Mocharla, V.P.; Lin, R.J.; Phelps, M.E.; Kolb, H.C.; Tseng, H.-R. Integrated Microfluidics for Parallel Screening of an In Situ Click Chemistry Library. Angew. Chem. 2006, 118, 5402-5407.
122. Melin, J.; Quake, S.R. Microfluidic Large-Scale Integration: The Evolution of Design Rules for Biological Automation. Annu. Rev. Biophys. Biomol. Struct. 2007, 36, 213-231.
123. Tseng, H.-R.; Kolb, H.C.; Wang, J.; Sul, G. Integrated microfluidics for parallel screening of chemical reactions. U.S. Patent 2011,013,652, 6th of September 2011.
124. Hartman, R.L.; Sahoo, H.R.; Yen, B.C.; Jensen, K.F. Distillation in microchemical systems using capillary forces and segmented flow. Lab Chip 2009, 9, 1843-1849.
125. Zhang, Y.; Kato, S.; Anazawa, T. Vacuum membrane distillation by microchip with temperature gradient. Lab Chip 2010, 10, 899-908.
126. Lam, K.F.; Cao, E.; Sorensen, E.; Gavriliidis, A. Development of multistage distillation in a microfluidic chip. Lab Chip 2011, 11, 1311-1317.
127. Hansen, C.L.; Classen, S.; Berger, J.M.; Quake, S.R. A Microfluidic Device for Kinetic Optimization of Protein Crystallization and In Situ Structure Determination. J. Am. Chem. Soc. 2006, 128, 3142-3143.
128. Gerds, C.J.; Elliott, M.; Lovell, S.; Mixon, M.B.; Napuli, A.J.; Staker, B.L.; Nollert, P.; Stewart, L. The plug-based nanovolume Microcapillary Protein Crystallization System (MPCS). Acta Crystallogr. D Biol. Crystallogr. 2008, 64, 1116-1122.
129. May, A.P.; Segelke, B.W. Efficient macromolecular crystallization using microfluidics and randomized design of screening reagents. Methods Mol. Biol. 2008, 426, 387-402.
130. Kralj, J.G.; Sahoo, H.R.; Jensen, K.F. Integrated continuous microfluidic liquid-liquid extraction. Lab Chip 2007, 7, 256-263.
131. Castell, O.K.; Allender, C.J.; Barrow, D.A. Liquid-liquid phase separation: Characterisation of a novel device capable of separating particle carrying multiphase flows. Lab Chip 2009, 9, 388-396.
132. Roman, G.T.; Kennedy, R.T. Fully integrated microfluidic separations systems for biochemical analysis. J. Chromatogr. A 2007, 1168, 170-188.
133. Li, Y.; Zhang, C.; Xing, D. Integrated microfluidic reverse transcription-polymerase chain reaction for rapid detection of food- or waterborne pathogenic rotavirus. *Anal. Biochem.* **2011**, *415*, 87-96.

134. Wang, Y.J.; Lin, W.Y.; Liu, K.; Lin, R.J.; Selke, M.; Kolb, H.C.; Zhang, N.; Zhao, X.-Z.; Phelps, M.E.; Shen, C.K.F.; Faull, K.F.; Tseng, H.-R. An integrated microfluidic device for large-scale in situ click chemistry screening. *Lab Chip* **2009**, *9*, 2281-2285.

135. Wang, J.; Bunimovich, Y.L.; Sui, G.; Savvas, S.; Wang, J.; Guo, Y., Heath, J.R.; Tseng, H.R. Electrochemical fabrication of conducting polymer nanowires in an integrated microfluidic system. *Chem Commun (Camb)*. **2006**, *29*, 3075-3077.

136. Oh, J.H.; Lee, B.N.; Nam, K.R.; Attla, G.A.; Lee, K.C.; Cjai, J.S. Design Features Of Microfluidic Reactor For [18F]FDG Radiopharmaceutical Synthesis. *AIP Conf. Proc.* **2011**, *1336*, 426-429.

137. Lee, C.C.; Snyder, T.M.; Quake, S.R. A microfluidic oligonucleotide synthesizer. *Nucleic Acids Res*. **2010**, *38*, 2514-2521.

138. Chen, Y.J.; Roller, E.E.; Huang, X.H. DNA sequencing by denaturation: experimental proof of concept with an integrated fluidic device. *Lab Chip* **2010**, *10*, 1153-1159.

139. Szita, N.; Polizzi, K.; Jaccard, N.; Baganz, F. Microfluidic approaches for systems and synthetic biology. *Curr. Op. Biotechnol*. **2010**, *21*, 517-523.

140. Bareither, R.; Pollard, D. A review of advanced small-scale parallel bioreactor technology for accelerated process development: Current state and future need. *Biotechnol. Prog.* **2011**, *27*, 2-14.

141. de Boer, A.R.; Bruyneel, B.; Krabbe, J.G.; Lingeman, H.; Niessen, W.M.; Irth, H. A microfluidic-based enzymatic assay for bioactivity screening combined with capillary liquid chromatography and mass spectrometry. *Lab Chip* **2005**, *5*, 1286-1292.

142. Fang, H.; Xiao, Q.; Wu, F.; Floreancig, P.E.; Weber, S.G. Rapid catalyst screening by a continuous-flow microreactor interfaced with ultra-high-pressure liquid chromatography. *J. Org. Chem.* **2010**, *75*, 5619-5626.

143. Kwapiszewski, R.; Skolimowski, M.; Ziółkowska, K.; Jędrych, E.; Chudy, M.; Dybko, A.; Brzózka, Z. A microfluidic device with fluorimetric detection for intracellular components analysis. *Biomed. Microdevices* **2011**, *13*, 431-440.

144. Ogawa, J.; Shimizu, S. Industrial microbial enzymes: Their discovery by screening and use in large-scale production of useful chemicals in Japan. *Curr. Op. Biotechnol* **2002**, *13*, 367-375.

145. Kumar, R.A.; Clark, D.S. High-throughput screening of biocatalytic activity: applications in drug discovery. *Curr. Op. Chem. Biol.* **2006**, *10*, 162-168.

146. Zimmermann, H.F.; Rieth, J. A Fully Automated Robotic System for High Throughput Fermentation. *JALA* **2006**, *11*, 134-137.

147. Bhambure, R.; Kumar, K.; Rathore, A.S. High-throughput process development for biopharmaceutical drug substances. *Trends Biotechnol.* **2011**, *29*, 127-135.

148. Kuswandi, B.; Nuriman; Huskens, J.; Verboom, W. Optical sensing systems for microfluidic devices: A review. *Anal. Chim. Acta* **2007**, *601*, 141-155.

149. Jokerst, N.; Royal, M.; Palit, S.; Luan, L.; Dhar, S.; Tyler, T. Chip scale integrated microresonator sensing systems. *J. Biophotonics* **2009**, *2*, 212-226.

150. Mark, D.; Haeberle, S.; Roth, G.; von Stetten, F.; Zengerle, R. Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. *Chem. Soc. Rev.* **2010**, *39*, 1153-1182.
151. Sin, M.L.; Gao, J.; Liao, J.C.; Wong, P.K. System Integration - A Major Step toward Lab on a Chip. *J. Biol. Eng.* **2011**, *5*, 6.

152. Jambovane, S.; Duin, E.C.; Kim, S.K.; Hong, J.W. Determination of kinetic parameters, $K_m$ and $k_{cat}$, with a single experiment on a chip. *Anal. Chem.* **2009**, *81*, 3239-3245.

153. Heijnis, W.A.; Wierenga, P.A.; Janssen, A.E.M.; van Berkel, W.J.H.; Gruppen, H. In-line quantification of peroxidase-catalyzed cross-linking of $\alpha$-lactalbumin in a microreactor. *Chem. Eng. J.* **2010**, *157*, 189-193 and references therein.

154. Asanomi, Y.; Yamaguchi, H.; Miyazaki, M.; Maeda, H. Enzyme-Immobilized Microfluidic Process Reactors. *Molecules* **2011**, *16*, 6041-6059 and references therein.

155. Togo, M.; Takamura, A.; Asai, T.; Kaji, H.; Nishizawa, M. An enzyme-based microfluidic biofuel cell using vitamin K3-mediated glucose oxidation. *Electrochim. Acta* **2007**, *52*, 4669-4674.

156. Zebda, A.; Renaud, L.; Cretin, M.; Pichot, F.; Innocent, C.; Ferrigno, R.; Tingry, S. A microfluidic glucose biofuel cell to generate micropower from enzymes at ambient temperature. *Electrochem. Comm.* **2009**, *11*, 592-595.

157. Miyazaki, M.; Kaneno, J.; Yamaori, S.; Honda, T.; Briones, M.P.; Uehara, M.; Arima, K.; Kanno, K.; Yamashita, K.; Yamaguchi, Y.; Nakamura, H.; Yonezawa, H.; Fujii, M.; Maeda, H. Efficient immobilization of enzymes on microchannel surface through His-tag and application for microreactor. *Protein Pept. Lett.* **2005**, *12*, 207-210.

158. Delattre, C.; Vijayalakshmi, M.A. Monolithic enzymatic microreactor at the frontier of glycomic toward a new route for the production of bioactive oligosaccharides. *J. Mol. Catal. B Enzym.* **2009**, *60*, 97-105.

159. He, P.; Greenway, G.; Haswell, S.J. Development of enzyme immobilized monolith microreactors integrated with microfluidic electrochemical cell for the evaluation of enzyme kinetics. *Microfluid. Nanofluid.* **2010**, *8*, 565-573.

160. Kataoka, S.; Takeuchi, Y.; Harada, A.; Yamada, M.; Endo, A. Microreactor with mesoporous silica support layer for lipase catalyzed enantioselective transesterification. *Green Chem.* **2010**, *12*, 331-337.

161. Schilke, K.F.; Wilson, K.L.; Cantrell, T.; Corti, G.; McIlroy, D.N.; Kelly, C. A novel enzymatic microreactor with *Aspergillus oryzae* $\beta$-galactosidase immobilized on silicon dioxide nanosprings. *Biotechnol Prog.* **2010**, *26*, 1597-1605.

162. Alam, M.N.H.Z.; Pinelo, M., Samantha, K.; Jonsson, G.; Meyer, A.; Gernaey, K.V.A. Continuous Membrane Microbioreactor system for Development of Integrated Pectin Modification and Separation Processes. *Chem. Eng. J.* **2011**, *167*, 418-426.

163. Zhang, Z.; Boccazzi, P.; Choi, H.; Perozziello, G.; Sinskey, A.J.; Jensen, K.F. Microchemostat—Microbial continuous culture in a polymer-based instrumented microbioreactor. *Lab Chip* **2006**, *6*, 906-913.

164. Hickey, A.M.; Ngamsom, B.; Wiles, C.; Greenway, G.M.; Watts, P.; Littlechild, J.A. A microreactor for the study of biotransformations by a cross-linked $\gamma$-lactamase enzyme. *Biotechnol. J.* **2009**, *4*, 510-516.

165. Wiles, C.; Hammond, M.J.; Watts, P. The development and evaluation of a continuous flow process for the lipase-mediated oxidation of alkenes. *Beilstein J. Org. Chem.* **2009**, *5*, 27-39.
166. Pierre, S.J.; Thies, J.C.; Dureault, A.; Cameron, N.R.; van Hest, J.C.M.; Carette, N.; Michon, T.; Weberskirch, R. Covalent enzyme immobilization onto photopolymerized highly porous monoliths. *Adv. Mat.* **2006**, *18*, 1822-1826.

167. Kundu, S.; Bhangale, A.S.; Wallace, W.E.; Flynn, K.M.; Guttman, C.M.; Gross, R.A.; Beers, K.L. Continuous flow enzyme-catalyzed polymerization in a microreactor. *J. Am. Chem. Soc.* **2011**, *133*, 6006-6011.

168. Ngamsom, B.; Hickey, A.M.; Greenway, G.M.; Littlechild, J.A.; Watts, P.; Wiles, C. Development of a high throughput screening tool for biotransformations utilizing a thermophilic L-aminoacylase enzyme. *J. Mol. Catal. B Enzym.* **2010**, *63*, 81-86.

169. Tudorache, M.; Mahalu, D.; Teodorescu, C.; Stan, R.; Bala, C.; Parvulescu, V.I. Biocatalytic microreactor incorporating HRP anchored onmicro-/nano-lithographic patterns for flow oxidation of phenols. *J. Mol. Catal. B Enzym.* **2011**, *69*, 133-139.

170. Luckarift, H.R.; Ku, B.S.; Dordick, J.S.; Spain, J.C. Silica-immobilized enzymes for multi-step synthesis in microfluidic devices. *Biotechnol. Bioeng.* **2007**, *98*, 701-705.

171. Thomsen, M.S.; Nidetzky, B. Coated-wall microreactor for continuous biocatalytic transformations using immobilized enzymes. *Biotechnol. J.* **2009**, *4*, 98-107.

172. Schwarz, A.; Thomsen, M.S.; Nidetzky, B. Enzymatic synthesis of β-glucosylglycerol using a continuous-flow microreactor containing thermostable β-glycoside hydrolase CellB immobilized on coated microchannel walls. *Biotechnol. Bioeng.* **2009**, *103*, 865-872.

173. Stojkovic, G.; Plazl, I.; Znidarsic-Plazl, P. L-Malic acid production within a microreactor with surface immobilised fumarase. *Microfluid. Nanofluid.* **2011**, *10*, 627-635.

174. Kataoka, S.; Endo, A.; Harada, A.; Inagi, Y.; Ohmori, T. Characterization of mesoporous catalyst supports on microreactor walls. *Appl. Catal. A Gen.* **2008**, *342*, 107-112

175. 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.

176. Stojkovic, P.; Znidarsic-Plazl, P. Immobilization of yeast cells within microchannels of different materials. *Acta Chim. Slov.* **2010**, *57*, 144-149.

177. Huang, T.T.; Mosier, N.S.; Ladisch, M.R. Surface engineering of microchannel walls for protein separation and directed microfluidic flow. *J. Sep. Sci.* **2006**, *29*, 1733-1742.

178. Kralj, J.G.; Sahoo, H.R.; Jensen, K.F. Integrated continuous microfluidic liquid-liquid extraction. *Lab Chip* **2007**, *7*, 256-263.

179. He, P.; Greenway, G.; Haswell, S.J. Development of a monolith based immobilized lipase microreactor for biocatalytic reactions in a biphasic mobile system. *Process Biochem.* **2010**, *45*, 593-597.

180. Koch, K.; van den Berg, R.J.F.; Nieuwland, P.J.; Wijtmans, R.; Schoemaker, H.E.; van Hest, J.C.M.; Rutjes, F.P.J.T. Enzymatic Enantioselective C–C-Bond Formation in Microreactors. *Biotechnol. Bioeng.* **2008**, *99*, 1028-1033.

181. Tisma, B.; Zelic, B.; Vasic-Racki, D.; Znidarsic-Plazl, P.; Plazl, I. Modelling of laccase-catalyzed L-DOPA oxidation in a microreactor. *Chem. Eng. J.* **2009**, *149*, 383-388.

182. Znidarsic-Plazl, P.; Plazl, I. Modelling and experimental studies on lipase-catalyzed isoamyl acetate synthesis in a microreactor. *Process Biochem.* **2009**, *44*, 1115-1121.

183. Swarts, J.W.; Vossenberg, P.; Meerman, M.H.; Janssen, A.E.; Boom, R.M. Comparison of two-phase lipase-catalyzed esterification on micro and bench scale. *Biotechnol. Bioeng.* **2008**, *99*, 855-861.
184. Pohar, A.; Plazl, I.; Znidarsic-Plazl, P. Lipase-catalyzed synthesis of isoamyl acetate in an ionic liquid/n–heptane two-phase system at the microreactor scale. *Lab Chip* 2009, 9, 3385-3390.

185. Liu, K.-K.; Wu, R.-G.; Chuang, Y.-J.; Khoo, H.S.; Huang, S.-H.; Tseng, F.-G. Microfluidic Systems for Biosensing. *Sensors* 2010, 10, 6623-6661.

186. Titchener-Hooker, N.J.; Dunnill, P.; Hoare, M. Micro biochemical engineering to accelerate the design of industrial-scale downstream processes for biopharmaceutical proteins. *Biotechnol. Bioeng.* 2008, 100, 473-487.

187. Bhambure, R.; Kumar, K.; Rathore, A.S. High-throughput process development for biopharmaceutical drug substances. *Trends Biotechnol.* 2011, 29, 127-135.

188. Shapiro, M.S.; Haswell, S.J.; Lye, G.J.; Bracewell, D.G. Design and characterization of a microfluidic packed bed system for protein breakthrough and dynamic binding capacity determination. *Biotechnol. Prog.* 2009, 25, 277-285.

189. Shapiro, M.S.; Haswell, S.J.; Lye, G.J.; Bracewell, D.G. Microfluidic Chromatography for Early Stage Evaluation of Biopharmaceutical Binding and Separation Conditions. *Sep. Sci. Technol.* 2011, 46, 185-194.

190. Meagher, R.J.; Light, Y.K.; Singh, A.K. Rapid, continuous purification of proteins in a microfluidic device using genetically-engineered partition tags. *Lab Chip* 2008, 8, 527-532.

191. Huh, Y.S.; Jeong, C.M.; Chang, H.N.; Lee, S.Y.; Hong, W.H.; Park, T.J. Rapid separation of bacteriorhodopsin using a laminar-flow extraction system in a microfluidic device. *Biomicrofluidics* 2010, 4, 14103.

192. Hu, R.; Feng, X.; Chen, P.; Fu, M.; Chen, H.; Guo, L.; Liu, B.F. Rapid, highly efficient extraction and purification of membrane proteins using a microfluidic continuous-flow based aqueous two-phase system. *J. Chromatogr. A* 2011, 1218, 171-177.

193. Nam, K.-H.; Chang, W.-J.; Hong, H.; Lim, S.-M.; Kim, D.-I.; Koo, Y.-M. Continuous-flow fractionation of animal cells in microfluidic device using aqueous two-phase extraction. *Biomed. Microdevices* 2005, 7, 189-195.

194. Tsukamoto, M.; Taira, S.; Yamamura, S.; Morita, Y.; Nagatani, N.; Takamura, Y.; Tamiya, E. Cell separation by an aqueous two-phase system in a microfluidic device. *Analyst* 2009, 134, 1994-1198.

195. SooHoo, J.; Walker, G. Microfluidic aqueous two phase system for leukocyte concentration from whole blood. *Biomed. Microdevices* 2009, 11, 323-329.

196. Znidarsic-Plazl, P.; Plazl, I. Steroid extraction in a microchannel system—mathematical modelling and experiments. *Lab Chip* 2007, 7, 883-889.

197. Znidarsic-Plazl, P.; Plazl, I. Development of a continuous steroid biotransformation process and product extraction within microchannel system. *Catal. Today* 2010, 157, 315-320.

198. Honda, T.; Miyazaki, M.; Yamaguchi, Y.; Nakamura, H.; Maeda, H. Integrated microreaction system for optical resolution of racemic amino acids. *Lab Chip* 2007, 7, 366-372.

© 2011 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).