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Microbioreactors for Process Development and Cell-Based Screening Studies

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Contents

Abstract Microbioreactors (MBRs) have emerged as potent cultivation devices enabling automated small-scale experiments in parallel while enhancing their cost efficiency. The widespread use of MBRs has contributed to recent advances in industrial and pharmaceutical biotechnology, and they have proved to be indispensable tools in the development of many modern bioprocesses. Being predominantly applied in early stage process development, they open up new fields of research and enhance the efficacy of biotechnological product development. Their reduced reaction volume is associated with numerous inherent advantages – particularly the possibility for enabling parallel screening operations that facilitate high-throughput cultivations with reduced sample consumption (or the use of rare and expensive educts). As a result, multiple variables can be examined in a shorter time and with a

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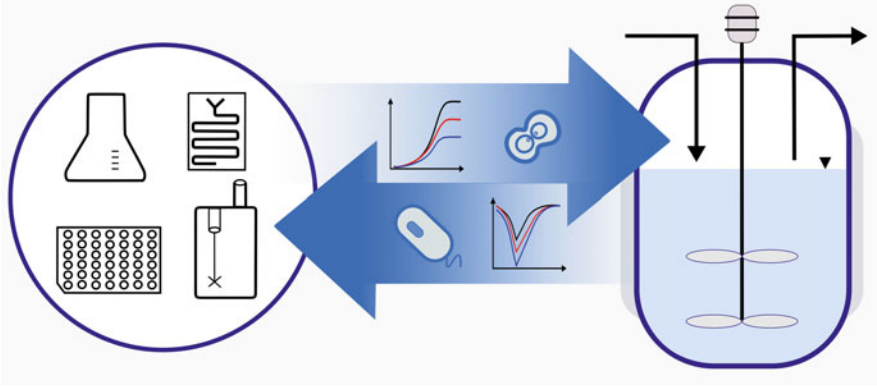
lower expense. This leads to a simultaneous acceleration of research and process development along with decreased costs.

MBRs range from simple miniaturized cultivations vessels (i.e., in the milliliter scale with limited possibilities for process control) to highly complex and automated small-scale microreactors with integrated sensors that allow for comprehensive screenings in very short time or a precise reflection of large-scale cultivation conditions. Progressive developments and improvements in manufacturing and automation techniques are already helping researchers to make use of the advantages that MBRs offer. This overview of current MBR systems surveys the diverse application for microbial and mammalian cell cultivations that have been developed in recent years.

Graphical Abstract



Keywords High throughput, Microbioreactor, Process development, Scale-up, Screening, Sensor integration

Abbreviations

μBC	Microbubble column-bioreactor
μ_{max}	Specific growth rate
CHO	Chinese hamster ovary
cwMBR	Capillary wave MBR
D	Dilution rate
DMF	Digital microfluidics
DO	Dissolved oxygen
EWOD	Electrowetting on dielectric
hMBR	Horizontally arranged plug flow-based microbioreactor
k_La	Volumetric liquid phase oxygen transfer coefficient
K_S	Monod constant

LHS	Liquid handling system
MBR	Microbioreactor
MTP	Microtiter plate
n_{crit}	Critical shaking frequency
OD	Optical density
OTR	Oxygen transfer rate
OUR	Oxygen uptake rate
P/V	Mean volumetric power input
PCR	Polymerase chain reaction
PDMS	Poly(dimethylsiloxane)
PMMA	Polymethylmethacrylate
q_P	Specific product formation rate
q_S	Specific substrate consumption rate
Re	Reynolds number
SAW	Surface acoustic waves
t_M	Mixing time
u_G	Superficial gas velocity
$Y_{X/S}$, $Y_{P/S,}$ $Y_{P/X}$	Biomass- and product-related yield coefficients from substrate/biomass, respectively

1 Microbioreactors for Cell Cultivation

To support and control a biologically active environment, any bioreactor must fulfill several elementary tasks – regardless of the reaction scale at issue [1]. In essence, it must create a mono-septic environment, where defined biological reactions can be performed within controlled ambient conditions. These basic requirements apply with equal force to microbioreactors (MBRs), which usually have a reaction volume of less than 1 mL. Other definitions describe a MBR as a small-scale cultivation system containing at least one microfluidic element. A MBR can generally be seen as a miniaturized device that sustains biology [2], combining high-throughput experimentation with profound bioprocess monitoring and control [3].

Working with MBR systems offers several advantages over more traditional systems. First, multiple simultaneous experiments can be conducted in parallel – allowing researchers to study more parameters affecting the cellular functions or process conditions at the same time. Due to this increase in high-throughput capability, biotechnological research and process development can be substantially accelerated. Additionally, lower amounts of samples, reagents, and consumables in general are required for each experiment – resulting in substantial increases in cost effectivity. The shorter distances and increased surface-to-volume ratio are also advantageous for more sensitive analytics and for improved heat and mass transfer. And finally, space requirements are reduced across the board.

These advantages are valuable for both industry and academia – a fact that is illustrated by the great variety seen in developed MBR systems as well as the rate at which they are being incorporated into modern biotechnological research.

Depending on the specific experimental needs, MBRs can be mixed via stirring, orbital and vertical shaking, or pumping or oscillating – with each mixing technique offering its own specific benefits. Besides high-throughput screenings and strain engineering, MBRs have extensively been applied for bioprocess development.

This chapter aims to provide a comprehensive overview of key available MBR technologies. First, different techniques for achieving MBR homogenization are described (Sect. 2). The mixing process in small-scale cultivation systems is a key requirement to ensure effective heat and mass transfer, to avoid creating unwanted gradients inside the reaction volume, and to keep cells in suspension. Various approaches have been developed in an attempt to tackle this challenge. The different fields of MBR applications are then reviewed (Sect. 3). For example, MBR systems have been developed for process development (aiming mainly at scale-up/down experiments) – contrasted with MBR systems that have been developed for analytical screening applications. Finally, future developments and fields of particular research interest are highlighted.

2 Homogenization of Microbioreactors

Ensuring rapid homogenization and sufficient mass transport is a key requirement of all bioreactors. Reproducible measurements generating conclusive and reliable data are only achievable if the creation of unwanted *pH*, temperature, and concentration gradients are effectively avoided, if cells are reliably supplied with sufficient amounts of nutrients, and if cell sedimentation is prevented [4, 5]. Inhomogeneity and heterogeneities are both major sources of measurement discrepancies and process variances [6–8]. Not only must the fluid phase itself be homogenized, but the mass transfer between liquid and gas phase must also be enhanced. In so doing, both the removal of metabolites and the oxygen transfer into the cultivation broth are intensified. Due to both the low solubility of oxygen in water and the high oxygen uptake of aerobic microorganisms with high specific growth rates, achieving adequate oxygen supply within the liquid phase is considered to be one of the greatest challenges in bioprocess development [9, 10]. Diffusive transport is generally considered to be insufficient for most purposes – necessitating the adoption of an adequate active mixing technique to avoid running into limitations that hinder biomass growth and/or product formation [11, 12]. Additionally, heat and mass transfer must be comparable across scales, if scalability during bioprocess development from micro- to lab- and pilot-scale is to be ensured [13].

Mixing small fluid volumes, however, comes with several inherent major challenges. As the specific systems dimension is reduced, capillary and viscous forces – caused by the enlarged surface-to-volume ratio – increasingly dominate over gravitational and inertial forces [14–16]. And due to the increased surface area, specific interactions between cells and the MBR walls must be carefully considered. Furthermore, the fluid flow in microsystems is by definition laminar, with low Reynolds numbers (mostly $<$1,000) [17]. As a result, the absence of turbulent flow or fluid

vortices can substantially impede proper mixing. To overcome these challenges and provide a wide operating window for particular bioprocess, various strategies have been reported that successfully assure suitable conditions for cell growth and/or product formation. Indeed, the sheer variety of published techniques aimed at solving these issues underscores the importance of ensuring rapid homogenization within biological applications.

Since fully turbulent conditions are very difficult to achieve within the confines of MBR systems, most mixing techniques instead aim to induce and increase chaotic advection and convection in order to enhance the mass transport [14, 17]. To ensure comparability across different mixing techniques, MBR setups, and reactor scales, researchers frequently designate the mixing time (t_M) – i.e., the duration of mixing necessary to achieve a certain homogeneity criteria [18–20]. In most cases, a 95% criterion is applied, meaning that t_M is the time until a homogeneity level of 95% is reached [21].

2.1 Mixing via Stirring

Following the common mixing technique of many lab-, pilot- and technical scale bioreactors, MBRs can be mixed via stirring. Under this method, using a centrically or eccentrically mounted stirrer shaft to blend the fluid and disperse gas into the liquid phase homogenization is achieved using rotating stirrers [22–26]. One advantage of stirred systems is the similarity to bioreactors of larger scale and the resulting analogies in the fluid motion and characteristics [27] – which can facilitate a subsequent scale-up. Additionally, systems with smaller fluid volumes have also been reported with integrated miniaturized stirrer bars or rod agitators [28–32].

2.2 Pumping

To avoid moving elements inside the reaction chamber, MBRs may instead be mixed via a pumping mechanism [33]. Using a digital hydraulic drive for pneumatic pumping, Tsai et al. [34] have developed a miniaturized and robust actuation system that can be connected to standard 96-well plates. The fluid is continuously mixed through up and down pumping, achieving a very gentile homogenization, which is why the main field of application is cell culture analysis.

2.3 Pneumatic Gassing

For increased gas exchange and oxygen transfer rates, MBRs can also be operated as bubble columns by inducing pressurized air at the reactor bottom [35–39]. Lladó Maldonado et al. [36, 37] have characterized the mixing performance of

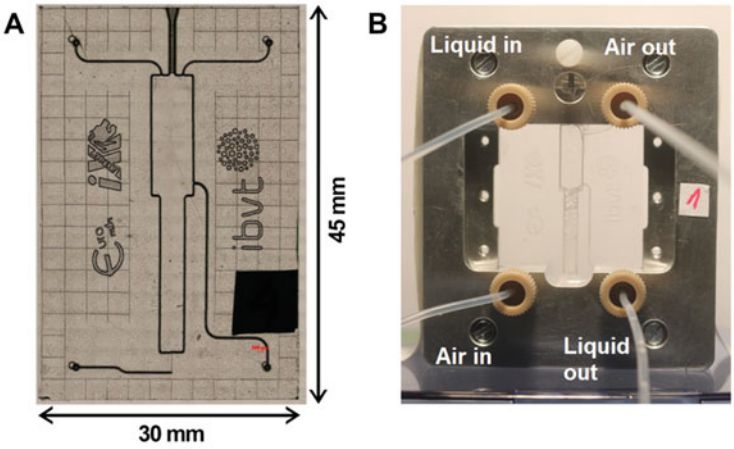


Fig. 1 (**a**) Borosilicate glass-based microbubble column-bioreactor (μBC), (**b**) μBC inside the supporting reactor holder [36] (© Copyright 2018 Elsevier B.V)

microbubble column-bioreactor (μBC) for biotechnological research. The μBC was manufactured with a reaction chamber (3 mm in width, 1 mm in depth, and 18 mm in height) and a funnel at the upper part (5 mm in width, 1 mm in depth, and 14 mm in height) for adequate phase separation (Fig. 1a). The μBC was also equipped with two inlets and two outlets, one for each phase (gas and liquid) (Fig. 1b).

Mixing experiments were performed at different airflow rates. Figure 2a shows an example of the sequence of images produced immediately after the injection of the fluorescent tracer pulse with a frame rate of ~1 fps at an aeration of a superficial gas velocity u_G of 1.3×10^{-3} m s^{-1}. In addition to mixing lab experiments, simulated tracer profiles were also calculated through transient simulations. As an example, time-lapse image series of the transient simulation with the same aeration of $u_G = 1.3 \times 10^{-3}$ m s^{-1} are shown in Fig. 2b with a frame rate of ~1 fps. When the experimental and simulated tracer profiles are compared for the same aeration rate, this model properly predicted the tracer profile distribution [36].

2.4 Orbital Shaking

The reaction volume of MBRs can also be shaken orbitally, inducing a circular motion of the cultivation broth due to inertial forces. This process is frequently used in micro titer plates (MTP) and shake flasks [40–43]. To overcome the surface tension of a fluid and induce motion, the appropriate critical shaking frequency (n_{crit}) must be exceeded. Due to the increased centrifugal force seen at higher orbital shaking frequencies, the liquid height rises at the outer reactor wall, and the hydrodynamic flow is changed [41, 44]. Hermann et al. [44] have proposed the following equation to calculate n_{crit}:

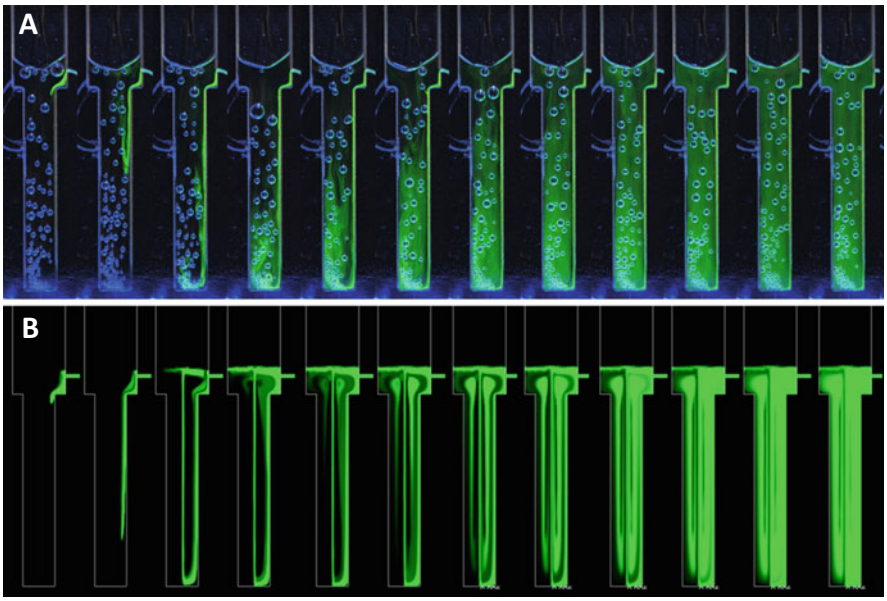


Fig. 2 Mixing time experiments and transient computational fluid dynamics (CFD) simulation in a microbubble column-bioreactor (μBC) (working volume 60 μL): (**a**) Time-lapse image series with a superficial gas velocity set at 1.3×10^{-3} m s^{-1} after the injection of a pulse of 2 μL of the fluorescent tracer solution through a needle pump and (**b**) the transient CFD simulation. The images are shown with a frame rate of ~1 fps [36] (© Copyright 2018 Elsevier B.V.)

$$n_{\text{crit}} = \sqrt{\frac{\sigma \cdot D_{\text{W}}}{4 \cdot \pi \cdot V_{\text{L}} \cdot \rho_{\text{L}} \cdot d_0}} \tag{1}$$

Here, σ is the surface tension, D_{W} is the well diameter, V_{L} is the liquid volume, ρ_{L} is the density of the fluid, and d_0 is the shaking diameter. For given fluid properties, n_{crit} is inversely proportional to V_{L} and d_0. Smaller fluid volumes of less than 50 μL are more difficult to mix – an observation which can be explained by reference to the larger surface forces seen in smaller volumes. As a result, more power must be expended to overcome these forces. It is important to note that orbital shaking only have limited practicability within MBR systems. For increasingly smaller systems, n_{crit} rises quickly – ultimately resulting in impracticable process conditions. However, increasingly smaller systems explicitly benefit from the advantages of MBRs, especially the saving of expensive substrates and facilitated opportunities for parallelization. Since diffusive species mixing is usually too slow to prevent mass transfer limitations in aerobic processes and inadequate for μL-scale volumes, suitable techniques for small scale mixing are required.

2.5 *Mixing of Droplet Microbioreactors*

To allow for manipulation of smaller fluid volumes below 10 µL, various strategies have been reported that effectively introduce advective transport. MBRs in the low µL range have been reported to be mixed using the application of an electrical potential by implementing a pair of electrodes, which can be configured either in a planar configuration or with a wire hanging from the top into the fluid. This technique is commonly referred to in the literature as *electrowetting on dielectric* (EWOD) [45–48]. By bringing electrostatic charges on the fluid, the latter can be manipulated, spread, moved, and ultimately mixed. EWOD is performed on a pair of insulator-coated electrodes which are covered by an insulating film working as the substrate for a conducting fluid [47]. The technique is characterized by an increased flexibility and possibility to manipulate the fluid volume, and as a result it has achieved wide application within droplet-based analysis systems [46, 49]. The fluid can alternatively be excited in resonance using piezoelectric transducers [50]. Creating frequencies above 10 kHz, the fluid is thereby effectively mixed via vibration. *Surface acoustic waves* (SAW) excited by acoustic streaming propagate through the liquid and set the liquid into motion, achieving extremely fast mixing within small volumes [14]. The technique and its advantages for the application in microfluidics are described in depth by Yeo and Friend [51]. In the latter techniques, mixing energy is being transmitted via oscillations that excite the phase boundary to resonate. Through the high-frequency oscillations and the resulting increased power input, however, the fluid temperature in small fluid volumes tends to rise, and cells are potentially disrupted [50]. Additionally, manufacturing these devices requires extensive efforts for shielding the electronics.

Another mixing technique for small fluid volumes that requires even less operational effort is the induction of capillary waves on the liquid surface via vertical oscillation [52]. If excited in resonance, a stationary wave is formed on the phase boundary of the liquid and the gas, due to competing inertia and surface tension forces [53–55]. This wave subsequently leads to rapid bulk mixing – which has been successfully used for mixing a 20 µL MBR [56] as well as a 7 µL system [57, 58] and achieving fast homogenization in less than 3 s using oscillations below 400 Hz. The capillary wave MBR (cwMBR) is shown in Fig. 3.

In the cwMBR setup, a Foturan® glass chip is used to form a defined fluid droplet with a reproducible interphase, which can be excited in resonance by vertical oscillation. Using four electromagnets, an oscillation table – where the reactor chip is mounted – is excited with specific oscillation conditions [58]. Here, oscillation in resonance leads to unique modes, which is defined by the number and position of the nodes [53, 55, 59].

In Fig. 4, different oscillation modes of a cwMBR filled with dyed water excited at resonance frequencies are illustrated. The resulting mode patterns are characteristic for each frequency.

Aside from the vertical displacement, excitation frequency is the decisive factor for resonance and the resulting power input. In Fig. 5, the inverse mixing time $1/t_M$ is

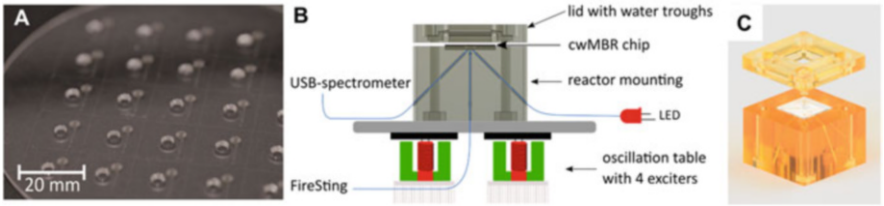


Fig. 3 Capillary wave MBR (cwMBR) setup: (**a**) 5 × 5 array manufactured on a 4 in. Foturan® wafer [57] (© Copyright 2019 MDPI AG). (**b**) Side view of reactor mounting with optical fiber side-in module and sensor assembly of optical measurements. (**c**) Perspective view on rendered cwMBR mounting with base element, cwMBR and lid, having four water troughs in the walls. All parts are clamped together using four screws [58] (© Copyright 2020 Elsevier B.V)

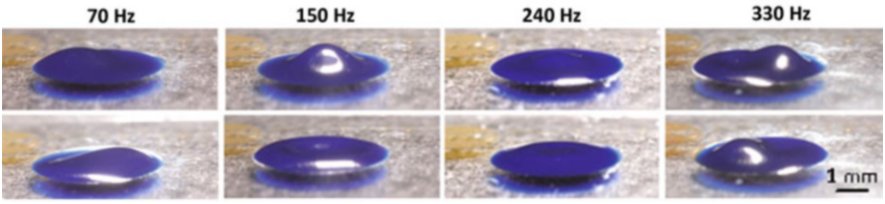


Fig. 4 Perspective view on the cwMBR filled with dyed water excited at its resonance frequencies. Different oscillation patterns are formed on the liquid surface, whereby the wavenumber increases for higher excitation frequency. Two characteristic time points are shown, where the amplitude of the oscillating liquid interface is the highest. Images were taken with a single-lens reflex camera (EOS 60d, Canon, Tokyo, Japan) connected to a micro-Nikkor objective (Nikon, Tokyo, Japan) with a focal length of 55 mm and a triggered ultrashort time flash with an exposure time of 1×10^{-5} s [58] (© Copyright 2020 Elsevier B.V)

shown for frequencies up to 400 Hz. The maxima of $1/t_M$, which corresponds to fast mixing, correlate well with the calculated resonance frequencies shown on top in red [58].

Finally, it is worth noting that mixing MBRs with continuous flow in enclosed micro-channels – which open up possibilities for massive high throughput – have also been described in detailed reviews [17, 59].

3 Application of Microbioreactors

There is a tremendous and growing demand for potent MBR systems that can be used to cultivate cells in the micro-scale and simultaneously permit automatized highly parallelized operations in which various process parameters can be independently modified. These systems are increasingly being applied in the field of bioprocess development, where they aim to mimic larger-scale (from lab- to pilot- and process-scale) cultivation systems. The knowledge acquired in these smaller-

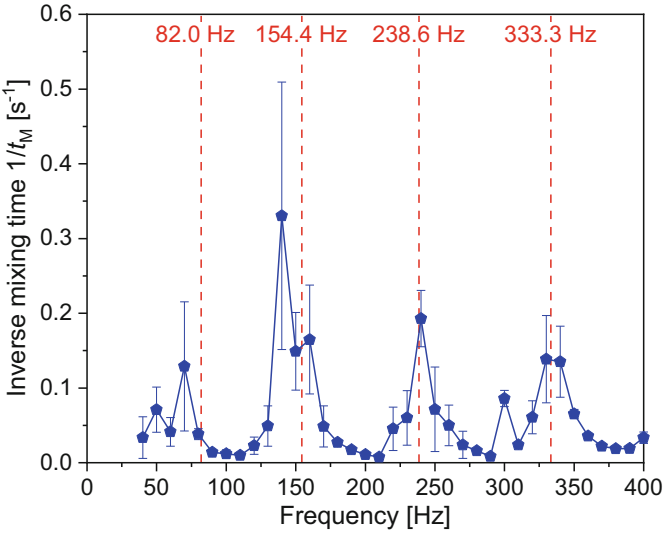


Fig. 5 Inverse mixing time $1/t_M$ for frequencies up to 400 Hz at excitation strength of 20%. Calculated resonance frequencies are shown on top in red. All data are given as mean and standard deviation ($n = 3$) [58] (© Copyright 2020 Elsevier B.V)

scale systems can then be transferred to the sequential process steps used to perform a process scale-up. But even aside from process development and the related scale-up, the advantages of MBRs are also increasingly being leveraged for screening applications – where these systems are used mainly for analytical purposes. In this context, the more pressing concern is the performance of cellular experiments in an automated high-throughput fashion for rapid generation of experimental data. It is therefore more important to sustain the cell population under carefully defined conditions and perform ongoing measurements, rather than to precisely mimic large-scale process conditions.

By applying MBR systems, lower sample consumption is achieved – which translates lastly into cost savings [60]. Additionally, the process development can also be substantially accelerated, since the number of samples processed in parallel is increased [61].

3.1 Microbioreactors for Process Development and Scale-Up

Bioprocesses are influenced by numerous factors that can significantly affect the performance efficiency of bioconversion. Profound knowledge of biological reaction kinetics is of crucial importance for high yield bioproduction to achieve optimal process conditions. For example, growth behavior, product formation, and yields are all heavily dependent on physicochemical parameters such as *pH*, temperature, and

nutrient availability, as well as overall media composition [62]. Additionally, ambient cultivation conditions in the bioreactor have a direct bearing on the entire process. Power input and the associated mixing performance are key parameters affecting nutrient availability and fluid homogeneity, as well as the operating shear stress appearing on the biocatalyst [10, 63, 64].

Accordingly, in order to effectively develop and improve a bioprocess, a holistic knowledge and insight into the entire bioprocess is a prerequisite. Acquiring this knowledge requires multiple experiments to thoroughly investigate all decisive factors in detail. In order to effectively reduce the applied substrates and volumes used per experiment, miniaturized cultivation systems are increasingly being applied to bioprocess development [3, 9, 65–68]. By reducing the reaction volumes required and applying small-scale cultivation systems instead, expenses can frequently be slashed – and, even more importantly, the degree of parallelization (i.e., simultaneous experiments) can be substantially increased [69].

In standard lab-scale bioreactors with a volume of 1 to 30 L, bioreactions and growth kinetics, product titer, and ultimate quality are also adequately representative to a pilot-scale reactor. But the achievable throughput is limited, and experimental operation is frequently elaborate. Especially in early stage process development, the number of variables that need to be examined often exceeds the capabilities of these lab-scale cultivation systems. As a result, it typically requires at least 50 experiments for characterization of a cultivation process – which can take up to 8 months to complete [69, 70]. Not surprisingly against the backdrop, systems that enable higher experimental throughput are in great demand.

A typical development of a biotechnological process is a sequence of consecutive steps. Starting from small reaction volumes with lower information content (but with a high number of variables to be examined), the reaction throughput decreases, and the information content gradually rises throughout the development process. The number of biological variables that need to be investigated is continuously being reduced in the course of the process development [3]. Initial screenings for an optimal production strain are often conducted in microtiter plates (MTPs) to enable the performance of up to 96 simultaneous reactions in parallel. Aside from the selection of the requested production strain, media development and adjustment can also be performed in this stage. The gathered information then informs the next larger-scale stage, which is mostly conducted in shaking flasks with a fluid volume of 10 to 100 mL. The number of parallel cultivation experiments that may be conducted in shaking flasks is already significantly limited, however, due to the labor-intensive nature of this phase of operations. Only the most promising cultivation conditions for the depicted production strain or host are then brought to the lab-scale bioreactor, where a level of process control comparable to that seen in the pilot scale is once again possible. In this pilot-scale stage, challenges related to the final large reaction volumes and scale-up must be addressed; these can include a higher hydrodynamic pressure, higher Reynolds numbers, higher mixing times, as well as more pronounced bioreactor inhomogeneities for increasing reaction volumes [65].

MBR systems aim to enhance and optimize this traditional process development workflow. The bioprocess itself is aimed to only minimally be affected by the scale-down and related changes in the cultivation scale. At the same time, the experimental throughput is ought to be enlarged maintaining the operational control of miniaturized cultivation systems. By mimicking a large-scale reactor, a quantitative characterization as well as a comparison across scales is supposed to be enabled.

For this purpose, a great variety of MBR designs and setups have been reported and applied, and several systems are currently commercially available. These systems differ significantly in the reaction volume required, the form of the cultivation vessel, the applied mixing techniques, and the gas supply – but shaking and stirred methods still constitute the majority of reported MBR systems.

3.1.1 Microtiter Plate-Based Microbioreactors

Addressing the lack of monitoring physiological parameters that characterize the early stages of process development, MTP-based cultivation systems with integrated online sensors (biomass, DO, *pH*, fluorescence) are prevalently applied [71]. These systems are mixed via orbital shaking, and the aeration is solely performed via the head space – resulting in volumetric liquid phase oxygen transfer coefficients ($k_L a$) up to 250 h^{-1} which enable even oxygen-demanding *Escherichia coli* cultivations, depending on the respective oxygen uptake rate (OUR) and the biomass concentration. To enlarge the oxygen transfer rate (OTR) and improve the mixing performance inside the micro-wells, a baffled MTP was also developed and applied for bioprocess development. With $k_L a$ values up to 600 h^{-1}, oxygen limitations can be avoided, and the OTR of large-scale cultivations can be modeled [42, 43, 72, 73]. The so-called *Biolector* system (m2p-labs, Baesweiler, Germany) was applied for the quantitative evaluation of media and nutrients, in order to detect differences in biomass and product yields [74, 75].

Due to their inherent enhanced degree of parallelization, MTP-based cultivation systems are well suited for screenings, determining optimal media compositions [76], growth conditions [77], and identifying optimal clones from strain libraries [78–82]. These working groups have therefore shown that they have developed versatile and major systems in high-throughput cultivation devices.

Besides microbial cultivations, MTP-based systems have also been applied as cultivation and process development tool for mammalian cells. The 24 deep square well plate (standard SBS (Society for Biomolecular Screening) format) system *micro-Matrix* (Applikon, Delft, Netherlands) was used for cell culture process development [83–85]. The system consists of 24 individual reaction elements, where each well can be individually controlled for *pH*, temperature, and dissolved oxygen (DO). Applying an additional feeding module, automated addition of substrates is enabled – which facilitates process development in the fed-batch mode. It can therefore be applied to optimize both feed and growth parameters [3]. Additional commercial systems are available from Pall (*micro-24*, New York, USA) featuring continuous aeration [86–89] and from Oy Growth Curves Ab (*Bioscreen C*,

Helsinki, Finland) with up to 200 reaction vessels in parallel [90–92]. Other systems have been reported by Harms et al. [93], Lamping et al. [85], and Zhang et al. [94].

Cultivation in MTP-based systems enables parallel examination of various reactions, although cultivation conditions (i.e., shaking frequency, temperature, etc.) can only be adapted for all wells in parallel. To individually manipulate separate culture wells, the shaking movement must be stopped – which can negatively affect the growth performance, due to oxygen transfer and decline of mass transfer [78, 87]. If more global parameters on the process performance are to be investigated, separate MTP cultivation runs must be performed, which also negatively effects the throughput [3].

Shaken MBR systems are advantageous due to the absence of movable parts inside the reaction chamber, which are prone to error and challenging in microfabrication. The fluid movement can also be compared to shake flasks with ease, which is often the next larger scale in process development workflow.

3.1.2 Microbioreactors with Rotating Mixers

In addition to the orbitally shaken systems, several stirred cultivation systems have been reported in the literature – and some are even coming onto the commercial market. These stirred systems can mimic the predominant conditions of lab-scale reactors even more precisely. For example, a single-use miniaturized stirred cultivation system with 24 or 48 parallel MBR, having a reaction volume of 10–15 mL, has been developed for cell culture applications, cell line development, and feed and growth parameter optimization [23, 24, 27, 95–97] – although it is also being applied for microbial process development [98]. The cultivation broth in the *ambr® 15* (Sartorius, Göttingen, Germany) is mixed via stirring by an eccentrically positioned stirrer. For monitoring the cultivation process, *pH* and DO can be measured quasi-continuously online via optodes, and the elevated reaction volume also enables some degree of sampling. The high-throughput capabilities of the system are fully amplified if the *ambr® 15* is operated by a liquid handling system (which enables automated cultivations). Besides batch and fed-batch processes, the *ambr® 15* can also be operated in a quasi-continuous perfusion mode. Using sedimentation for cell retention, a scale-down perfusion cell culture reactor was applied to predict viable cell concentrations of human cell lines [95]. When compared to 1 and 1,000 L approaches, this miniaturized system showed accurate prediction of product quality attributes – especially glycosylation profiles. Yet the cell culture media requirements were reduced 80-fold, and the daily operator time was halved – resulting in a massive cost saving and facilitation of much more resource-efficient process development. Extensively applied in process optimization and scale-up, the *ambr® 15* has proven to be an excellent scale-down model for large-scale bioreactors [27, 99].

A miniaturized cultivation system (V_L = 10 mL) with a magnetically driven one-sided paddle impeller is reported by Hortsch et al. [25, 100]. This system is specifically designed to promote the growth of mycelium forming microorganisms. The rotating stirrer forms a liquid lamella to effectively prevent wall growth or

foaming. The system was characterized in terms of k_La coefficients and volumetric power input, to compare its performance to a 2 L lab-scale bioreactor. For a given mean volumetric power input, the maximum local energy dissipation in the stirred MBR was reduced (compared to the lab-scale bioreactor) and showed a more uniform power distribution into the reaction medium for the smaller scale. Despite these discrepancies, similar power consumption characteristics were found on both systems – proving a reliable scale-up possibility with the miniaturized bioreactor. To enhance the applicability for enzymatic processes, the stirrer setup was further optimized and applied to the hydrolysis of suspended plant cells [101]. Additionally, when the stirred miniaturized reactor systems were applied for the microbial expression of recombinant proteins in a reactor cascade setup, they outperformed a continuous process [102, 103].

Downsizing the reaction volume of cultivation systems primarily restricts their ability to monitor and control the cultivation process – which is why MBR systems for process development are generally limited to the upper µL or lower mL range, where the information content and its validity are somewhat higher [104–106]. But smaller systems are still being developed in an effort to continue to refine and optimize the potential benefits of miniaturization.

The use of miniaturized stirrers to achieve an active mixing technique in liquids at the µL range certainly still poses challenges. It was successfully reported by Szita et al. [28] in a system made of fused layers of polymethylmethacrylate (PMMA) and poly(dimethylsiloxane) (PDMS) housing a liquid volume of 150 µL. Monitoring DO and *pH*, this system can log elementary procedures in the small reaction chamber which is fed by microfluidic channels connected to fluidic ports. With agitation frequencies up to 700 min^{-1}, batch cultivations of *E. coli* were performed which corresponded well with similar cultivations performed in 500 mL lab-scale reactors (SixFors®, Infors, Bottmingen, Switzerland), as well as in shake flasks [32]. Using the microfluidic inlets for reagent feeding, chemostat cultivations were also performed [107]. After modifying the system setup slightly (by increasing the vessel height and reinforcing a membrane barrier), this system was also applied for gene expression studies of *Saccharomyces cerevisiae* and *E. coli* in glucose and galactose media [29, 30].

A considerably smaller system for batch or continuous cultivations of suspension cells with a cylindrical reaction volume of 100 µL was reported by Schäpper et al. [31]. It aims to combine the advantages of MTPs (small working volume) with more versatile bench-top reactors. In this system, homogenization is ensured and cell sedimentation prevented by use of a stirrer bar that is left to freely float within the reaction chamber. Similar to many previously reported systems, DO and *pH* can be monitored online in the presented MBR. Additionally, via absorbance measurements, the cell density and cell growth can be determined.

3.1.3 Microbioreactors Without Movable Mixing Elements

Taking a similar approach, and in an effort to accommodate biological reaction kinetics from stationary process data of a chemostat cultivation, Edlich et al. [108] have developed a horizontally arranged plug flow-based microbioreactor (hMBR) where the main flow direction is perpendicular to the lift force. The hMBR foregoes movable parts inside the reaction chamber and operated with a reaction volume of 8 µL. The hMBR was made of glass and PDMS manufactured by soft lithography technology and had integrated sensors for optical density (OD) and DO. The oxygen was diffused into the cultivation broth through the PDMS membrane. Here, the concentration gradients of the limiting substrate, metabolites, and products occur not only over the length of the reactor but also along the hMBR height in terms of cell distribution and the oxygen supply. However, this system came with one significant disadvantage: bubbles that arose in the mixture could remain in the system, thereby displacing the liquid and/or influencing or even completely blocking the liquid flow and disturbing optical measurements.

An alternative and improved operation was developed by Peterat et al. [35], in a vertical configuration featuring active pneumatic gassing and material surface hydrophilization to ensure planktonic cultivation of microorganisms and to prevent wall growth [109]. By inducing a continuous fine bubble stream at the reactor bottom, Peterat et al. [35] created a µBC made of borosilicate glass with a reaction volume of 70 µL [35, 38], which was further developed by Lladó Maldonado et al. [36] (compare Figs. 1 and 2). By harnessing pneumatic aeration with pressurized air, several key challenges in MBR development were addressed: namely, inadequate homogenization and related mass transfer limitations. This MBR setup with the particular mixing technique has proven to support an environment favorable for growth of yeast cells, which was monitored via absorbance and optical DO measurement.

In this µBC, Krull and Peterat [38] carried out cultivations with the Crabtree-positive yeast *S. cerevisiae* CCOS 538 in chemostat cultivation by varying the dilution rate D between 0.12 and 0.42 $h^{(-1)}$. The values for the parameters of the reaction kinetic model were determined analytically, using experimental data for the stationary concentrations of biomass, substrate, and ethanol as primary product on the micro-scale. The maximal specific growth rate (μ_{max}) and the Monod constant (K_S) were calculated using linearization methods (Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf). Considering the empirical model of Luedeking and Piret for product kinetics, the yield coefficients $Y_{X/S}$, $Y_{P/S}$, and $Y_{P/X}$ were determined from plots of the specific substrate consumption rate $q_S = f$ (dilution rate, D) and the specific product formation rate $q_P = f(D)$, respectively. The kinetic reaction model was in agreement with the experimental data – and hence it provided a solid mathematical description of the biotechnological process (Fig. 6).

Considering the Crabtree effect on yeast metabolism, the two following validity ranges of the kinetic model were discussed in detail: (a) $\mu = D < D_{crab}$, applied to purely oxidative metabolism, in which glucose was completely converted into

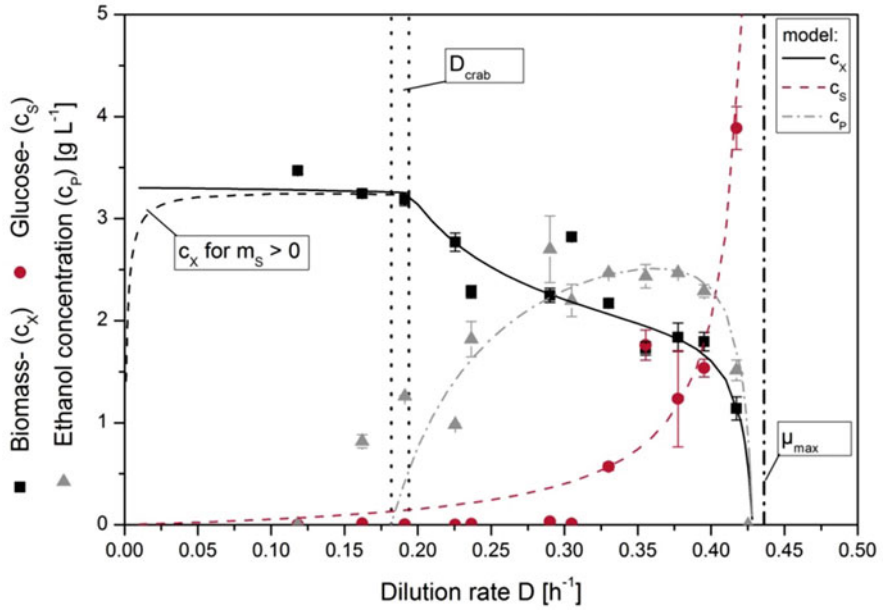


Fig. 6 Comparison of the values obtained using the reaction kinetic model, using estimations of the steady-state glucose (c_S, - - -), biomass (c_X, —), and ethanol concentrations (c_P, – · –) and the experimental data (c_S, •), (c_x, ■), and (c_P, ▲) for the continuous cultivation of *S. cerevisiae* in the μBC as a function of the dilution rate D. The parameters used in the reaction kinetic model were as follows: $c_{S,in} = 10$ g$_S$ L^{-1}, $0.182 \leq D_{crab} \leq 0.194$ h^{-1}, (a) $\mu = D < D_{crab}$ (purely oxidative metabolism) $\mu_{max} = 0.436$ h^{-1}, $K_S = 0.182$ g$_S$ L^{-1}, $Y_{X/S} = 0.335$ g$_{CDW}$ g$_S^{-1}$, $m_S = 0$ and c_X, − with m_S (maintenance coefficient) $= 0.004$ g$_P$ g$_{CDW}^{-1}$ h^{-1}, and $Y_{P/X} = 0$; and (b) $\mu = D > D_{crab}$ (oxido-reductive metabolism with an active Crabtree effect), the same values as above for μ_{max}, K_S and $Y_{X/S}$, $Y_{P/S} = 0.715$ g$_P$ g$_S^{-1}$, $Y_{P/X} = 2.637$ g$_P$ g$_{CDW}^{-1}$, and $m_S = 0$ [38] (© Copyright 2016 Elsevier B.V)

biomass or was used for endogenous maintenance metabolism and no ethanol was generated, and (b) $\mu = D > D_{crab}$, applied during oxido-reductive metabolism occurring under the Crabtree effect, in which ethanol was formed at the expense of biomass generation under aerobic conditions. This production of ethanol was strictly coupled to the metabolic activity occurring and was growth-associated. The data obtained using the μBC was then compared with the results obtained in chemostat experiments conducted on a macro-scale in stirred tank reactors (2.5 and 2.85 L) by Rieger et al. [110] and von Meyenburg [111, 112], respectively. Despite the fact that the volumes in question differed by a factor of ~50,000, the values determined using the microsystem were of the same order as the values for the kinetic constants of the published experimental data from laboratory scale and thus validate the applicability of the μBC as a suitable screening tool for aerobic submerged cultivations [38].

To facilitate the integration of miniaturized sensors, another prototype was also developed. Based on similar reaction geometries combined with the active pneumatic bubble aeration, a reaction setup with 550 μL made of polystyrene was equipped with additional online sensors [37] (Fig. 7).

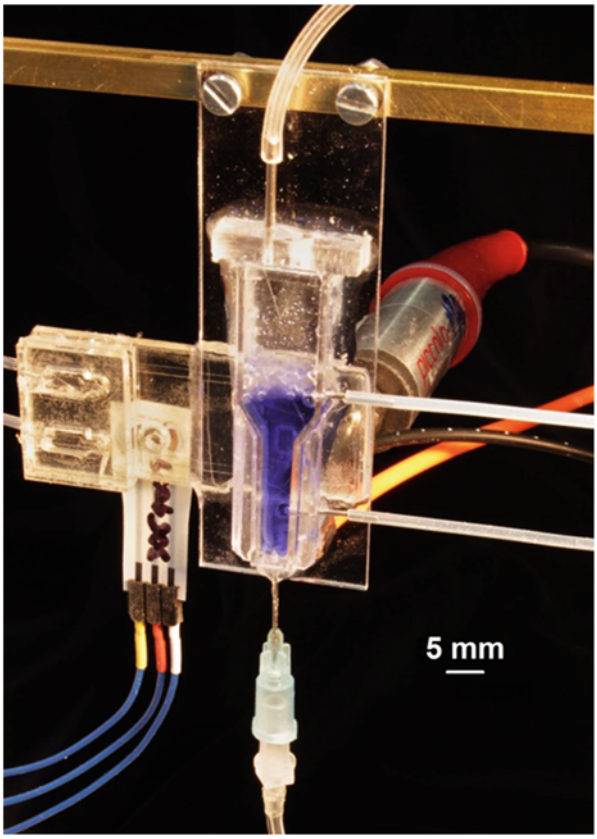


Fig. 7 Picture of the μBC with the microfluidic flow chip and glucose biosensor; the inlets and outlets of the liquid and gas phases; and the integrated sensors for *pH*, dissolved oxygen, and optical density with their associated glass fibers [113] (© Copyright 2019 Wiley)

To avoid sampling, glucose as the limiting carbon source was measured with a microfluidic chip housing an electrochemical biosensor. The addition of online *OD*, *pH*, and *DO* sensors facilitated a holistic evaluation of the biological process. The system was then applied for multiphase chemostat cultivations in the μBC to determine reaction kinetics of *Staphylococcus carnosus* proving its applicability for submerged cell cultivations by achieving steady-state biomass and substrate concentration in chemostat mode for various dilution rates [113]. Additional microbubble column bioreactors were also reported by Doig et al. [114, 115], Betts et al. [89], and Weuster-Botz [116].

3.1.4 Challenges in Upscaling of Processes Evaluated in Microbioreactors

The aforementioned MBR systems aim to scale-down bioprocesses and thereby provide effective platforms to execute efficient process development. However, due to differences in fluid dynamic properties, mass gradient profiles, and

inhomogeneities, a large-scale bioprocess can only ever be mimicked partially and insufficiently by miniaturized cultivation systems. As a result, any MBR system will always remain (at best) an approximation of the corresponding large-scale bioprocess it is supposed to mimic. The further research must therefore be especially attuned to the potential for process confounding variabilities, making a surround understanding of the entire process of crucial importance [117, 118]. This leads to a diverging physiology and productivity of the production organism when it is evaluated in the scale-down experiment. Miniaturizing the reaction volume is always also accompanied by an increase in surface area-to-volume ratio, which results in disproportional effects of capillary and viscous forces compared to gravitational and inertial forces [14–16]. Consequently, greater efforts must be made to ensure homogenization and to prevent mass transfer gradients requiring higher agitation speeds for mixing. The risk of wall growth is also comparatively greater, due to the altered fluid dynamics and the higher tendency of microsystems to form eddies. Another phenomenon with enhanced effect in small-scale systems is liquid evaporation – and the resulting dilution and concentration effects [69, 119, 120]. By contrast, large-scale bioreactors exhibit higher hydrostatic pressures by nature, which cannot be imaged insufficiently in small-scale systems. Fluid dynamics in microfluidics cannot by definition exceed transitional regimes – whereas turbulent flow is mostly aimed to achieve for homogeneous process control [121]. Furthermore, mixing times in large scales also tend to be larger, which frequently results in the formation of concentration and temperature gradients. Microorganisms or cells therefore often experience oscillating environmental conditions, which can substantially impact growth kinetics, product formation, and quality [27, 122].

3.1.5 Scaling Parameters

To obtain similar environments across scales, several scaling parameters are reported in the relevant literature which must be kept constant for increasing the reaction scope [123]. Relevant scaling parameters can refer to either mean volumetric power input (P/V) or power consumption [63, 64]. Notably, however, the power input also influences multiple other parameters – affecting mass transfer and mixing, as well as shear stress and (resultingly) cell morphology and viability [25, 89, 124]. Because oxygen transfer into the liquid phase is for aerobic bioprocesses one of the most important transport process [9, 10], a common scaling parameter is the volumetric liquid phase oxygen transfer coefficient $k_L a$ [11, 125–127]. Oxygen is poorly soluble in aqueous solutions and therefore constitutes a limiting factor for cell growth. The $k_L a$ value represents the capacity of a system to transport oxygen from the gas to the liquid phase, which is kept constant across scales to ensure similar oxygen supply. If the oxygen driving force, being the concentration gradient between gas and liquid phase, is also considered, then the oxygen transfer rate (OTR) can be used as an additional scaling parameter [11].

$$\mathrm{OTR} = k_{\mathrm{L}}a \cdot \left(c^{*}_{\mathrm{O_2,L}} - c_{\mathrm{O_2,L}}\right) \tag{2}$$

Here, $c^{*}_{\mathrm{O2,L}}$ is the DO saturation constant and $c_{\mathrm{O2,\ L}}$ is the apparent DO concentration in the liquid phase. If the driving force across the scales is not equal (due to the hydrostatic pressure or other effects increasing the oxygen saturation concentration), then scaling down of a bioprocess to the MBR scale by keeping the $k_{\mathrm{L}}a$ constant will result in an altered OTR [65]. Hence, the oxygen availability and supply for cells in the reaction volume are affected – which may result in differences in cell growth and bioprocess kinetics.

To avoid creating unwanted concentration and/or temperature gradients inside the reaction volume, mixing and mass transfer must be ensured at all scales. The mixing time t_{M} can therefore be used to compare process scales, keeping homogenization constant. t_{M} tends to increase for large-scale bioreactors, however [126, 128]. Another vulnerable scaling parameter is the Reynolds number (*Re*), being the relation of inertial to viscous forces. It appears that turbulences of geometrical similar bodies are identical at the same Reynolds numbers – meaning that flow conditions can be compared via *Re*. But as a result of enhanced viscosity and surface forces dominating inertia and gravity at small scales, velocity and flow conditions cannot be accurately mimicked by keeping *Re* constant across scales [14–16]. Since *Re* refers to the characteristic system dimension, it tends to be small and under-predicted, resulting in a laminar flow for microfluidics by definition [14]. These missing turbulent flows can impede proper mixing as the system dimension is reduced, thereby hampering the scale-up process. Aside from $k_{\mathrm{L}}a$, OTR, t_{M}, or *Re*, stirrer tip speed for stirred systems [129, 130] or the superficial gas velocity u_{G} [131] for actively aerated systems can also be used as scaling parameters.

A great variety of studies are now being reported in which bioprocesses are scaled up based on a single process parameter, while other parameters are not taken into account. To truly develop a holistic image of a large-scale process using miniaturized cultivation systems, however, a much more complex characterization of the process parameters is critical. This requires the consideration of multiple scale-up parameters – all of which exert a strong influence on the process performance. Tajsoleiman et al. [65] report one such illustrative case study about the influence of different scaling parameters on the success of the process transfer. Here, different scaling parameters overlap in a pilot-scale bioprocess mimicking a 100 m^3 bioreactor, but lie far away from each other in a MBR system.

For an additional and more comprehensive view on small-scale cultivation systems for process development, in-depth reviews from Breslauer et al. [132], El-Ali et al. [133], Schäpper et al. [66], Bareither and Pollard [69], Hegab et al. [67], Kirk and Szita [9], Lattermann and Büchs [68], Krull et al. [4], Hemmerich et al. [3], as well as Junne and Neubauer [134] are all recommended.

3.2 *Droplet Bioreactors as Analytical Screening Tool*

The application of MBR systems to accelerate the optimization of bioprocesses has become an increasingly common practice in industrial and academic research fields – which has led to a substantial rise in the number of commercially available miniaturized cultivation devices. But aside from process development and scale-up, MBRs are also potent analytical tools for obtaining deeper insight into cellular processes and microbial physiology [135]. Compared to the application in bioprocess development (where the main focus lies on mimicking a larger-scale cultivation system), MBR systems can help to analyze the internal cellular physiological and to perform specific monitoring tasks. These advantages are particularly potent in facilitating early screening procedures or strain selections with extensive probes and variables requiring a high degree of parallelization [65].

Screenings with enhanced throughput are routinely performed in MTP formats. Offering between 6 and 1,536 reaction cavities, simultaneous experimentation can potentially accelerate the rate of biotechnological research and development with minimal manual intervention [136]. Having said that, sensor integration, monitoring, and control are more difficult and limited in comparison with larger cultivation systems – in which can in turn negatively affect the quality and validity of the generated data. The evaluation and analysis of experimental data consequently becomes a highly important consideration.

MTPs have been used to screen for specific microbial strains with a desired capacity concerning the metabolism, displaying a novel enzyme activity, or having certain adaptive capacities to environmental conditions [136]. Here, in-house or external cell libraries can be routinely tested for biocatalytic activity [78]. Additionally, cell lines can be improved using directed evolution and a variety of genetic techniques to enhance enzymatic specificity. After cell line development has been completed, the apparent process conditions, as well as media compositions, bioprocess kinetics, cell growth, yields, and oxygen requirements, can all be optimized [136]. Genome engineering of *E. coli* for enhanced growth rate and a reduced lag time was conducted in a MTP-based system [82, 137]. An improvement of product yields and formation rates in *Bacillus subtilis* was also performed by Motta dos Santos et al. [138]. Furthermore, for synthetic biology approaches, *Corynebacterium glutamicum* was modified, and irrelevant gene clusters were deleted [139]. Using a complementary *respiration activity monitoring system* (*RAMOS*), comprehensive data about growth behavior and product formation was achieved [74]. MTP systems have also been applied for clone screening and optimization of feeding strategies [81].

The application of MTPs has greatly improved the capabilities to perform multiple experimental preparations – whether in a metabolic assay or a cultivation process – in parallel. Compared to classical laboratory shaking flask experiments, generation of valuable experimental data can be massively accelerated – but MTP-based systems can also involve a certain lack of flexibility and versatility and also pose challenges in fluid manipulation and operation.

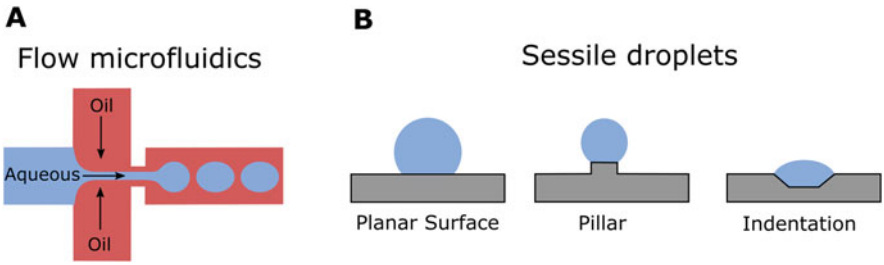


Fig. 8 Operation modes in droplet microfluidics in cross-sectional view. (**a**) Generation of droplets in an enclosed microchannel/capillary with a continuous fluid flow for droplet convection. Here, the droplets are generated via flow focusing. Additional method to generate droplets in flow is via a T-junction or a co-flow in a capillary (adapted from [141]). (**b**) Sessile droplets either pinned to a planar surface, placed on a pillar (adapted from [60, 142]), or positioned into an indentation of a flat surface ([57], adapted from [58])

Droplet-based cultivations systems are striving to fill this gap and meet the increasing demand for experimental data in biopharmaceutical research with a particular focus on versatility and high throughput. Each droplet is a separate fluid element and can therefore be seen as an individual reactor vessel [60]. For droplet-based microfluidics, two distinct operation modes have been developed: the droplets are either (a) generated in an enclosed microchannel or capillary and conveyed via a continuous fluid flow (see Fig. 8a) or (b) formed on an open and planar surface where they are shaped due to the surface tension of the fluid and interactions at the liquid-solid interphase (see Fig. 8b) [46, 60, 140].

A holistic outline on droplet microfluidics is given in the chapter *Droplet Microfluidics for Biotechnology* (M. Agler-Rosenbaum) within this book. Here, the primary focus lies on an operation mode, which is also referred to as "sessile droplets." The form of these sessile droplets can range from rather flat puddles and spherical shapes – mostly depending on the hydrophobicity of the solid surface and the resulting contact angle of the droplet forming fluid [143]. The droplets can be positioned on a flat surface, on a pillar, or in an indentation to edging it and define the location. While these droplets are generally non-mobile and fixed to a defined position, different techniques have also been employed to control and move droplets on the solid surface. This droplet manipulation can be performed using acoustic or electric actuation. If droplets are manipulated via electrowetting on an array of electrodes, it is referred to as *digital microfluidics* (DMF) [45, 46, 60]. Sessile droplets are mostly in the nL to µL range – larger than droplets in flow microfluidics, where mostly pL to nL droplets are handled. This facilitates the generation of sessile droplets, what can be performed using regular pipettes or piezoelectric transducers [144]. In contrast to flow-based microfluidics, each droplet in DMF can be controlled individually, without the need for microfluidic channels, pumps, or valves [46]. Due to the small fluid volumes in DMF and sessile droplet systems, the capacity for parallelization and automation is increased, sample consumption is decreased, and integration of straightforward analytical techniques is facilitated [145]. These

advantages all render sessile droplets a particularly versatile platform that can serve as a potential alternative to microplates [60].

Sessile droplet systems were applied for cell-based testing of active pharmaceutical ingredients [146, 147]. This system has been utilized (among others) for droplet manipulation by transferring, splitting, fusion, deposition, and mixing; cell culturing and drug testing can be performed on a droplet array. Using a PDMS droplet operation array, drug screening assays on lung cancer cells were reported by Du et al. [148]. Over a period of 11 days, cell culture experiments were performed in 500 nL droplets. Another cell-based assay device has been reported by Fang et al. [149]. This device is able to perform 3D cell culture, cell co-culture, and cell migration assays. Sessile droplet systems have also been applied for chemical and enzymatic synthesis, to study reaction kinetics or discover new compounds [49, 150, 151]. Since the droplet bioreactor systems require less consumables and have a lower sample or reagent consumption, these systems are particularly advantageous in situations where only small amounts of samples are available. This holds true for analytical applications including immunoassays [152–155]; nucleic acid amplifications or other DNA-based assays such as polymerase chain reaction (PCR), sequencing, hybridization, and extraction [156–161]; and clinical diagnostics [153, 162].

In addition to the operation mode of sessile droplet bioreactors, there is also another operation mode which is closely related to sessile droplets on planar surfaces – only with a flipped arrangement. In such systems, the droplet is pinned to a solid surface (via surface forces which exceed gravitational force). This has been applied for cell spheroid cultivation, creation of organoids, and cell migration assays [60, 149]. Both operation modes can also be combined, as showed by Ma et al. [149], in systems where upper sessile droplet is separated via a horizontal membrane from a lower hanging droplet. In-depth description of further applications of sessile droplets and DMF has also been reported by Choi et al. [46], Ng et al. [45], and Garcia-Cordero and Fan [60].

One inherent challenge of sessile droplet systems is fluid evaporation – which leads to proportionally high losses of fluid in relation to the droplet volume. Evaporation, and the related drying out of the droplet, can begin immediately after the droplet generation and exposure to a gaseous atmosphere [163]. Through this fluid loss, concentrations in the droplet are raised, leading to sample enrichment – which subsequently affects data accuracy and reduces the duration for potential experiments. The rate of evaporation kinetics is influenced by many factors, including fluid properties (i.e., volatility, surface tension, viscosity), the properties of the solid surface in question (wettability, roughness, thermal conductivity), and the properties of the surroundings (temperature, relative humidity, and pressure) [60, 164]. Without adequate countermeasures, a typical droplet with an almost spherical shape dries in between 200 s and 3 h [165, 166]. If the cultivation media (i.e., the fluid properties) cannot be modified, then the experimental surroundings and droplet environment must be adjusted. Since the evaporation rate is higher at the edges of the droplet – where the fluid height is smaller – the aim should be to induce a spherical droplet shape. The humidity of the droplet atmosphere can also be increased to achieve a vapor-saturated environment. Evaporation can also be

reduced through additional installations, including restricting the headspace above the droplet [56] or covering the droplet with (mineral) oil. Finally, the liquid level of the droplet can simply be closely monitored, and evaporative losses can be compensated using microfluidic feeding channels or *liquid handling systems* (LHS). In some circumstances, evaporation can also be used as an advantage – i.e., by implementing it into the experimental procedure itself. For example, in diagnostics with very low analyte concentrations, for example, evaporation can be applied to perform a sample enrichment, enhance sensitivity, and/or accelerate measurements due to lower traveling distances of an analyte to the sensor area [60, 167, 168].

Sessile droplets can be analyzed using image acquisition and appropriate analysis software to monitor cell concentrations or conditions. MBR-related research can gain a deeper insight into metabolic processes using fluorescent microscopy or spectroscopy. A great variety of biosensors or plasmonic nano-sensors to perform Raman spectroscopy have reported [168–173]. Droplet-based arrays can also be connected to matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy, to perform analysis of droplets without the need for analyte labeling [174]. In sessile droplet approaches, comprehensive experiments and cell-based assays can be performed in very low fluid volumes and in a highly parallel fashion – although the level of monitoring and control in droplet-based MBR systems remains restricted.

Even though droplet-based systems provide very small reaction elements, usually a vast number of cells are cultivated per droplet resulting in the analysis of a profile of a cell population. The overall growth kinetics of a cell cultivation is highly affected by the variability of physiology and the phenotypic behavior [175, 176]. Specific devices, however, are increasingly being developed to cultivate single cells and analyze their phenotypic heterogeneity and its impact on growth behavior [177, 178]. Using single-cell MBRs, cellular interactions can be monitored, and the history of cells can be tracked over time – which is not possible using other techniques, such as flow cytometry [135].

4 Conclusions and Future Perspectives

The great variety of reported MBR applications illustrates both the growing importance and the immense potential benefits that this technology holds for biotechnological research. Studies have been conducted in an attempt to improve bioprocess development, scale-up processes, cultivation optimization, and survey many different cellular assays as well as analyze reaction kinetics, metabolic fluxes, and toxicity screenings.

This article has aimed to provide an overview of the MBR setups reported in the relevant literature and to give the reader a sense of the breadth and versatility of their practical deployment. On the one hand, there are rather simple reaction elements which come with limited monitoring and control but offer increased possibilities for parallelization and high-throughput experimentation. On the other hand, there are extensively equipped cultivation devices which can effectively mimic larger-scale

bioreactor systems, providing in-depth and valid experimental data especially for process development. Current research is increasingly aimed at bridging these two fields, facilitate even greater parallel experimentation while simultaneously, progressively decreasing reaction volumes of MBR systems.

However, there remains a great (and currently unmet) demand for effective MBR systems in which process parameters can be varied individually, to allow for highly parallelized experimentation with precise analytics. Consistent standards among reaction platforms would further encourage and promote the implementation of MBR technology for general biotechnological research. With improving analytics, the fields of application and the achieved outcomes will only continue to increase.

For future applications, achieving increased throughput is a point of special interest – because it facilitates even faster research progression. Extensive sensor integration for more informative and significant data, even in smallest scale, will also be required to facilitate this goal. The next steps must be to generate significant cross-scaling criteria, in order to be able to map large-scale processes on a small-scale, and vice versa. Adequately mimicking large-scale process conditions using MBRs still poses tremendous challenges, and improving this key metric demands a more thorough understanding in order to securely perform process scale-up operations. Since certain particular process conditions of the large-scale process cannot be realized in the MBR, holistic models imaging these conditions are urgently required. In order to achieve more efficient implementation and more widespread use of MBR technology in process development, the challenge of bringing multiple scaling parameters together must be even more actively addressed moving forward. But if these demands are met, then it is almost certain that MBR technologies will become indispensable tools for daily laboratory operations across a wide range of applications.

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