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A Falling-Film Microreactor for Enzymatic Oxidation of Glucose

Sabine Illner,[a] Christian Hofmann,[b] Patrick Löb,[b] and Udo Kragl*[a]

Many oxidation processes require the presence of molecular oxygen in the reaction media. Reactors are needed that provide favorable conditions for the mass transfer between the gas and the liquid phase. In this study, two recent key technologies, microreactor technology and biotechnology, were combined to present an interesting alternative to conventional methods and open up excellent possibilities to intensify chemical processes in the field of fine chemicals. An enzyme-catalyzed gas/liquid phase reaction in a falling-film microreactor (FFMR) was examined for the first time. The test reaction was the oxidation of β-D-glucose to gluconic acid catalyzed by glucose oxidase (GOx). Various factors influencing the biotransformation, such as oxygen supply, temperature, enzyme concentration, and reaction time were investigated and compared to those in conventional batch systems. The most critical factor, the volumetric mass-transfer coefficient for the efficient use of oxygen-dependent enzymes, was determined by using the integrated online detection of dissolved oxygen in all systems. The extremely large surface-to-volume ratio of the FFMR facilitated the contact between the enzyme solution and the gaseous substrate. Hence, in a continuous bubble-free FFMR system with a residence time of 25 seconds, a final conversion of up to 50% in enzymatic oxidation was reached, whereas conversion in a conventional bubble column resulted in only 27%. Finally, an option for scale-up was shown through an enlarged version of the FFMR.

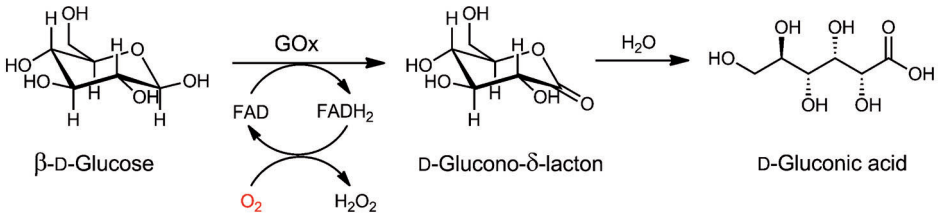
Introduction

High yields, safe operation conditions, small environmental impacts and costs, and fast and flexible process development characterize efficient production processes for chemical substances. Continuous processes using microstructured devices can fulfill a certain number of these demands.[1] Particularly in comparison with conventional batch systems, microreactors are characterized by shorter mixing times, an improved heat and mass transfer, precise residence times, and a small liquid hold-up. Microreactors were built to realize difficult or unfeasible-to-control procedures of highly exothermic or fast reactions with toxic compounds for which the reaction volume has to be as low as possible.[2,3] We found that the advantages of microscale reaction technologies for biotransformation are similar to those in chemocatalysis. This is mainly the very large surface to volume ratio in, e.g., a falling-film microreactor (FFMR). Uniform bubble-free gas entry with high oxygen utilization coefficients can resolve the well-known problem of gas-restricted biocatalysis at higher concentrations. In particular, oxygen is generally known as a limiting factor for numerous oxidation processes, aerobic fermentations, and wastewater treatment.[4–6] Many processes have been developed and improved significantly regarding oxygen entry through membrane technology,[7] thin silicon tubes,[8,9] or cyclone reactors[10] for aeration. Dispersive methods potentially inactivate the enzymes by the complex effects of hydrodynamic shear stress and the gas/liquid interface.[11] Bubble-free oxygenation in an FFMR, developed by IMM (now Fraunhofer ICT–IMM, Germany), provides a solution to prevent the inactivation of enzymes. The use of an FFMR with a large specific phase–boundary surface for bubble-free oxygenation has not yet been tested for oxygen-dependent biocatalysts and especially for oxidoreductases so far. The FFMR has, however, found several successful applications in the field of fast exothermic gas/liquid reactions.[12–17] Generally, for enzyme catalysis in microreactors, several factors, such as the complete solubility of all reaction components and high enzyme activity and stability, must be taken into account. The major challenge is the immobilization of active cells or enzymes in or on microchannels. There are a number of examples in the field of analytical biochemistry, e.g., microsensors with immobilized enzymes or lab-on-a-chip biosystems for the detection of glucose in blood or wine.[18–20] However, the adaptation of microreactors for biocatalytic reactions in industrial processes is not yet visible. A key reason for the lack of implementation is the fact that both key technologies are still very young and must overcome their own difficulties. Some recent articles, such as that of Matsuura et al., have reported enzyme-encapsulated microreactors for efficient synthesis.[21] Zhang et al. used an open tubular microreactor with an enzyme-functionalized microfluidic channel for the amperometric detection of glucose.[22] The linear range for the detec-

[a] S. Illner, Prof. Dr. U. Kragl
Department of Chemistry
University of Rostock
Albert Einstein Str. 3a, 18059 Rostock (Germany)
Fax: (+49) 381-498-6452
E-mail: udo.kragl@uni-rostock.de

[b] C. Hofmann, Dr. P. Löb
Continuous Chemical Engineering Department
Fraunhofer ICT-IMM
Carl-Zeiss-Strasse 18–20, 55129 Mainz (Germany)

tion of glucose was 0.05–7.5 mmol L^{-1} with a detection limit of 23 μmol L^{-1}. In most cases, researchers worked with very low concentrations because high activities in closed channels are still not attainable by covalent immobilization. Hence, microchannels were packed with encapsulated enzymes and called "packed-bed micro-bioreactors."[21,23,24] In this paper, we demonstrate that a continuously operated microreactor system can be used for oxidative enzyme catalysis even without immobilization. For the appropriate test reaction, the biocatalyst should have high activity and a moderate price. All reaction components should be soluble in high concentrations (up to 200 mmol L^{-1}) making the oxygen transfer the limiting factor. All these points are united in the glucose oxidase (GOx, EC 1.1.3.4) catalyzed conversion of β-D-glucose under oxygen consumption (Scheme 1).



Scheme 1. High-specific GOx-catalyzed reaction of glucose to gluconic acid under oxygen consumption for cofactor recycling.

GOx, a flavoprotein from *Aspergillus niger*, can oxidize β-D-glucose to D-glucono-δ-lacton, which spontaneously hydrolyzes to gluconic acid ($pK_a = 3.76$). The prostetic group flavin adenine dinucleotide (FAD) reacts thereby to $FADH_2$. Molecular oxygen, as a hydrogen acceptor, ensures FAD recycling under the formation of hydrogen peroxide (Scheme 1). The consumption of dissolved oxygen or the conversion of glucose is coupled stoichiometrically with the production of gluconic acid. Clearly the limiting factor is the low oxygen solubility of 8.11 mg L^{-1} (25 °C, 1013 hPa) or 0.25 mmol L^{-1} in water and, therefore, requiring efficient methods for oxygen supply in the reaction mixture.

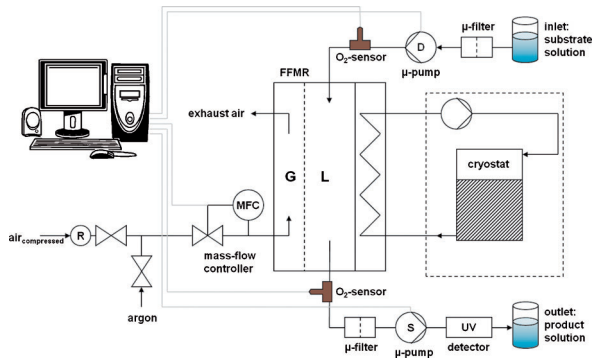


Figure 1. Flow scheme of continuously operated FFMR.

In Figure 1, the setup used to operate the FFMR is shown; details can be found in the literature.[12,25] The FFMR has a low liquid hold-up at open channels in the front and an efficient heat exchanger in the back part and is combined with online measurement technology for oxygen control and mass transfer. In further experiments, this standard FFMR-S was replaced by a large FFMR-L to test the scale-up conditions. To evaluate this novel microreactor system, we compared this system with conventional batch systems. A theoretical evaluation of continuous-flow microreactors versus conventional processing options for glucose oxidation was described by Dencic et al. in 2012.[26] With respect to these calculations, it must nevertheless be noted that the assumption of an enzyme immobilization in an FFMR is aggravated by the fact that the residence time is in the range of seconds, and for high yields, the enzyme activity in every open channel has to be more than 100 units for a preparative scale. There is ongoing work to develop such systems by surface modification of the reactor parts directing the liquid flow. For demonstrating the feasibility of the system, the soluble enzyme was used. Another approach would be to use an ultrafiltration membrane to retain the enzyme in the reactor.[5]

Results and Discussion

Oxygen mass transfer characteristics of the compared systems

Oxygen is passed from air with the entry essentially determined by the phase boundary, temperature, pressure, turbulence, and air movement. First, the determination of the volumetric mass-transfer coefficient (k_La) in the compared reactors is crucial for evaluating aeration efficiency and quantifying the effects of the operating variables. The increase of the oxygen concentration in the liquid c_L over time can be described as:

$$\frac{dc_{O_2}}{dt} = k_L a(c^*_{O_2} - c_{O_2}) \quad (1)$$

in which (c^*-c) defines the driving force and k_La the volumetric mass transfer coefficient.

As shown in Figure 2, the logarithmic plot of oxygen concentration versus time forms a straight line determining the k_La in conventional batch systems. The dissolved oxygen concentration profile was measured three times for each set of operating conditions after the liquid in the reactor was deoxygenated by argon sparging and the stirrer or air inflow was started. With a surface-to-volume ratio of 28 $m^2 m^{-3}$ and a volumetric mass transfer coefficient of 1.13 ± 0.004 h^{-1} for oxygen, the fast oxidation reaction in the stirred beaker (200 rpm) will be highly limited. For the bubble column (BC), a large range for the k_La value can be found in the literature because of the

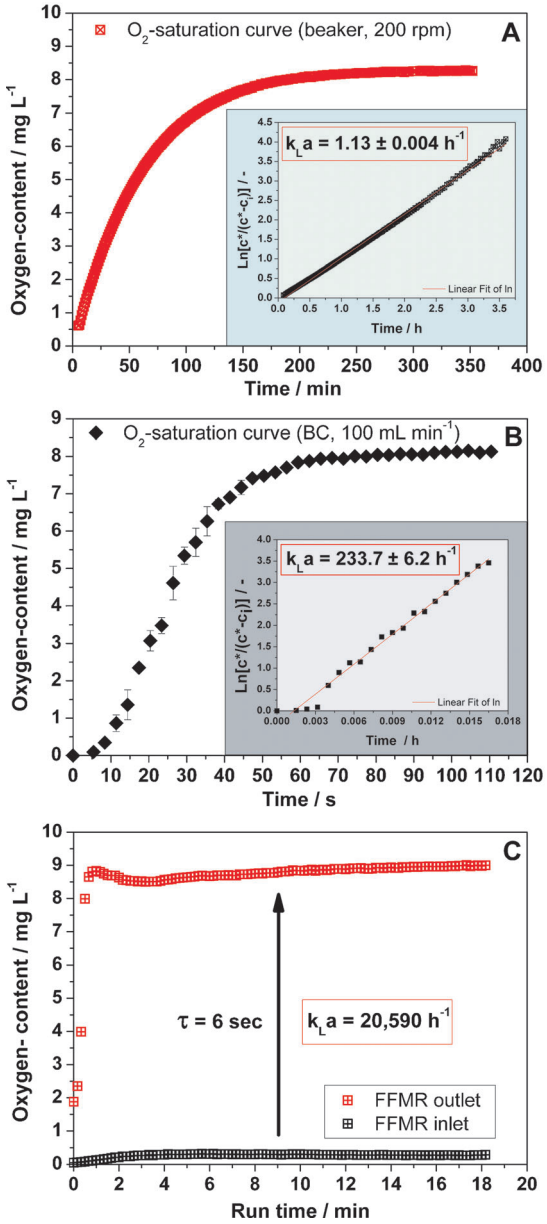


Figure 2. Determination and comparison of the volumetric mass-transfer coefficient for oxygen without enzyme using A) a beaker, stirred at 200 rpm; B) BC, air flow rate: 100 mL min^{-1}; C) FFMR-S, 64-channel reaction plate (tempered at 25 °C), liquid flow: 1 mL min^{-1}, compressed air counterflow: 100 mL min^{-1}. Conditions: c^* = 8.11 mg L^{-1} (25 °C, 1013 mbar), volume of 25 mL previously degassed with argon, 0.1 mol L^{-1} glucose in phosphate buffer (pH 7) at 25 °C.

differences in gas-distributor designs, liquid properties, and column dimensions. Garcia-Ochoa and Gomez provided a comprehensive overview of the oxygen transfer rate in various bioreactors.[27] Accurate bubble-size measurements for theoretical calculations are difficult to obtain. The intensity of the turbulence and shearing fields determine the size of the bubbles and thereby the size of the boundary surface of the phase. Thus, for the experimental determination of k_La, we measured the dissolved oxygen content over time with optical oxygen sensor spots (Presens, Germany) for different gas volume flows. Weuster-Botz et al. reported k_La values of up to 576 h^{-1} for a superficial air velocity of $\leq$0.6 cm s^{-1} in small-scale BCs.[28] BCs have, in general, higher volumetric oxygen-transfer coefficients (250 to >500 h^{-1}) than shaken vessels ($\approx$140 h^{-1}).[29] With an air velocity of 0.24 cm s^{-1}, we found a k_La value of 234 h^{-1} in the BC. However, the k_La values of conventional batch systems can in no way be compared to those achievable in the FFMR system. The surface-to-volume ratio of up to 20 000 m^2 m^{-3}[30] is several orders of magnitude larger than in the beaker and much higher than in the BC. In the FFMR an oxygen saturation of 8.9 mg L^{-1} is reachable in less than six seconds, which was chosen as the residence time for the liquid. Under these conditions the k_La value was estimated to be 20 590 h^{-1} (6 s^{-1}). This is in good agreement with previously published data. Yeong et al. reported k_La values from 3 to 8 s^{-1} corresponding to 10 800 to 28 000 h^{-1} depending on liquid flow.[31] Owing to the setup (pumps, diameter of tubing), shorter residence times were not possible in our case.

The huge volumetric oxygen mass transfer coefficient results from the large surface-to-volume ratio and the almost exclusively laminar boundary layer at the grooves, guaranteeing unsurpassable bubble-free oxygen entry (Table 1). The initial increase in the measured values was only attributable to the start-up response of the FFMR and the oxygen flow-through cells.

Bioconversion experiments

The glucose oxidation catalyzed by GOx was investigated in an FFMR and the two batch systems at reaction times of approximately 5–37 s or 7200 s (2 h), respectively, at 25 °C. In the FFMR, different residence times were obtained by changing the flow rate, with lower flow rates giving longer residence times and vice versa (see Figure 6 A). In batch mode, samples were taken at predetermined intervals. The values of glucose conversion versus time were measured by using HPLC. First, regarding the batch systems, the conversion of glucose to gluconic acid is compared in Figure 3 after a reaction time of 120 min using several enzyme concentrations.

The conventional systems reveal different trends of product concentration versus time depending on the enzyme concentration used. Despite the same high enzyme amounts, the conversion in the beaker is strongly limited. Immediately after the addition of GOx, the oxygen content decreases within less

Table 1. A comparison of oxygen supply in the beaker, the bubble column, and the FFMR.

Reactor type	Gas flow rate [mL min^{-1}]	Superficial air velocity [cm s^{-1}]	k_La [h^{-1}]	OTR_{max}[a] [g O_2 L^{-1} h^{-1}]
Beaker	0	0	1.13 ± 0.004	≈0.01
Bubble column	15	0.035	38.8 ± 0.4	0.32
	50	0.118	137.3 ± 2.2	1.13
	100	0.236	233.7 ± 6.2	1.93
FFMR[b]	100	0.012	20 590 ± 500	170

[a] OTR: Oxygen transfer rate; $c(O_2)^*$ = 8.11 mg L^{-1}. [b] 64 channels.

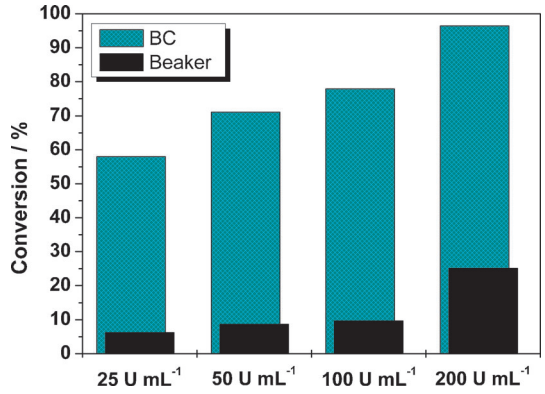


Figure 3. Comparison of conversions in the batch mode after 120 min.

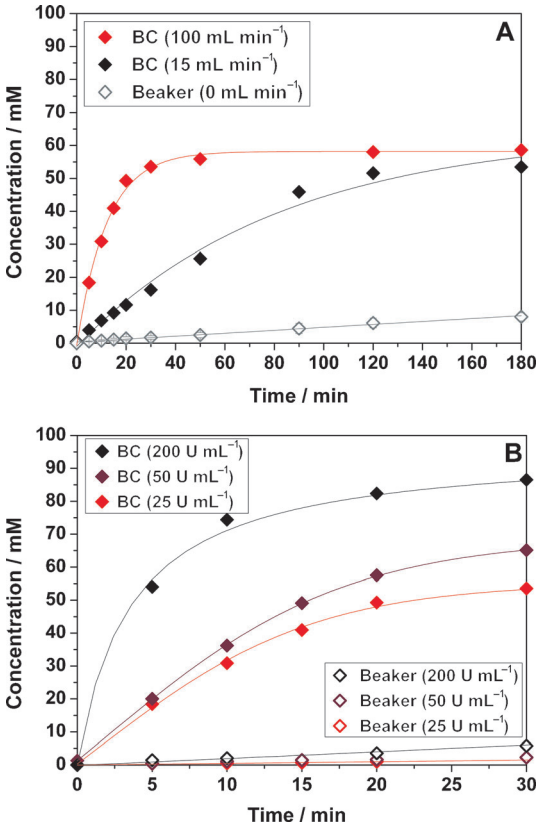


Figure 4. Conversion of glucose to gluconic acid as a function of reaction time at 25 °C using A) different gas flows, conditions: 0.1 mol L^{-1} glucose in phosphate buffer (pH 7), 25 U mL^{-1} GOx (2.96 mg), 25 mL, beaker: 200 rpm; B) various GOx concentrations (U mL^{-1}) under otherwise the same conditions and 100 mL min^{-1} gas flow in BC.

than a minute to 0 g L^{-1}. Complete conversion was reached after approximately 20 h using an enzyme activity of 50 U mL^{-1}. In the BC, glucose was completely converted to gluconic acid within approximately 2 h by using high enzyme activities (200 U ml^{-1}) and without the formation of byproducts as shown by HPLC analysis. However, in bubble column experiments the formation of foam was observed caused by protein denaturation at the gas/liquid interface. This foam formation can be reduced by reducing the gas flow, but, at the same time, reducing the reaction velocity as a result of the limiting oxygen supply (Figure 4 A). Despite the reduced gas flow, the foam forming is not completely prevented. With the native enzyme, this is a great disadvantage of an otherwise successful batch system regarding the high volumetric oxygen mass transfer coefficients and low system costs. In Figure 4 B, the conversion is shown during the first minutes of the reaction under variation of the enzyme concentration. The results clearly show that high concentrations of GOx are necessary to compare the beaker and BC with the falling film microreactor. Owing to the design developed for fast and exothermic chemical reactions, only short residence times of less than one minute are possible in the FFMR. Under initial rate conditions without any limitations, the full conversion of 2.5 mmol glucose should be achieved in approximately 30 s with an enzyme concentration of 200 U mL^{-1}. After 24 h, complete conversion in all setups with high enzyme concentrations was achieved.

The low effective initial activities determined from the slope of the linear part of the gluconic acid production curve can be correlated with the determined k_La values for oxygen. These investigations in conventional batch systems still offer considerable scope for optimization and impressively confirmed that the oxygenation limits the reaction rate at high substrate concentrations.

To overcome the oxygen limitation, we used a continuously operating FFMR system. This reactor guarantees permanent oxygen saturation in the reaction solution even at high reaction rates resulting from high enzyme concentrations. The very short residence times of only a few seconds limits the achievable conversion. The results obtained in the continuously operated FFMR cannot be compared directly through the plots in Figure 4 with the reaction times in the batch systems. For example, the reaction times to provide the same conversion differ between hours in the beaker and seconds in the FFMR. Hence, improvements in the continued supply of oxygen are better reflected in the volumetric productivity or space–time yield (STY) calculated by using Equation (2):

$$\mathrm{STY} = \frac{m_0\, C\, S}{V_R\, \tau} = \frac{c_0\, M\, C\, S}{\tau} \qquad (2)$$

in which m_0 stands for the amount of glucose, C for conversion, S for selectivity, τ for residence time, M for the molecular mass of glucose, and V_R for the reactor volume or volume of the liquid film.

The necessary periods for emptying, cleaning, filling, and maintenance of the batch systems were not considered, but the advantage of the continuous system is obvious. However, it also has to be considered that a comparison of batch and continuous systems is difficult owing to huge differences in reactor volume and residence time. The liquid film thickness amounts to 25–100 μm, and the film volume was calculated over the liquid flow rate Φ_L multiplied by the residence time τ using Equation (3):

$$V_F = \Phi_L\, \tau \qquad (3)$$

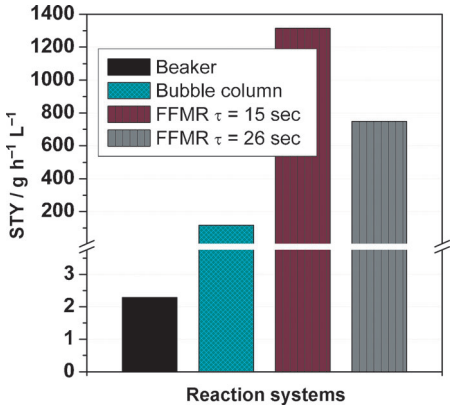


Figure 5. Comparison of STY in different reaction systems at 30% conversion. Conditions: 0.1 mol L^{-1} glucose, phosphate buffer (pH 7), 25 mL, FFMR/BC: compressed air cross-flow: 100 mL min^{-1}, FFMR 32 channels, beaker: 200 rpm.

The significantly higher STYs in the microreactor (780 and 1300 g h^{-1} L^{-1}) than in the beaker (2.3 g h^{-1} L^{-1}) and the bubble column (117 g h^{-1} L^{-1}) are highlighted in Figure 5, reflecting the improvements in oxygen supply and shortened reaction time. This result corresponds to an increase in STY by a factor of 300 in the microreactor relative to the beaker system allowing more efficient use of the enzyme activity.

The reaction time for a conversion of 30% (5.4 g L^{-1} glucose) differs from 142 min in the beaker or 3 min in the BC to some seconds in the FFMR. Thus, we have successfully demonstrated that a continuous-flow microreactor is a promising tool for the development of efficient enzyme reaction systems requiring an effective supply of a gaseous substrate. A defined and constant yield between 20 and 40% can be reached within seconds depending on the chosen enzyme concentration and the residence time on the microstructured reaction plates (Figure 6B). By reducing the flow rate or the reactor angle of inclination from 90° to 45° and varying the channel geometry and number on the reaction plate from 32 channels with 600 μm × 200 μm cross-section to 64 channels with 300 μm × 100 μm cross-section, the residence time can be further enhanced, and a conversion rate of 50% was reached (data not shown). However, higher or full conversion remains problematic because of the low volumetric flow necessary. In addition, diffusion processes within the liquid phase might become limiting as well. Also, the process of mutarotation of the glucose can be a further restriction. The timescale for reaching the equilibrium between α- and β-D-glucopyranose (34% and 66%, respectively, in the equilibrium at 25 °C) is also in the range of minutes. The rate constant for a noncatalyzed reaction of the [α]→[β] form is calculated at 0.0135 min^{-1} from kinetic measurements following a first-order kinetics.[32] GOx converts only the β-D-glucopyranose. The prevention of enzyme inactivation at the liquid/gas interface is another big advantage of bubble-free oxygenation in an FFMR.

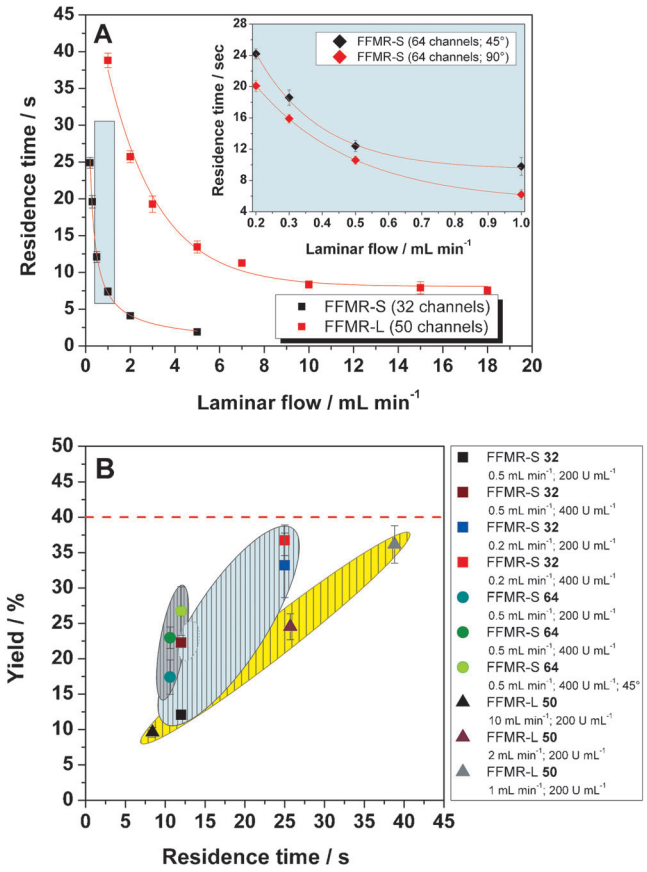


Figure 6. Comparison of FFMR-S and FFMR-L: A) range of varying residence times in FFMR-S (32 channels) and FFMR-L (50 channels) with an inset graph for FFMR-S (64 channels); conditions: averaged residence time of 10-fold measurement with isopropyl alcohol as the liquid phase; B) yield with respect to gluconic acid concentration at high enzyme concentrations at different flow rates in standard FFMR-S with 32 (squares, blue-shaded area) and 64 (circles, gray-shaded area) channels and large FFMR-L with 50 channels (triangles, yellow-shaded area); conditions: 0.1 mol L^{-1} glucose, phosphate buffer (pH 7); compressed air cross-flow: 100 mL min^{-1}; note: a product concentration of 50 mmol L^{-1} complies to a yield of 50%.

Scale-up from FFMR-S to FFMR-L

To investigate longer residence times and a first step for an upscale of the volume, a repetition of the experiments in the FFMR-L was performed. This reactor bears a larger reaction plate with a tenfold larger surface than the FFMR-S working with a cross-section of the 50 channels of 1200 μm × 400 μm (Figure 7). Measurement of the residence-time distribution yielded the highest residence time of approximately 40 s with a flow rate of 1 mL min^{-1} in the FFMR-L. Regarding the strong broadening in residence time distribution, a lower flow rate is not recommended. For many biotransformations, the range of residence times between 1 and 25 seconds in FFMR-S or 7 and 40 s in FFMR-L is far too small. Only with highly active enzymes and high catalyst concentration, high conversion is possible. In Figure 6B, the yield is shown if varying the reactor, the channel geometry, and the residence time. All the FFMR experiments exhibit unusually small standard deviations through well defined contact times between the liquid and the gas phase. The yield of gluconic acid depends linearly on the residence time

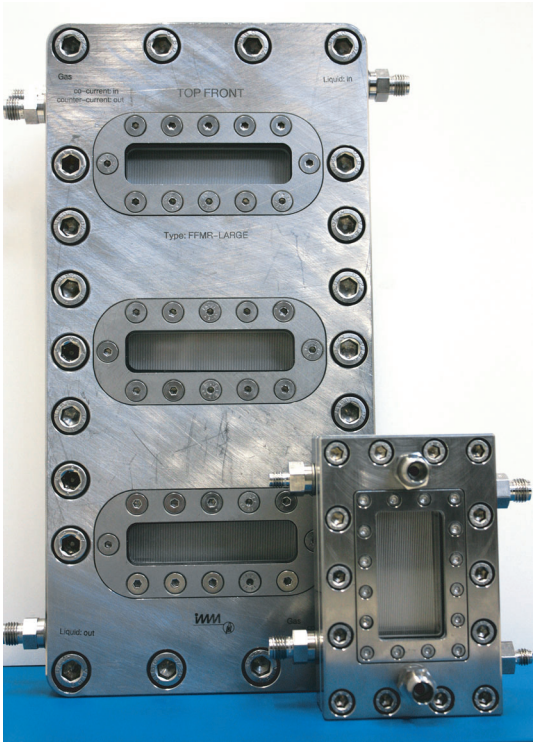


Figure 7. Falling-film microreactor developed by IMM; left: FFMR-S, right: FFMR-L.

up to 50%. Despite longer reaction times in the FFMR-L, the conversion is also limited to 40% at an initial glucose concentration of 100 mM. The highest yields at an enzyme concentration of 200 U ml^{-1} are obtained with a flow rate of 1 mL min^{-1} in the FFMR-L. This confirms the previously described limitation through slow diffusive processes during decreased glucose concentrations or high conversion. The simple transfer of experiments of FFMR-S to FFMR-L (Figure 7) confirmed an especially risk-free scale-up, as often described in the literature.[12,16,33] The conversion in different FFMRs is nearly constant, as the production rate in the reactor increases through higher capacity and flow rates.

Conclusions

For the first time, an enzyme-catalyzed oxidation in a falling-film microreactor was investigated. Microstructured devices are new and flexible tools that show high potential to enabling chemical reactions without mass- or heat-transport limitations with additional low risks in process up-scaling. Thus, it is not necessary to substitute all process steps through microreactor technology, but only if this represents an advantage for the whole process; the same applies to biotechnology. In the FFMR system, significantly higher specific interfacial areas improve the oxygen transfer rate. The space–time yields exceed those obtained with conventional reactors by orders of magnitude. Herein, we describe a system that allows continuous conversion of the substrate up to 50% in less than 10 seconds of residence time. The initial reaction rate in the FFMR was 12 times faster than that in the bubble column and 588 times higher than in the beaker using equal amounts of enzyme. Full conversion as observed in the bubble column is not possible because of the not yet optimized reactor geometry for longer residence times. The initial concentration of α-D-glucose lies in the range of 50 to 200 mmol L^{-1}, proving that the system is indeed suitable for preparative-scale synthesis. This result opens the door for the application of continuously operating microbioreactors for the flexible biochemical processing of fine chemicals.

Experimental Section

Chemicals and materials

All chemicals, such as isopropyl alcohol, phosphate buffer salts, β-D-glucose, and D-glucono-δ-lacton, were obtained from Merck or Sigma–Aldrich. Glucose oxidase (EC 1.1.3.4 from *A. niger*, type X-S, lyophilized powder, 211 U mg^{-1}) was purchased from Sigma–Aldrich. All chemical reagents used in the experiment were of analytical grade.

Experimental setup and procedure

The biotransformation was performed in a stirred beaker (B), a bubble column (BC) constructed of glass with sintered glass bottom as a gas diffuser, and a standard falling-film microreactor FFMR (IMM, Mainz, Germany). Beaker and BC as conventional systems were each characterized by a diameter of 3 cm and a reaction volume of 25 mL. A preparation of 0.1 mol L^{-1} glucose (18 g L^{-1}) in a phosphate buffer solution (pH 7) was converted under varying enzyme concentration (50–400 U mL^{-1}). The reaction in the continuous FFMR system occurred along three vertical microstructured reaction plates (16 parallel open microchannels, width×thickness: 1200×400 μm; 32 channels, 600×200 μm; 64 channels, 300×100 μm) with a size of 76×25.6 mm. The liquid film of glucose–enzyme solution was transported within the grooves by the action of gravitational forces, and the open form ensured contact with the gas phase. The two pulsation-free pumps were necessary to provide a constant liquid-film thickness, typically in the range of several dozens of micrometers. The liquid hold-up in the FFMR was varied and adjusted depending on the reaction plate. The volume before and after the FFMR was kept to a minimum, resulting in a total volume of approximately 5 mL. In front of the grooves was a gas-phase chamber. In this chamber, a counter-flow of compressed air or argon was controlled by a pre-pressure regulator (Parker, Porter 4000, 0–60 psi) and a digital mass-flow controller (Bronkhorst, El-Flow Select F-201CV). For online oxygen detection, we used optical oxygen sensor spots (B, BC) and flow-through cells (FFMR) from Presens GmbH. Directly before and after the FFMR were two oxygen flow-through cells and two microannular gear pumps (HNP, mrz-2942) with a particle filter (Swagelok, 7 μm) combined through Swagelok connections in a 1/8" periphery. Negative effects of shearing forces on enzyme stability by pumping through the FFMR system were not observed. Stability experiments (closed-loop circulation) displayed no loss of enzyme activity over 22 h. The FFMR was temperature-controlled by a cryostat (Huber, CC 3). In the BC, the gas flow rate was equally controlled. In the beaker, a stirring rate of 200 rpm was adjusted without additional gassing. Samples (0.5 mL) were taken at the outlet of the FFMR system, with the number of samples based on the liquid flow rate in the FFMR. All samples were immediately heated to 99 °C resulting in

a complete loss of enzyme activity (Eppendorf, Thermomixer comfort, holding time: 10 min, stirrer speed: 300 rpm) and measured with HPLC.

Analytical procedure

The concentration of gluconic acid was determined by HPLC using an Aminex HPX-87H column (Biorad, 300×7.8 mm) and a Cation-H Refill Cartridge (Supleco, 30×4.6 mm) as pre-column at 65 °C. The eluent was a 6 mM sulfuric acid with a flow rate of 0.8 mL min^{-1}, coupled with an RI and UV detector in a Knauer HPLC system. The injection volume was 20 μL, and the retention time of the gluconic acid was 6.8 min. Gluconic acid was calibrated through its peak area ($\lambda = 210$ nm), and the initial concentration of glucose was measured by RI detector.

Large-FFMR experiments

Apart from the standard FFMR, a large FFMR was used with a 10-fold increase in the structured surface area on the reaction plate. Therefore, the length and the number of channels were increased by a factor of $10^{0.5}$. In the general design, the FFMR-L was relatively similar to that of FFMR-S. Prior to the biotransformation, the residence time distribution in the FFMR-L was measured. All other steps are identical to the previous FFMR-S procedure.

Measurement of residence time in microreactor systems

Experimental measurement of the mean residence time (τ) was achieved through a sight window in the upper part of the housing. Detection through video images was not possible over the whole microstructured plate through a limited frontal view, therefore, manual measurements with a stopwatch and additional lighting covering the complete plate was favored. The flow distribution of isopropyl alcohol while wetting was approximately representative of a stationary flow condition. For the statistical evaluation of flow-front measurements, 10-fold repetition was performed. Theoretical simulations or mathematical calculations of flow distribution have been described previously in the literature.[15,34]

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Keywords: enzyme catalysis · continuous processes · gluconic acid · microreactor · oxygen transfer

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