Chapter

Design and Operation of Fixed-Bed Bioreactors for Immobilized Bacterial Culture

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Abstract

Fixed-bed processes operated in perfusion, where cells are immobilized within macroporous carriers, are a promising alternative to processes with suspended microbial or mammalian cells. Their potential has been demonstrated for many purposes. Nevertheless, the number of industrial fixed-bed processes is quite small. To some extent, this is due to the lack of process development tools for fixed-bed processes. To fill this gap, a strategy was developed for the design and evaluation of relevant process parameters of fixed-bed processes. A scale-up concept is presented in order to evaluate the performance as part of process design of fixed-bed processes. This comprises fixed-bed reactors on three different scales, the smallest being the downscaled Multiferm with 10 mL fixed-bed units, the second a 100 mL fixed-bed reactor, and the third a pilot-scale reactor with 1 L fixed-bed volume. The performance of this concept will be discussed for fixed-bed cultures of lactic acid bacteria. Furthermore, a reaction kinetic model for the design of fixed-bed reactors will be presented.

Keywords: fixed-bed, bacterial culture, scale-up, modeling, lactic acid bacteria

1. Introduction

Technologies for immobilization of biocatalyst, e.g., microbial or mammalian cells, are increasingly being considered for biotechnological processes due to many advantages compared to cell suspension culture such as continuous operation, accelerated reaction rates, high volumetric productivity, retention of plasmid-bearing cells, prevention of interfacial inactivation, stimulation of production and excretion of secondary metabolites, and protection against turbulent high-shear environment, reduced susceptibility of cells to contamination, improved production efficiency, and reduced risk of washout [1–3]. Especially the increased importance of productivity for industrial processes due to restriction of production time and final product volume has drawn the attention to immobilization techniques in recent years, as they allow overcoming most of the limitations of commonly applied suspension cultures [2]. A summary of advantages and disadvantages for suspension cultures (stirred tank reactors) and immobilized cultures (fixed-bed reactors) is given in **Table 1**.

Various immobilization techniques such as the entrapment of cells in stable porous gels (e.g., alginate, agarose, collagen, chitosan, cellulose, κ-carrageenan, or

	Advantages	Disadvantages
Stirred tank/ suspension	Known technology	Aeration difficult at high cell densities (relevant for aerobic cells)
	Good mass transfer	Cell damage by shear and aeration (e.g., mammalian cells)
	Good mixing	Foaming (relevant for aerobic cells)
	Cell count possible	Low cell density and volumetric productivity Cell retention required for perfusion culture, techniques insufficient for long-term culture
	High potential for scale-up	
Fixed-bed/ immobilized cells	High cell density and productivity per unit	Concentration gradients
	Easy exchange of medium	Nonhomogeneous
	High productivity over long periods of time	Cell count impossible
	Low-shear rates (relevant for mammalian cells)	

Table 1.
Summary of advantages and disadvantages of stirred tank (suspended cells) and fixed-bed reactors (immobilized cells).

- High volumetric cell density and high productivity
- Low-shear stress environment for mammalian and tissue cells
- Easy medium exchange and separation of cells and product simplifying downstream processing

Table 2.
Characteristics of fixed-bed reactors used for cultivation of microorganisms or mammalian/tissue cells (adapted from Pörtner and Märkl [5]).

gel-matrix polymers such as polyacrylamide-hydrazide) or hydrogels or immobilization in solid macroporous carriers have been developed and are applied in both laboratory and industrial scales for different purposes, e.g., food, dairy, and beverage industry, production of drugs, wastewater treatment, agricultural industry, and biodiesel production [2–4].

Bioreactors for immobilized biocatalyst are mostly operated continuously in perfusion mode. Here continuous stirred tank reactors with cell retention and fixed-bed (packed bed) or fluidized-bed bioreactor systems can be applied. The following remarks focus on fixed-bed reactors, which consist of a packed column of macroporous carriers wherein cells are immobilized, as they have been used very successfully for a wide range of applications [4]. The advantages of fixed-bed reactors with immobilized cells (**Table 2**) are mainly with respect to general productivity and operational flexibility [6]. The volumetric productivity of immobilized cells is generally higher than the corresponding free cell fermentations [6]. This higher productivity can be explained by the fact that the microenvironments offered by the carrier are more stabilizing for the organisms, which generally show optimal activity only in a narrow range of physical conditions. Due to cell retention, it is possible to run fixed-bed bioreactors in a perfusion mode at a steady state with dilution rates higher than the maximum specific growth rate of the used strain. By this, very high volume-specific productivities can be reached and maintained for long periods of time and greatly facilitate recycling or reuse of

microorganisms [6]. Consequently, both the operational stability of the immobilized organisms and the productivity are improved.

Despite the obvious advantages of fixed-bed bioreactor systems, the number of industrial fixed-bed processes is quite small [2–4]. To some extent, this is due to the lack of process development tools for fixed-bed processes and meaningful concepts for design and operation of fixed-bed reactors on a large scale. To fill this gap, strategies for the design and evaluation of relevant process parameters of fixed-bed processes are required, and a scale-up concept is introduced.

In the following, the characteristics of fixed-bed bioreactors as well as a design concept for layout and scale-up will be introduced. Examples for macroporous carriers will be given. The design strategy for fixed-bed reactors will be discussed in detail for immobilized cultures of lactic acid-producing bacteria (LABs). Finally, a reaction kinetic model is introduced which allows evaluation of the culture performance. Conclusions complete the text.

2. Fixed-bed reactor systems

2.1 Principle

Fixed-bed bioreactors consist of a mostly cylindrical column containing macroporous carriers, wherein cells are immobilized (**Figure 1A**). The column is permanently perfused with fresh medium [4, 7]. If required, the medium can be circulated in a loop (**Figure 1B**). This might be useful if appropriate flow rates and medium supply rates vary significantly.

For small fixed-bed volumes with a height of approximately 10 cm, the medium can be pumped axially through the bed. In this case, at the outlet the oxygen concentration in the case of aerobic cells, e.g., mammalian cells [8], or the pH in the case of acid-producing anaerobic cells, e.g., LABs [6], should remain in a physiological range. A further increase of the length would result in too low oxygen or pH values in the upper zones of the bed. This can be overcome by applying a radial medium flow as shown in **Figure 1C**, where the radius determines the length of the oxygen or pH gradient, not the height of the column. This concept was successfully applied for mammalian cell culture [8] and lactic acid bacteria, as discussed in the following.

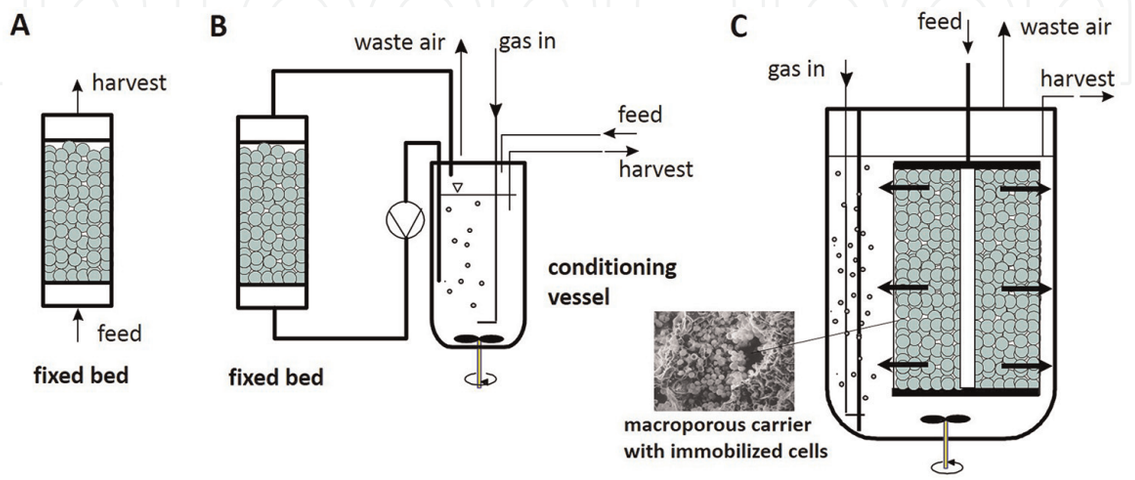


Figure 1.
Examples for design concepts of fixed-bed reactors. (A) Axial-flow fixed bed with plug flow. (B) Axial-flow fixed bed with external conditioning vessel. (C) Fixed bed with radial flow integrated in conditioning vessel, plug flow.

Cell immobilization in fixed-bed reactors with macroporous carriers is fairly simple compared to other methods such as entrapping in gels (e.g., alginate). Cell loading is often carried out by simply pumping a cell suspension through the bed of carriers, and cells are kept under same physiological conditions for the immobilization. As only the natural properties of the surface and cells interact, there are no toxic effects arising from activating reagents compared to cell entrapment within polymers. Additionally, high load of cells can be avoided by desorption of cells from the solid surface to the cell suspension.

2.2 Concept for design and operation

Process parameters that have to be optimized during process development comprise selection of carriers, medium selection, appropriate flow velocity, and long-term performance, among others. All these information are required to evaluate the overall performance, e.g., productivity, and to layout the scale-up strategy. In the following, a platform for development of processes for immobilized cells is introduced (**Figure 2**). As a start, suitability of different carriers can be compared in a small-scale multi-well system. After this, bioreactor systems of different sizes can be used to work out the required process parameters. The first, very small scale of 10 mL working volume is the multi-fixed-bed bioreactor "Multiferm" [9]. The next step is an axial-flow 100 mL fixed-bed system, which can be operated continuously with reasonable effort to investigate the performance and long-term stability of the culture [6]. As a first approach for scale-up, a radial-flow 1 L fixed-bed reactor is applied [6, 10]. Even if this is probably not the final industrial scale, the reactor system has already been the main characteristics of a large-scale system, mainly the radius. For further increase of the volume, just the height has to be increased [11]. For all three systems, a "proof of concept" has been shown before [10]. In Chapter 4 the performance of these three fixed-bed systems is compared for fixed-bed cultures of LABs.

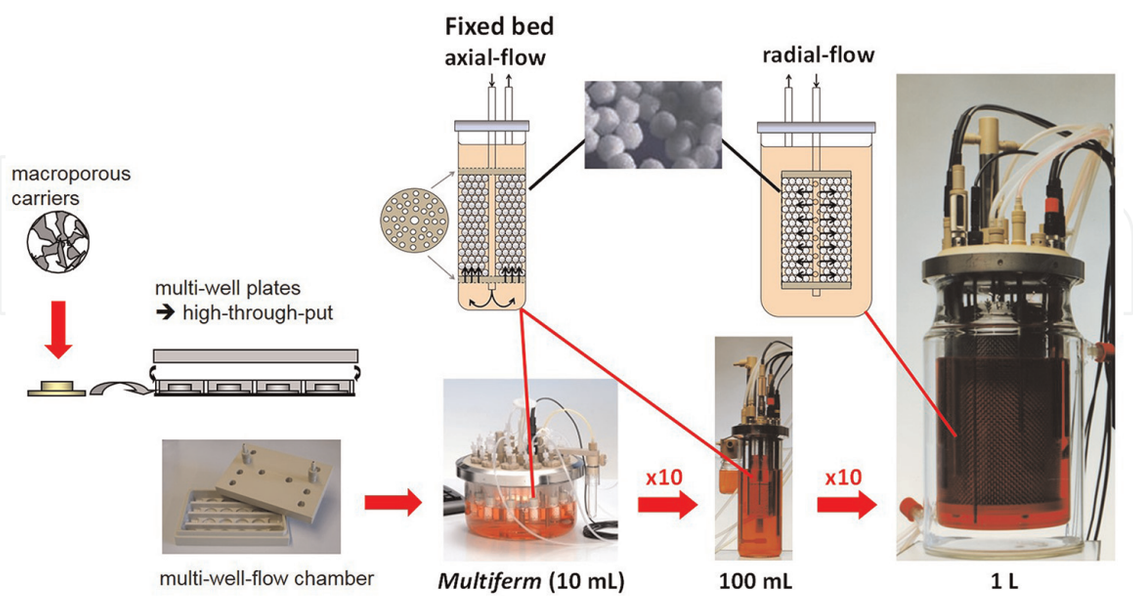


Figure 2.
Platform for development of processes for immobilized cells. From left to right: multi-well plates with special inserts for the first evaluation of appropriate carriers under high-throughput, static conditions; multi-well flow chamber for evaluation of carriers under flow conditions; multi-fixed-bed reactor "Multiferm" (max. 12 small fixed-bed units containing approx. 10 mL carriers for evaluation of carriers under different process parameters (type of carrier, flow rate, medium, oxygen concentration, pH, etc.)); 100 mL axial-flow fixed-bed for long-term continuous culture under steady-state conditions; and 1 L radial-flow fixed-bed representative for a pilot scale for long-term continuous cultures under steady-state conditions.

3. Carriers

In cell immobilization, properties of the carrier materials play an important role. This type of immobilization on solid synthetic materials firstly has the advantage that the microorganisms attach independently to the carrier (interaction with the surface) and thus no additional process steps and reagents are required for immobilization. At this point, carrier materials have to demonstrate several certain characteristics. Atkinson et al. [12] and Pörtner and Märkl [5] summarized these properties for cell immobilization such as simple and nontoxic material, high cell loading capacity, mechanical stability, stable at appropriate operational pH values, autoclavable, resistant to microbial degradation, cost appropriate to the application, density appropriate to reactor type used, as well as reusable, if possible. Examples are given in [4, 6]. In our own studies, carriers made of glass [Siran (QVF, Mainz, Germany), VitraPOR® (ROBU® Glasfilter-Geräte GmbH)] or ceramics [(CERAMTEC EO 19/30 (CeramTec, Marktredwitz, Germany) (**Figure 3**) or Sponceram (Zellwerk, Oberkrämer, Germany)] were applied. All carriers showed similar results with respect to immobilized cell density and lactic acid productivity for immobilized LAB strains [6].

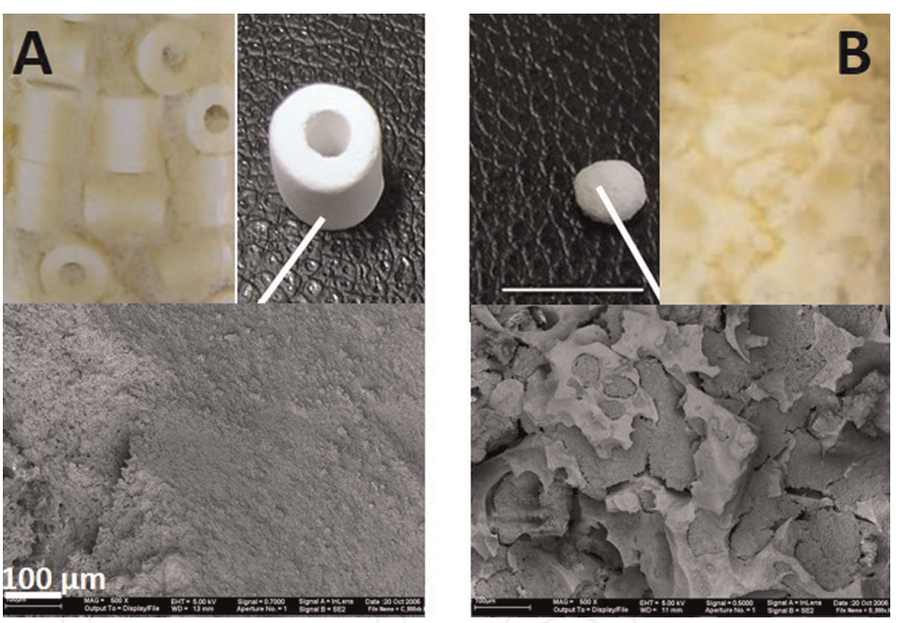


Figure 3.
Examples for carriers applied in fixed-bed cultures: (A) CERAMTEC EO 19/30 (α-aluminum, ring, diameter 3–8 mm, height 8 mm, porosity 65%; manufacturer, CeramTec; up-right, carrier; up-left, carrier in fixed-bed cultivation of Lactococcus lactis*; down, SEM of carrier); (B) VitraPOR® (glass, sphere, diameter 4 mm; manufacturer, ROBU; up-left, carrier; up-right, carrier in fixed-bed cultivation of* L. lactis*; down, SEM of carrier).*

4. Case study: fixed-bed cultivation of LAB strains

4.1 Overview on immobilization techniques used for LAB strains

Lactic acid bacteria are commonly used in the production of fermented dairy products as well as for production of lactic acid, antimicrobial substances (bacteriocins), and biodegradable polymers, among others [13–15]. Industrial processes use mostly conventional batch or fed-batch fermentation with suspended cells. Reactor volumes go up to 100 m^3, and process time varies between several hours and days depending on the strain and the process strategy [14]. Even if high cell and product

concentrations can be reached, the known drawbacks such as low productivity, product inhibition, and also the variation from batch to batch remain [16–18].

Since the immobilization of LAB has many advantages, it has been examined extensively, e.g., for the production of lactic acid; the production of starter cultures; the production of bacteriocins, e.g., nisin; and the formation of aromatic compounds (reviewed in [6]). Different methods have been used for immobilizing LAB: physical entrapment in polymeric networks, microencapsulation, attachment or adsorption to a carrier, and membrane entrapment [6]. The purpose of all these techniques is either to keep high cell concentrations within the bioreactor or to protect cells from a hostile environment. In many applications of cell entrapment, droplets of thermal (κ-carrageenan, gellan, agarose, gelatin) or ionotropic (alginate, chitosan) gels are used to produce spherical gel biocatalysts, and these controlled-size polymer droplets are produced using extrusion or emulsification, under mild conditions (reviewed in [6]). However, although promising on a laboratory scale, the large-scale production of beads under aseptic conditions still has difficulties [19].

Another immobilization technique is to immobilize LAB cells onto solid macroporous carriers and apply these in fixed-bed bioreactors. Examples are given in [6]. In the following, our recent work in this area will be discussed.

4.2 Fixed-bed cultures of LAB strains

4.2.1 Examples for fixed-bed cultivation on different scales

For all three fixed-bed bioreactor systems (*Multiferm* 10 mL, axial flow 100 mL, radial flow 1 L), a "proof of concept" has been shown before [6, 9, 10]. More infos on Materials and Methods can be found there. In the following, the main results are highlighted. Both *Lactococcus lactis* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* could be cultivated successfully in the fixed-bed reactors. As expected, the lactate concentrations in the harvest flow were in a similar range or slightly lower as in the corresponding batch cultures. The yield of lactate depended on the type of strain and the used medium. The cell concentration in the harvest flow was considerably lower as in the corresponding batch culture, especially in case of *L. bulgaricus*. This is probably due to the short duration of most experiments (50–100 h per perfusion rate). In longer experiments considerably higher cell concentrations in the harvest flow were found.

The volume-specific lactic acid and cell productivity increased with increasing perfusion rate (see below).

Parallel cultivation in the *Multiferm* bioreactor system showed a very high reproducibility [9]. Standard deviation for lactate concentration from different parallel runs was below 5%, indicating a high reproducibility of the system. Therefore, the system is well suited for evaluation of process parameters in a very small scale with reduced effort.

The microbiological and mechanical stabilities of continuous cultivations during prolonged fermentations are critical properties of an immobilized cell process, and industrial applications are largely dependent on these properties. Therefore, we focused on the examination of long-term (52 days) continuous cultivation of *L. lactis* immobilized on ceramic carriers in an axial-flow 100 ml fixed-bed reactor (**Figure 4**) [6]. This proved that the continuous immobilized cell fermentation with *L. lactis* demonstrated a high biological stability longer than 50 days. The viability of cells in the harvest flow was usually around 90%, and the growth rate of cells re-cultivated as batch was similar to the corresponding batch. This indicates that functional cells can be harvested continuously from the fixed-bed.

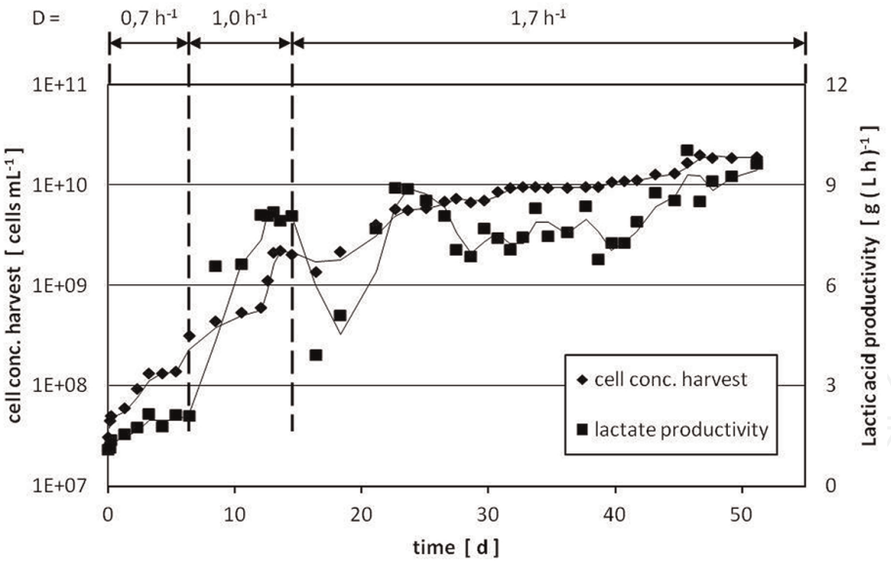


Figure 4.
Long-term cultivation of Lactococcus lactis *in an axial-flow fixed-bed (100 mL, Medorex) filled with macroporous carrier CERAMTEC EO/90 at different perfusion rates D in MRS medium (data from* S. *Zengen (TU Hamburg), not published). Cell concentration in harvest flow (left) and volume-specific lactate productivity versus cultivation time.*

The scale-up from 100 mL to 1 L fixed-bed (**Figure 5**) was successful, as similar productivities could be obtained in both systems (see below). As a conclusion, the continuous cultivation of immobilized LAB strains in fixed-bed reactors shows a high biological stability as well as cell and lactate production in long-term fermentation.

4.2.2 Comparison of suspension and fixed-bed systems on different scales

Fixed-bed and suspension cultures of *L. lactis* were compared with respect to the volume-specific lactate productivity (**Figure 6**) [10]. Continuous suspension culture in chemostat mode showed the expected course [20]. At first the productivity increases with increasing dilution rate up to a maximum. When the dilution rate

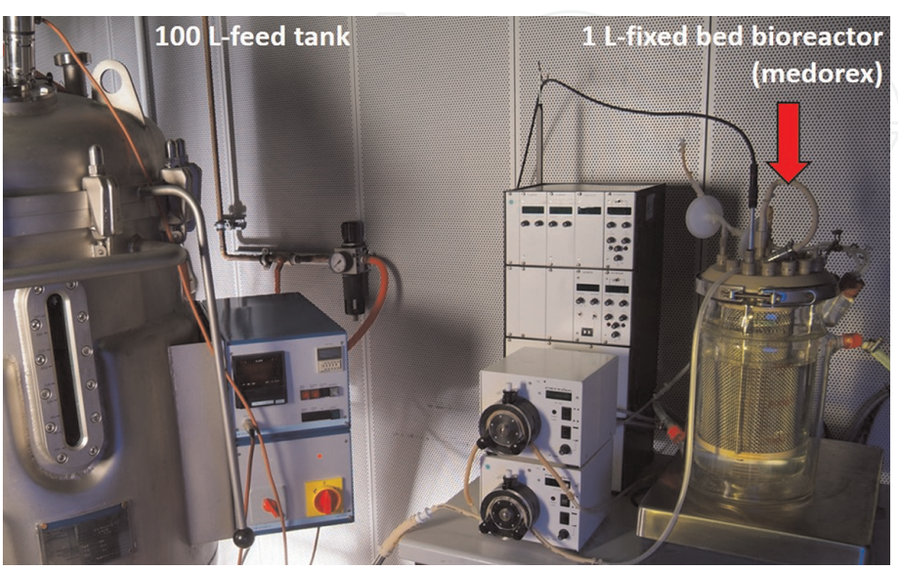


Figure 5.
Pilot plant fixed-bed bioreactor system consisting of 1 L radial-flow fixed-bed reactor (Medorex), feed and harvest pumps, feed tank (100 L), and control unit (temperature, pH, oxygen, depending on the type of microorganism (aerobic or anaerobic)).

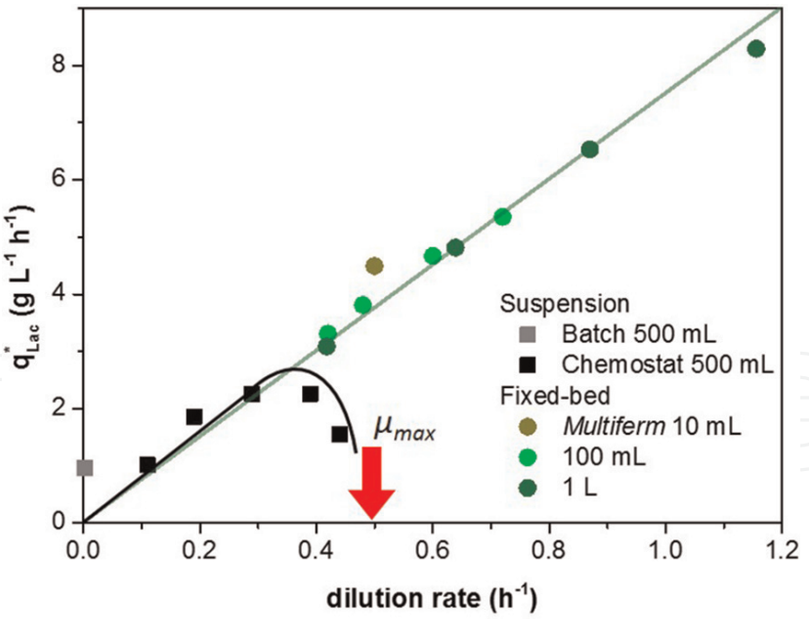


Figure 6.
Comparison of suspension and fixed-bed cultures for Lactococcus lactis*. Volume-specific lactate productivity q^*_{Lac} versus dilution rate D. M17 medium (Difco) with 5 g L^{-1} lactose; carrier, 10 mL Multiferm; 100 mL, VitraPOR® 4 mm; 1 L, VitraPOR® 8 mm. The red arrow indicates the maximum specific growth rate of the strain.*

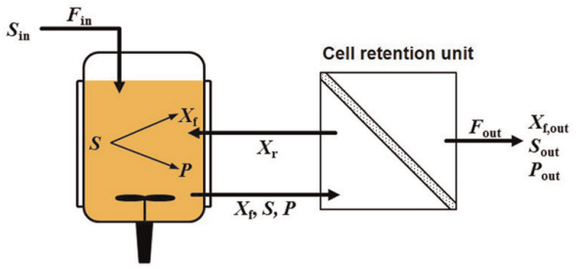
gets close to the maximum specific growth rate μ_{max}, the productivity decreases, as washout of cells occurs.

For the fixed-bed-cultures, the productivity increases further due to cell retention in the carriers. The highest value determined here is approx. 3–4 times higher than the maximum in chemostat cultivation. Obviously the maximum for fixed-bed cultures has not been reached so far.

All fixed-bed systems used here can be described by the same spline. This is very important with respect to scale-up, as obviously data from small-scale systems can be used to predict the performance on a larger scale (for more details on scale-up, see [20]).

4.3 Reaction kinetic model for start-up of fixed-bed reactors

For establishment of mathematical process model, biomass formation, lactose consumption, and lactate production during start-up of fixed-bed cultures with immobilized *L. lactis* were investigated experimentally and described by a reaction kinetic model [21]. Appropriate modeling and simulation of fixed-bed processes require biomass data. Therefore, a low-volume multiple fixed-bed reactor system (*Multiferm*) was used to investigate biomass formation of a *L. lactis* strain during the start-up phase of fixed-bed cultivation. The generation of data in parallel experiments was fast and easily compared to larger single reactor systems. Biomass data obtained from both fractions, retained and free suspended biomass, was used for modeling and simulation, together with data for lactose and lactate. The underlying Luedeking-Piret-like model structure was developed based on the results from suspension cultivations with the same strain. The fixed-bed system was described as perfusion culture with cell retention (**Figure 7**). For this, merely four additional parameters had to be defined to extend the suspension model to fixed-bed cultures. Experimental trends and steady states of both biomass fractions besides substrate and product could be described very well. Thus, this model could be used for process layout during process development.



$$\mu = \mu_{max} \frac{S}{K_S + S}$$

$$\frac{dX_f}{dt} = X_f \cdot \mu - D \cdot (1 - R) \cdot X_f$$

$$\frac{dX_r}{dt} = D \cdot R \cdot X_f - k_{lys} \cdot X_r$$

$$\frac{dS}{dt} = -\frac{X_f \cdot \mu}{Y_{x/s}} - m_r \cdot X_r + D \cdot (S_{in} - S)$$

$$\frac{dP}{dt} = \alpha \cdot X_f \cdot \mu + \beta \cdot X_f - \gamma \cdot X_f \cdot S + \beta \cdot X_r \cdot \eta_P - D \cdot P$$

Parameter from suspension culture (chemostat)

μ_{max} (h^{-1})	K_s ($g \cdot L^{-1}$)	$Y_{x/s}$ ($g \cdot g^{-1}$)	α ($g \cdot g^{-1}$)	β (h^{-1})	γ ($L \cdot g^{-1} \cdot h^{-1}$)
0.48	0.24	0.18	10.22	0.12	0.53

Parameter from fixed bed culture (continuous)

R (-)	k_{lys} (h^{-1})	m_r (h^{-1})	η_p (-)
0.37	$2.92 \cdot 10^{-3}$	0.21	6.16

Figure 7.
Reaction kinetic model for fixed-bed cultures with immobilized microorganisms. For details see text and [21].

5. Conclusions

The goal of the studies was to evaluate the performance of fixed-bed bioreactor systems on different scales compared to suspension culture. The suggested concept for development of fixed-bed processes could be confirmed. The multi-fixed-bed bioreactor *Multiferm* provides an ideal downscaled and economical system that can be used for basic studies with low requirements on medium and cells. Here, questions such as optimal carrier design, appropriate medium, and process parameters (e.g., technique for immobilization, initial cell density, flow rate, temperature, oxygen, pH) can be evaluated. Especially the start-up phase can be investigated. The next step, a 100 mL fixed-bed system, provides data on the performance and long-term stability of the culture. Problems that might not have been shown up in the *Multiferm*, e.g., insufficient long-term stability, can be detected here. The 1 L fixed-bed can be regarded as a pilot scale already because medium requirement was already at 27.6 L per day at the highest dilution rate. Additionally, the radial-flow geometry can be easily scaled up further.

As expected, fixed-bed bioreactors could be operated in a perfusion mode at a steady state with dilution rates higher than the maximum specific growth rate. By this, very high volume-specific productivity with respect to lactate can be reached and maintained for long periods of time. The fixed-bed processes with lactic acid bacteria on macroporous carriers could be transferred on a pilot scale without loss in productivity. Furthermore, the productivity could be described by a spline, indicating that the maximum growth rate was not reached in this study.

Therefore, a process development tool for fixed-bed processes is now at hand that will pave the way for an industrial application of this promising technology.

Abbreviations and symbols

D	Dilution rate (h^{-1})
F	Flow rate ($L \cdot h^{-1}$)
F_{in}	Inlet flow rate ($L \cdot h^{-1}$)

F_{out}	Outlet flow rate ($L \cdot h^{-1}$)
j	Running index
k_{lys}	Lysis rate (h^{-1})
K_S	Substrate saturation constant ($g \cdot L^{-1}$)
LAB	Lactic acid bacteria
m_r	Maintenance rate of retained cells (h^{-1})
P_{out}	Product concentration at the outlet ($g \cdot L^{-1}$)
R	Fraction of retained biomass in perfusion fermentation (−)
S	Substrate concentration ($g \cdot L^{-1}$)
S_{in}	Substrate concentration at the inlet ($g \cdot L^{-1}$)
S_{out}	Substrate concentration at the outlet ($g \cdot L^{-1}$)
X_f	Free suspended biomass concentration ($g \cdot L^{-1}$)
$X_{f,out}$	Free suspended biomass concentration at the outlet ($g \cdot L^{-1}$)
X_r	Retained biomass concentration ($g \cdot L^{-1}$)
Y_j	Concentration of either biomass, substrate, or product ($g \cdot L^{-1}$)
$Y_{X/S}$	Biomass yield coefficient from substrate ($g \cdot g^{-1}$)
α	Growth-associated product formation rate ($g \cdot g^{-1}$)
β	Nongrowth-associated product formation rate (h^{-1})
γ	Constant for unspecific substrate loss ($L \cdot g^{-1} \cdot h^{-1}$)
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
η_P	Effectiveness factor production (−)

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