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Microscale and miniscale fermentation and screening

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Small-scale bioreactors in the microliter and milliliter range gained more importance in recent years. For the characterization of mass transfer, the volumetric mass transfer coefficient k_La and the oxygen transfer rate OTR_{max} are considered. k_La values up to 1440 hour^{-1} are reported for small-scale bioreactors. The OTR_{max} is strongly influenced by the liquid film thickness and, finally, by the liquid viscosity. Optical on-line methods, such as fluorescence and scattered light measurements, are applied to monitor pH, dissolved oxygen tension (DOT), product formation and biomass. Recently, single cell microfluidics are used to obtain new insights into microbial behavior at changing operating conditions. Finally, novel fed-batch techniques are applied to assimilate the cultivation conditions between screening and production scale.

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Introduction

In modern biotechnological process development, microbioreactors and minibioreactors play an essential role in high-throughput screening and small-scale cultivation experiments. The application of such bioreactors accelerates process development, which is usually slow and expensive [1]. To comprehensively characterize bioreactors, knowledge on mass transfer and power input is essential. New studies in these fields have been published. In recent years, new monitoring technologies and control strategies for small-scale cultivation systems as well as novel bioreactor systems have been developed. Currently, a trend toward decreasing working volumes and automated cultivation platforms is noticed. Simultaneously, new feeding techniques for small-scale bioreactors help to diminish the gap between operating conditions in screening (batch) and production (fed-batch) scale.

In this article, the latest results of microscale and miniscale bioreactors development of the last two years (2012–2014) are presented. For microbioreactors we use the definition of a working volume <1 mL. Miniscale bioreactors are considered as bioreactors with a working volume up to 100 mL. Among others, microtiter plates, shake flasks and microfluidics will be considered in this article.

Mass transfer and power input

Mass transfer and power input strongly influence the performance of bioreactors. In particular, a sufficient oxygen supply is mandatory for the successful screening and cultivation of aerobic microorganisms. The mass transfer in bioreactors is described by the volumetric mass transfer coefficient k_La. Even if there is already published an immense number of studies, research is still continuing in this field. Two excellent reviews about this topic have been published recently [2•,3•]. Kirk and Szita sum up the properties and values of the volumetric mass transfer coefficient k_La of microbioreactors and minibioreactors [2•]. It is stated that microbioreactors (<1 mL) show generally a higher k_La compared to minibioreactors (1–10 mL, definition of Kirk and Szita). The highest k_La of 1440 hour^{-1} in that study is reported for a 48 parallel stirred minibioreactor system published by Puskeiler *et al.* [4]. Klöckner and Büchs focus on important engineering parameters and present basic correlations regarding mass transfer and power input in shaken bioreactor system [3•]. Another study has been published by Betts *et al.* [5]. A 24-well based parallel miniature reactor system μ24 (Pall, Port Washington, USA and Applikon, Delft, The Netherlands, working volume: 3–7 mL) was used to describe the oxygen transfer for different aeration strategies of a fed-batch cultivation of Chinese Hamster Ovary cells. k_La values up to 53 hour^{-1} have been determined. The mass transfer of carbon dioxide in microalgae cultivation was investigated in a 96-well microtiter plate by means of an optical pH indicator [6].

Mass transfer in bioreactors is generally influenced by several parameters. Klöckner and Büchs point out that, among others, the surface properties and the degree of baffling have a strong influence on the oxygen supply into shaken bioreactors [3•]. Baffling in microbioreactors was systematically investigated for shaken 48-well microtiter plates [7]. An empirical correlation was developed to calculate the maximum oxygen transfer capacity OTR_{max} dependent on current shaking parameters. The relative

perimeter of the wells, which reflects the degree of baffling, is used as a geometric key parameter. As a result, an optimum OTR_{max} is found at a relative perimeter of 1.1. To reduce the experimental effort, a mechanistic model is proposed by Klöckner *et al.* for unbaffled cylindrical shaken bioreactors [8]. This model enables the calculation of the volumetric oxygen transfer area and volumetric power input and agrees with computational fluid dynamics (CFD) simulations. Thus, it is suitable to predict the OTR_{max}, depending on the prevailing operating conditions. However, the model is based on the assumption that the vessel diameter is larger than the shaking diameter.

Another parameter which influences the mass transfer is the liquid viscosity. In particular, the liquid film on a vessel wall is influenced by the viscosity. With increasing viscosity, the film thickness and the oxygen transfer increases. Research studies on gas-liquid oxygen transfer in bioreactors are often based on the assumption of water-like viscosities. Giese *et al.* investigated the influence of the viscosity on oxygen transfer in shake flasks [9••]. It was numerically shown that the oxygen concentration in thin liquid films is not always 0 mol/m^3. As a consequence, the driving oxygen concentration gradient, and thus, oxygen transfer is reduced. Additionally, Higbie's film theory is not applicable if the liquid oxygen concentration is >0 mol/m^3 [9••]. The influence of viscosity on the oxygen transfer was also investigated by Wilming *et al.* who identified oxygen limited cultivation conditions in shaker flask experiments at elevated viscosity [10]. If the oxygen supply is not sufficient, the cultivation conditions and the performance of the microorganisms drastically change. Marín-Palacio *et al.* observed a morphological change and an increased product formation of *Streptomyces lividans* in shake flasks cultivation at oxygen limitation [11]. The liquid viscosity is influenced by the effective shear rate. Giese *et al.* developed a shear rate correlation for shake flasks as a function of important shaking parameters (valid for 50–1000 mL shake flasks) [12]. For shake flasks, at least 1.55 times higher effective shear rates compared to stirred tank bioreactors were identified, depending on the broth's flow behavior index. As a consequence, the viscosity in shake flasks might be up to 50% lower compared to stirred tank reactors at the same volumetric power input.

Monitoring and control

Monitoring and control of microbioreactors is a complex task due to spacial limitations, small operating volumes and, thus, possible influences of the measurement itself. Therefore, non-invasive on-line monitoring methods, such as, for example, fluorescence measurements, are often applied. Ge and Rao showed the application of optical disposable luminescent patches to determine pH, dissolved oxygen tension (DOT) and CO_2 in shake flasks [13]. Electrochemical respiration activity measurements are applied in the head space of a shaken bioreactor to determine the oxygen transfer rate OTR of microorganisms. This RAMOS technique is commercially available (HiTec Zang GmbH, Herzogenrath, Germany and Kühner AG, Birsfelden, Switzerland; 250 mL shake flasks) and enables the determination of the respiration activity during cultivation in shake flasks. A new mathematical method is presented to increase the amount of available data during OTR measurements [14]. In recent years, novel sensors and control strategies have been developed. Suarez *et al.* demonstrate that the absorbance measurement in a 96-well microtiter plate is enhanced by inserting a glass fiber filter punch at the plate bottom [15]. A novel ratiometric biosensor based on Förster resonance energy transfer (FRET) was developed by Potzkei *et al.* [16]. This sensor enables oxygen determination in the cytosol of living cells and consists of an oxygen-insensitive flavin mononucleotide (FMN) based fluorescent proteins (FbFP) donor domain and an oxygen sensitive enhanced yellow fluorescent protein (YFP) acceptor domain. Fluorescence measurements, however, might provide wrong results, if other fluorescent proteins are present in the fermentation broth. Kunze *et al.* report about incorrect measuring signals from pH and DOT spots in microtiter plates in the presence of GFP, YFP, FbFP or MCherry proteins. Moderate measurement errors, however, could be compensated by a mathematical correction function [17]. This example illustrates the challenges of microbioreactor control. Gernaey *et al.* published an expert opinion on the development needs in the monitoring and control of microbioreactors [18•]. To control microbioreactors successfully, the authors suggest the introduction of mathematical methods into microbioreactor systems, including software tools for the design of experiments, biostatistics, trend analysis and data evaluation. However, also other technical challenges exist considering microbioreactor control. For example, individual temperature control in shaken high-throughput screening systems is often ambitious and cost-intensive. Commonly, thermostat-controlled incubation chambers are used to adjust one temperature for all reaction vessel. Other solutions, such as thermostated bioreactors, exist as well. Nunes *et al.* presented a 24-well microtiter, which is linked to a thermoregulator device [19]. This microtiter plate was used for enzymatic bioconversion studies and can be placed on any shaking table without any temperature controlled environment. A technically sophisticated device is the micro-Matrix system from Applikon (Delft, Netherlands), which consists of a 24 parallel-operated bioreactor system with individual temperature control and 1–7 mL working volume.

New bioreactor systems

Today, several microscale and miniscale bioreactors, that show capabilities of on-line monitoring, are available. Two relevant stirred minibioreactor systems are commercially available, the bioREACTOR 48 system (2mag,