Microbioreactors with microfluidic control

A. Buchenauer

buchenauer@iwe1.rwth-aachen.de

In this research project microbioreactors based on standard microtiter plates are developed. Microfluidic devices are integrated in order to dispense two different fluids individually into the wells for controlling the pH of the fermentations or for supplying with nutrient solutions. The microfluidic devices are fabricated in polydimethylsiloxane (PDMS). Each microfluidic device can supply four wells from two reservoirs and four devices can be integrated in one microbioreactor. The microbioreactor is used in pH controlled fermentations of *Escherichia coli.* During the fermentations, the pH is measured optically by optodes. In case of variations from the pH set point of 7.2, ammonia solution and phosphoric acid are dispensed. The pH in the controlled culture can be sustained within 7.0 and 7.3 while the pH of an uncontrolled reference culture varies from 6.5 to 9.0. This microbioreactor demonstrates the possibility of pH-controlled fermentations in micro-scale with a user-friendly experimental setup.

The majority of screening and process development in biotechnology is performed in microtiter plates (MTP). But fermentations performed in this environment are often not comparable to industrial processes because of the lack of active pH control or substrate feeding. Other setups dealing with controlled microcultures mostly consist of complex and expensive systems, limiting their application for higher throughputs [1-3]. Recent developments in optical sensor technologies provide the possibility of online-monitoring even in the microscale format of a microtiter plate. The BioLector® technology, first described by Samorski et al. [4] and available from the company m2p-labs (www.m2p-labs.com), is a fiber-optical measurement system for monitoring all relevant culture parameters. Therefore, it is the ideal base for our integration of microfluidic process control into microtiter plates. The individual dispensing of small volumes into each well of a microtiter plate can be carried out by microfluidic devices with integrated valves and pumps. Softlithography is a suitable fabrication technique for the realization of active microfluidic components and has little requirements in laboratory equipment. Polydimethylsiloxane (PDMS), as a material often used in softlithography, fulfils the requirements of good chemical and mechanical durability and optical transparency [5]. Pneumatically actuated microvalves made of PDMS enable small dispensing volumes, short actuation times and high reliability under alternating loads [6-9].

The microbioreactor system consists of the main components microtiter plate (MTP) and microfluidic device. The size and arrangement of the wells are based on standard 48 well MTPs (figure 1 a). The transparent microfluidic device forms the bottom of the wells and is clamped to the MTP by a transparent polymethylmethacrylate (PMMA) plate. The MTP made of PMMA contains four rows with four wells and two reservoirs each. The diameter of the wells is 12 mm, the height is 20 mm. Reaction volumes of 0.2 ml to 1 ml for each well are intended. The reservoirs contain a volume of about 2 ml. The reservoirs are pressurized to transport fluids through the microfluidic channels to the wells.

The microfluidic device is made of polydimethylsiloxane (PDMS). It consists of three layers. The first layer, the fluid layer, carries the fluid channels to

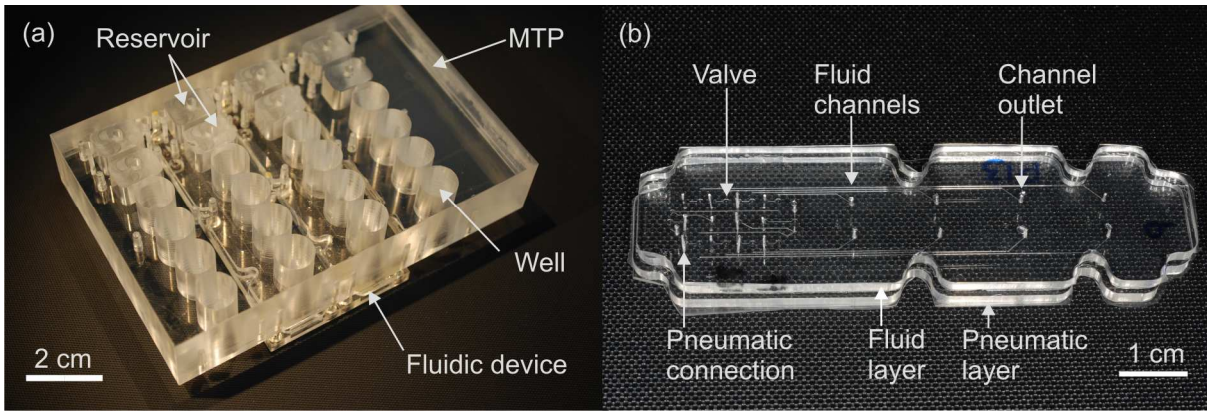

Fig. 1. (a) Microbioreactor made of PMMA with two microfluidic devices. (b) Microfluidic device made of PDMS

guide the acids and bases in two different channel systems. The channels are 100 µm wide and 140 µm deep. Every channel leading to a reaction well is controlled by a valve. The eight valves are placed below the reservoirs in two rows (figure 1 b). The pitch of the valves is 4 mm. In the fluid layer, a valve is defined by a step with a width of 50 µm which interrupts the channel. Detailed descriptions of the microfluidic device and the complete experimental setup are given in [10,11].

The microbioreactor has been used to cultivate *Escherichia coli* in Terrific Broth (TB) medium. Each well was filled with 700 µl cell suspension. During the cultivation, the pH and the scattered light signal are monitored online as described in [4]. The microfluidic device dispenses ammonia solution and phosphoric acid (both 2 mol/l) to control the pH in two wells. The other wells served as references.

The fermentation of an Escherichia coli K12 culture has been monitored for 18 h. The scattered light signal and the pH are shown for a pH uncontrolled well (figure 2) and for a controlled well (figure 3).

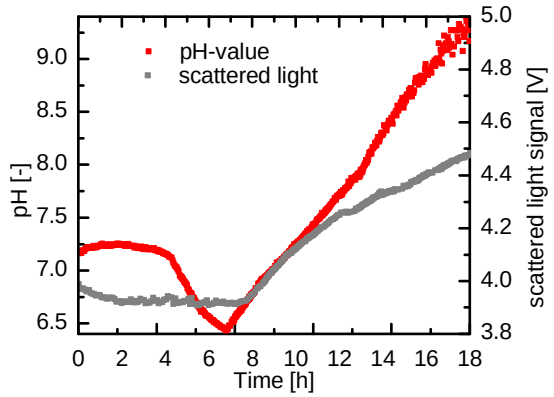

Fig.2. The pH-value und the scattered light signal of a pH uncontrolled culture.

Between 4 h and 7 h the first growth phase of the bacterial culture is visible, reflected by an increase of the scattered light signal of both cultures.

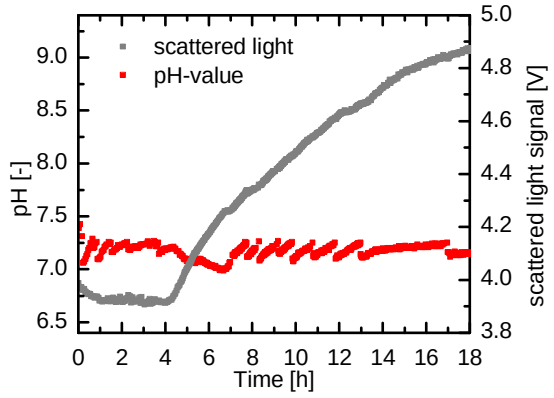

Fig.3. The pH-value und the scattered light signal of a pH controlled culture.

During this time, the pH of the uncontrolled culture decreases significantly to a value of 6.5. The pH of the controlled cultivation is slightly decreasing but stays always above 7.0.

At approximately 7 h an inflection point indicates the start of a second growth phase. In this second phase, the scattered light signal further increases. The pH of the uncontrolled culture increases significantly to a value of 9 until approx 15h, where the end of the bacterial growth in this well is indicated by a stagnation of the scattered light signal. In this second growth phase the pH of the controlled culture is held accurately in-between the dead band of ± 0.05 pH-units around the set point of 7.2.

The results of the fermentation experiment show the ability of the microbioreactor to control the pH of the cultivation. The TB medium was chosen because it results in a decrease as well as an increase of pH. While the pH of uncontrolled cultures varies in the range of 6.5 to 9, the pH in controlled cultures can be sustained within 7 to 7.3 by utilizing a PI-controller. The described measurement setup offers the possibility to perform micro scale pH-controlled experiments in parallel to gain much information with acceptable effort. Further information about the pH controlled fermentations is given in [10,11].

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