Development of a multiplexed microbioreactor system for high-throughput bioprocessing

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A multiplexed microbioreactor system for parallel operation of multiple microbial fermentation is described. The system includes miniature motors for magnetic stirring of the microbioreactors and optics to monitor the fermentation parameters optical density (OD), dissolved oxygen (DO), and pH, *in-situ* and in real time. The microbioreactors are fabricated out of poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS), and have a working volume of 150 μl. Oxygenation of the cells occurs through a thin PDMS membrane at the top of the reactor chamber. Stirring is achieved with a magnetic spin bar in the reactor chamber. Parallel microbial fermentations with *Escherichia coli* are carried out in four stirred microbioreactors and demonstrate the reproducible performance of the multiplexed system. The profiles for OD, DO, and pH compare favourably to fermentations performed in bioreactor systems with multiple bench-scale reactors. Finally, the multiplexed system is used to compare two different reactor designs, demonstrating that the reproducibility of the system permits the quantification of microbioreactor performance.

1. Introduction

Microbial fermentation plays an important role in agriculture, food processing, health care, and environmental management. Advances in chemical, metabolic, and *in vitro* engineering expand the range of existing and potential fermentation products in a large variety of applications.[1–6] The increasing number of genetic and process permutations needed to screen for new products and process optimizations drives a strong demand for obtaining accurate biological data in short time frames. Performing a large number of fermentation experiments in parallel, with bioreactors that offer real-time process parameter data, would help to address this issue. However, this is a challenge with current bioreactor technology.

The high cost and labor required to operate stirred bench-scale reactors limits the ability to perform a large number of fermentations in parallel. Fermentation efforts encompass the assembly of the reactor parts, such as sensor electrodes and fluidic connectors, their sterilization, and disassembly and cleaning. While some fermentation parameters such as dissolved oxygen and pH can be obtained in real time, many other parameters, for example cell biomass, are still largely measured off-line, requiring frequent sampling of the cell culture. This sampling increases the risk of contamination and significantly reduces the working volume during the course of an experiment, altering the process conditions. Finally, bench-scale bioreactors have volumes between 0.5 and 10 l, and thus consume significant, often expensive resources.

Tubes and shake flasks, which account for 90% or more of all cell culture experiments in biotechnology,[7] can easily be operated in parallel and with smaller volumes. However, they are rarely instrumented and growth data are typically obtained as endpoint measurements.

Efforts have been made to overcome these limitations and to accelerate the acquisition of high-throughput fermentation data. Bench-scale bioreactor systems containing several stirred reactors with automated monitoring and control of dissolved oxygen (DO) and pH have been realized. The "Sixfors" (Infors, Switzerland), for example, operates six reactors in parallel, and the "Fedbatch-Pro" (DASGIP AG, Germany) includes up to sixteen culture vessels. However, the necessity of off-line measurements of biomass and individual preparation of each reactor imply that the effort scales approximately with the number of reactors. Moreover, with working volumes of a few hundred milliliters, the consumption of resources remains significant. The recently introduced "Cellstation" (Fluorometrix, MA, USA) accommodates stirred tube-shaped bioreactors with working volumes up to 35 ml. Sequential monitoring includes OD, DO and pH and is performed on a rotary device, currently limiting the maximum number of bioreactors to 12. Finally, microtiter plates offer a larger number of reactor wells combined with greatly reduced working volumes. Sensor integration has been shown for either a DO[8,9] or a pH[10] measurement. However, mixing is generally achieved by shaking the entire plate, which reduces mixing efficiency due to small sample volumes. Furthermore, shaking the whole plate complicates optical and fluidic integration to control the fermentation parameters and medium evaporation.

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Microfabricated bioreactors with integrated sensors can be scaled out to reactor arrays, and are thus better suited to acquire high-throughput fermentation data. Fabricated in polymers, such a platform can be made disposable, thus reducing assembly, cleaning, and sterilization efforts. At the same time, microbioreactors require fewer resources and produce less waste, which is of interest for bioassays in general, and specifically for investigations such as continuous culture assays and studies of expensive enzymes.

The potential to obtain valuable growth kinetics data from bacterial fermentation processes from microbioreactors has been demonstrated previously. Kostov *et al.*[11] realized a stirred microcuvette with a working volume of 2 ml, and the OD, DO and pH profiles compared favorably to a 1 l bioreactor. A multiplexed system was also proposed, but not demonstrated.[12] Lamping *et al.*[13] presented OD and DO data in a stirred bioreactor with a working volume of 6 ml. The miniaturized bioreactor was machined in Plexiglas and the growth data mirrored fermentation data from 15 l bioreactors. Maharbiz *et al.*[14] realized a microbioreactor array based on microtiter plate wells with working volumes of only 250 µl. Electrodes for electrolytic oxygen generation, as well as on-board optics to monitor OD, were incorporated into the array. Cell growth for different oxygenation rates was demonstrated, but did not include comparative data to multiple bench-scale reactor systems. Kim *et al.*[15] presented a microbioreactor chip machined in silicon with sensors for DO, pH and glucose. The microbioreactor chip could in principle be scaled to large reactor arrays, but no growth data were presented. We have previously shown single disposable microbioreactors in poly-(dimethylsiloxane) (PDMS) with working volumes from 5 to 50 µl and demonstrated that the OD, DO and pH data from bacterial fermentations are similar to 500 ml bench-scale reactors.[16] The microbioreactors were used for both off-line HPLC[16] and global gene expression analysis.[17] Moreover, the microbioreactors could be scaled out to arrays using soft lithography techniques.

While single disposable microbioreactors with integrated sensors greatly simplify the effort per experiment, parallel operation of multiple microbioreactors needs to be demonstrated to make the technology viable for high-throughput data acquisition. This requires a platform on which disposable microbioreactors can be set-up rapidly and with which real-time information of fermentation processes can be obtained. In addition, the fermentation data from multiple reactors must be reproducible. In this contribution, we investigate a multiplexed system which can accommodate up to eight instrumented microbioreactors. We use microbioreactors made of poly-(methylmethacrylate) (PMMA) and PDMS with a working volume of 150 µl, which are similar to a previously reported microbioreactor design.[18]

2. Materials and methods

2.1. Multiplexed microbioreactor system

The multiplexed microbioreactor system was built for parallel operation of one to eight microbioreactors, and all the necessary parts were enclosed in an aluminium housing (Fig. 1). The microbioreactors were held on an aluminium platform which rested on support rails mounted to the sidewalls of the housing. Pins in the support rail maintained proper alignment and could be used to quickly exchange one reactor platform for another, thus accommodating different microbioreactor types and configurations. The platform also included openings at the bottom for optical access. A bracket mounted to a slider on a single-axis stage (Unislide, P/N MA2512K2-S2.5, Velmex, NY, USA) held optical fibers (custom-made PMMA fibers, NA = 0.5, RoMack Fiberoptics, Williamsburg,VA) above and below the microbioreactors. The stage was attached to the rear wall of the housing, and the slider motion was controlled with a stepper motor (Type 23T1, Velmex) which permitted an axial step resolution of 5 µm for the optical bracket. Outboard limit switches (P/N 3-8515, Velmex) prevented over-travel of the bracket. The optics bracket scanned over the microbioreactors in stop-and-go sequences executed by computer control algorithms. The process parameters were measured and recorded for each reactor using lifetime fluorescence and absorbance methods. In a typical monitoring sequence, the optical bracket would sequentially read all four microbioreactors and then return to its initial home position, waiting for the start of the next cycle. A total of three bifurcated optical fibers were attached to the bottom of the bracket to monitor DO (PSt3, PreSens, Precision Sensing GmbH, Regensburg, Germany) and pH (HP2A, Precision Sensing) sensors at the bottom of the microbioreactors, and to introduce light for the OD (600 nm) measurement. An optical fiber collected the transmitted light above the reactors. To maximize signal intensity, the z-position of the fibers both above and below the microbioreactors was adjustable. This was performed with cylinders which held the fibers, and were able to slide inside vertical bores machined within the bracket. The positions of the fibers with respect to the cylinders, and the position of the cylinders with respect to the bracket, were fixed with set screws. In the case of the DO and pH sensors, which are based on phase-modulated lifetime fluorimetry, the bifurcation of the fibers served to guide the excitation light from the different LEDs to the sensors with one branch, and to lead the fluorescent emission light from the sensors to photodetectors with the other branch. For the bottom OD_{600} fiber, the bifurcation served to obtain a reference signal, which was used to compensate for intensity fluctuations of the LED. A detailed explanation of the working principle of the OD_{600}, DO and pH monitoring, including the calibration of the DO and pH sensors, is given by Zanzotto *et al.*.[16]

Four magnetic stirrers were attached underneath the reactor platform. The stirrers were comprised of a DC torque mini motor (Faulhaber1212 E 006, Instech Laboratories, Plymouth Meeting, PA) with a gear (Spur gear head 12/3, transmission ratio 9.17:1, Instech) and a permanent magnet (long neodymium iron boron, diameter 6.3 mm, length 6.3 mm, Instech) attached to the rotating shaft. The gear was chosen to permit stirring between 200 and 1500 rpm. Individual motor controllers (MC50, Instech) allowed independent stirring of each reactor. The total height of motor, gear and shaft was approximately 47 mm with a diameter of 10 mm. The motors were held on a separate plate, which could be exchanged to quickly reconfigure the number of stirrers and their distance

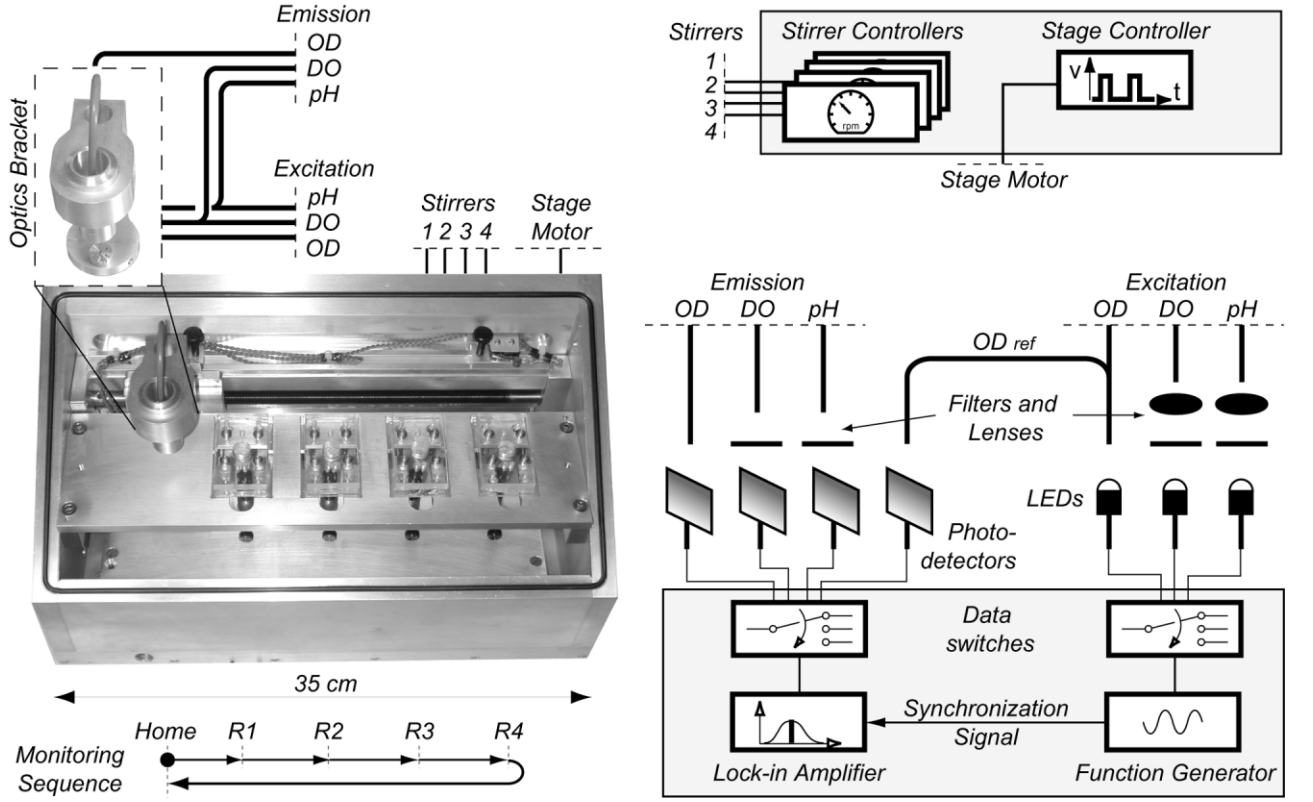

Fig. 1 Photograph of the multiplexed microbioreactor system embedded in a schematic of the instrumentation. The multiplexed system is loaded with four stirred microbioreactors made out of PMMA and PDMS. The optics bracket (detailed photographic view) contains the optical fibers for monitoring of the fermentation parameters OD_{600}, DO, and pH. The fibers connected to LEDs and photodetectors (for purposes of figure clarity, the fibers and wires are drawn as split paths, and the connections are indicated by virtual interfaces with dashed lines and corresponding labels). Signal excitation and emission are controlled with a function generator and a lock-in amplifier, and the signals are multiplexed to the LEDs and photodetectors with data switches. The optics bracket is attached to a slider on a stage and sequentially reads the microbioreactors from reactor R1 to R4, starting from the home position. Stage and stirrer controllers operate the motors for magnetic stirring and stage motion. The shaded boxes indicate the instruments controlled by a LabVIEW program routine.

from each other. The magnetic flux obtained with these permanent magnets was strong enough to perform reliable magnetic stirring from a distance of approximately one inch.

The housing of the multiplexed system was sealed with a top lid (not shown in Fig. 1). Inlet and outlet ports on the side walls of the housing allowed flushing of the platform with the desired gas concentration. The optical and electrical lead-throughs were placed in the rear wall of the housing below the motorized stage. Swagelok tube fittings (SS-6-UT-1-4BT, Swagelok, OH, USA) were mounted to the rear wall, and used to clamp the optical fibers at the fiber break-out (*i.e.* at the location where the fibers bifurcated) to the rear wall. The break-out consisted of an aluminium cylinder with an outer diameter of 9.5 mm, which matched the inner diameter of the tube fitting bore. For the electrical wires of the magnetic motors and the limit switches, provisions in the rear wall were made for circular plastic connectors (Tyco Electronics P/N 1445759-1, Newark InOne, MA, USA). System-wide temperature control was achieved by flowing water from an external heated bath (Thermostat C10-B3, Haake, MA, USA) through the base of the housing.

The multiplexed system was fully automated and controlled under a LabVIEW software routine (National Instruments, Austin, TX). The function generator and the lock-in amplifier were controlled through a GPIB interface card (PCI-GPIB, National Instruments). The data switches (8037, Electro Standards Laboratories, Cranston, RI), which multiplexed the signals for the three LEDs (465 nm with NSPB500S, and 505 nm with NPE590S, Nichia America Corp., MI, USA; 600 nm with L600-10V, Epitek, Kyoto, Japan) and the four photodetectors (PDA-55, Thorlabs, Inc., NJ, USA), as well as the stepper motor controller (VXM-1, Velmex) for the stage, were controlled through RS232 ports. One analog port (Analog output card PCI 6722, National Instruments) was assigned to each of the four motor controllers enabling individual control of the stirring speed. A graphical user interface allowed the setting of experimental parameters, such as the sampling rate, the total run time, as well as all instrument parameters.

2.2. Design and fabrication of the microbioreactors

The first reactor design consisted of two 3 mm thick PMMA layers with two PDMS layers sandwiched in between (Fig. 2a and b). The bottom PMMA layer contained a reactor chamber with a depth of 2 mm and a diameter of 10 mm, yielding a total

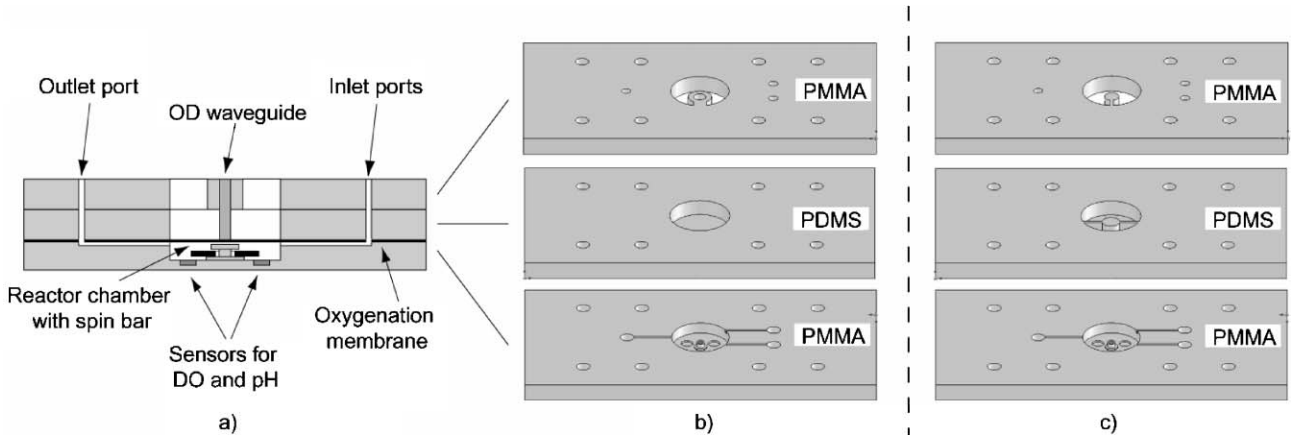

Fig. 2 (a) Schematic of longitudinal section of the microbioreactor with a PMMA fiber as OD waveguide. The cell culture in the microbioreactor is stirred with a magnetic spin bar, which rotates around a vertical post in the center of the reactor chamber. A cap at the top of the post and a shoulder at the bottom holds the spin bar at the desired height within the chamber. Oxygenation takes place through a thin PDMS membrane, indicated in the schematic by a thick line. (b) Solid models of the four layers for the microbioreactor design using a PMMA waveguide. The two bonded PDMS layers are shown as one layer. (c) Solid models of the three layers of the microbioreactor with the PDMS post as OD waveguide. (Solid models drawn to scale)

volume of approximately 150 μl. Adjacent to the reactor chamber, three 500 μm × 500 μm channels were milled into the PMMA. The channels served for culture inoculation, water or nutrient feed, and waste outlet. At the end of each channel, a deeper recess was drilled to facilitate the fluidic connection. For mixing, a magnetic spin bar with a length of 6 mm and a thickness of 0.5 mm (Engineered Concepts, Vestavia Hills, AL, USA) rotated in the center of the reactor chamber. The rotation axis was defined by a free-standing vertical post (height of 800 μm, diameter of 1.35 mm), and the vertical position with respect to the bottom of the chamber was determined by a shallow shoulder (height of 200 μm, diameter of 2.2 mm) machined into the post. To keep the spin bar at the bottom of the reactor, a cap made of PMMA was glued to the top of the post. Two recesses (diameter of 2 mm, depth of 300 μm, 2.7 mm radial distance to the post) in the bottom of the reactor accommodated the pH and DO sensors. The DO sensor foil (PSt3, Precision Sensing GmbH, Regensburg, Germany) was cut with a custom-made punch to the appropriate outer diameter and embedded in the recess by means of silicone grease (high vacuum grease, Dow Corning, Midland, MI). For the pH sensor, approximately 1 μl of liquid pH-sensitive dye (HP2A, Precision Sensing) was dispensed into the drilled recess and allowed to dry.

Oxygenation of the cell culture occurred through a 100 μm PDMS membrane.[16] A 3 mm thick PDMS layer was bonded to the thin PDMS layer, which hermetically sealed the reactor. The thin PDMS membrane was spin-coated on a separate silicon wafer, which had previously been silanized (T2492 KG, United Chemical Technologies, PA, USA) just before the spin-coating process to prevent the sticking of the membrane. To obtain the thickness of approximately 100 μm, 5 ml of liquid PDMS was spun at 1200 rpm for 20 s. For the thick piece, the PDMS was cast on a negative master made of aluminium, and then cured in an oven at 80 °C for 1 h. It included a through-hole of 10 mm diameter directly above the reactor well to permit gas exchange through the PDMS membrane. The thick piece of PDMS was placed on top of the uncured PDMS membrane, and then cured in an oven at 70 °C for 2 h. The last curing step also provided the bonding of the two PDMS layers. Finally, the thin PDMS membrane was cut along the edges of the thicker PDMS piece, and the two-layer PDMS structure was lifted off the wafer.

In the top PMMA layer, an unjacketed PMMA fiber with a diameter of 2 mm (R02-549, Edmund Industrial Optics, NJ, USA) and a length of approximately 6 mm was glued (Weld-on 4, IPS Corp., CA, USA) into a bore machined into an arm that extended into the reactor opening of the top PMMA layer. The fiber had been cut to size by means of an optical fiber cutting block (R54-013, Edmund Industrial Optics) and a hot razor blade. The resulting waveguide was positioned directly on top of the thin PDMS membrane to capture the transmitted OD_{600} light, thereby keeping the optical path-length inside the reactor chamber constant and independent of the bulging of the PDMS membrane. At the same time, this waveguide facilitated the coupling of the OD_{600} light into the optical fiber at the top of the scanning bracket. The top PMMA layer also included openings for the fluidic ports. All layers had bores to accommodate screws with which the layers were clamped together and attached to the reactor platform.

In the second reactor design, the reactor bottom was identical to the first design. The only design differences were related to the OD_{600} measurement, and influenced the design and fabrication of the PDMS layers and the top PMMA layer. For this reactor type, the two PDMS layers were obtained in one compression-molding step over a polycarbonate master, and the PDMS structure included a post, which was directly attached to the PDMS membrane. To avoid bubbles during casting of the PDMS post, the polycarbonate master included grooves to create two ridges, with a width of 0.5 mm, adjacent to the post. The polycarbonate master was measured to give a membrane thickness of 150 μm, and the measured thicknesses of the membrane were approximately 300 μm. For the reactor top, the PMMA arm did not include a bore, yet matched the

location and footprint of the underlying PDMS post (Fig. 2c). Therefore, after traveling through the reactor, the transmitted light of the OD_{600} reading first crossed a PDMS waveguide and a PMMA waveguide before being collected in the top OD fiber of the optics bracket.

2.3. Culture conditions and experimental procedure

Escherichia coli strain FB 21591 (thiC::Tn5 -pKD46, Kan R), a kanamycin resistant derivative of the strain K12, was used in all experiments, and can be obtained from the *E. coli* Genome Project at the University of Wisconsin. Luria–Bertani medium was used and contained: 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract (Difco Laboratories, Becton Dickinson, NJ, USA), and 5 g l^{-1} NaCl. To this was added 8 g l^{-1} glucose and 0.1 M MES buffer (pH 6.9). One hundred micrograms per milliliter of kanamycin (final concentration) was added.

Cell cultures were prepared according to a previously reported protocol[16] to ensure that the bacterial cultures had the same physiological conditions for each experiment. A single colony from an LB agar plate was repeatedly transferred to fresh medium, first from agar plate to tube (37 °C, 60 rpm), then from tube to shake flask (37 °C, 150–200 rpm), and finally from shake flask to Falcon tube, yielding an inoculum with a starting OD_{600} of $\sim$0.05. For each experiment, all microbioreactors received the same inoculum prepared from a single colony.

Before inoculation, the microbioreactors were primed with carbon dioxide to facilitate bubble-free loading.[19] The microbioreactors were then filled with de-ionized and filter-sterilized water with added kanamycin (same final concentration as in the medium). In order to rapidly perform this priming procedure for all four microbioreactors simultaneously, we used an external valve manifold (Five Stopcock Manifold, Harvard Apparatus, MA). The fluidic connection between the manifold and the microbioreactors was made with polyethylene tubing (Intramedic 427410, Becton Dickinson, NJ). On the microbioreactor side, a short steel tube (Gauge 23, Small Parts, FL, USA) was attached to the polyethylene tubing and pierced through the thick PDMS layer of the microbioreactor. Sterile hypodermic needle stubs with luer lock (23G × 3/4in., Precision Glide 305143, Becton Dickinson, NJ, USA) connected the polyethylene tubing to the outlet ports of the manifold. One inlet port of the manifold was connected to a carbon dioxide gas tank, and the other inlet port was connected to a small water container.

The microbioreactors were inoculated through the second inlet port with a sterile syringe equipped with a needle (26G × 3/8in., Precision Glide 305110, Becton Dickinson) and approximately 3 ml of inoculum. The reactors were stirred throughout the inoculation procedure, which took approximately 20 min. At the end of the inoculation, the tubing at the outlet and the sample port was removed, and a tapered silicone rubber plug (9277K41, McMaster-Carr, NJ, USA) was pressed into the top holes of the top PMMA layer to seal the reactors. The tubing at the inlet port of the reactor was not removed and the corresponding manifold valves were set to connect the microbioreactors with the small water container. The container was raised about 200 mm above the level of the reactors. The resulting water feed compensated for the medium evaporation in the microbioreactors, and the water pressure also reduced the bubble formation during fermentation.

After inoculation, the LabVIEW routine for the multiplexed system was initiated. The control algorithm executed the stop-and-go sequences for the optics bracket. Starting from the home position (Fig. 1), the bracket stopped at each reactor, such that the optical fibers from the optics bracket would align with the OD_{600} waveguide from the reactor top layer and the DO and pH sensors in the reactor bottom, and the respective fermentation parameters were measured. The travel speeds in between the measurements and for the travel from and to the home position were 1 mm s^{-1}. One scanning sequence took approximately 7 min to complete. We chose a sampling rate of 10 min. This rate was sufficient to determine growth kinetics of even fast growing bacterial species, such as *Escherichia coli*,[16] which have doubling times of approximately half an hour. The scanning time can be reduced (or increased) by changing the stage speed, and the sampling rate can be adjusted accordingly. At the end of the experiment, the cell cultures from each reactor were harvested and a reference OD_{600} reading was taken with an external spectrophotometer (Spectronic® 20 Genesys™, Spectronic Instruments Inc., NY, USA). This reading was used to scale the OD_{600} data.

3. Results and discussion

We have previously demonstrated fermentation data from microbial processes in single microbioreactors with a working volume of 5 μl, 50 μl and 150 μl, in batch and fed-batch fermentation modes.[16,18] The objectives of this study was to establish a reliable multiplexed microbioreactor system with which reproducible and parallel fermentation experiments can be performed, and thus to demonstrate that high-throughput fermentation data can be obtained in real time from microbioreactors. For our experiments, we used four stirred microbioreactors with a working volume of 150 μl and with integrated sensors for on-line monitoring of the fermentation growth parameters.

3.1. Microbioreactors with a PDMS post as OD waveguide

Fig. 3 summarizes the growth parameters of four parallel *E. coli* fermentations in the microbioreactors with a PDMS post (Fig. 2c). For this experiment, the microbioreactors were stirred at 300 rpm.

After inoculation, the *E. coli* cells start to grow immediately, rapidly entering exponential growth phase. They reach stationary phase within 4 to 5 h. After 6 h, an average OD_{600} of 3.5 with a coefficient of variation (CV, standard deviation divided by the mean) of 6% is obtained (Fig. 3a). Consistent with the strong cell growth at the beginning of the fermentation, the oxygen concentrations in the culture medium rapidly decrease and have completely depleted within 1.5 h. Oxygen starts to recover after 10 h when the cells enter the late-stationary phase and become limited in nutrients (Fig. 3b). The starting pH values are between pH 6.6 and 6.8 and the pH reaches a minimum of 5.3 (CV $<$ 1.5%) after 5.5 h (Fig. 3c).

The time courses of OD_{600}, DO, and pH during growth in each of the four microbioreactors are reproducible. During

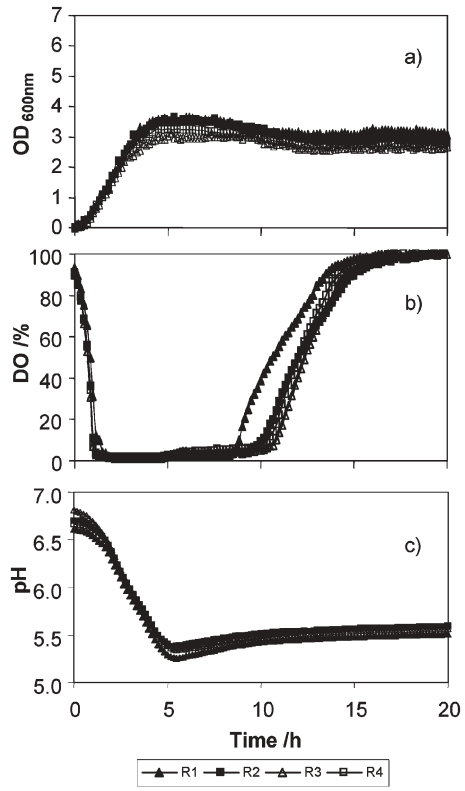

Fig. 3 Parallel fermentations of *E. coli* performed in four microbioreactors equipped with a PDMS post, and real-time measurements of: (a) OD_{600}, (b) DO, and (c) pH.

exponential growth phase, the calculated standard deviations are less or equal than 0.15 for OD_{600}, 12% of oxygen concentration for DO, and 0.1 for pH. The comparatively high number for DO is a result of the steep oxygen depletion curve. In fact, after oxygen is depleted, the calculated standard deviations for DO are less than 2% of oxygen concentration until oxygen recovery starts.

While the reproducibility demonstrates the robustness of both the multiplexed system and the microbioreactors, the final biomass was lower than expected. With an average OD_{600} of 3.5, the biomass from these four microbioreactors is only half that obtained from previous microbioreactor and bench-scale reactor fermentations, which were conducted with the same strain and under the same growth conditions.[16] At the same time, the oxygen depletes faster in this microbioreactor configuration, indicating a severe oxygen limitation, which may explain the lower than expected cell growth. Therefore, oxygenation had to be improved to obtain higher biomass, which led to a different microbioreactor design.

3.2. Microbioreactors with a PMMA fiber as OD waveguide

For this experiment, we used four microbioreactors with a PMMA fiber as an OD waveguide (Fig. 2a and b). In contrast to the PDMS post, which requires PDMS ridges to stabilize its position and to facilitate the fabrication process, the PMMA fiber is held in the top reactor layer. Therefore, this OD waveguide does not compartmentalize the headspace above the reactor chamber, and reduces the obstruction of the oxygenation area to a minimum. Moreover, the spin coating of the membrane provides better control over the thickness than the molds used previously so that thinner membranes can be fabricated. Finally, to further improve oxygenation, the microbioreactors were stirred at 700 rpm.

In the microbioreactors with the PMMA fiber as the OD waveguide, the *E. coli* cells reach the stationary phase after 6 h and achieve an average OD_{600} of 5.6 with a CV better than 6% (Fig. 4a). This represents biomass increase of 60% compared to the microbioreactor with the PDMS post configuration. The increased cell growth is sustained by better oxygenation as evidenced by the delayed oxygen depletion, which occurs over an hour later than in the microbioreactors with the PDMS post. The oxygen concentrations remain low for a much shorter period of time, indicating an earlier depletion of nutrients, hence a more rapid conversion of nutrients into biomass. After approximately 12 h, oxygen recovers to 100% air saturation (Fig. 4b). Explaining this sudden increase in oxygen concentration would require more investigation. However, the aim of this contribution is not to detail the cell biology for this particular strain, rather to present microsystems which allow parallel investigation of cell growth. Moreover, this surge occurs in the late stationary phase, which is of less importance for the strain physiology than the growth phase. The starting pH values are between pH 6.6 and 6.9, and change little in the first hour of the microbial fermentations, most likely as a result of the buffering capacity of the MES (Fig. 4c). The pH then drops significantly for approximately

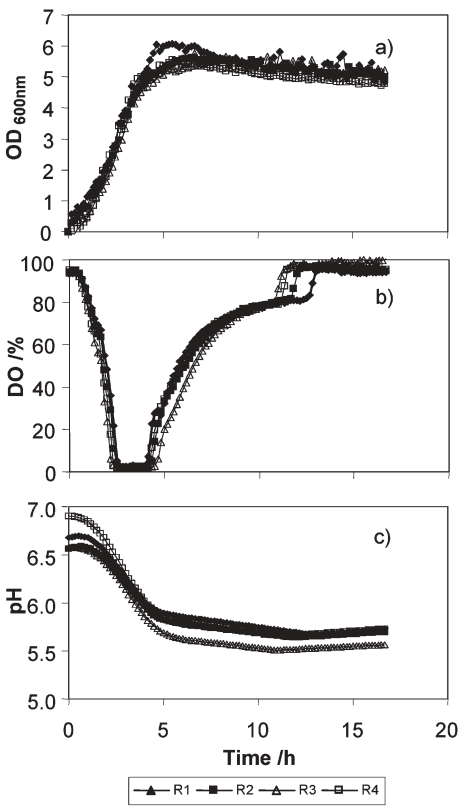

Fig. 4 Parallel fermentations of *E. coli* performed in four microbioreactors equipped with a PMMA waveguide, and real-time measurements of: (a) OD_{600}, (b) DO, and (c) pH.

4 h to below pH 6, and finally reaches a minimum of 5.6 (CV < 1.5%) after approximately 12 h.

The four microbioreactors again show reproducible performance in terms of the measured process parameters. During exponential growth phase, the calculated standard deviations are less than or equal to 0.38 for OD_{600}, 14% of oxygen concentration for DO, and 0.16 for pH. After the oxygen is depleted, the calculated standard deviations for DO are less than 1% of oxygen concentration until oxygen recovery starts.

The fermentation data from the multiplexed microbioreactor system compares favorably with experimental data obtained with a commercial multiple bioreactor systems (Fig. 5). The microbial fermentations in the multiple bench-scale reactor systems were undertaken with the same *E. coli* strain and with the same growth conditions, and yielded similar profiles and reproducibility for OD_{600}, DO and pH. Finally, with a working volume of 150 μl, the reactors provide sufficient biomass to allow for several off-line endpoint investigations, such as HPLC and gene expression analysis.[16,17]

4. Conclusions

We have realized a multiplexed microbioreactor system for the simultaneous operation of up to eight microbioreactors. The system includes miniaturized motors for magnetic stirring of the reactors, and optics for measuring the fermentation parameters. Optical density is determined with a transmittance measurement through the reactor chamber, and *in-situ* measurements of dissolved oxygen and pH are obtained with fluorescence lifetime sensors embedded in the bottom of the reactor chambers. The multiplexed microbioreactor system monitors and records the process parameters in real time for each microbioreactor.

Four microbioreactors fabricated of PMMA and PDMS were used to perform parallel batch culture fermentations with *E. coli*. Fermentation data indicated healthy and expected cell growth behavior with good reproducibility of the OD_{600}, DO, and pH profiles. Moreover, the data compare favorably with microbial fermentations undertaken with the same strain and under the same conditions in multiple bioreactor systems at the bench scale. Robust operation of the multiplexed system was demonstrated with 20-h-long fermentation experiments, during which no user interaction was required.

The multiplexed system can easily be reconfigured to work with different types of microbioreactors and can for example be used to investigate different reactor designs, facilitating rapid design iterations for reactor optimization. We compared two reactor designs with different waveguide structures for the optical density measurement. The quality and reproducibility obtained from parallel fermentations with four microbioreactors of each design demonstrated a distinct difference in the oxygenation pattern and the final biomass level.

The real-time monitoring of parallel fermentation processes provided by the multiplexed system provides information on

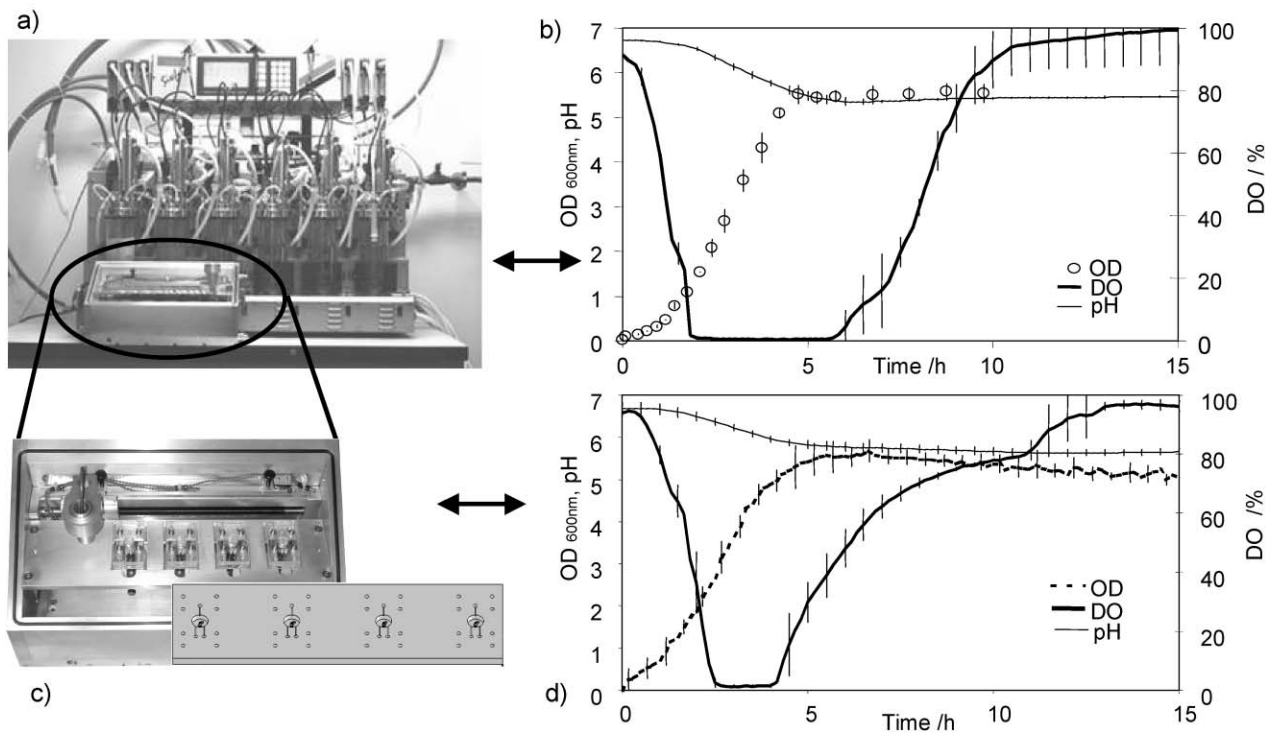

Fig. 5 (a) Photograph showing the multiplexed microbioreactor system in front of the "Sixfors" bioreactor system containing six bench-scale reactors (Infors, Switzerland). (b) Fermentation data obtained with the Sixfors. The markers for the OD graph indicate the uptake of culture medium as opposed to the on-line measurement of the DO and pH, which are represented with lines. The error bars represent the calculated standard deviation from three parallel fermentations. (c) Photograph of the multiplexed system with four stirred microbioreactors and an illustration of an integrated microbioreactor cassette. (d) Fermentation data obtained with the multiplexed microbioreactor system. The error bars represent the calculated standard deviation from the four parallel fermentations shown in Fig. 4.

cell growth physiology within hours, which makes the system a valuable tool for rapid screening of microbial strains and new products, and for process optimizations. Moreover, with a working volume of 150 μl, the microbioreactors offer a significant reduction of resources, while producing enough biomass to perform additional off-line analysis such as HPLC or global gene expression analysis at the end of a fermentation process, which permits the combination of high-throughput growth physiology with metabolic and genomic analysis. This microbioreactor system, therefore, presents a significant departure from multiple shake flask cultures with their limited information per experiment, and is a step towards a high-throughput data acquisition system for bioprocessing application at a miniaturized laboratory scale.

In the future, the four microbioreactors could be integrated into one single cassette of four or more reactors (Fig. 5). With self-sealing fluidic ports, an automated inoculation procedure could be performed with an external pipetting robot, which would further reduce sterilization and preparation efforts. The cassette could include microbioreactors for batch or fed-batch fermentations, and could be mass-fabricated with injection molding techniques. Finally, optical monitoring of additional fermentation parameters, such as carbon dioxide within the medium, or oxygen and carbon dioxide[20] in the reactor headspace, could be integrated into the optics bracket, allowing the closing of the carbon balance of fermentations.

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