Design and Fabrication of A Polymer-based, Instrumented Microbioreactor for High-Throughput Continuous Microbial Cell Cultures

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Abstract

In continuous culture, microbial cells are grown at a steady state where cell biomass production, substrates and products concentrations remain constant. These features make continuous culture a unique and powerful tool for biological and physiological research. However, continuous culture experiments in conventional stirred-tank bioreactors are time- and labor intensive. We present new microbial cell cultivation microsystems for high-throughput screening as well as for bioprocess development.

Different from batch or fed-batch processes, continuous culture requires a dynamic balance of medium feeding and cell growth to avoid “wash-out” or overpopulation of cells in the microbioreactor. Additional challenges for realizing chemostat performance in microsystems include avoiding cell growth on the reactor walls and avoiding chemotaxis of bacteria into microfluidic channels during long growth runs. We present a polymer-based microbioreactor system that addresses theses issues and integrates optical density, pH, and dissolved oxygen optical sensors. *Escherichia coli* cells are continuously cultured in a 150μL, well-mixed microbioreactor by pressure-driven feeding of fresh medium through a microchannel. Steady state cell cultivation in the microbioreactor at different dilution rates is demonstrated.

Microbioreactor Setup

The microbioreactor consistsof four PMMA layers and two PDMS layers (see Figure 1). The 150 μL microbioreactor chamber and three connecting channels are fabricated in three PMMA layers and thermally bonded. A thin layer (100 μm) of spin-coated PDMS cover the reactor chamber and serves as the aeration membrane. This PDMS layer is anchored by a thick PDMS gasket layer to facilitate device assembly and hermetical sealing. A top

PMMA layer is used to provide a rigid support for mechanical assembly. In the reactor chamber, two recesses at the bottom of the bioreactor chamber accommodate pH and DO fluorescence lifetime sensors. A small ring-shape magnetic stir bar provides active mixing. The rotation of the stir bar is defined horizontally by a free-standing vertical post in the center of reactor chamber. DO, pH, and OD are measured by optical methods; bifurcated optical fibers lead into the chamber from both the top and the bottom and are connected to LEDs and photodetectors to perform the optical measurements. Both dissolved oxygen and pH are measured using phase modulation lifetime fluorimetry. OD data are obtained from an absorbance measurement using an orange LED.

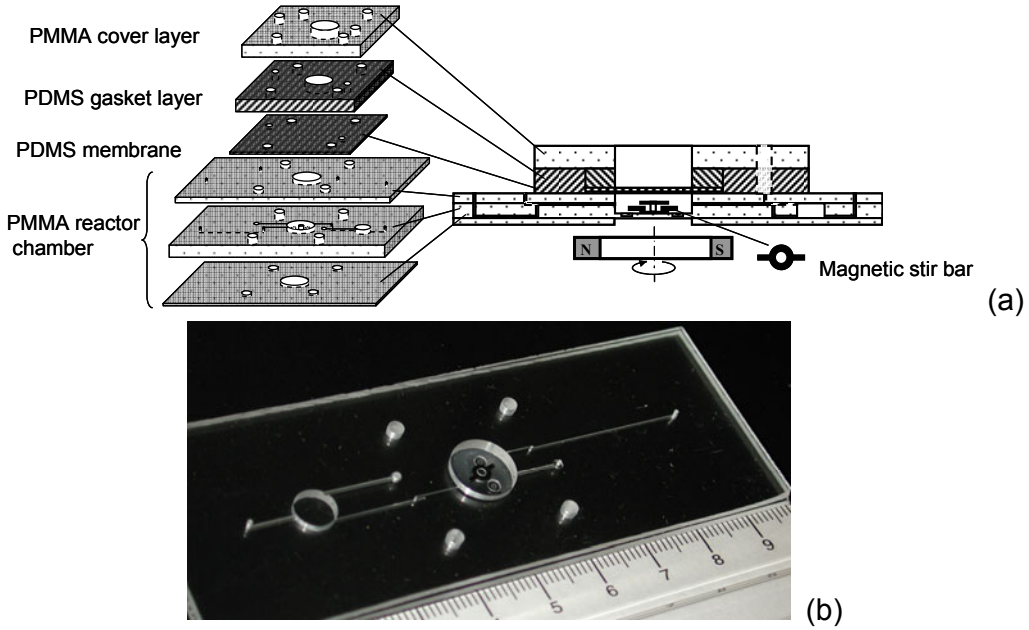

Figure 1: a – Schematic view of the longitudinal section of the microbioreactor;

b –Photograph of the empty PMMA chamber of the reactor (middle layer for reactor chamber)

In the microchemostat liquid continuously flow through the microbioreactor to eliminate potential disturbances in flow rates caused by surface tension effects at small scales. As a consequence, mobile bacteria, e. g. *E. coli* cells, could swim upstream into feeding the channel for food. Typical flow rates in the microchannel range from 0.5 µL/min to 2 µL/min, corresponding to average linear flow rates of 130 ~ 500 µm/s in the microchannel, significantly higher than the average migration speed of *E.coli* cells (20~80 µm/s). As a result, only a very small faction of cells should be able to migrate up the feeding

channel. To further reduce the potential for cells migration, a local thermoelectric heater is used to raise the temperature in the feed line to ~70 ^{o}C and thereby reverse the driving force for chemotaxis. At the exit side of the chemostat, a Peltier thermoelectrical cooler reduces the local temperature of a 40 µL effluent reservoir to 4 ^{o}C to keep cells at low temperature and significantly reduce metabolic activity to facilitate off-line sampling for further analysis.

A PAA-g-(PEG-r-PPG) polymer coating on both PDMS and PMMA surface are developed to reduce cell adhesion. PAA-g-(PEG-r-PPG) graft copolymer is synthesized using an amidation reaction to graft H_2N-(PEG-*r*-PPG)-OCH_3 chains to the carboxylic acid groups on the PAA backbone with a grafting ratio of 50%. The surface modification protocols start with O_2 plasma treatment for PDMS and reduction with $LiAlH_4$ for PMMA to generate surface hydroxyl groups. PDMS and PMMA surfaces are then react with N-(6-aminohexyl)aminopropyltrimethoxysilane (ethanol solution) and then coated by PAA-*g*-(PEG-*r*-PPG) (aqueous solution).

E. coli FB21591 (thiC::Tn5 -pKD46, Kan^R) is used to culture in MOPS minimal medium with 1 g/L glucose as the sole carbon source.

Results and discussion

Figure 2 shows an example of continuous culture experiments with *Escherichia coli*. At the beginning of the reaction, cells from metabolically active inoculation utilize all available oxygen to build up biomass, as a result, DO level drops to zero in hours. The pH level of the culture broth decreases as a result of acidic byproducts accumulation due to anaerobic metabolism, and then recovers when the DO level recovers and fed with fresh medium. At about 60 hours DO, pH, and OD reach stable levels and chemostat conditions are established. The steady conditions balance medium feed rate at 0.5 µL/min, and relative slow cell growth rate characterized by DO level of ~ 95%. After the dilution rate increases to 1 µL/min, the cell growth rate is significantly faster and causes DO to drop to ~ 78%. Stable cell culture at 1.5 µL/min dilution rate corresponds to DO of ~ 56%. In this experiment aerobic metabolism dominates in the steady state conditions at different dilution rates, and no significant pH variation is observed. OD_{600} level stabilized at ~ 1 (~0.45 g dry/L) despite of different dilution rates; this is consistent to bioprocess stoichoimetry observed in conventional bioreactors when glucose is the only carbon source for *E. coli* aerobic cultivation. Cell wall growth in the microbioreactor is also investigated. After 7 days of cell cultivation, PEG surface coating effectively reduces cell adhesion on both PDMS and PMMA surfaces for more than 90% by cell counting.

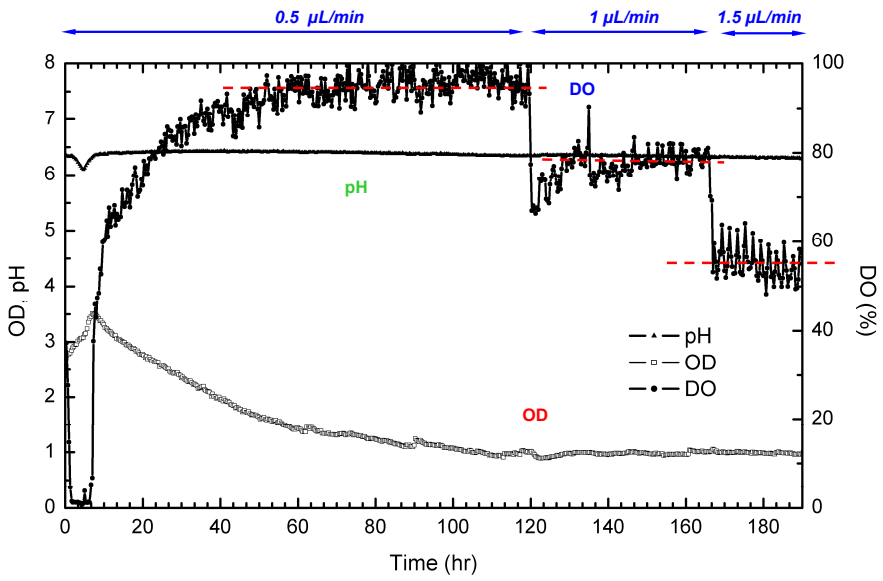

Figure 2, Steady state continuous culture at various dilution rates.