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Cultivation of yeast in diffusion-based microfluidic device

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ABSTRACT

The capacity to create a diffusive chemical concentration gradient in microfluidic systems has the potential to improve the study of microbial processes. These tools allow the evaluation of microbial cell performance under different and controlled conditions. Diffusion-based gradient generators, in particular, have the capacity to maintain spatiotemporally constant gradient concentrations necessary to evaluate cell behavior in a precise environment. This work uses a known microfluidic device capable of generating a diffusive glucose concentration gradient to evaluate for the first time the behavior of *Saccharomyces cerevisiae* ATCC 7754 inside a microchannel. The cell growth along the microfluidic microchambers was observed and the kinetic parameters determined, with values statistically similar to those of conventional batch cultivation. Monod kinetic parameters could also be determined in the microfluidic device using small substrate concentrations. These results show the potential of this microbioreactor to investigate yeast growth with microliter samples and to evaluate experiments in triplicate performed and in parallel. The diffusive concentration gradient in a microfluidic device allowed the acquisition of results in a more practical way when compared to conventional techniques.

1. Introduction

An essential step for different industrial applications, such as biofuel [1,2], enzyme production, pharmaceutical, food and textile industry [3] involves obtaining products from microbial metabolism and/or enzyme activities. In this case, important parameters, such as optimal limiting substrate concentration [4,5], define the efficiency of the bioprocess. On an industrial scale, bioprocess reactions have been operated on bioreactors with volumes that reach thousands of liters. However, ideal conditions for microbial growth and product formation are determined on a laboratory scale [4]. Furthermore, the determination of the ideal bioprocess conditions is normally laborious, requiring the development of screening tools and approaches capable of reducing time and facilitating the study of the fundamental bioprocesses' conditions.

In this context, microfluidics emerges as a promising technology that operates in the micro-scale using low amounts of reagents and samples. Microfluidics has the potential to innovate the way modern biology is conducted [6], allowing the development of new tools and devices that significantly contribute to biological and medical areas [7]. It facilitates the investigation of cell behavior and the study of cellular mechanisms and biochemistry in different dynamic situations [8]. These characteristics have increased interest in the use of microfluidics, more specifically, in the field of concentration gradient generators.

Microfluidic concentration gradient generators can be made by different biocompatible materials and geometries based on the desired research goals and applications [9]. It has been applied mainly to animal cells, to investigate ideal concentrations of molecules of interest [10,11], to evaluate cell growth, cellular differentiation and to perform single-cell analysis [12–15], and to understand the behavior of adherent cells [16].

Concentration gradient generators can be diffusion or convection-based [17]. Convection-based gradient generators use laminar flow mass transport in the microchannels. These generators have previously been used to evaluate microbial growth and viability [18], identify factors affecting enzyme reaction rate [19], and study chemotaxis with different microchannel geometries [20–23]. However, in convective systems a major challenge is the necessity of constructing a microchannel network,

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in order to uniformly distribute the flow and to obtain proper gradient generation [17]. Moreover, these systems have the added drawback of possibly dragging free cells [21].

On the other hand, transport of matter in diffusion-based concentration gradient generators is purely diffusive, with the concentration distributed linearly in the microchannel and without any fluid velocity in the region where the gradient is created [17,24]. These characteristics permit the study of non-adherent cells since they are not dragged along the microchannel [17,21] and allow for optimization of bioprocesses in a single device.

Despite the high potential of diffusion-based gradient generators, in terms of microbial cells, the majority of the scientific literature reports the application of conventional microbioreactors without concentration gradients. These microbioreactors are mostly employed for microbial cultivation, to evaluate biocatalysis reactions [5,25,26] and to monitor the morphology and cell development under certain conditions [27]. Despite the fact that diffusion-based concentration generators show high potential to improve bioprocess development, studies with free cells are yet under explored.

The present work shows that a diffusion-based concentration gradient microfluidic device is an effective strategy to select the ideal growth profiles of free cells, allowing the evaluation of *Saccharomyces cerevisiae* growth in different glucose concentrations. This system was also able to determine the kinetic Monod parameters using low glucose concentrations; which is normally unreliable in batch processes due to consumption of the limiting substrate over time. Therefore, this technique can contribute to the evaluation of yeast growth in different substrate concentrations and is a more practical and fast method than conventional techniques of submerged cultivations.

2. Material and methods

2.1. Microbial cultivation

S. cerevisiae ATCC 7754 was grown in YPD medium (Yeast–Peptone–Dextrose) containing 1% yeast extract; 2% peptone and 2% dextrose; in a pH 5.0 solution in shaking flasks at 30 °C for 24 h. The cell suspension was serially diluted in PBS (phosphate-buffered saline) buffer to obtain an initial cellular concentration of about 50 cell/μL for the microbial growth to avoid any obstruction in the microchannel. The presence of cells in microchambers was confirmed, in one test, by the fluorescent reagent FUN 1 at a concentration of 20 μM using the Live/Dead® Yeast Viability kit (Molecular Probes, Invitrogen).

2.2. Microfluidic device fabrication

The two-level design of the microfluidic device was based on the work of Atencia et al. [16]. Basically, a Y-shape geometry with two independent inlets of culture medium in different limiting substrate concentrations converge to a main channel where the streams flow in parallel on the top level flow (Figs. 1 and 2). The diffusive concentration gradient was generated in the microchannel at the bottom level, where the microbial cell growth occurs. This section of the device was named microchamber (Figs. 1 and 2).

The top and bottom levels of this microfluidic device were made out of glass to avoid gas exchange, with a glass coverslip (Fig. 1a) at the bottom to assure proper working distance for optical microscopy observation, while the top consisted of a common glass slide. The middle part was built with a laminated sheet of PDMS (polydimethylsiloxane) of 0.51 mm thickness (Stockwell Elastomerics, USA), bordering the microchamber walls. The design included three replicates of the microchamber, allowing for operation in parallel. Each microchamber presented a volume of 0.23 μL. The laminated sheet of PDMS also promoted the adhesion between the bottom and top glasses (Fig. 1b).

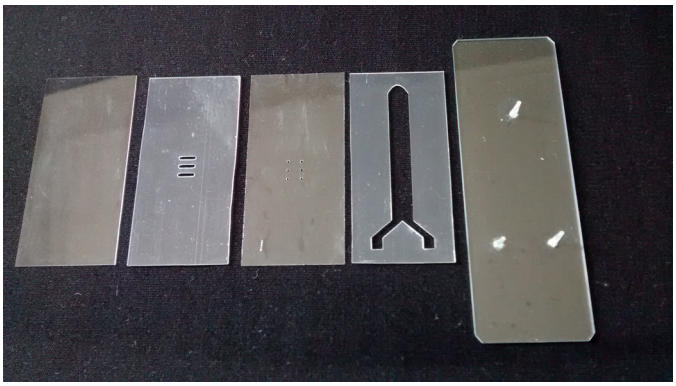

Fig. 1. Components of the microfluidic device. Bottom level: (a) glass coverslip; (b) laminated sheet of PDMS molding the microchambers wall. Top level: (c) glass coverslip with holes (to close microchambers and allow contact with the upper streams); (d) laminated sheet of PDMS in Y-shape, delimiting the upper stream wall and, (e) glass slip with 2 inputs (medium with different limiting substrate concentration) and one output.

The microchannel geometries in the laminated sheet of PDMS were designed using the Corel draw® software and fabricated by a CO_2 laser ablation machine (CO_2 laser of 10.6 μM, power of 35 Watts and wavelength of 630–680 nm, L-Solution 100, Gravograph, USA). Then, the laminated sheet of PDMS was washed with neutral detergent and dried in an oven at ~40 °C. The microchambers were closed with a glass coverslip with three pairs of holes (~0.5 mm diameters) in parallel. The alignment was carried out by manually adjusting the holes (glass coverslip, Fig. 1c) with the microchambers wall (laminated sheet of PDMS, Fig. 1b). These holes were made by a wet etching process, keeping the glass coverslip in contact with hydrofluoric acid (HF) for 20 min under stirring. Therefore, the microchambers have base and top walls composed of glass (hydrophilic) and walls made by a laminated sheet of PDMS (hydrophobic). Considering that most of the surface area of the microchambers is made of glass, the device was considered to primarily exhibit hydrophilic characteristics. The glucose concentration gradient on the bottom level was generated by the parallel flow of two streams with a different glucose concentration on the top level (Y-shaped) (Fig. 1d). The device was closed with a glass slide with two inputs and one output (~1 mm diameters) made with a rotary tool (Dremel®, series 3000). Micropipette tips (10 μL) were used to apply a glass adhesive to connect the tubes bonded at the edges of the (Fig. 1e).

The microfluidic device was sealed by the adhesion of the laminated PDMS and assembled in 4 steps, as shown in Fig. 2. We tested for leakages and gas bubbles inside the microchannel, as well as, the ability of the device to generate the concentration gradient and exhibit flow stability.

The system did not present leakage and the top and bottom of glass prevented gas exchange inside the microchannel.

2.3. Cultivation of S. cerevisiae in microfluidic device

Every microdevice piece was first sanitized with neutral detergent and washed with distilled water followed with 70% ethanol (v/v). Then, they were dried in an oven (40 °C for 40 min) and sterilized by irradiation (UV light for 1 h). The device was assembled in a laminar flow equipment, including the step of inoculation of yeast suspension into the microchamber (with a known cell concentration).