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Ethanol fermentation bioreactor for microfluidic actuation☆

Wolfgang Hilber*, Bernhard Jakoby

Institute for Microelectronics and Microsensors, Johannes Kepler University, Linz, Austria

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ABSTRACT

We present a concept for autonomous, long-term and high-pressure fluid transport on microfluidic chips, which is fully bio-compatible and, in principle, also compostable. The actuation principle is based on ethanol fermentation, a well-known biological process in which microorganisms such as yeast cells convert sugar molecules into cellular energy and thereby produce ethanol and carbon dioxide (CO_2) as metabolic waste products. A two-chamber fluidic system separated by a flexible membrane is proposed for active fluid transport utilizing a bio-reactor. One chamber, connected to the outside via a pressure-sensitive valve, contains the fluid to be actuated, the other one the culture medium for the yeast cells. Once the yeast cells are injected into the culture medium, ethanol fermentation and thus the production of CO_2 starts, which builds up pressure on the membrane and hence also on the fluid chamber. As soon as the switching point of the pressure sensitive valve is reached, fluid transport at a predefined and constant flow rate starts.

1. Introduction

Microfluidics is a highly interdisciplinary research field associated with manipulation and control of small amounts of fluids [1]. Typically active fluid transport on microfluidic chips is realized by the integration of microfluidic pumps [2] which require either the connection to external power supplies or external stimulation e.g. by electromagnetic fields. Common concepts are reciprocating displacement pumps, where movable membranes induce pressure strokes on a confined fluid volume and due to unidirectional valves at the inlet and outlet a net flow evolves in the system [3], or pump structures utilizing the electroosmotic effect, where the spontaneous formation of an electric double layer at the solid to liquid interface and an applied electric field is used to create a plug flow [4]. Concepts for autonomous and controllable fluid transport, particularly suited for mobile and disposable lab-on-a-chip (LOC) applications, are rare. Classically autonomous fluid transport at small scales is achieved by exploiting capillary phenomena which result from the competitive interplay between surface tension of the liquid and geometry and surface chemistry of the solid interfaces in the microchannel. The spontaneous minimization of the free energy between solid, liquid, and gas phase can be utilized to fill microchannels, direct and pump liquids along surfaces [5]. However, capillary systems are not suitable if high pressure fluid transport, possibly even for long periods of operation, is required. Furthermore, capillary forces are based on surface chemistry and modification which is naturally subjected to degradation in time and also during operation. Other actuation mechanisms suited for autonomous and high pressure fluid transport reported so far are based on polymer hydrogels, utilizing, e.g., the inherent swelling behavior in response to water/solvent stimuli [6], or on actuators made of phase change materials [7] and shape memory polymers and alloys [8]. With the exception of polymer hydrogels, which utilize the natural swelling behavior, these actuation concepts require temperature stimulus, either provided by the surrounding environment, which is difficult to control, or by integrated heaters, which makes the system useless for autonomous operation without external power supply. Furthermore, fabrication and integration of these materials into microfluidic structures requires considerable technological effort. In this contribution we investigate the suitability of a biological ethanol fermentation process for long-term autonomous fluid transport in disposable LOC applications [9]. Based on this idea we designed a functional demonstrator, capable of pushing fluid through a microfluidic system at a predefined and constant flow rate, fully bio-compatible and easy to fabricate.

2. Operation principle

Fig. 1 shows a sketch of the suggested fluid actuation concept. It consists of a bioreactor with two chambers separated from each

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* Corresponding author.
E-mail address: wolfgang.hilber@jku.at (W. Hilber).

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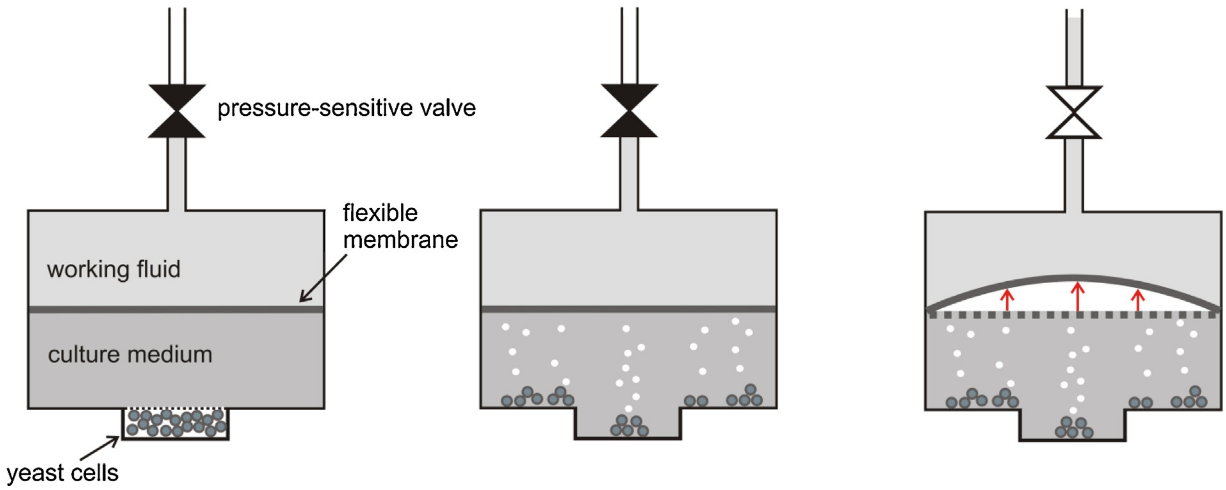

Fig. 1. Fluid actuation concept by ethanol fermentation: in the initial state the yeast cells are separated from the culture medium (left). Once the seal (dotted line) is broken, ethanol fermentation starts producing CO_2 which exerts pressure on a deformable membrane (middle). As soon as the pressure in the reactor exceeds the switching point of the pressure-sensitive valve, fluid is pushed through the outlet at a predefined, constant rate (right).

other by a flexible but gastight membrane. One of the chambers, hereafter referred to as reaction chamber, contains the culture medium for the ethanol fermentation process, the other one the freely selectable working fluid which is connected to an arbitrary microfluidic network via a pressure sensitive valve. Prior to the activation of the bioreactor, the yeast cells are isolated from the culture medium, for instance, by a breakable seal. Once the seal is broken, e.g. by finger pressure, yeast cells are injected into the culture medium and the production of ethanol and the metabolic waste product CO_2 starts. The reaction rate of the fermentation process can be adjusted by choosing the composition of the culture medium and the amount of yeast cells. In simple terms, the more sucrose and yeast cells are in the culture medium, the faster is the process of ethanol and CO_2 production. The increased amount of gas in the reaction chamber increases the pressure in the whole reactor, also in the working fluid chamber separated by the membrane. As soon as the switching point of a pressure-sensitive valve is reached, working fluid is pushed through the outlet at a certain rate.

3. Basic model system

The basic behavior of the device can be studied using a simplified model as shown in Fig. 2, comprising a movable piston in a cylindrical tube. The increasing gas pressure in the reaction chamber

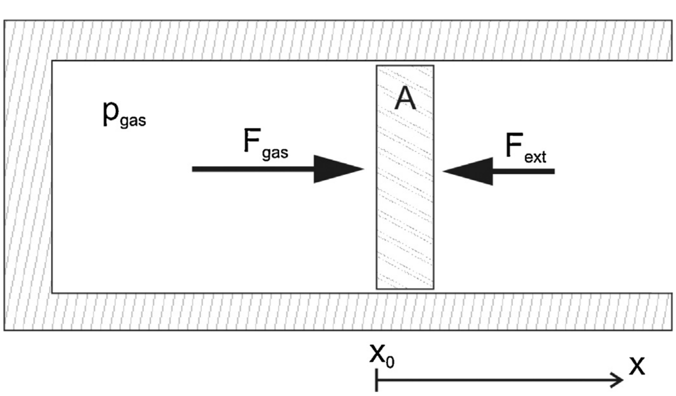

Fig. 2. Model system consisting of a movable piston in a cylinder illustrating the basic functionality of the proposed actuator. The pressure in the reaction chamber due to the ethanol fermentation process pushes a piston (membrane) outward against external forces.

pushes the piston, representing the flexible membrane, outward against external forces, which, in the simplest case, can be ambient pressure or, more generally, the back pressure of a connected microfluidic network. Thus, the model essentially resembles the outstroke of a pneumatic cylinder.

The force due to the gas pressure in the chamber acting on the moveable piston (membrane) can be described by a linear model

$$F_{gas} = F_{gas,0} - k \cdot \Delta V, \tag{1}$$

where $F_{gas,0}$ denotes the force on the piston (membrane) generated by the gas if the piston were fixed at the reference position x_0 (corresponding to no membrane deformation), k the effective spring constant representing the viscoelastic properties of the flexible membrane and the compressibility of the working gas, and $\Delta V = \Delta x \cdot A$ the volume increase due to piston displacement (or membrane deformation in the real system). Dividing by the surface area A of the piston (membrane) (1) can be written in terms of pressure as

$$p_{gas} = p_{gas,0} - k \cdot \Delta x, \tag{2}$$

A particular state of fermentation corresponds to specific values of the model parameters $p_{gas,0}$ and k. Two the extreme cases of (2) can be considered: (i) maximum pressure/force with zero deformation and (ii) maximum deformation, i.e. by letting the system expand until the pressure balances the ambient pressure. For stationary conditions of the reactor, the model parameters could by determined experimentally from these two cases. However, we note that in reality the forces and pressures due to the membrane and the compressibility of the gas will be a non-linear function of the volume or the membrane displacement. As the experimental results below indicate, the influence of the gas compressibility on k is fairly small, though. Moreover, since we only use a very small fraction of the bioreactor's gas production capability for our demonstration device, the observed membrane deformations can be expected to still yield restoring forces in the elastic range, and thus the linear approximations in (1) and (2) are feasible to obtain an estimate for the effective spring constant of the model system.

4. The yeast cell bioreactor

4.1. Ethanol fermentation process

Ethanol fermentation, also referred to as alcoholic fermentation, denotes a biological process induced by microorganisms in