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A microfluidic concentrator for cyanobacteria harvesting

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ABSTRACT

The dilute conditions under which microalgae suspensions are cultured have negatively impacted economical routes for cell harvesting using conventional approaches. A promising alternative has arisen, for which a microfluidic concentration approach has been developed for harvesting Cyanobacterium *Synechocystis* sp. PCC 6803. This method uses passive hydrodynamic forces that include inertial focusing and Dean flow, and has a number of advantages over existing methods for concentrating dilute suspensions of microalgae; the method is truly passive, and does not require additional reagents, or external fields or electronics. After injecting a cyanobacteria suspension into an optimized microchannel configuration at a pre-determined flow rate, intrinsic inertial forces generated from the structure and geometry of the microfluidic network move the cyanobacteria laterally toward a known equilibrium position in the channel cross-section. Once focused, the cyanobacteria stream can be readily separated from culture medium to obtain a concentrated product. The system design and materials used in the new device are chosen with an eye toward low-cost, large-scale commercial manufacture. The operating parameters for inertial focusing of the cyanobacteria suspension have been optimized to achieve high-throughput and high-efficiency harvesting. The maximum recovery efficiency achieved in a single microchannel device is 98.4 ± 0.2% (mean ± standard deviation). For those conditions, initial results yield a concentration factor of 3.28 for a single pass, which is 98.5% of the maximum possible value for the current design. In addition, the calculated power consumption is less than or equal to that of tradition harvesting methods for a wide range of concentration factors. It is anticipated that this highly parallelizable, robust harvesting approach will prove to be economically feasible for concentrating microalgae/cyanobacteria at commercial scales.

1. Introduction

Although there is great opportunity and potential advantages for microalgae/cyanobacteria to replace significant quantities of petroleum-based fuels, these biofuels are not yet economically feasible at commercial scales. There remain a number of technical challenges to address in order for microalgae-based biofuels to become commercially viable [1]. These challenges include the production of sufficient algal biomass in bioreactors [2–3], metabolic engineering of new strains of that are sufficiently robust and productive [4–5], creation of a low-cost method to harvest the microalgae/cyanobacteria [1,6–8], and development of a life-cycle assessment tool to evaluate the sustainability, economics, and scalability of the entire production process [9]. Among the different processing steps, cell harvesting is a critical economic component, because it can account for 20 to 30% of the total cost [1,7,8]. The primary challenge for harvesting is the need to concentrate a suspension of dilute, micron-sized cells from 0.5 g/L (0.05 wt%) to 200 g/L (20 wt%), after which lipid extraction may be carried out with relatively high efficiency [10,11]. Many harvesting methods have been developed, such as gravity sedimentation, filtration and centrifugation. However, the cost of the traditional harvesting methods still failed to be economically feasible. The costs for the dewatering technologies include both capital and operational costs (e.g., power consumption, maintenance). The base case capital costs of settling ponds, membranes, and centrifuges discussed in a National Renewable Energy Laboratory (NREL) report are \$5.80/(m^3/day), \$178/(m^3/day) and \$478/(m^3/day), respectively [1]. Thus, viable harvesting methods that are economically feasible and scalable for commercial use still need to be identified. Recently, there are a number of new or improved technologies under development, such as electrocoagulation [11], magnetic separation [7], and ultrasonic separation [1]. The investigation of emerging technologies may yield a harvesting strategy that meets needed efficiencies, costs, and longevity.

Microfluidic platforms provide a set of fluidic unit operations that enable the miniaturization, integration, and parallelization for specific applications. In contrast to isolated application-specific solutions, microfluidic platforms are designed for ready integration within a well-defined fabrication technology, allowing easy, fast, and cost-efficient implementation for a wide variety of different applications

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[12–14]. In addition, microfluidics technology is readily scalable to high volume, high throughput processes due to its intrinsic properties of miniaturization and parallelization [15–18]. An important characteristic of these platforms is that validation of a single microfluidic device's working principles and subsequent optimization of its operating conditions is sufficient to demonstrate intrinsic scalability of the platform via a multilayer, parallelized design.

Inertial focusing in microfluidic devices is an emerging and robust approach to concentrate and isolate particles from a liquid medium based on size. The general approach capitalizes on microscale hydrodynamic physics resulting from flow through microchannels [19–22]. Due to the advantages of low device fabrication and operation costs, and throughput scalable to macroscale system requirements, inertial focusing microfluidics has been demonstrated as a promising approach for the continuous concentration of mammalian cells, and has replaced the need for centrifugation [23]. However, in contrast with most of the devices designed for operation with mammalian cells, which typically have a length scale greater than 10 μm, concentrating cyanobacteria-sized bio-particles (~2 μm in size for Cyanobacterium *Synechocystis* sp. PCC 6803) remains a challenge using inertial microfluidics. Our previous work has demonstrated that inertial focusing could be applied to efficiently focus and isolate polystyrene microspheres with a size comparable to Cyanobacteria, in devices where substantial changes were made in microchannel design and fabrication [19–22].

In this study, a unique approach is presented that integrates microfluidics and cyanobacteria concentration, and which has significant potential for process throughput scale up that is required to meet large-scale biofuel production needs. The critical feature of this technology is its intrinsic scalability. Although the focus of this study is proof of principle, fabrication of a massively parallel microchannel network for processing large volumes, with multiple sequential stages for increased concentration factors, is anticipated to be a straightforward process. In this study, to demonstrate and quantify the new approach, the mechanism for concentration of 2 μm cyanobacteria is presented. Then, the specific design for harvesting cyanobacteria is described in detail and the fabrication steps for this polymeric prototype are outlined. High-speed camera images of the concentration and cell isolation process have been recorded, and were subsequently used to analyze cyanobacteria distribution patterns across the microchannel width. The associated effects of operating parameters such as flow rate and inlet suspension concentration were characterized based on the data from the video; from that data the harvest efficiency was evaluated using the measured concentration factor and recovery efficiency. Since the cost of algal harvesting is a major factor in the overall economics of large-scale operation, device fabrication cost and energy consumption (pumping cost) have been calculated and presented in this article.

2. Material and methods

2.1. Device fabrication

The inertial focusing microfluidic device was fabricated using soft lithography [24] with thermoset polyester (TPE). First, the channel pattern was designed using AutoCAD (AutoCAD 2014, AutoDesk, Inc). Following that step the CAD design was printed at 20,000 dots per inch (dpi) onto a photomask. Then, a mold was fabricated in a single step process under a UV lamp (OmniCure S2000, Lumen Dynamics Group Inc., Ontario, Canada).

Before fabricating the microfluidic device, the mold was exposed to hexamethyldisilazane (HMDS) (Sigma-Aldrich, MO, USA) for 4 h at 60 °C. A well-mixed and degassed resin (TAP Clear-Lite Casting Resin, CA, USA) with MEPK catalyst (TAP plastics, CA, USA) (resin: catalyst = 10:1 w/w) was poured on the mold and surrounded by a piece of PDMS, which confined the mix within the mold. A piece of transparency film (3M Scotchpak 9744, MN, USA) was used as a top cover over the mix to ensure a flat surface. Then, the mold with resin was baked for 8 min at 60 °C, after which the TPE replica was peeled from the master. A biopsy punch (Technical Innovations, FL, Inc. USA) was used to create 1.5 mm diameter inlet and outlet ports.

To generate an enclosed channel, the TPE replica and a piece of glass substrate (or a piece of coverslip for high-speed camera imaging) are placed in a plasma chamber and pumped down to 200.3 mTorr, followed by purging with O_2 gas for 20 s. Then the pieces are exposed to oxygen plasma (Plasma Etch, NV, USA) for 1 min. After removal from the chamber, the TPE piece is brought into contact with the glass, and left to cure in a 60 °C oven for 5 min. To achieve pressure-driven liquid flow of the suspensions in these TPE-glass hybrid devices, tubing connectors (Nanoport, WA, USA) are attached to the ports on the chip using room temperature cured epoxy.

2.2. Cyanobacteria source and cultivation

Cyanobacterium *Synechocystis* sp. PCC 6803 was grown in liquid BG-11 medium [25]. The strain was inoculated at an initial concentration of 10^6 mL^{-1} and cultured in a 250 mL Erlenmeyer flask with 50 mL culture medium in an INNOVA 44 Incubator Shaker (New Brunswick Scientific, NJ, USA) at 30 °C and a speed of 225 rpm, and an average light intensity was $100 \pm 9\,\mu mol \cdot m^{-2} \cdot s^{-1}$. These 50 mL suspensions were cultured to a concentration between 2×10^8 and 5×10^8 mL^{-1} for future use, at which time the culture was diluted or concentrated to the desired cell density for the inertial focusing tests.

2.3. Experimental setup and method

The inlet of the device was connected to one syringe using Tygon tubing. The cyanobacteria culture in a syringe with known concentration (2.4×10^7 mL^{-1}; 2.4×10^8 mL^{-1} or 2.4×10^9 mL^{-1}) was delivered into the inertial focusing microfluidic chip at specified flow rates using a syringe pump (New Era Pump Systems, NY, USA) to generate stable and continuous volumetric flow. The inlet concentration of the cyanobacteria suspension was measured with a hemocytometer (Hausser Scientific Partnership, PA, USA) three times.

2.4. High-speed Imaging and data analysis

The TPE-glass hybrid device was mounted onto the stage of an Olympus IX 71 Inverted optical Microscope (Olympus, Japan), and connected to a high-speed camera (Fastcam SA3, Photron, USA). Using the high-speed camera the cyanobacteria concentration process in the isolation region of the chip was recorded at 2000 frames per second (495 μs interval) with a 5 μs shutter speed. After images were acquired and stored, analysis conducted using ImageJ (http://rsb.info.nih.gov/ij/) counted the number of cyanobacteria in each frame and recorded the horizontal *xy* location of each cell. Performance of this chip for cyanobacteria harvesting was assessed using the measured recovery efficiency and concentration factor.

3. Theory

The concept of inertial migration has been introduced in this discussion to provide a qualitative background for the physics of system operation, and to indicate how modifications may be made to existing design to facilitate focusing of micron-sized cyanobacteria. The first studies of inertial migration involved macro-scale systems in the laminar flow regime, and were demonstrated by Segré and Silberberg [26] in 1961. Their experiments in tubes of circular cross section showed that rigid, 1 mm diameter spherical particles migrated to an annular region located approximately six-tenths of the distance from the axis to the wall in a 1 cm diameter cylinder. Di Carlo et al. first carried out studies to accomplish inertial focusing of smaller particles in a microfluidics format under laminar flow conditions [27]. Those results demonstrated the potential for concentrating micron-sized particles/bio-particles in a microfluidic chip without externally applied forces or fields.

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