

MICROFLUIDICS FOR CONTROL IN SYNTHETIC BIOLOGY

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ABSTRACT

We report the microfluidic control of a synthetic biological system using a one milliliter volume microbioreactor. Precise fluid injection and mixing enable control schemes such as rapid medium introduction in continuous culture conditions. A *Saccharomyces cerevisiae* strain with a dual inducible fluorescent reporter system was tested with the microbioreactor cultivation for precise induction control. Flow cytometry data suggests that both higher gene expression levels and tighter population distributions were readily achievable with the microbioreactor compared to test tube induction.

KEYWORDS: Microbioreactor, Synthetic biology, Continuous cell culture platform

INTRODUCTION

Microfluidic systems with integrated analysis and control of environmental parameters can enable precise control of metabolic activity and protein expression. Here, we couple *microfluidic controls* in a polycarbonate-PDMS microbioreactor [1] (Figure 1 (a)) with closed-loop control of temperature, pH, dissolved oxygen, and cell density [2] to the *biochemical control* enabled by synthetic biology [3]. *In situ* measurements of optical density (OD) were used to calibrate flow rates and peristaltic mixing times in the microbioreactor growth chamber. Long-term culture requires precise injection and extraction of fluid from the 1 mL growth chamber. In this case, 831.3 nL injections were used for fluid addition/extraction via peristaltic metering valves [2]. Such high precision and control fidelity enable the development of accurate numeric models as well as control strategies for the microfluidic system. As an example, a control algorithm was used to dynamically alter the concentration of the injected glucose/galactose to reduce the required time to reach target concentration to ~ 1 hour, achieving control of the chemical environment faster than the doubling time in continuous culture conditions. Figure 1 (b) shows the experimental verification of this control scheme with a dye solution using the online OD sensor, which measures the *in situ* light scattering/absorption through 1 mm path length at the optical port in Figure 1 (a).

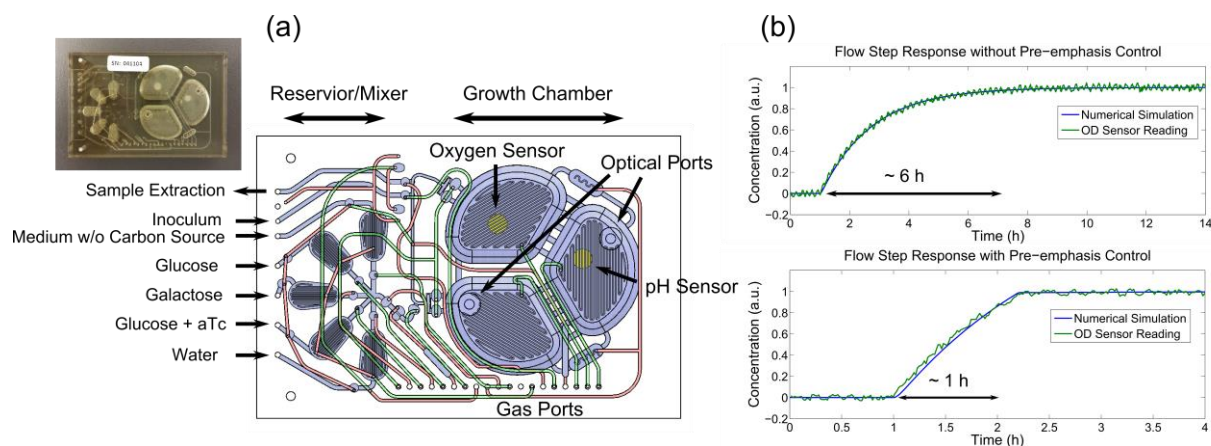


Figure 1: (a) A schematic diagram showing the plastic-PDMS culture chip configuration. The actual device photo is shown on the top left side of the figure. (b) Flow calibration experiment with online optical sensor showing a pre-emphasis microfluidic injection control for rapid medium introduction under constant flow rate conditions typical for yeast cell culture. The input medium had two times higher concentration than the target concentration in the pre-emphasis control experiment.

EXPERIMENTAL

A *Saccharomyces cerevisiae* strain with dual inducible fluorescent reporter proteins was constructed for this study. The construct allows for induction of either red fluorescent protein (RFP) or green fluorescent protein (GFP) depending on the carbon source used for growth and whether a small molecule inducer is provided. As illustrated in Figure 2, expression of RFP is induced in the presence of galactose but repressed in glucose, while GFP is expressed in the presence of both glucose and anhydrotetracycline (aTc, a small molecule inducer). The ability to independently control expression of either protein arises because RFP can *only* be expressed using galactose, while although GFP can be expressed using *either* galactose or glucose, aTc is also required. In order to test this independent control of gene expression, a continuous turbidostat culture (where the microbioreactor automatically adjusts the flow rate to maintain a constant cell density in log phase) was used with alternating glucose (without aTc) or galactose carbon sources (Figure 3 (a)). Samples were extracted from the microbioreactor after steady-state was reached under glucose (at 24 hours), after the carbon source was switched from glucose to galactose with dynamic control of injected concentrations (at 48 hours), and after the carbon source was switched back to glucose (at 72 hours). Induction of GFP expression with glucose + aTc was performed in the turbidostat separately under similar environmental control. The flow cytometry histograms in Figure 3 (b) indicate the fluorescence protein distributions for samples taken from the microbioreactor and from comparison experiments with test tube cultivation of the same strain with 24 hours as the induction period.

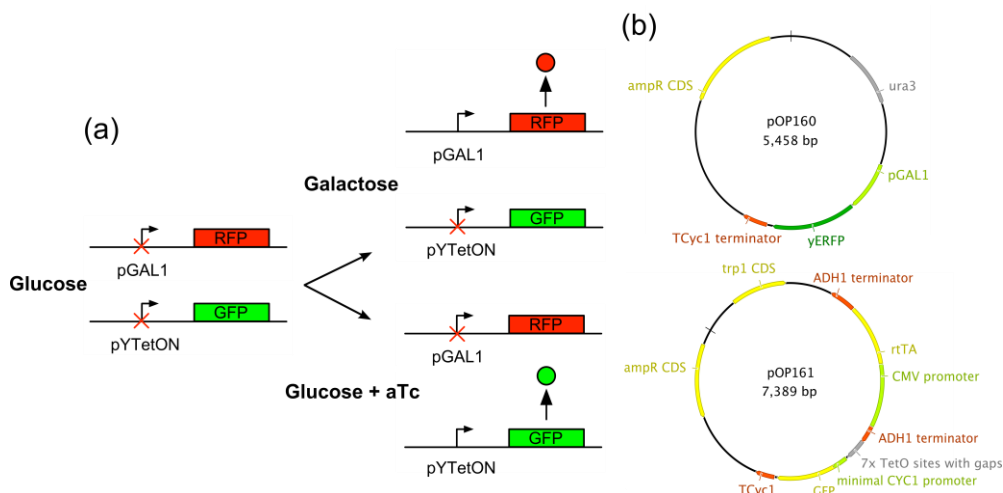


Figure 2: (a) A schematic diagram showing the two-input and two-output synthetic biology system used in this study. pGAL1 is a galactose inducible promoter. pYTetON is a glucose + aTc inducible promoter. RFP and GFP are the reporter proteins for these two promoters respectively. The pYTetON system comprises the CMV promoter driving rtTA, the minimal CYC1 promoter with 7x TetO binding sites upstream, and the ADH1 terminator between the CMV promoter and the 7x TetO sites. (b) Plasmid maps for the galactose inducible system, pOP160 (top) and the glucose + aTc inducible system, pOP161 (bottom).

RESULTS AND DISCUSSION

As can be seen from Figure 3 (a), sequential and reversible on-and-off switching can be achieved during the microfluidic galactose induction experiment. Examination of the flow rate (which is equal to the cell growth rate under constant OD control) indicates a clear lag phase when switching from a glucose to galactose feed. The complete induction cycle also demonstrates the effectiveness of the medium switch-over with microfluidic control. A closer comparison with the test tube induction data in Figure 3 (b) suggests that fold induction with the synthetic circuits in the microbioreactor was much higher compared to test tube cultivation, with over 8-fold (from ~50x to ~410x) and over 3-fold (from ~50x to ~160x) greater induction increases for the galactose and glucose + aTc inducible systems, respectively. Furthermore, the data also indicates that the protein expression throughout cell populations was more uniform and tightly distributed grown in the microbioreactor compared to test tube cultivation. The normalized standard devi-

ation, which is defined as the ratio of the standard deviation over the mean, of cell fluorescence intensity decreased from 0.98 to 0.47 for galactose induction and decreased from 0.87 to 0.37 for glucose + aTc induction. The improved homogeneity of the cellular distribution is likely due to well-mixed cultivation conditions and the constant supply of fresh nutrients. It also demonstrates the importance of environmental control for the reduction of population-level noise in synthetic gene circuits.

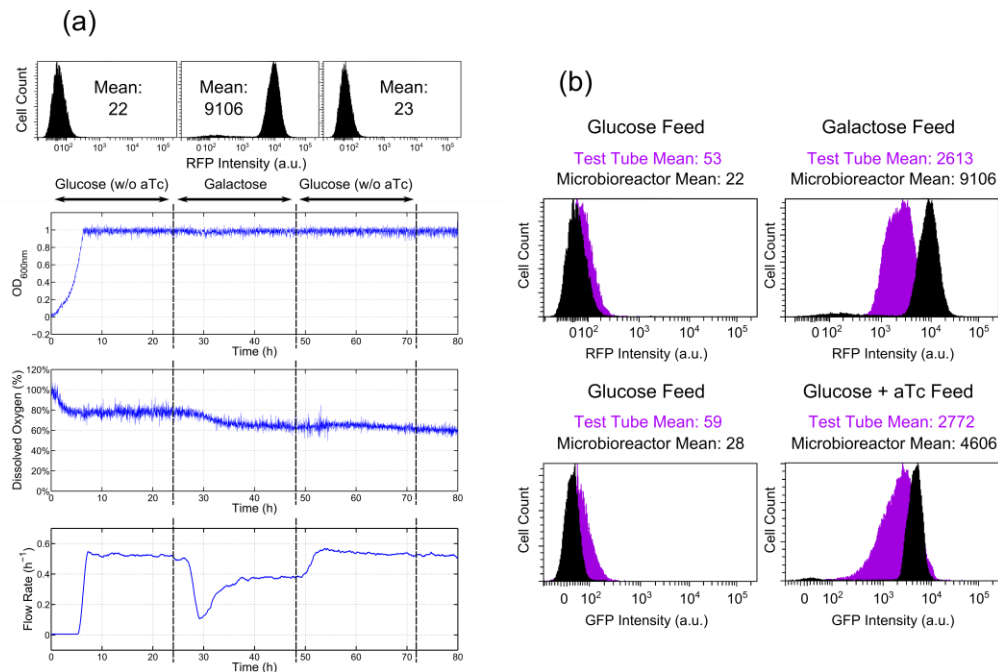


Figure 3: (a) The OD, dissolved oxygen, and flow rate (equivalently growth rate under the turbidostat) plot for the microbio reactor cultivation. The temperature was controlled within 30 ± 0.5 °C and the pH was self-maintained within 6.7 ± 0.2 throughout the entire cultivation period. The pre-emphasis injection control shown in Figure 2 was used for rapid medium switching. The flow cytometry histograms showing the RFP induction level at steady-state (24, 48, and 72 hours, before medium switching) are plotted alongside the OD plot. (b) Flow cytometry histograms showing the comparison of fluorescence induction levels between microbio reactor and test tube cultivations.

CONCLUSION

We have demonstrated flexible and tight microfluidic control over an artificial gene circuit. With improved and better characterized performance of synthetic circuits under controlled cultivation, our device creates new avenues for controlling, characterizing, and standardizing synthetic biology parts and devices.

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