Integrated microbioreactor for culture and analysis of bacteria, algae and yeast

Sam H. Au · Steve C. C. Shih · Aaron R. Wheeler

Published online: 14 September 2010 © Springer Science+Business Media, LLC 2010

Abstract We introduce a micro-scale bioreactor for automated culture and density analysis of microorganisms. The microbioreactor is powered by digital microfluidics (DMF) and because it is used with bacteria, algae and yeast, we call it the BAY microbioreactor. Previous miniaturized bioreactors have relied on microchannels which often require valves, mixers and complex optical systems. In contrast, the BAY microbioreactor is capable of culturing microorganisms in distinct droplets on a format compatible with conventional bench-top analyzers without the use of valves, mixers or pumps. Bacteria, algae and yeast were grown for up to 5 days with automated semi-continuous mixing and temperature control. Cell densities were determined by measuring absorbances through transparent regions of the devices, and growth profiles were shown to be comparable to those generated in conventional, macro-scale systems. Cell growth and density measurements were integrated in the microbioreactor with a fluorescent viability assay and

Sam H. Au and Steve C. C. Shih contributed equally

S. H. Au · S. C. C. Shih · A. R. Wheeler
Institute for Biomaterials and Biomedical Engineering, University of Toronto,
164 College St.,
Toronto, ON M5S 3G9, Canada

S. H. Au · S. C. C. Shih · A. R. Wheeler Donnelly Centre for Cellular and Biomolecular Research, 160 College St., Toronto, ON M5S 3E1, Canada

A. R. Wheeler (⊠)
Department of Chemistry, University of Toronto,
80 St George St.,
Toronto, ON M5S 3H6, Canada
e-mail: aaron.wheeler@utoronto.ca

transformation of bacteria with a fluorescent reporter gene. These results suggest that DMF may be a useful new tool in automated culture and analysis of microorganisms for a wide range of applications.

Keywords Digital microfluidics · Microorganism · Bacteria · Algae · Yeast · Cell culture · Microbioreactor · Absorbance · Optical density

1 Introduction

Microorganisms such as bacteria, algae, and yeast are important for a wide range of applications. For example, bacteria and yeast are used extensively for protein production (Cereghino and Cregg 1999; Demain and Adrio 2008; Swartz 2001) and genomic studies (Koonin and Galperin 1997; Piškur and Langkjær 2004), and algae is a potential source of biofuel production (Chisti 2008; Yu et al. 2009). These types of cells are cultured in specialized growth media, often accompanied by active mixing and temperature control, and algae cultures have an added requirement of light as an energy source. A common method for monitoring growth profiles is to measure the absorbance of the culture at a specific wavelength. As biomass accumulates, the absorbance increases in a manner that is predictable and correlated with the density of cells in suspension.

In commercial applications, microorganisms are often grown in bioreactors with volumes up to thousands of liters, but prior to large-scale culture, smaller systems (for example, microwell plates bearing hundreds of microliters) are used to screen for optimum conditions for growth and analyte production (Humphrey 2008). There is great interest in developing miniaturized culture systems to further reduce the costs of consumables,

increase throughput and reduce manual labour requirements. Most such efforts have relied on enclosed networks of microchannels; for example, microfluidic devices have been developed to grow bacteria and yeast with integrated sensors (Walther et al. 2000; Zhang et al. 2006) and/or the ability to precisely control media delivery rates (Balagadde et al. 2005; Groisman et al. 2005). Moreover, there is great potential for integrating channel-based microorganism culture systems with other operations such as dielectrophoretic sorting (Lapizco-Encinas et al. 2004; Vahey and Voldman 2008) or even single-cell analysis (Peng and Li 2004; Ryley and Pereira-Smith 2006). However, a disadvantage of microchannel-based culture systems is that they are not well-suited to absorbancebased cell density measurements because of short pathlengths. Although devices with integrated optics (Kee et al. 2008; Liang et al. 1996; Llobera et al. 2007; Verpoorte et al. 1992) or systems that allow for direct counting of cells (Faley et al. 2008, 2009) offer some relief from these problems, fabrication of such devices is complicated and time-consuming, and can lead to high fabrication costs. In addition, parallelization in microchannel-based systems is challenging, especially for perfusion systems (Balagadde et al. 2005; Groisman et al. 2005; Zhang et al. 2006).

Here, we introduce a proof-of-principle microbioreactor relying on digital microfluidics (DMF) (Wheeler 2008). In DMF, fluid droplets are controlled in parallel on an open surface by applying electrical potentials to an array of electrodes coated with a hydrophobic insulator [for a comprehensive review of device geometries and fabrication techniques, see (Abdelgawad and Wheeler 2009)]. DMF has become a popular tool for biochemical applications, including mammalian cell-based assays (Barbulovic-Nad et al. 2010, 2008; Shah et al. 2009), enzyme assays (Miller and Wheeler 2008; Srinivasan et al. 2004a, b), immunoassays (Sista et al. 2008a, b), protein processing (Chatterjee et al. 2010; Jebrail and Wheeler 2009; Jebrail et al. 2009; Luk and Wheeler 2009; Moon et al. 2006; Wheeler et al. 2005), the polymerase chain reaction (Chang et al. 2006), and clinical sample processing and analysis (Mousa et al. 2009). However, there is only one report of the use of DMF with microorganisms-Son and Garrell demonstrated that droplets containing yeast could be moved on a DMF system (Son and Garrell 2009). In the present work we have built on these preliminary results, demonstrating a system capable of automated growth and density analysis of several different types of microorganisms. To validate the new technique, the growth characteristics of bacteria, algae, and yeast were measured and compared to those of microorganisms grown and analyzed using conventional macroscale techniques. Furthermore, a viability assay and a genetic transformation were implemented on-chip to illustrate how the platform can be integrated with down-stream analyses after up-stream culture and density measurement.

2 Methods

2.1 Reagents and materials

Unless specified otherwise, reagents were purchased from Sigma-Aldrich (Oakville, ON). *Escherichia coli* DH5 α were generously donated by Prof. Kevin Truong (Institute of Biomaterials and Biomedical Engineering, University of Toronto). *Saccharomyces cerevisiae* BY4741 (S288C Background) were generously donated by Prof. Igor Stagilar (Department of Medical Genetics and Microbiology, University of Toronto). *Cyclotella cryptica* (CCMP 332) algae and associated culture reagents were purchased from the Center for Culture of Marine Phytoplankton (Maine, NE).

2.2 Macroscale cultures

Bacteria and yeast were grown in 3-mL aliquots of media (LB broth and YPD broth, respectively) in vented tubes in a shaking incubator (37°C/225 rpm and 30°C/ 200 rpm/45° inclination, respectively). To generate growth curves, 0.3 mL aliquots of saturated culture $(OD_{600}=2.76\pm0.02$ for bacteria and $OD_{600}=6.95\pm0.14$ for yeast) were inoculated into 2.7 mL fresh broth, and absorbance at 600 nm of diluted aliquots were measured periodically using a UV/Vis spectrophotometer (Eppendorf, Westbury, NY). Algae was grown in 30-60 mL aliquots of f/2 medium (CCMP, Maine, NE) supplemented with biotin and cyanocobalamin (2 nM final concentration ea., CCMP) in vented bottles at 14°C with agitation by magnetic stir bar (60 rpm), with continuous illumination by a 60 W lamp positioned 20 cm from the culture. Algae were maintained by weekly subculture at inoculation densities of $\sim 9.0 \times 10^5$ cells/mL. To initiate growth curves, exponentially proliferating algae were harvested by centrifugation (2000 g, 12 min) and inoculated in medium at a density of 7.0×10^4 cells/mL, and absorbance at 660 nm was measured periodically using a UV/Vis spectrophotometer (Shimadzu, Burlington, ON). All cultures were evaluated by microscopy (Leica DM2000, Leica Microsystems Canada, Richmond Hill, ON) and were grown and evaluated in triplicate.

2.3 Device fabrication

Devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) fabrication facility. Fabrication supplies included parylene-C dimer from Specialty Coating Systems (Indianapolis, IN), Teflon-AF from DuPont (Wilmington, DE), and A-174 silane from GE Silicones (Albany, NY). Silane solution comprised isopropanol, DI water, and A-174 solution ($50:50:1 \nu/\nu/\nu$).

Glass substrates bearing patterned chromium electrodes (used as bottom plates of DMF devices) were formed by photolithography and etching as described previously (Barbulovic-Nad et al. 2008) using photomasks printed with 20,000 dpi resolution by Pacific Arts and Design (Toronto, ON). After patterning, devices were primed for parylene coating by immersing them in silane solution for 15 min, allowing them to air-dry and then washing with isopropanol. After priming, devices were coated with Parylene-C (6.9 µm) and Teflon-AF (235 nm). Parylene was applied by evaporating 15 g of dimer in a vapor deposition instrument (Model PDS 2010 LABCOTER® 2, Specialty Coating Systems, Indianapolis, IN), and Teflon-AF was spin-coated (1% w/w in Fluorinert FC-40, 2000 rpm, 60 s) and then post-baked on a hot-plate (160°C, 10 min). To facilitate the application of driving potentials, the polymer coatings were locally removed from the contact pads by gentle scraping with a scalpel. Unpatterned top plates were formed by spin-coating indium tin oxide (ITO) coated glass substrates (Delta Technologies, Stillwater, MN) with Teflon-AF (235 nm, as above).

2.4 Device operation

As depicted in Fig. 1, the BAY microbioreactor comprises a reactor region (four 10.5×9.5 mm electrodes arranged in

a 2×2 array) mated to a sample region (three rows of eleven 3×3 mm electrodes) and a reservoir region (three 6×6 mm electrodes, one for each sample row). Each of the droplet actuation electrodes (shown) are connected by microfabricated wires to contact pads (not shown for clarity) to facilitate application of driving potentials. Each of the three rows includes an L-shaped electrode which defines a 1.5×1.5 mm transparent window for absorbance measurements. Prior to an experiment, reagents were pipetted onto the appropriate electrodes on a bottom plate, and then an unpatterned, transparent top ITO/Teflon-AFcoated plate was positioned onto the device, sandwiching the droplets between the two plates. The spacing between the two plates was defined by 350-µm thick spacers formed from five-high stacks of double-sided tape. Driving potentials of 400-500 V_{rms} were generated by amplifying the output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 1-5 kHz, and droplets were actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate.

Droplet motion was managed using an automated control system. Briefly, a computer running a custom LabVIEW (National Instruments, Austin, TX) program interfaced to a DAQPAD 6507 (National Instruments, Austin, TX) controls the states of a network of highvoltage relays (RT424012F, Tyco Electronics, Berwyn, PA). The inputs of the relays are connected to the function generator/amplifier (see above), and the outputs of the relays mated to the contact pads on the bottom

Fig. 1 Schematic of BAY microbioreactor. A reactor region contains the mother drop, from which daughter droplets are dispensed for analysis in the sample region or mixed with reagents dispensed from the reservoir region. L-shaped electrodes in the sample region define 1.5×1.5 mm transparent windows which are used for absorbance measurements



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plate of a device via a 40-pin connector. In practice, the user manually loads reagents into the microbioreactor and then inputs a series of desired droplet movement steps, after which all droplet actuation is controlled automatically by the system.

2.5 Microscale cultures

The media used for microscale culture were identical to those used for macroscale culture (see 2.2), but supplemented with Pluronic F68 (bacteria and yeast 0.1 % w/v, algae 0.02%). We regularly use pluronic additives to reduce adhesion of cells (Barbulovic-Nad et al. 2010, 2008) and proteins (Luk et al. 2008) to DMF device substrates and have found no detrimental effects on cell vitality or proliferation. Prior to use, devices were sterilized by rinsing in 70% ethanol, and microorganisms were grown in $\sim 70 \ \mu L$ aliquots termed "mother drops" in the reactor region. During culture, the devices were stored in a humidified chamber (a sealed Petri dish saturated with water vapor), and the mother drops were actuated in a circular pattern at programmed intervals. Temperatures were controlled by means of a digital hot-plate (bacteria and yeast) or by storage in a chilled room (algae). Algae cultures were positioned under a 60 W lamp (at a distance of 20 cm) for continuous illumination. The parameters for each type of culture are listed in Table 1.

To generate growth curves, microbial cultures were initialized by inoculating culture fluid into fresh media, using identical procedures and densities to those used in the macroscale (see 2.2). Mother drops containing bacteria, algae, or yeast were then grown with automated semi-continuous mixing. For absorbance measurements, three \sim 7 µL daughter droplets were dispensed from the mother drop onto the sample region and were driven to the L-shaped electrodes at designated intervals (see Table 1). The microbioreactors were then positioned onto the tops of transparent 96 well-plates and inserted into a PHER-Astar microplate reader (BMG Labtech, Durham, NC) for absorbance measurements at 600 nm for bacteria/yeast and 660 nm for algae. The absorbances were collected using a

well-scanning program, in which 8 separate measurements were collected from pre-determined spots in a $\sim 2.25 \text{ mm}^2$ area. The absorbances of the three daughter droplets were averaged together and were background-corrected by subtracting the average absorbance (collected once at the beginning of each experiment) measured from droplets containing only media on the same devices. After measuring the optical densities, the daughter droplets were translated by DMF actuation back to the reactor region where they were re-combined with the mother drop for continued culture.

2.6 Growth curve generation

To generate growth curves for each microorganism, optical density (OD) measurements from the well plate reader and benchtop spectrometers were plotted in natural log scale. The data were then baseline corrected (subtracting the lowest value) and re-scaled (dividing by the highest, corrected macroscale value) to generate growth curves in the range of 0–1 for comparison between macroscale and microscale profiles. For each data point, three replicate measurements were obtained and the average and standard deviations were plotted as a function of time.

Doubling times, T_d , were calculated as

$$T_d = (t_2 - t_1) \frac{\log(2)}{\log\left(\frac{d_2}{d_1}\right)}$$

where d_1 and d_2 correspond to the biomass densities as determined by absorbance at times t_1 and t_2 respectively. Times t_1 and t_2 were defined during early log phase growth and were compared using a two-tailed t-test.

2.7 Cell death assays

The viability of *S. cerevisiae* yeast grown in BAY microbioreactors was assayed using the nucleic acid dye Ethidium homodimer-1 (EthD-1) (Invitrogen Molecular Probes, Eugene, OR). Prior to operation, a 20 µL mixture

 Table 1 Parameters used for microfluidic culture and analysis of bacteria, algae, and yeast

E. coli S. C. cryptica cerevisiae Growth Media LB YPD broth f/2 supplemented with biotin and broth cyanocobalamin 37 14 Temperature (°C) 30 Mixing Frequency (min) 2.5 2.5 120 Absorbance Measurement 1 2 24 Frequency (h)

of 2 μ M EthD-1 in PBS supplemented with 0.05% F68 was added to one reservoir and another mixture of 2 μ M EthD-1 in PBS supplemented with 0.05% F68 and 0.05% (*v*/*v*) Triton X-100 was added to another. Yeast were then inoculated as described above and incubated at 30°C with automated mixing for 4 h before the assay was started. The assay was completed by dispensing daughter droplets of yeast from the mother drop and merging each of them with droplets containing Triton X-100 and dye dispensed from the reservoirs. The combined droplets were mixed in the sample region by actuation along the linear path 10 times. The microbioreactor was incubated at 30°C for 1 h and then visualized for fluorescence over a square sample electrode.

2.8 Transformation

E. coli bacteria were transformed in a BAY microbioreactor with a pTriEx vector encoding yellow fluorescent protein (YFP) and ampicillin resistance (generously donated by Kevin Truong, University of Toronto). Prior to operation, a 20 µL mixture of 200 ng plasmid DNA and 0.20 M CaCl₂ in LB broth without antibiotic supplemented with 0.05% F68 was added to a reservoir. Bacteria (without ampicillin resistance) were then inoculated as described above and incubated at 37°C with automated mixing for 1 h before transformation. After confirming that the cultures were at early log phase densities (as above), the microbioreactor was placed on ice for 5 min, after which a daughter droplet was combined with an equal volume droplet dispensed from the reservoir and mixed in the sample region by actuating the droplet in the sample region approximately 10 times. The microbioreactor was chilled on ice for 1 h, rapidly heated on a hot plate at 42°C for 50 s and then cooled on ice for an additional 1 min. The microbioreactor was incubated at 37°C for 1 h with automated mixing after which the droplet containing transformed bacteria was spread on an LB agar plate containing ampicillin at 100 mg/L and incubated overnight at 37°C to allow colony formation.

3 Results and discussion

3.1 BAY microbioreactor

A wide range of applications for microorganisms, particularly those involving screening of different conditions, would benefit from automated, micro-scale culture techniques. Here, we present an automated microbioreactor using digital microfluidics that is capable of culture, analysis and transformation of microorganisms in droplets. We call this device the "BAY" microbioreactor, after the three microorganism species used here: bacteria (E. coli), algae (C. cryptica) and yeast (S. cerevisiae).

The primary function of the BAY microbioreactor is longterm cell culture. As shown in Fig. 1, the device was designed such that culture takes place in a $\sim 70 \ \mu L$ aliquot of media called a "mother drop." In conventional bioreactors, cultured cells are gently and continuously mixed to ensure uniform distribution of dissolved gases, nutrients, and the cells themselves (Groisman et al. 2005; Lapizco-Encinas et al. 2004). In the BAY microbioreactor, this function was accomplished by manipulating the mother drop in a circular path at regular intervals. As has been reported elsewhere (Lu et al. 2008; Paik et al. 2003a, b), when droplets are actuated in similar paths in array-based DMF systems, droplet contents are mixed at rates up to $10-50\times$ faster than by diffusion alone. As shown in Fig. 2(a), in the current work, each circular mix step comprised a sequence of four movements between adjacent electrodes. While future designs may be developed for more efficient mixing [using more complex DMF movement paths such as figure-eights (Paik et al. 2003a,b)], in this work, a simple circular path was observed to be adequate.

To facilitate automated microorganism culture, the BAY microbioreactor was designed to be compatible with absorbance measurements, which serve as an indicator of cell density. As shown in Fig. 2(b), in each such measurement, three daughter droplets were dispensed successively from the mother drop and were driven onto L-shaped electrodes for analysis using a well plate reader. The frequency of these measurements (i.e., every 1 h for bacteria, 2 h for yeast and 24 h for algae) was determined by the growth rates of the organism. After the measurements, the daughter droplets were returned to mother drops to continue incubation (thus maintaining the volume of the culture). The L-shaped electrodes were designed with transparent regions that are one quarter (1/4) of the area of the square electrode. Other ratios of window to electrode area (e.g. 1/2 and 1/8) were evaluated, but were found to be sub-optimal, as droplets either were not reliably moved over the window (1/2) or the window was too small for reproducible measurements in the plate reader (1/8). While the strategy of using an L-shaped electrode worked well in the current design, future devices might be formed using transparent driving electrodes for simultaneous incubation and analysis.

A significant advantage of the new technique is the simplicity of the analysis, especially when evaluation of many different conditions is required. The L-shaped electrodes in BAY devices were designed to be 9 mm from each other, matching the pitch of a 96-well plate, and absorbances were measured by inserting devices into a multiwell plate reader. As described elsewhere for other applications (Barbulovic-Nad et al. 2008; Luk and Wheeler 2009; Miller and Wheeler 2008), we propose that

Fig. 2 Sequence of images from a movie of the BAY microbioreactor in action. In (a), the mother drop was mixed by an automated control mechanism. The drop was moved in a circular pattern on the four large electrodes (which facilitates active mixing) at specific time intervals (i.e., every 2.5 min for bacteria and yeast and every 2 h for algae). In (b), daughter droplets were dispensed from the mother drop to facilitate absorption measurements and were returned to the mother drop after measurement



compatibility with off-the-shelf optical detectors is an attractive feature of DMF-based systems. The optical pathlength for absorbance measurements in the BAY devices is determined by the spacer thickness between top and bottom plates in the device. A 350 μ m spacer was used for the work reported here, but DMF is compatible with inter-plate spacers of up to several millimeters (data not shown).

Other advantages of this method are the ease with which active mixing can be incorporated and the inherent batch mode of operation. For the former advantage (mixing), many microscale reactors rely on diffusion for

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mass transport, which is inefficient for cell-sized particles (Fowler et al. 2002). Specially designed micromechanical mixers can provide some relief for this limitation in microchannels (Lee et al. 2006; Zhang et al. 2006); in contrast, simple repetitive manipulation of droplets in the DMF microbioreactor is sufficient for active mixing without added complexity. For the latter advantage (batch mode), although the current generation of BAY devices was designed for a single culture, the format, which matches the pitch and dimensions of multiwell plates and detectors, is likely scalable in future generations for analysis of different culture and analysis conditions in parallel. We propose that future generations of BAY systems may be useful for growth and screening of many populations of organisms [e.g. *S. cerevisiae* gene deletion libraries (Giaever et al. 2002)].

3.2 BAY culture

To compare the growth rates of microorganisms grown in BAY microbioreactors to those cultured by conventional means, bacteria, yeast and algae grown in both systems (micro- and macro-scale) were interrogated with absorbance measurements over 8, 12 or 120 h of culture. As described in the Section 2, the culture conditions in both systems (macro-scale or BAY) were similar. Cell proliferation with minimal clumping was observed in both systems for all three microorganisms over the course of the experiments (Fig. 3). As shown in Fig. 4 and Table 2, the growth rates of bacteria, algae, and yeast were similar for the macro- and micro-scale; this is striking, given the significant differences between the systems (volumes, electrostatic actuation, detectors, etc.).

We speculate that the variations in the growth rates between the micro- and macro-scale systems may be caused by a number of factors. The most likely is temperature differences since small fluctuations in incubation temperature can result in vastly different growth rates in bacteria, algae and yeast (Fogel and Brunk 1998;



Fig. 3 Photomicrographs of daughter droplets positioned on L-shaped electrodes containing (**a**) *E. coli* at 0 and 8 h, (**b**) *C. cryptica* at 0 and 5 days, and (**c**) *S. cerevisiae* at 0 and 12 h in BAY microbioreactors. Scale bars are 500 μm



Fig. 4 Representative growth curves of (a) *E. coli*, (b) *C. cryptica*, and (c) *S. cerevisiae* grown in macroscale (*circles*) or in BAY microbioreactors (*triangles*). Absorbance measurements were taken at 600 nm for bacteria/yeast and 660 nm for algae and were normalized to the highest value. Macroscale measurements were conducted using benchtop UV/Vis spectrophotometers while microscale measurements were conducted on BAY microbioreactors using a well-plate reader. Samples were evaluated in triplicate and error bars represent one standard deviation

Montagnes and Franklin 2001; Ratkowsky et al. 1982). This difference between the macro- and micro-scale systems is most relevant during absorbance measurements—in the macro-scale system, small aliquots were collected from the main culture, measured for density, and then disposed (while the main culture remained at the temperature listed in Table 1). In the micro-scale system, the entire device (including the main culture) was exposed to room temperature (in the plate reader) during density measurements. In the future, a plate reader with temper
 Table 2 Exponential doubling times of bacteria, yeast and algae in macro and micro-scale formats. P-values compare the difference between macro- and micro-scale

	E. coli	S. cerevisiae	C. cryptica
Macro-scale Doubling Time (h)	0.79 ± 0.06	1.80 ± 0.24	37.0±1.2
Micro-scale Doubling Time (h)	1.23 ±0.43	1.88 ± 0.15	42.6±2.4
<i>P</i> -value	0.08	0.31	0.02

ature control might be used to correct for this. Other potential sources of variation may include differences in mixing efficiency and imprecise temperature control on hot plates in comparison to incubators.

The greater variances in the optical density measurements in the microscale are most likely a result of the combination of shorter path lengths in the microscale (350 μ m versus 1 cm) and differences in the analysis tools (well-plate reader versus benchtop spectrophotometer).

3.3 BAY downstream processing/analysis

A key advantage of microfluidic systems is the potential for integration of multiple processes onto a single platform. To illustrate this point with the BAY microbioreactor, two different downstream processes were integrated: a fluorescent viability assay of yeast and genetic transformation of bacteria. In the former, the viability of S. cerevisiae grown in the BAY microbioreactor was assayed on-chip with a fluorescent nucleic acid stain (Ethidium homodimer-1). Dye with or without the surfactant, Triton X-100, was loaded into device reservoirs, the yeast were grown for 4 h and then daughter droplets dispensed and mixed with reagents dispensed from reservoirs. Figures 5(a-c) are representative images collected in this assay, which demonstrates the toxicity of Triton-X 100 at this concentration. Here, fluorescence was used a read-out for cell death; in the future, we propose that many other probes or assays relying on fluorescence or luminescence are likely compatible with the BAY microbioreactor.

In the latter application, genetic manipulation was implemented on-chip; this is particularly relevant for the BAY reactor, as this process is typically performed in early to mid-log phase growth (Mandel and Higa 1970). To demonstrate this integrated process, *E. coli* were grown and their optical densities were measured to confirm that they were in early to mid-log phase. The droplets containing the bacteria were then transformed with a YFP reporter gene in a step-wise process involving several thermal cycling steps and exposure to calcium chloride (which facilitates gene uptake). After transformation, the Fig 5 Representative images of yeast viability assay and bacterial transformation. Yeast grown for 4 h in BAY microbioreactor were incubated with 2 µM Ethidium homodimer-1. (a) and (b) are brightfield and fluorescence images of yeast not exposed to Triton X-100, and (c) is a fluorescence image of yeast exposed to 0.05% (v/v) Triton X-100. Brightfield images of yeast exposed to Triton X-100 were similar to (a) and are not shown. Comparison of the images reveals that over 99% of the yeast were non-viable after treatment with Triton X-100. (d) is a fluorescent image of an LB agar plate spread with YFPtransformed bacteria. Scale bars are 60 µm



bacteria were spread on an ampicillin agar plate overnight to confirm successful transformation (Fig. 5(d)).

In this work, we have demonstrated a device architecture that can be used for microliter-scale culture of bacteria, algae, and yeast, and integration with downstream processing. But we propose that the principle of using digital microfluidics for microorganism culture, analysis and manipulation could extend to an even wider range of organisms and high-throughput technologies (e.g. two-hybrid screens). Devices can be configured to accommodate droplets ranging from nanoliters to milliliters (Abdelgawad et al. 2008), and electrodes can be arranged into virtually unlimited numbers of spatial configurations. For example, future designs might be developed for self-contained culture of limited supply or dangerous species, yielding information on gene expression, protein interactions, and biological interactions within living cells, microarray or parallel-scale culture (e.g., on 384-well or 1536-well formats) (Bailey et al. 2002, 2004; Ziauddin and Sabatini 2001), or integration with microchannels (Abdelgawad et al. 2009; Watson et al. 2010) for other types of analyses (Mousa et al. 2009; Peng and Li 2004; Ryley and Pereira-Smith 2006).

4 Conclusion

A platform for the integrated growth and cell density analysis of microorganisms in distinct microdroplets has been developed using digital microfluidics (DMF). These microbioreactors operate with automated semi-continuous DMF-driven mixing and are compatible with a diverse range of organisms and processes. The new technique may be beneficial for microbial applications that require miniaturization or parallelization in highly customizable formats.

Acknowledgements We thank Prof. Kevin Truong (Institute of Biomaterials and Biomedical Engineering, IBBME, University of Toronto) for generously donating E. coli and plasmid DNA, Prof. Igor Stagjlar (Department of Medical Genetics and Microbiology, University of Toronto) for generously donating S. cerevisiae and Prof. William Ryu (Department of Physics, University of Toronto) for the use of the cold room. We also thank Evan Mills (IBBME, University of Toronto) and Dawn Edmonds (Department of Medical Genetics and Microbiology, University of Toronto) for their assistance with bacterial transformation and yeast culture. We thank Kamlesh D. Patel and Pam Lane (Sandia National Laboratories, Livermore, CA) for discussion and assistance with algae culture. We thank the Canadian Institutes of Health Research (CIHR) for financial support. SHA and SCCS thank NSERC (Natural Sciences and Engineering Research Council) for graduate fellowships, and ARW thanks CRC for a Canada Research Chair.

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