

## Aerobic Fermentation of *Saccharomyces cerevisiae* in a Miniature Bioreactor Made of Low Cost Poly(Methylmethacrylate) (PMMA) and Poly(Dimethylsiloxane) (PDMS) Polymers

(Fermentasi Aerobik *Saccharomyces cerevisiae* dalam Bioreaktor Mini Dhasilkan daripada Polimer Poli(Metilmetakrilat) (PMMA) dan Poli(Dimetilsiloksana) (PDMS) Kos Rendah)

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### ABSTRACT

*In this paper, a minibioreactor platform made of low cost polymers is presented. The minibioreactor prototype was designed as an alternative solution for carrying out microbial fermentation experiments in laboratory. The minibioreactor prototype has a working volume of 1.5 mL and was fabricated from poly(methylmethacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) polymers. Cell density was measured online whilst agitation rates and the temperature of the reactor content can be tightly controlled to desired set-point values. As proof-of-concept, various *S. cerevisiae* fermentation experiments were conducted. In every experiment, the minibioreactor operated stably for the entire length of operation which was nearly 40 h with very minimal volume loss i.e. about  $2.8 \mu\text{L}\cdot\text{h}^{-1}$  at  $37^\circ\text{C}$ . The minibioreactor has the maximum oxygen transfer rate (OTR) of  $16.6 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  under the agitation rate of 300 rpm. Under these conditions, cell specific growth rate as high as  $0.291 \text{ h}^{-1}$  was obtained. The experimental data in the minibioreactor operation was also reproducible using shake flask where similar growth profiles were attained under a similar growth conditions.*

*Keywords: Bioreactor; miniature bioreactors; online UV detection; scale down; yeast fermentation*

### ABSTRAK

*Dalam kertas ini, minibioreaktor yang dihasilkan daripada polimer berkost rendah telah dibincangkan. Prototip minibioreaktor ini direka bentuk sebagai alternatif dalam menjalankan eksperimen fermentasi di dalam makmal. Prototip minibioreaktor ini mempunyai isi padu kerja sebanyak 1.5 mL dan difabrikasi daripada polimer poli(metilmetakrilat) (PMMA) dan poli(dimetilsiloksana) (PDMS). Ketumpatan sel diukur secara dalam talian sementara kadar pengadukan dan suhu kandungan reaktor boleh dikawal pada nilai yang ditentukan. Untuk membuktikan konsep, beberapa eksperimen fermentasi *S. cerevisiae* telah dijalankan. Pada setiap eksperimen, minibioreaktor beroperasi secara stabil selama eksperimen berlangsung hampir 40 jam dengan kehilangan isi padu yang minimum iaitu sebanyak  $2.8 \mu\text{L}\cdot\text{h}^{-1}$  pada  $37^\circ\text{C}$ . Minibioreaktor ini mempunyai kadar pemindahan oksigen maksimum  $16.6 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  pada kadar pengadukan 300 rpm. Pada keadaan ini, kadar pertumbuhan sel khusus setinggi  $0.291 \text{ h}^{-1}$  diperolehi. Data eksperimen dalam operasi minibioreaktor juga boleh diperolehi menggunakan kelalang goncang dengan profil pertumbuhan yang sama dicapai pada keadaan pertumbuhan yang serupa.*

*Kata kunci: Bioreaktor; bioreaktor bersaiz mini; fermentasi yis; menskalakan ke bawah; pengesanan UV dalam talian*

### INTRODUCTION

*Saccharomyces cerevisiae* is one of the most commonly studied microorganisms at both industry and academic levels and a major producer of commercial ethanol. It was usually found in plant material including fruits, vegetables and grains (Shuler & Kargi 2002). In regards to the production of ethanol i.e. from industrial perspectives, *S. cerevisiae* is physiologically stronger to bacteria, other yeasts and filamentous fungi (Tesfaw & Assefa 2014). *S. cerevisiae* could tolerate acidic medium with pH optimum in the range between pH 3 and 5 and thereby, makes the fermentation process of *S. cerevisiae* less vulnerable to infection than bacteria (Lin et al. 2012; Ortiz-Muñiz et al. 2010). Report also showed that *S. cerevisiae* has a higher

tolerance to inhibiting compounds compared to other ethanol producing microorganisms (Almeida et al. 2007; Prasertwasu et al. 2014). Another added advantageous is that *S. cerevisiae* is generally regarded as safe (GRAS) for human consumption (Tesfaw & Assefa 2014). Optimization of *S. cerevisiae* fermentation processes typically involved a huge number of experimental works. These experimental works were needed to investigate which culture conditions would give the fastest reaction rate or the highest product yield and for determination of cells growth kinetics e.g. cells specific growth rate,  $\mu_m$  and saturation-constant,  $K_s$  (Lin et al. 2012; Ortiz-Muñiz et al. 2010).

At laboratory scale, optimization of fermentation processes are generally performed using shake flasks and

the bench-scale bioreactors. In the early fermentation process development, microbiologists often rely on parallel experimentation in shaken flasks to determine optimal medium composition and/or to screen for the suitable microbial strain (Betts & Baganz 2006). In a more advance stage, where it is necessary to quantify important engineering parameters e.g. the oxygen transfer coefficient,  $k_L a$ , volumetric power consumption,  $P/V_L$  and/or to assess the impact of fundamental engineering aspects such as mixing and heat transfer rate on the reactions, bench scale bioreactors are usually utilized (Stanbury et al. 1999). In general, shake flasks operations are much easier to set-up and they require low volumes of culture medium (~50-200 mL). Medium consumptions can be further reduced with the use of microtitre plates i.e. a miniature size shaken reactor with working volumes of few hundreds microlitre such as between 100-200  $\mu\text{L}$  (Standbury et al. 1999).

However, with this approach, experiments are normally carried out in batch mode and control of process parameters under shaking conditions is rather difficult. Bioreactor, on the other hand is a more sophisticated experimental tool. Culture conditions can be precisely controlled and it can be operated with a working volume as low as 180 mL ("Multifors 2" bioreactor system, Infors, Switzerland). Nevertheless, performing multiple fermentation experiments at few hundreds of mL per run is still relatively costly. Furthermore, extra laboratory work is usually needed for preparation and cleaning of the bioreactor (Schäpper et al. 2009).

In this work, we report the establishment of a miniature bioreactor system with working volume of 1.5 mL as alternative tools for studying *S. cerevisiae* fermentation experiments. The main aimed was to design a miniature scale bioreactor system that can be fabricated inexpensively and easy-to-use in a laboratory environment. The concept of easy-to-use is referred to simple and quick installation of the reactor - similarly to a typical shake flask (or microtitre plate) operation and disposal i.e. no cleaning needed in post reactor operation. In making the minibioreactor system inexpensive, main components for the reactor process control were built on separate platform such that there are reusable. Poly(dimethylsiloxane) (PDMS) and poly(methylmethacrylate) (PMMA) polymers were used for the reactor fabrication and only the temperature and the stirring speed were controlled. Moreover, cell density was measured online using optical fibres and humidified air was actively supplied into the reactor to keep a reasonable level of oxygen transfer rate for the cell growth. The use of optical fibres for cells detection allowed for the observation of the fermentation progress in real time and eliminate the necessity for sampling. It is expected that the propose minibioreactor prototype would be a suitable solution (or as a teaching tool) for studying the fundamentals of *S. cerevisiae* fermentation processes in laboratory.

## MATERIALS AND METHODS

### MATERIALS AND REACTOR FABRICATION

The materials used for the reactor fabrication were the poly(dimethylsiloxane) (PDMS) and the poly(methylmethacrylate) (PMMA) polymers. The reactor parts made of PMMA were machined using a computer-numerical-control (CNC) milling machine (Roland MDX-40A CNC machine, Roland DGA Corporation, Barranca Pkwy Irvine, CA, USA) where else the parts made of PDMS polymers were fabricated using a soft lithography procedure (Becker & Gaertner 2008). The latter was performed by curing a pre-polymer PDMS liquid solution in a mold made of PMMA at 70°C for 2 h. The PDMS liquid solution was prepared by mixing 10 parts silicone and 1 part curing agent (Sylgard 184, Dow Corning). The mixture was thoroughly mixed and bubbles were first removed from the mixture before pouring onto the mold. After curing, the cured PDMS polymer was left to cool in a room temperature before removing it from the mold with great care. Additionally, a three-dimensional (3D) printer (Up plus 3D printer, 3D Systems, Inc, RH, SC, US) was used to fabricate various parts for the reactor operation. These include the syringe holder, the magnetic motor platform and a placement board for the optical components. For the 3D printing, rolls of round filament made of polylactide (PLA) polymers ( $\varnothing$  1.75 mm) was utilized as materials for the fabrication.

### MINIBIOREACTOR DESIGN AND PROCESS CONTROL

The minibioreactor prototype presented has been realized as a miniature stirred tank reactor. The setup of the minibioreactor prototype is presented in Figure 1. The prototype consisted of 8 mm PMMA layer that was pressed between a 3 mm PDMS layer and a 1 mm PMMA layer by M2.5 stainless steel screws. Fluidic connections for transport of liquid to and from the minibioreactor was established by using a standard polytetrafluoroethylene (PTFE) tubing ( $\varnothing_1$  2 and  $\varnothing_0$  2.5 mm). All fluidic ports were realized on the top PMMA layer.

The outer diameter of the tube is 0.5 mm larger than the size of the fluidic port. This 'mis-match' ensured a tight connection and warrants a leak-free operation. Such tightness could also withstand certain pressure resulted from the reaction chamber during the reactor operation - at least up to  $0.6 \times 10^6$  bar (Perozziello et al. 2008). The reaction chamber is located at the center of the reactor and is 20 mm in diameter and 8 mm deep (total working volume is approximately 2500  $\mu\text{L}$ ). The outer diameter of the reactor is 40 mm and thus, provides a relatively thick wall thickness (10 mm). This is essential in order to reduce the heat dissipation rate to the surroundings through the side walls (Zainal Alam et al. 2010). The bottom PMMA layer is 1 mm thick and formed the base of the minibioreactor. A thickness of 1 mm was chosen as a much thinner layer (i.e. < 1 mm) would not be able to withstand the pressure from the M2.5 screws in the final assembly. In the final

assembly, all layers were aligned and carefully pressed by using two M2.5 screws. A small amount of PDMS solution was spread around the edge of the bottom layer in the final assembly to achieve a leak-free sealing.

Due to the small working volumes of the minibioreactor, sampling is not possible in the minibioreactor experiments. Monitoring of the concentration dynamics of cells was

relied on the UV absorption method via optical fibres. In the proposed scheme, a bright yellow LED (L600-10V, 600 nm, Epitex, Kyoto, Japan) was modulated with a sine wave of 47 Hz (5V current signal) and was transmitted into the minibioreactor by using an optical probe (Figure 1). The probe consisted of 7 optical fibres ( $\varnothing 1$ , 57-097 Edmund Optics, Singapore) that were bundled together neatly into a

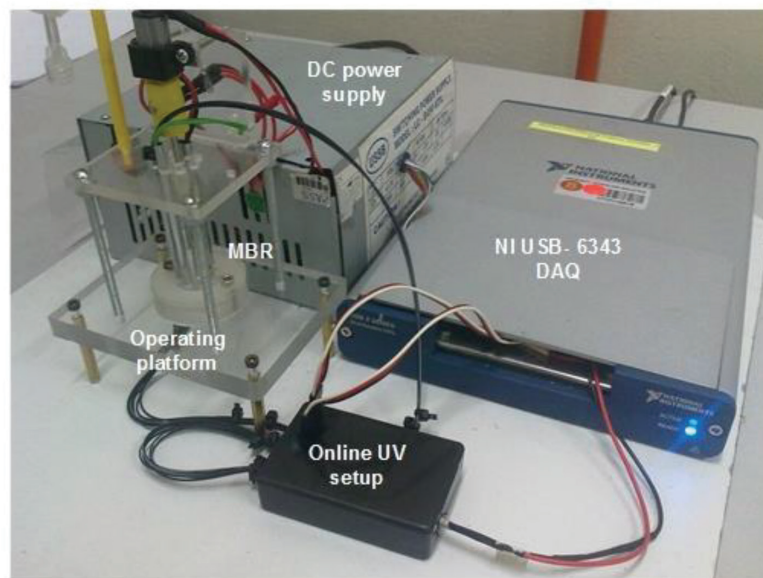
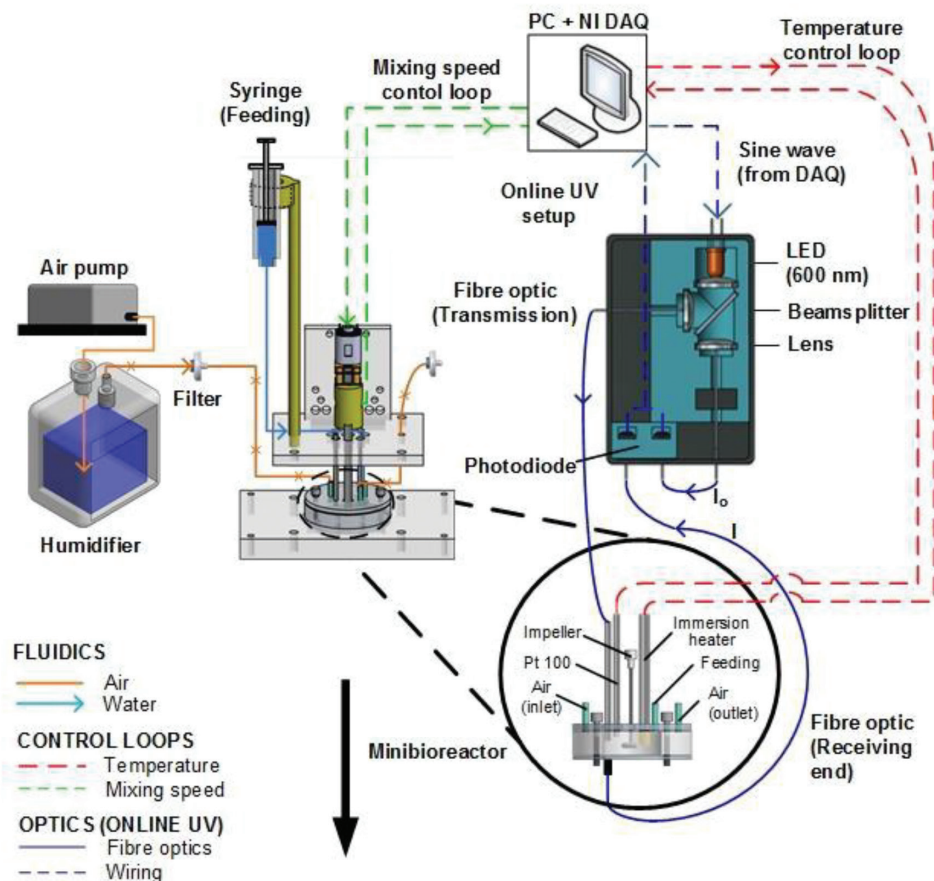


FIGURE 1. Detail schematic (top) and the actual image of minibioreactor set up (bottom)

hollow cylindrical steel. In this manner, the OD optical lines (probe) can be easily disconnected, cleaned and reused repeatedly. Lens was used to focus lights to and/or from optical fibers and a photo-detector (FDS-100, Thorlabs, US) was utilized to collect lights on the receiving end of the transmission. The transmission and the receiving lines were positioned vertically along z-axis i.e. from the top and at the bottom of the reactor content to obtain an optical length of approximately 2.5 mm.

Mixing for the minibioreactor is achieved by means of a miniature size stainless steel rod. The rod is 2 mm thick and 10 mm long. It was rotated by a direct current motor (SPG 10- 30K, Cytron, Malaysia) that was mounted on the top of the reactor. The rotating energy for the mixing was delivered from the motor to the impeller by a connecting shaft. A proportional-integral (PI) control algorithm was implemented to control the rotational speed of the impeller. As for the temperature control, a simple on/off control algorithm proposed by Zainal Alam et al. (2010) has been realized. The temperature of the reactor content was measured by using a miniature size Pt 100 sensor (CLS 181-2012 Correge, France) whilst heating was achieved using a custom made resistive heater (heat transfer area  $\sim 90 \text{ mm}^2$ ). The effectiveness of both the stirring and temperature controller was assessed through set-point tracking experiments. Experiments were carried out using a distilled water with continuous aeration. Contrary to shake flask or microtitre plate operation, a small air pump ( $50 \times 80 \times 30 \text{ mm}$ , 0.016 MPa, E-JET 168) was used to deliver a continuous air supply into the headspace of the minibioreactor. The air was first sparged into a container filled with cold water ( $\sim 18\text{-}20^\circ\text{C}$ ) and filtered by using a  $0.2 \mu\text{m}$  nylon syringe filter (Nalgene<sup>TM</sup>). This is a necessity in order to supply a humidified and sterile air into the reactor chamber. Air was delivered into the headspace of the reactor and not sparged into the base of the reactor as to maintain a bubble-free environment within the reaction chamber (Duetz et al. 2000). Another  $0.2 \mu\text{m}$  nylon syringe filter (Nalgene<sup>TM</sup>) was placed at the gas outlet port in order to ensure that the reactor content will remain in sterile condition (Gill et al. 2008). A continuous air flow and a slight back pressure due to the differences of the size of the inlet and outlet gas ports ( $\text{Ø}_{\text{inlet}} \gg \text{Ø}_{\text{outlet}}$ ) created the necessary driving force for the diffusion of the oxygen into the reactor content. The dissolved oxygen was distributed evenly by the rotating impeller to maximize the oxygen transfer capacity. The oxygen transfer capacity of the reactor was assessed using the sulfite method test (Linek & Vacek 1981). The test measures the maximum oxygen transfer rate ( $\text{OTR}_{\text{max}}$ ) under specific aeration and agitation rates.

All sensing and control routines for the above mentioned variables were programmed using the LabVIEW<sup>TM</sup> Academic Premium Suite software (National Instruments, Austin, TX, USA). The program was executed by interfacing it to a data acquisition card (NI USB-6343, National Instruments, Austin, TX, USA) for in-/output of signals. All data were measured on-line and written in

text files for further processing by using Matlab v7.0 (The Mathworks, Natick, MA, USA).

#### SACCHAROMYCES CEREVISIAE FERMENTATION EXPERIMENT

In order to demonstrate the workability of the proposed minibioreactor system, series of *S. cerevisiae* batch fermentation experiments were performed. A single colony of instant baker's yeast (*S. cerevisiae*, Mauri-pan, UK) was first grown in a standard Yeast Peptone Dextrose (YPD) agar media as stock culture. The YPD agar media consisted of 20 g/L glucose (Sigma-Aldrich, US), 10 g/L peptone (Sigma-Aldrich, US), 5 g/L yeast extract (Sigma-Aldrich, US) and 20 g/L of agar powder. During medium preparation, glucose solution was sterilized separately at  $110^\circ\text{C}$  for 10 min to prevent caramelization. Yeast was cultured in a plate at  $37^\circ\text{C}$  for 24 h. The starting pH of the YPD medium was adjusted to pH5.4 using 2 M sulfuric acid,  $\text{H}_2\text{SO}_4$  and 2 M sodium hydroxide, NaOH solutions. For the inoculum development, a small amount of the stock culture was transferred to a 150 mL Erlenmeyer flask that contained 100 mL YPD medium and incubated overnight (i.e. 10-14 hours) in an orbital shaker ( $37^\circ\text{C}$ , 200 rpm). The inoculated culture was then diluted with a fresh YPD medium to produce an inoculum with an initial cell concentration of 0.1 g/L. The inoculum volume was 10% (v/v) of the total working volume of the minibioreactor (1.5 mL). For comparison purposes YPD medium was also prepared to carry out yeast fermentation experiments in the shake flask (150 mL). All fermentation experiments were carried out at  $37^\circ\text{C}$  for  $\sim 40$  h until cells growth reach a stationary phase. Cells optical density in the minibioreactor was measured on line (at 600 nm) where else for the shake flask cell optical density was measured off line using a spectrophotometer (Jenway 6300, Bibby Sc. Ltd., UK). For this purpose, a YPD medium was used as blank and 2 mL of samples were taken out every hour from the shake flask. Also, the final concentration of the glucose in the reactors was determined using a DNS reagent method suggested in Saqib and Whitney (2011).

## RESULTS AND DISCUSSION

#### MINIBIOREACTOR OPERATION AND PERFORMANCE

The basic functionality of the minibioreactor prototype was evaluated using a distilled water. A total volume of 1.5 mL distilled water was loaded using a syringe and the reactor was operated with a slight headspace such that bubbles would rise into the head space and do not coalesce within the reaction chamber. The presence of bubbles is not desirable as it may perturb the stirring capacity of the micro impeller and interfere with the optical density measurements. The way the minibioreactor was operated is contrary to most micro scale bioreactor design with volume less than 1 mL where a bubble-free conditions were often preferred (Lee et al. 2006; Schäpper et al. 2010; Szita et al. 2005). The minibioreactor prototype was also connected to

a syringe drive to assess the reactor capacity to operate in continuous mode. The reactor was filled continuously at various feed rates i.e. between 1-5 reactor residence times and no leakages were found during loading and/or during the continuous mode operation. This clearly indicated that the polymer reactor was securely sealed and any excessive pressure in the reactor had been pushed through the outlet fluidic port.

Figure 2(a) and 2(b) shows the results of the temperature and the stirring control set-point tracking experiments. Based on the results, a satisfactory degree of control was achieved for the temperature parameter. The heating step was relatively fast as it took only a couple of minutes to increase the reactor temperature by 10°C. Although humidified air at 18°C was continuously supplied to the system (process disturbance), the controller operation remained stable with a measurement accuracy of about 0.2°C from the temperature set point values.

There was no volume loss during the heating step and the controller responded to each step change in a couple of milliseconds (no delay). The cooling step however, was much slower as there was no cooling element installed and cooling was completely depending on the heat loss to the surroundings. Based on the results, it was found that the cooling rate was decelerated as the reactor temperature asymptotically reaching the room temperature. The cooling rate was decelerating from 100°C·min<sup>-1</sup> (i.e. the value of cooling rate from 50°C to 40°C) to about 22°C·min<sup>-1</sup> when the set point value was adjusted from 30°C to room temperature. This was to be expected because the driving force for the thermal change of the reactor content was the temperature difference ( $\Delta T$ ) between the temperature of the reactor and the ambient. The proposed temperature control method was viable for our application as rapid temperature change i.e. as seen in most microfluidic polymerase chain reaction chips (Zhang & Xing 2007) was not needed and the reactor temperature always operated above the temperature of the ambient. Figure 2(b) shows the performance of the minibioreactor stirring control. A PI control algorithm was chosen and not an on-off control

algorithm. The on-off controller was not suitable because of two reasons. First, the measured values was oscillating too large from the set-point values and secondly, a continuous stirring was required and not a pulsating stirring in order to ensure that the cells remain in suspension state with no (or very minimal) 'dead-zones'. The auto-tuning feature included in the PID Autotuning subVI (a readily available sub program in the LabVIEW library) allowed the software to automatically calculate the PI controller constants based on the measured signals and the desired set-point values. At the chosen operating range i.e. between 0 and 300 rpm, the proportional,  $K_c$  and the integral,  $T_i$  constants were calculated to be 0.3 and 0.017, respectively. Despite a slight overshoot, the controller has a relatively fast response time (i.e. approximately 1 millisecond) and the stirring speed can be precisely controlled at the accuracy of  $\pm 2$  rpm of the set-point values. Through a brief tracer test experiment at the agitation rate of 300 rpm ( $NRe = 245$ ), the mixing time was in the order of 3 s. The value of the mixing time attained was comparable to most polymer-based microbioreactor design that operated at the same agitation rate (Schäpper et al. 2009; Szita et al. 2005; Zainal Alam et al. 2010). Zanzaotto et al. (2004) demonstrated through a computational fluid dynamic (CFD) analysis that at this operating range, a homogenous mixture required for fermentation experiments can be achieved.

The optical cell density measurement of the reactor was calibrated using yeast cells suspended in distilled water. The absorbance value i.e. the voltage ratio between the intensity of the light that passed through the reactor content,  $I$  and the reference signal,  $I_0$  was correlated to the actual cells concentration using the Beer Lambert's law equation. It was found that the voltage ratio (measured OD signal) has a linear correlation with the cells density between 0 and 20 g·L<sup>-1</sup>. This linear relation is depicted in Figure 2(c). A long optical path length (2.5 mm) was chosen in the minibioreactor design in order to increase the cells detection accuracy. It has been reported that longer path length would increase measurement accuracy especially when running experiment with low cells concentration (Schäpper et al. 2009).

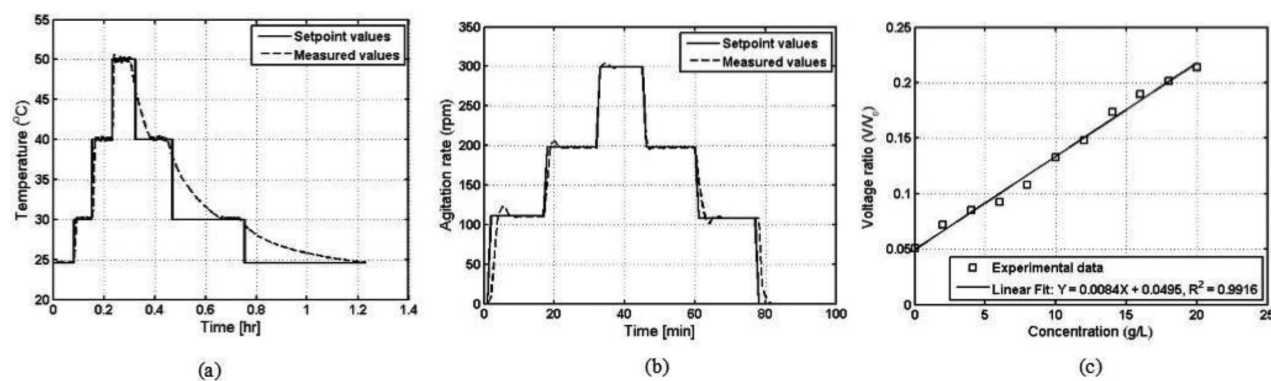


FIGURE 2. a) Results of the temperature control set-point tracking experiment for three different temperature set-point values (30°C, 40°C and 50°C), b) Results of the stirring control set-point tracking experiment for three different stirring speed set-point values (100, 200 and 300 rpm) and c) Calibration curve for the optical cell density measurement

PROOF OF CONCEPT EXPERIMENT: BATCH CULTIVATION OF *SACCHAROMYCES CEREVISIAE*

As a proof of concept, various aerobic fermentation experiments with the *S. cerevisiae* were performed in the mini-bioreactor prototype. In each experiment, the reactor was first sterilized under an ultraviolet (UV) light for 20 min. This took place in a laminar flow hood cabinet (air flow was switched-off during sterilization step). Once sterilized, the reactor was placed on the operating platform where it will be connected to all the sensors, actuators and the fluidic lines. The experiments were initiated once the cells inoculum has been injected into the reaction medium. Three sets of *S. cerevisiae* fermentation were carried out where the agitation rate was varied (i.e. at 100, 200 and 300 rpm) whilst the air supply was set for  $937.5 \mu\text{L}\cdot\text{h}^{-1}$ . The resulting time course profiles of the culture growth curve are shown in Figure 3(a). The result showed that after a couple of hours of lag phase, the cells grew exponentially (indicated by the sharp increase of the OD readings) before came to a stationary phase after 25 h of cultivation. The deceleration of the cell growth was probably due to the depletion of glucose i.e. the main substrate for the cultivation (Boccazzi et al. 2006; Wee et al. 2010). This was also being from our analytical analysis where the glucose level at the end of each experiment was found to be less than  $0.8 \text{ g/L}$ . Cells specific growth rate ( $\mu$ ) was found to be at the highest i.e. at  $0.291 \pm 0.02 \text{ h}^{-1}$  when the experiment was carried out at agitation rate of 300 rpm. At agitation rate of 100 rpm, the cells specific growth rate ( $\mu$ ) was only about  $0.186 \pm 0.023 \text{ h}^{-1}$ . Apparently, by increasing the agitation rates, the oxygen transfer rate (OTR) of the reactor was also improved (Figure 3b) and thus, led to a greater cell growth. Higher OTR is indeed essential to prevent cells starvation due to the limited oxygen feed.

Each fermentation experiments have been carried out for nearly 40 h and the reactor variables i.e. the stirrer speed and the reactor temperature have been successfully controlled within the desired set-point values during this period (Figure 3(b) and Figure 3(c)). An unexpected disturbance was encountered by the temperature controller around  $t = 10 \text{ h}$  in one of the experiments (Figure 3c); which led to a slight overshoot in the temperature readings i.e. by nearly  $3^\circ\text{C}$ . This mere disturbance however was overcome automatically by the controller to maintain the reactor temperature close to the set-point value for the rest of the reactor operation. Water evaporation was also very minimal i.e. in the order of  $2.8 \mu\text{L}\cdot\text{h}^{-1}$ . Additional fluidics line to compensate the volume loss was not necessary as the total volume loss for the entire length of operation at  $37^\circ\text{C}$  was only  $100 \mu\text{L}$ . This was less than one tenth of the total working volume of the reactor and the remaining reactor content ( $\sim 1.4 \text{ mL}$ ) was more than enough to allow for several off-line post analysis e.g. HPLC and protein expression analysis, if necessary. Moreover, significant cell sedimentation or wall-growth by the microbe was not observed in the end of every experiments; presuming that cells were completely mixed and in suspension state

throughout the entire operation. The growth profiles attained in the mini-bioreactor was also comparable to the growth profiles of *S. cerevisiae* fermentation performed in a shake flask where the max OTR and the specific growth rate,  $\mu$  were calculated to be  $10 \text{ mM}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  and  $0.253 \text{ h}^{-1}$ , respectively (Figure 4). The results attained further demonstrate the validity of the results obtained using the polymer-based mini-bioreactor prototype. This also means that similar experimental results typically achieved in larger scale of operation can indeed be produced using a disposable miniature scale bioreactor platform.

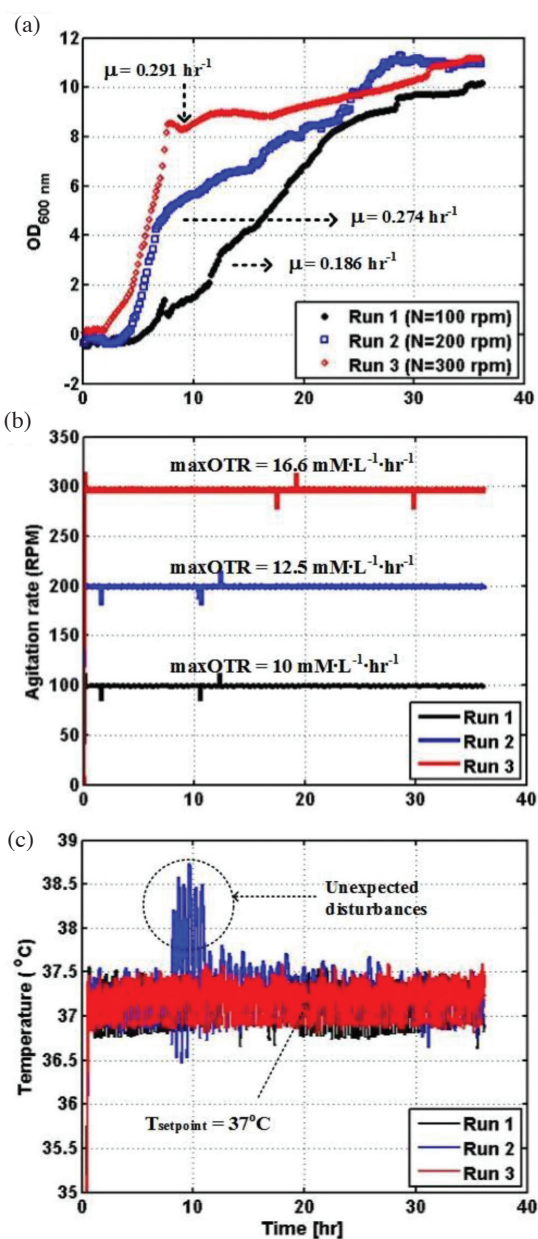


FIGURE 3. a) Growth profiles for the *S. cerevisiae* fermentation experiments performed in the mini-bioreactor at three different agitation rates, b). Mini-bioreactor maximum oxygen transfer rate at different agitation rates and c) Online data of the reactor temperature in each experiment

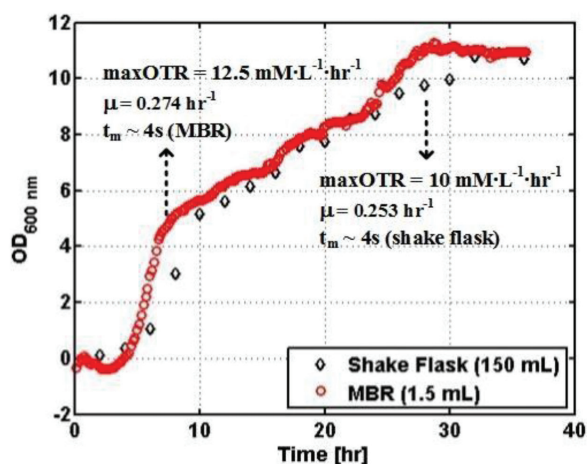


FIGURE 4. Comparison of growth profiles for the *S. cerevisiae* fermentation experiments in shake flask and in the mini-bioreactor prototype

TABLE 1. Comparison of acquisition cost for mini-bioreactor and 150 mL flask

	MBR	150 mL flask
Main module / DAQ unit	€1900 <sup>a</sup>	€2500 <sup>b</sup>
Reactor cost	€3 <sup>c</sup>	€15 <sup>d</sup>
Sensors & Actuators	€70 <sup>e</sup>	€3 <sup>f</sup>
Medium cost per run	<€1	€35
Total cost <sup>g</sup>	€1974	€2553

a-NI USB 6343 DAQ unit. b-commercial orbital shaker with embedded heater. c-materials needed for fabrication. d-price of 150 mL flask. e-Temperature, stirring and optical density. f-stirrer bar. g-approximate cost

Table 1 shows comparison of the acquisition between the mini-bioreactor platform and the 150 mL shake flask operation. As evident, readily available data acquisition (DAQ) unit for automation and online measurement of the proposed mini-bioreactor platform were costly i.e. almost as expensive as a standard orbital shaker module. However, a more cost-effective solution to carry out similar process automation tasks is also available. For example, the use of microcontroller platform where a decent board (including discrete electronic components) usually cost only about €100. This would significantly reduce the capital cost of the entire setup. Most importantly, the material cost needed to fabricate a single mini-bioreactor unit and running cost per experiment are very low compared to the 150 mL shake flask. The latter was indeed beneficial as waste produced per run was also largely reduced.

## CONCLUSION

The paper presents the establishment of a mini-bioreactor platform made of low cost PDMS and PMMA polymers as alternate solution for studying the basic of fermentation processes (or as a teaching tool) in laboratory environment. The clamp-n-play reactor configuration and the plug-n-play fluidics interconnect design result in a modular prototype that was easy to use. Cells optical density was measured

online and thus, eliminates the need for sampling. A low standard deviation shows that experimental data were highly reproducible between batches especially when a new piece of reactor was used in every experiment. Cells specific growth rate ( $\mu$ ) attained under this condition i.e.  $0.291 \pm 0.02 \text{ h}^{-1}$  was also within a typical range for *S. cerevisiae* yeast cultivation. It was also demonstrated that the growth profiles of *S. cerevisiae* in the mini-bioreactor platform are representative for the results obtained in the 150 mL shake flask - a reactor system that is 100 fold larger in volume compared to the size of the mini-bioreactor prototype. In the current version of the mini-bioreactor, basic requirements to facilitate microbial fermentation experiment were met. Nevertheless, the proposed mini-bioreactor design can still be improved. As the next development step, the mini-bioreactor system is to be embedded with low cost pH and dissolved oxygen sensor spots and integrated with a suitable micropump to increase reactor flexibility and data quality.

## ACKNOWLEDGEMENTS

We would like to express our gratitude to the Ministry of Higher Education (MOHE), Malaysia and Universiti Teknologi Malaysia for funding the project. The project was funded by Research University Grant Scheme (vote number, 05J26).

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Received: 3 September 2015

Accepted: 8 December 2016