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A Low Cost Stirring Platform with Integrated Temperature Control Scheme for Microbioreactor Operation

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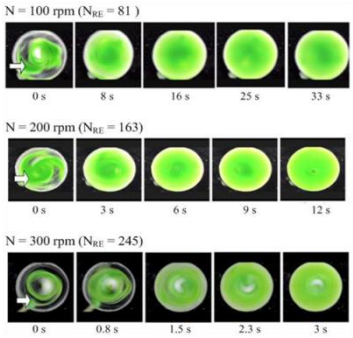
Graphical abstract



Abstract

In this paper, we presented the establishment of a cheap and simple stirring platform integrated with on/off temperature controller for microbioreactor operation. The stirring platform was designed to provide necessary mixing via magnetic stirrer bar for a microbioreactor setup. The microbioreactor (volume ~ 300 microliter) used in this investigation was fabricated out of the poly(methylmethacrylate) (PMMA) polymer material via micromachining. The reactor was deliberately designed to work under bubble-free conditions and limited only to batch operation. The paper first described the details of the mechanical design of the stirring platform and the microbioreactor prototype used in the work. These include the dimensions of the reactor and the stirring platform, positioning of the sensors and actuators employed, wiring connections, and the process control algorithm. Secondly, experimental results obtained to assess the mixing quality of the reactor and to characterize the performance of the controller (stirring and temperature) in terms of control accuracy and system responses were presented. We show that by implementing a rather straight forward control algorithm, the mixing quality and the temperature of the microbioreactor can be accurately controlled within an acceptable range of the set point values and provide a good response (i.e. in the range of few seconds). Results also showed that (1) at agitation rate of 300 rpm, mixing time as fast as 3 seconds was obtained and (2) reactor temperature can be tightly controlled at ±0.15 °C of the set point value.

Keywords : Microbioreactors; mixing, process automation; temperature control and micro-stirrer bar

Abstrak

Dalam kertas kerja ini, penghasilan pelantar pengadukan integrasi bersama sistem kawalan suhu buku/tutup untuk operasi mikrobioreaktor ditunjukkan. Pelantar pengadukan ini direkabentuk untuk menyediakan operasi pengadukan dengan menggunakan pengaduk magnet mikro bagi sistem mikrobioreaktor. Mickrobioreaktor (isipadu ~ 300 microliter) yang digunakan dalam ujikaji ini telah diperbuat daripada bahan polimer poly(methylmethacrylate) (PMMA) secara pembuatan-mikro. Reaktor tersebut direkabentuk untuk digunakan dalam keadaan bebas-buih dan terhad pada operasi berpukal sahaja. Kertas kerja ini pertamanya akan menerangkan tentang rekabentuk mekanikal pelantar pengadukan dan prototaip mikrobioreaktor yang digunakan dalam ujikaji ini. Ini termasuk dimensi reactor dan pelantar pengadukan, perletakkan pengesan-pengesan dan penggerak-penggerak, penyambungan wayar, dan algoritma proses kawalan. Keduanya, keputusan-keputusan eksperimen yang diperolehi untuk menilai kualiti pengadukan dan untuk menguji keberkesanan unit kawalan (pengadukan dan suhu) dari segi ketepatan kawalan, dan tindakbalas unit kawalan akan ditunjukkan. Kami tunjukkan bahawa dengan menggunakan algoritma kawalan yang yang mudah, kualiti pengadukan dan suhu mikrobioreaktor boleh dikawal dengan tepatnya dalam lingkungan yang boleh diterima dan memberikan tindakbalas yang bagus (iaitu dalam lingkungan beberapa saat). Keputusan menunjukkan bahawa (1) pada kadar pengadukan 300 rpm, masa pencampuran sepantas 3 s diperolehi dan (2) suhu reactor boleh dikawal dengan rapatnya pada ±0.15 °C daripada nilai yang disasarkan.

Kata kunci: Mikrobioreaktor; pengadukan; proses automasi; kawalan suhu dan pengaduk magnet mikro

1.0 INTRODUCTION

Microbioreactors are miniature size reactor system that generally fabricated to facilitate studies associated with bioprocessing e.g. fermentation experiments to screen for optimal growth conditions for cells [1-3], assessing novel enzyme processes [4-6], etc. Microbioreactors are indeed a new emerging technology and have been receiving increasing attention from the academia and

industry due to their numerous advantages in assessing biological processes. These include significant reduction in substrates consumption due to small working volumes (i.e. less than 1 milliliter), high throughput, remarkably fast mass and heat transfer rates, rich-information system and offer a good process control capabilities comparable to the bench scale bioreactor systems [7].

In microbioreactor operation, a good mixing scheme is imperative for (1) the transport and/or homogenous mixing of nutrients and substrates (e.g. oxygen, carbon sources, etc.), (2) keeping cells in suspension and finally, (3) to promote adequate mass and heat transfer rates through-out the reactor. Hence, allowing the system to handle a larger amount of biomass with increased reaction rates [7]. On a standard bench scale bioreactor system, intense mixing is normally applied to achieve necessary turbulence conditions (i.e. $N_{Re} > 2000$) for a good mixing [8]. On contrary, in a microliter scale operation, due to the low Reynolds number; liquid motion in the reactor is always in the laminar flow regime (i.e. $N_{Re} < 100$) [7,9] and often relies on the molecular diffusion rather than turbulence. Mixing in this state is terribly slow and inefficient. Therefore, alternatives to improve mixing condition in such a small reactor system are generally aimed at increasing the contact/interfacial area and/or reduce the diffusion lengths between two or more fluids [7,9].

Mixing in microbioreactors can be achieved by either passive mixing or active mixing schemes [7]. Table 1 shows advantages and disadvantages of various types of mixing schemes that have been implemented in the microbioreactor world to provide sufficient mixing for microbioreactor operation. In passive (or static) mixing schemes, no moving parts are applied and mixing is achieved by pumping the reactor content through a well-structured groove that is normally machined into a shallow microchannel. During mixing, fluid streams are repeatedly split and combined along the microchannel to achieve a homogenous mixing [10]. Indeed, seemingly like a viable solution but necessary three-dimensional microstructures for a sufficient mixing e.g. the staggered herringbone mixer [10] can be extremely difficult to fabricate. Moreover, such passive method does not guarantee that the cells will be in suspension state during operation. In the active mixing schemes, moving parts (e.g. orbital shaker [11], magnetic stirrer bar [1,3,5,9,12], micropumps and microvalves [2], syringe pump [13], etc.) are normally utilized for mixing of the reactor content. Active mixing schemes can be activated on demand and generally deployed to induce mixing inside the microbioreactor reaction chamber.

Table 1 Advantages and disadvantages for different mixing scheme options for microbioreactor operation

Mixing schemes	Types	Advantages	Disadvantages
Passive	T/Y shape and serpent-like mixers [4]	• Easy to fabricate • Simple reactor design • No moving parts • Continuous operation: possible	• Pump needed • Less effective for viscous medium • Microchannel can be excessively long • Parallel operation: costly if external pumps are used
	Staggered herringbone mixer [10]	• Simple reactor design • No moving parts • Shorten microchannel's length for mixing • Continuous operation: possible	• Difficult to fabricate • Difficult to scale-up • Pump needed • Parallel operation: costly if external pumps are used
	Shaker [11]	• Simple solution for lab • Parallel operation: possible (i.e. if user satisfied with the same mixing speed for every reactors)	• Not feasible for reactor with no headspace (i.e. induce little mixing) • Batch operation only • Impossible to scale-up
Active	Magnetic stirrer bar [1,3,5,9,12]	• Better for viscous medium • Prevent any dead zones • Easy to scale-up • Continuous operation: possible • Simple reactor design	• Parallel operation: costly (i.e. magnetic stirrer for each reactor) • Difficult to fabricate stirrer bar (except for the commercial ones)
	High velocity recirculation flow [2]	• Simple reactor design • Continuous operation: possible • High recycle flow improved mixing	• Pump needed • Slow mixing in the tubes • Complex fluidic connections for parallel operation
	Air cushions [13]	• Parallel operation: possible at different mixing speed • Prevent any dead zones • Continuous operation: possible	• Difficult to fabricate • Difficult to scale-up

One of the most commonly used active mixing methods for mixing in microbioreactor operation is the use a magnetic stirrer bar [1,3,5,9,12]. In the microbioreactor design of Zhang *et al*. [9], a ring-shape magnetic stirrer bar (6 mm arm length, 0.5 mm diameter) was fabricated and mounted on a hub (i.e. a rigid vertical post) for a steady mixing on a fixed rotational axis. Whilst this system creates defined liquid movement in the reaction chamber volume, it suffers from a couple of drawbacks

as well. First, both the stirrer bar as well as the hub needed to mount the impeller requires a precise fabrication which can be complicated and secondly, it cannot guarantee that there are no dead zones in the reactor: the corners between the vertical walls and the horizontal floor are typically the most critical point [7]. An alternative solution and perhaps a simpler approach is to use a commercially available micro magnetic stirrer bar [5,12]. Zainal Alam *et al*. [5] and Schäpper *et al*. [12] used a micro magnetic stirrer bar (3 mm length, 1.2 mm diameter, Sigma) and actuated it above a magnetic stirrer platform to create a random chaotic motion inside the reactor chamber. Thus, eliminates any possible dead zones whilst keeping the microbial cells in suspension. Since the magnetic stirrer bar is not mounted on any hub, it often hits the reactor walls and bounce back to the center of the reactor chamber in an irregular pattern. This makes it rather difficult to simulate with standard engineering software e.g. COMSOL, Fluent, etc. for optimization purposes. In any case, the use of magnetic stirrer bar is truly advantageous as it could create an intense local mixing and provides the necessary updraft lifting force to keep the microbial cells in suspension. Intense mixing created larger interfacial area and increase contact times for mixing. Reactor design is also simpler [5,9,12] and hence; minimize the reactor fabrication steps required.

In this paper, we presented the establishment of a low cost and simple stirring platform with integrated temperature control scheme for microbioreactor operation. The stirring platform was designed to provide necessary mixing via magnetic stirrer bar with inclusion of a cheap temperature control scheme for a microbioreactor setup. In our application we aimed at developing disposable single-use microbioreactors with minimal or no electrical connections on the single-use part of the reactor. Thus, keeping a low fabrication cost and decreasing the risk of handling errors during starting-up of the microbioreactor operation. The paper first described the details of the mechanical design of the stirring platform and the microbioreactor prototype used in the work. These include the dimensions of the reactor and the stirring platform, positioning of the sensors and actuators employed, wiring connections, and the process control algorithm. Secondly, experimental results obtained to assess the mixing quality of the reactor and to characterize the performance of the controller in terms of control accuracy and system responses were presented.

2.0 MATERIALS AND METHODS

2.1 Microbioreactor Design and Fabrication

The microbioreactor prototype presented in this work was fabricated specifically to facilitate two different types of experiments. The reactor was used (1) to assess the mixing quality of the reactor and (2) to evaluate the workability of the integrated temperature control scheme. It was designed to work with constant working volume, with no head space and under bubble-free conditions.

The prototype (Figure 1a) consisted of a reaction chamber (centered in the middle of the reactor) and a trapezoid shape side stand. The reaction chamber has a depth of 2.5 mm and a diameter of 12 mm, giving a volume of about 300 microliter. The bottom of the reaction chamber was covered by a thin poly(methylmethacrylate) (PMMA) layer (thickness ~ 0.5 mm) where else the top of the reaction chamber was left open. A magnetic stirrer bar with a length of 7 mm and thickness of 2 mm was placed on the reactor's floor for mixing. The side wall of the prototype was extended into a trapezoid shape side stand (thickness = 1 mm and surface area ~ 132 mm^2) to keep the reactor in balance (i.e. rigid position) during operation. In this prototype, there are neither microchannels nor additional ports for loading of the reactor content. The reactor content was filled by injecting a known amount of desired working solution from the top of the reactor which is already-opened. The reactor design was kept simple as (1) it will only be used for a proof-of-concept study and, (2) to minimize the fabrication steps needed.

The prototype was completely made of poly(methylmethacrylate) (PMMA) and was fabricated by using computer-numerical-controlled (CNC) milling machine (MDX-40A Benchstop Milling Machine, DGA Corporation, CA, USA). The scheme of the experimental setup for the microbioreactor prototype is illustrated in Figure 1b.

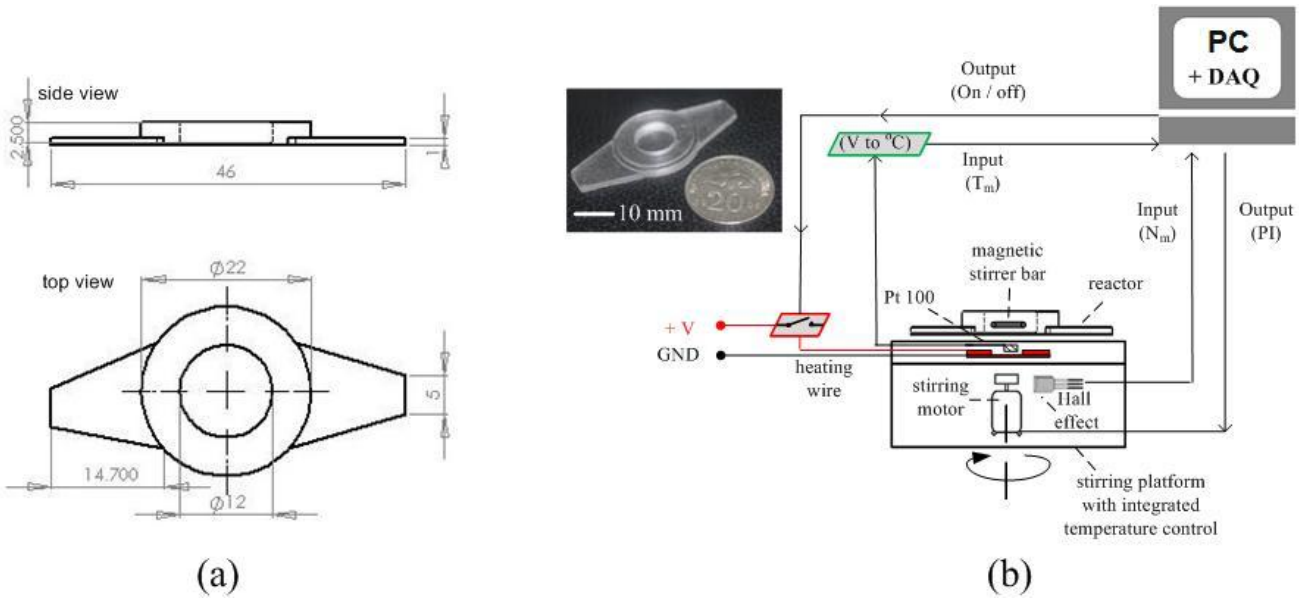


Figure 1 (a) Geometrical dimensions of the microbioreactor prototype, (b) Scheme of the experimental setup for the microbioreactor prototype. Inset is the actual image of the microbioreactor prototype

2.2 Stirring Platform: Mechanical Design

A cheap stirring platform with integrated temperature control scheme was customized to provide necessary mixing and temperature control capacity for the microbioreactor prototype (Figure 2a). For this purpose, a 12 cm (length) x 8 cm (width) x 5 cm (thickness) Polyvinyl chloride (PVC) housing was utilized. The platform was designed as a modular component containing a magnetic stirrer (built in inside the PVC housing) and a temperature controller plate (fixed via screws on the top of the PVC housing).

For the magnetic stirrer subcomponent, a neodymium earth magnet (i.e. salvaged from a computer hard drive) was milled and glued on a small plastic disc (diameter ~ 10 mm) before it was mounted on the shaft of a 6V direct current (DC) micro gear motor (SPG10-30K, Cytron Technologies Sdn. Bhd., Malaysia). In the setup also contained a Hall Effect sensor (A1301EUA-T, RS-Components). The Hall Effect sensor was used to measure the magnetic stirrer speed and it was placed as close as possible to the magnet on the motor in order to provide strong and persistent measurement signal. The distance between the tips of switch and the edge of the magnet is approximately 1 mm.

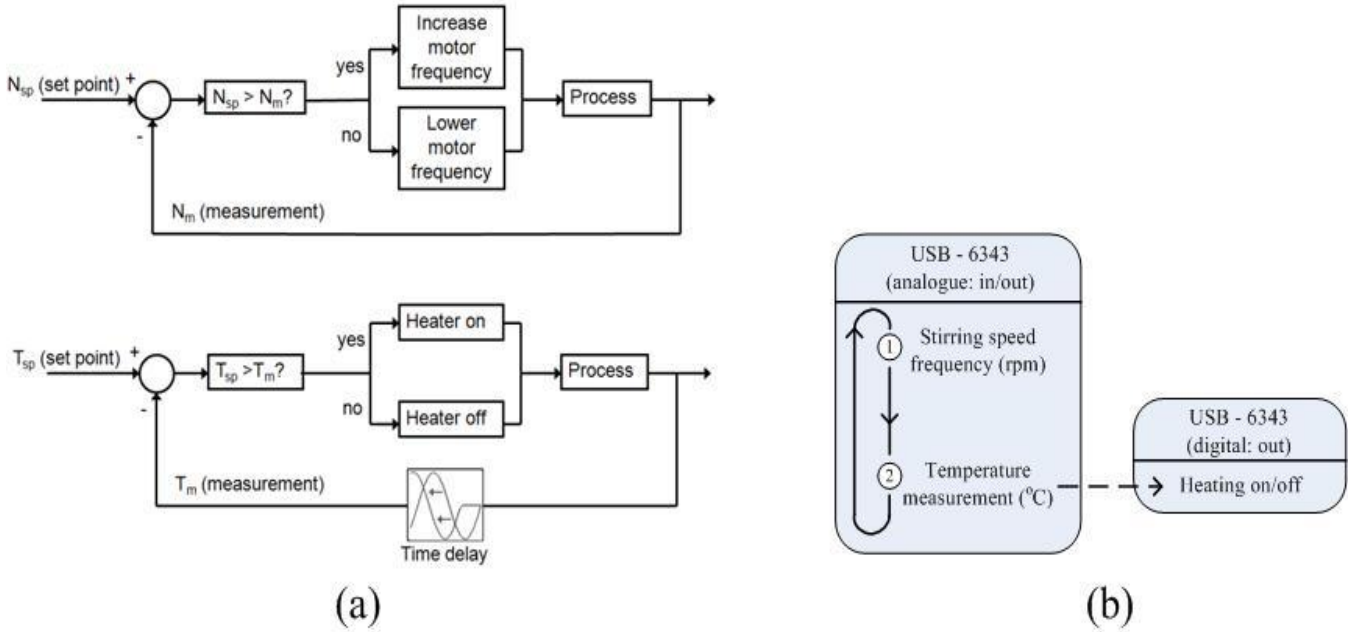


Figure 2 (a) Block diagram of the magnetic stirrer PI controller (top) and the on/off temperature controller (bottom), (b) The operational scheme of the magnetic stirrer and the temperature control routines

As for the temperature controller plate, it includes a miniature Pt 100 temperature sensor (PCA 1.2005.1L, JUMO GmbH & Co., Fulda, Germany) and a sub-miniature heater (12 mm x 6.7 mm x 0.6 mm, RS 615-1564, RS-Components) for temperature measurement and heating of the reactor content, respectively. The temperature control scheme realized for this setup was adopted from Zainal Alam *et al.* [14]. Figure 2b shows the exact positioning of the Pt 100 sensor (5 × 2 × 1.3 mm) and the microheater on the temperature controller plate. The heater was integrated in this manner in order to provide an even heat distribution into the reaction chamber. In the setup, the heater was connected to a DC power supply (converted from a standard ATX computer power supply with 3.3, 5 and 7 V outputs) for heating. The surface containing the sub-miniature heater was covered with a cell phone screen protector foil to prevent from excessive heat transfer to the reactor which may compromise the physical condition of the polymer substrate.

As shown in Figure 1, both the magnetic stirrer and the temperature controller plate were fixed directly underneath the microbioreactor's reaction chamber. Additionally, the sticky surface of the screen protector foil warrants a strong adhesion of the reactor to the platform and ensures that the reaction chamber will always be in direct contact with the heater. As demonstrated in Schäpper *et al.* [12], the additional thin layer on the bottom of the reactor offers virtually no thermal resistance and thus allows for the precise temperature measurement and heating of the reactor content.

2.3 Process Control Algorithm

Programs for the control routines were written in LabVIEW™ Academic Premium Suite software (National Instruments, Austin, TX, USA), and were implemented by interfacing the LabVIEW™ programs with a data acquisition (DAQ) card (NI USB-6343, National Instruments, Austin, TX, USA) for data logging and sending signals to actuators. In the microbioreactor prototype presented here, only the stirring frequency of the magnetic stirrer and the temperature were measured and controlled. Further analysis of the on-line measurement data (i.e. the stirring speed frequency and temperature measurement values) was done using Matlab v7.0 (The Mathworks, Natick, MA, USA).

2.3.1 Mixing

Mixing was achieved by means of a magnetic stirrer bar and the rotational speed of the magnetic stirrer was measured by using an analog Hall Effect sensor. The Hall Effect sensor produces a square wave output signal depending on the amount of magnetic field it encounters. In our application, the output square wave

signal of the Hall Effect sensor was based on the number of passes made by the magnetic stirrer i.e. in revolution per minute (rpm). Sampling frequency used to obtain the square wave signal (via LabVIEW) was 1500 Hz (samples per second). Additionally; the square wave pulses generated by the Hall Effect sensor were confirmed by using an oscilloscope (data not shown). The magnetic stirrer stirring frequency was adjusted (i.e. either increase or decrease of stirring frequency) by a proportional-integral (PI) control algorithm whose error signal was based on the deviation between the measured and the desired stirring frequency values (Figure 2a). A power transistor was connected between the DC power supply and the DC motor to amplify the analog output signal from the DAQ as the analog outputs do supply enough current to drive the DC motor during operation. The magnetic stirrer PI controller was tuned using the Ziegler-Nichols tuning method [15]. A set point tracking experiment was also performed to assess the performance of the PI controller based on its accuracy, response time and settling time.

2.3.2 Temperature control

The temperature of the reactor content was controlled by an on/off controller which was adopted from Zainal Alam *et al*. [14]. The operation of the temperature controller is presented in Figure 2a. In this feedback temperature controller, analog voltage signals received from the temperature sensor (Pt 100) were linearized and converted to temperature values (^oC) via a transmitter (JUMO dTRANS T04, JUMO GmbH & Co., Fulda, Germany). Deviation (error) between the desired set point value T_{sp} and the measured value T_m will then be computed by LabVIEW program. Heating will only be activated if the T_m value goes below the desired T_{sp} value (i.e. if the error is positive, $T_{sp} > T_m$), else the heater will remain in off state. Heating is achieved by passing an electrical current at fixed voltage supply through the resistance wire embedded on the stirring platform. A latching relay was used to connect the DC power supply to the heating wire for heating. A 1 s delay was introduced in the routine to prevent rapid switching of the relay output [14]. The on/off temperature control scheme was tuned by applying various power inputs (i.e. at 3.3, 5 and 7 V) at different temperature set point values between 25^{o}C and 50^{o}C. The tuning step was necessary to achieve optimal on/off controller settings (good accuracy and fast response).

Both of the control routines presented here were set to operate sequentially in continuous loop where it involves the use of analog in-/output (AI/AO) channels and digital output channel of the DAQ card. The operational scheme of the LabVIEW program is presented in Figure 2b. The LabVIEW program was initiated by first performing the measurement and control of the magnetic stirring frequency via the AI/AO ports. In sequence, the program then read the temperature measurement data through a different AI port and gives out the resulting digital output value for the temperature control part. The routine was then looped back to the first step to repeat the whole cycle again. One complete cycle runs for approximately 3 s before restarting (excluding the time consumed for heating). All experiments were repeated twice to check data reproducibility.

2.4 Mixing Test: Fluorescent Dye Visualization Technique

The mixing quality of our microbioreactor prototype was assessed using the fluorescent dye visualization technique [5]. The experiment was performed by first filling the reactor with the desired working solution, which was then followed by adding a drop of fluorescent solution (prepared by diluting a concentrated fluorescein green solution with distilled water at mixing ratio 1:1) with a syringe. Mixing effects were observed and images of dispersion of the fluorescent solution in the reactor were recorded by a digital camera (Nikon D90, Nikon Corporation, Japan). Mixing time was estimated at various agitation rates (i.e. at 100 rpm, 200 rpm and 300 rpm) by using water as the model solution. Duplicates were made for each condition tested. Mixing time was recorded as the time for the fluorescent dye to be completely dispersed.

A rather straight forward image analysis was also performed to graphically assess the homogeneity of the reactor content at various mixing conditions. Recorded images were uploaded and analyzed via ImageJ software (Wayne Rasband, National Institute of Health, USA) [16]. Analysis was based on the difference between the background colour and the colour intensity of the concentrated dye injected into the microbioreactor chamber. The colour difference was computed and the homogeneity level in the microbioreactor chamber was represented as total area covered by the concentrated dye (i.e. percentage area covered).

3.0 RESULTS AND DISCUSSIONS

3.1 Controller Performance

Figure 3 shows the response of the microbioreactor PI stirring controller and the on/off temperature controller for a series of step changes in the agitation and temperature set point values, respectively. For the PI stirring controller, the agitation rate, N (rpm) was raised from an off-state condition (i.e. initial condition at zero voltage supply) until it reached the agitation rate, N of approximately 600 rpm which is the maximum speed achievable by the DC motor used in this microbioreactor setup. As previously mentioned, the controller was tuned according to the Ziegler-Nichols tuning method. The controller constants attained were 0.3 and 0.017 for the proportional, K_c and integral, T_i constants, respectively. Since a readily available sub-VI (a sub program attained from the LabVIEW software library) for the PI stirring controller was used, the controller constants can also be automatically adjusted and/or refined to give better approximations of the PI stirring controller i.e. if needed so. Based on the data obtained (Figure 3a), it was found that the PI controller responded nearly instantaneously in every step change applied with a recorded response time of about 0.001 ms. A slight overshoot and/or undershoot ($\underset{\sim}{2}$ rpm from the set point values) with settling time of approximately 8 s when increasing and/or decreasing the agitation rate are expected behavior for such PI controller. As presented in Figure 3a, the accuracy attained for the PI stirring controller was $\pm$ 1 rpm of the set point value. For the on/off temperature controller, the controller was tuned to operate at heater pulse length of 1 s and connected to voltage supply of 5 V. It was found that by implementing such settings the temperature can be controlled to within ±0.15 ^{o}C of the set point value. This is illustrated in Figure 3b. Moreover, no delay was observed when regulating the temperature set point values from one set point to another. This is mostly due to the high surface to volume ratio, S/V (1100 m^{-1}) of the microbioreactor which facilitates a fast thermal response. The fluctuating measured signal (as seen in Figure 3b) is a typical behavior of the on/off controller. The heating rate and the cooling rate of the reactor were calculated to be 0.36 $^oC \cdot s^{-1}$ and 0.16 $^oC \cdot s^{-1}$, respectively. The cooling step was rather slow compared to the heating capacity of the controller as there is no cooling element was realized in the setup and cooling was

merely depends on heat dissipation to the surroundings. Eventually, room temperature will be reached asymptotically as the driving force is the temperature difference itself. Our results are consistent with what have been reported by Zainal Alam *et el.* [14].

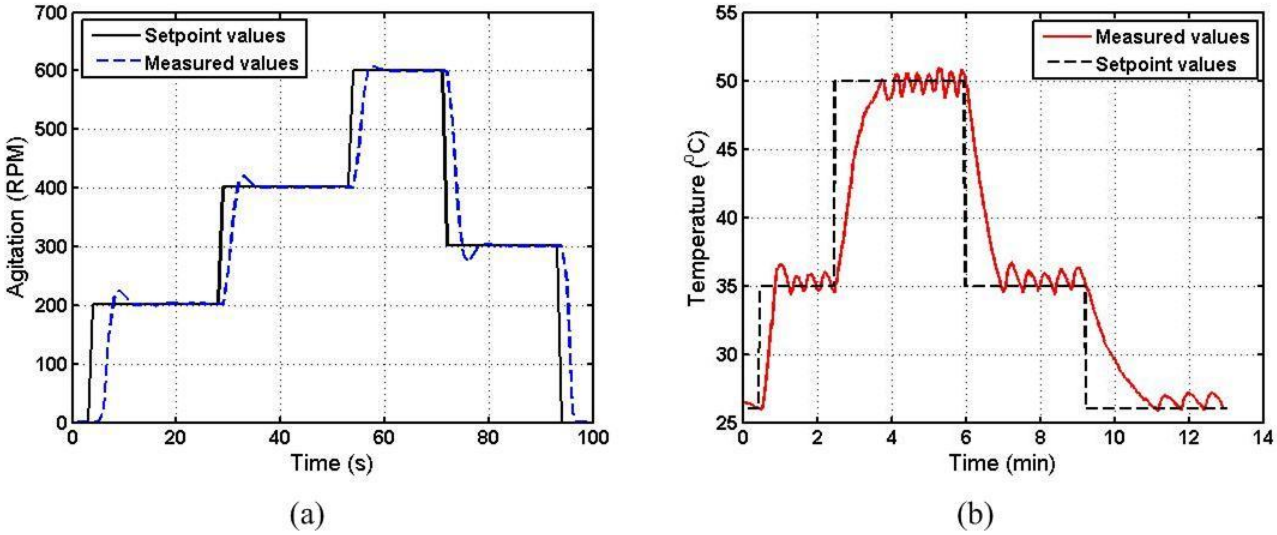


Figure 3 Figure 3. Response of the microbioreactor PI stirring controller (left) and on/off temperature controller (right) for a series of step changes in the agitation and temperature set point values, respectively

3.2 Mixing Quality And Homogeneity

The mixing quality of the microbioreactor was evaluated by using a simple tracer method i.e. by visualizing the dispersion rate of a very small amount of concentrated fluorescent solution. Such method enabled us to visualize the mixing pattern inside the reactor and predict the mixing time at various agitation rates. Results obtained from the mixing test experimentation are depicted in Figure 4. Based on the time-course images in Figure 4, it can be seen that the fluorescent solution immediately unwound and disperse uniformly throughout the reaction chamber due to the stirring action of the magnetic stirrer bar. Regardless of the agitation rate applied, uniform color distribution was eventually obtained after a certain period of time. The obvious concentration difference between the concentrated fluorescent solution and the mother solution (water) led to diffusion of the concentrated dye into other regions within the reaction chamber where else the vigorous stirring action of the magnetic stirrer bar breaks the concentrated dye to create larger interfacial area for mixing. A longer mixing time was recorded for slower agitation rate i.e. at *N*=100 rpm mixing time is approximately 33 s but at higher agitation rate (i.e. at *N*=300 rpm), the mixing accelerated and a mixing time of about 3 s was recorded. A faster mixing was achieved at higher agitation rates because at increasing rates, the degree of local turbulence also increases which promotes better mixing. The degree of local turbulence was represented by the Reynolds number, N_{RE}. Reynolds number can be defined as a dimensionless number of that gives a measure of the degree of turbulence in a confined space [17]. It was found that the turbulence conditions within the microbioreactor reaction chamber was shifting from laminar flow regime (N_{RE} = 81) to transition phase (N_{RE} = 245) when the agitation rate was increased from *N*=100 rpm to *N*=300 rpm, respectively. Results attained in this study are comparable with data from published investigations on mixing in microbioreactor setup via magnetic stirrer bar. Zhang *et al.* [9], integrated a ring-shape magnetic stirrer bar (6 mm arm length, 0.5 mm diameter) and a mixing time of 30 s was achieved when operating at agitation rate, *N* of 180 rpm (the microbioreactor volume was 150 microliter. Zainal Alam *et al*. [5] used a commercial magnetic stirrer bar (3 mm length, 1.2 mm diameter, Sigma) to stir a sugar beet pectin solution (solution concentration = 10 g/L) and uniformity was obtained in less than 30 s when operated at agitation rate, *N* of 500 rpm (the microbioreactor volume was 200 microliter). Similarly in Schäpper *et al.* [12], a commercial magnetic stirrer bar (3 mm length, 1.2 mm diameter, Sigma) was used and a complete mixing was observed after 1.2 s (the microbioreactor volume was 100 microliter).

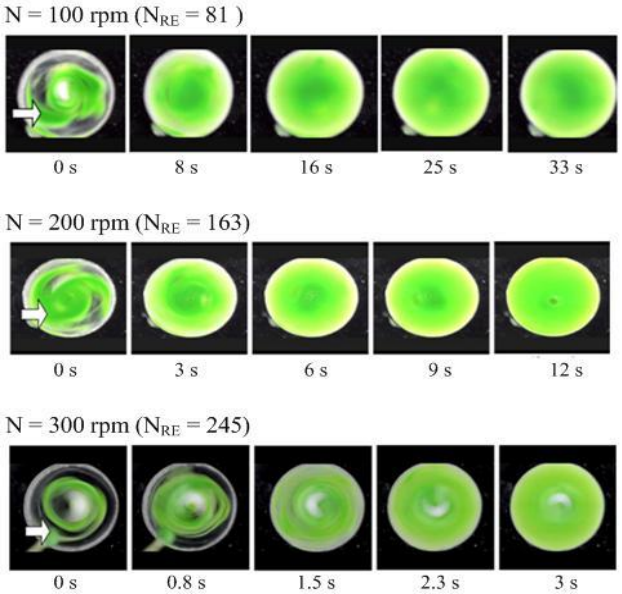


Figure 4 Images of mixing of the fluorescent dye solution in the microbioreactor at various stirring speed i.e. *top*: *N* =100 rpm (*NRE= 81*), *middle*: *N*=200 rpm (*NRE= 163*) and *bottom*: *N*=300 rpm (*NRE= 245*). Arrows indicate the point of injection

From the results in Figure 4, uniform colour distribution seemingly indicated that the reactor content was well-mixed without any "dead zones". However, to confirm this, we assessed the quality of the image by using the graphical analysis software (ImageJ software, Wayne Rasband, National Institute of Health, USA) [16]. If the homogeneity level is approximately or greater than 95% (i.e. based on the difference of colour intensity), it can be assumed that a well-mixed condition was successfully achieved. This was however not the case in our microbioreactor setup when the agitation rate was set at stirring speed 200 rpm and below (Figure 5). At this condition, the reactor content was only 80-85% homogenous. As evident in Figure 5c, a nearly perfect homogenous solution was sufficiently obtained when operating at agitation rate of 300 rpm. The results further suggested that for our microbioreactor operation, conducting the microbioreactor experiments at agitation rate in the range between 300 rpm to 400 rpm is sufficient to achieve a good and the necessary homogenous reactor content. Although the micro magnetic stirrer bar was not attached to a fixed axis and rotating in an irregular movement, it did not create any limitations for our microbioreactor operation. On contrary, it seems that such irregular rotating motion eliminates the unnecessary 'dead-zones' within the microbioreactor chamber particularly on the edge and corners of the reaction chamber walls.

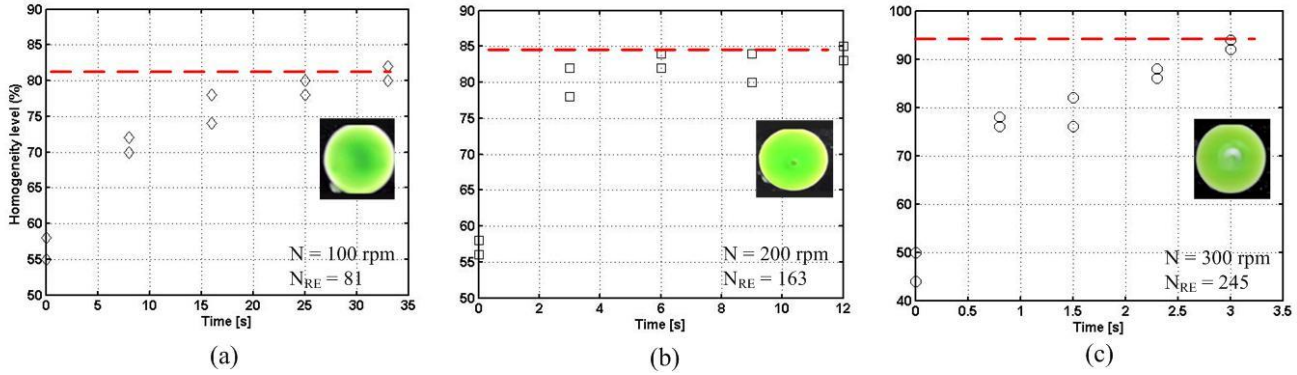


Figure 5 Results of image analysis representing the level of homogeneity of the microbioreactor content at various stirring speed i.e. (a) N=100 rpm (N_{RE}= *81*),. (b) N=200 rpm (N_{RE}= *163*) and (c) N=300 rpm (N_{RE}= *245*). Dashed red lines representing the level of homogeneity achieved at given conditions and inset illustrates the final state of the reactor content

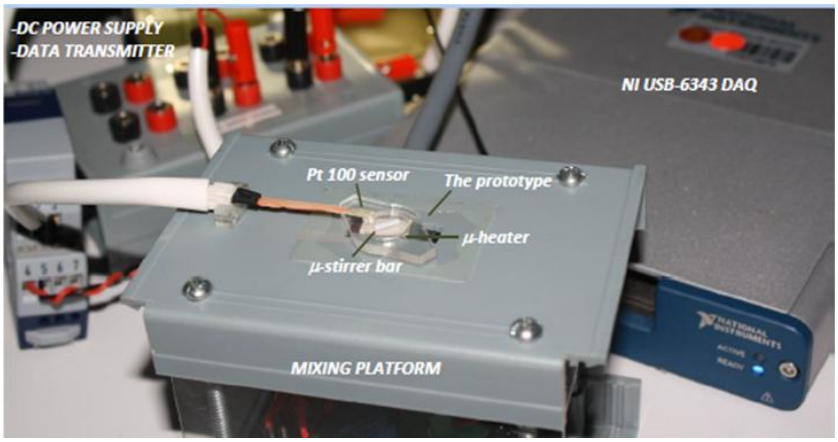


Figure 6 Picture of the actual microbioreactor setup established in the Process Systems Engineering Centre (PROSPECT), UTM hands-on laboratory

3.3 Practical Feasibility Of The Microbioreactor Setup

The whole idea of the work was to fabricate a low cost and feasible operating gear for the microbioreactor operation. In our microbioreactor system, the plan was to keep the microbireactor design as simple as possible and separate the essential sensors and actuators to control the microbioreactor operation on a separate platform. As a separate unit made completely from a cheap poly(methylmethacrylate) (PMMA) polymer that featured only a reaction chamber and a micro magnetic stirrer bar; the microbioreactor can definitely be made disposable. Additionally, the microbioreactor would be easy-to-handle as no cleaning nor sterilization steps needed when starting new experiment.

The operating platform was customized by using parts and electronic components that are commercially available inexpensively in any hardware and/or electronic store. The capital cost was approximately 100-125 € / setup. Despite its simplicity, the operating platform allows users to vary the agitation and the temperature set point values accordingly. The agitation rate can be adjusted by changing the frequency of the DC motor (possible operating range between 0 to 600 rpm) and the temperature of the reactor content can be varied by controlling the on/off pulse length of the micro-heater (possible range between room temperature and 50^{o}C). Such flexibility provides one to carry out experimentation and/or investigations by using the microbioreactor setup at various experimental conditions. The microbioreactor at its current state is only suitable to cater batch biocatalyst processes under buffered

conditions. Surely, future improvements is needed e.g. integration of on line UV detection method, dissolved oxygen control, etc., such that the microbioreactor setup can also be used to carry out fermentation experiments. Based on the experimental results (Figure 3 and Figure 4), fast mass and heat transfer rates i.e. in the range of few seconds demonstrated the main advantage of conducting experimentation in sub-milliliter range. The only minor drawback for our setup is the bulky and the expensive National Instruments (NI) data acquisition card (DAQ) for execution of the process control routines. The unit is however imperative for the microbioreactor operation and a cheaper NI data acquisition cards are also available which could perform similarly to what was used in this microbioreactor setup i.e. the NI USB 6343 (Figure 6).

■4.0 CONCLUSIONS

A cheap and simple stirring platform integrated with on/off temperature controller for microbioreactor operation was developed. The microbioreactor prototype was fabricated from poly(methylmethacrylate) (PMMA) polymer and separated from the stirring platform. This was deliberate as the plan was to bring forth the single-use (i.e. disposable) microbioreactor concept. It was demonstrated that the set point values for the agitation rate and the reactor temperature can be precisely controlled within acceptable range of the set point values. The controller is also user-friendly as to operate the microbioreactor system, one need only to adjust the frequency of the DC motor for mixing and vary the heating pulse length for the temperature control. Further improvements are necessary in order to push the capacity of the microbioreactor technology such that it can facilitate fermentation experiments rather than limiting its usefulness only for biocatalyst processes.

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