

## Overview on Design Considerations for Development of Disposable Microbioreactor Prototypes

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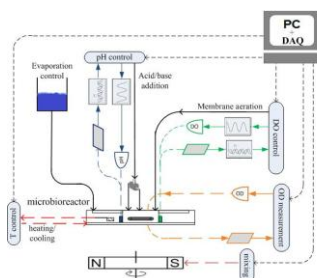
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### Abstract

Microbioreactors are a miniaturized scale bioreactor system normally designed for bioprocess development. Such a microbioreactor design offer a new platform in carrying out cheap fermentation experiments under well controlled conditions – comparable to that of typical bench scale bioreactors. Additionally, by adapting polymer technology, microbioreactor can for example be made disposable. Thus, eliminates the need for cleaning of the reactor at the end of every experiment. Since typical working volumes of microbioreactors are less than 1 mL, this furthermore reduces substrate and utility consumption per experiment. Additionally, often a microbioreactor system is interfaced with optical measurements to acquire a real-time experimental data and thus, increases the amount of information gained per experiment. To design a microbioreactor system, one must consider the design of the entire system that drives the reactor and not just restrict the design only to the mechanical aspects of the reactor. In this paper, important design considerations as well as technical challenges for establishment of a microbioreactor to facilitate a typical aerobic fermentation processes are discussed. These include reactor operating feature and size, reactor mechanics (materials, fabrications, and mixing), reactor fluidics (connections, aeration, evaporation and feeding strategy), process control of physical parameters (temperature, pH, and dissolved oxygen level) and detection methods for measuring the cells concentration.

**Keywords:** Fermentation; microbioreactor; microfabrication; process control

### Abstrak

Mikrobioreaktor adalah sistem bioreaktor skala minatur yang kebiasaannya direkabentuk untuk pembangunan bioproses. Rekabentuk mikrobioreaktor seperti ini memberikan pendekatan yang baru dalam menjalankan eksperimen-eksperimen fermentasi berkos rendah pada keadaan terkawal – menghampiri pada keadaan yang secara tipikalnya diperolehi di bioreaktor skala makmal. Malah, dengan mengadaptasikan teknologi polimer, mikrobioreaktor boleh dijadikan sebagai bahan pakai-buang. Secara tidak langsung ini membasmi langkah yang diperlukan untuk pembersihan reaktor di akhir setiap eksperimen. Oleh sebab isipadu kerja yang tipikal bagi mikrobioreaktor adalah kurang dari 1 mL, ini akan mengurangkan penggunaan bahan dan utiliti bagi setiap eksperimen. Sistem mikrobioreaktor juga sering dilengkapi dengan cara pengukuran optik untuk mendapatkan data-data eksperimen benar dan ini seterusnya meningkatkan maklumat-maklumat yang boleh diperolehi setiap eksperimen. Untuk merekabentuk sistem mikrobioreaktor, adalah perlu untuk menilai rekabentuk bagi keseluruhan sistem dan bukan menghadkan rekabentuk hanya pada ciri-ciri mekanikal reaktor sahaja. Dalam kertas kerja ini, dibincangkan penilaian rekabentuk yang penting dan ciri-ciri teknikal bagi penghasilan mikrobioreaktor untuk proses fermentasi aerobik. Ini termasuk saiz dan cara operasi reaktor, ciri-ciri mekanik reaktor (bahan-bahan, fabrikasi dan pengadukan), ciri-ciri bendalir reaktor (penyambungan, pengudaraan, pemeruapan dan strategi suapan), proses kawalan parameter fizikal (suhu, pH, dan oksigen terlarut) dan cara-cara untuk pengukuran kepekatan sel.

**Kata kunci:** Fermentasi; mikrobioreaktor; mikrofabrikasi; kawalan proses

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## 1.0 INTRODUCTION

Bioprocess development typically involved series of fermentation experiments to screen for a new strain/bioproduct and/or bioprocess optimization for higher yield and productivity. Currently, fermentation experiments are normally performed in either shake flasks or bench scale bioreactor systems. Shake flasks can easily operate in parallel and with small volumes but the attainable level of control and the flexibility in achievable operation modes (i.e. typically operate in batch) are rather limited. Furthermore, shake flask operations require frequent sampling which increases risks of cells contamination. Bioreactor on the contrary is a more versatile experimental tool and offers a tight control over reactor variables (dissolved oxygen level, pH and temperature). Nevertheless, low throughput (i.e. numbers of experiment can be performed in a single run) and high substrates consumption (typical reactor volume is approximately 1 L or more) limits its usefulness for bioprocess development. Moreover, conducting experiments with bench-scale bioreactors is laborious due to the efforts required for preparation and cleaning of the reactor [1]. In this respect, microbioreactor technology offers several distinct advantages for carrying out fermentation processes.

Firstly, microbioreactors operate with very small volumes (i.e. L to nL range) – even when operate in continuous mode – thus, significantly reduced the volume/amount of substrates and enzymes used per experiment. Secondly, microbioreactors have a very high surface to working volume ratio,  $S/V$  (i.e. in the order of 1000) which resulted in an increased in heat and mass transfer rates. Third, microbioreactor is often interfaced with optical measurements to acquire a real-time experimental data and thus, increases the amount of information gained per experiment. Microbioreactors can also be integrated with sensors and actuators to achieve satisfactorily level of control for carrying out fermentation processes. Additionally, by adapting polymer technology, microbioreactor can for example be made disposable. Thus, eliminates the need for cleaning of the reactor at the end of every experiment. Finally, microbioreactors can be scaled out to platforms of multiple reactors, thus greatly increasing throughput for elucidating strain behavior under various relevant bioprocessing conditions [2]. An increasing demand for a high through-put cultivation technology has indeed become the driving force for the development of microbioreactors in the bioprocess engineering field.

To design a microbioreactor system is however not so straight forward. One must consider the design of the entire system that drives the reactor and not just restrict the design only to the mechanical aspects of the reactor. In this paper, important design considerations as well as technical challenges for establishment of a microbioreactor to facilitate a typical aerobic fermentation processes are briefly discussed. These include reactor operating feature and size, reactor mechanics (materials, fabrications, and mixing), reactor fluidics (connections, aeration, evaporation and feeding strategy), process control of physical parameters (temperature, pH, and dissolved oxygen level) and detection methods for measuring the cells concentration. The focus of this paper is on the type microbioreactors that allow fermentations with microorganisms growing in suspension, because the majority of industrial fermentations are based on this type of process.

## 2.0 REACTOR OPERATING FEATURE AND SIZE

Due to small operating volumes, microbioreactors often designed to work under bubble-free conditions [2]. This means that, the

reactor will be completely filled with liquid (i.e. no head space) and operates at a constant volume (i.e. no volume increase during operation,  $dV/dt = 0$ ). Bubbles are not desirable in such a small reactor system because their relatively large size i.e. compared to the size of the microchannels (1) would easily clog the microchannels or even block fluidic ports with possibility of compromising the leak-free operation of the reactor, (2) occupy a large volume of a microbioreactor reaction chamber which would perturb the stirring/pumping motion for mixing and finally, (3) the presence of bubbles would also disturb any *on-line* measurements in microbioreactors – consequently, rendering them useless. Formation/presence of air bubbles is indeed a major obstacle for successful microbioreactor operation, and it is crucial that they are either; removed via an integrated bubble-trap [3], prevented from entering the system via a passive bubble filter [4] or purged out of the system before initiating the experiment [5].

The size (i.e. working volume) of a typical microbioreactor system is certainly a debatable issue. Nevertheless, the microbioreactor size is normally less than 1 mL with characteristic dimensions in a range of 10-1000  $\mu\text{m}$ . The advantage in keeping the reactor size small besides significantly reducing the amount of substrate consumption per experiment is that firstly, smaller reactor size guarantees an excellent mass and heat transfer rates due to a high surface area to volume,  $S/V$  ratio and secondly, smaller reactor size would maintain a small reactor footprint. Nevertheless, take note that as size decreases, the less instrumented the reactor will be [6]. This is due to the limited ability to manipulate essential reactor variables within a small working volume. Moreover, one also needs to provide sufficient spacing for placement of sensors for *on-line* measurements of reactor variables e.g. cells optical density, pH, temperature, dissolved oxygen level, etc. Generally, microbioreactors are designed to take the shape of a typical cylinder (i.e.  $\text{volume} = \pi r^2 h$ ). This is an important consideration as it will ease later scaling-up step to a larger scale of operation.

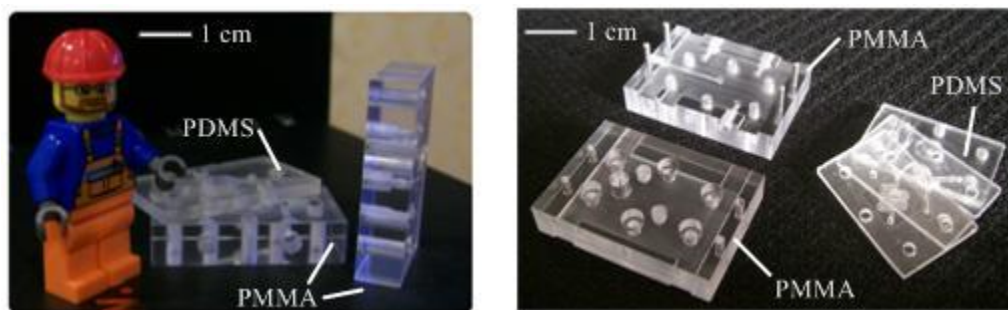
## 3.0 REACTOR MECHANICS

### 3.1 Materials and Fabrications

Microbioreactors are generally fabricated by using polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS) polymers as substrates for fabrication. Both PMMA and PDMS polymers are cheap materials for microfabrication, easy to handle and offers the possibility to fabricate two- (2D) and three-dimensional (3D) microfluidic geometries via casting and micro-machining procedures [7]. Examples of 2D and 3D microfluidic geometries made of PMMA and PDMS polymers are illustrated in Figure 1. In terms of microbioreactor operation, both PMMA and PDMS (1) have a good optical quality (i.e. optically transparent in visible wavelengths) which facilitates optical measurements, and (2) biocompatible in the sense that microbioreactor can be made disposable and thus reduce preparation efforts and eliminate the need to clean the reactor after every experiment [2].

Despite these advantages, PMMA and PDMS polymers have several limitations. Firstly, a high gas permeability of thin PDMS layer which promotes good oxygen permeation for aerobic fermentation process can also lead to unwanted levels of water vapor evaporation. Nevertheless, this drawback can be dealt with by either operate the reactor system in continuous mode or passively replenish the evaporated water vapor [2,9]. Secondly, both PMMA and PDMS substrates are not compatible with organic solvent and have poor resistivity towards strong acids and base solutions [7]. This is however not a major issue for microbial

fermentation process as water (not organic solvents) is the commonly used solvent in preparing fermentation broth.

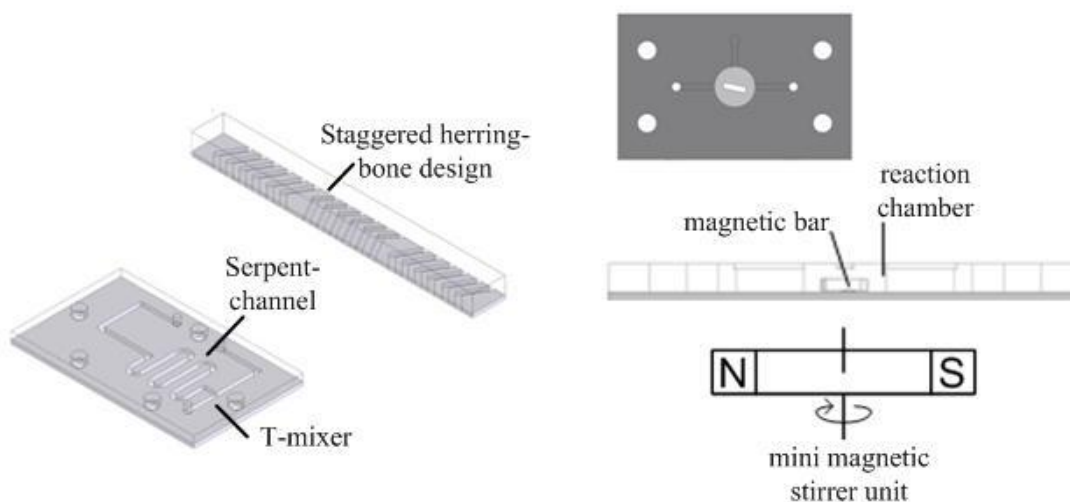


**Figure 1** Examples of possible microfluidic geometries made of PDMS and PMMA fabricated via casting and micro-machining procedures [8]

### 3.2 Mixing

Mixing is crucial in microbioreactor operation as (1) it promotes uniform heat distribution, (2) keeping the cells in suspension and (3) for the transport of nutrients and substrates (i.e. oxygen, carbon source, etc.) through-out the reactor. In microbioreactors, liquid motion in the reactor is always in the laminar flow regime (i.e.  $N_{Re} < 100$ ) and mixing often relies on molecular diffusion rather than turbulence. Mixing in microbioreactor can be achieved either by passive or active mixing schemes. In passive (or static) mixing schemes, no moving parts are involved. Mixing is achieved by pumping/passing unmixed volumes of liquids into 3D microfluidic geometries. Often, shallow and well-structured grooves e.g. T/Y-shape mixer, serpent-like channel, staggered herringbone design [10-11], etc. are fabricated into a microchannel to facilitate passive mixing

schemes (Figure 2a). Although these 3D microstructures can be very difficult to fabricate but these complex structures are necessary to develop a strong chaotic advection mixing. In active mixing schemes, different actuation mechanisms are implemented to create larger molecular interfacial-area for mixing. Mixing is achievable either by (1) changing the reactor boundaries by creating a peristaltic motion of the liquid inside a reactor chamber via on-board micropumps [12] (2) pumping the reactor content back and forth between to serially connected reactors [13] or by (3) stirring the reactor content with a magnetic stirrer bar [8]. The use of magnetic stirrer bar is advantageous and perhaps the most straight forward solution for the lab (Figure 2b). By using a magnetic stirrer bar, hypothetically, an intense local mixing is created and diffusion distance is minimized. Thus, promote a better mixing of the microbioreactor content.



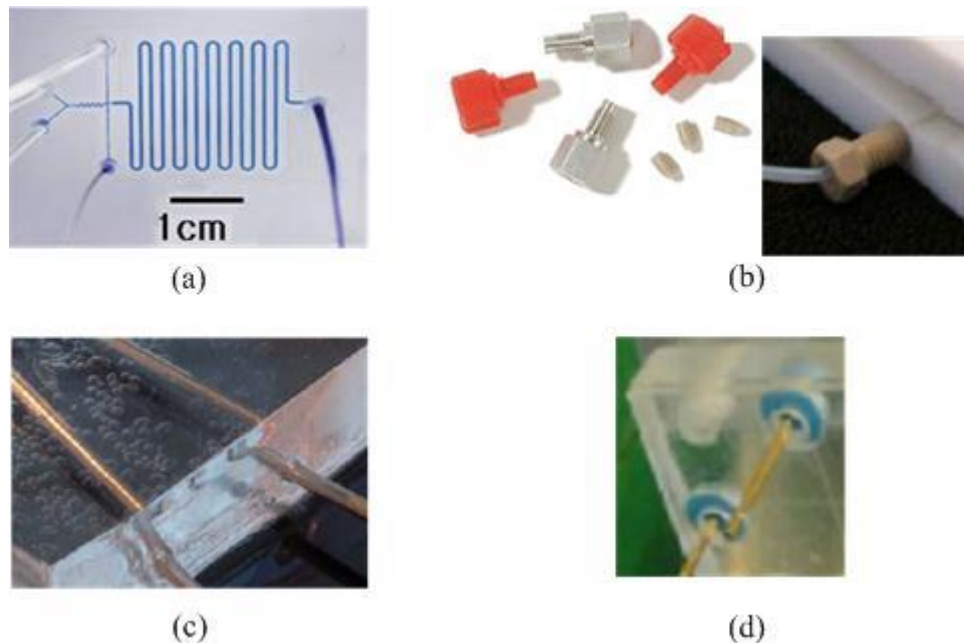
**Figure 2** (a) Examples of 3-D microfluidic channels to facilitate passive mixing schemes [10,11] .(b) Active mixing via magnetic stirrer bar. Inset is the top view of the microbioreactor [8,9]

## 4.0 REACTOR FLUIDICS

### 4.1 Fluidics Connections

Often, a microbioreactor system is interfaced to its macro world counter parts e.g. syringe pump, feed reservoir, etc. during operation for delivery of liquid to and/or from the

microbioreactor system. Reliable fluidic connections are essential and satisfactorily solution is always a compromise between easy handling, cost and specific system requirements (e.g. operating temperature, pH, etc). Examples of various types of fluidic interconnects that may be suitable to be implemented as microbioreactor fluidic interconnects is illustrated in Figure 3.



**Figure 3** Examples various fluidic interconnects that have been implemented in microbioreactor world. (a) Gluing tubes into fluidic ports [14]. (b) Mounting standard chromatography fittings (e.g. VICI Jour, Upchurch Scientific, etc) on microbioreactor surface [13]. (c) Piercing needle through PDMS substrates [2] (d) Metal ferrules and O-rings fluidic connections [16]

For a simple (and fast) solution, one could directly glue standard size chromatography tubing into a fluidic port or hole that has been fabricated on the interface of the microbioreactor system [14]. Technically, the size of the fluidic port is normally one-tenth smaller than the outer diameter of the tube and adhesives (i.e. glue or epoxy) is normally applied around the fluidic connection to achieve a water-tight connection. Such solution seemed like a conclusion drawn from personal experience rather than a scientific method but gluing has been reported as the commonly used method for fluidic interconnection of microstructures [15]. Despite its simplicity and inexpensive fabrication cost, such permanent connection is not so reliable and can easily disconnect from the fluidic port during experiments if steady connections are not guaranteed. A more versatile solution is to connect the tube into a metal ferrules instead of directly glue the tube to the fluidic port [16]. Leak-free operation is ensured with the use of soft intermediate element such as O-rings. Concentric shape grooves can be fabricated around the connection holes to facilitate proper alignment of the O-ring. Combination of O-rings and the mechanical stress applied to the connections guarantee a water-tight fluidic interconnect. Additionally, this type of connection allows for tubing to be connected and/or disconnected repeatedly i.e. a reversible connection. Commercial fittings/connectors (e.g. from Upchurch Scientific, Vici Jour, etc.) can also be used to establish a reliable fluidic connections for microbioreactor system [13] – particularly for reactor that is made of thermoplastic polymers such as Polyetherether ketone (PEEK), polymethylmethacrylate (PMMA), etc. A threaded port can be fabricated on microbioreactor interface to fit these specialized connectors. Although these commercial fittings are relatively expensive and occupy a large area when mounted to the reactor, it offers a *plug 'n' play* solution for the microbioreactor system, inert, have a low dead volume and leak-proof over a wide pressure range.

#### 4.2 Aeration, Evaporation and Feeding Strategy

Aeration is critically important for aerobic fermentation and in most bubble-free microbioreactor system; it is achieved by diffusion of oxygen through a thin semi-permeable polydimethylsiloxane (PDMS) membrane (i.e. in the order of approximately 50 to 100  $\mu\text{m}$ ). The use of a thin PDMS membrane is indeed an ideal solution for aeration (i.e. oxygen supply) in bubble-free microbioreactor operation since only gas molecules may pass through the membrane. But, unfortunately, such a thin PDMS membrane also allows for diffusion of water vapour which consequently, could lead to undesirable level of water evaporation. Evaporation rates in the order of 5  $\text{L}\cdot\text{hr}^{-1}$  (water at 37°C) have been reported [17]. Water evaporation is a major concern in microbioreactor operation and if not all in most microsystems. If the evaporated water is not replenished or prevented, the reactor content would eventually dries out and bubbles would occur in the reactor system. The presence of bubbles – as previously mentioned – could significantly interfere with the optical measurements installed in the microbioreactor system.

In most bench-scale bioreactor system, the reactor is inoculated by injecting a known volume of inoculum (normally 5-10% of the reactor working volume) into one of its inlet fluidic ports. This technique is not very suitable to be adopted into microbioreactor system with a constant working volume and operates under bubble-free conditions as it will only introduce more bubbles into the system. Plus, without a precise control, it is rather difficult to keep such a small inoculum volume constant in every experiment. Inconsistency of inoculum volume may to the certain extent affect the quality of the end results. Thus, a different feeding strategy needs to be pursued. An elegant solution would be to install on-board micropumps and multiple microvalve systems. It has been demonstrated – by most cell culture microbioreactor systems [18] – that the use of on-board micropumps and microvalves



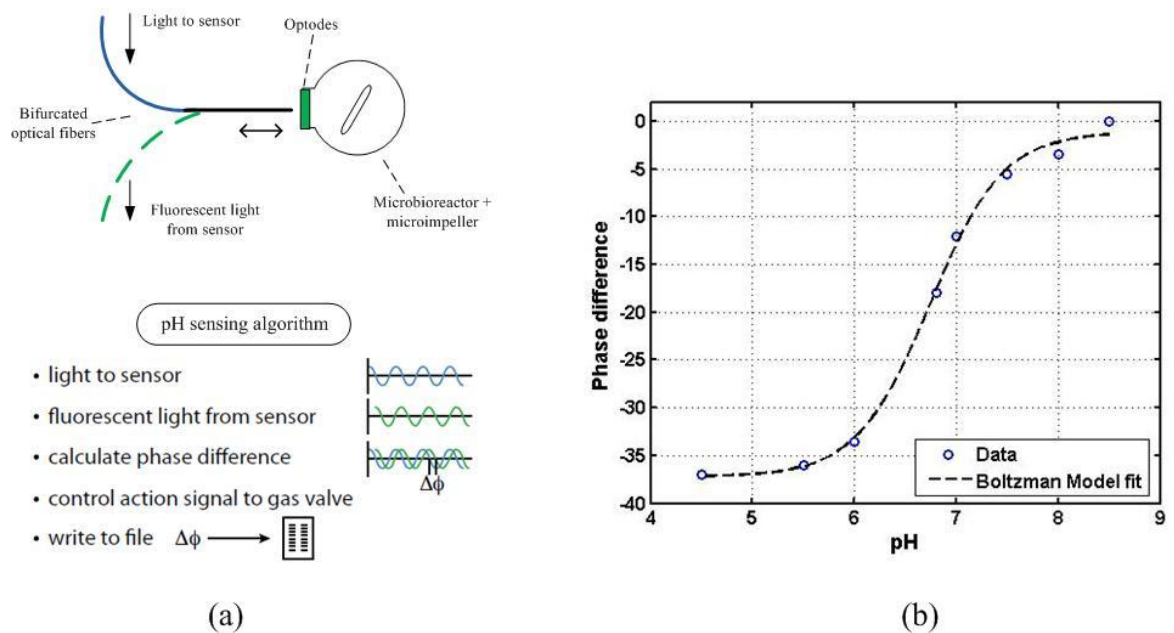
enable a tight control over individual flow rates to/from the reaction chamber for either continuous mode of operation or for intermittent feeding of substrates. Whilst this obviously complicates reactor fabrication steps, it ensures consistent inoculum volumes in every experiment and may ease the process for multiplexing of the microbioreactor systems (i.e. for parallel reactor operation).

## 5.0 PROCESS CONTROL OF PHYSICAL PARAMETERS

Microbial growth rates and the successful of fermentation experiments are highly dependent on environmental factors such as temperature, pH and the dissolved oxygen level. It is therefore essential to equip the microbioreactor system with necessary sensors and actuators for *on-line* measurements and process control over these physical parameters. Additionally, such reactor features will also increase amount of information gained per experiment.

Miniature size sensors are normally preferable for *on-line* measurements in microbioreactor systems [2]. One also opt for sensors that is non-reactive, relatively cheap, good responses, broad measurement range and can easily be embedded into the reactor without compromising the microbioreactor design or complicates the reactor fabrication steps. For example, temperature in microbioreactors is typically measured with the use of Pt 100 or Pt 1000 sensors (i.e. resistance temperature detectors made of platinum with nominal resistances of 100 and 1000  $\Omega$  at 0°C, respectively). Pt 100 (or Pt 1000) sensors are commercially available in small sizes (e.g. 5 x 2 x 1.3 mm),

have linear responses over a broad temperature range (e.g. 0 °C – 100°C) and have a good measurement accuracy i.e. to  $\sim 0.1^\circ\text{C}$  [2]. As for pH and dissolved oxygen (DO) level, measurements are mostly performed via optical sensing methods with the use of a fluorescent sensor spot or ‘optodes’ [11,16]. In every measurement, a sine (or square)-modulated light at specific wavelengths (e.g. 465 nm and 505 nm for pH and DO level measurements, respectively) and frequencies is shone onto the optodes which is normally placed inside the reactor (i.e. in direct contact with the reaction medium). This sensor spot in turn emitted fluorescent lights with the same frequency as the incoming light, but with a phase lag. The measured phase shift between the outgoing and the incoming signal is then correlated to either pH values or DO levels depending on the application. Examples illustrating the phase difference once the optical light is detected/sent and the relationship between phase difference and pH level are shown in Figure 4 [19-21]. Bi-furcated optical fibers are usually used for transmission and collection of light signals [11,16]. Optodes has a measurement accuracy of approximately 0.01 of the measurement units with a response time ( $t_{90}$ ) of less than 90 s [2]. With respect to measurement range, for pH sensor spots, it has a rather limited dynamic measurement range i.e. normally between pH 5.5 and 8. Contrary to the pH sensor spot, DO sensor spot can work for the entire saturation values, from 0 to 100% and their sensitivity is optimal at low DO levels, which is the relevant range for fermentation experiments [2]. Miniature size sensors are indeed useful in microbioreactor system as they allow for local monitoring of desired reactor variables – a highly desirable feature in a process control routine.



**Figure 4** Examples illustrating (a) the phase difference once the optical light is detected/sent [19] and, (b) the relationship between phase difference and pH level according to Boltzman model [20,21]

Process control is a standard requirement for any reactor system. In order to fully support aerobic fermentation processes, a microbioreactor needs to be designed such that it can achieve a tight control over important reactor variables such as temperature, pH and the dissolved oxygen level. In microbioreactors, micro-heaters are generally installed to regulate the reactor temperature [11,13]. Other options include

performing microbioreactor experiments in an incubator [5] or circulating thermostated water through the reactor [16]. Since microbioreactors are not thermally insulated, heat loss (i.e. to the surroundings by natural convection) is inevitable. On the one hand, this might impose some technical challenges as continuous heating is necessary to first, compensate the heat losses and secondly, to provide sufficient heat load to induce a

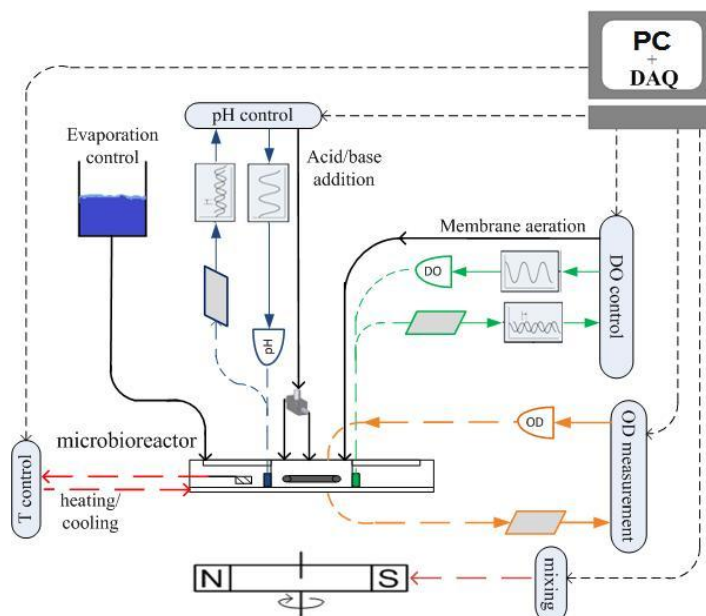
temperature change. On the other hand, continuous heat loss is advantages as cooling of the reactor can be achieved by taking advantage of the heat dissipation to the surroundings [8]. This would simplify the microbio reactor design (i.e. no cooling element) and reduces fabrication cost. Moreover, one need to bear in mind that microbio reactor systems have a high surface area to volume,  $S/V$  ratio – compared to the conventional bench scale bio reactor – which warrant a large and fast heat transfer rate.

In a typical bench scale bio reactor system, acid or base are added to either decrease or increase the pH level of the reactor. Such method is however difficult to implement for a microbio reactor system that operates under bubble-free conditions, fixed working volume and without any headspace. Nevertheless, efforts have been made to adapt this acid-bass addition method in microbio reactors but its application is hampered by the limited volume of the reactor [11,16]. A simple solution for the lab would be to use a buffered solution. But, the use of buffer is always not sufficient for the entire fermentation experiment due to the limited buffering capacity where for example a pH drop by nearly 2 pH units has been reported in a buffered system due to the acidification during the fermentation process [22]. This limitation can technically be circumvented by operating the reactor continuously (i.e. continuous feeding of fresh and buffered substrates/medium) [13]. Finally, one could also choose to establish a gaseous pH control strategy where for example ammonia,  $\text{NH}_3$  and carbon dioxide,  $\text{CO}_2$  gases are fed through a semi-permeable poly(dimethylsiloxane) (PDMS) membrane to induce pH changes [23]. The practical feasibility of this gaseous pH control scheme has been demonstrated in larger reactor operation [24]. Furthermore, introduction of gases via a thin PDMS membrane ensure a constant reactor volume throughout the entire operation.

Oxygen is one of the key substrates for an aerobic fermentation process. It is therefore necessary to keep the saturation oxygen concentration at desired set-point values to avoid any undesirable oxygen-limiting conditions during operation. Contrary to the bench scale bio reactor system –

where oxygen is supplied into the reactor via a ring-sparger – in microbio reactors; oxygen supply is supplied via membrane aeration. Through this method, formation of bubbles are prevented and oxygen transfer coefficient,  $k_L a$  as high as  $500 \text{ hr}^{-1}$  have been reported for microbio reactor operation [11]. Since oxygen is sparingly soluble in water ( $\sim 7.8 \text{ mg}\cdot\text{L}^{-1}$  at room temperature ( $28^\circ\text{C}$ ) and at atmospheric pressure of 760 mm Hg, [25]), it is crucial (1) to maintain a high interfacial area for gas transfer rate through the PDMS membrane, and (2) promote good mixing to increase the level of dissolved oxygen content in the microbio reactor.

Realizing a well-functioning process control loop in microbio reactor setup is a very challenging task. Besides the need to carefully design necessary microfluidic networks for precise heating or dosage of oxygen, and choosing suitable sensors and actuators, one also need to establish a feedback control configuration that best suits their applications. Typically, a complete microbio reactor system consisting of several measurements and process control loops. These include optical cells density measurement, evaporation control, feeding, and control loops for temperature, pH and dissolved oxygen level (Figure 5). Take note that each measurements and/or control loops run at different timing. Thus, it is imperative that the system is programmed in such a way that all measurements and control loops compliments each other (total cycle time is governed by the slowest operating loop) and can operate reliably (i.e. software does not crashed during reactor operation) for a certain period of time (e.g. 24 hours). Programs for measurement and control routines are normally implemented by interfacing the program/software with suitable data acquisition card (DAQ) for data logging and sending signals to actuators. When all the above-mentioned measurements and control subcomponents are in place, it is then worth to put a serious thought on parallel reactor operation. This is indeed a very challenging task as fluidic handling (e.g. feeding strategy), and local reactor monitoring and control become increasingly complex when the number of microbio reactors increases from one to many units.



**Figure 5** Schematic illustrating a complete microbio reactor system consisting of optical cells density (OD) measurement, evaporation control, fluidic connections for feeding, and control loops for temperature, pH and dissolved oxygen level. Dashed and/or colored lines indicating either optical fibers or wiring whilst black and solid lines indicates the fluidic connections. This schematic was created according to information obtained from various published works on microbio reactor systems [8,9,16,21,22]

## 6.0 CELLS OPTICAL DENSITY (OD) MEASUREMENT

Since microbioreactors generally designed to have a working volume less than 1 mL, sampling is not possible in most microbioreactor experiments, and monitoring of the concentration dynamics of cells typically relies on *on line* optical measurement systems, e.g. UV absorption method. The UV absorption method is a simple transmission measurement system (i.e. based on the Beer Lambert absorption law) and is commonly applied in microbial cell-based microbioreactors to measure, e.g. optical density (OD) of cells. *On line* OD measurement method is indeed an essential feature for microbioreactors as it allows for obtainment of real time measurement of reaction dynamics and most importantly it does not take up any volumes of the microbioreactors in each measurement [2].

*On line* OD measurement in microbioreactors is realized with the use of optical fibers [11,15,18]. Light from light-emitting diodes (LED) is sent into the reaction chamber (i.e. space with defined optical path length containing the reactor content/sample) and then guided into a photo-detector for measurement. Transmission and collection (i.e. on the opposite side of the transmitted light) of light signals are all done with the use of optical fibers. Signal obtained is usually correlated with cells dry weight or number of cells per volume (e.g. g cells·L<sup>-1</sup>). Visible light wavelength ranging between 400 to 700 nm is normally chosen in most microbioreactor setups for cells OD measurement. For example, OD<sub>600nm</sub> is generally used for OD measurement in fermentation experiments involving *E.coli* and *S. cerevisiae* strain [2]. Sensitivity and accuracy of the OD measurement is greatly depending on the fixation of the optical fibers and the optical path length provided (in the reactor) for the OD measurement.

Though OD measurement via optical probes has proven to be the most suitable method of estimating cell density in microbioreactors; its implementation is however not so straight forward. One need to (1) isolate the ambient light signal (typically done by utilizing modulated light signal) which may corrupt signal collected for OD measurement, (2) properly aligned the optical components to minimize signal loss, and (3) prevent any unpredictable interferences during operation for examples interference caused by the presence of bubbles, stirring motion of the magnetic bar used for mixing, etc.

## 7.0 CONCLUSION

This paper presented important design considerations for establishment of a microbioreactor system to facilitate a typical aerobic fermentation process. These include reactor operating feature and size, reactor mechanics (materials, fabrications, and mixing), reactor fluidics (connections, aeration, evaporation and feeding strategy), process control of physical parameters (temperature, pH, and dissolved oxygen level) and detection methods for measuring the cells concentration. Practical solutions as well as limitations for each of the reactor subcomponents are also briefly discussed. Obviously, microbioreactor design is a challenging task as it requires one to resolve not only mass and heat transfer rate issues, but also needed to realize suitable process control routine (data logging and sending signals to actuators) for the reactor operation – all while keeping the fabrication cost low. A good engineering practice would be to first determine essential reactor features that would fulfill basic requirements in conducting specific fermentation experiments e.g. mixing, optical density measurement, temperature control, etc. followed by,

implementing relevant solutions to achieve specific reactor features step by step before finalizing the whole microbioreactor design. Finally, it can be confirmed that it is possible to design a microbioreactor system that is applicable for aerobic fermentation experiments based on existing micro-technology.

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