Jurnal Teknologi

Full paper

Prototyping of Polymer based Microbioreactors: Micromachining by using Poly(Methyl Methacrylate) and Poly(Dimethylsiloxane) Polymer Materials

Hazwan Halimoon[a], Muhd Nazrul Hisham Zainal Alam[a, b*]

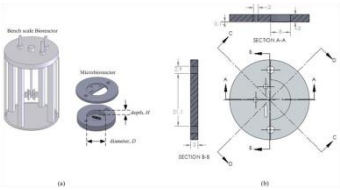
[a]*Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[b]*Process Systems Engineering Centre, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: nazrul@cheme.utm.my

Article history

Received :22 December 2012
Received in revised form :
18 February 2013
Accepted :15 May 2013

Graphical abstract



Abstract

Polymers have been widely accepted as materials for the fabrication of microbioreactor prototypes. In this work, microfabrication strategies namely the micromachining and casting (soft lithography) with the use poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) polymers as substrates for fabrications were discussed in details. A step-by-step illustration (including examples on digital prototyping of the microbioreactor by using a computer-aided-design (CAD) software) for the above mentioned micromachining procedures, and discussions on the necessary design considerations were presented as well. In the work, we showed the simplicity of such machining procedures for the fabrication of microbioreactor prototypes. It was confirmed that through micromachining, microbioreactor prototypes can be fabricated by using poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) polymers with high precision (down to one tenth of mm). It was also demonstrated that the processing time for the fabrication of the microbioreactor prototypes was in the order of few hours and maybe days for a complex reactor design.

Keywords: Microbioreactor; polymers; microfabrication; casting, and micromachining

Abstrak

Polimer telah pun diterima secara luasnya sebagai bahan dalam fabrikasi prototaip mikrobioreaktor. Dalam kertas kerja ini, strategi fabrikasi mikro iaitu pemesinan mikro dan penuangan (lithograf halus) dengan menggunakan polimer-polimer poly(methyl methacrylate) (PMMA) dan poly(dimethylsiloxane) (PDMS) sebagai suapan untuk proses fabrikasi dibincangkan dengan lanjut. Illustrasi terperinci (termasuk contoh-contoh pada pembikinan prototip digital mikrobioreaktor dengan menggunakan perisian rekabentuk-bantuan-komputer (CAD)) bagi langkah-langkah pemesinan mikro di atas dan perbincangan pada unsur-unsur rekabentuk yang perlu dipertimbangkan juga ditunjukkan. Dalam kertas kerja ini, kami menunjukkan cara-cara pemesinan mikro yang mudah bagi proses fabrikasi prototaip mikrobioreaktor. Secara pemesinan mikro, prototaip mikrobioreaktor boleh dibentuk dengan menggunakan polimer-polimer poly(methyl methacrylate) (PMMA) dan poly(dimethylsiloxane) (PDMS) pada keteparan yang tinggi (iaitu pada satu persepuluh dari unit mm). Masa pemprosesan bagi pembentukan prototaip mikrobioreaktor dalam lingkungan beberapa jam dan mungkin beberapa hari bagi rekabentuk reaktor yang complex juga ditunjukkan.

Kata kunci: Mikrobioreaktor; polimer; mikrofabrikasi; dan pemesinan mikro

1.0 INTRODUCTION

Microbioreactors are a microfabricated chip with characteristic dimensions in the range between 50 to 1000 micrometer (i.e. submillimeter range) designed to facilitate specific biochemical analyses and/or bioreactions i.e. biocatalytic and fermentation processes [1]. Microbioreactors are indeed a new emerging technology and have been receiving increasing attention from both the academia and industry due to numerous advantages offered by these microsystems compared to their macro scale counterparts in biochemical processing (i.e. biocatalyst screening, production of fine chemicals, synthesis of organic chemicals, etc.). First, the microbioreactors operate with very small volumes (i.e between microlitre and nanolitre range) and thus, significantly reduced the volume/amount of medium and biocatalysts used per experiment. Secondly, microbioreactors have a very high surface to working volume ratio, *S/V* i.e. in the order of 1000 m^{-1} compared to a typical bench scale bioreactors

which is approximately 100-150 m^{-1}. A high *S/V* values significantly increased the heat and the mass transfer rates of microbioreactors. Additionally, reduced reactor dimensions also promote homogenous reaction conditions and increases process safety. Such a condition is indeed beneficial as it allows bioreactions to be performed under more aggressive conditions (i.e. non ideal state) with possible higher yields than that of traditional bioreactors. Third, since microbioreactors are often integrated with sensors and actuators and can include series of inlet and outlet microchannels, these microreactors are ideally suited for a continuous mode of operation under well-controlled experimental conditions that is relevant for actual industrial processes. And finally, scale-up to production stage is achievable through scaling out step i.e. replication of microbioreactor unit by numbers. This approach is advantageous as it could bypass the scaling-up step from pilot scale to production scale which is costly, time consuming and often technically difficult [1,2].

Based on the data from current literature, poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) are the most commonly used polymer materials for the fabrication of microbioreactor prototypes [3-9]; of which few will be presented here as examples. Zhang *et al*. [5] fabricated a microbioreactor prototypes consisting of multilayers of PDMS and PMMA substrates that were sealed together by a thermal bonding at elevated temperature. Schäpper and his co-workers [7] sealed a couple of PDMS layers by curing the layers using PDMS pre-polymer solution at 70°C for an hour to produce a microbioreactor prototype that is completely made of PDMS polymer. Zainal Alam *et al* [9] on contrary, fabricated a membrane microbioreactor prototype out of PMMA and PDMS polymers. A water-tight sealing was acheived by pressing the alternate PMMA-PDMS-PMMA layers by using stainless steel screws.

PDMS is a elastomeric material [10,11]. This means that with the presence of load (external force) PDMS substrates can stretch elastically and instantaneously returning back to its original shape once the load is removed. Becker *et al*. [10] reported that PDMS can technically exhibits at least 200% elastic elongation. PDMS is also favorable as materials for fabrication of microbioreactors because of its high gas and/or vapor permeability features [12]. It was found that most microbioreactor designers especially the one designing the systems to facilitate fermentation experiments would utilize a thin PDMS layer (thickness ~ 100 micrometer) as aeration membrane for oxygen supply to cells. Data on oxygen transport and oxygen uptake rate by cells through such PDMS membrane were typically provided as well [3-8]. On contrary, PMMA is a thermoplastic material that can be structured and reshaped above its glass transition temperature (T_g) by using a replication techniques such as the injection molding and the hot embossing methods. By definition, below T_g, polymer materials behaves similarly like a rigid and solid amorphous glass. However, above T_g, the polymer becomes distinctively soft and flexible [10]. This feature is indeed beneficial and can be manipulated to achieve various purposes in microfabrication of microlfuidic devices.

Despite the obvious differences between these two materials, both polymer materials are relatively cheap material for microfabrication, possess good optical qualities (i.e. optically transparent in visible spectrum; 350 nm – 750 nm) [13], non-toxic to most fermentation medium [1] and there are easy-to-handle. By using either the PDMS or PMMA materials, two- (2D) and/or three-dimensional (3D) microfluidic (microbioreactors) geometries can be easily fabricated via rather straight forward microfabrication strategies e.g. micro machining and casting (soft lithography). Inexpensive polymer substrates coupled with relatively simple fabrication methods offer the possibility for mass production of disposable microbioreactor prototypes.

In this work, we presented a step-by-step micromachining (i.e. drilling via computer-numerical-controlled (CNC) milling machine) and casting (soft lithography) procedures for fabrication of microbioreactor prototype by using poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) as materials for fabrication. Such inputs are very important is fabrication of microbioreactors especially in laboratory environment where necessary machine tools for fabrication is often a limitation. Additionally, we discussed the necessary design considerations during the conceptual phase i.e. sketching via a suitable computer-aided-design (CAD) and provided means on how to bond/seal various PDMS/PMMA layers together forming the desirable microbioreactor prototype. We focus on the fabrication of microbioreactor prototypes that are suitable for submerged microbial fermentation processes.

2.0 MATERIALS AND METHODS

2.1 Materials

Poly(methyl methacrylate) (PMMA) and Poly(dimethylsiloxane) (PDMS) were the only polymer materials used in this work. PMMA materials were obtained from a local supplier (SAMN USAHA JAYA ENTERPRISE). The polymers were prepared in a form of a square shape slab with 100 mm (width) x 100 mm (length) at various thicknesses i.e. 1.5 mm, 2 mm, 3 mm and 5 mm. The PDMS polymer and its curing agent (Dow Corning Sylgard 184 Silicone Elastomer Kit) used in this work was supplied by CELTITE Sdn. Bhd. Physical and chemical properties of both of these polymers are tabulated in Table 1.

Table 1 Properties of Poly(methyl methacrylate) (PMMA) and Poly(dimethylsiloxane) (PDMS) [10]

Name (Trade name)	Density (g/cm^3)	T_g (°C)	Water absorption (%)	Refractive index	Young's Modulus (MPa)	Resistant against	NOT Resistant against	Organic solvent stability
PMMA (Perspex, Plexiglass)	1.19	110	2	1.492	3200	Acid, bases (med conc.), oil, petrol	Alcohol, acetone, benzole, UV Radiation	Attacked by most solvents (e.g. benzene, acetone)
PDMS (Sylgard)	1.03	-120	0.1	1.43		Weak acid and bases	Strong acids, hydrocarbons	

2.2 Fabrication Methods

2.2.1 Prototyping and Micromachining

Fabrication of the microbioreactor prototypes was done through the prototyping and the micromachining procedures with the use of PMMA polymers. The first step in this procedure was the digital prototyping phase where a 3D model of the microbioreactor prototype was drawn by using three-dimensional (3D) CAD software Solidwork™. Such CAD software allows for the generation of 3D models and enables one to design and/or visualize the mechanical design (geometries, dimensions, etc.) of the microbioreactor prototype before machining. Next, G-code for machining step was generated by the CNC program. Coding was based on the CAD data produced earlier. Finally, the 3D model as previously drawn using the CAD software was machined accordingly. Processing time depends on the complexity of the design.

2.2.2 Casting (Soft Lithography)

The starting point of the casting procedures (also known as the soft lithography method) was the fabrication of the replication mold (master of the PDMS replica). In our work, the negative image of the intended PDMS layer was machined onto a PMMA substrate. Next, a pre-polymer liquid PDMS solution containing 10 parts silicone and 1 part curing agent was poured into the mold. To remove any air bubbles that formed during the preparation, the pre-polymer liquid PDMS solution was placed into an exsiccator for degassing period for approximately 20 minutes. Then, the mold (containing the bubble-free pre-polymer liquid PDMS solution) was cured in an oven at 70°C for 2 hours. Alternatively, curing step can also be done at room temperature for 48-72 hours. The cured PDMS layer was then removed from the mold by gently peeling it off by using a scalpel. The PDMS casting process is illustrated in Figure 1.

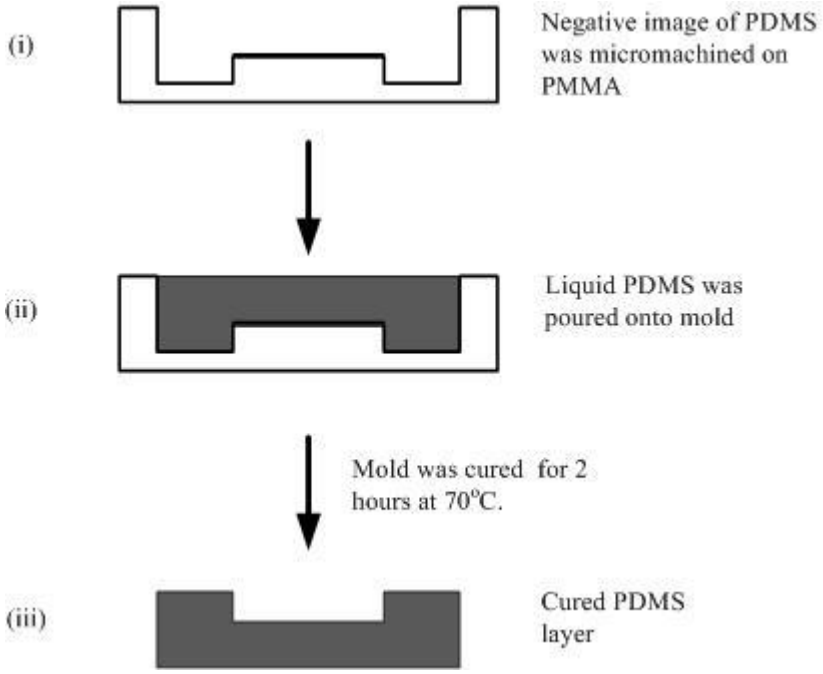
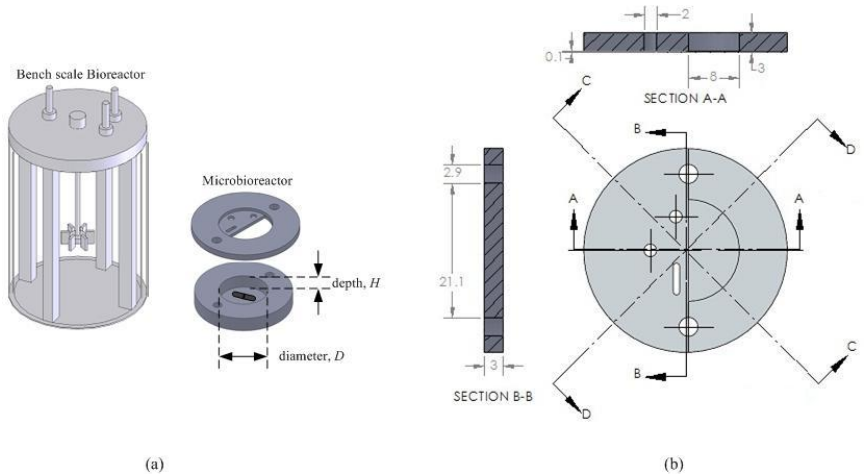


Figure 1 Diagram illustrating PDMS casting processes

■3.0 RESULTS AND DISCUSSIONS

3.1 Digital Prototyping, Micromachining and Casting Step

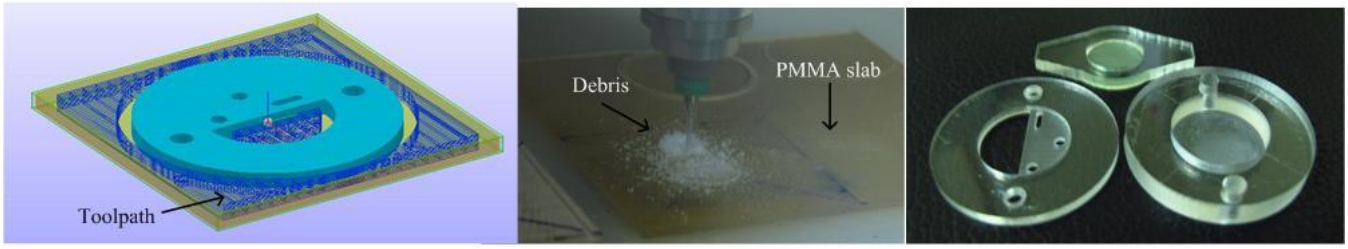
Micromachining i.e. drilling by using a bench stop computer-numerical-controlled (CNC) milling machine (or a 3-D printer) is the most commonly employed method for the fabrication of microbioreactor prototypes made of thermoplastic polymers [3-5]. Typical polymer substrates used include PMMA, Polycarbonate (PC), Polyetheretherketone (PEEK), etc. In this work PMMA polymers were used. As previously mentioned, the first step in fabrication of microbioreactor prototype via micromachining procedure, was the digital prototyping phase where the geometry and the dimensions of the microbioreactor prototype was sketched by using a CAD software namely SolidWork™. Figure 2 presents the example of possible 3D model (generated from the CAD software) and the example of possible sectional cut features in CAD software SolidWork™. It can be seen that the 3D microbioreactor model can either be presented in isometric projection (Figure 2a) or as a two-dimensional (2D) engineering drawing with additional info on sectional views (Figure 2b). A sectional view is generally used to visualize a hidden part/component of an object by removing or cutting away portion of that object. As shown in Figure 2b, in SolidWork™, sectional view can be performed by cutting through the object either horizontally (section A-A), vertically (section B-B) and/or diagonally (section C-C and section D-D). Additionally, dimensioning of the microbioreactor geometry during sketching was also possibly in such CAD software.



(a) (b)

Figure 2 a) Example of possible 3D model generated from a CAD software SolidWork™; b) Example of possible sectional cut features in a CAD software SolidWork™

In general, most sketches made in the CAD software will be saved in a standard graphic format (DXF files) before proceeding to the machining step. This is essential as most computer-aided-manufacturing (CAM) software will only read DXF format files (in 2D or 3D) when creating the tool path for the CNC equipment. The tool path indicates the action of the drill bits during machining step. It shows which parts to be drilled first, the step size, speed of the drill bit, etc. The tool path is generally represented by G-codes i.e. codes used to describe actual machine movement in simple steps. Example of this tool path is illustrated in Figure 3a. During machining, debris (fine polymer particles) often accumulates surrounding the drill bits and on the surface of the drilled parts (Figure 3b). In order to minimize extensive build-up of the debris, it is strongly recommended to either link the tips of the drill with a blower such that the debris will be blown away during milling operation or simply place the entire polymer substrate in a water reservoir. The latter is the simplest approach to passively wash away the debris during the milling operation.



(a) (b) (c)

Figure 3 a) Example of typical tool path created by computer-aided-manufacturing software for milling via CNC equipment; b) Image illustrating the micromachining step and the accumulation of polymers debris during milling operation; c) Desired polymer parts machined via micromilling

In our practice, during machining, we set the CNC equipment to first drill a simple component of the 3D model e.g. through holes. Through hole normally needed for screws (typically one tenth smaller the outer diameter of a standard stainless steel screw), and/or through hole for fluidics connections, etc. Secondly, we would start with a less complicated drilling step such as the milling of the reaction chamber and finally, the finishing step that is removing the 'almost-completed' part from the polymer substrates. If smaller drill bits were used (e.g. drill bits with outer diameter of 0.5 mm), than the drilling speed is often set at higher rate than drill bits with larger outer diameter. A mismatch between edge of square shape polymer substrates and the drill bits is expected particularly when a larger drill bits were used. Figure 4 illustrates the example of typical design constraints and expected mismatch during the machining step.

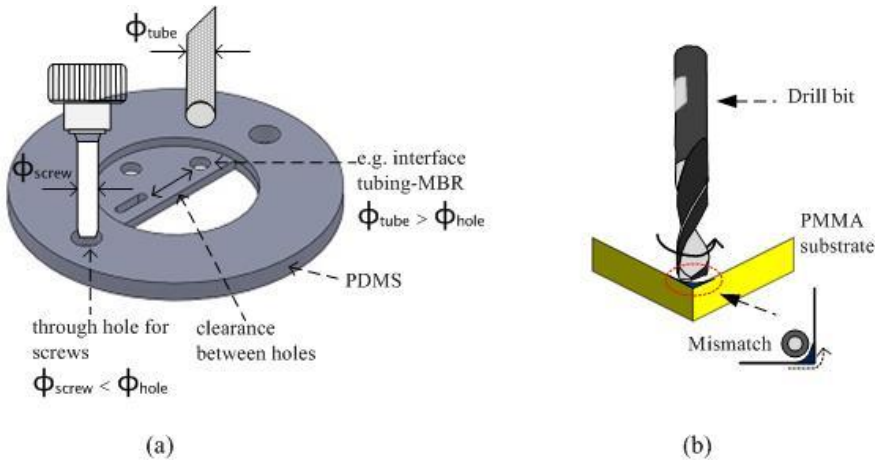


Figure 4 a) Design constraints for through holes; b) Image illustrating typical mismatch between drill bits and edge of square shape polymer substrates

The first step in the casting procedure implemented in our work was the fabrication of the replication mold. This was done via micromilling step by using PMMA polymer substrates. Figure 5a presents the replication mold made of PMMA polymers that was fabricated through the above mentioned micromachining technique. It was found that by applying such machining procedures, a replication mold with precision down to one tenth of millimeter of a scale can be made possible. It took approximately five to six hours to fabricate the mold. The fabrication time was very lengthy because a small step size (distance covered by drill bits during milling operation) was introduced and thus, preventing from breaking down the drill bits during milling operation. PDMS layers are important in our microbioreactor design as these layers function as a gasket in achieving the water-tight sealing for the prototype. During the fabrication step, the PDMS layer was carefully peeled off from the mold (Figure 5b). If the PDMS layer were not carefully removed from the mold, it could easily tear apart. We also ensured that there were no bubbles or air trapped within the mold during the curing stage. If bubbles were not completely removed, the end product of the PDMS layer will end with a defect. This is illustrated in Figure 5b. The use of PMMA and PDMS polymers also allows for a rather straight forward bonding procedures. PDMS layer can easily be compressed between two PMMA layers with metal screws to obtain a water-tight seals. Alternatively, pre-polymer PDMS solutions can be spread on the contact surface between the two polymers and cured at either room temperature or at elevated temperature (Figure 5c). Low material cost couple with a simple and cheap fabrication methods are ideal features for rapid prototyping and mass production of microbioreactor prototypes.

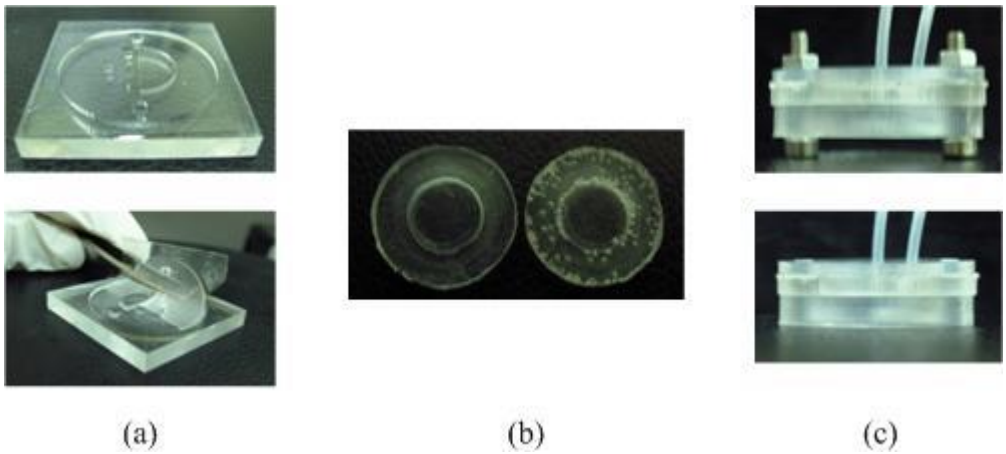


Figure 5 a) Image of a replication mold made of PMMA polymer (top) and procedure in peeling off cured PDMS layer from the mold (bottom); b) Image illustrating possible defect in a PDMS layer if bubbles are not properly removed; c) Image representing sealing of multiple PDMS-PMMA layers by using screws and PDMS pre-polymer solution

3.2 Design Considerations

When sketching and designing the microbioreactor prototype, several factors were taken into considerations. These include (i) microbioreactor size and shape, (ii) fluidics connections, and (iii) the potential microfluidics components to be integrated into the microbioreactor prototype.

3.2.1 Microbioreactor Size and Shape

Microbioreactor prototypes –especially the ones fabricated to facilitate fermentation experiments and biocatalysts processes– are mostly realized as direct copies of a bench scale bioreactor setup with working volumes less than 1 milliliter (Figure 2). For simplicity in the fabrication steps, and also for a possible

scaling-up of the design to a larger operating scale, the geometrical shape a microbioreactor prototype reaction chamber often designed to take the shape of a typical cylinder with height to diameter ratio, *H/D* of approximately 1:3, respectively (Figure 2a). Szita *et al*. [3] multiplexed microbioreactor platform contained a reactor chamber with a depth of 2 mm and a diameter of 10 mm yielding a working volume (i.e. under bubble-free conditions) of 150 microliter. Zhang *et al*. [14] microbioreactor design consisted of a shallow reaction chamber with 1 mm depth and a diameter of 10 mm. Schäpper *et al*. [7] fabricated a single-use PDMS microbioreactor consisting of a cylindrical shape reaction chamber with a depth of 2 mm and a diameter of 10 mm. Zainal Alam *et al*. [9] realized a membrane microbioreactor setup to facilitate biocatalyst degradation of pectin substrates that contained a 100 microliter reaction chamber with a depth of 2.5 mm and a diameter of 7 mm. Figure 2c illustrates a typical cylindrical shape of a microbioreactor reaction chamber design. It is also important to note that whilst the *H/D* ratio was maintained in the order 1:3, the depth of the reaction chamber is often limited within couple of millimeters for a high oxygen transfer rate. Contrary to the bench scale bioreactor setup, where oxygen is supplied via a ring sparger [15], aeration for microbial fermentation process in microbioreactor platform is normally provided via surface aeration through a thin semi-permeable PDMS membrane (i.e. membrane thickness ranging between 50 and 100 micrometer [3-5,7]). This is to prevent unnecessary bubbles formation inside the reaction chamber during reactor operation. Bubbles are undesirable in microsystems as they may for example perturb the rotational of mini magnetic stirrer bar and/or potentially interfere with any online measurements installed in the reaction chamber [1]. Nevertheless, in order to maintain a reasonably high oxygen transfer coefficient, k_La, it is crucial to keep the depth of the microbioreactor chamber within practical limit e.g. not more than 3 mm. This is because during aeration, mass transfer is not limited by diffusion through the PDMS membrane (oxygen permeability through a thin PDMS membrane has been reported to be about 6×10^{-8} cm^3 (STP) cm cm^{-2} s^{-1} $cmHg^{-1}$ [12]) but by molecular transport through the reactor content. This technically means that the gas transport efficiency (i.e. from the membrane surface to the bottom of the reaction chamber) is inversely proportional to the depth (height) of the microbioreactor reaction chamber [14].

3.2.2 Fluidics Connections

Another important aspect when sketching the geometry and the dimension of a microbioreactor prototype is the consideration on the space needed for the fluidic connections. Fluidics connection is an interface between the microbioreactor platform and its macro world counterparts e.g. syringe pump, valves, etc. for delivery of liquid into and/or from the microbioreactor. With respect to fluidics connections for microbioreactors, there are numbers of option available. These include by gluing a tube into fluidic ports [12], metal ferrule-O ring interconnects [5] and a standard tube-nut assembly [9]. Each of these designs imposes different constraints and system requirements. For example, gluing a tube into fluidic ports is indeed a very simple approach and doable by utilizing materials commonly available in the lab. Moreover, only small area is needed for fabrication but gluing a tube into place is risky as glue can potentially clog the fluidic connections if it is not carefully handled. In the metal ferrule-O ring interconnects, O-rings are often placed in a concentric groove around the interconnection holes to achieve water-tight connections [5]. This type of connection is reversible as tube is connected to a rigid tube (ferrule). Contrary to the above mentioned fluidic connections, tube-nut assembly connections are realized with the use of commercially available chromatography fittings. Such fittings has a low dead volume, able to withstand a high pressure build-up (if any) and applicable to most microfluidics setup made of plastic. A tube-nut assembly connection is relatively expensive and requires a larger spacing as a threaded port needs to be fabricated for the placement of the fittings. In our design, we inserted a standard perfluoroalkoxy (PFA) tubing into a hole made of PDMS. The size of the PDMS hole is one tenth larger than the outer diameter of the tube and hence, achieving tightness (Figure 3).

3.3.3 Integrated Microfludics Components

Additional spaces are also needed to include necessary microfluidic components namely micro mixer, micro pump, etc. These integrated features are essential to support microbioreactor operation. For example, vigorous local mixing is imperative to keep cells in submerged conditions and also for efficiency of molecular transport within the reaction chamber. Lee *et al*. [6] realized a micropump underneath the reaction chamber to create the necessary sequential pumping motion for mixing purposes. Edlich *et al*. [8] integrated a passive micro mixer at the inflow of the reaction chamber to create larger interfacial area for mixing. Schäpper *et al*. [7] and Zainal Alam *et al*. [9] placed a mini stirrer bar to induce mixing inside the reaction chamber. All in all, it is important to conclude that the size (volume) of the microbioreactor reaction chamber influence the type of mixing scheme applicable for the microbioreactor prototype, and vice versa. Often, extra room is also needed for integration of miniature size sensors for on line monitoring of physcial parameters such as temperature, dissolved oxygen concentration, pH, etc. as extensively reviewed by Schäpper *et al*. [1].

■4.0 CONCLUSIONS

The use of PMMA and PDMS polymers as substrates for fabrication reduces time, cost and complexity for prototyping. PDMS and PMMA substrates are relatively cheap and fabrication can be done via micro-milling and casting fabrication methods, thus avoiding the need to access any specialized facilities e.g. a clean room facility. Due to the relatively inexpensive and rather straightforward fabrication method (i.e. micro-milling and casting), re-designing and fabrication of a new microbioreactor prototype (if at all necessary) is achievable in a very short period of time. This is indeed advantageous because development of such a microbioreactor prototype is an iterative process, where a series of refinements or adjustments of the prototype are often needed until the final design aim is achieved.

Acknowledgement

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

References

[1] Schäpper, D., Zainal Alam, M. N. H., Szita, N., Lantz, A. E., Gernaey, K. V. 2009. Application of Microbioreactors in Fermentation Process

Development: A Review. *Analytical and Bioanalytical Chemistry*. 395: 679–695.

[2] Xie, D. 2012. Using an Advanced Microfermentor System for Strain Screening and Fermentation Optimization. *Methods in Molecular Biology*. 834: 217–231

[3] Szita, N., Bocazzi, P., Zhang, Z., Boyle, P., Sinskey, A. J., and Jensen, K. F. 2005. Development of A Multiplexed Microbioreactor System for High-Throughput Bioprocessing. *Lab on a Chip*. 5: 819–826.

[4] Lee, H. L., Bocazzi, P., Ram, R. J., and Sinskey, A. J. 2006. Microbioreactor Arrays with Integrated Mixers and Fluid Injectors for High Throughput Experimentation with Ph and Dissolved Oxygen Control. *Lab on a Chip*. 6: 1229–1235.

[5] Zhang, Z., Perozziello, G., Boccazzi, P., Sinskey, A. J., Geschke, O. and Jensen, K. F. 2007. Microbioreactors for Bioprocess Development. *Association for Laboratory Automation*. 12: 143–151.

[6] Bower, D. M., Lee, K. S., Ram, R. J. and Prather, K. L. J. 2012. Fed-Batch Microbioreactor Platform for Scale Down and Analysis of a Plasmid DNA Production Process. *Biotechnology & Bioengineering*. 109(8): 1976–1986.

[7] Schäpper, D., Stocks, S. M., Szita, N., Lantz, A. E., and Gernaey, K. V. 2010. Development of a Single-use Microbioreactor for Cultivation of Microorganisms. *Chemical Engineering Journal*. 160: 891–898.

[8] Edlich, A., Magdanz, V., Rasch, D., Demming, S., Zadeh, S. A., Segura, R., Ka¨hler, C., Radespiel, R., Bu¨ttgenbach, S., Franco-Lara, E and Krull, R. 2010. Microfluidic Reactor for Continuous Cultivation of Saccharomyces Cerevisiae. *American Institute of Chemical Engineers*. 26(5): 1259–1270.

[9] Zainal Alam, M. N. H., Pinelo, M., Samantha, K., Jonsson, G., Meyer, A., and Gernaey, K. V. 2010. A Continuous Membrane Microbioreactor System for Development of Integrated Pectin Modification and Separation Processes. *Chemical Engineering Journal*. 167: 418–426.

[10] Becker, H. and Gärtner, C. 2008. Polymer Microfabrication Technologies for Microfluidic Systems. *Analytical and Bioanalytical Chemistry*. 390: 89–111.

[11] Mcdonald, J. C. and Whitesides, G. M. 2012. Poly(dimethylsiloxane) as a Material for Fabricating Microfluidic Devices. Accounts of Chemical Research. 35(7): 491–499.

[12] De Jong, J., Lammertink, G. H., and Wessling, M. 2006. Membranes and microfluidics: a review, *Lab on a Chip*. 6: 1125–1139.

[13] Starr, C. 2005. *Biology: Concepts and Applications*. Thomson Brooks/Cole. ISBN 0-534-46226-X.

[14] Zhang, Z., Szita, N., Boccazzi, G., Sinskey, A. J. and Jensen, K. F. 2005. A Well-Mixed, Polymer-Based Microbioreactor with Integrated Optical Measurements. *Biotechnology and Bioengineering*. 93: 287–296.

[15] Shuler, M. L. and Kargi, F. 2002. *Bioprocess Engineering: Basic Concepts*. 2nd ed., Prentice Hall, New Jersey, US.