OPTIMIZATION OF OPERATING VOLUME OF MICROBIOREACTOR BY USING ATTAINABLE REGION

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OPTIMIZATION OF OPERATIN VOLUME OF MICROBIOREACTOR BY USING ATTAINABLE REGION APPROACH

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Adissertation submitted as fulfilment of the
requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical Engineering
UniversitiTeknologi Malaysia

JUNE 2013

Dedicated to my beloved family members

ACKNOWLEDGEMENTS

I own a great deal of thanks to Allah and to many people who have contributed towards the successful completion of this thesis. It has been a long and difficult journey, but also a fruitful and enjoyable one because of these people.

I sincerely acknowledged the support of my research project supervisor, Dr. MohdKamaruddin Abdul Hamid. He undoubtedly deserves the greatest thanks for his assistance and tireless effort toward the completion of this project. Without his guidance and support, this project would not have been accomplished. He has always been more than generous with his time and ideas, not withstanding his busy schedule.

My sincere appreciation also to my co-supervisor, Dr MohdNazrulHisham. ZainalAlam for his contributions. His critical questions and comments have helped to make the project a reality and relevant to bioprocess engineering.

Another party that most not be forgotten is the microbioreactor research group in the microbioreactor laboratory.My good friend, HazwanHalimoon. Thank you for all the assistance. I will never forget your help.

To all my fellow colleagues from Process Systems Engineering (PSE) research group, VahidAkbariBaghbani, Phoon Li Yee, Hazy, Bolaji Ibrahim, and Suraya thanks for your outstanding ideas during our lively discussions. I appreciate every moment at Prospect lab.

Again, I like to acknowledge the management of Kaduna Polytechnic for their financial support.Last but not the least; I would like to express my gratitude to

my heartthrob DR. KudratHamza and my entire family member (SalimLawal, YasirLawal, Aisha Lawal, and Fatima Lawal) for their unconditional love, prayer and support.

ABSTRACT

This research project presents the development of a model-based methodology in identifying a suitable (i.e. appropriate) operating volume for a microbioreactor system. Optimization of the microbioreactor operating volume is formulated as a generic optimization problem and solved by decomposing it into five hierarchical stages: (i) pre-analysis, (ii) design target selection, (iii) process design analysis, (iv) final selection and fabrication, and (v) result validation. In the first stage, the concept of attainable region is used to locate the optimal process design solution in terms of optimal condition of operation and economic point of views. The target for this optimal solution is defined and selected at the maximum point of the attainable region diagram. Accordingly the solution target at the maximum point of the attainable region shows the highest value of the objective function, hence the optimal solution for determine the optimum operating volume for the microbioreactor system. Finally, the size and dimension of the optimum microbioreactor is determined and fabricated by using the optimum design value (A = 1.2 ml, 464 μL). The validity of the proposed model-based methodology is confirmed by performing series of fermentation experiments (i.e. conversion of glucose to lactic acid) in threemicrobioreactors setup that was fabricated according to the operating volume suggested at three points located on the attainable region diagram (A= 1.2 ml, B = 1.45 ml, and C = 1.07 ml). The analysis of the biomass concentration profile of glucose in each of the microbioreactor shows that the microbioreactor with design value of point A (i.e. maximum point of attainable region diagram) produced the highest concentration at any interval of fermentation period of six hours. Hence, optimum volume of lactic acid is produced with microbioreactor A. The results obtained confirm the suitability of the proposed model decomposition-based approach (i.e. attainable region approach) for determine the optimum operating volume of microbioreactor before fabrication phase and clear justification for sizes of microbioreactors.

ABSTRAK

Penyelidikaniniadalahberkenaantentangpembinaanmetodologiberdasarkan model untukmengetahuiisipaduoperasisistemmikro-bioreaktor yang sesuai.Permasalahanpengoptimumanisipaduoperasibagimikro-bioreatorinidiformulasidengancaradefinisioperasi yang umumdandiselesaikandengancaraNyah-Komposit yang dibahagikankepada lima peringkatiaitu (i) Pra-Analisis, (ii) Pemilihanmatlamatrekabentuk ,(iii) Analysis rekabentuk process, (iv) PemilihanAkhirdanFabrikasidan(v) Pengesahan. Dalamperingkatpertama,konsep*Attainable Region*digunakanuntukmengesanlokasi optimal rekabentuk proses. Kondisi optimal yang perludiselesaikaniniadalahdalamkerangkaoperasidanekonomi.Matlamatpenyelesaiani nidedefinisidandipilihpadatitikmaksimumdalam rajah *Attainble Region*.Matlamatrekabentukiniadalahpadatitikmaksimum*Attainable Region*daninimenunjukkannilaitertinggifungsiobjektif.Olehituiaadalahpenyelesaian optimal isipaduoperasisistemmikro-biorektortersebut.Langkahseterusnyaialahsaizdandimensi yang optimal ditentukandandifabrikasidenganmengunakkannilairekabentuk optimal (A= 1.2ml, 464μL). Pengesahanmetodologi yang diutarakaniniadalahdenganmenjalankanujikajipenapaian (contoh: menukarkanglukosakepadaasidlaktik) dalamtigapersediaan yang berbezamengikutoperasiisipadu yang dicadangkanpadatigatitik di rajah *Attainable Region* (A=1.2 ml, B = 1.45ml, dan C= 1.07ml). Analisisdilakukandengancaramengambilsampel di setiapmikro-bioreaktordankepekatansampeltersebutdianalisis. Analisisprofilglukosakepekatanbiojisimpadamikro-bioreaktordengannilairekabentuktitik A (contoh: Padatitik yang maksimumdalam rajah *Attainable REgion*) telahmenghasilkankepekatan yang paling tinggidalammasaenam jam proses penapaian. Keputusan yang

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LIST OF ABBREVIATIONS

OD	=	Optical Density
DO	=	Dissolved Oxygen
ISFET	=	Ion-Sensitive Field Effect Transistor
PDMS		=Polydimethysiloxane
PMMA	=	Polymethyl methacrylate
PEEK	=	Polyetheretherketone
PC	=	Poly Carbonate
MTP	=	Micro Titer Plate

CHAPTER 1

INTRODUCTION

1.1 Introduction

One area of interest in biotechnology is the parallelization of biological processes in a small-scale reactor system such as microbioreactor. Microbioreactors are generally defined as a very small bioreactor system in which bioprocess operation takeplace under controlled operating conditions (Steinhaus*et al*., 2007). Microbioreactor working volumes are usually between 50 to 800µL and due to their size, they offer a number of cost-reducing advantages for conducting fermentation processes. Advantages offered by microbioreactor include lower running cost of substrate and utilities, reduced space requirements for parallel experiments, and reduced labour and effort required to prepare fermentation experiment (Zainal Alam*et al.*, 2010).

The capability of parallel operation couple with high through-put screen that can be achieved with microbioreactor system for specific experiment in bioprocess has been behind their transfer to large-scale application (Ivan *et al.,* 2004). Due to the small size of microbioreactors, the fluid flow and the process conditions in the microbioreactorpositively improved thetransport phenomena and efficiency of themicrobioreactor system. The microbioreactor system is classically used in process

development of bioprocess and fermentation processes. These processes can either be aerobic or anaerobic. Microbioreactors are generally fabricated by using polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS) polymeric materials. The advantages of fabricating microbioreactor with these materials include: they are cheap materials for microfabrication, easy to handle, and they offers the possibility to fabricate two-dimensional and three dimensional microfluidic geometries through a rather straight forward fabrication procedures e.g. casting and micromachining (ZainalAlam*et al*., 2012).

Microbioreactor design is a complex engineering task. Many researchers have developed different versions of microbioreactors with working volumes as low as 5μL. Lee *et al.* (2006) presented a 100μL microbioreactor fabricated with PDMS which allow better control condition of the variables. Zhang *et al*. (2006a) have described a polymer-based microbioreactor with good control over the microbioreactor operation condition and capable of producing *E. coli* at different dilution rate. Microbioreactors are often designed to work under bubble-free condition which means that, the reactor will be completely filled with liquid and operates at a constant volume. Therefore, it is important to consider and determine the total operating volume of the microbioreactor before fabrication phase. Generally, the shape of microbioreactors are designed in cylindrical form (i.e. volume = $\pi r^2 h$) and the operating volume is equal to the volume of the cylinder. In spite of the significant progress in microbioreactor technology, the working volume of a typical microbioreactor system is still a debatable issue. Until now, there is no well-established method for identifying optimal volume for microbioreactor system. Many of the researchers mention above designed and fabricated their microbioreactor without clear justification on the final volume selected. This research project develops a model-based methodology for optimization of microbioreactor volume by using attainable region approach. This method will enable microbioreactor designer to determine the optimum volume of the microbioreactor prior to fabrication phase.

1.2 Research Motivation

Research has shown that the use of microbioreactor for studying bioprocess operation in order to obtain experimental data has the ability to overcome problems which are usually encounter when using large scale reactors. The advantages of using microbioreactors for bioprocess development experiment would include: the reduction in the quantity of often expensive synthetic substrates required for process development; rapid generation of design data for use in process and economic model, and a more rapid translation of processes of discovery to pilot plant scale. Research has also shown that microbioreactor systems are an advanced system with the advantage of improvingthe performance efficiency, improve safety, reduce cost, reduce experiment time, and lower reagent use in bioprocess and biochemical operations.(Chin and Linder, 2007).

Presently, most of the bioprocesses development are been carried out by the use of largescale bioreactors with typical volumes between 0.5 and 10L (Zhang *et al.,* 2006b). These reactors have many problems which include the following: 1) process procedures are usually difficult and time wasting and consuming; 2) higher cost of maintenance; 3) difficult to carry out multiple experiments; 4) contamination of samples; 5) a large quantity of reagents is required; 6) high quantity of waste production ; and 7) small output (Pattanathu *et al*., 2008).

Several research works have been carried out to address these issues, some of which are presented here: Buchs (2001) and Zanzotto *et al.* (2004) have both established that microbioreactor systems provided goodoperational conditions, which are suitable for effective bioprocess and biochemical operations. Pattanathu *et al.*(2008) develop a microbioreactor system which was used for the production of model microorganism. They established that the reactor system compared favorably with the conventional processes. Zanzotto *et al.* (2004)fabricated a low cost microbioreactor system from poly dimethylsiloxane (PDMS), polymethylmethacrylate (PMMA) and glass. Sun *et al*. (2008) have used micro-capillary reactor to produced biodiesel and obtained a yield of 99.4 %. Lee *et al*.

(2008) further developed the micro-capillary reactor by reducing the mass transfer area and produced biodiesel with a yield of 99.5 % with residence time of 28s. This is the fastest residence time reported when compared with the time around 1hr for large scale reactors. Steinhaus *et al.* (2007) develop a microbioreactor to study the optimization of process variables for microorganism growth. The studied demonstrated that microorganism (i.e. E. *coli*) grow favorably in the microbioreactor. Schapper *et al.* (2010) developed a disposable microbioreactor for growing cells. The microbioreactor is fabricated with PDMS. The microbioreactor shows a good performance when it was compared with conventional reactors.

From the studies of the work of researchers mentioned above, it was obvious that they arbitrary chose the size and the volume of their designed microbioreactor without any clear justification or method on the final volume selected hence; there is the urgent need of a methodology for selecting the size and dimension of a microbioreactor system prior to fabrication phase

The main objective of this research work is to develop a model-based methodology for optimization of operating volume of microbioreactor and to determine the optimum operating volume of microbioreactor where design objectives of the microbioreactor system is satisfied for optimal throughput screening. The method will provide the advantage of clear justification for selection of size and dimension for microbioreactor system. The method has the additional advantage that it also offers clear justification for size and dimension of designed microbioreactor.

1.3 Problem Statement

Microbioreactors are often designed to work under bubble-free condition which means that, the reactor will be completely filled with liquid and operates at a constant volume. Therefore, it is important to consider and determine the total operating volume of the microbioreactor before fabrication phase. In spite of the significant progress in microbioreactor technology, the working volume of a typical microbioreactor system is still a debatable issue. Until now, there is no well-established method for identifying optimal volume for microbioreactor system.

1.4 Research Objective

The main objective of this research is to develop a model-based methodology for optimization of operating volume of microbioreactor by using attainable region method.

1.5 Research Scope

In order to achieve the above mentioned objective, the following scope has been identified:

- Review state-of-the-art on the existing microbioreactor design platform, design and fabrication practice.

- Development of a new model-based methodology for determination of optimum operating volume of microbioreactor where design objective of the microbioreactor system is satisfied.
- Selection of the optimal design solution at the maximum point of the attainable region.
- Design and fabrication of microbioreactor system
- Validation of the result with experimental work.

1.6 Research Contribution

The key specific contributions that have emerged from this work include:

- The development of model-based decomposition methodology for determines optimum operating volume of a microbioreactor system.
- The development of attainable region diagram for fermentation of glucose to lactic acid.
- The establishment of an optimum operating volume of microbioreactor for fermentation of glucose to lactic acid.
- Design and fabrication of microbioreactor for fermentation of glucose to lactic acid.

1.7 Outline of the Research Work.

This thesis is divided into five chapters. The first chapter contains background of the research and it includes introduction, motivation, research objectives and scope. The second chapter contains the detail literature review which includes reviews on state-of-the-art on design platform and fabrication of the microbioreactors system. The third chapter presents the proposed model-based methodology for optimization of operating volume of microbioreactor system using attainable region approach. The results and discussions of result are presented in the fourth chapter. The fifth chapter presents the conclusion on this research project.

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