State Of The Art and Integrated Process Design for Microbioreactor Systems-A Review

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Abstract - Over the years, microbioreactors have been equipped with a variety of tools for online monitoring and measuring of environmental conditions and assessing the physiological state of microorganisms. Microorganisms have played an essential role in producing everyday commodities, such as alcohol, beverages, enzymes. Micro scale system such as microbioreactor offers the potential to develop disposable and miniaturized version of conventional bioreactor. Microbioreactors systems combine the ease of handling of shake flask operations while preserving the online sensing and control capabilities of stirred tank bench-scale bioreactors. It offers academia and the industry the capacity to acquire real-time experimental data through cheap and high-throughput experimentation under well-controlled conditions. However, in spite of development of microbioreactor technology, new challenges still exist. One obvious challenge is the need for efficient strategies for determining the optimal operating condition where both the design and control objectives of obtaining optimal throughput screening in a microbioreactor can be best achieved. Such strategy will increase the efficiency of the reactor and reduce its working hours drastically in addition to other advantages of performing experiments in a microbioreactor system. Current microbioreactor process design and control is analyzed in this review paper, focusing on state-of-the-art and proposing a new strategy for determining the optimal operating conditions where both the design and control objectives of obtaining optimal throughput screening with a microbioreactor can be achieved.

Index Terms—microbioreactors, attainable region, Oxygen demand, Dissolved oxygen

I. INTRODUCTION

A. Integrated Process Design of Microbioreactor

The early generation of microbioreactor was designed to increase levels of complexity, with Poly (dimethylsiloxane) and Poly (methyl methacrylate) (PDMS), by micro machining and multilayer thermal compression bonding procedures. Online optical measurements of optical density, pH, and dissolved
oxygen are integrated. Active mixing is made possible by a miniature magnetic stir bar. Plug-in-and-flow microfluidic connectors and fabricated polymer micro-optical lenses/connectors are integrated in the microbioreactors for fast set up and easy operation.

Since 2004, researchers at Massachusetts Institute of Technology, U.S.A (MIT) have developed different versions of microbioreactors with working volumes as low as 5µL. A membrane aerated microbioreactor was constructed out of PDMS and glass and was integrated with embedded optical sensors for measuring and controlling pH, DO, and optical density (OD) [1]. The top layer of the PDMS serves as the aeration membrane through which oxygen diffused into the reactor. Fermentation carried out in this microbioreactor compared favorably with fermentation processes carried out in the conventional reactor. In fact, advance in microbioreactor technology has been progressing tremendously in the Bioprocessing industries to a realm of designing Nano liters microbioreactor by using two-level soft lithography fabrication techniques. [2] construct a 4 cm^2 microbioreactor with 64 unit array of the 3nL individual reactor. The c-shape reactors were designed with the opening smaller than the cell size. The cells are trapped within the reactor and micro fluidic controlled media flows in the rings surrounding the reactor and allowing the nutrients to diffuse through the opening. The drawback of this system is that the array is not equipped with sensors thus, its transparency allowed visualization after fluorescent staining. [3] constructed a parallel microbioreactor array of two 8-well strips. Each of the 250µL wells was incorporated with optical sensors that continuously reports OD, temperature, and oxygen input. Oxygen is individually controlled and supplied to each reactor by electrolytic generation in the lower strip. Temperature is monitored and controlled in each well by film thermistors and heaters. The design of the microbioreactor allows adding sensors. A solid-state pH sensor chip was added to the 8th well of the strip during an E. coli fermentation studies. [4] developed tube spin cell culture centrifugal tubes. The tubes are commercially available through Techno plastic products (Trasadingen, Switzerland). A novel 2mL microbioreactor was constructed by [5] out of a standard cuvette. It was integrated with optical sensors for monitoring OD, pH, and DO concentration. The reactor was stirred with a magnetic stir bar and was aerated by sparging. Table 1 below shows the state-of-the-art of microbioreactor platforms and their basic characteristics.

Table 1: Current microbioreactor design Platform

olume (µL)	Organism and fermentation mode	mixing	Minimization Of evaporation	k_La (hr^{-1})	OD max.	OD meas.	DO meas.	control	pH meas.	control	Temp. regulation	Source
5-50	E.coli, batch	Diffusion	Humid air	60	8	transmittance	Optode	None	Optode	buffered	Heated chamber	Zanzotto et al. (2004)
50	S. cerevisie, batch	Stirrer on axis	Water replenishment from elevated reservoir	-	6.87	transmittance	Optode	None	Optode	-	Heated chamber	Boccazzi et al. (2006)
100	E. coli, batch	Peristaltic	Oxygen feed humidified	500	40	transmittance	Optode	Oxygen conc. In mixer	Optode	Fluidic injection	Foil heater on base plate	Lee et al. (2006)
250	E.coli, batch	Shaken steel bead	Sealed	150	2.5	transmittance	None	No control, electrical generation	ISFET	Electr. CO_2 generation	Thermistor and resistive heating	Maharbiz et al. (2004)
150	E.coli and S.cerevisie batch	Stirrer on axis	Water replenishment from elevated reservoir	20-75	6	transmittance	Optode	None	Optode	Buffered	Heat chamber	Zhang et al. (2006) and Szita et al. (2005)
700	E.coli, batch	Shaken	Sealed	-	-	Scattered light	None	None	Optode	Fluidic injection	Incubator	Buchenauer et al.(2009)
20	E.coli,batch	Shaken	Water	-	-	Transmittance	Optode	None	Optode	Fluidic Injection	Incubator	Purkeiler et al. (2005)
-	S. cerevisie	Stirrer on Axis	-	-	-	-	None	No control, Electrical generation	-	-	Incubator	Muzzio et al. (1999)

B. Microbioreactor Process Control

It is important to measure and control critical process variables online, especially parameters that are used in closed control loops. There are several means to measure and control these common parameters, with the advantages of each method.

In practice, temperature, pH, and dissolved oxygen are the most widely measured and controlled in situ parameters. Sensors for these are typically inserted into specially designed port on the vessel [6].

1) Temperature Control in Microbioreactor

Temperature measurement is easy to achieve in microbioreactor system. The control of temperature in a microbioreactor system is rather a difficult task to achieve. This is as a result of the high surface area of working volume ratio (S/V) in a microbioreactor system. Hence, the heat transfer in a microbioreactor system is large and very rapid. Another reason is the location of the heating element. It is important to place the heating element in a position where it will not create a large temperature gradient. This is particularly important for microbioreactor fabricated from PMMA and PDMS, because these materials have very low thermal conductivities of about 0.2 $Wm^{-1}K$ (Assa et al., 2008)[7] and 0.17 W m^{-1} K (Shin et al., 2003) [8]respectively.

Uniformity of temperature of temperature in the chamber of microbioreactor is also another problem in establishing a temperature control in microbioreactor system. Different techniques exist for achieving temperature uniformity in bioreactor chamber. First, several temperature sensors can be placed at different spots within the microbioreactor chamber to evaluate the temperature distribution. Secondly, monitoring heat images of thermal distribution patters by using a thermo or infrared camera in microbioreactor chamber (Liu et al., 2003[9] and [10]). Thirdly, a model describing mass and heat transfer in a microbioreactor can be used to stimulate a three-dimensional (3D) temperature distribution profile in a reactor chamber by using the finite element method which is now a standard engineering tool in software packages. Finally, an adequate and efficient mixing system that can keep cells in suspension can also provide uniformity of temperature in micro bioreactor system.

Numerous ways are available for efficient control of microbioreactor temperature. The most common and simplest method is to carry out the experiment with the microbioreactor in a temperature-controlled room or in an incubator (Vervliet et al., 2008[11] and Van et al., 2009[12]). It was shown that the temperature variation in the microbioreactor can be kept to within 1.5^0C of the desired set point in a temperature controlled room. The limitation of this temperature control method is that it is not feasible for parallel operation in a micro bioreactor at different operating temperatures. Another method of controlling temperature in microbioreactor system is to link the microbioreactor to a water bath and circulate thermostated water through the microbioreactor base. These

methods have presented in the work of [13], [14], [1] and [15] have shown a reliable way of controlling temperature. Again, the drawback of this method is the limitation it imposes on the parallel operation of reactor at different temperatures. The addition of fluidic system increases the risk of liquid leakage and this can lead to failure of temperature control in the system. Microbioreactor developed by [2], [3], [16] and [17] have all demonstrated that temperature control in microbioreactors can also be achieved by integrating electrical micro heater embedded onto the microbioreactor. The temperature was controlled by maintaining the temperature of the copper base plate at a constant level by using a foil heater (Lee et al., 2006) [2]. Microbioreactor with incorporated heater onto a printed circuit board which was adhered to the base of the microbioreactor was demonstrated by Maharbiz [3] and [17] and their results show that the method can be used to control temperature in microbioreactor. [16] control temperature in a microbioreactor by embedding two electrode heaters into the side walls of the microbioreactor chamber. A low-cost temperature control method relying on the use of resistance wires as heating elements in microbioreactor contents has been developed by [18]. It was demonstrated that an on/off temperature controller applying a voltage to resistance wires embedded in the polymer results in accurate temperature control of the microbioreactor and provided a good disturbance rejection capability. The method of embedding and integrating heater onto the microbioreactors has shown to be the best method for controlling temperature in microbioreactor system. The method is simple and easy to incorporate into the microbioreactor. In addition, the method allows for parallel operation at different temperatures when thermal insulation preventing thermal transfer between the micro bioreactors is provided. It is important to provide temperature control loop because the micro bioreactor chamber is not thermally insulated and the surface area to work volume ratio is higher.

Table 2 shows the state-of-the-art for temperature sensing and control in micro bioreactor.

source	Type of MBR	Working volume(µL)	T sensor	Measurement approaches	Operating T (^{0}C)	T regulation	Control precision
Vervliet et al. (2008)	Loop reactor	150-650	Pt100	*ex situ*	25	T control room	±1.5 ^{0}C
Zanzotto et al. (2004)	Stirred tank	50-150	Thermo-couple	*ex situ*	37	Reactor base heated with thermosted water	N/A
Maharbiz et al. (2004)	Micro well	250	Thermistor and heater	*in situ*	25-55	Micro-heater	±2^{0}C
Lee et al. (2006)	Stirred tank	100	Pt100	*ex situ*	37	Reactor base heated with foil heater	N/A
Petronis et al. (2006)	perfusion	50	Thermistor	*in situ*	37	Micro heater	±0.26^{0}C
Krommenhoek et al. (2007)	Micro well	200	Pt100	*in situ*	30	Micro heater	±0.4 ^{0}C
Yamamoto et al. (2002)	Micro tank	1.25	RTD	*in situ*	20-90	Micro heater	±0.4 ^{0}C
Zainal Alam et al. (2010)	Stirred tank	100	Pt100	*in situ*	30-50	Resistance wire	±0.1^{0}C
Schapper et al. (2010)	Single use micro MBR	100µL	Pt100	*in situ*	28	Resistance wire	±0.2 ^{0}C

MBR= microbioreactor; T= temperature; N/A = data not available

C. pH Control in Microbioreactor

Optimal cell growth depends heavily on tight pH control and many cell produce acids as a metabolic by-product. Steam-sterilizable glass electrodes remain the state-of-the-art for use in microbioreactor systems. Their low mechanical stability has limited their use and spurred research development in optical sensors based on absorbance or fluorescence from pH-sensitive dyes. Optical sensors typically suffer from a narrow operating range, but this limitation is balanced by increased sensitivity near pKa of the dye. Single optical sensor generally cannot be used to monitor all bio-processes; fermentation can successfully be monitored by a sensor match to the pH range of the process [6]. The most frequently applied miniature pH sensors are optical sensors based based on fluorescence sensor spots or optodes and solid-state, ion-sensitive, field-effect transistor (ISFET) pH sensor chips. Optical sensors are the most preferable sensor in a microbioreactor system for control and monitoring of pH due to their noninvasive nature and flexibility for integration [19]. The optodes do not usually require any reference element to perform pH measurements. Because of this property coupled with the fact that they are cheap, they are a good alternative for a disposable microbioreactor. The pH sensing is performed through either fluorescence intensity or by fluorescence lifetime measurements. The fluorescence sensor spots are usually affected by photo bleaching effect. This reduces their lifetimes and pH range. The measurement accuracy and response time of optical sensor is 0.01 pH unit and less than 90s respectively. The operating temperature for optodes are

between 0-50 0C. The measurement range of most pH sensor spots is from pH 4 to 9 and they have a nonlinear response [19]. The ISFET pH sensors have a wider range of pH value (pH 2 to 12) and have a linear response that is similar to that of a standard pH probe and have a measurement accuracy of about 0.01 pH units with a response time of less than a second. ISFET pH sensors have wide temperature range of -45 0C to 120 0C. The drawback of ISFET pH sensor includes measurement drift and sensitivity to the surrounding light. Another drawback of this sensor is that the pH sensor chip required a reference electrode (Ag/AgCl reference electrode) to perform the pH measurement. This will imply that the micro bioreactor have to be designed such the embedded integrated ISFET pH chip is reusable so that the cost of the micro bioreactor is minimized. In spite of these limitations both sensors have provided adequate and rapid pH measurements in a micro bioreactor system over the last decade. Therefore, real time pH monitoring and measurement in microbioreactor is feasible.

Control of pH in a microbioreactor is still undergoing development in the Bio-processing industry. One of the early attempts to control pH in a microbioreactor was by either using a buffered system or by intermittently injecting base or acid [20]. The simplest method of controlling pH in a microbioreactor is the use of buffers. But the use of buffer is not usually sufficient to maintain a constant pH level. This is because buffers have a limited buffering capacity and can only compensate for a few numbers of ions before losing their resistance to pH changes. The limitation on the buffer capacity can be overcome in a continuous culture microbioreactor system. Perfusion microbioreactor system with continuous feeding of freshly buffered nutrient medium enabled the reactor pH to maintain constant to within ±0.02.

pH in microbioreactor can also be controlled by the method of the intermittently injecting base or acid into the microbioreactor. The decrease in the pH value as a result of metabolic activity of the growing microorganisms in the microbioreactor can be adjusted by intermittently injecting a base solution. This method is limited by the microbioreactor volume. However, [21] (in Tab. 3) has shown that this limitation can be overcome by injecting gas into the microbioreactor. This gaseous pH control has been applied in a 24-well 4-6 ml microbioreactor system where NH_3/CO_2 was used as the injecting gas. An in situ electrolytic gas generation where CO2 gas was dosed from underneath microbioreactor chamber through the semi permeable silicone membrane has also been developed by [3]. [22] demonstrated that the pH of a fermentation process can be controlled by injecting CO_2 gas and NH_3 vapor into microbioreactor thus, both are yet to optimize their methods. [21] have optimized this method and showed the potential of introducing gases through a membrane to control pH in microbioreactor system. Table 3 describes the state of the art for pH control and monitoring in a micro bioreactor system.

Table 3: Current pH measurement and control methods in microbioreactor

Source	pH sensor	Measurement Approach	pH regulation	Advantages	Disadvantages
Bylund et al. 2000	pH optode	in situ	Buffer system	Simple. Easy handling.	Limited buffer capacity. Scale-up: not feasible.
Koncki, 1998	pH optode	in situ	Acid/base addition	pH can be maintained longer. Scale-up: feasible.	Limiting reactor volume. Concentrated acid – base leads to local high/low pH.
Sotomayor et al. 2001	pH ISFET	In situ	Gas diffusion through the PDMS membrane	Not limited by reactor volume. Scale-up: feasible	Complicated fluidics for gas supply.
Zaina Alam et al. 2012	pH optode	In situ	On/off controller	Not limited by reactor volume. Scale-up: feasible	Complicated fluidics for gas supply.

II. METHODOLOGY

Integrated Process Design and Control Methodology

Microbioreactors technology offers the potential to develop disposable and miniaturized versions of bench-scale bioreactors for carrying out fermentation experiments. They are the easiest and cheapest means for performing parallel and controlled fermentation process. They combine the ease of handling of shake flask operations while preserving the online sensing and control capabilities of stirred-tank reactor. Microbioreactor technology thus offers academia and industry the capacity to acquire real-time experimental data via cheap and high throughput experimentation under well-controlled conditions. Although microbioreactors have been

developed and are commercially available, other problem still exists. For example a strategy for finding the optimal parameters of microbioreactor which gives the best performance of the reactor where both design and control objectives are best satisfied. The recent trend in microbioreactor technology development in both academia and industry has necessitated the need for a strategy for screening microbioreactor for optimal throughput. Hence, in this review paper the concept of attainable region (AR) for IPDC as presented by [23] is adopted with some modification for screening microbioreactor for optimal throughput. The aim of this approach is to determine the optimal operating condition where both the design and control objectives of obtaining optimal throughput screening in a microbioreactor can be best achieved. The method consisted of four stages to find the optimal parameters which give the best performance of the microbioreactor [23]. 1) Pre-analysis 2) steady-state analysis 3) dynamic analysis 4) evaluation stage. Stage one, of this approach involves generating equilibrium data and locating the maximum point and value of the attainable diagram. Stage two, the established target is validated by finding the feasible values of design-control parameters. The third stage, the selected optimal operating conditions identified in the previous stage together with the corresponding design-control variables are analysis in terms of performance indices. This is to verify whether the selected point performance best satisfied the minimum value of the derivative of AR. The minimum value of derivative determines the sensitivity and controllability of the system. The last stage of this approach involves suggesting different ways of operating the microbioreactor to achieve optimal throughput. Figure 1 below describes the steps of the approach diagrammatically.

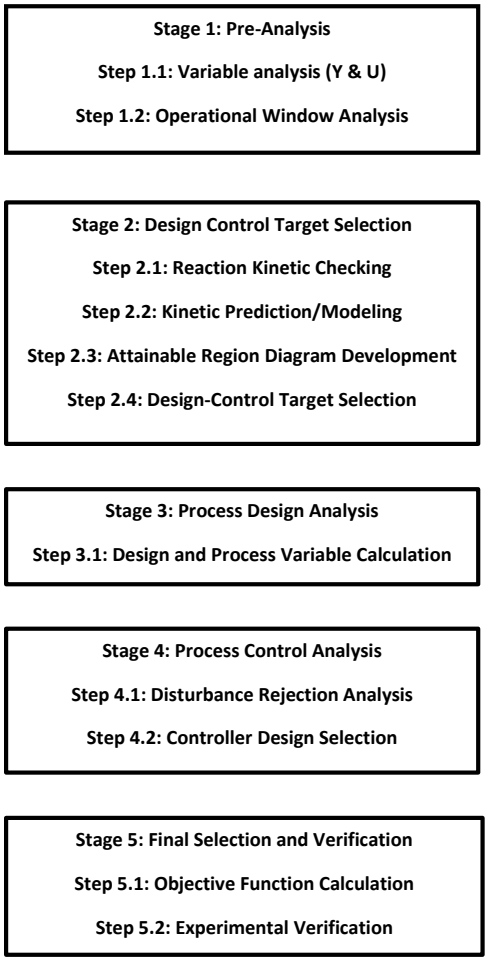

Fig.1: Algorithm of Integrated Process Design and Control for Micro bioreactor

III. CONCLUSION

Clearly, advances in microbioreactor technology have changed what was once a strikingly unusual laboratory technology into the mainstream. Importantly, the microbioreactor technology has proven to be scalable, so the data generated at the small scale is representative of larger scale operations. The platform provides almost no limit to the combinatorial number of experimental conditions that can be examined. It is now possible to conduct high throughput Bioprocess experimentation under highly control and monitor condition in a microbioreactor. However, in spite of the development of microbioreactor technology, new challenges still exist. One obvious challenge is the need for efficient strategies for determining the optimal operating condition where both the design and control objectives of obtaining optimal throughput screening in a microbioreactor can be best achieved. These challenges can be overcome by the above proposed method and the strategy will increase the efficiency of the reactor and reduce its working hours drastically in addition to other advantages of performing experiments in a microbioreactor system.

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