To the Graduate Council:

I am submitting herewith a thesis written by Bo Huang entitled "DEVELOPMENT OF AN ON-LINE BIOSENSOR FOR OPTIMIZATION AND CONTROL OF CONTINUOUS BIOREACTOR PROCESS." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

T.W. Wang, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
and Dean of The Graduate School

DEVELOPMENT OF AN ON-LINE BIOSENSOR FOR OPTIMIZATION AND CONTROL OF CONTINUOUS BIOREACTOR PROCESS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Bo Huang

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To my parents and sister

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ABSTRACT

During the operation of any bioreactor process two of the most crucial elements are the microbial biomass products and their metabolic state of growth. One of the major inhibiting factors in the development and implementation of effective bioreactor control regimes has been the lack of suitable on-line sensors for measuring such parameters and variables. The aim of the work is to develop and evaluate an on-line biosensor for a continuous bioreactor based on the light emission capabilities of the recombinant host cell culture. The cells have been genetically engineered to include the *lux* gene operon, originally isolated from bioluminescent marine bacteria, such as *Vibrio fischeri*. The *lux* gene codes for products that lead to the bioluminescence phenomenon. The focus of this work is to derive an on-line growth and biomass estimation scheme based on the intensity of emitted light. Experimental data is presented, and it appears that bioluminescence level corresponds to the relative dilution rate which is equal to the growth rate at steady state. It was found that higher dilution rate leads to higher bioluminescence level. It confirmed the batch experimental results that bioluminescence is a reflection of the relative rate of growth rather than the absolute amount of biomass.

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Control and optimization of bioreactor is strongly influenced by the quality of the sensors available for crucial response variables. Direct on-line sensors are generally available for the physical variables, such as dissolved oxygen level, temperature, speed of agitation, and pH. Since these sensors respond relatively quickly, proportional plus integral feedback controllers are often suitable for the control of these variables. However, on-line sensor for the composition (e.g. concentrations of biomass, substrates, nutrients, products, ions) and those for the physiological state of biomass are either not available or are relatively unreliable. In many cases, these variables are analyzed off-line resulting in a significant gap between the time when samples are taken and the time when the results are available.

Future biological processes engaged in large volume applications will move more and more towards continuous modes of operation [13]. Continuous operation of microorganisms maintain a culture in a constant state of growth. The advantage of continuous operation is that it allows one to separate and define parameters which are independent in batch fermentation, such as, growth rate and cell density. For the engineers who attempt to optimize the industrial-scale production of biomolecules, the control of these parameters in continuous operation can lead to more economical maximizing productivity over batchwise fermentation.

The potential maximum rate of biomass or product synthesis during fermentation is determined partly by two variables: 1) biomass concentration and 2) the metabolic state of the cells. Since those state variables cannot be measured on-line with currently available sensors, on-line estimation of these states have been relied upon to yield most likely estimates to be used in the optimization and feedback control of the reactor process. The development of new reliable on-line sensors allowing measurement of such parameters is urgently needed.

1.2 Statement of the Problem

The inadequacy of available on-line biosensors for crucial bioreactor variables suggests a problem that can be stated as follows, for the case in which no cellular product is produced :

> *Develop an on-line biosensor, based on the intensity of light emitted by the microorganisms in a continuous bioreactor process, that can provide indirect, correlated on-line information on the metabolic state of growth and the cell biomass concentration.*

This on-line measurement will provide the necessary information for the implementation of feedback control strategies required for the optimization of the bioprocesses.

CHAPTER 2

Background

2.1 Review of Biosensors

2.1.1 General Review of Biosensors

Biosensors are required to supply information essential for the effective control of bioreactor operations, such as fermentation processes. A biosensor is defined as a sensor that detects biological material as a primary recognition system and signal generator [1]. Biosensors for on-line process control offer the potential of implementing control techniques, but present a very difficult design challenge. Several review papers on biosensors are available [28, 52, 57, 64, 71]. Briefly, biosensors can be divided into: off-line, such as gas chromatography and mass spectrometry; and on-line, which can be further divided into three categories:

- Intermittent sensors that require injection of a portion of a sample stream into the instrument;
- Continuous sensors that permit the sample to flow continuously through the instrument;
- Non-invasive sensors in which the chemical analysis is done *in situ*, directly inside the process line, using a probe that is chemically sensitive.

The disadvantage of using flow-through on-line sensors is their inherent need to divert part of the reactor broth through a flow loop in order to present it to the analyzing instruments. The diversion constitutes a disturbance to the overall system in that delays, contaminations, or variations in the process characteristics may be introduced. These problems are further complicated by the on-line sensor's need to have separate hardware to perform the sampling, concentration, separation, and sensing/identification steps. A major limitation to the successful application of existing biosensors is that cleaning procedures are required to maintain sterility, especially in large-scale industrial bioreactor systems. Therefore, the problem to be overcome centers on the ability of on-line sensors to be maintained sterile without loss of sensitivity.

The noninvasive sensors do not come into direct contact with the culture system, thereby eliminating many sampling and sterilization problems. Examples of noninvasive sensors are the fluorescence sensors which use fiber optics [30], near-infrared(NIR) spectroscopy from 700nm to 1000nm [48], and ultrasound [65]. Of these methods, the fluorescence sensor is the most applicable to the bioreactor process.

Several attempts at developing new on-line biosensors have been reported. They include enzyme embedded probes [41, 46, 47] that would react with one of the reaction broth components to yield an indirect measurement of the concentration of that component. Another optical-based approach is the on-line turbidimetric measurementment approach in which a broth stream is cycled through a spectrophotometer which measures the optical density (OD) of the broth. Through correlation, the biomass concentration can be inferred. A drawback in this approach is in its nonspecificity in that all particles present in the broth, such as dead cells and lysis debris, would all contribute to the OD reading. Of the above mentioned. A developing methods, that of fluorescence is

most similar to the role of bioluminescence in yielding indirect biomass concentration information. They are reviewed below.

2.1.2 General Review of Fluorescence Sensor

The principle behind using fluorescence for the inferring biomass is similar to the approach taken here in using bioluminescence. Therefore, it will be reviewed in more detail below. For the last ten years, much effort has been focused on the design of instruments capable of on-line measurement of biomass concentration and growth rate. A number of significant advances have been made in spectroscopic analyses, specifically fluorescence and infrared spectroscopy. These advances are often coupled with advances in fiber optics and in computer-aided analysis. Among several approaches, the measurement of NADH-dependent culture fluorescence appears to be promising. Since the discovery by Duysens and Amesz (1957) [14] of culture fluorescence, fluorescent measurements have been attempted by several investigators. Humphrey's group at Lehigh University began using fluorometry to monitor intracellular NAD(P)H levels 20 years ago [25]. Zabriskie and Humphrey (1978) [79] showed that the fluorescence signal has the potential to be used as an estimate of biomass concentration. Meyer and Beyeler (1984) [43] developed process control strategies based on the NADH content of microbial cells. During the last ten years, two different types of fluorometers, designed for the *in situ* monitoring of microbial cells, have appeared on the market (BioChem Technology, Malvern, PA, USA and Ingold AG, Urdorf, Switzerland). Ristroph et al. [54] determined the timing of substrate feed in a fed-batch reactor by monitoring the culture fluorescence kinetics. Einsele et al. [17] used the fluorometric approach to determine mixing times in a bioreactor as well as to study the kinetics of substrate uptake. In most cases when the environmental

conditions are controlled, a linear correlation can be usually obtained between the log of the biomass concentration and the log of the fluorescence. Under the controlled conditions, this correlation has the considerable potential as a method to provide the on-line biomass estimates for process control and optimization since the fluorescence data is obtained on-line. More research continues in the investigation of the feasibility of this approach [67, 76].

The fluorometric approach includes several drawbacks [37]: The interpretation of an NAD(P)H fluorescence signal from the whole culture broth is complex and complicated, depending on the medium, the oxygen level and the substrate. Also, the cells and the growth media contain a number of fluorophores, such as pyridoxine, tryptophan, and riboflavin, in addition to NAD(P)H. The behavior and fluorescence intensity of various fluorophores differ in the various fermentation systems. Applying fluorometric methods to a bioreactor is a complex installation process. Miniaturization and standardization of a fluorescence sensor must be performed in order to equip a fluorescence probe for every standard bioreactor.

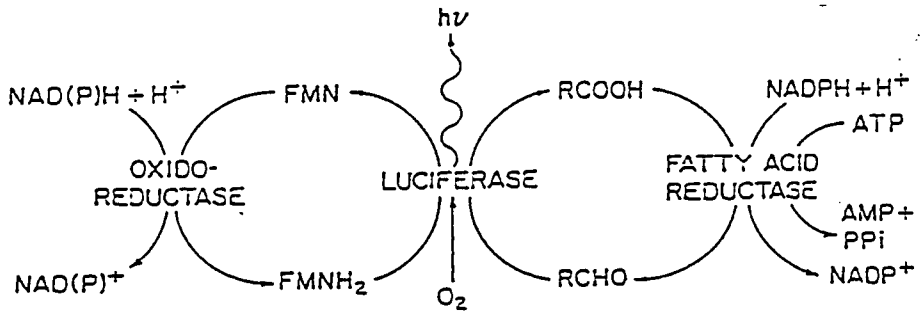
2.2 Review of Bioluminescence

During the past few years, the bioluminescent *lux* genes originally isolated from either the firefly *Photinus pyralis* or marine bacteria *Vibrio, Photobacterium, Alteromonas,* and *Xenorhabdus* have been cloned into several microorganisms for applied measurement purposes [3, 6, 18, 45, 69]. Bioluminescence has excited the curiosity of people over centuries for its unique beauty, although the practical applications of this naturally occurring phenomenon have been developed only recently.

The *lux* A and B genes of the *lux* structure genes code for the α and β subunits of the luciferase enzyme. The *lux* C, D and E genes code for the polypeptides of the fatty acid reductase system responsible for the regeneration of the aldehyde substrate for luciferase from the fatty acid product [9], [23], [29]. *Luciferase*, a mixed function oxidase, catalyzes light emission of the *lux* system. In bioluminescent bacteria, the luciferase catalyzes the oxidation of a long-chain aldehyde and $FMNH_2$, resulting in the emission of visible blue-green light at 490 nm wavelength. The reactions are presented below, and the details are shown in Figure 2.1.

$$R-CHO+FMNH_2+O_2 \stackrel{LuxAB}{\longrightarrow} R-COOH+FMN+H_2O+h\nu$$

$$R-CHO+NADP+AMP+PPi+H_2O \stackrel{luxCDE}{\longleftarrow} R-COOH+NADPH_2+ATP$$



(From Showalter, R.E. J. of Bacterial, June 1990, p2947)

Figure 2.1: Components of the bioluminescence reactions in *Vibrio fischeri*; R-CHO - a long chain aldehyde substrate; RCOOH - a long chain fatty acid, $h\nu$ refers to light.

The native bioluminescent reaction system is most active between 23°C and 30°C. In general, the bioluminescent activity is regulated, among others, by the endogenous synthesis and accumulation of the so-called autoinducer molecules [15, 18]. A study by Stouthamer [55] showed that the cellular energy required to support the bioluminescence is negligible, when compared to the overall cellular demand for ATP.

The strain RB1151, used in our experiment, is an *E. coli* DH5α strain containing the pUTK4 plasmid, constructed by Robert Burlage of the Center for Environmental Biotechnology at the University of Tennessee, Knoxville. The pUTK4 plasmid was created using the pUCD615 *lux* fusion plasmid [56], and the pUC18 plasmid (generally used as a *lacZ* fusion plasmid), containing the *lac* promoter, a strong promoter when derepressed. The construction is shown in Figure 2.2. In this strain, the *lac*I repressor gene is absent, thus making the *lux* gene activity constitutive, such that the bioluminescence gene is always active. Further, construction is such that the *lux* gene is capable of de-novo synthesis of the aldehyde, a substrate required for the bioluminescent reaction. The ligated plasmids were transformed into *E. coli* DH5α. Selection was on LB agar with ampicillin. The light-producing colonies were easily seen after the plates were cooled down to 25°C. The orientation of the pUC18 insert was ascertained using restriction enzyme digestion and agarose gel electrophoresis.

Prior work with cells harboring the *lux* gene cassette suggests that there is a direct correlation between the amount of biomass and the amount of emitted light [36]. If such a correlation can indeed be established, then it has the potential to be used as an on-line sensor to provide biomass concentration information during bioreactor operation. In addition, when a gene product is the desired final product, the *lux* gene may provide a convenient way of monitoring its synthesis progress. For example, the *lux* gene may

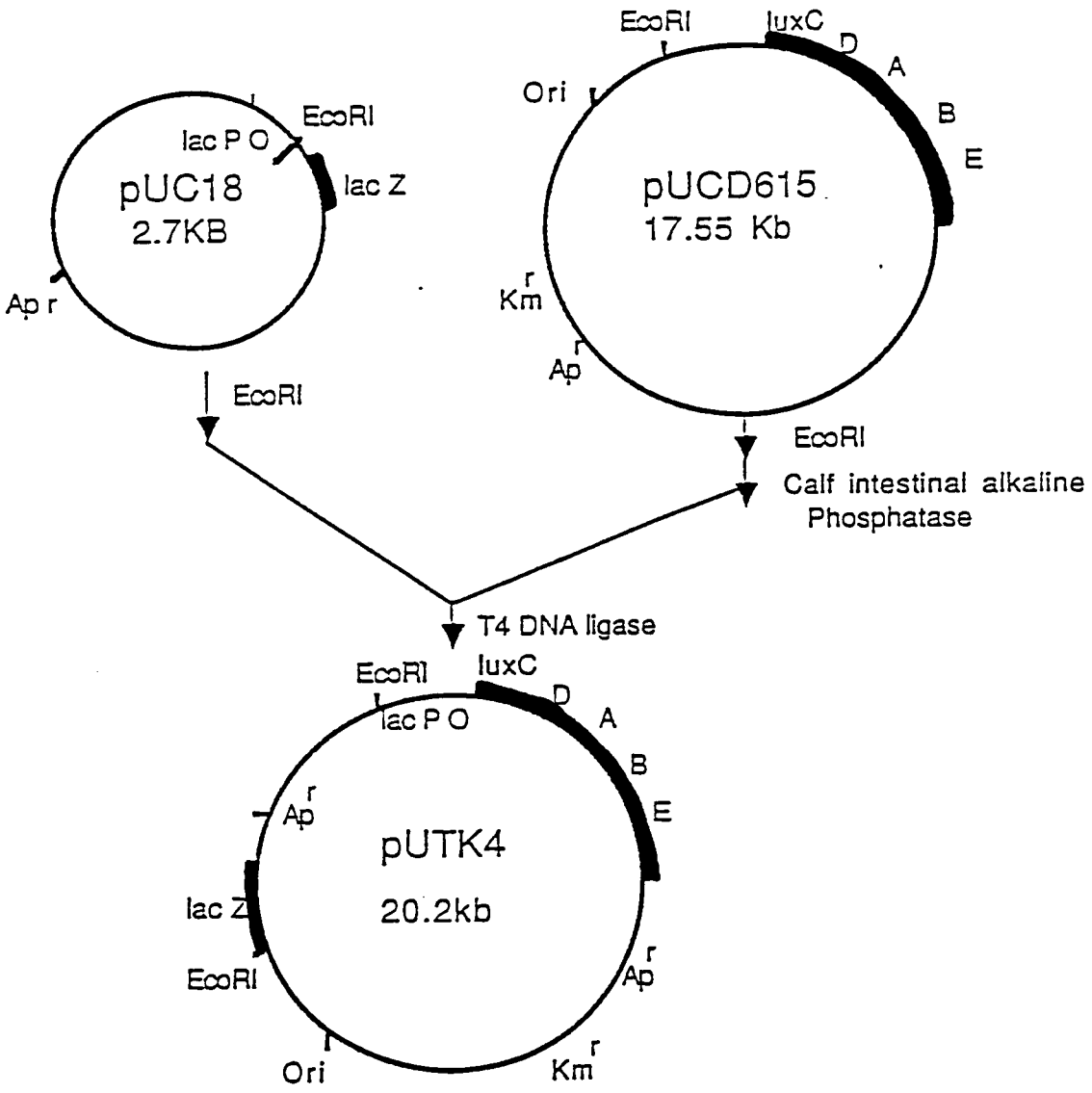


Figure 2.2: Construction of bioluminescent plasmid pUTK4, including the cloning strategy. Km^r denotes Kanamycin resistance; Ap^r denotes Ampicillin resistance; ori denotes origin of replication.

be cloned adjacent and be subjected to the control of the protein gene of interest, such that when the transcription and translation of the protein gene becomes active, bioluminescence genes also become active. Monitoring the kinetics of bioluminescence would provide a convenient handle for monitoring the synthesis of the protein of interest. Any disturbance to its synthesis would lead to a shift in the corresponding bioluminescence profile. Finally, feedback control of the protein synthesis would then be achieved through the bioluminescence measurement. Of course, it is recognized that initial cloning work is required to introduce the *lux* gene cassette at the appropriate site in the host cell of interest. With the increasing employment of recombinant cells in the production of pharmaceutical, food, and agriculture products, the cloning work involved in introducing the *lux* gene may not prove to be too inhibitive. If successful, the increased profit margin derived from using such on-line information may very easily offset the added cost involved.

2.3 Application of Bioluminescence

Bioluminescent micro-organisms have shown to have potential in industrial and environmental applications. Shimon Ulizur was the first to commercially exploit the industrial potential of bioluminescent bacteria. His work with *Photobacterium phosphoreum* led to the development of the Microtox system for detecting environmentally toxic agents [31]. The principle of application is that *in vivo* bioluminescence depends on a functional intracellular biochemistry. Substances which inhibit that biochemistry result in a reduction of light output. In consequence, the killing effect of biocidal agents can be measured in real time as a decrease in the light output of a bioluminescent culture.

Korpela and Karp [36] described the use of bioluminescent *E. coli* for the detection of the heavy metal cadmium. The advantage of using recombinant bacterial *lux* system is that it allows the use of hypersensitivity mutants specifically targeted for the detection of a particular environmental toxicant. Stewart and Denyer [68] had described the use of bioluminescence *E. coli* as a model system for biocide monitoring. They observed a close correlation between the viable count evaluation (24 h) and bioluminescence (10 min interval of evaluation) for the antimicrobial phenol at in-use concentrations, indicating that in this context bioluminescence is an excellent indicator of cell viability. The ability to monitor on-line the physiological status of cells, means that individual component strains in complex microbial systems can be studied. It can further be applied to food system, biofilms, and fermentors. Lloyd [38] developed an oxygen sensor using luminescent bacteria, where concentrations of oxygen lower than 1 μM are to be measured. This low concentration was too low to be measured by choices of other techniques.

The second application of bioluminescence is in the study of mass transfer rate in biochemical processes [74, 75]. Since the measurement of mass transfer in a highly viscous pseudoplastic broth is difficult to obtain by conventional methods and little data is available. Their work presented the method which uses bioluminescence for mass transfer measurements of oxygen and substrate components in biochemical reactors by monitoring the cell bioluminescence response. Mass transfer in continuous operation was studied at steady state conditions over a wide range of parameters. They found that the optimal specific rate of production is nearly double of that in a batch and transient growth.

2.4 Continuous Culture System

Continuous fermentation offers the means in obtaining a cell population which grows indefinitely in an unchanging environment. This is accomplished by continuous feeding of carbon-enriched medium to a bioreactor and continuously removing culture broth to maintain a fixed volume. In general, the growth rate of a bacterial strain may be within a very wide range, independent of the concentration of a given factor; but since at zero concentration the growth rate is zero, there must of necessity exist, at sufficiently low concentration of the growth factor, a region in which the growth rate falls with falling concentration of growth factor. Therefore, it should be possible to maintain a bacterial population over an indefinite period of time growing at a rate lower than normal simply by maintaining the concentration of one or more growth factors at a sufficiently low level, while the concentration of all other growth factors may at the same time be maintained at high level. In a chemostat, cell growth is limited by selected nutrient. Thus the rate at which the medium is supplied dictates the final steady state growth rate of the organism, and the concentration of the limiting substrate in the nutrient feed determines the final cell density [12, 13, 20, 58].

During the course of a bioreactor operation, the value of cell and substrate concentrations as a function of time are important indicators of the state of the system. In addition, the specific growth rate, μ, defined as the rate of cell concentration growth per unit cell concentration with units of $time^{-1}$, describes the dynamic state of growth of the microbial system. Therefore, these three variables are chosen as the state variables:

b: the biomass concentration (mass/volume)

s: the substrate concentration (mass/volume)

μ: the specific growth rate (/time)

During continuous operation, there are two variables that can be operated at will in the laboratory: D, the dilution rate, and S_f, the substrate concentration in the feed. They are independent from each other.

dependent variables: b, s;

independent variables: D, s_f

For a continuous reactor based on the conservation of mass component, the biomass and substrate balances are described below (if one assumes the Monod equation for growth rate):

2.4.1 Biomass Balance

The balance of biomass around the bioreactor is given by

$$Biomass_{in} - Biomass_{out} + Biomass_{growth} - Biomass_{death} = Biomass_{accumulation} \quad (2.1)$$

Each term in 2.1 refers to a rate term. In this process, the feed is sterile. Therefore, the first term is 0. Because the growth rate is generally much greater than the death rate, the fourth term can be ignored. Then, the corresponding mathematical equation is

$$\dot{b} = \mu b - Db \quad (2.2)$$

where $\mu = \frac{db}{dt}\frac{1}{b}$, $\frac{db}{dt}$ refers to growth only.

If the reactor is well mixed, dilution rate (D) is defined as:

$$D = \frac{F}{V_R} = 1/\tau \quad (2.3)$$

where V_R stands for the reactor volume and τ stands for the retention time.

2.4.2 Substrate Balance

The balance of substrate around the bioreactor is given by

$$Substrate_{in} - Substrate_{Out} - Substrate_{Consumed} = Substrate_{accumulation} \tag{2.4}$$

The dynamic equation is:

$$\dot{s} = D(s_f - s) - \frac{\mu b}{y_s} \tag{2.5}$$

where y_s is the biomass yield with respect to substrate, and s_f is the substrate concentration in the feed.

Generally the Monod equation is used to describe the growth dependence on the substrate:

$$\mu = \frac{\mu_{max} s}{K_s + s} \tag{2.6}$$

where K_s is a constant in the Monod equation and μ_{max} is the maximum specific growth rate. Both of these parameters depend on the ease of utilization of the substrate by the biomass.

At steady state condition,

$$\frac{db}{dt} = \frac{ds}{dt} = 0 \tag{2.7}$$

Therefore, the substrate and biomass concentration at steady state can be expressed as the following:

$$s_{s.s} = \frac{K_s D}{\mu_{max} - D} \tag{2.8}$$

$$b_{s.s} = y_s(s_f - s) \tag{2.9}$$

$$\mu = D \tag{2.10}$$

where the specific growth rate μ can be controlled by manipulating the dilution rate D. For our experiment, we can control the dilution rate at where the bioluminescence

is optimal. It is expected that in a continuous reactor, with substrate limited growth, logarithmic growth is always maintained. Further, with the cells always growing at logarithmic rate, a maximum level of bioluminescence can be expected from our previous experience.

CHAPTER 3

EXPERIMENTAL SETUP AND METHODS

3.1 Experimental Setup

All continuous culture studies were conducted with setup described as Figure 3.1. The bioreactor was a 3-litre (working volume 1.8-litre) glass and stainless steel tank, manufactured by Applikon of Foster City, California. The instrumentation has the capability of controlling pH, temperature, dissolved oxygen and foam through simple feedback control loops. Agitation was provided by two, four-bladed, turbine impeller mounted on a centered vertical shaft. Sterile air was directly introduced through a simple hole sparger. For the purpose of data acquisition and future process control implementation, the bioreactor was interfaced to a Sun workstation. On-line data acquisition at one minute intervals of light intensity, pH, dissolved oxygen level, and flow rate was started after inoculation. A 5-ml biomass sample was taken for off-line Klett unit measurements for optical density intermittently. The experiment was carried out usually for about 10 days.

In a chemostat, the medium goes to the reactor and the product has to be removed to achieve a constant level. Two pumps were used to keep the volume constant: one for medium inlet and the other for outlet broth syphoning off. outlet. The outlet was achieved with a fixed speed drive (20 rpm) and pumpheads (Masterflex Model 7015-20). The inlet needs through a pump with a variable speed drive (1 - 100 rpm) and pumpheads (Masterflex Model 7014-20). One has to be certain that the outletflow is at least as great as the inlet flow when choosing a pumphead.

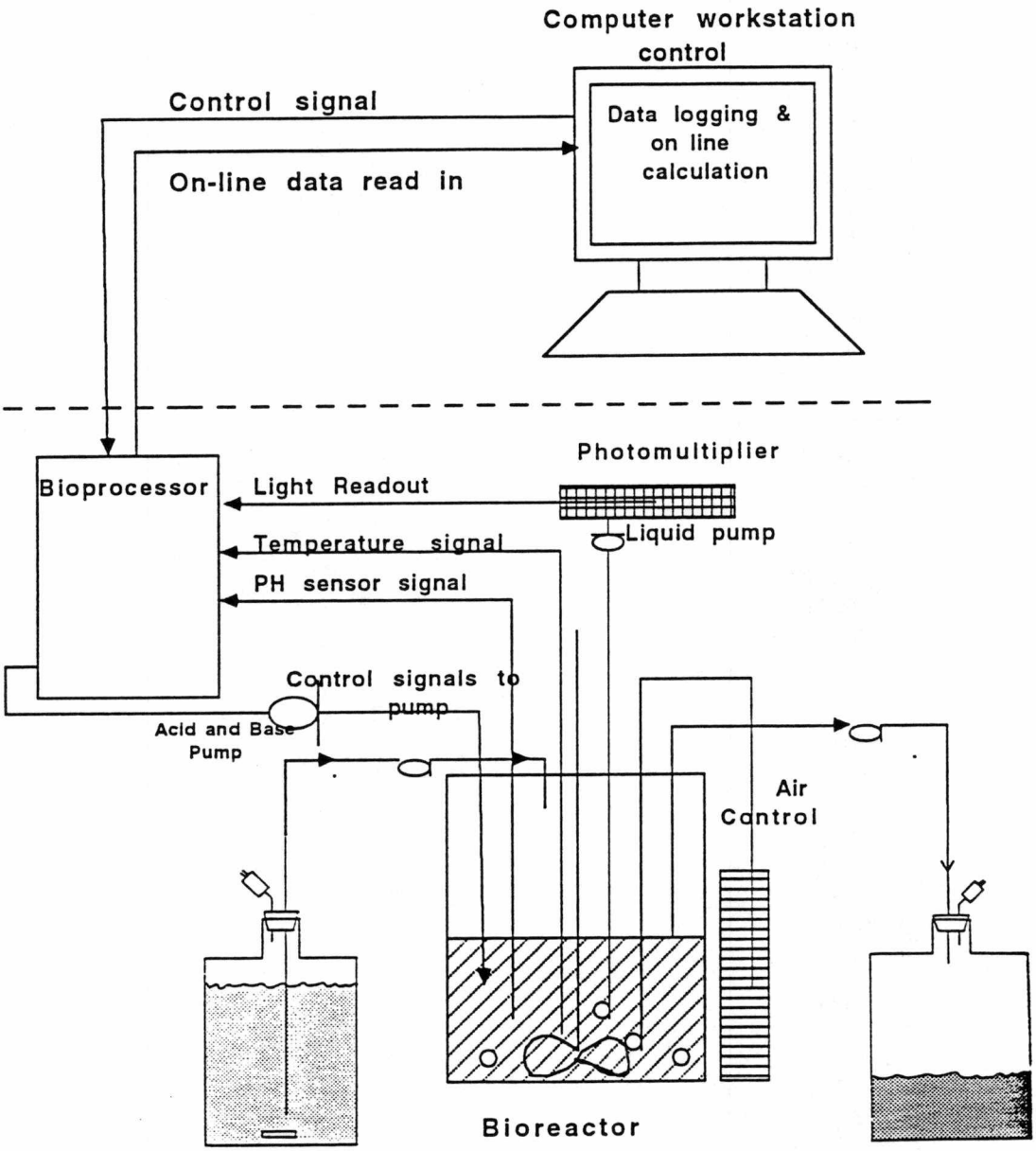


Figure 3.1: Diagram of bioreactor, controller and computer analysis system

3.2 Data Acquisition and Control

3.2.1 Hardware

The experiment was monitored by a SUN Workstation 3.5 interfaced to an Applikon Bio-Processor. The Bio-Processor is a modular designed instrument, used for measuring and control of operating parameters during a bioreactor run. In fact, it can be considered to be a hierarchical control system, containing several levels in ascending order of flexibility and supervisory potential. It can be seen from Figure 3.2.

Level 1 and level 2 are required for measurement and control of the parameters involved in a bioprocess. Level 1 contains the basic equipment necessary for a bioprocess. It includes the reactor itself, the sensors and electrodes and the hardware modules such as pumps, valves, heater, etc. Input signals from the sensors/electrodes in level 1 are amplified by the electronics in the parameter module. The major component of level 2 is the parameter module, which is basically a control instrument that performs the controller job with input and output cards. Output signals, generated by the parameter modul, are passed to the service module for implementation.

Level 3 can be added for bi-directional data transfer and advanced control purposes with a computer interface. The computer can be used to support or overrule the control action of the parameter module.

Level 4 is the top level for data manipulation in the host computer.

In the experiments described in this document, only the capabilities of level 1 and 2 are utilized.

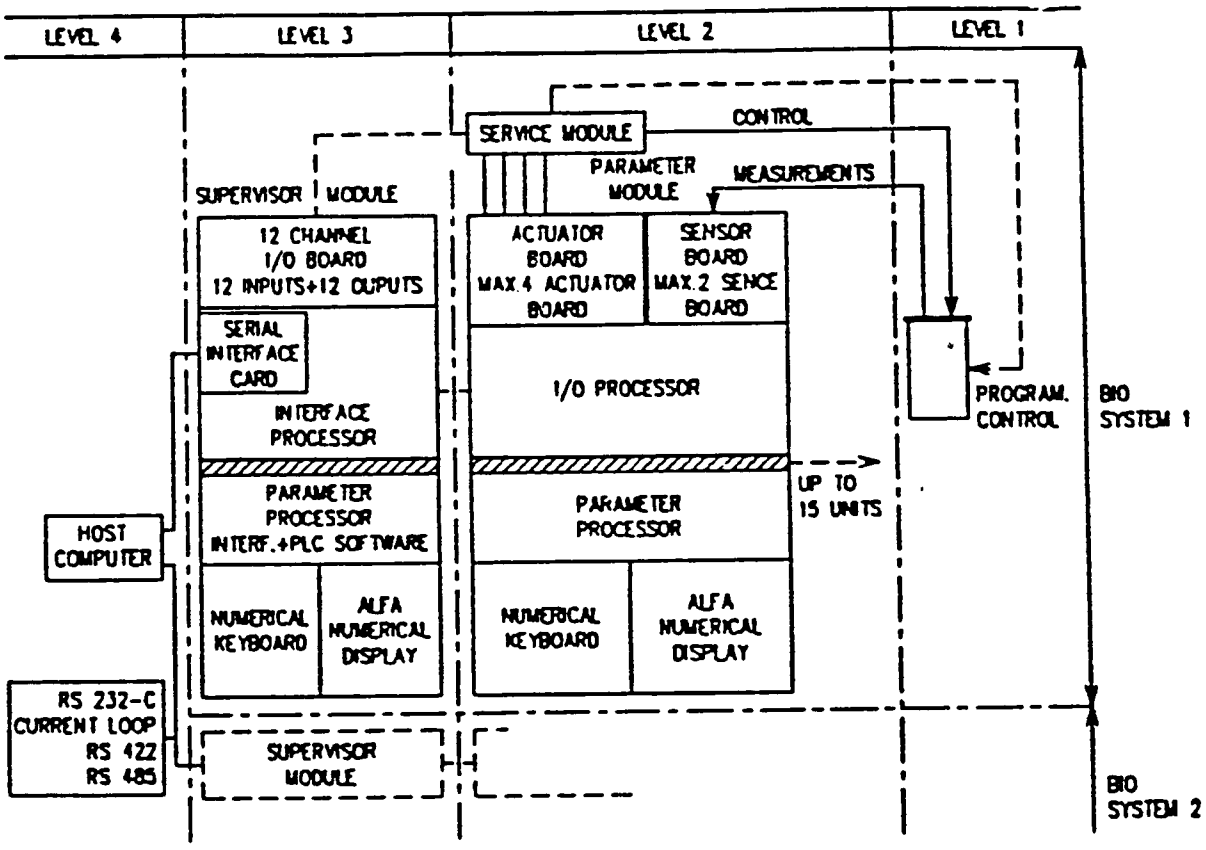


Figure 3.2: Bio-Processor Configuration

3.2.2 Data Acquisition

The computer system was set up to automate data acquisition. It was operated under the UNIX system. The computer acquires its information from Bio-Processor via internally loaded programs written in C (Appendix).

A real time clock provided accurate time for the system. It was interfaced to the computer as a memory based device.

3.2.3 Control Programs

The Bio-Processor has the built-in parameter control module software, which is able to perform several different PID based control strategies. The controllers were tuned empirically based on the dead time and time constant of the process to be controlled. The variables of dead time and main process time were determined by a step experiment. The advanced possibilities in data acquisition and process control are offered by the use of the supervisor module of the Bio-Processor as an interface between the parameter module and a host computer. Hence the supervisor module can be used as a Programmable Logic Controller (PLC) which offers great benefits in process automation.

3.3 Materials and Methods

Bacterial strain: The *E.coli* strain RB1151 used here is *E. coli* DH5α (*rec lac* Nal^r, Bethestha Research Laboratory). It contains the pUTK4 plasmid, constructed by Robert Burlage of the Center for Environmental Biotechnology at the University of Tennessee, Knoxville (Shown on Figure 2.2).

Medium: Glucose M9 salt medium was used for all culture work. It consisted of (per liter): 750 ml sterile deionized water, 200 ml M9 salts solution (See Table 3.1) , 0.5-20 ml 20 % glucose solution, 1 ml of sterilized 1 M $MgSO_4$, 0.1 ml of sterilized 1 M $CaCl_2$, 1ml trace element stock solution (See Table 3.2 [49]), 0.5 ml vitamin stock solution (See Table 3.3 [13]). Finally, filter-sterilized ampicillin was added to the medium to give a final concentration of 50 mg/l. pH was adjusted to 7.0 by using 1 N NaOH solution.

Table 3.1: $5 \times M9$ salts Composition (1 liter)

Ingredient	*gram*
$Na_2HPO_4 \cdot 7H_2O$	64
KH_2PO_4	15
$NaCl$	2.5
NH_4Cl	5.0
divided into 200 ml aliquots and sterilized	

Growth conditions: Overnight RB1151 cultures were grown in M9 salt medium with 0.2-0.5 g/l glucose in 250-ml flasks, in a 26°C water bath with constant shaking at 250 rpm with antibiotic selection. Cell growth was monitored by light scattering, using a Klett meter (Klett Manufacturing Co., New York).

Operating parameters: Each experiment was performed in a 3-litre bioreactor (Applikon Inc.) at an initial broth volume of 1.5 litre. The reactor was first filled with 1.5 liters of medium and then inoculated with the over night culture. A 150-ml exponential phase growing culture (at 50 Klett units) was used to inoculate the sterilized Glucose

Table 3.2: Trace Elements Composition (from *Methods in Microbiology*)

Ingredient	*mg*
$CuCl \cdot 2H_2O$	50
$MnSO_4$	10
$ZnNO_3(or ZnO)$	70(45)
$CoCl_2 \cdot 2H_2O$	10
MoO_3	10
$FeCl_3 \cdot 6H_2O$	100
add 1 liter water and being sterilized by autoclave as stock solution.	

M9 medium at 1:10 dilution. During growth, temperature was maintained at 26°C, pH at 7.0, and pure oxygen flow at 0.05 LM (l/min); impeller speed ranges from 300 to 800 rpm (under computer control). The dissolved oxygen level was controlled by a simple feedback controller loop through the bioprocessor at 80% or above of saturation level. The dissolved oxygen level was sensed by a dissolved oxygen probe (the auxiliaries of the applikon bioreactor). A low dissolved oxygen level was compensated first by increasing the impeller stirring speed up to a specified allowable maximum rate. If the desired dissolved oxygen level was still not met after the impeller speed had been increased to the maximum allowable speed, for instance, during the cell exponential growth phase, then the bioprocessor increases the flow rate of the inlet oxygen. A typical continuous run was operated for a period of 10 to 20 days. Intermittent sampling of the cell density (Klett units) was performed using aseptic techniques.

Table 3.3: Vitamin Stock Formula (per 100 ml)

Ingredient	*mg*
Biotin	4
Aminobenzonic Acid	10
Folic Acid	4
Pantothenic Acid	10
Nicotinic Acid	10
Vitamin B_{12}	0.2
Thiamine hydro	10
Pyridoxine hydro	20
Thiotic Acid	10
Riboflavin	1
sterilized by filtration	

Data analysis software: All numerical and simulation work was carried out with the software Matlab [40] on a Sun workstation. The computer programs are documented in the Appendix.

3.4 Experimental Preparation and Start-up

Continuous processing of the biological system placed additional demands on aseptics and biocontainment in terms of equipment design, instrumentation, and operation. Bioreactor systems have an additional element of complexity when compared to chemical reactor: microbial contamination must be prevented. Bioreactor design requires that a single bacterium or virus not be allowed to penetrate the reaction over a period of 10 to 20 days.

In order to prevent or reduce the chance of contamination, several preventive measures were taken. All connections are made air-tight. Any attempt to open the system to the air was done quickly and carefully. For example, when taking off-line samples, the surrounding and the connection of the sample port were always flamed.

The culture was allowed to grow for about 10 hours in batch mode before switching into the continuous mode at the dilution rate of 0.14 hr^{-1}. It usually takes about 40 hours for the system to come to steady state after switching to the continuous mode.

3.5 Biomass Measurement

Off-line biomass measurement provides a baseline against which on-line bioluminescence is to be correlated, several methods can be used for the off-line measurement of the biomass. We have chosen to use the Klett meter measurement because of its convenience.

Every 2 to 10 hours, about 5 ml of sample was withdrawn from the reactor through the sampling port for off-line measurement. Several precautions in withdrawing the sample from the bioreactor were taken. Such as, the general area around sample port was flamed to prevent contamination. Furthermore, the culture residing inside the sample tube has

to be purged back into reactor before withdrawing the sample in order to get.

3.6 Light Detection System

Light production from growing cultures in the bioreactor was measured by a photomultiplier system via a flow-through chamber, designed at the Chemical Engineering Department (UTK). It was essentially a light-tight steel cylinder containing a photomultiplier tube that was juxtaposed with a flow-through glass cuvette (0.75 ml Suprasil Micro Spectrophotometer Cell, with a light path of 10mm $\pm$ 0.01, and an aperture (mm) of 11$\times$6.5). The culture broth was continuously pumped to and from the reactor through the cuvette by a variable speed pump (flow rate from 10 to 100 ml/min). The tube connections were made so that the creation of turbulent flow or bubbles was minimized. The output of the photomultiplier tube was in the form of a voltage potential. The power supply voltage used to drive the photomultiplier tube was set at 1000 volts, and the amplification was implemented to the photomultiplier for adjusting the signal range of light emission as we need. Usually, the background noise of the detection system was around 0 to 5 mv. The flow-through cuvette and all the connecting tubings of the detection system were autoclaved prior to usage to maintain sterility.

CHAPTER 4

COMPUTER SIMULATION

Results obtained from the numerical studies were first carried out in order to further understand and anticipate the process kinetics. The objective of the numerical study was to examine the general dynamic properties of bacterial continuous reaction process and to determine the experimental parameters.

A series of numerical simulations were carried out with the purpose of better understanding the continuous process and to role of the process parameters (μ_{max}, Y_s, K_s) on the final steady state levels. Thus one can predict what the optimal feeding substrate concentration (s_f) that can be used to reach the desired steady state at suitably chosen operating conditions and what the transient dynamics as well as the trend of final steady state biomass and substrate will be after varying the dilution rate.

The numerical simulation was performed as follows: understructured models were assumed for the growth structures; the monod equation was used for the specific growth rate μ. These bioreactor design equations (Eq. 2.2 and Eq. 2.5) were then integrated for various values of the operating parameters to produce a variation of the state variables b and s as a function of time.

4.1 K_s Determination

A functional relationship between the specific growth rate μ and the essential compounds concentration was proposed by Monod in 1942. The Monod equation is one of

the equations which was used in the simulation study. Therefore, we must estimate the parameters beforehand. The Monod equation states that

$$\mu = \frac{\mu_{max} s}{K_s + s} \tag{4.1}$$

where μ_{max} is the maximum growth rate achievable when $s >> K_s$ and with the concentrations of all other essential nutrients unchanged. In our case, μ_{max} was determined to be 0.3 $hour^{-1}$ based on the batch experimental data. K_s is the value of the limiting nutrient concentration at which the specific growth rate is half its maximum value. As mentioned in Bailey and Ollis's book [2], K_s values for *E. coli* strains growing in glucose-limited media are 0.22×10^{-4}M, which is about 0.004 g/L. Gaudy indicated that K_s was extremely low for most species of microorganisms growing on simple substrates [20].

It is apparent that the Monod equation is a great oversimplification, but we used it in the simulation to approximate the interrelationship between the state variables. A reasonable range of these parameters was made. The Monod model was employed for $\mu(s)$ with $\mu_{max} = 0.3 hour^{-1}$ and $K_s = 0.005 g/l$ together with a constant substrate yield Y_s of 0.5 g/l of substrate. Figure 4.1 shows the effect of K_s on the relationship between the substrate concentration S (lower case) and specific growth rate μ under different assumed K_s. It can be seen that a small difference in s (at very low value of s) will evoke a large change in μ. Figure 4.1 was used as a reference chart for choosing the initial substrate concentration s_0 and the feeding substrate concentration s_f.

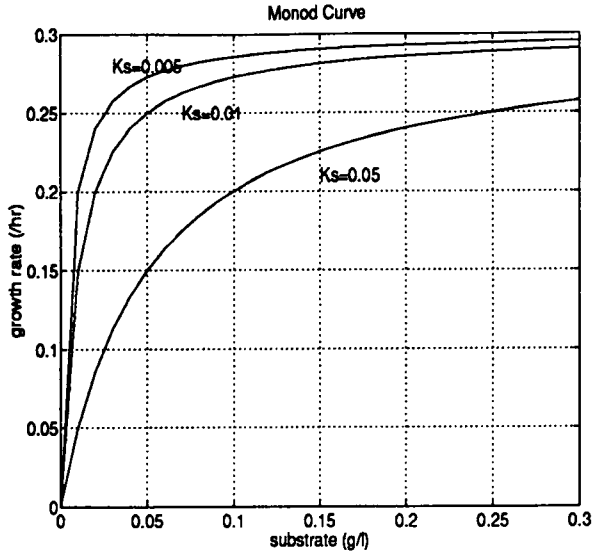


Figure 4.1: Monod curve under the assumed K_s. Assumed condition: μ_{max} =0.3 hr^{-1}.

4.2 The Effect of Initial Condition on Final Steady State Values

The transient dynamics toward the final steady state will be determined partially by the initial inoculum and substrate concentration (b_0 and s_0). Figure 4.2, 4.3, and 4.4 show that different b_0 and s_0 lead to the same steady state value of b, s, and μ even though the initial substrate concentrations were different. In this case, 0.05g/l was assumed as the initial inoculum in the study of the effect of initial substrate concentration on final steady state values during the operation of continuous bioreactor. The value is close to the amount of the inoculum used in the experiment which was derived from the correlation between Klett Unit and dry weight (g/l). It was assumed that the process was run under the batch mode for 5 hours then switched to continuous mode at $D = 0.1hr^{-1}$. After switching to the continuous mode, the feeding substrate

concentration was assumed to be 0.1 g/l. For all cases, all the parameters in simulation were based on $\mu_{max} = 0.3\ hour^{-1}$ and $K_s = 0.005 g/l$ together with a constant substrate yield Y_s of 0.5 g/l of substrate. Figure 4.5, 4.6, and 4.7 show the transient as well as steady state values of b, s, and μ at different initial inoculum. The initial feeding substrate concentration was fixed to be at 0.1 g/l in order to test the effect of initial inoculum on transient and steady state values. In the assumed condition, it can be seen from the figures that the system will take about 25 hours to reach the same steady state level no matter what the initial condition. It indicates that at the same D the steady state performance of the bioreactor system is independent of the initial state variable values (s_0 and b_0). However they differ in the transient behavior

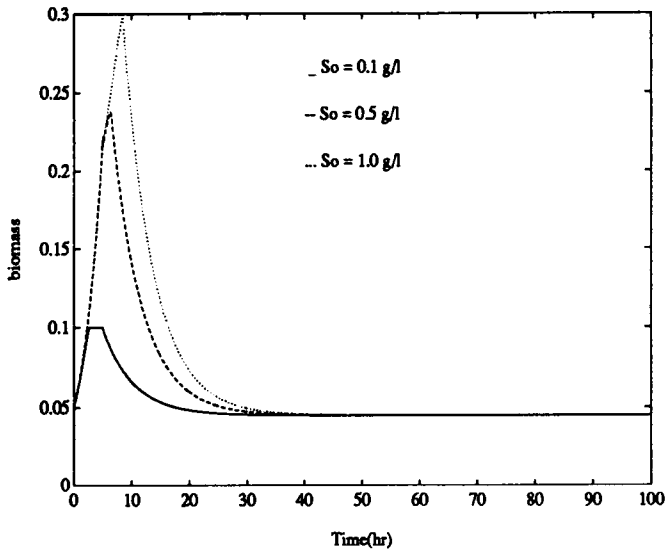


Figure 4.2: Effect of initial substrate concentration in the medium on biomass dynamics at $D = 0.1 hr^{-1}$.

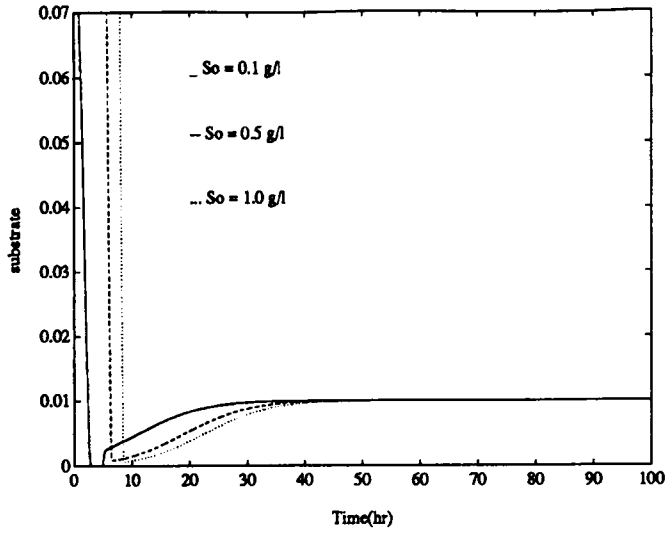


Figure 4.3: Effect of initial substrate concentration in the medium on substrate concentration at $D = 0.1hr^{-1}$.

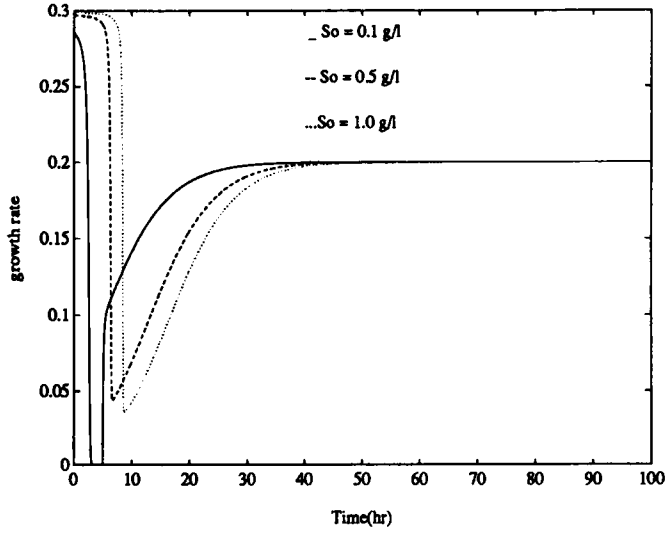


Figure 4.4: Effect of initial substrate concentration in the medium on the specific growth rate at $D = 0.1hr^{-1}$.

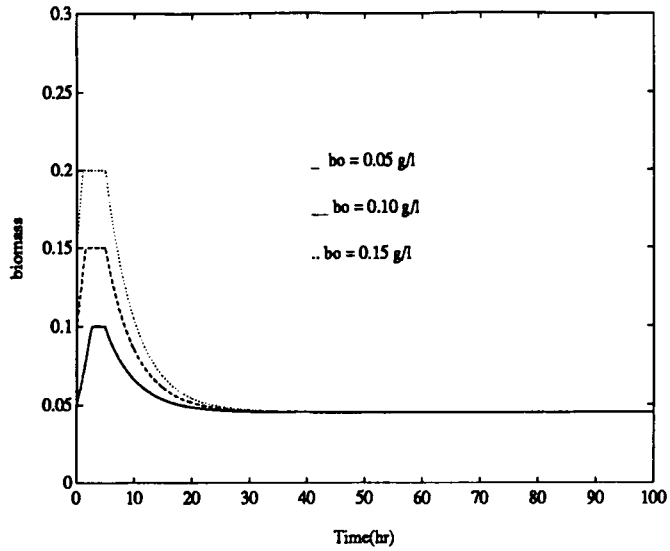


Figure 4.5: Effect of initial inoculum on biomass dynamics at $D = 0.1hr^{-1}$.

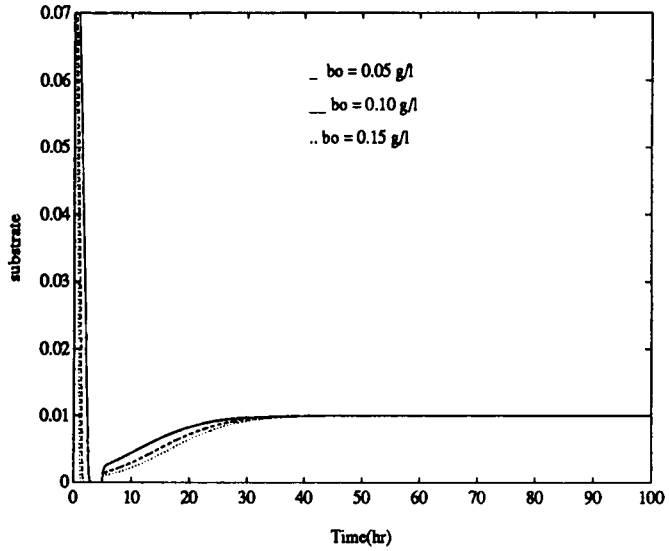


Figure 4.6: Effect of initial inoculum on substrate concentration at $D = 0.1hr^{-1}$.

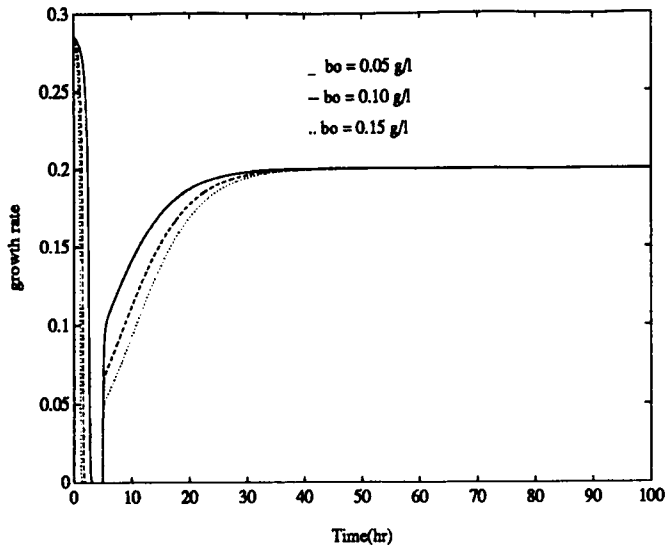


Figure 4.7: Effect of initial inoculum on specific growth rate at $D = 0.1hr^{-1}$.

4.3 Effect of the Time to Switch to Continuous Run

Before operating the process, it is vital to determine the optimal time to switch to continuous run from batch mode. Figure 4.8 showed the effect on biomass and growth rate if switching to continuous mode is at 3 hours, 5 hours, and 10 hours of batch run. It indicates that the steady state level is independent of the time point of switching the operating mode. But it suggests that it is better to switch to continuous run before substrate is exhausted to avoid starving the cells. The optimal switching time is when cells are growing during exponential phase of the batch growth. Because of the continuing depletion of nutrients and accumulating dead cells during the stationary phase of growth, it takes longer time for system to reach steady state at this stage.

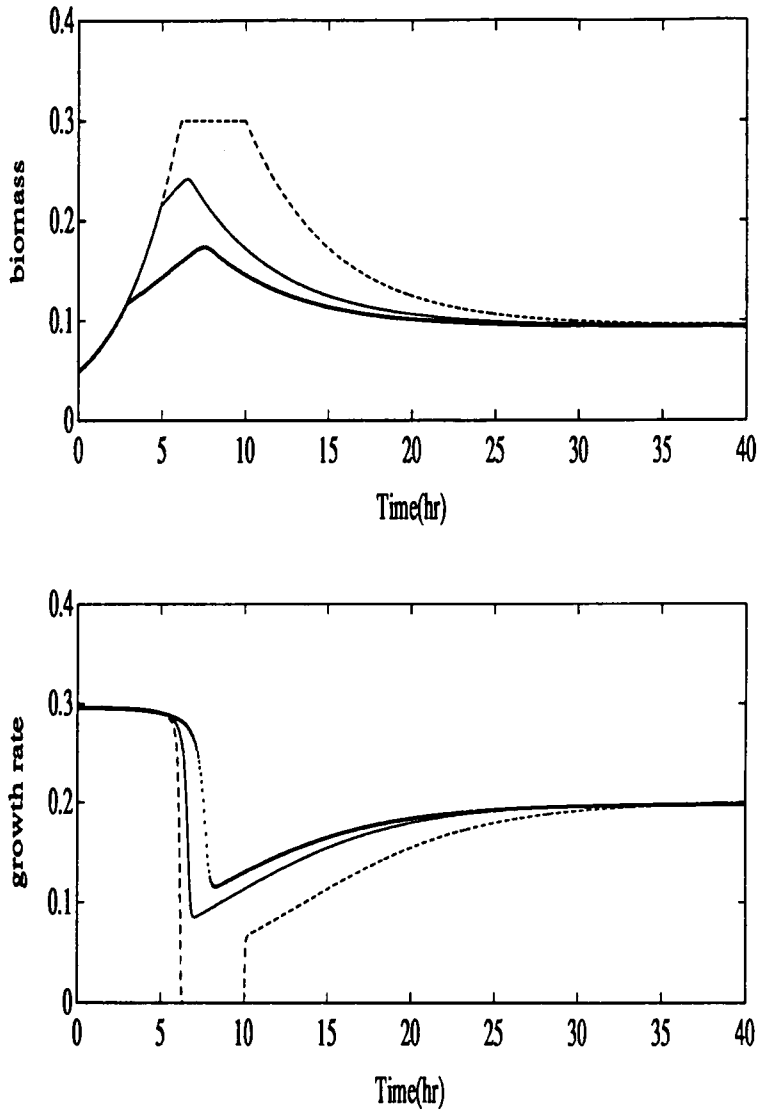


Figure 4.8: Effect of of switching time from batch to continuous. Solid line indicates the switch to continuous run at 5 hours of batch run; dash line (–) indicates the switch at 10 hours of batch; dot line (...) indicates the switch at 3 hours of batch.

4.4 Influence of Dilution Rate on the Steady-state Variables

The operation of a chemostat was simulated under the conditions in which the steady state specific growth rate was controlled by the dilution rate. Figures 4.9, 4.10, and 4.11 show the response of the values of biomass and growth rate in correspond to a series of step changes of the dilution rate in a chemostat. They showed the specific growth rate tracks the series of step changes in the dilution rate from 0.05 hr^{-1} to 0.10 hr^{-1}, then to 0.15 hr^{-1}. It can also be seen that the steady state substrate concentration increases slightly when D increases in these intervals. It can be explained from Equation 2.8 that the steady state level of substrate is only dependent on the dilution rate D, and a higher value of D leads to a higher level of steady state substrate concentration. We simulated the responses of state variables to a series of step changes in D at different s_f (0.1, 0.2, 0.005 g/l). In the case of very low s_f of 0.005 g/l, the system took longer time to reach a steady state growth rate in response to a shift of dilution rate D. Results are shown in Figure 4.11.

At our case of low K_s, the dependence of μ on b is very slight, thus biomass concentration did not change much with the shift of dilution rate. It confirms the pattern of changes in biomass b and substrate concentration s as a function of the dilution rate as shown in Figure 4.12 (From D.W. Tempest's book [70]). If K_s is very low, as was observed by Monod and some of the early workers in continuous culture, the cells dilute out at D close to the value of μ_{max}, and there is little change in substrate and biomass concentration levels over a wide range of value of D. While at higher values of K_s, the substrate concentration in the effluent begins to rise at progressively lower values of D [20]. At low feeding substrate concentrations comparing with low K_s at 0.005, the

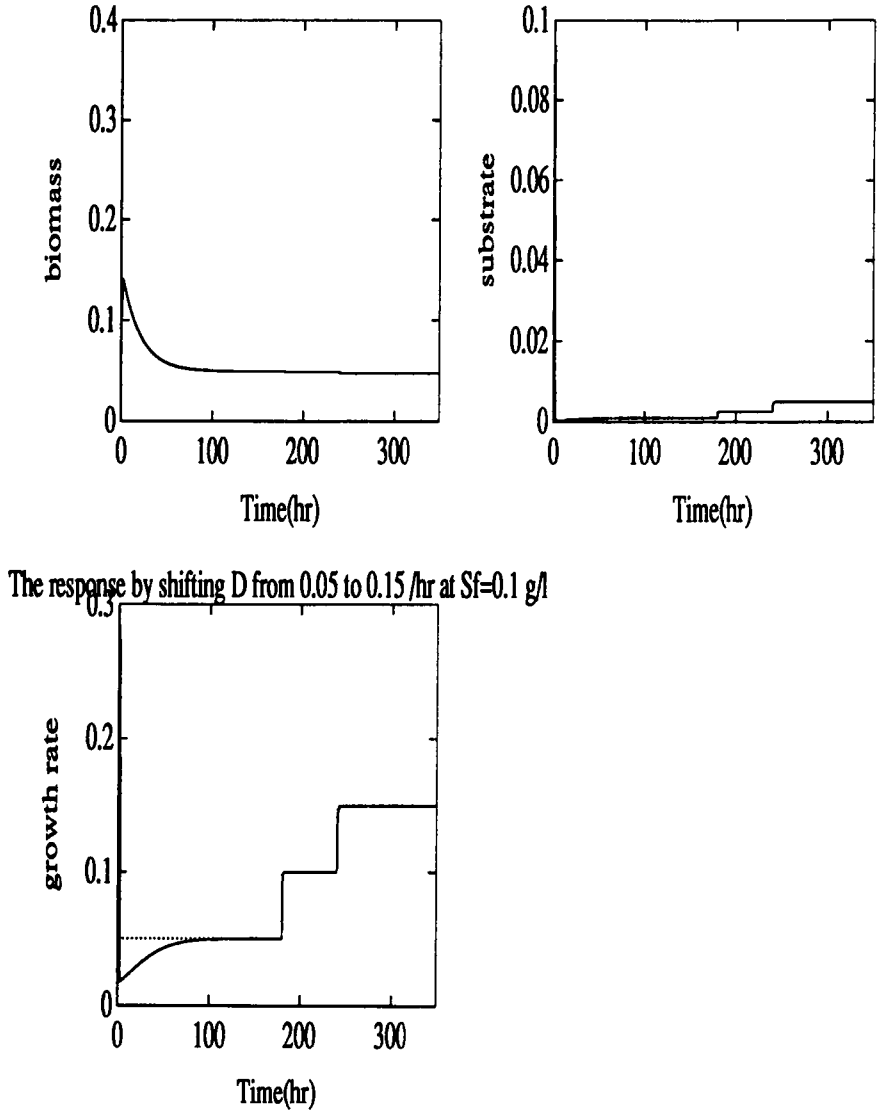


Figure 4.9: Response of state variables to a series of step changes in the dilution rate for continuous reactor at $s_f = 0.1g/l$.

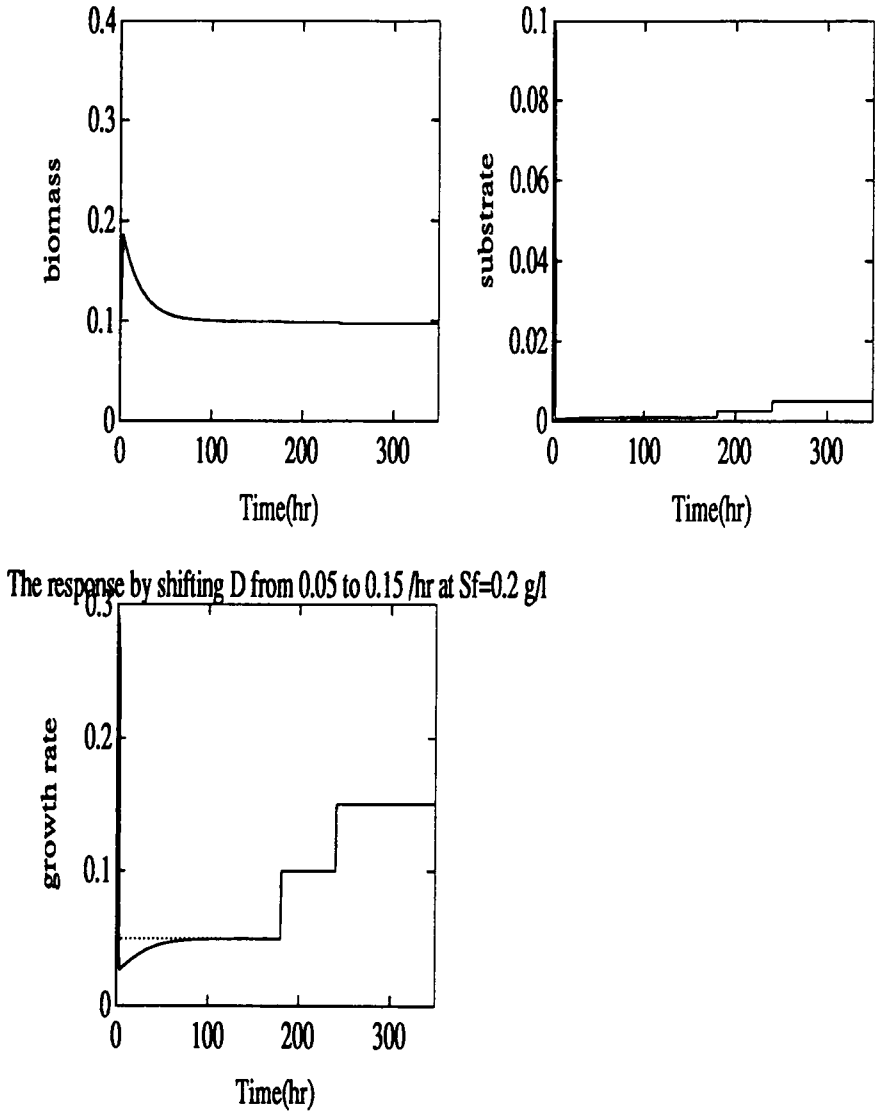


Figure 4.10: Response of state variables to a series of step changes in the dilution rate for continuous reactor at $s_f = 0.2g/l$.

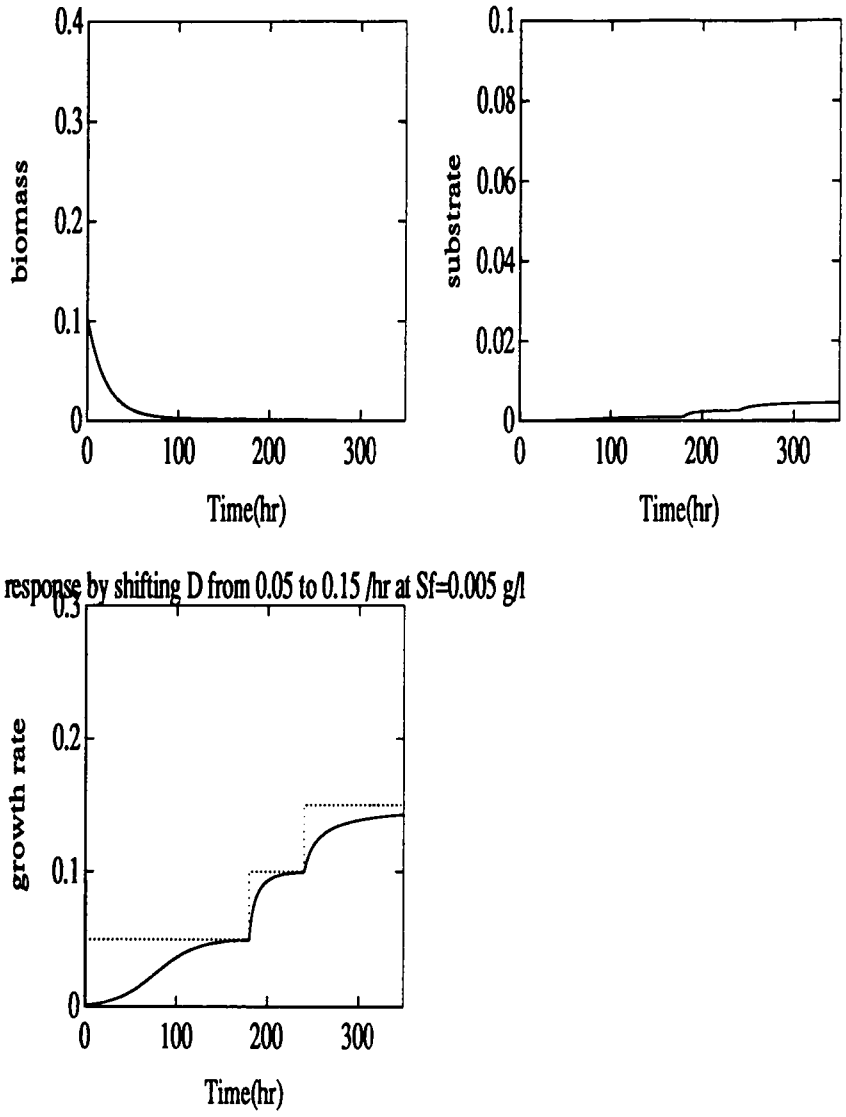


Figure 4.11: Response of state variables to a series of step changes in the dilution rate for continuous reactor at $s_f = 0.005g/l$.

concentration of biomass in continuous culture can be varied by increasing the feeding substrate concentration s_f, while substrate concentrations in the effluent remain more or less the same, as can be seen in the simulation plot of Figure 4.13.

4.5 Summary of Simulation Studies

Results presented in this chapter are representative of a variety of numerical simulations carried out in order to shed light on the proper design of the proposed continuous experiment. The results of the experimental work will be presented in Chapter 5. We proposed to do continuous runs to reach desired steady state at suitably chosen operating conditions, in a reasonable amount of time, by doing dilution rate shift-up and shift-down experiments, in order to study the pattern of the corresponding bioluminescence. The purpose of the simulation is to predict the relative changes in the state variable values as they vary during the course of an experiment. An important feature of a good simulation is the ability to reliably predict the relative changes or variations as they may develop during the course of process operation. Simulation results show that steady state values of specific growth rate will be equal to to the dilution rate, and in the case of low K_s, μ is almost independent of the corresponding biomass concentration, as described by Eq. 2.9. Since from theory, one would predict that at constant feed substrate concentration, s_f, the steady state biomass concentration level is independent of the dilution rate, whereas the specific growth rate would track the dilution rate at steady state. The corresponding changes in the steady state bioluminescence level as D changes will give an indicator as to whether bioluminescence is related more to biomass concentration or to the growth rate.

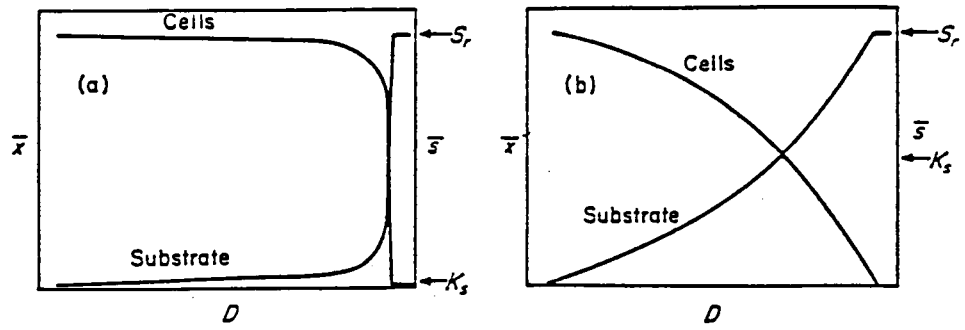


Figure 4.12: Influence of dilution rate (D) on the steady-state concentration of biomass and growth-limiting substrate in a chemostat culture: (a) When K_s is small relative to s_f and (b) where K_s is large relative to S_f

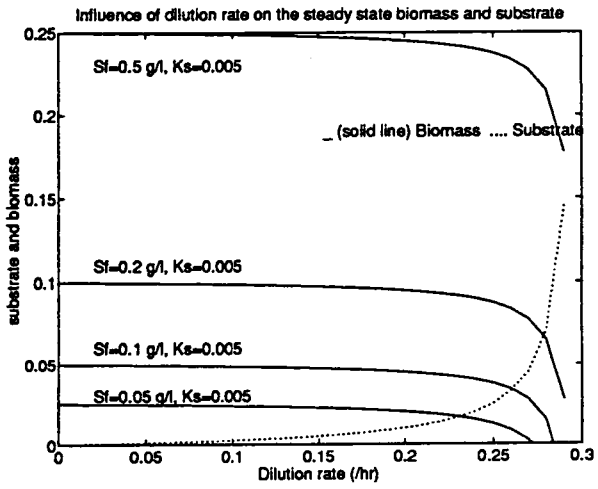


Figure 4.13: Effect of s_f on the patterns for biomass and substrate concentration as dilution rate is increased

In order to test the relationship among bioluminescence biomass, and specific growth rate, the dilution rate shift-up and shift-down analysis will be given in the real system. The transient behavior of the system that was invariably observed is given the relative estimation scheme to operate the real system. It gives clear ideas to handle the transient stage and avoid mistakes by the trial and error. It also suggests to switch to continuous mode from batch mode during the exponential growth phase.

CHAPTER 5

Experimental Results and Discussion

5.1 Preliminary Studies

Biomass-bioluminescence correlation work has been carried out previously in a batch operated reactor [22]. Cell mass and bioluminescent intensity information were monitored and recorded over a period of about 24 hrs during batch runs of *E. coli* strain RB1151 in LB broth. A total of 11 runs were carried out. Figure 5.1(a) shows the growth profiles of three of the experiments at 1:20 inoculation dilutions. Figure 5.1(b) shows the corresponding bioluminescence profiles. Figure 5.2 is a typical light emission profile superimposed onto the corresponding biomass growth curve. The biomass curve has the typical sigmoid-shaped kinetics associated with batch growth. In contrast, light emission starts from a low level, goes through a maximum, then rapidly declines. Toward the late log phase, it is evident that only a very low level of light was observed, in spite of the fact that biomass concentration was reaching maximum. Comparing the two curves, it is immediately evident that the two curves, as they stand, do not have a direct correlation, but rather that the light emission curve appears to have a more direct correlation with the slope (or first derivative) of the biomass curve. The slope of the biomass curve reflects the *rate* of biomass growth. The slope of the biomass curve shall be referred to as the *growth rate* curve. This correlation hypothesis is based on the observation that light emission starts and ends at a low level, corresponding to the

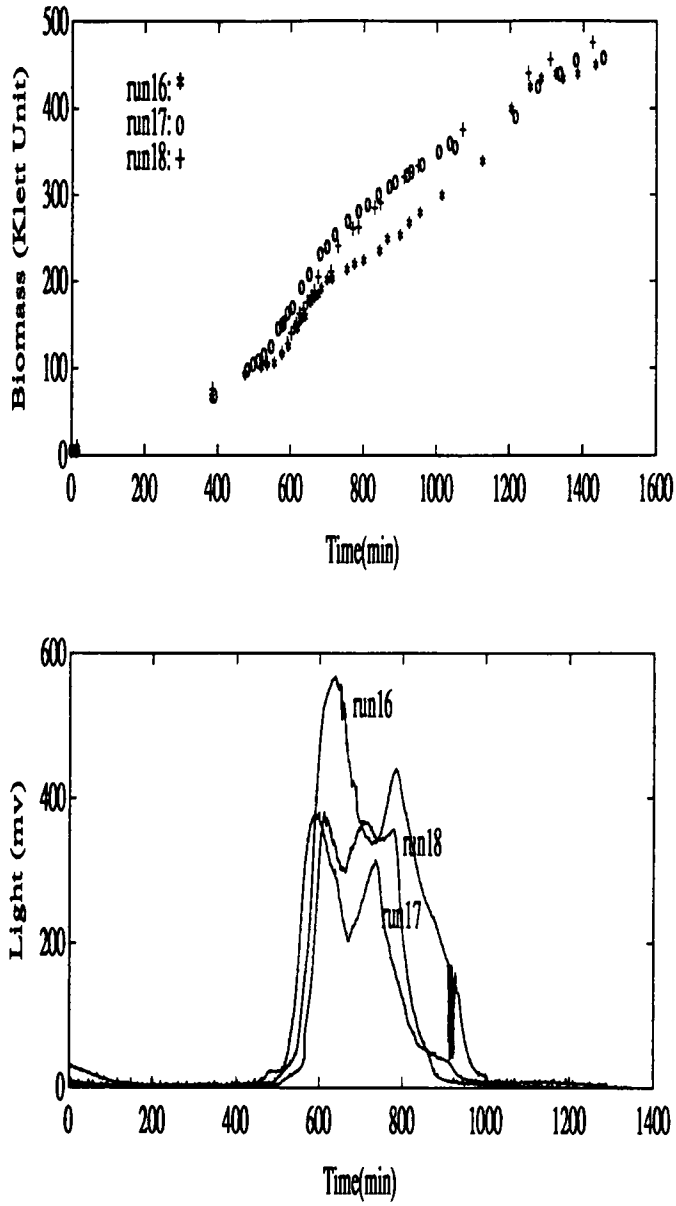


Figure 5.1: (a): Growth curves for *E.coli* RB1151 at pH 7.2, temp 26.0 °C. (b): Bioluminescence comparisons under identical operating conditions of three batch runs.

relatively low growth rate regions of the lag and late log phases of growth. The emission goes through a maximum corresponding to the region of maximum growth rate. It suggests that we can take the first derivative of the biomass kinetics to obtain the growth rate profile to see if the light intensity is growth rate related. Figure 5.3 shows a comparison of the derivative of the biomass growth kinetics and the bioluminescence profile of one batch run. From a mathematical point of view, the maximum point of the biomass derivative curve is the point of inflection of the biomass curve, and it turns out to correspond to the point of maximum light emission. The above observation implies that the light emission phenomenon is correlated with the process of growth, and not to the total amount of biomass itself. This is physiologically plausible, taking into account the underlying mechanism of bioluminescence emission, in that reactions involving the luciferase enzymes, oxygen, NADPH and the flavin mononucleotide, $FMNH_2$ and ATP are responsible for producing the light emission, and that the intensity of emitted light would be dependent upon the relative production and turnover rates of these enzymes and molecules. The relative turnover rates, in turn, are directly related to the rate of growth of the biomass. When the net growth rate is close to zero, as in the stationary phase, the net turnover rate of these enzymes would also be close to zero. As a result, no light emission would be evident. It can be seen further that the greatest demand for oxygen is at the time of maximum growth rate or maximum light emission. If, during this time, dissolved oxygen level is not adequate, one observes an immediate decline in the emitted light intensity.

Due to the varying base line of the photomultiplier output signal, we normalized the light emission curves of four experiments and attempted to correlate the normalized light curves to the first derivative of the corresponding growth curves via fitting with

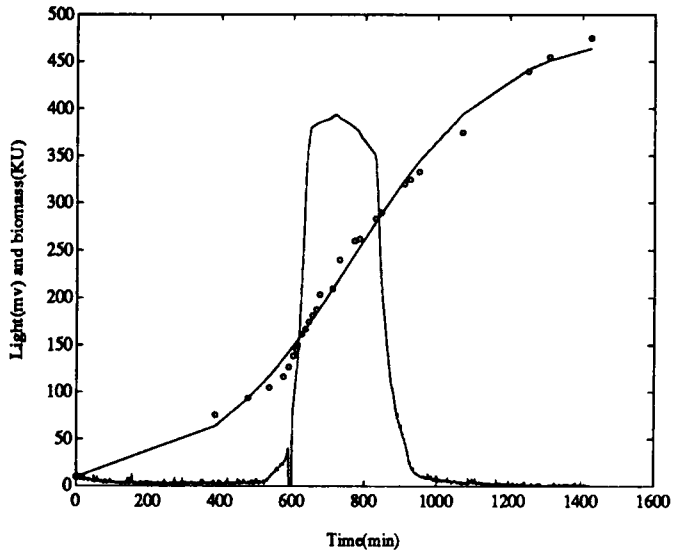


Figure 5.2: Growth and bioluminescence kinetics of *E. coli* RB1151 grown in LB medium with ampicillin. Growth measured in Klett unit (open circle) and light intensity as millivolts. Circles indicate growth and solid line indicates light emission.

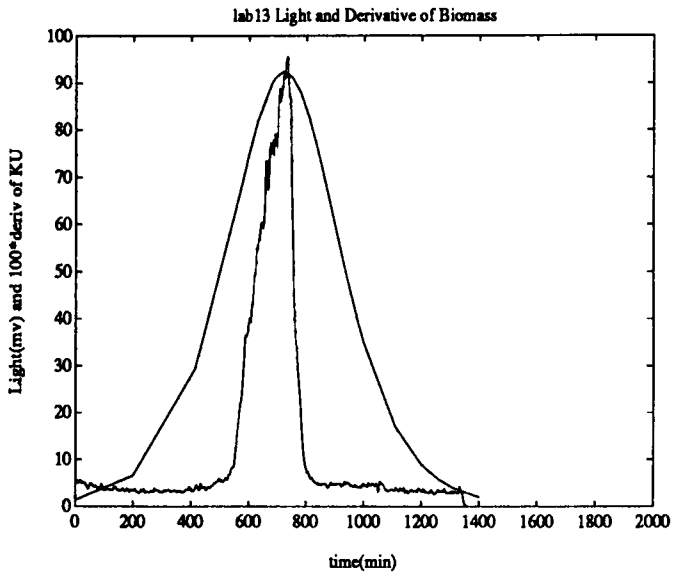


Figure 5.3: Comparison of the derivative of growth curve and bioluminescenc curve. (The upper curve is the derivative of the growth curve, the lower is the bioluminescence curve).

Gaussian probability distribution functions, which are characterized by two parameters, the mean μ, and the variance, σ. The values for the parameter μ for the two fitted curves coincide because the maximum of the light emission curve almost always corresponds to the point of maximum growth rate. A nearly constant ratio between the two σ's of the fitted Gaussian curves was indeed derived from the normalized data. Finally, this correlation was validated on an independent experimental data set (normalized). Figure 5.4 compares the actual and the model-predicted results. Light emission intensity data was logged at one minute intervals. For this validation study, with each normalized light measurement, the corresponding growth rate is calculated from the correlation model. Then, the growth rate is integrated discreetly to yield the corresponding total biomass level. The fit appears to be compatible. The ability to derive a consistent correlation when normalized but not raw data is used suggests that an inherent correlation does exist (albeit a nonlinear one) between the light emission level and the biomass rate of growth.

We examined the correlation in the logarithmic growth region of the batch runs. A linear relationship between biomass and bioluminescence does appear to be evident for this region. This observation suggests that a more clear-cut linear correlation may be expected to be derived for cell systems growing in the exponential growth phase, which is the predominate growth phase in a continuous stirred tank bioreactor, in which the dilution rate would determine the final steady-state specific growth rate. In addition, continuous reactors have the potential to yield much higher level of output products.

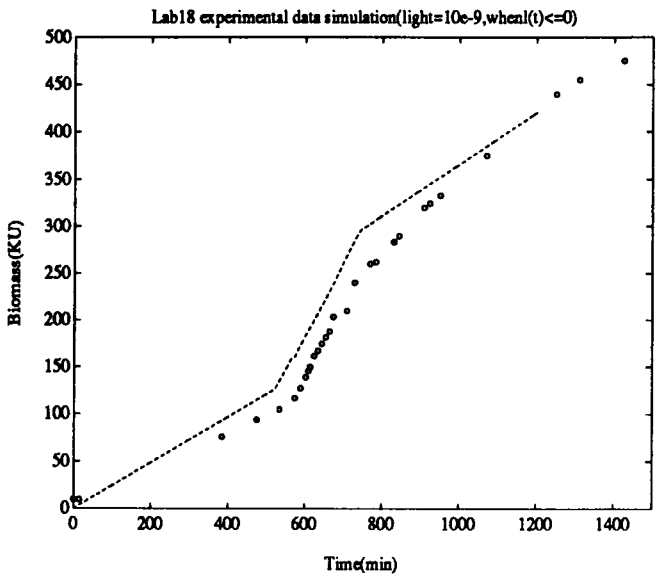


Figure 5.4: On-line prediction of biomass using the fitted Gaussian distribution curve based on the measured light intensity during the batch culture of *E. coli*; (o) experimental biomass measured off-line, (–) biomass predicted from the correlation model.

5.2 Results and Discussion

The objective of this phase of work is to investigate the correlation pattern between growth kinetics and bioluminescence in a continuously operated bioreactor. It is accomplished by applying dilution rate shift-up and shift-down experiments and allowing the system to come to a steady state after each shift. Three continuous runs were carried out. Cell mass and bioluminescence intensity information were monitored and recorded over a period of 8 to 15 days during continuous runs of *E. coli* strain RB1151 grown in glucose medium. From the simulation results shown in the last chapter, when glucose substrate concentration s_f in the feeding is fixed and changing the dilution rate D will change the growth rate of the culture up and down accordingly; while biomass would not change appreciably by shifting D as K_s is relatively small. Furthermore, the batch experimental results appear to suggest that bioluminescence was a reflection of the relative rate of growth rather than the absolute amount of biomass. Thus we expect that the bioluminescent response will follow the dilution rate shift-up and shift-down in a continuous run under constant environmental operating conditions. A continuous bioluminescent culture system was perturbed by shifting the dilution rate up and down, which caused the cell growth to go up or down, and the response of bioluminescence of the system was monitored. The system response can then be used to draw inference concerning the correlation between bioluminescence and cell growth state itself.

Shown in Figure 5.5 through Figure 5.11 are the profiles from three runs of on-line bioluminescence and off-line biomass concentration over a eight to fourteen-day period of monitoring. Each experiment includes several hours of batch culture operation and 10-13 days of continuous operation. Results indicate that bioluminescence response

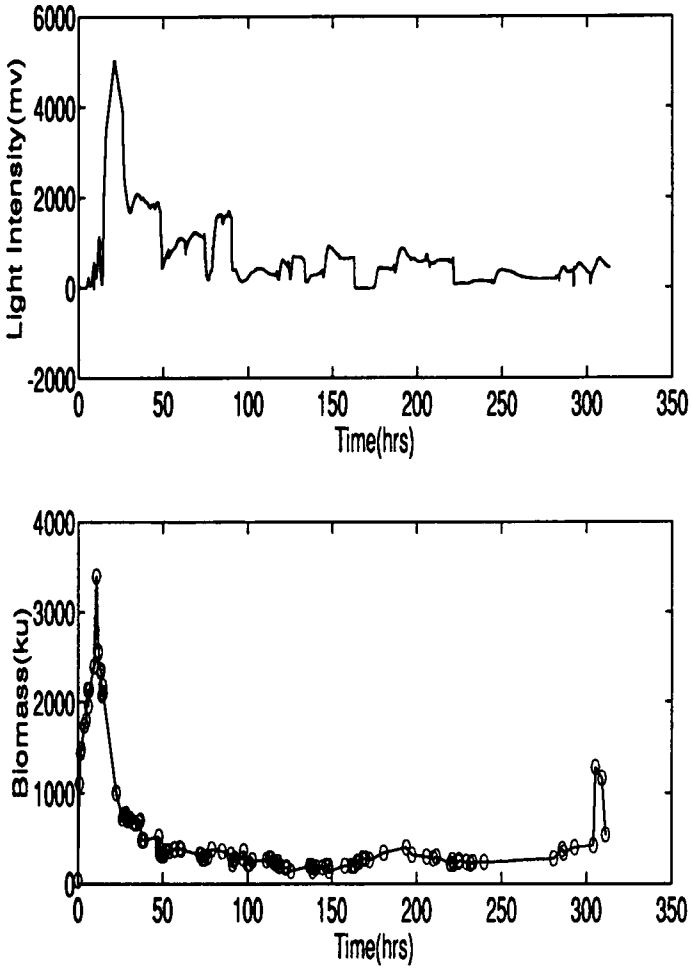


Figure 5.5: The overall bioluminescent and biomass profiles of *E. coli RB1151* under the operation of run #1.

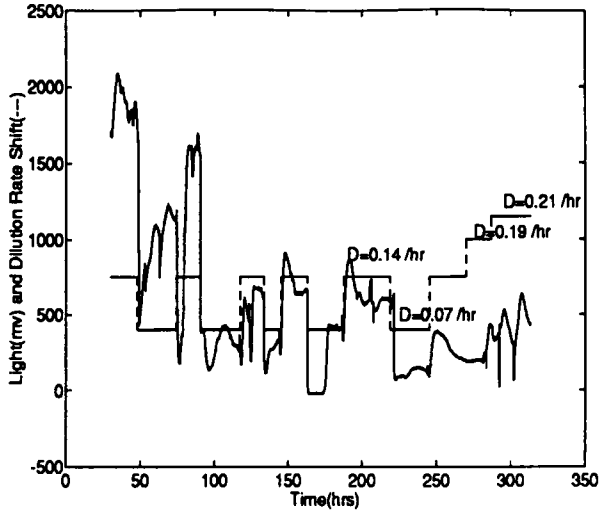


Figure 5.6: Bioluminescent profile of *E. coli RB1151* controlled by shift-up and shift-down dilution rate under the continuous operation of run #1.

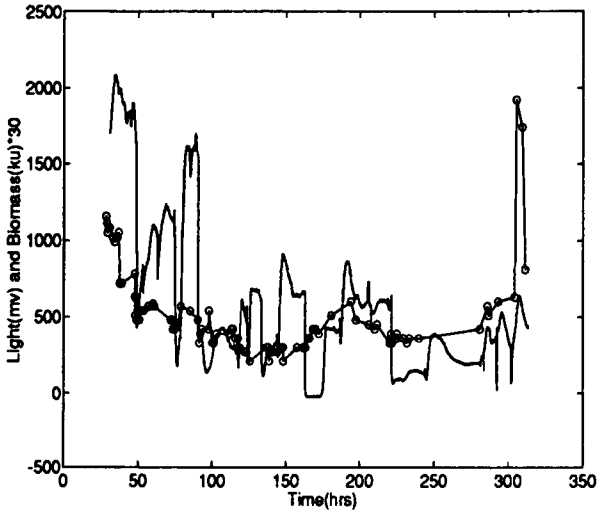


Figure 5.7: The comparison of Bioluminescent profile and biomass profile under the continuous operation of run #1.

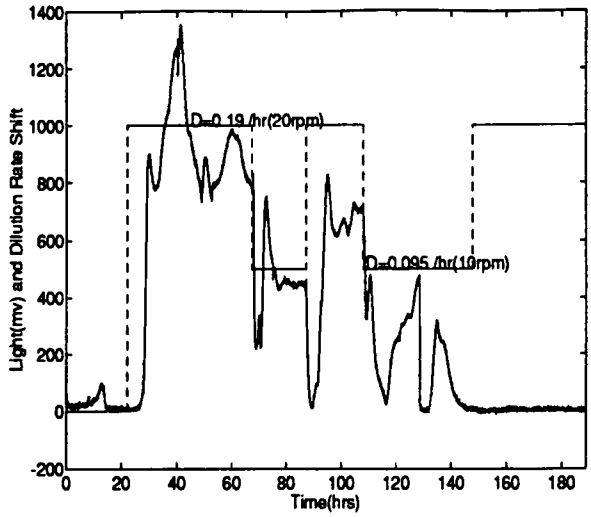


Figure 5.8: Bioluminescent profile of *E. coli RB1151* controlled by shift-up and shift-down dilution rate under the continuous operation of run #2.

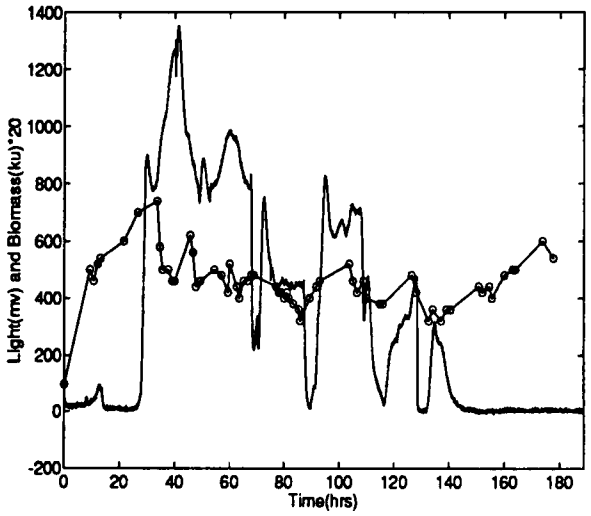


Figure 5.9: The comparison of Bioluminescent profile and biomass profile under the continuous operation of run #2.

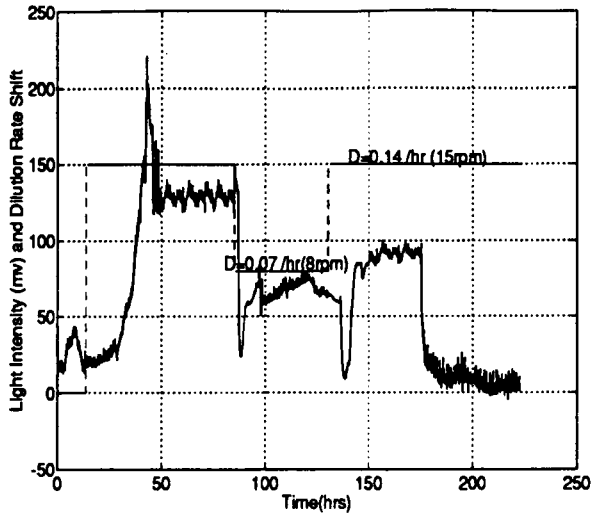


Figure 5.10: Bioluminescent profile of *E. coli RB1151* controlled by shift-up and shift-down dilution rate under the continuous operation of run #3.

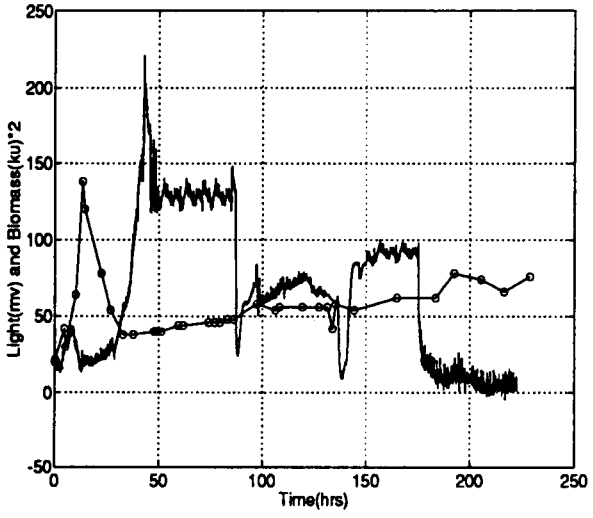


Figure 5.11: The comparison of Bioluminescent profile and biomass profile under the continuous operation of run #3.

was not so closely tied to biomass concentration and there was little change in biomass for significant changes in bioluminescence. Figure 5.6 and Figure 5.7 give a closer view of the bioluminescence profile and detailed changes of biomass kinetics. The system dynamics was controlled by shifting D up to 0.14 hr^{-1} and down to 0.07 hr^{-1} periodically. It can be seen in Figure 5.6 that steady state light intensity from bioluminescent cells tracks the ups and downs of the dilution rate. It can be observed always that light intensity exhibits an undershoot right after the dilution rate is changed and then was followed by an overshoot before the light level reaches steady state. Taking into account the underlying mechanism of bioluminescence emission, the intensity of emitted light is dependent upon the relative production and turnover rates of luciferase enzymes and essential cofactors (such as $NADPH$ and $FMNH_2$, etc.). The relative turnover rates, in turn, are directly related to the rate of growth of bacteria. Thus it is not surprising that steady state bioluminescence level appears to be closely related to the dilution rate which is equal to the specific growth rate at steady state. The level of the activity of the *lux* enzymes depends partially on the relative rates of the transcription and translation of the genes coding for the α and β subunits of luciferase, which in turn depends on the relative metabolic rate of the cells, and on the relative rate of the electron transport redox reactions. The undershoot of the light intensity level may be explained that the process of the transcription and translation of the genes coding for luciferase was disturbed by a change in dilution rate, therefore the light emission was sharply down, immediately after to conserve energy perhaps. Then the enzyme system adapts to the environmental changes and adjusts the rate of bioluminescence emission. The turnover rates and the rate of transcription and translation reach a new equilibrium level concordant with the new dilution rate.

It seems that the light intensity level stablized around the range of 600 to 700 mv when D was at 0.14 hr^{-1} and stablized around the range of 300 to 400 mv when D was at 0.07 hr^{-1} after two cycles of shift up and down in Run #1. Then after five cycles of shift, the bioluminescence system did not correspond to further shift in dilution rate even when we shifted the dilution rate up to 0.19 hr^{-1} and then to 0.21 hr^{-1}. The failure to respond may be caused by internal effects, but the cause may not be ascertained without additional experiments. The reduced bioluminescence level as time goes on is not due to the loss of the plasmid by the cells. This is evidenced by the fact that cells taken from the continuous culture broth at a time of low bioluminescence, when plated on glucose plates, showed almost invariably uniform expression of bioluminescence.

It can also be noticed that there are two sharp drops in the bioluminescence profile of Run #1. The first drop occurs at 160 hours and was most likely caused by a break in the continuously feeding of the glucose medium for five minutes. It took hours for the culture to come back to the original steady state level of bioluminescence. The second drop occurs at 220 hours and was perhaps caused by a temperature drop of 4 °C accidentally during a half hour span. As can be seen, the bioluminescent system appears to be sensitive to even a short period of disturbance in the environmental operating conditions. We wanted to ascertain that the bioluminescence does reach a true steady state eventually after each shift in D. Therefore we lengthened the interval before we bring another switch in D in the following experiment.

Figures 5.8 and 5.9 show the profile of bioluminescence and biomass concentration of a second continuous operation which repeated the trend observed in the first run. Steady state bioluminescence corresponded closely to the change in dilution rate. It is evident that bioluminescence is related more to the specific growth rate rather than to

the absolute amount of biomass concentration. Figure 5.9 shows that biomass is held at more or less a steady level for every shift in D. But after three shifts, the bioluminescence system did not respond accordingly as was observed in the previous run. The cells near the tail-end of the run were again tested by plating on glucose plate and was found that even though the bioluminescence system appeared to have been shut down in the continuous culture, cells bioluminescence continued at the original level when plated onto the glucose plates. Evidently, there are some internal physiological effects which can not be explained by the limited number of experiments, although the bioluminescent enzyme system appears to be functioning properly.

The aim of the third run is to check the stability of the bioluminescent system. The system was allowed to run sufficient time to reach steady state under each shift of dilution rate, and the steady state response of bioluminescence was monitored. Again it can be seen that the response of bioluminescence system was a stable indicator of the rate of growth. However, we were not able to carry out more shift ups or downs because the bioluminescence again disappeared almost totally after only 150 hours of operation. However it can be observed that the relative steady state levels of bioluminescence do correspond to the relative levels of the dilution rates and that the response of bioluminescence remained stable for hours at the same dilution rate.

5.3 Conclusion and Future Work

The objective of this research was to attempt to derive a correlation between the emitted light intensity and the biomass concentration of a bacterial culture during continuous operation in order to investigate the feasibility of using the bioluminescence as

a sensor to give biomass concentration information. Although a quantitative correlation between the light emission and biomass concentration can not be achieved, an apparent qualitative correlation was discovered to exist between the light emission level and the specific growth rate at steady state of the continuous operation. From three recent 10-day continuous experiments, consistent pattern of the bioluminescence kinetics as a function of the shifting ups and downs of the dilution rate was observed. Thus monitoring of bioluminescence may provide on-line information for the relative specific rate of growth.

From the analysis of the three sets of experimental results, several general qualitative conclusions can be drawn:

- It appears that steady state bioluminescence level corresponds to the specific growth rate. The higher the specific growth rate, the higher the corresponding bioluminescence level, thus confirming the hypothesis derived from the batch run observation.

- It can be observed that light intensity level almost always exhibits an undershoot immediately after the dilution rate is changed and often this is followed by an overshoot before the light level reaches steady state. The undershoot is possibly due to the culture dilution effect; whereas the overshoot may be due to the adaptation of the bioluminescence enzyme systems to the change in dilution rate.

- The kinetics of light emission from bioluminescent bacteria for continuous runs appears to be sensitive to the environmental operating conditions, such as pH, dissolved oxygen, and temperature. Even a short disturbance, for instance, in temperature changes or in substrate depletion, may lead to quite a few hours of

delay for the system to be restored back to the original steady state light emission level.

- It was observed that steady state light emission level became gradually lower at the same D but at different time intervals. The reason to this observation is uncertain due to the limited number of our experiments.

For a continuous bioreactor operation, the bioluminescence emission profile appears to be better suited to act as a sensor to detect any anomalies in the metabolic growth of cells because of its apparent sensitivity to the environmental condition. Since it is very sensitive to environmental disturbances, such as a change in temperature, pH, dissolved oxygen level, any inhibitory factor that results in a variation in the specific growth rate, will manifest itself in an altered bioluminescence emission profile. Hence, it appears that on-line information of bioluminescence can be used as an indicator for perturbations to the growth system. The ability to monitor on-line specific rate of cell growth can be applied to the system with high-valued products, where payoff for more efficient operation would be much higher.

Results obtained in this work are very encouraging and should be extended with more experiments. There are still a number of unsolved problems in our continuously operating process. The decline of steady state light emission after each shift to the same D may be solved by the addition of arginine or aldehyde or other stimulator [42] to see if the bioluminescence is subsequently stimulated. Quantitative correlation between the bioluminescence and the steady state specific rate of growth can then be obtained if reproducible bioluminescence profiles are obtained after addition of bioluminescence stimulation.

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APPENDIX

CHAPTER 6

COMPUTER PROGRAMS

6.1 Matlab Program for Bioreactor Process Simulation

6.1.1 Monod Curve

```
%=========================================================================
%
%              Program to Plot Monod Curve
global umax ks ys sf
%=========================================================================
%
 %
 % Process parameters
 %
 %
      umax=0.3;     %  Maximum specific growth rate
      umaxm= umax*.9;
      ks=0.005;      %  A constant in the Monod equation
      ksm=ks;
      ys=0.5;       %  Biomass yield with respect to substrate
         s=0:0.01:0.3;
         miu = umax*s ./ (ks+s);
   plot(s,miu,'w');
   xlabel('substrate (g/l)');
   ylabel('growth rate (/hr)');
   title('Monod Curve ')
 %  ========================================================================
 %
 %     THE END
 %
 %  ========================================================================
```

6.1.2 Step Response of Dilution Rate in Continuous Run

```
%=========================================================================
%
%   Program to test state variables to reach steady state by series
%   step change of dilution rate
%
%=========================================================================
clear;
global umax ks ys sf diu_fn
 % Process parameters
 %
      umax=0.3;     %  Maximum specific growth rate
      umaxm= umax*.9;
```

```
      ks=0.005;      %  A constant in the Monod equation
      ksm=ks;
      ys=0.5; %-0.5*0.15;  %Biomass yield with respect to substrate ys 0.5
      ysm=ys;
      sf=0.1;        %  Substrate concentration in the feed
      sfm=sf;
      umaxim=0.2;  %  Maximum dilution rate
      umin=0;        %  Minimum dilution rate
 %
 %=========================================================================
 %
 % Definition of the time interval
 %
     to=0;
     dt=0.05;
     tpc = round(350/dt);
     ttc = tpc*dt;
     tic = to:dt:ttc; %time interval for the following 70 hrs continuous
      tin=tic;
     ntime  = length(tin);
  %
%=========================================================================
%
        b(1) = 0.1; % 0.1 is biomass at steady state at this condition,
                    %0.05 is initial biomass 5 - 10 klett unit, the relation is
                   %biomass(g/l)=4.68e-2 + 6.3e-4*(klett unit)
        s(1) = 0.1;    %  substrate at steady state
        xin = [b(1); s(1)];
        diu = zeros(1,ntime);
%        b = zeros(1,ntime);
%        s = zeros(1,ntime);
        xin = [b(1); s(1)];
          jstep1 = 180/0.05;
         for j = 1:jstep1;
            diu(j)=0.05;
            diu_fn=0.05;
         end
            [tout,xout] = lsodar('pcon_fn',tin(1:jstep1),xin);
            nout = length(tout);
            b = xout(:,1);
            s = xout(:,2);
            miu = umax*s ./ (ks+s);
            jstep2 = 240/0.05;
          for j =jstep1+1: jstep2
            diu(j)=0.1;
            diu_fn=0.1;
          end
            [tout2,xout2] = lsodar('pcon_fn',tin(jstep1+1:jstep2),..
                                   xout(nout,:));
          b2 = xout2(:,1);
          s2 = xout2(:,2);
           miu2 = umax*s2 ./ (ks+s2);
          for j = jstep2+1 : ntime
            diu(j)=0.15;
            diu_fn=0.15;
          end
```

```
            nout2 = length(tout2);
            [tout3,xout3] = lsodar('pcon_fn',tin(jstep2+1:ntime),..
                                   xout2(nout2,:));
            b3 = xout3(:,1);
            s3 = xout3(:,2);
           miu3 = umax*s3 ./ (ks+s3);
        miu = [miu' miu2' miu3'];
        b = [b' b2' b3'];
        s = [s' s2' s3'];
        tout = [tout tout2 tout3];
%=========================================================================
 %
 % Initial values of the vector used to plot results (yp(1): calculated
 % biomass)
 %
 %   yp(1)=xi(1)*umax*xi(2)/(ks+xi(2)),
 %=========================================================================

   subplot(221);
   axis([0 350 0 0.4])
   plot(tout,b,'w');
   ylabel('biomass');
   xlabel('Time(hr)');
   axis([0 350 0 0.1])
   plot(tout,s,'w');
   ylabel('substrate');
   xlabel('Time(hr)');
   axis([0 350 0 0.3])
   plot(tout,miu,tout, diu,':');
   ylabel('growth rate');
   xlabel('Time(hr)');
 %  ======================================================================
 %
 %     THE END
 %
 %  ======================================================================
      function dxdt = pcon_fn(t,x)
%
%     System equations
%
      b = x(1);
      s = x(2);
      miu = umax*s/(ks+s);
      dxdt(1) =  (miu-diu_fn)*b;
      dxdt(2) = diu_fn*(sf-s) - miu*b/ys;
```

6.1.3 Program for Simulation the Experimental Process

This program simulate the experimental process which initiated from batch run and switched to continuous mode after a few hours batch running. It can be used to predict the availability of the experiment under assumed conditions and time period to reach steady state.

```
%=========================================================================
```

```
clear;
global umax ks ys sf
%=========================================================================
 %
 % Process parameters
 %
 %
      umax=0.3;     %  Maximum specific growth rate
      umaxm= umax*.9;
      ks=0.005 %-0.05*0.5;      %  A constant in the Monod equation
      ksm=ks;
      ys=0.5; %-0.5*0.15;   %Biomass yield with respect to substrate ys 0.5
      ysm=ys;  % if ys fixed to 0.5, in batch run the biomass of 100 to 150
               % KU (0.68-0.99 g/l) will reach half Umax,
      sf=0.2;       %  Substrate concentration in the feed
      sfm=sf;
      umaxim=0.2;  %  Maximum dilution rate
      umin=0;       %  Minimum dilution rate
 %
 %=========================================================================
 %
 % Definition of the time interval
 %
     to=0;
     dt=0.05;
     tpp = round(10/dt);
     ttb = tpp*dt;
     ti = to:dt:ttb;  %time interval for 5 hrs batch
     tpc = round(150/dt);
     ttc = tpc*dt;
     tic = ttb:dt:ttc; %time interval for the following 90 hrs continuous
     tin=ti;
     tin2=tic;
     ntime  = max(size(tin));
  %
 %=========================================================================
%  Initial Conditions for the batch simulation
%
%
         b(1) = 0.05;%0.05 is initial biomass of 5 - 10 klett unit,
             % the relation
              % is biomass(g/l)=4.68e-2 + 6.3e-4*(klett unit)
         s(1) =0.2;   % initial substrate
         xin = [b(1); s(1)];
         [tout,xout] = lsodar('subst',tin,xin);
         b = xout(:,1);
         s = xout(:,2);
         miu = umax*s ./ (ks+s);
         xin2 = [b(tpp);s(tpp)];
         [tout2,xout2] = lsodar('subcc', tin2,xin2);
         b2 = xout2(:,1);
         s2 = xout2(:,2);
         miu2 = umax*s2 ./ (ks+s2);
%=========================================================================
%      Combine the data from batch and continuous run
         tout=[tout tout2];
```

```
        b = [b' b2'];
        s = [s' s2'];
        miu= [miu' miu2'];
%=========================================================================
 subplot(211)
   axis([0 100 0 0.3])
   plot(tout,b,'--');
   ylabel('biomass');
   xlabel('Time(hr)');
   axis([0 100 0 0.07])
   plot(tout,s,'w');
   ylabel('substrate');
   xlabel('Time(hr)');
subplot(212)
   axis([0 100 0 0.3])
   plot(tout,miu,'--');
   ylabel('growth rate');
   xlabel('Time(hr)');
 %  ========================================================================
 %
 %     THE END
 %
 %  ========================================================================
 %    Function used in batch process
      function FX = subst(t,x)
%
%     System equations
      b = x(1);
      s = x(2);
      miu = umax*s/(ks+s);
      FX(1) =  miu*b;
      FX(2) = -miu*b/ys;
%=========================================================================
%
%     Function used in continuous process
      function dxdt = subst(t,x)
%
%     System equations
%
      b2 = x(1);
      s2 = x(2);
      miu2 = umax*s2/(ks+s2);
      diu=0.2; %miu(tpp);
      dxdt(1) =  (miu2-diu)*b2;
      dxdt(2) = diu*(sf-s2) - miu2*b2/ys;
```

6.2 C Program For Data Acquisition and Process Control

These sets of programs are used for process monitoring and control and data acquisition.

```
/*********************************************************************
*            Main Process Monitoring and Acquisition File
* C Program to read and write those process variables (time, temperature,
* PH, dO2, mass flow rate, light intensity, etc.), then save these vectors
* to fp file for linking to Matlab program. It will be used on process
* monitoring and on-line ploting.
*********************************************************************/
#include <stdio.h>
enum {
    LWSPACE, LWO2_8, LWMEAS_ERR, LWO2_10, LWEMPTY
};
main() {
    FILE *fp, *fopen();
    double ftime, btime[1], xc, tx, xt[22000], xtp[22000], xtck;
    double xlight[22000];
    double xph[22000], yt[22000], xdate[1], xi[1], xdo[22000], xmass[22000];
    double flow0;
    static char *lite_warn[] =
        {" ",
" ***--> O2 To 8+ !!",
" ***--> Measmt. Err??",
" ***--> O2 To 10+ !!",
        ""};
    int lwn;
    int error;
    int i = 0;
    xc = 0.0;
    flow0 = 0.5;
    lwn = LWSPACE;
    biowrite("massflow", "", &flow0, &error);
    if (error != 0) {
pbioerror("demo");
exit(1);
    }
    bioread("time", "", &btime[0], &error);
    if (error != 0) {
pbioerror("demo");
exit(1);
    }
    do {
sleep(60);
bioread("time", "", &xt[i], &error);
if (error != 0) {
    pbioerror("demo");
    exit(1);
}
xdate[0] = xtck;
fp = fopen("foot.mat", "w");
bioread("temp", "", &xtp[i], &error);
if (error != 0) {
    pbioerror("demo");
    exit(1);
}
bioread("ph", "", &xph[i], &error);
```

```
if (error != 0) {
    pbioerror("demo");
    exit(1);
}
bioread("do2", "", &xdo[i], &error);
if (error != 0) {
    pbioerror("demo");
    exit(1);
}
if (xdo[i] < 95) {
    flow0 += 0.2;
    if (flow0 >= 5) {
flow0 = 5;
    }
    biowrite("massflow", "", &flow0, &error);
    if (error != 0) {
pbioerror("demo");
exit(1);
    }
}
bioread("massflow", "", &xmass[i], &error);
if (error != 0) {
    pbioerror("demo");
    exit(1);
}
bioread("CO2", "", &xlight[i], &error);
/*  Unit to check status of Light mV o/p  */
if ((xlight[i] >= 60) && (xlight[i] <= 200))
    lwn = LWO2_8;
else {
    if (xlight[i] >= 900)
lwn = LWMEAS_ERR;
    else {
if ((xlight[i] >= 201) && (xlight[i] <= 450))
    lwn = LWO2_10;
else
    lwn = LWEMPTY;
    }
}
/*  End Unit */
        if (error != 0) {
         pbioerror("demo");
         exit(1);
        }
        printf("time          : %12.6f \n", xt[i]);
        printf("Temp (deg C)  : %g \n", xtp[i]);
        printf("pH            : %g \n", xph[i]);
        printf("D_02 (rel %)  : %g \n", xdo[i]);
        printf("massflow(l/m): %g %s \n", xmass[i], lite_warn[lwn]);
        printf("light (mV)    : %g \n", xlight[i]);
        printf("sample #      : %d \n", i);
        xi[0] = i;
/*=====================================================================
*
*          Save for linking Matlab Program
*
*====================================================================*/
```

```
        savemat(fp, 1000, "xt", 22000, 1, 0, xt, (double *) 0);
        savemat(fp, 1000, "xdate", 1, 1, 0, xdate, (double *) 0);
        savemat(fp, 1000, "btime", 1, 1, 0, btime, (double *) 0);
        savemat(fp, 1000, "xi", 1, 1, 0, xi, (double *) 0);
        savemat(fp, 1000, "xph", 22000, 1, 0, xph, (double *) 0);
        savemat(fp, 1000, "xtp", 22000, 1, 0, xtp, (double *) 0);
        savemat(fp, 1000, "xdo", 22000, 1, 0, xdo, (double *) 0);
        savemat(fp, 1000, "xmass",22000, 1, 0, xmass, (double *) 0);
        savemat(fp, 1000, "xlight", 22000, 1, 0, xlight, (double *) 0);
        fclose(fp);
        i++;
    }
        while (i < 22000);
        return 0;
}
%=============================================================================
%
%       THE END
%
%=============================================================================
/*=============================================================================
 *      savemat.c File
 * savemat - C language routine to save a matrix in a MAT-file.
 *
 * Here is an example that uses 'savemat' to save two matrices to disk,
 * the second of which is complex:
 *
 * FILE *fp;
 * double xyz[1000], ar[1000], ai[1000];
 * fp = fopen("foo.mat","wb");
 * savemat(fp, 2000, "xyz", 2, 3, 0, xyz, (double *)0);
 * savemat(fp, 2000, "a", 5, 5, 1, ar, ai);
 *      fclose(fp);
 *
 * ============================================================================
 */
#include <stdio.h>
typedef struct {
     long type;   /* type */
     long mrows;  /* row dimension */
     long ncols;  /* column dimension */
     long imagf;  /* flag indicating imag part */
     long namlen; /* name length (including NULL) */
} Fmatrix;
savemat(fp, type, pname, mrows, ncols, imagf, preal, pimag)
FILE *fp;       /* File pointer */
int type;       /* Type flag: Normally 0 for PC, 1000 for Sun, Mac, and  */
/* Apollo, 2000 for VAX D-float, 3000 for VAX G-float    */
/* Add 1 for text variables.  */
/* See LOAD in reference section of guide for more info. */
int mrows;      /* row dimension */
int ncols;      /* column dimension */
int imagf; /* imaginary flag */
```

```
char *pname;    /* pointer to matrix name */
double *preal;  /* pointer to real data */
double *pimag;  /* pointer to imag data */
{
Fmatrix x;
int mn;
x.type = type;
x.mrows = mrows;
x.ncols = ncols;
x.imagf = imagf;
x.namlen = strlen(pname) + 1;
mn = x.mrows * x.ncols;
fwrite(&x, sizeof(Fmatrix), 1, fp);
fwrite(pname, sizeof(char), (int)x.namlen, fp);
fwrite(preal, sizeof(double), mn, fp);
if (imagf) {
    fwrite(pimag, sizeof(double), mn, fp);
}
}

/*=========================================================================
*
*                    loadmat.c
*
 * loadmat - C language routine to load a matrix from a MAT-file.
 *
 * Here is an example that uses 'loadmat' to load a matrix from a MAT-file:
 *
 * FILE *fp;
 * char name[20];
 * int type, mrows, ncols, imagf;
 * double *xr, *xi;
 * fp = fopen("foo.mat","rb");
 * loadmat(fp, &type, name, &mrows, &ncols, &imagf, &xr, &xi);
 *      fclose(fp);
 * free(xr);
 * if (imagf) free(xi);
 *
 * The 'loadmat' routine returns 0 if the read is successful and 1 if
 * and end-of-file or read error is encountered.  'loadmat' can be called
 * repeatedly until the file is exhausted and an EOF is encountered.
 *
 * ========================================================================
 */
#include <stdio.h>

typedef struct {
    long type;   /* type */
    long mrows;  /* row dimension */
    long ncols;  /* column dimension */
    long imagf;  /* flag indicating imag part */
    long namlen; /* name length (including NULL) */
} Fmatrix;

loadmat(fp, type, pname, mrows, ncols, imagf, preal, pimag)
FILE *fp;       /* File pointer */
int *type;      /* Type flag: see reference section of guide */
int *mrows;     /* row dimension */
int *ncols;     /* column dimension */
```

```
int *imagf;      /* imaginary flag */
char *pname;     /* pointer to matrix name */
double **preal;  /* pointer to real data */
double **pimag;  /* pointer to imag data */
{
char *malloc();
Fmatrix x;
int mn, namlen;
/*
 * Get Fmatrix structure from file
 */
if (fread((char *)&x, sizeof(Fmatrix), 1, fp) != 1) {
return(1);
}
*type = x.type;
*mrows = x.mrows;
*ncols = x.ncols;
*imagf = x.imagf;
namlen = x.namlen;
mn = x.mrows * x.ncols;
/*
 * Get matrix name from file
 */
if (fread(pname, sizeof(char), namlen, fp) != namlen) {
return(1);
}
/*
 * Get Real part of matrix from file
 */
if (!(*preal = (double *)malloc(mn*sizeof(double)))) {
printf("\nError: Variable too big to load\n");
return(1);
}
if (fread(*preal, sizeof(double), mn, fp) != mn) {
free(*preal);
return(1);
}
/*
 * Get Imag part of matrix from file, if it exists
 */
if (x.imagf) {
if (!(*pimag = (double *)malloc(mn*sizeof(double)))) {
printf("\nError: Variable too big to load\n");
free(*preal);
return(1);
}
if (fread(*pimag, sizeof(double), mn, fp) != mn) {
free(*pimag);
free(*preal);
return(1);
}
}
return(0);
}
/*=====================================================================
```

```
*
*          bioread.c
*  C language routine to read those process variables
*
*=========================================================================*/
#include <stdio.h>
#include <string.h>
#include "biocmd.h"

main(argc, argv)
int argc;
char *argv[];
{
char *c;
int error;
double reply;

if (argc < 2) {
fprintf(stderr,"usage: bioread <function> [<parameter>]\n");
exit(1);
}
if (argc == 2) {
if ((c=strchr(argv[1], (int)','')) != (char*)NULL)
*c = '\0';
bioread(argv[1], "", &reply, &error);
}
else {
if ((c=strchr(argv[2], (int)','')) != (char*)NULL)
*c = '\0';
bioread(argv[1], argv[2], &reply, &error);
}
if (error) {
pbioerror("bioread");
}
else
printf("current value: %13.6f\n", reply);
}

/*=========================================================================
*
*          biowrite.c
*  C language routine to write those process variables
*
*=========================================================================*/
#include <stdio.h>
#include <string.h>
#include <math.h>
#include "biocmd.h"

main(argc, argv)
int argc;
char **argv;
{
char *c, func[BUFSIZE], param[BUFSIZE];
int error;
double reply, value;

if (argc < 3) {
    fprintf(stderr,"usage: biowrite <function> [<parameter>] <value>\n");
    exit(1);
}
if ((c=strchr(*++argv, (int)','')) != (char*)NULL)
```

```
*c = '\0';
if (strlen(*argv) >= BUFSIZE-1) {
fprintf(stderr, "unknown function : %s\n", *argv);
exit(1);
}
strcpy(func, *argv);
if (argc == 3) {
*param = '\0';
} else {
if ((c=strchr(*++argv, (int)',')) != (char*)NULL)
*c = '\0';
if (strlen(*argv) >= BUFSIZE-1) {
fprintf(stderr, "unknown parameter : %s\n", *argv);
exit(1);
}
strcpy(param, *argv);
}
bioread(func, param, &reply, &error);
if (error) {
pbioerror("biowrite");
exit(1);
} else
printf("old value: %13.6f\n", reply);
value = atof(*++argv);
biowrite(func, param, &value, &error);
if (error) {
pbioerror("biowrite");
exit(1);
}
bioread(func, param, &reply, &error);
if (error) {
pbioerror("biowrite");
exit(1);
} else
printf("new value: %13.6f\n", reply);
}
/*=====================================================================
*
*          biotime.c
*  C language routine to read clock time
*
*=====================================================================*/
#include <stdio.h>
#include <sgtty.h>
#include <sys/ioctl.h>
#include <sys/file.h>
#define BUFSIZE 50
char com[] = "\002M5,F40\r";
/*
char com[] = "\002M5,F41,880317.113200\r";
*/

main()
{
int i;
```

```
FILE *ch;
int cc;
char c, *s;
char ans[BUFSIZE];
struct sgttyb tty, rtty;
int fd;
extern int errno;

if ((fd = open("/dev/ttya",O_RDWR)) == -1)
fprintf(stderr,"bad open\n");
/* set up exclusive use */
if (ioctl(fd,TIOCEXCL,NULL) == -1) {
fprintf(stderr,"bad TIOCEXCL\n");
fprintf(stderr,"errno = %d\n",errno);
}
/* odd parity */
tty.sg_flags = ODDP|CBREAK;
tty.sg_ospeed = B9600;
tty.sg_ispeed = tty.sg_ospeed;
/* set up with params in tty struct */
if (ioctl(fd,TIOCSETP,(char *)&tty) == -1) {
fprintf(stderr,"bad TIOCSETP\n");
fprintf(stderr,"errno = %d\n",errno);
}
for (s=com; *s != '\0'; s++) {
write(fd, s, 1);
}
for (s=ans; s<&ans[BUFSIZE-2]; s++) {
if ((cc = read(fd, s, 1)) == -1) {
fprintf(stderr,"\nbad read: errno = %d\n",errno);
}
if (*s == '\n') {
*++s = '\0';
break;
}
}
fprintf(stderr, "%s", ans);
/* release exclusive use */
if (ioctl(fd,TIOCNXCL,NULL) == -1) {
fprintf(stderr,"bad TIOCNXCL\n");
fprintf(stderr,"errno = %d\n",errno);
}
if (close(fd) == -1)
fprintf(stderr,"bad close\n");
}

/*=====================================================================
*
*  C language routine to list the location and name of each function
*  modul in Applikon Bio-Processor
*
*=====================================================================*

#include <stdio.h>
#include "biocmd.h"
```

```
struct noparam noparamtable[] = {
{"PH", "M1,F1"},
{"TEMP", "M1,F2"},
{"RELAY_PH", "M1,F21"},
{"ANALOG_T", "M1,F22"},
{"DO2", "M2,F1"},
{"FOAM", "M2,F12"},
{"RELAY_DO", "M2,F21"},
{"RELAY_FOAM", "M2,F22"},
{"ANALOG_DO", "M2,F23"},
{"MASSFLOW", "M2,F23"},
{"ANALOG_REC", "M2,F24"},
{"O2", "M3,F1"},
{"CO2", "M3,F2"},
{"ANALOG_O2", "M3,F21"},
{"ANALOG_CO2", "M3,F22"},
{"OUTLET_CO2", "M4,F1"},
{"FEEDPUMP", "M4,F21"},
{"CONCPUMP", "M4,F22"},
{"TIME", "M5,F40"},
{EOLIST}
};
struct funcspec ftable[] = {
{"PH", "M1", '1'},
{"TEMP", "M1", '2'},
{"RELAY_PH", "M1", '1'},
{"ANALOG_T", "M1", '2'},
{"DO2", "M2", '1'},
{"FOAM", "M2", '2'},
{"RELAY_DO", "M2", '1'},
{"RELAY_FOAM", "M2", '2'},
{"ANALOG_DO", "M2", '3'},
{"MASSFLOW", "M2", '3'},
{"ANALOG_REC", "M2", '4'},
{"O2", "M3", '1'},
{"CO2", "M3", '2'},
{"ANALOG_O2", "M3", '1'},
{"ANALOG_CO2", "M3", '2'},
{"OUTLET_CO2", "M4", '1'},
{"FEEDPUMP", "M4", '1'},
{"CONCPUMP", "M4", '2'},
{"TIME", "M5", '4'},
{EOLIST}
};
struct paramspec ptable[] = {
/* control block parameters */
{"SETPOINT", "F1", '0'},
{"SP", "F1", '0'},
{"KP", "F1", '1'},
{"TI", "F1", '2'},
{"TD", "F1", '3'},
{"T_DEAD", "F1", '4'},
{"T_PROCESS", "F1", '5'},
{"D_ZONE_-", "F1", '6'},
{"D_ZONE_+", "F1", '7'},
```

```
{"TO_OUTPUT", "F1", '8'},
/* input block parameters */
{"CALIBRATE", "F2", '0'},
{"BUFFER_1", "F2", '1'},
{"BUFFER_2", "F2", '2'},
{"SLOPE", "F2", '3'},
{"OFFSET", "F2", '4'},
{"CONTR_OFFSET", "F2", '5'},
{"USE_INPUT", "F2", '6'},
{"DEFAULT_VALUE", "F2", '7'},
{"BIO_REACTOR", "F2", '8'},
/* dose monitoring block parameters */
{"R1_DOSED_VALUE", "F3", '0'},
{"R1_FACTOR", "F3", '1'},
{"R1_TO_OUTPUT", "F3", '2'},
{"R2_DOSED_VALUE", "F3", '3'},
{"R2_FACTOR", "F3", '4'},
{"R2_TO_OUTPUT", "F3", '5'},
/* input control block parameters */
{"ALARM_TO_OUTPUT", "F4", '0'},
{"LOW_ALARM", "F4", '1'},
{"HIGH_ALARM", "F4", '2'},
{"LOW_ALARM_TO_OUTPUT", "F4", '3'},
{"HIGH_ALARM_TO_OUTPUT", "F4", '4'},
/* output control block parameters */
{"LOW_LIMIT", "F5", '0'},
{"HIGH_LIMIT", "F5", '1'},
{"ENG_UNIT", "F5", '2'},
{"SUFFIX_CODE", "F5", '3'},
/* time parameters */
{"READ", "F", '0'},
{"SET", "F", '1'},
{EOLIST}
};
```

6.3 Matlab Program For On-line Data Acquisition and Analysis

```
%=========================================================================
%                Main Matlab Program
%
% This matlab file is used to check the state of the bioprocessor
% the available vectors are:
%      xtv=xt     : The bioreactor time (min)
%      xphv=xph   : the pH in the bioreactor
%      xtpv=xtp   : The temperature of the bioreactor (C)
%      xdov=xdo   : The DO2
%
%=========================================================================
false=0;
true=1;
footness=false;
for i=1:22000
  while footness==false
       status = exist('foot.mat');
```

```
      if (status == 2)
           footness = true;
      end
  end,
footness = false;
   load foot;
   kf=xi;
   kb=kf-12000;
   if kb <= 0;
       kb=1;
   end,
   time_start=btime,
   yearb=round(btime/10000-.5);
   mesb=round(btime/100-.5)-yearb*100;
   dayb=round(btime-.5)-mesb*100-yearb*10000;
   hourb=round((btime-dayb-mesb*100-yearb*10000)*100-.5);
   minb=round((btime-hourb/100-dayb-mesb*100-yearb*10000)*10000-.5);
   secb=round((btime-minb/10000-hourb/100-dayb-mesb*100-yearb*10000)*1000000-.5);
   xtv=xt(kb:kf);
   for i=kb:kf
       year=round(xtv(i)/10000-.5);
       mes=round(xtv(i)/100-.5)-year*100;
       day=round(xtv(i)-.5)-mes*100-year*10000;
       hour=round((xtv(i)-day-mes*100-year*10000)*100-.5);
       min=round((xtv(i)-hour/100-day-mes*100-year*10000)*10000-.5);
       sec=round((xtv(i)-min/10000-hour/100-day-mes*100-year*10000)*1000000-.5);
       if abs (day-dayb) > 0.01
            hour=hour+24;
       end
       xtv(i)=(hour-hourb)*60+(min-minb)+(sec-secb)/60;
   end,
   xphv=xph(kb:kf);
   xtpv=xtp(kb:kf);
   xdov=xdo(kb:kf);
   xmassv=xmass(kb:kf);
   xlightv=xlight(kb:kf);
%=========================================================================
%
%           Plot Results
%
%=========================================================================
   subplot(231)
   axis([xtv(kb) xtv(kf) 0 110]);grid on;
   plot(xtv,xdov);
   title('Bioreactor DO2');
   ylabel('DO2');
   xlabel('Time(min)');
   hold on
   subplot(232)
   axis([xtv(kb) xtv(kf) 0 1.5]);grid on;
   plot(xtv,xmassv);
   title('Air flow rate');
   ylabel('Mass Flow (L/min)');
   xlabel('Time(min)');
   hold on
```

```
    subplot(234)
    axis([xtv(kb) xtv(kf) 0 5000]);grid on;
    plot(xtv,xlightv);
    title('Light');
    ylabel('Light (mv)');
    xlabel('Time(min)');
    hold on
%    figure(2)
    subplot(233)
    axis([xtv(kb) xtv(kf) 6.95 7.05]);grid on;
    plot(xtv,xphv);
    title('Bioreactor pH');
    ylabel('pH');
    xlabel('Time(min)');
    grid on;
    subplot(235)
    axis([xtv(kb) xtv(kf) 27.2 28.5]);grid on;
    plot(xtv,xtpv);
    title('Bioreactor Temperature');
    ylabel('Temperature C');
    xlabel('Time(min)');
    grid on;
    pause(9);
end,

%=========================================================================
%
%           THE END
%
%=========================================================================
```

VITA

Bo Huang was born on August 2, 1967 in China. She was attracted by the mysterious realm of molecular biology when she was in high school. She majored in biology and obtained her B.S. degree in June of 1989, when she still dreamed of entering the spherical kingdom of the double helix. Subsequently, She came to the United states with her dream on December of 1989.

In the spring of 1990, she started graduate studies in Microbiology at the University of Tennessee, Knoxville. After one year of theoretical studies, she started to hunt for something more practical. Finally, she found her interests in the field of biotechnology and started her research work in biosensors in the Department of Chemical Engineering. She continued her graduate studies in Bioengineering, and was awarded the Master of Science degree in Microbiology in August, 1992 and the Master of Science degree in Chemical Engineering in August, 1993.