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# FIRST PRINCIPLES, UNSTRUCTURED, DYNAMIC MODEL FOR REGULATING CO<sub>2</sub> AND pH IN BICARBONATE BUFFERED PERFUSION BIOREACTOR

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FIRST PRINCIPLES, UNSTRUCTURED, DYNAMIC MODEL FOR REGULATING CO<sub>2</sub> AND  
pH IN BICARBONATE BUFFERED PERFUSION BIOREACTOR

BY

ASHISH GAURAV AIRAN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
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IN  
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UNIVERSITY OF RHODE ISLAND

2013

MASTER OF SCIENCE THESIS  
OF  
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UNIVERSITY OF RHODE ISLAND

2013

## ABSTRACT

Since its inception, the biotechnology industry has faced the problem of pH control and CO<sub>2</sub> management. This research paper explores the challenges of developing a first principles, unstructured, dynamic, nonlinear mathematical model to maintain pH and control carbon dioxide levels in an aerobic Chinese hamster ovary (CHO) cell culture in a perfusion bioreactor. Perfusion bioreactor is an extremely complex bioreactor to model because the cells grow in a quasi-steady state system. Cell growth, feed, substrate consumption, by-product formation and product formation are all time dependent, equations for which can only be solved through numerical methods. Added complexity comes from presence of stiffness in solving the non-linear equations due to the different time scale of each set of equations. Equations related to pH that involve acid/base ionization have reaction rates on the order of nano- or pico-seconds. CO<sub>2</sub> or O<sub>2</sub> hydration and bubble dynamics involve the reaction rates on the order of seconds and cell growth equations are on the order of days. This research paper also presents a framework for software development to solve these equations without conducting experiments, except as a final trial before using the results of the model on the manufacturing floor.

## PREFACE

Father of the term, “biotechnology,” Károly (Karl) Ereky, a Hungarian scientist, published *Biotechnologie* in 1919 in Berlin, Germany (Bud, 1993). In that book, Ereky related biotechnology to nucleic acids and observed that both plant and animal proteins contain the same amino and nucleic acids (Fári and Kralovánszky, 2006). At about the same time, in 1921, Canadian physiologists, Sir Frederick Grant Banting and Charles Herbert Best, extracted insulin from the pancreatic tissue of dogs with the help of a British physiologist, John James Rickard Macleod (Microsoft Student, 2008). The Canadian biochemist, James Bertram Collip, then isolated pure insulin that could be injected into humans. In this way, insulin became the first protein to be administered as a drug to humans and in 1965, became the first human protein to be synthesized for medicinal applications (Microsoft Student, 2008).

The journey of development of the biotechnology industry, which produces therapeutic proteins from genetically engineered living cells, is quite fascinating. This nascent industry gained much needed boost in the United States in 1980 when the Supreme Court of the United States ruled in favor of General Electric genetic engineer, Ananda Mohan Chakrabarty and granted him the right to patent a genetically engineered bacterial cell pursuant to a patent application he had filed in 1972. *Diamond v. Chakrabarty*, 447 U.S. 303 (1980). Still in its infancy, the new age biotechnology industry, however, played second fiddle to the pharmaceutical

companies because besides insulin, the market did not have many biopharmaceutical products with medical promise.

Before the widespread availability of biopharmaceuticals and in the absence of any treatment or cure, patients inflicted with the rare diseases had to simply learn to live with that disease. All that changed when biopharmaceutical companies began seeing some tangible progress and began discovering drugs for diseases that inflicted a small number of people for which no treatment was not available. For example, when Abbey Meyers could not find any drug to treat her son's Tourette's syndrome, she lobbied the United States Congress to change the law to give companies incentives to develop drugs for rare diseases (Anand, 2005). In 1983, her efforts led the United States Congress to enact the Orphan Drug Act, which in addition to tax credits, entitled the orphan drugs to market protection that was in some respects better than a patent. *Id.* This law provided the necessary fortitude that catapulted the genetic engineers from their "garage laboratories" to executives in increasingly confident biotechnology companies in the marketplace. Thus began a new era of biotechnology companies that produced biopharmaceutical drugs that act similar to naturally occurring proteins in the human bodies. Today, the biotechnology industry is at the forefront of developing treatments for difficult diseases for which the pharmaceutical drugs are not readily available (Hopkins *et al.*, 2007).

Proteomic scientists discovered that scientific advances enabled geneticists to identify propitious traits in living organisms which could have biopharmaceutical applications. Biotechnologists physically manipulated the genetic structure of living cells to create genetically engineered living cells programmed to manufacture proteins that promise to improve human life. Bioprocess engineers exploited a variety of technologies to manipulate the artificial growth environment of these genetically modified mammalian cells to produce commercial quantities of desirable biopharmaceutical protein (Shuler and Kargi, 1992). However, unlike process engineers in the chemical industry who can reasonably rely on established scientific and mathematical principles to predict a process and its outcome, even small changes in pH, temperature, pressure, ionic strength or even genetic mutations, render a bioprocess unpredictable. The biological environment of the cells determines the rate, extent and type of biological processes that take place in a growth medium. Consequently, bioprocess engineering challenges involve optimizing the growth environment of genetically engineered cells to maximize product formation and eventually harvesting the product by separating protein from cells and cell debris.

This research paper presents a first principles, unstructured, dynamic, nonlinear mathematical model to maintain pH and control carbon dioxide levels in aerobic Chinese hamster ovary (CHO) cell culture in a perfusion bioreactor. CHO cells are preferred hosts for mass production of recombinant proteins because of all

animal cells, CHO cells grow more rapidly, have higher stability and are efficient in foreign gene expression (Matsunaga, 2009a). CO<sub>2</sub> is preferred for regulating cell culture pH because

- (i) CO<sub>2</sub> reduces high pH quickly
- (ii) At approximately neutral pH, CO<sub>2</sub> is self-buffering
- (iii) Unlike strong acids, CO<sub>2</sub> does not produce local areas of very low pH, which could harm the sensitive mammalian cells
- (iv) CO<sub>2</sub> is cheaper and safer to handle in a manufacturing plant because it is not corrosive and its handling can be completely automated.

Chapter 1 discusses the need for the current thesis, its scope and limitations. This chapter also discusses the statement of the problem and the hypothesis. Chapter 2 discusses the background theories relevant to the current project. It gives a brief history of cell culture perfusion systems, types of mathematical models, and then evaluates parameters that influence a mammalian cell culture. Chapter 3 provides methodology employed to solve the problem of CO<sub>2</sub> management and pH control including material balance equations. Chapter 4 provides MATLAB® simulation details and chapter 5 concludes this thesis and makes some suggestions for future work.



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## NOTATION INDEX

a	gas-liquid interfacial area ( $a = \frac{6\varepsilon}{d_B}$ ) (m <sup>2</sup> )
A	Volumetric agitated gas-liquid interfacial area at the top of the liquid as defined in Gray <i>et al.</i> (1996)
B	Cell broth bleeding rate from the vessel (L/min) as defined in Gray <i>et al.</i> (1996)
$C_{CO_2}^T$	Total aqueous concentration of CO <sub>2</sub> (M)
$C_{CO_2}$	Aqueous concentration of CO <sub>2</sub> (M)
$C_{O_2}$	Aqueous concentration of O <sub>2</sub> (M)
$C_{CO_2}^*$	Aqueous concentration of CO <sub>2</sub> in equilibrium with the gas phase CO <sub>2</sub> (M)
$C_{O_2}^*$	Aqueous concentration of O <sub>2</sub> in equilibrium with the gas phase O <sub>2</sub> (M)
$C_L$	Dissolved oxygen concentration in the bulk liquid (mmol/L)
$C^* - C_L$	Concentration difference across the film at the interface, 'driving force' (mmol/L)
$C_{Gln}$	Concentration of glutamine in the culture (mM)
$C_{NH_3}$	Concentration of NH <sub>3</sub> in the culture (mM)
$C_{NH_3}^{in}$	Concentration of NH <sub>3</sub> in the feed (mM)
$C_{O_2}$	Concentration of oxygen in water (mg/L)
$d_B$	Bubble diameter (m)
D	Medium exchange rate (L/hr)
$F_0$	Feed flow rate going into the bioreactor (L/hr)
$F_1$	Recycle flow rate from cell separator device into bioreactor (L/hr)
$\mathcal{H}_{CO_2}$	Henry's Law constant for CO <sub>2</sub> (L.atm/mol)
$\mathcal{H}_{O_2}$	Henry's Law constant for O <sub>2</sub> (L.atm/mol)
$k_A$	CO <sub>2</sub> transfer coefficient at liquid surface, as defined in Gray <i>et al.</i> (1996)

- $k_{a2}$  Equilibrium constant for CO<sub>2</sub> hydration,  $5.2 \times 10^{-7} M$  as defined in Gray *et al.* (1996)
- $k_{CO_2}^{surface}$  Surface aeration contribution to  $k_L a$  (1/hr) as defined in Zupke and Green (1998)
- $k_{CO_2}^{sparge}$  Sparger contribution to  $k_L a$  (1/hr) as defined in Zupke and Green (1998)
- $k_{O_2}^{surface}$  Surface aeration contribution to  $k_L a$  (1/hr) as defined in Zupke and Green (1998)
- $k_{O_2}^{sparge}$  Sparger contribution to  $k_L a$  (1/hr) as defined in Zupke and Green (1998)
- $k_L$  Individual mass transfer coefficient for liquid phase, based on concentration difference (m/s)
- $K_L$  Overall mass transfer coefficient for liquid phase, based on concentration difference (m/s)
- $k_L a_{CO_2}$  Volumetric CO<sub>2</sub> transfer coefficient (L/min) for Gray *et al.* (1996) equations and CO<sub>2</sub> gas-liquid mass transfer coefficient (L/hr) as defined in Zupke and Green (1998)
- $K_S$  Saturation constant (g/L)
- $[O_2]_{eq}^{surface}$  Aqueous concentration of O<sub>2</sub> in equilibrium with headspace gas (M)
- $[O_2]_{eq}^{sparge}$  Aqueous concentration of O<sub>2</sub> in equilibrium with sparge gas (M)
- P Medium withdraw rate from the cell separator (L/min) as defined in Gray *et al.* (1996)
- $P_B$  Pressure at the bottom of the tank (atm) as defined in Zupke and Green (1998)
- $P_g$  Power consumption the presence of gas (W)
- $P_T$  Pressure at the top of the tank (atm) as defined in Zupke and Green (1998)
- Q Gas flow rate (L/min)

$$\langle Q \rangle = \frac{X_V - k_{O_2}^{surface} ([O_2]_{eq}^{surface} - [O_2])}{k_{O_2}^{sparge} (-[O_2])}$$

$\langle Q \rangle$	Average gas sparge rate (L/min)
$Q_{max}$	Maximum gas sparge rate (L/min)
$q_{CO_2}$	Specific CO <sub>2</sub> production rate (mmol/cell/hr)
$q_{O_2}$	Specific O <sub>2</sub> production rate (mmol/cell/hr)
$q_P$	Specific product production rate (mmol/cell/hr)
$q_S$	Specific substrate consumption rate (mmol/cell/hr)
$Q^{overlay}$	Bioreactor headspace overlay gas flow rate (L/min)
R	Universal gas constant (L.atm/mol.K)
$r_x$	Rate of cell growth (hr <sup>-1</sup> )
S	Substrate concentration in bioreactor (g/L)
$S_0$	Substrate concentration in feed going into the bioreactor (g/L)
T	Temperature (°C)
V	Cell culture (liquid) volume in the bioreactor (L or m <sup>3</sup> , will be specified)
$V_L$	Volume of liquid in bioreactor (m <sup>3</sup> )
$V_h$	Volume of headspace in the bioreactor (m <sup>3</sup> )
$V_g$	Superficial gas velocity (m/s)
X	Cell concentration in the bioreactor (g/L)
$X_v$	Viable cell density (cells/mL)
$x_{CO_2}$	Mole fraction of CO <sub>2</sub>
$x_{CO_2}^{overlay}$	Mole fraction of CO <sub>2</sub> in headspace overlay
$x_{CO_2}^{sparge}$	Mole fraction of CO <sub>2</sub> in sparge gas
$x_{O_2}$	Mole fraction of O <sub>2</sub>
$x_{O_2}^{overlay}$	Mole fraction of O <sub>2</sub> in headspace overlay
$x_{O_2}^{sparge}$	Mole fraction of O <sub>2</sub> in sparge gas
$X_0$	Cell concentration in the feed stream going into bioreactor (g/L)



- $X_1$  Cell concentration in the recycle stream going into bioreactor (g/L)  
 $X_2$  Cell concentration in effluent stream from the cell separator (g/L)

### **Greek Letters**

- $\epsilon$  Fractional gas hold up (%)  
 $\mu$  Specific growth rate ( $\text{hr}^{-1}$ )

### **Superscripts**

- \* At equilibrium; dimensionless quantities as used in Hill 2006

### **Subscripts**

- 0 Initial value  
aq Aqueous  
eff Effective  
F Feed as used in equations by Gray *et al.* (1996)  
L Liquid phase  
LM Log mean  
m Maximum  
T Total

### **Abbreviations**

- CER CO<sub>2</sub> Evolution Rate  
CPR CO<sub>2</sub> Production Rate  
CTR CO<sub>2</sub> Transfer Rate  
OUR O<sub>2</sub> Uptake Rate  
OTR O<sub>2</sub> Transfer Rate  
sCPR Specific CO<sub>2</sub> Production Rate; (mol/cell/min – for Gray *et al.*, 1996 equations) and (mmol/cell/hr for Zupke and Green, 1998 equations)

sLPR Lactic acid production rate (lactate/cell/hr)

VCD Viable Cell Density

## CHAPTER 1

### INTRODUCTION

Biopharmaceutical drug manufacturing process is very different from the small molecule classical drug manufacturing process. Under appropriate living conditions, live cells with recombinant DNA produce a protein, which is purified for use as a safe and effective treatment for serious diseases. These cells are cultivated in bioreactors, which provide optimum living conditions for the drug producing cells. As discussed in Section 2.2 and depending on the cell line, these bioreactors can be operated in batch, fed-batch or perfusion modes with the goal of increasing yield of protein drug per volume by providing optimum amount of nutrient and removing waste. These bioreactors can cultivate anchorage-dependent or suspension cell cultures.

Since mammalian cells are extremely sensitive to physiological growth conditions, ongoing operation of these bioreactors required precise and careful CO<sub>2</sub> management and pH maintenance strategies. This thesis provides a mathematical model for maintaining steady CO<sub>2</sub> concentration and pH levels in a perfusion bioreactor growing Chinese hamster ovary cells in a suspension culture.

In the biotech industry, a precise mathematical description of drug producing cells is immensely valuable as such a model, in addition to saving

significant capital, will significantly aid process development both to start out initial drug manufacturing trials and subsequent troubleshooting during production. Such a model forms the backbone of adaptive control strategies, which require complete knowledge of the process including complete analytic expressions (Van Impe and Bastin, 1995). Researchers have presented many different kinds of modeling strategies, for example, neural networks (Karim *et al.*, 1997; Nagy, 2007), data based modeling (Karim *et al.*, 2003), and stochastic simulation (Li *et al.*, 2008). However, none of these strategies have been applied to CO<sub>2</sub> balance and pH control.

One of the primary and well-known challenges of a bioreactor operation is CO<sub>2</sub> management and pH control. Optimum growth conditions, including CO<sub>2</sub> concentration and pH levels, highly cell line dependent. If two companies use Cho cells but different cell lines, then growth condition requirements are different for each cell line. Nevertheless, the published scientific literature has a dearth of articles on modeling CO<sub>2</sub> concentration and pH control in the bioreactors (Yoon *et al.*, 2005). Such a model would lead to the development of a control strategy to maintain CO<sub>2</sub> concentration and pH level.

Today, biopharmaceutical industry routinely produces therapeutic proteins from aerobic mammalian cells. Obviously then, the industry has addressed the challenge of CO<sub>2</sub> management and pH control but each company has knowledge regarding its own particular cell line. Conceivably, these individualized solutions to

the CO<sub>2</sub> management and pH control issues exist in the proprietary knowledgebase of these respective corporations. Consequently, there is a dire need for a publicly available first principles model of CO<sub>2</sub> concentration and pH control in bioreactors that can be applied to multiple cell lines. This thesis fills this void by presenting a way to model CO<sub>2</sub> concentration and control pH in an perfusion bioreactor.

Methodology developed in this thesis can be modified for application to batch and fed batch modes of operation of bioreactors.

### 1.1. Statement of the Problem

Cell growth in a bioreactor requires steady state conditions i.e. steady nutrient levels and a steady physiological environment. However, in a bioreactor, viable and total cell counts fluctuate appreciably during cultivation due to known and unknown causes and as such, a bioreactor system is unlikely to be in a true steady state (Vits and Hu, 1992). Thus, in a bioreactor, cells exist in a quasi-steady state and the equilibrium continuously shifts. For example, with the passage of time, cells divide and number of cells increase. Each of these mammalian cells alters the pH of the fermentation broth by releasing metabolic by-products – ammonia, CO<sub>2</sub> and lactic acid (Wu *et al.*, 1993). The goal of this model is to develop a mathematical strategy to maintain pH and CO<sub>2</sub> within a specified narrow range.

High-density mammalian cell cultures that use CO<sub>2</sub> to regulate pH have three sources of CO<sub>2</sub>/CO<sub>3</sub><sup>2-</sup>: (1) CO<sub>2</sub> that the aerobic cells excrete as part of their normal growth process (Krebs cycle); (2) HCO<sub>3</sub><sup>-</sup>/CO<sub>3</sub><sup>2-</sup> that is added to regulate

(raise) pH; and (3) CO<sub>2</sub> sparged into the bioreactor to regulate (lower) pH (Zanghi *et al.*, 1999). Excess CO<sub>2</sub> can be defined as the cell line dependent amount of CO<sub>2</sub> concentration above which cell growth is appreciably negatively impacted. Excess CO<sub>2</sub> is not only toxic to the mammalian cells, it may also affect the quality of protein products (Gray *et al.*, 1996; Matsunaga, 2009b). This thesis presents a mathematical strategy to regulate CO<sub>2</sub> levels in a perfusion bioreactor.

In addition to multiple sources of CO<sub>2</sub>, aerobic cell cultures with high VCD pose their own unique challenges because as VCD increases, so does cell stress due to environmental conditions. Cells experience stress if the physiological growth conditions deviate optimum growth conditions. For example, cells can experience stress in if the broth is highly acidic or highly basic or due to excessive agitation in the bioreactor. For these mammalian cells, the nature of the stress is key to product formation because certain stress would induce the cells to produce the protein-product while other types of stress may inhibit cell growth and/or protein synthesis.

Cell cultures with high VCD produce large amounts of CO<sub>2</sub> and require large quantities of O<sub>2</sub> for respiration. If high VCD causes anorexic and/or low O<sub>2</sub> conditions, then in response, the CHO cells may begin to excrete lactic acid, which lowers pH and eventually the CHO cells switch to metabolizing the lactic acid instead of glucose (Wu *et al.*, 1993). CHO cell growth on lactic acid is undesirable because such growth adversely affects the quality of the protein-product.

In high density mammalian cell cultures, perfusion systems address the problem of accumulation of highly soluble CO<sub>2</sub> and the high demand for sparingly soluble O<sub>2</sub> in the bioreactor fermentation broth, by exchanging spent media with oxygen-rich fresh media and by recycling the viable cells into the bioreactor. Appropriate agitation of the fermentation broth can alter local concentrations of CO<sub>2</sub> and O<sub>2</sub>, as higher agitation rates lead to more homogeneous growth conditions (Arjunwadkar, 1998). However, the mammalian cells in bioreactors must be gently agitated because as the agitation rates increase, risk of cell lyses also increases.

As cells grow in the perfusion bioreactor, increasing VCD increases the amount of NH<sub>3</sub>, CO<sub>2</sub> and lactic acid in the cell culture broth; and correspondingly the cells consume increasing amounts of nutrients and O<sub>2</sub>. Depending on the amount dissolved in the fermentation broth, CO<sub>2</sub> can be both – a nutrient and a toxin. CO<sub>2</sub> is toxic in high concentrations but is also essential for survival and for general well-being of the cells.

## 1.2. Research Goals of the Current Thesis

Specific goals of this thesis project are:

1. To understand and model the effect of carbon dioxide concentration on specific growth rate of CHO cells.

2. The solution to the equations must maintain steady state in terms of pH and concentrations of  $\text{NH}_3$ ,  $\text{CO}_2$ , lactic acid and  $\text{O}_2$  in the cell culture broth.
3. To develop a framework for a computer program that is easily adaptable for any cell line by changing few characteristic constants that are specific to a particular cell line.
4. To optimize the addition of sodium carbonate ion and  $\text{CO}_2$  gas to regulate pH.
5. To incorporate carbon dioxide stripping due to its subsurface sparging into the mathematical model.

### 1.3. Scope or Limitations of the Problem

Biological processes involve intricate and interdependent reaction pathways. In this thesis, non-linear, dynamic equations describe an unstructured, first principles mathematical model of perfusion bioreactor. Certain simplifying assumptions help solve these modeling equations. A more detailed description of the assumptions used to solve these equations is presented alongside the solutions to those equations.

Fermentation stability is of paramount importance for a viable cell culture. However, time-dependent increases in CHO cell density in a bioreactor under dissolved oxygen undergo spontaneous bifurcations losing stability. (Chung *et. al.*, 2003). This loss in stability means sustained and amplified perturbation in the



bioreactor dissolved oxygen concentration and in oxygen gas flow rate to the bioreactor. *Id.* In this mathematical model, the high-density CHO cell culture is assumed to be inherently stable.

Cell cultures grown at constant hydrostatic pressures of  $10^5 - 9 \times 10^5$  Pa, show little variation in cell growth or specific glucose consumption rates (Takagi *et al.*, 1995). However, as the pressure is increased (to  $\sim 5 \times 10^5$  Pa), specific lactate production rate slightly decreases and specific glutamine consumption and ammonia production rates increase. *Id.* Perfusion bioreactors considered in this thesis typically contain 3-4 m<sup>3</sup> of fermentation broth and CHO cells. For this reason, hydrostatic pressure effects because of liquid height in a bioreactor are ignored as negligible (for example, approximately 3,000 L bioreactor filled to a height of 1.5 m with water, exerts a pressure of  $\sim 1.5 \times 10^{-4}$  atm or  $\sim 15$  Pa at the bottom of the tank).

Heat balance is not considered. Heat is transported into and out of a perfusion bioreactor broth through convective and molecular transport when new feed is added to the system and through media exchange. Additionally, each cell produces and consumes heat because of various intra- and intercellular reactions (Riet, 1983). Similarly, mechanical energy or power applied to propel the agitator does work on the system, which degrades into the broth as thermal energy: as the agitator rotates, each shell of broth that is adjacent to the agitator blade rubs against that agitator blade. This friction between adjacent layers of fluid and the

wall of the agitator blades produces heat. Finally, the electric wires around the bioreactor may produce electric and magnetic fields that may affect the temperature of the broth. All these sources and sinks of heat are ignored. The mathematical model assumes that a heat jacket around the reactor keeps the temperature inside the bioreactor steady i.e. the system is diabatic or non-adiabatic. In solving the modeling equations, the temperature of the broth is assumed to be within the range of 36.5 °C to 37.5 °C.

From inoculation until the time when the downstream processes begin, many generations of CHO cells produce the desired protein product. It is conceivable that genetic mutations are introduced over several generations that can alter the metabolism of the cells. This model assumes that number of cells with such mutations is very small to have any material effect on the behavior of the bulk of the bioreactor. Likewise, this thesis ignores the effects of microgravity (Anderson, 2004) and various mechanical and environmental factors on cell behavior and gene expression.

Bubble dynamics play an important role in gas transport and cell survivability in a bioreactor. However, effects of bubbles that are produced as a result of CO<sub>2</sub> and O<sub>2</sub> sparging and as a result of agitation are not considered (Wang *et al.*, 1994; Meier *et al.*, 1999; Ma *et al.*, 2006). Similarly, position dependence of agitators is neither considered nor investigated, which has been shown to have an impact on oxygen and carbon dioxide transfer rates (Arjunwadkar, 1998).

Eddy dynamics affect material transport and diffusion into and out of gas and liquid phases in a bioreactor (Croughan and Wang, 1989). This model assumes that mixing between randomly dispersed eddies is instantaneous. Dynamics of mixing within eddies is ignored. Finally, a bioreactor has many coupled reactions. That is, they require co-substrates or co-factors, which are either recycled or regenerated by another companion reaction. This unstructured model also ignores these coupling effects.

#### 1.4. Hypothesis

Contois equation (Contois, 1959) best describes substrate-limited specific growth rate of CHO cells in a perfusion bioreactor that has a high viable cell density (Shuler and Kargi, 1992).

Maintaining CO<sub>2</sub> concentration and pH at a steady state would require dynamic CO<sub>2</sub> material balance and dynamic control of pH.

#### 1.5. Justification for and Significance of the Study

Biopharmaceutical drugs are prohibitively expensive due to high research costs and long development time that is required to understand and then develop the drug manufacturing process. A mathematical model that addresses a primary challenge of a mammalian cell culture would greatly reduce process development related time and costs. The fact that work described in this thesis is highly sought

after and timely can be gauged from the fact that even though bicarbonate management is a long standing problem, there is no effective solution available.

In 1957, Dr. Theodore T. Puck initiated the use of Chinese hamster ovary cells for research in mammalian somatic cell genetics (Gamper *et al.*, 2005). Since then, CHO cells have become the most popular industrial cells that are used to produce tissue plasminogen activator, erythropoietin, granulocyte colony stimulating factor, factors VIII and IX, deoxyribonuclease I, glucocerebrosidase, beta-interferon, MAb against GPIIb/IIIa, CD20, tumor necrosis factor alpha, tumor necrosis factor receptor and HER2 (Xie and Zhou, 2006). Today, CHO cells produce nearly 70% of all recombinant protein therapeutics that account for \$30 billion in annual sales (Jacob *et al.*, 2009). Manufacture of these blockbuster biopharmaceutical therapies from mammalian cell cultures is capital intensive and requires implementation of robust and reliable bioprocesses that consistently produce uniform product. Such production in turn requires a detailed understanding of dynamics and interplay amongst critical bioprocess parameters such as temperature, dissolved CO<sub>2</sub> and O<sub>2</sub> and concentrations of lactic acid and ammonia.

Increasing the product titer requires extensive manipulation of growth conditions inside a bioreactor (Leist, 1990). And although this manipulation is routinely performed in the industry, it is usually a closely guarded trade secret (Yoon *et al.*, 2005; Frick and Junker, 1999). Similarly, despite their numerous applications for producing therapeutic proteins, publications on CHO cell culture

processes are quite limited (Xie and Zhou, 2006). Rarer still are publications and purported solution to the problem of carbon dioxide/bicarbonate management in a bioreactor even though bicarbonate management is an overarching issue that biotechnologists deal with every day. Currently, empirical models exist in the industry that are cell line specific, in which, cells are grown under well-characterized conditions. This research thus fills a major void and advances the knowledge about CHO cells growth processes by focusing on mathematically modeling one of the primary challenges that such cultures face – accumulation of CO<sub>2</sub> in bioreactors (Frick and Junker, 1999).

A perfusion bioreactor requires elaborate i.e. expensive support system as compared to a batch or fed-batch bioreactor. The goal of such a support system is to ensure optimum growth conditions for CHO cells while minimizing conditions that are damaging to cell health and growth. Mathematical and computer models aid in estimation of capital outlay by forecasting bioprocess system needs. Frequently changing culture conditions can influence the consistency of protein product. However, consistent process performance and product quality is a regulatory requirement (21 C.F.R. part 211; Woodside *et al.*, 1998). Thus, in the highly regulated biopharmaceutical manufacturing industry, compliance and regulatory promises and not just economic considerations often dictate bioprocess choices.

Ideally, an accurate representation of cell growth in a bioreactor accounts for every conceivable variation in the process. Not surprisingly, such a description

would be computationally onerous and financially impractical (Haag *et al.*, 2005).

This thesis has a direct impact on such considerations by examining the relationships between the critical parameters by focusing specifically on CO<sub>2</sub> management.

## CHAPTER 2

### LITERATURE REVIEW

Living cells produce proteins that have become blockbuster biopharmaceutical drugs like Enbrel® and Remicade®. Chinese hamster ovary (CHO) cells are primary host cells that the biopharmaceutical industry uses to produce recombinant therapeutic proteins. Manufacture of these proteins in bioreactors consists of two distinct processes: upstream process during which the viable cell density (VCD) increases to a predetermined level and downstream process during which the biopharmaceutical protein that the living cells have produced is purified and isolated. As with any other manufacturing process, protein production through living cells is rife with opportunities for improvement.

A longstanding problem that the biotechnology industry faces is carbon dioxide/bicarbonate management especially in mammalian cell cultures. This thesis focuses on developing a first principles, unstructured, dynamic mathematical model for regulating carbon dioxide and pH in a bicarbonate buffered perfusion bioreactor for aerobic Chinese hamster ovary (CHO) cell system. A perfusion bioreactor is the focus of this research because it is the established bioreactor system in large-scale recombinant protein commercial production facilities. However, perfusion systems are significantly more complex, hence more expensive, and more prone to contamination than batch or fed-batch systems (Fenge and Lüllau, 2006).

## 2.1. Cell Culture Perfusion Systems

Production of biopharmaceutical protein molecules from mammalian cells begins with a single genetically engineered living cell when it is inoculated into a bioreactor containing favorable growth media and environmental conditions. According to Graff and McCarty (1958), a cell culture is a highly artificial expedient in which host influences are abolished but can be simulated. Suitably then, bioprocess engineers induce the genetically engineered cells to multiply and produce desired protein drug by maintaining optimum growth conditions. In addition to mechanical characteristics of a bioreactor, the growth conditions and critical parameters include optimum concentrations of ammonia, dissolved carbon dioxide, dissolved oxygen, lactic acid, osmolality, pH, substrate and temperature and ionic strength of the fermentation broth. Controlling all these parameters at the same time to ensure optimum environment for the cells is a challenging task that requires simultaneous calculations and real time decision-making. As one scientist noted, “[e]ven the simplest living cell is a system of such forbidding complexity that any mathematical description of it is an extremely modest approximation” (Bailey, 1998).

As the aerobic mammalian cells grow, they consume nutrients and  $O_2$  and excrete  $CO_2$ ,  $NH_3$  and lactic acid. As the cell density increases, the concentration of excreted by-products progressively increases in the medium, which can lead to unnatural extremes. Both, dissolved carbon dioxide and lactic acid are mild acids



and tend to lower the pH of the biological medium, which is unfavorable for cell growth. Thus, bioprocess engineers must closely monitor dissolved carbon dioxide and nutrient levels.

## 2.2. Bioreactor Operation Modes: Batch, Fed Batch and Perfusion

A bioreactor can be operated in one of three different modes based on the how often cell culture media is replenished and removed: (a) batch; (b) fed-batch; (c) continuous or perfusion with cell recycle.

### 2.2.1. *Batch and Fed Batch Bioreactors*

In a batch bioreactor, where there is neither inflow nor outflow of cells or media, the cells grow undisturbed in the initially supplied media. Frequently, oxygen is sparged. Thus, only pH, temperature and aeration are controlled in batch reactors. However, over time, local cell environment constantly changes as the cells multiply and consume available nutrients and excrete by-products like CO<sub>2</sub>, NH<sub>3</sub> and lactic acid.

In a fed-batch operation, a substrate feed maintains the concentration of the nutrients at a predetermined steady state. However, there is no outflow of cells or media. The disadvantage of fed-batch operation is that accumulation of metabolic by-products such as lactate and ammonia limit cultivation times (Vits and Hu, 1992).

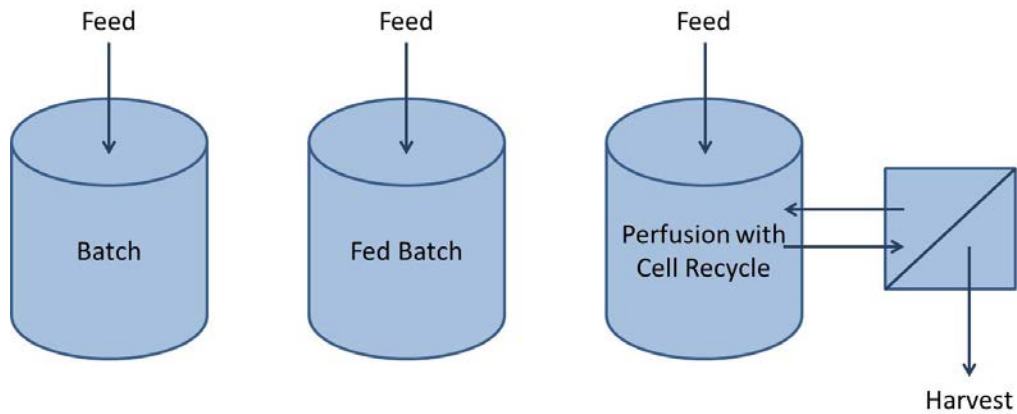


Figure 1: Types of Bioreactors

### 2.2.2. *Perfusion Bioreactor Systems*

Perfusion systems are characterized by media exchange. These perfusion systems can either be “open” in which fresh, enriched media is continuously added and spent media is correspondingly removed; or the perfusion system can be closed in which the spent media is withdrawn and pumped through an “oxygenator” before being re-circulated back into the bioreactor (Griffiths, 1990; Hu and Wang, 1986).

In addition to replenishing dissolved oxygen in the bioreactor, the exchange of media in perfusion bioreactors removes metabolites like  $\text{NH}_3$ ,  $\text{CO}_2$  and lactic acid that can become toxic in high concentrations (Riley, 2006). According to Hu and Wang, assuming oxygen consumption rate of 1.5 mmol/L.h in the cell culture, a media recirculation rate (or perfusion rate) of approximately 8 volumes/hr is needed to avoid oxygen limitation. In this manner, by varying the perfusion rate

according to the demands of the cell population, a perfusion bioreactor avoids alternate periods of “famine and feast” in a cell culture, which are characteristic of fed batch reactors (Graff and McCarty, 1958; Nahapetian, 1986).

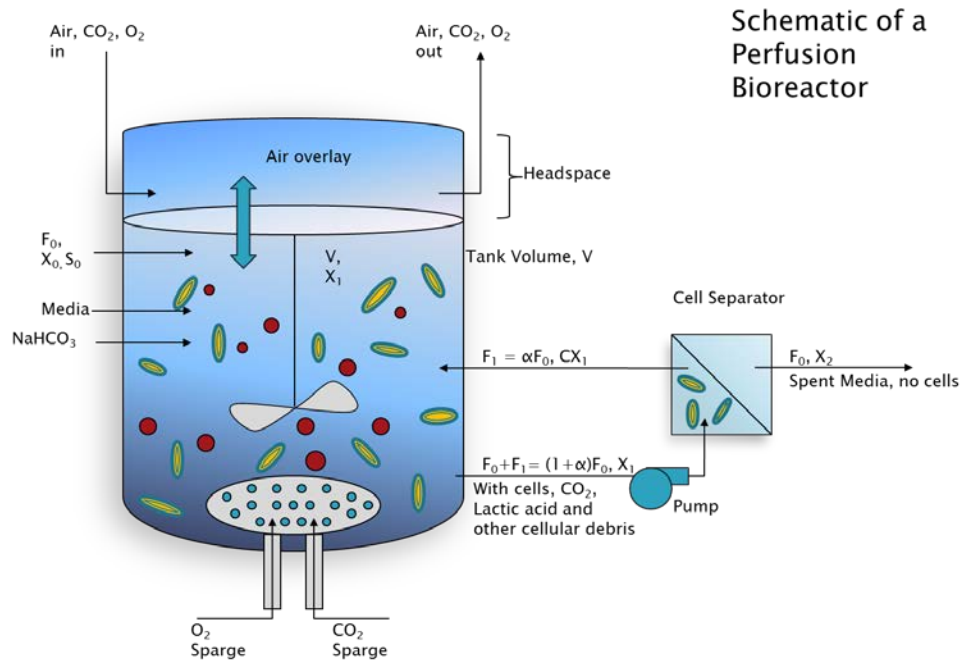


Figure 2: A Typical Perfusion Bioreactor

Any media exchange in a suspension culture requires a cell retention device and can consist of either submerged spin filters or membrane filtration devices outside the bioreactor (Woodside *et al.*, 1998). Perfusion bioreactor systems offer a relatively constant culture environment over months of operation, a short product residence time and increased product concentration, while operating at a high cell density of  $10^6$ - $10^7$  cells/mL (Drouin *et al.*, 2007; Kumar *et al.*, 2007).

### 2.3. Cell Cultivation: A Brief History

Perfusion systems are characterized by a bioreactor with a filtration system that retains cells during media exchange. Today, the biopharmaceutical industry employs perfusion systems to cultivate high-density cell cultures by providing, controlling and maintaining for the cells, a steady state homogenous environment that attempts to closely approximate the cells' natural physiological growth conditions. However, the perfusion systems were initially developed for medical applications to examine animal tissues *in vitro* under the microscope and to cultivate organs outside of an organism.

#### *2.3.1. Early Development of Perfusion Techniques*

As early as 1907, Ross Granville Harrison of Johns Hopkins University grew a nerve cell outside the animal body (Patterson, 1975). Subsequently, a young medical student, Montrose Burrows and Harrison, together developed a perfusion system as part of their studies involving tissues from frog embryos in which they used blood plasma as growth medium (Butler, 1986; Friedman and Friedland, 1998).

In 1912, French physician, Alexis Carrel of the Rockefeller Institute of Medical Research, New York, who won the Nobel Prize in Medicine that same year, described a method for cultivating a large quantity of tissue for extended observation under a microscope (Carrel, 1912a). That same year, Carrel reported

cultivating cell suspension culture and a heart of an eighteen-day-old chick fetus (Carrel, 1912b). Finally, in 1935, American aviator, Charles A. Lindbergh developed one of the first perfusion systems and Carrel perfected the techniques for cultivation of whole organs in that perfusion system (Lepicard, 2008).

Parenthetically, Friedman and Friedland's investigation into history of cell cultivation revealed that Carrel had delegated his laboratory duties to Albert Eberling who had modified Carrel's original tissue culture technique. Eberling cultured the heart by placing it on the floor of a glass culture vessel and feeding it a drop of chick blood plasma and a drop of watery extract of chicken embryonic tissue. Once the cell culture used up all the nutrients in the clot, Eberling would repeat the process with half the resultant grown tissue. Eberling claimed that this heart, the size of a match head (called an explant), was alive until two years after Carrel himself died in 1944. However, Friedman and Friedland found that Eberling was continuously adding new cells to the culture.

The 1950s was an eventful decade in the advancement of perfusion systems, cell culture medium development and biotechnology in general. In 1953, James Watson and Francis Crick published the structure of deoxyribonucleic acid (DNA) in *Nature*. Graff and McCarty (1957 and 1958) published their articles on perfusion cell cultures wherein they described method for controlling pH by controlling "CO<sub>2</sub> tension."

In 1953, Christiansen *et al.* and Danes *et al.* published their twin studies on continuous tissue culture; Harry Eagle published a series of papers from 1955 to 1960 reporting results regarding amino acids, vitamins, ionic species, energy sources and other factors essential for the survival and growth of mammalian cells in culture (Darnell *et al.*, 2005; Eagle, 1977). Together these discoveries laid the foundation for biotechnological advances by making large-scale high-density cell cultures possible. 1960s saw the development of cell culture techniques that had direct application for large-scale high-density cell cultures. In 1969, Himmelfarb *et al.*, described a perfusion system using a spin filter in suspension culture. Using this spin filter, they achieved cell densities close to  $10^8$  cells/mL.

### 2.3.2. *Foundation for Modern Biotechnology*

1970s saw major advancements in the biotechnological techniques including the developments in bioreactors, monitoring technologies and of course applied recombinant DNA techniques. In 1975, Köhler and Milstein developed hybridoma technology, which allowed production of monoclonal antibodies. In 1988, Riechmann *et al.* developed a technique to generate humanized antibodies with 90-95% human content. All these advancements laid the foundation for eventual FDA approval in 1986 of the first therapeutic monoclonal antibody, Orthoclone® OKT3® (Ortho Biotech) and of first humanized diagnostic monoclonal antibody, *Arcitumomab*® (Immunomedics/Pharmacia, Inc.) in 1996.

From the Biologics Control Act of 1902 to the modern day Food, Drug and Cosmetics Act, regulatory framework has also advanced with the developments in drug manufacturing technologies. Of course, discussion of statutory and legal framework is outside the scope of this work. However, it must be mentioned that U.S. laws, like Orphan Drug Act have played a pivotal role in development of the entire biopharmaceutical drug industry.

#### 2.4. Types of Mathematical Models

Depending on application and the level of desired detail, different types of models have been proposed that characterize a suspension cell culture in a perfusion bioreactor.

##### 2.4.1. *Black Box, Gray Box and White Box Models*

Black box (or empirical or input-output) models link input factors with output responses. These models are constructed when the mechanism underlying a process is not understood sufficiently well, or is too complicated, to allow an exact model to be postulated from theory (Lübbert and Jørgensen, 2001; Box *et al.*, 2002; van Lith, 2002). These models are straightforward and their results can be readily interpreted.

White box (or first principles or fundamental or mechanistic) models are based on known biological, chemical and physical laws (van Lith, 2002; Lübbert and Jørgensen, 2001). Such models are typically nonlinear and thus formulated in

terms of ordinary or partial differential equations with supporting linear algebraic equations. These models are employed when understanding a process or a system is essential to progress or when the state of the art is sufficiently advanced to make a useful white box model easily reliable (Box *et al.*, 2002). Consequently, these models require a high level of effort and can be constructed even when the system itself is not yet constructed (van Lith, 2002). Thus, a first principles model is mathematically rigorous albeit computationally expensive.

As the name suggests, gray box models are hybrid models, which are a combination of first principles and empirical models. Models that are based on heuristic knowledge are termed gray box models to distinguish them from the purely data driven black box models and models based on mechanistically completely understood mathematical models (Lübbert and Jørgensen, 2001). Today, most models are gray box models to some extent (van Lith, 2002).

#### *2.4.2. Dynamic and Static Models*

Static models can generally be described by  $y = f(u)$  where the value of the independent variable does not change with time. Dynamic models on the other hand, seek to describe the behavior of independent variables with respect to time. In a dynamic model, values of parameters can vary discretely or continuously with time. A continuous variable can take any value within a time interval. A discrete variable can take only a certain number of values within a time interval. Many techniques are available to mathematically manipulate discretised form of dynamic



control functions, for example, orthogonal collocation method or control vector parameterization (CVP). However, added complexities emerge when the dynamic problems are stiff.

#### *2.4.3. Linear and Nonlinear Models*

Simultaneous linear algebraic equations describe a linear model. For such equations, condition for existence and uniqueness of the solution is trivial. These equations can be solved by explicit numerical methods like vector/matrix operations or various elimination methods (Constantinides and Mostoufi, 1999). A linear algebraic model will have a unique solution if it is well defined (Beers, 2007). However, biological systems are seldom well defined and seldom completely defined only by linear models.

A nonlinear model is at least partially described by nonlinear equations wherein the output is a nonlinear function of the input. All nonlinear equations have the form  $f(x) = 0$ , where  $x$  is a single variable that can have multiple roots that satisfy this equation. For example, the function  $f(x)$  may assume many nonlinear functionalities like higher order polynomial, trigonometric, exponential and logarithmic terms. Methods of solution of nonlinear systems and differential equations use the technique of linearization of the models, thus requiring the repetitive solution of sets of linear algebraic equations (Constantinides and Mostoufi, 1999). Thus, nonlinear equations require implicit numerical solution like

finite difference methods, Newton-Raphson method, Runge-Kutta methods, Eigenvalue method etc.

#### *2.4.4. Segregated and Non-segregated Models*

A segregated model considers individual cells that are different from one another depending on some distinguishable characteristic (Shuler and Kargi, 1992). Non-segregated models consider the population as lumped into one biophase that interacts with the external environment so that a single variable can describe the cell concentration (Blanch and Clark, 1996).

#### *2.4.5. Structured and Unstructured Models*

The concept of structure arises when considering the detailed reactions occurring within the cell (Blanch and Clark, 1996). For example, a structured model may consider the kinetics of compounds involved in a Tricarboxylic Acid (TCA) or Krebs cycle and describe reactions and ATP, CO<sub>2</sub> and O<sub>2</sub> consumption and production in detail. That is, structured models break the population into distinct subcomponents (Shuler and Kargi, 1992). Thus, structured models require a thorough knowledge of intracellular reactions and their regulation mechanisms (Zeng and Bi, 2006).

Unstructured models on the other hand, consider cell as an entity and models its interactions with the environment assuming fixed cell composition (Shuler and Kargi, 1992). These models are based on fundamental observations of

biological processes (Zeng and Bi, 2006). They can be used to qualitatively and quantitatively describe important features of cell culture, for example, effect of pH on cell growth. However, since the unstructured models quantify cell mass as a single component, they cannot describe transient behavior very well (Shuler and Kargi, 1992).

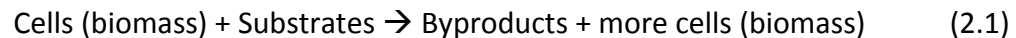
#### *2.4.6. Transient and Steady State Models*

A steady state of a dynamic system is defined as one in which the time derivatives of each state variable are zero (Beers, 2007). In other words, over time, the input and output parameters of a system are held constant. A steady state is stable, if following every infinitesimal perturbation away from a steady state,  $x_s$ , the system returns to  $x_s$ . If any infinitesimal perturbation causes the system to move away from  $x_s$ , then a steady state  $x_s$  is unstable. Finally, a neutrally stable steady state exists when a perturbation neither grows nor decays with time (Beers, 2007).

A transient state exists when a bioreactor is brought into operation or is being shut down so that concentrations, pH, temperature and other parameters continually change with time. Alternatively, an unstable steady state can be nudged into a transient state due to some perturbation caused by changes in environment or some feedback control.

## 2.5. Biomass, Cell Growth Kinetics and pH Control

The primary objective of every step in the production of protein from mammalian cells is to increase titer – the protein concentration. Amount of titer can be increased by increasing (1) the cell mass; (2) the productivity of each cell; and (3) time. Given ample supply of nutrients, growth of cells in a bioreactor is an autocatalytic process. The amount of change in cell population at any given time is proportional to the initial cell concentration. Thus, the following overall reaction describes cell growth in unstructured models:



$$\mu = \frac{1}{X} \frac{dX}{dt} \quad (2.2)$$

where  $\mu$  is specific cell growth rate ( $\text{hr}^{-1}$ ),  $X$  is cell mass concentration ( $\text{g/L}$ ) and  $t$  is time ( $\text{h}$ ) (Shuler and Kargi, 1992). Under favorable growth conditions in an ideal system, a cell population increases exponentially.

As VCD increases, aerobic CHO cells consume  $\text{O}_2$  and produce  $\text{CO}_2$  and lactic acid. Equation (2.2) includes a multitude of cellular reactions that produce and consume various acids and bases, altering pH in a bioreactor. However, ideal growth conditions require steady state operation at desired pH, temperature and control of other parameters. To maintain a bioreactor at a certain pH, an acid or a base is added at commercial production facilities where mammalian cells are cultivated in a series of bioreactors. However, because of the variability associated

with each individual cell, it is difficult to predict how much acid or base must be added.

#### *2.5.1. Criteria, Limitations and Considerations for Modeling Equations*

The model in this research assumes balanced growth of the CHO cells by assuming a fixed cell composition for all CHO cells and by treating the entire cell culture in the bioreactor as homogeneous. In doing so, this black box model disregards the microscopic expression of cells and ignores stoichiometry and the behavior of various organelles *inside* each cell.

The following sections describe the roles and significance of four compounds – CO<sub>2</sub>, NH<sub>3</sub>, lactic acid and O<sub>2</sub>, in CHO cell growth cycle in a bioreactor. The mathematical model will then address the individual challenges of maintaining steady state by focusing on the interactions of CO<sub>2</sub> with the concentration profiles of NH<sub>3</sub>, lactic acid and O<sub>2</sub>.

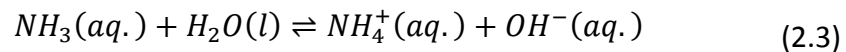
#### *2.5.2. Ammonia/Ammonium Ion*

In a 1996, Schneider *et al.* published a survey of research work done on the toxic and inhibitory effects of ammonia/ammonium ion in mammalian cell cultures. Ammonia is one of the two major metabolic by-products of mammalian cell growth (the other is lactate) that accumulates in high-density mammalian cell cultures as a result of glutamine metabolism and its spontaneous decomposition (Xie and Wang, 1996; Yoon *et al.*, 2005; Gódiá and Cairó, 2006). Ammonia negatively influences cell

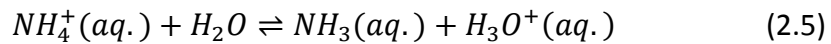
growth, recombinant protein productivity of CHO cells and protein quality (Chen and Harcum, 2005).

### 2.5.2.1. $NH_3/NH_4^+$ in Aqueous Solutions

Ammonia, which due to its polarity, is highly soluble in water, exists in two forms in aqueous solutions. At low pH (below ~8.75), ammonia exists as ammonium ion ( $NH_4^+$ ) and as the pH rises above ~9.75, the ammonium ion converts to un-ionized form of dissolved ammonia ( $NH_3$ ). Thus, in CHO cell fermentation broth which is at pH 7.0, ammonia exists as ammonium ion. Total ammonia in an aqueous solution is the sum of ammonium ion and ammonia concentrations.



$$K_b = \frac{[NH_4^+][OH^-]}{[NH_3]} = 1.8 \times 10^{-5} \text{ at } 25^\circ\text{C} \quad (2.4)$$



$$K_a = \frac{[NH_3][H_3O^+]}{[NH_4^+]} = \frac{1 \times 10^{-14}}{1.8 \times 10^{-5}} = 5.6 \times 10^{-10} \quad (2.6)$$

As the above equations demonstrate, dissolved ammonia acts as a weak base and the ammonium ion acts as a weak acid in aqueous solutions. Since at the targeted pH of 7.0, about 99% of total concentration of ammonia/ammonium is present as  $NH_4^+$ , in aqueous solutions, the following equation describes the ammonia and ammonium equilibrium:

$$pH = pK + \log \frac{[NH_3]}{[NH_4^+]} \quad (2.7)$$

where pK has a value of 9.3 at 37°C (Schneider *et al.*, 1996).

#### 2.5.2.2. Toxic and Inhibitory Effects of Ammonia/Ammonium

High concentration of ammonium ion in the fermentation broth is toxic to CHO cells because ammonium ion rapidly diffuses into the cytoplasm of a cell through the cell membrane forcing ammonia out into the extracellular broth. This release of cellular ammonia causes cytoplasmic acidification and extracellular alkalization (Martinelle, 1996). Such a ammonium ion transport highway causes cell death – both through necrosis and through apoptosis. *Id.* Moreover, ammonium ion partially inhibits the TCA cycle shifting metabolic pathways (Lao and Toth, 1997). Ammonium ion also affects the quality of the protein product (Xing *et al.*, 2008; Harcum, 2006). For these reasons, one of the major design parameters of CHO cell bioreactor includes reducing ammonium ion formation and/or removing ammonium ion from the bioreactor.

Just like any other physiochemical parameter in a bioreactor, the toxic and inhibitory effects of ammonia/ammonium ion are highly cell line dependent (Schneider *et al.*, 1996). For example, Kurano *et al.* (1990b) reported a 50% reduction in the growth of CHO cells at 8 mM ammonium ion concentration. Hansen and Emborg's study in 1994 corroborated this observation when they reported that in continuous cultures like perfusion bioreactors, CHO cells

experience inhibition of growth at ammonium ion concentration above 8 mM. However, according to Xing *et al.* (2008), ammonium ion levels above 5.1 mM inhibit CHO cell growth and Takagi *et al.* (2001) reported even lower threshold of 4mM of ammonium ion concentration.

The extent of toxic and inhibitory effects of ammonium ion depends on whether the cells have had time to adapt to the new environment. For instance, Xie and Zhou (2006) reported substantial growth inhibition in CHO cells for 2 mM ammonium ion at inoculation. However, they did not observe growth inhibition for up to 12.5 mM ammonium ion concentration when the cells had extended time to adapt to the culture.

Mammalian cells produce ammonia in direct proportion to their glutamine consumption (Faraday *et al.*, 2001). At low glutamine concentrations, adding non-essential amino acids into the CHO cell media minimizes ammonia production (Xie and Wang, 1996; Chen and Harcum, 2005). Consequently, the strategies to reduce ammonium ion accumulation involve reducing glutamine concentration in the fermentation broth and lowering or eliminating the CHO cell demand for glutamine (Xie and Zhou, 2006; Zeng and Bi, 2006).

### 2.5.3. Carbon Dioxide

Carbon dioxide (CO<sub>2</sub>) concentration – both in the fermentation broth and in the headspace above the broth, is one of the most important parameters in a



mammalian cell bioreactor. For aerobic CHO cells, the process of CO<sub>2</sub> management in a bioreactor is complicated because CO<sub>2</sub> is a metabolic by-product and in small quantities, a nutrient when it is required for synthesis of pyrimidines, purines and fatty acids in animal cells (Ma *et al.*, 2006; Zeng and Bi, 2006). For example, during carboxylation of pyruvate to oxaloacetic acid, CO<sub>2</sub> acts as a nutrient even for aerobic cells (Gódia and Cairó, 2006). However, in large quantities, not only is CO<sub>2</sub> toxic, it also adversely affects the quality of the protein product (e.g. Gray *et al.*, 1996; Xing *et al.*, 2008). For this reason, bioengineers must design bioreactors with active CO<sub>2</sub> management systems.

#### 2.5.3.1. Sources and Effects of CO<sub>2</sub> in Fermentation Broth

In a bicarbonate buffered medium, there are three sources of CO<sub>2</sub> – the aerobic mammalian cells, bicarbonate buffer used to regulate pH and sparged CO<sub>2</sub>. At low cell densities, the buffer capacity of the media compensates for the variations in cell culture pH due to minor changes in concentrations of ammonia, CO<sub>2</sub> and lactic acid that the mammalian cells release into the medium. However, as VCD increases, these three metabolic by-products can significantly alter the pH of the medium easily overwhelming the buffer capacity of the CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>/CO<sub>3</sub><sup>2-</sup> buffer system. Addition of sodium bicarbonate can restore the buffer capacity and thus the pH of the medium. Unfortunately, accumulation characterizes HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> buffered media thereby affecting the CO<sub>2</sub> balance, increasing the pH to above the optimum level of 7.0 – 7.4 (Zanghi *et al.*, 1999; Neeleman, 2000). For this reason, it

is necessary to sparge CO<sub>2</sub> gas, which helps lower the pH. The sparged CO<sub>2</sub> adds to the complexity of the CO<sub>2</sub>/water system because of the specific reactions that CO<sub>2</sub> undergoes with water even though a small fraction (<1%) of dissolved CO<sub>2</sub> converts to carbonic acid (H<sub>2</sub>CO<sub>3</sub>).

CO<sub>2</sub>, a non-polar molecule that is 25 times more soluble in fermentation broth as compared to O<sub>2</sub>, readily diffuses across cell membranes and lowers the intracellular pH (Frick and Junker, 1999; Pattison *et al.*, 2000). Significantly, methods that serve to enhance O<sub>2</sub> transfer to the broth also enhance CO<sub>2</sub> transfer rate (Frick and Junker, 1999).

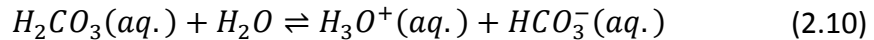
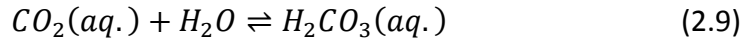
Higher intracellular CO<sub>2</sub> concentration affects the activity of intracellular enzymes and alters cellular metabolism (Nyberg, 1999). Mammalian cells produce relatively low quantities of protein (approximately several pg/cell). Protein quantity and quality is related to cell growth, maintenance and metabolism (Nyberg, 1999). If the concentration of CO<sub>2</sub> is higher in the media, then cells grow under stress. In such conditions, cells produce an undesirable quality of protein. Thus, during the cell growth phase, bioengineers take care to reduce the stress that cells experience.

Increasing the cell density will cause the dilution and perfusion rates to increase requiring active control of CO<sub>2</sub> and O<sub>2</sub> sparging regime. Increasing the CO<sub>2</sub> sparge rate will strip CO<sub>2</sub> from the cell culture broth causing it to drop below the ideal limit of 5% thus raising the pH. However, it is expected that increased VCD will also cause more CO<sub>2</sub> to be released into the broth and lower the pH. In fact, the

local CO<sub>2</sub> concentrations could become so high that CO<sub>2</sub> will become toxic to the cells requiring a higher agitation speed, which in turn must be balanced against the shear tolerance of the CHO cells.

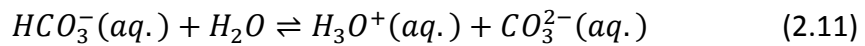
### 2.5.3.2. CO<sub>2</sub> Reactions

Concentration of different species of CO<sub>2</sub> in the aqueous phase vary as a function of pH and other factors. In the aqueous solution of a cell culture, following reactions involving CO<sub>2</sub> may take place:



At 37°C (Goudar *et al.* 2011)

$$K_1 = 10^{-6.30}$$



At 37°C (Goudar *et al.*, 2011)

$$K_2 = 10^{-10.23}$$

At low pH, [HCO<sub>3</sub><sup>-</sup>] dominates, while at high pH, [CO<sub>3</sub><sup>2-</sup>] dominates. [H<sub>2</sub>CO<sub>3</sub>] and [CO<sub>3</sub><sup>2-</sup>] concentrations are negligible as compared to [HCO<sub>3</sub><sup>-</sup>] and [CO<sub>2</sub>] concentrations because H<sub>2</sub>CO<sub>3</sub> almost instantly hydrolyses into HCO<sub>3</sub><sup>-</sup> and H<sub>3</sub>O<sup>+</sup>. Consequently, at the target pH of 7.0, reactions 2.9 and 2.10 predominate (Goudar *et al.*, 2011).

As these reactions demonstrate, sparging CO<sub>2</sub> will lower the pH of the cell culture. Additionally, maximum amount of CO<sub>2</sub> dissolved in water is a function of pH because the fraction of total dissolved CO<sub>2</sub> in aqueous solution changes as the pH of the solution changes. Thus, total CO<sub>2</sub> concentration affects the pH of the cell culture broth according to the following equations (Gray *et al.*, 1996; Ma *et al.*, 2006):

$$C_{CO_2} = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \quad (2.12)$$

Since at the target pH of 7.0, carbon dioxide is primarily present as [HCO<sub>3</sub><sup>-</sup>], equation (2.12) simplifies to:

$$C_{CO_2} = [HCO_3^-] + [CO_3^{2-}] \quad (2.13)$$

And according to Gray *et al.* (1996), accumulation of [HCO<sub>3</sub><sup>-</sup>] can be characterized as:

$$\frac{dC_{CO_2}}{dt} = (k_L a_{CO_2} + k_A A)(C_{CO_2}^* - C_{CO_2}) \quad (2.14)$$

Gray *et al.* derived a model to predict pCO<sub>2</sub> level in perfusion bioreactor. They predicted that the following equations describe the steady state dissolved CO<sub>2</sub> concentration in the perfusion cell culture broth, C<sub>CO<sub>2</sub></sub>:

$$C_{CO_2} = \frac{\gamma V(k_L a_{CO_2} \cdot C_{CO_2}^* + k_A A \cdot C_{CO_2}^* + sCPR \cdot X) + \gamma F_0 [C_{CO_2}^T]_F}{\gamma V(k_L a_{CO_2} + k_A A) + P + B} \quad (2.15)$$

where,

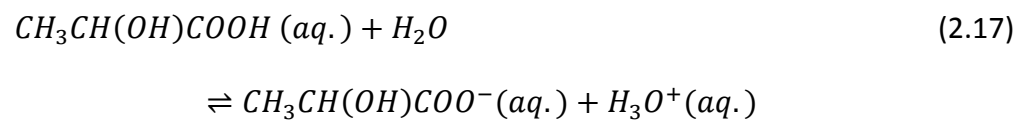
$$\gamma = \frac{10^{-pH}}{10^{-pH} + k_{a2}} \quad (2.16)$$

However, their equations are not applicable when either  $[HCO_3^-]$  or  $[CO_3^{2-}]$  is used to adjust cell broth pH. Consequently, equations (2.15) and (2.16) are not applicable to the model developed in this thesis. Relevant equations, that account for  $[HCO_3^-]$  or  $[CO_3^{2-}]$  addition, are presented in Section 3.2.3.

#### 2.5.4. Effect of Lactic Acid/Lactate in Fermentation Broth

CHO cells produce lactate as a result of their inefficient metabolism of glucose consumption (Faraday *et al.*, 2001; Hinterkörner *et al.*, 2007). As VCD increases, glucose consumption increases leading to production of large amounts of lactate (Gramer and Ogorzalek, 2007). High concentration of lactic acid in the cell fermentation broth inhibits CHO cell growth by decreasing specific Adenosine Triphosphate (ATP) production rate and ATP yield from glutamine (Takagi *et al.*, 2001; Hinterkorn *et al.*, 2007). Modeling lactic acid concentration in the mammalian cell fermentation broth is complicated by the fact that although lactate is an inhibitory metabolic product, under high stress i.e. in glucose limiting conditions, CHO cells switch over from glucose to using lactate as a carbon and energy source (Takagi *et al.*, 2001; Tsao *et al.*, 2005).

A weak acid, lactic acid partially hydrolyses into lactate and hydronium ions according to the following equation:



Or the above equation can be written as,



$$K_a = 1.38 \times 10^{-4} \quad (2.18)$$

$$pK_a = 3.86$$

Since the fermentation broth for the CHO cells is typically maintained at a pH ~7.0 at 37°C (Shuler and Kargi 1992), majority of lactic acid hydrolyses and is present in the fermentation broth as lactate. In a CHO cell culture, the specific rate of lactate production (sLPR) goes from 70 pg lactate cell<sup>-1</sup> hr<sup>-1</sup> at the beginning of the culture to about 20 pg lactate cell<sup>-1</sup> hr<sup>-1</sup> after 5 days of cultivation (Godoy-Silva *et al.*, 2009).

According to Gramer and Ogorzalek (2007), at the pH of approximately 7.0, 1 mol of lactic acid produced by the cells requires addition of 1 mol of base.

Assuming that lactic acid is the dominant metabolite contributing to pH change and that primarily pH is raised by base addition, then 1 mole of lactic acid that the cells produced will consume 1 mol of base, which must then leave the bioreactor in the form of CO<sub>2</sub> because in this model, bicarbonate is used as a base.

#### 2.5.5. Oxygen and Volumetric Mass Transfer Coefficient

Dissolved oxygen (DO) in the fermentation broth is a vital substrate for optimum growth of aerobic CHO cells. Oxygen is sparingly soluble in water and can easily become a growth limiting substrate as the VCD increases (Shuler and Kargi, 1992). Additionally, due to CO<sub>2</sub> and O<sub>2</sub> sparging regimes employed to maintain pH

and O<sub>2</sub> levels respectively, O<sub>2</sub> (and CO<sub>2</sub>) can be stripped from the fermentation broth due to sparging if the gas flow rate is too high.

Blanch and Clark, in their 1996 study observed that the solubility of O<sub>2</sub> in water can be correlated with temperature according to the following equation:

$$C_{O_2} = 14.16 - 0.394T + 0.007714T^2 - 0.000646T^3 \quad (2.19)$$

where  $C_{O_2}$  is in mg/L and T is in °C.

Jorjani and Ozturk (1999) quantitatively studied the effect of temperature on oxygen consumption rate (now referred to as oxygen uptake rate, OUR) on three different cell lines – baby hamster kidney (BHK), murine hybridoma and CHO. They reported 10% decrease in OUR for each 1% decrease in cell culture temperature. For this thesis, Specific Oxygen Uptake Rate,  $q_{O_2}$ , for CHO cells is taken to be  $1.99 \times 10^{-13}$  mol cell<sup>-1</sup> hr<sup>-1</sup> and the Respiratory Quotient is assumed to be 1.

In summary, the beneficial effects of low culture temperature on specific productivity depend on cell types and target proteins (Tang *et al.*, 2009) and the benefits are especially pronounced for high density perfusion cultures of mammalian cells (Zeng and Bi, 2006).

#### 2.5.6. Culture pH

Cell culture pH is arguably the most important parameter that affects cell growth in the bioreactors. Elaborate process analytical technology (PAT) systems and hardware are dedicated to controlling and maintaining broth pH. Mammalian

cells grow over a narrow pH range, typically  $\pm 0.1$  pH units, significant deviations from which profoundly impact cell growth, cell metabolism and protein biosynthesis (Ozturk and Palsson, 1991; Tang, 2009). This impact results in altered substrate and product formation rates and if not addressed, may cause cell damage.

Slightly acidic pH in a CHO cell culture reduces glucose consumption and increases lactic acid buildup (Ozturk and Palsson, 1991; Tsao *et al.*, 2005). Lactic acid is one of the contributors to localized pH deviation from steady state. Additionally, the region where base is added to raise pH also experiences a local spike in pH that causes cell damage (Langheinrich and Nienow, 1999). Another metabolic by-product,  $\text{CO}_2$  also causes pH deviations as mentioned in Section 2.5.3. For example, although the physiological range of partial pressure of  $\text{CO}_2$  in the fermentation broth,  $p\text{CO}_2$ , is 31 – 54 mmHg, typical range of  $p\text{CO}_2$  in a high-density bioreactor is 150 – 200 mmHg High  $p\text{CO}_2$  levels causing cell growth inhibition if the pH is not actively controlled (deZengotita *et al.*, 2002; Goudar *et al.*, 2006).

Similar to the buffer system in human blood, a  $\text{CO}_2$ -bicarbonate buffer system maintains pH in the CHO cell culture in a bioreactor. The Henderson-Hasselbalch equation relates the constituents of the  $\text{CO}_2$ -bicarbonate buffer system per equation (2.10):

$$pH = pK_a + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \quad (2.20)$$



$$pH = pK_a + \log \frac{[HCO_3^-]}{\mathcal{H}_{CO_2} * pCO_2} \quad (2.21)$$

where  $[HCO_3^-]$  is the bicarbonate concentration in the cell culture;

$[H_2CO_3]$  is the carbonic acid concentration in the cell culture;

$\mathcal{H}_{CO_2}$  is Henry's Law constant; and

$pCO_2$  is the partial pressure of  $CO_2$  in the cell culture broth. According to Zeng and

Bi, the following expression relates the equilibrium bicarbonate concentration, pH

and  $pCO_2$  at 37 °C:

$$\log[HCO_3^-] = pH + \log[pCO_2] - 7.543 \quad (2.22)$$

Assuming that the activity coefficients of the ions is approximately equal to 1 and the value of  $K_a$  is constant over around the temperature of 37 °C  $\pm 0.1$  °C , the following equation gives the buffer capacity,  $\beta$ , of the  $CO_2$ -bicarbonate buffer system:

$$\beta = \frac{[HCO_3^-]}{d(pH)} \quad (2.23)$$

$$\beta = 2.303 \left( [H^+] + \frac{[H_2CO_3]K_a[H^+]}{(K_a + [H^+])^2} + \left[ \frac{K_w}{[H^+]} \right] \right) \quad (2.24)$$

To control pH, two separate control loops are needed – one to raise pH and one to lower pH. Such a control loop must avoid excessive control impulses

otherwise the increase in CO<sub>2</sub> flow will increase cell culture osmolality, which impedes cell growth.

#### 2.5.7. Temperature

Temperature is a cell line specific critical variable in cell growth kinetics that affects product yield and product quality (Kumar *et al.*, 2007). Bioreactors cultivating the CHO cells traditionally tend to simulate the normal body temperature of a Chinese hamster of around 37°C (Kurano *et al.*, 1990a; Tang *et al.*, 2009).

CHO cells are sensitive to variations in temperature. If the bioreactor temperature rises above the optimal temperature range of a particular cell line, then the growth rate decreases and thermal death of the cells may occur leading to a net decrease in VCD (Shuler and Kargi, 1992). To guard against possible excursions from optimal range of temperature, bioprocess engineers use growth media fortified with d-glucose, d-galactose or d-mannose, which increase the survival of CHO cells at higher temperatures in a concentration-dependent and time-dependent manner (Henle *et al.*, 1984). Lower temperature, on the other hand, results in a more complex physiological response from the CHO cells.

Chuppa *et al.* (1997) reported that in a high-density perfusion cell culture, where oxygen may become limited, reducing the temperature allows the bioreactor to be operated at a lower perfusion rate and simplify pH control regime while

improving product quality. Similarly, while investigating the production of erythropoietin (EPO) by CHO cells, Ahn *et al.* (2008) observed that lowering the perfusion culture temperature to below 37°C increased cell viability for a longer period of time and resulted in higher cumulative EPO production. They cited high shear resistant characteristics of mammalian cells at low temperatures as one of the possible reasons for this behavior. Additionally, they reported that the quality of EPO as measured by glycosylation also improved at a lower temperature. *Id.* On the other hand, Chen *et al.* (2004) reported that lowering the temperature only had a marginal effect on glucose and lactate metabolism.

Variations in temperature of a cell culture must be minimized to obtain desirable culture performance. A perfusion bioreactor poses unique challenges in maintaining a constant temperature by virtue of its design and purpose. Perfusion cell cultures achieve high cell density ( $10^6$ - $10^7$  cells/mL), which may lead to very high local temperatures in the broth (Lara *et al.*, 2006). Agitation together with cooling jackets with feedback control loop easily solves this problem. A cell retention device that allows the removal spent medium and secreted product (if present), while retaining the cells in the bioreactor, characterizes a perfusion bioreactor. Consequently and if the cell separator device is outside the perfusion bioreactor, then the whole apparatus must be designed to maintain the cell culture at the same constant temperature that the cells experience inside the perfusion bioreactor. Although short term, transient exposures to high temperatures are not

deleterious for CHO cells a bioreactor must be operated within a well-defined range of cyclic temperatures that have no significant impact on cell productivity and growth rate (Drouin *et al.*, 2007; Lee *et al.*, 2008).

Varying solubility of various gases at different temperatures adds a new dimension to the analysis of cell growth kinetics. For example, with an increase in temperature from 30°C to 40°C, the solubility of CO<sub>2</sub> decreases by approximately 25% (Pattison *et al.*, 2000). Moreover, although concentrations of both CO<sub>2</sub> and NH<sub>3</sub> in fermentation broth must be regulated, since dissolution of CO<sub>2</sub> in aqueous ammonia is endothermic ( $\Delta h > 0$ ), with rising temperature, the solubility of CO<sub>2</sub> in ammonia solution also increases (Pazuki *et al.*, 2006). However, the model presented in this research does not consider the variations in solubility with variations in temperature for carbon dioxide and oxygen.

#### 2.5.8. Cell Growth Kinetics

Scientists have proposed many equations to model cell growth kinetics (e.g. Gomes and Menawat, 2000). The Monod equation is the most commonly used cell growth equation. This thesis uses Contois equation (Contois, 1959) to model CHO cell growth in perfusion bioreactors because the Contois cell growth model depends on the concentration of both the substrate and the cells with the cell growth being inhibited at high cell density. The Contois equation is used to quantify the growth kinetics of exponential and balanced growth of CHO cell culture:

$$\mu = \frac{\mu_m S}{K_S X + S} \quad (2.25)$$

where  $\mu_m$  is the maximum growth rate,  $S$  is substrate concentration and  $K_S$  is saturation constant (Shuler and Kargi, 1992). The saturation constant, which is proportional to the cell concentration, describes the substrate-limited growth at high cell densities. Thus, the Contois equation predicts that the specific growth rate decreases with decreasing substrate concentrations eventually becoming inversely proportional to the cell concentration in the medium. Substituting equation (2.2) into the cell growth equation (2.25) gives the rate of cell growth expression:

$$r_X = \frac{dX}{dt} = \mu X = \frac{\mu_m S}{K_S X + S} X \quad (2.26)$$

In a perfusion bioreactor, viable and total cell counts fluctuate appreciably during cultivation due to unknown causes and as such, the system is unlikely to be in a true steady state (Vits and Hu, 1992).

## 2.6. Tank Geometry and Hydrodynamics

Current work focuses on the high-density perfusion cultures of CHO cells wherein oxygen transfer is quite often the rate-limiting step. As mentioned in Section 2.5.6, solubility of oxygen is low in aqueous solutions and even more so in the presence of ionic salts. In such situations, increasing the productivity of mammalian cell cultures requires, in addition to prolific cell lines, a vigorous fermentation process that incorporates an efficient bioreactor that can achieve a uniform gas-liquid mass transfer coefficient,  $k_{L,a}$ .

A number of variables affect bioreactor performance (e.g. Kompala and Ozturk, 2006; Lara *et al.*, 2006). Many researchers have published reviews of bioreactor design for cultivating mammalian cells (e.g. Prokop and Rosenberg, 1989; Lübbert and Jørgensen, 2001). Maximizing  $k_La$  values for gas transfer and minimizing any “dead zones” – areas exhibiting poor local mass transfer, is one of the primary performance parameters for the mechanical construction and hydrodynamics inside the bioreactor. Many techniques have been proposed to minimize these “dead zones,” which are more pronounced in high-density perfusion cultures (Gogate and Pandit, 1999). These techniques include optimizing  $k_La$  by altering aspect ratio, tip speed, type, location and number of impellers, etc. Each of these techniques has its advantages and limitations and must function under its own set of constraints. Although a complete modeling of these parameters is outside the scope of this thesis, this chapter briefly explores the impact of these techniques on cell growth in a bioreactor.

### 2.6.1. $k_La$

In a perfusion reactor or continuous stirred tank reactor with cell recycle, the goal of the agitation scheme is to improve gas-liquid mass transfer coefficient,  $k_La$ . Oxygen is an important substrate for CHO cells. Since oxygen is sparingly soluble in water, it is often a growth limiting substrate in the bioreactors. Consequently, bioreactors are designed to ensure that the  $k_La$  is as high as possible.

Zeng and Bi (2006) describe static gassing out method and dynamic gassing out method of measuring oxygen uptake rate (OUR) and  $k_La$  for a particular bioreactor. In the static method, the cell culture is grown to a known cell density and then killed. Oxygen is removed from the headspace and aeration is turned on at the typical gas flow rate and the impeller is operated at a typical level. The increase in oxygen concentration is followed until oxygen saturation ( $C^*$ ) is reached. Change in dissolved oxygen concentration is given by

$$\ln\left(1 - \frac{C}{C^*}\right) = -k_La * t \quad (2.27)$$

where C is concentration of oxygen in solution

$C^*$  is equilibrium solubility of oxygen (oxygen saturation); and

t is time.

A plot of  $\ln(1-C/C^*)$  versus time gives a slope of  $-k_La$ . In Zeng and Bi's dynamic method, aeration to an active culture is briefly turned off and the unsteady state mass balance of oxygen is tracked while taking care that oxygen concentration does not drop so as to negatively impact the cell growth. This is so that rate of oxygen uptake is independent of oxygen concentration. In this method,

$$k_La = \frac{\ln\left(\frac{C_S - C_1}{C_S - C_2}\right)}{t_2 - t_1} \quad (2.28)$$

where  $C_S$  is the steady state dissolved oxygen concentration. Zeng and Bi (2006) describe that the OUR can be calculated from

$$OUR * X = k_L a * (C^* - C_S) \quad (2.29)$$

where X is cell density (cells/L)

OUR is oxygen uptake rate (g O<sub>2</sub>/10<sup>6</sup> cells-hr).

The k<sub>L</sub>a is characteristic of each cell line and bioreactor configuration and is a proprietary value and a closely guarded trade secret. In this thesis, only publicly available values are used which may not be typical of the bioreactor modeled.

### 2.6.2. Agitation

In 2005, Puthli *et al.* reported that under steady state conditions, k<sub>L</sub>a values increase from single impeller to dual impeller to triple impeller. Their studies revealed that the triple impeller configuration exhibited good uniform dispersion, good mass transfer rate and consumed least amount of power. Additionally, k<sub>L</sub>a values increase as impeller speed increases. Based on their experiments, Puthli *et al.* proposed the following correlations for k<sub>L</sub>a based on the number of impellers in a bioreactor:

Single impeller:

$$k_L a = 1.38 \times 10^{-4} \left( \frac{P_g}{V} \right)^{0.58} (V_g)^{0.43} \quad (2.30)$$

Dual impeller:

$$k_L a = 1.36 \times 10^{-4} \left( \frac{P_g}{V} \right)^{0.61} (V_g)^{0.43} \quad (2.31)$$

Triple impeller for simulated media that includes salts:



$$k_L a = 2.93 \times 10^{-6} \left( \frac{P_g}{V} \right)^{0.98} (V_g)^{0.53} \quad (2.32)$$

where  $P_g$  is power consumption in presence of gas (W)

$V_g$  is superficial gas velocity (m/s)

$V$  is volume of liquid ( $m^3$ )

Following are the design considerations:

1. Impeller tip speed is limited by amount of shear stress and rate of shear cells can handle.
2. As impeller speed increases, it increases frictional forces, shear stress and rate of shear. Thus, increase in impeller speed increases temperature of the broth.
3. The distance between any two impellers must be greater than their diameter otherwise the individual impellers will generate liquid streams which are inclined towards each other and combine halfway between the impellers acting as a impeller producing a radial outflow (Puthli *et al.*, 2005).

Although the Overall Volumetric Mass Transfer Coefficient ( $K_L a$ ) for carbon dioxide in a well-mixed bioreactor may be available in literature, such values are typically highly specific to the actual experimental conditions. For this reason, it may be best to calculate the  $K_L a$  values as suggested by Hill (2006):

$$K_L a = 33.9 + 7.96T^* + 15.7Q^* + 18.8RPM^* + 6.46Q^{*2} + 8.25T^*Q^* \quad (2.33)$$

where,

$$T^* = \frac{T - 27.5}{7.432} \quad (2.34)$$

$$Q^* = \frac{Q - 1.1}{0.5351} \quad (2.35)$$

$$RPM^* = \frac{RPM - 375}{133.8} \quad (2.36)$$

### 2.6.3. Bioreactor Parameters: Gas Hold-up, Bubbles and Sparging

Two kinds of hydrodynamic forces are constantly at work in a bioreactor – direct forces like agitator or bubble bursting causing cell lysis and indirect forces like microgravity and chronic exposure to energy dissipation rate (EDR), which is used to quantify local mixing performance in stirred tanks (Godoy-Silva *et al.*, 2009). CHO cell lines are sensitive to lower values of energy dissipation rate (EDR) (relative to the values needed for cell lysis in one exposure), if the exposure to such levels of EDR is chronic (Godoy-Silva *et al.*, 2009).

An important hydrodynamic design parameter of any bioreactor, fractional gas hold up,  $\epsilon$ , is defined as the ratio of the gas phase volume to total volume in a bioreactor (Arjunwadkar *et al.*, 1998b). Fractional gas hold up, together with mean bubble diameter, determines the gas-liquid interfacial area ( $a = \frac{6\epsilon}{d_B}$ ) (Arjunwadkar *et al.*, 1998a). Consequently, fractional gas hold-up determines the mass transfer coefficient,  $k_La$ . Arjunwadkar *et al.*, (1998a) have observed approximately 30% decrease in actual gas hold-up as compared to simple air-water system. They attribute this behavior to larger mean bubble diameter, which has higher rise velocity and correspondingly lower residence times as compared to bubbles with smaller diameters.

#### 2.6.4. Viscosity

As viable cell density increases, the rheology of fermentation broth, which is initially similar to water becomes viscous and non-Newtonian (Moilanen *et al.*, 2006). At a constant agitation rate, changes in viscosity do not significantly affect the metabolic activity of CHO cells (Moreira *et al.*, 1995). Increased viscosity, however, has a detrimental effect on the mass transfer coefficient,  $k_La$  (Puthli *et al.*, 2005). For example, in 1987, Schumpe and Deckwer reported that in aerobic fermentations, the oxygen transfer rates into viscous broths are low in all fermentor types.

Puthli *et al.* (2005) note that with increase in the viscosity, the resistance to the mass transfer increases. They reason that only turbulent eddies with sufficiently high energy can overcome the resistance of the viscous layer to cause bubble break-up or gas-solute transfer, resulting in an overall decrease in the  $k_L a$ , which is more than that expected on the basis of variation in fractional gas phase hold-up alone. According to Dahod (1993), actual  $\text{CO}_2$  dissolved in fermentation broth can far exceed its value calculated from the assumption of an equilibrium between the broth and the air leaving the fermentor. The departure from the equilibrium value increases as the broth viscosity increases.

## CHAPTER 3

### METHODOLOGY

A large number of factors affect cell growth in a high-density perfusion bioreactor. Although each individual cell in a bioreactor experiences a unique physiochemical environment, technology has enabled bioprocess engineers to collect data regarding various growth factors and predict cell behavior under given set of physiochemical conditions. This multivariate analysis is most suited for modeling cell growth, requires simultaneous solution to partial differential equations and differential algebraic equations (Xing *et al.*, 2008). In this thesis, the mathematical model considers the impact of various correlated variables.

*An a priori* mathematical description provides insight into the physical interactions and the nonlinearities involved in the variables that impact cell growth. Ideally, a mathematical model of a typical perfusion bioreactor containing trillions of cells for biopharmaceutical drug production, should contemplate every single reaction occurring within each one of those trillions of cells. Such a model would contain large number of model parameters – identification and estimation of which would be an arduous task. Apart from being unwieldy, such a model would be unappealing because results from such a complex mathematical model would distract from and fail to discriminate crucial parameters from the noise that affect the kinetics of cell death, growth, substrate consumption, and metabolite and

product formation. To be useful, a mathematical model must explain parameter expression and predict cell behavior in a straightforward manner.

### 3.1. Model Development

Primary challenge in modeling bioprocesses is discriminating between biologically significant and trivial information because it is extremely difficult to pinpoint the exact cause of perturbations that have a measurable impact on biologically significant parameters. For this reason, a typical mathematical model describing a typical bioprocess focuses only on select processes while entirely ignoring the impact or consequence of other processes. Chapter 2 describes the interaction of some of these parameters with  $\text{CO}_2$  however, it does not present an exhaustive list of these parameters. Following flow diagram describes steps to take to calculate pH.

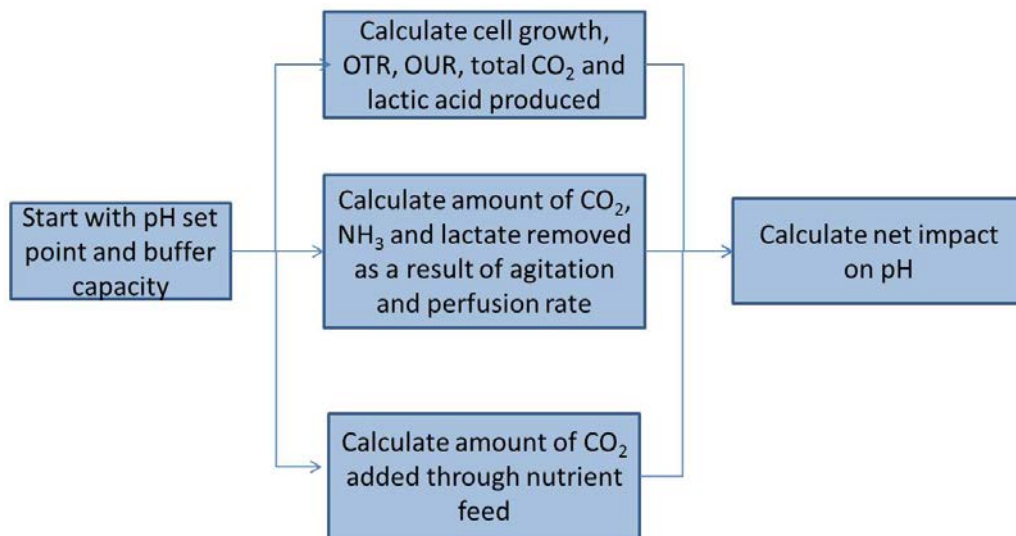


Figure 3: Steps to take to calculate pH

### 3.1.1. Design Considerations and Modeling Approach

The mathematical model developed in this thesis is based on recently published research involving CHO cell cultures in a perfusion bioreactor. Since bioreactor modeling involves a substantial number of parameters, published articles discuss only a specific cell behavior. This thesis brings all of that research together as a black box model, which takes inputs like cell density, growth rate, specific glucose uptake rate, etc. and provides an output of growth profile, product concentration profile, and acid/base addition profile to maintain bulk pH within a given range. The current thesis project does not involve any laboratory experiments.

### 3.1.2. Model Objective

In a CHO cell culture, the model seeks to maintain steady state CO<sub>2</sub> and pH profiles as a function of time as described in the picture below:

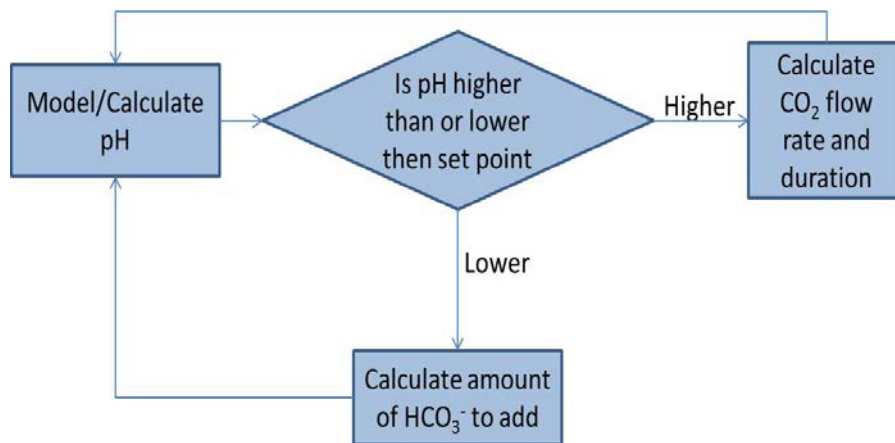


Figure 4: A Basic Modeling Approach

### 3.1.3. Modeling Inputs and Outputs

Modeling inputs include initial concentrations of  $[\text{NH}_3]$ ,  $[\text{CO}_2]$ , [lactic acid] and  $[\text{O}_2]$ , CER, OUR,  $\text{CO}_2$  and  $\text{O}_2$  sparge rates, perfusion and feed rates, feed composition, power input and cell growth. Additionally, a complete understanding of all the specific rates, rate constants etc. is needed. In practice, such complete information about a bioprocess is never available necessitating a need for simplifying assumptions (Van Impe *et al.*, 1995). The figure below depicts the input and output parameters.

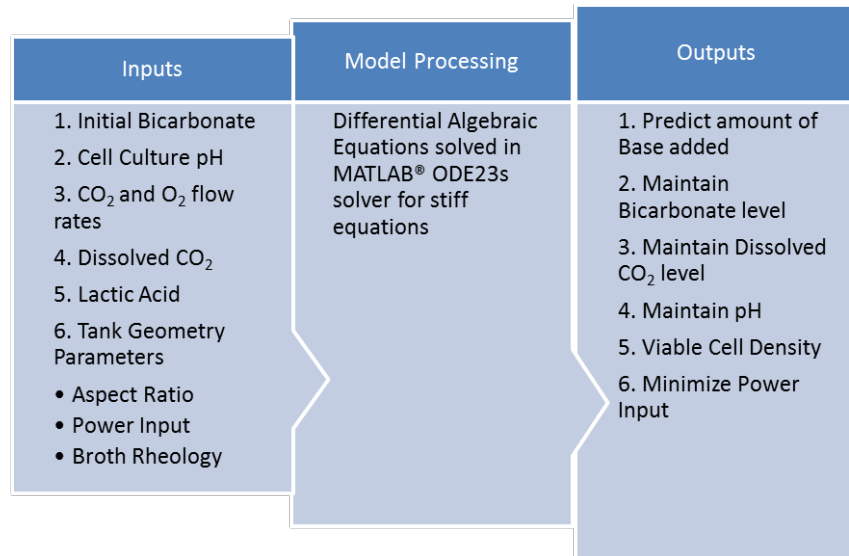


Figure 5: Primary Input and Output Parameters for Carbon Dioxide and pH Profiles

Inputs and outputs can be categorized into two groups: cell kinetics parameters like cell growth rate and tank geometry/ physical bioprocess parameters like aspect ratio, impeller configuration. Both types of parameters are equally important in fully describing dynamics inside the bioreactor. Since this thesis focuses on cell



kinetics parameters, a few simplifying assumptions make the modeling more manageable.

#### 3.1.4. *Simplifying Assumptions*

A rigorous first principles model must be computationally efficient to be practical. As the parameters in a model are varied, the qualitative nature of an equation and of its numerical solution can change (Beers, 2007). The goal of the assumptions is to simplify the field by partial differential equations of mass balances into simultaneous differential algebraic equations.

Following assumptions were made in deriving the mathematical model:

1. Bioreactor is completely homogenous, ideally well mixed so that the species concentration and temperature are uniform throughout the bioreactor. Thus, tank geometry, like aspect ratio, power input are not considered in the model.
2. All CHO cells are fully-grown cells of the same size. This assumption is made because big cell diameter and the low surface to volume ratio are limiting factors of the internal mass transfer and uptake rate of nutrients thus influencing cell growth and metabolic rates (Leist *et al.*, 1990).
3. CHO cell division does not result in random genetic mutations that have any material impact on their predictable behavior in the cell culture.

4. CHO cells do not exhibit shifting metabolic pathways in the middle of the growth cycle, which results in diauxic growth (Shulder and Kargi, 1992).
5. As part of pH control mechanism, the dissociations of  $\text{NH}_3$  and  $\text{H}_2\text{O}$ , the first and second dissociation of  $\text{CO}_2$ , and the formation of carbonate ion are the most important chemical reactions in the  $\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}/\text{H}_2\text{O}$  and  $\text{NH}_3/\text{NH}_4^+/\text{H}_2\text{O}$  systems (Pazuki *et al.*, 2006).

### 3.2. Material Balance in a Perfusion Bioreactor

For a perfusion cell culture (or a continuous culture with cell recycle or a chemostat with cell recycle), material balance is performed on three systems: the bioreactor, the recycle system and both the bioreactor and the recycling system combined.

#### 3.2.1. Biomass and Substrate Balance Using Contois Equation

Material balance on biomass in a bioreactor with cell recycle gives:

$$\begin{array}{cccc} \text{Rate of Mass} & - & \text{Rate of Mass} & + & \text{Rate of Mass} & = & \text{Rate of Mass} \\ \text{In} & & \text{Out} & & \text{Generation} & & \text{Accumulation} \end{array}$$

Based on this principle, Fenge and Lüllau (2006) have proposed the following equations for mass balance for an ideal perfusion bioreactor, which assumes no cell death, no cell lysis, no accumulation of product and no product degradation. From equation (2.26),

$$\frac{dX}{dt} = \mu X = \frac{\mu_m S}{K_S X + S} X \quad (2.26)$$

where S represents substrate like glucose and glutamine and other symbols have the same meaning as described in Section 2.5.8. The Contois equation has saturation constant proportional to cell concentration that describes substrate-limited growth at high cell densities (Shuler and Kargi, 1992). According to this equation, the specific growth rate decreases with decreasing substrate concentrations and eventually becomes inversely proportional to the cell concentration in the cell culture. *Id.*

Fenge and Lüllau (2006) have proposed the following equation for substrate consumption:

$$\frac{dS}{dt} = -q_S X + D(S_0 - S) \quad (3.1)$$

$$D = \frac{F_0}{V} \quad (3.2)$$

And the following equation provides product formation:

$$\frac{dP}{dt} = q_P X - DP \quad (3.3)$$

where, D is medium exchange rate (L/hr)

F is feed flow rate into the bioreactor (L/hr)

P is product concentration (mol/L)

S<sub>0</sub> substrate concentration in the feed (mol/L)

S is substrate concentration in the bioreactor

$q_s$  is specific substrate consumption rate (mmol/cell/hr)

$q_p$  is specific product production rate (mmol/cell/hr)

### 3.2.3. Mass Balance

Material balance of carbon involves balancing inorganic carbon that exists in the bioreactor in the form of hydrated and gaseous carbon dioxide. In 1996, Gray *et al.* modeled the CO<sub>2</sub> concentration in a perfusion bioreactor. Their equations are presented in Chapter 2. However, Zupke and Green (1998) presented a more intuitive model that takes into account the headspace gas composition, sparging, surface and bubble mass transfer and generation by mammalian cells.

Zupke and Green suggested the following equation for headspace gas composition in which the first parenthesis represents gas flow into the headspace, the second parenthesis represents gas flow out of the headspace and the third term represents surface mass transfer.

For CO<sub>2</sub> in headspace gas,

$$\begin{aligned} \frac{dx_{CO_2}}{dt} = & \frac{1}{V_h} (x_{CO_2}^{overlay} Q^{overlay} + x_{CO_2}^{sparge} \langle Q \rangle) \\ & - \frac{x_{CO_2}}{V_h} (Q^{overlay} + x_{CO_2}^{sparge} \langle Q \rangle) \\ & - \frac{RTV_L k_{CO_2}^{surface}}{P_T V_h} \left( \frac{x_{CO_2} P_T}{\mathcal{H}_{CO_2}} - C_{CO_2} \right) \end{aligned} \quad (3.20)$$

For O<sub>2</sub> in headspace gas,

$$\begin{aligned} \frac{dx_{O_2}}{dt} = & \frac{1}{V_h} (x_{O_2}^{overlay} Q^{overlay} + x_{O_2}^{sparge} \langle Q \rangle) \\ & - \frac{x_{O_2}}{V_h} (Q^{overlay} + x_{O_2}^{sparge} \langle Q \rangle) \\ & - \frac{RTV_L k_{O_2}^{surface}}{P_T V_h} \left( \frac{x_{O_2} P_T}{\mathcal{H}_{O_2}} - C_{O_2} \right) \end{aligned} \quad (3.21)$$

where the following equation gives the value of  $\langle Q \rangle$  in which Zupke and Green

(1998) further described the gas sparging requirements:

$$\langle Q \rangle = Q_{max} \frac{q_{O_2} X_V - k_{O_2}^{surface} ([O_2]_{eq}^{surface} - [O_2])}{k_{O_2}^{sparge} ([O_2]_{eq}^{sparge} - [O_2])} \quad (3.22)$$

According to Zupke and Green (1998), the following equation gives the liquid phase CO<sub>2</sub> composition wherein the first parenthesis gives the surface mass transfer, the second term accounts for the bubble mass transfer and the final term, CPR, represents CO<sub>2</sub> generation by cells.

$$\begin{aligned} \frac{dC_{CO_2}^T}{dt} = & k_{CO_2}^{surface} \left( x_{CO_2} \frac{P_T}{\mathcal{H}_{CO_2}} - C_{CO_2} \right) \\ & + k_{CO_2}^{sparge} (C_{CO_2}^* - C_{CO_2})_{LM} + CPR \end{aligned} \quad (3.23)$$

Where,

$$\mathcal{H}_{CO_2} = 35 \frac{L \cdot atm}{mol} \quad (3.24)$$

When CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> are at equilibrium, then

$$C_{CO_2} = \frac{C_{CO_2}^T}{1 + \frac{k_{eq}}{10^{-pH}}} \quad (3.25)$$

where  $k_{eq}$  is the combined equilibrium constant of equations (2.10) and (2.11).

Zupke and Green (1998) also presented an implicit solution for bubble composition for CO<sub>2</sub> and O<sub>2</sub> gases sparged into the bioreactor broth as needed to maintain pH:

$$C_{CO_2}^* = \frac{x_{CO_2}^{sparge} P_T}{\mathcal{H}_{CO_2}} \quad (3.26)$$

$$C_{CO_2,0}^* = \frac{x_{CO_2,0}^{sparge} P_B}{\mathcal{H}_{CO_2}} \quad (3.27)$$

$$C_{O_2}^* = \frac{x_{O_2}^{sparge} P_T}{\mathcal{H}_{O_2}} \quad (3.28)$$

$$C_{O_2,0}^* = \frac{x_{O_2,0}^{sparge} P_B}{\mathcal{H}_{O_2}} \quad (3.29)$$

$$x_{CO_2}^{sparge} = \frac{\frac{x_{CO_2,0}^{sparge} P_B Q}{RT} + r_{CO_2}}{\frac{P_B Q}{RT} + r_{CO_2} + r_{O_2}} \quad (3.30)$$

$$x_{O_2}^{sparge} = \frac{\frac{x_{O_2,0}^{sparge} P_B Q}{RT} + r_{O_2}}{\frac{P_B Q}{RT} + r_{CO_2} + r_{O_2}} \quad (3.31)$$

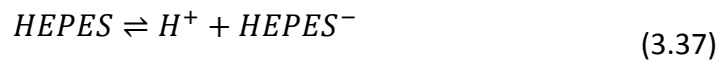
$$r_{CO_2} = V_L k_{CO_2}^{sparge} \frac{C_{CO_2}^* - C_{CO_2,0}^*}{\ln\left(\frac{C_{CO_2}^* - C_{CO_2,0}^*}{C_{CO_2} - C_{CO_2,0}^*}\right)} \quad (3.32)$$

$$r_{O_2} = V_L k_{O_2}^{sparge} \frac{C_{O_2}^* - C_{O_2,0}^*}{\ln \left( \frac{C_{O_2}^* - C_{O_2,0}^*}{C_{O_2} - C_{O_2}^*} \right)} \quad (3.33)$$

where r represents rates of transfer of respective species in mol/hr. Finally, the CO<sub>2</sub> gas (to lower pH) or base (to raise the pH) requirements can be calculated from the following equilibrium equation:

$$\begin{aligned} \Delta[H^+]^2 + \Delta[H^+](\text{acid produced}) + [HCO_3^-]_0 + [H^+] \\ + K_{eq}) + [HCO_3^-]_0(\text{acid produced}) \\ - K_{eq}CPR = 0 \end{aligned} \quad (3.34)$$

Here the quantity “acid produced” requires elaboration. Following equations contain the species that have a material direct or indirect impact on the cell culture pH broth:







To calculate the instantaneous pH, the concentration of each of these species must be known at that instant. At any given time, their concentration is impacted by

1. Feed coming into the bioreactor
2. Feed leaving the bioreactor and into the cell separator
3. Feed exiting the system product is removed from the cell separator
4. Feed leaving the cell separator and into the bioreactor
5. CO<sub>2</sub> and O<sub>2</sub> sparged into the bioreactor
6. Base feed added to raise pH

Equation (2.12) provides the mass balance for carbon dioxide species in the cell broth. To calculate the pH of the cell culture broth, mass and charge balance for all the species in equations (3.37) through (3.40) must also be calculated which requires solving simultaneous partial differential kinetic equations. Such an analysis is outside the scope of this research project. Consequently, the term “acid produced” in equation (3.34) is not calculated in this thesis.

### 3.3. Solution Method

In developing an unstructured mathematical model for this thesis, following steps were taken:

1. Describe cell growth in a perfusion bioreactor using Contois equation – described in Section 3.2.1.
2. Describe steady state – described in Section 3.2.2.
3. Write unstructured material balance equations for CO<sub>2</sub> – described in Section 3.2.3.
4. Model change in CO<sub>2</sub> and pH profiles as a function of cell growth and time.

#### 3.3.1. Key Parameter Limits

All the constraints, constants, linear and nonlinear inequalities and linear and nonlinear equations must be identified before simulation can begin. The following equations (from Xing *et al.*, 2008) provide some of the constraints and limits that apply to a CHO cell culture:

$$6.95 \leq pH \leq 7.05 \quad (3.41)$$

$$[AMM] < 5.1 \text{ mM} \quad (3.42)$$

$$35 < C_{CO_2}^T < 111 \text{ mmHg} \quad (3.43)$$

$$[LAC] < 58 \text{ mM} \quad (3.44)$$

$$\text{Osmolality} < 382 \frac{\text{mOsm}}{\text{kg}} \quad (3.45)$$

$$\text{Osmolality} \left( \frac{\text{mOsm}}{\text{kg}} \right) = 0.31 C_{CO_2}^T (\text{mmHg}) + 122 \quad (3.46)$$

According to Goudar et al. (2011), following are the specific carbon dioxide production and specific oxygen uptake rates:

$$sCPR = 6.25 \frac{\text{pmol}}{\text{cell. day}} \quad (3.47)$$

$$sOUR = 5.53 \frac{\text{pmol}}{\text{cell. day}} \quad (3.48)$$

These rates will be multiplied by instantaneous cell count to get CPR and OUR for that instant.

For IMDM medium, which is considered in this thesis the following equations provide the limits of substrate present (Burgener and Butler, 2006):

$$K_{I[AMM]} = 15 \text{ mM} \quad (3.49)$$

$$K_{I[LAC]} = 90 \text{ mM} \quad (3.50)$$

Assume (3.51)

$$[AMM]_0 = 0$$

Assume (3.52)

$$[LAC]_0 = 0$$

$$[GLC]_0 = 25 \text{ mM} \quad (3.53)$$

$$[GLN]_0 = 4 \text{ mM} \quad (3.54)$$

$$[HCO_3^-]_0 = 36 \text{ mM} \quad (3.55)$$

$$[HEPES]_0 = 25 \text{ mM} \quad (3.56)$$

$$pK_{a,HEPES} = 7.3 \quad (3.57)$$

### 3.3.2. Solving the Equations in MATLAB®

The model equations presented in this thesis are stiff. Stiffness arises when the control functions vary unevenly with time. In these systems, small and large time constants occur in the same system – small time constant controls the earlier response, whereas the large constant controls the tailing response (Rice and Do, 1995). This model contains equations with three different time scales corresponding to three different reaction rates of the equations modeled in this thesis:

1. For some equations, which are primarily related to pH, e.g. water, base or  $\text{H}_2\text{CO}_3$  ionization, reaction rates are very high – on the order of nano- or pico-seconds.
2. Some equations, e.g.  $\text{CO}_2$  or  $\text{O}_2$  hydration, the reaction rates are slower, on the order of seconds.
3. Cell growth equations are on the order of days.

This presence of fast changing and slow changing components in simultaneous equations render some numerical methods unstable leading to the requirement of very small step sizes to obtain numerically stable solutions.

The model developed in this thesis is solved using MATLAB® computing language (MathWorks, Inc., Natick, MA). The equations to be solved are contained in various .m files that the user accesses via a graphical user interface (GUI), which

is developed and programmed for easy manipulation of the parameters. Appendix 1 contains the code from various .m files.

This mathematical model contains differential algebraic equations, which can be solved using MATLAB® stiff solvers ODE15s, ODE23, ODE23s and ODE45. ODE15s is a multistep variable order solver while ODE23s is a one-step solver based on Rosenbrock formula of order 2, which is better suited at crude tolerances. They both dynamically vary the step size based on local error and mathematically generate Jacobians. MATLAB® solvers ODE45 and ODE23 solve the stiff equations by employing variable step Runge-Kutta integration methods. ODE23 uses 2<sup>nd</sup> and 3<sup>rd</sup> order pair of formulas for medium accuracy and ODE45 uses a 4<sup>th</sup> and 5<sup>th</sup> order Dormand-Prince pair for higher accuracy.

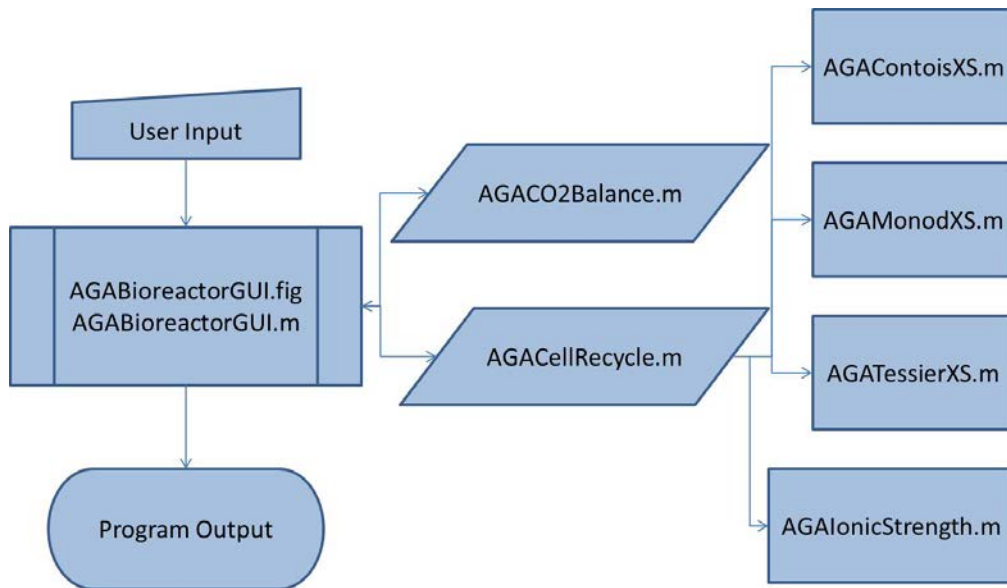


Figure 6: Software Flowchart

The computer code presented in Appendix 1 demonstrates how the code could be written to solve these equations. However, mathematical modeling and not the computer programming was the focus of the current thesis. The mathematical model presented in this thesis can be properly solved by employing the full power of MATLAB® especially the Partial Differential Equation Toolbox® and the Optimization Toolbox®. Per MATLAB®, when using the Optimization Toolbox®, the following steps must be taken:

1. Separate bounds, linear equalities, linear inequalities, nonlinear equalities, and nonlinear inequalities
2. Combine all variables into one vector ( $x$ )
3. Write vectors for lower and upper bounds ( $lb$ ,  $ub$ )
4. Write matrix and vector of inequalities ( $A$ ,  $b$ )
5. Write matrix and vector of equalities ( $A_{eq}$ ,  $beq$ )
6. Write nonlinear constraint function
7. Write the objective function or vector,  $f$
8. Call the solver

As mentioned in section 3.2.3., the term “acid produced” in equation 3.34 is not calculated in this thesis because calculation of pH involves PID control strategies and computer programming complexities that are outside the scope of this thesis. Additionally, any simulation that is run must be verified against experimental data,

which is not available for this thesis. For this reason, a demonstrative MATLAB® code is written for the purposes of this thesis.



## CHAPTER 4

### FINDINGS

Researchers have reported a large number of factors that affect cell growth, metabolism, product and toxin formation, and cell death. Chapter 2 discusses some of these factors. However, optimal growth of the cells in a bioreactor may or may not involve intricate interactions amongst all these factors. A robust mathematical model must take care to not overly define a bioreactor problem. For example, although both lactate and ammonium adversely affect cell growth, for most cell cultures, lactate and ammonium cannot be the dominant factors determining growth rate (Zeng and Bi, 2006).

In line with the first hypothesis stated in Section 1.4, the Contois equation adequately describes the cell growth in the perfusion bioreactor. As the second hypothesis predicted, maintaining  $\text{CO}_2$  concentration and pH at a steady state does require dynamic  $\text{CO}_2$  material balance and dynamic control of pH. As discussed in Chapter 3, determining instantaneous  $\text{CO}_2$  concentration and pH requires experimentation that is not part of current research even though the equations can be adequately solved using MATLAB®.

#### 4.1. Simulation in MATLAB®

To solve the mathematical equations, the following graphical user interface (GUI) was developed using MATLAB® GUIDE®:

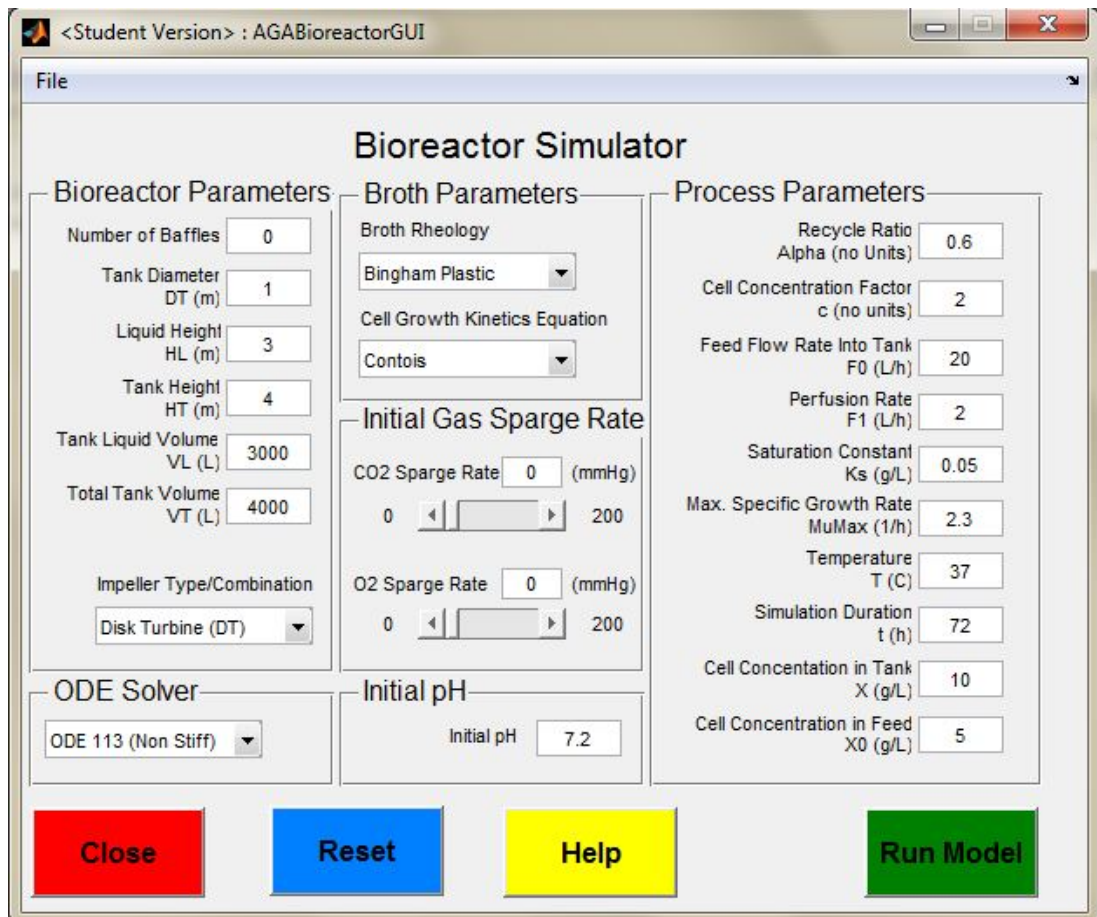


Figure 7: GUI Interface

The GUI then calls a set of .m files, which in turn call a subset of .m files. This structure ensures maximum possible clarity in the computer code. The code is presented in Appendix 1 in its entirety.

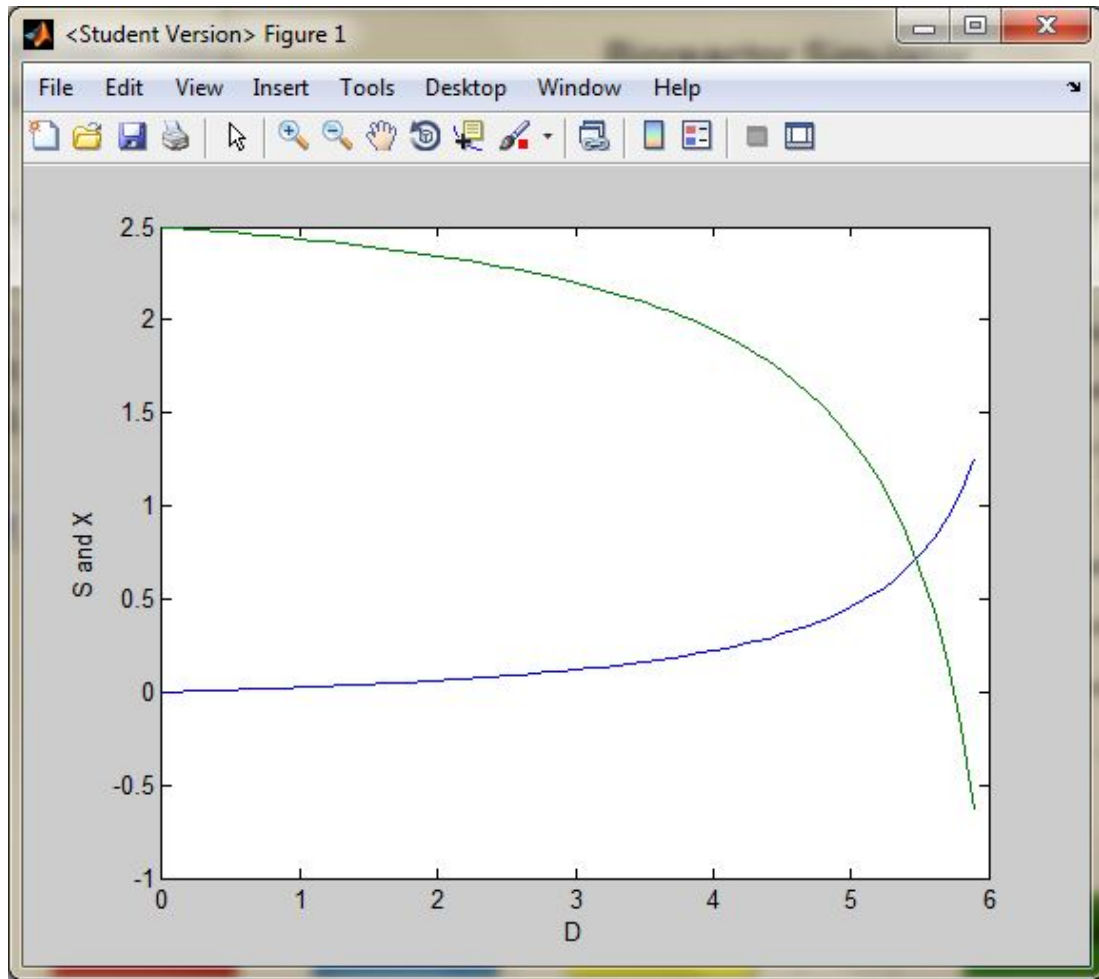


Figure 8: Substrate,  $S$ , and Cell Concentration,  $X$ , as a function of Dilution Rate,  $D$

The computer program uses a set of simulated data to generate the graph in Figure 5 that presents the depletion in substrate level as the cell population increases with dilution rate in a perfusion bioreactor. Since calculation of pH involves PID control strategies and complex computer programming, “acid produced” term in equation (3.34) was not calculated. Deschênes *et al.* (2006) have developed guidelines for controller parameter tuning through linear approximation

of the closed loop dynamics. The code presented in this research is representative of how the mathematical model that is presented in this thesis can be solved.

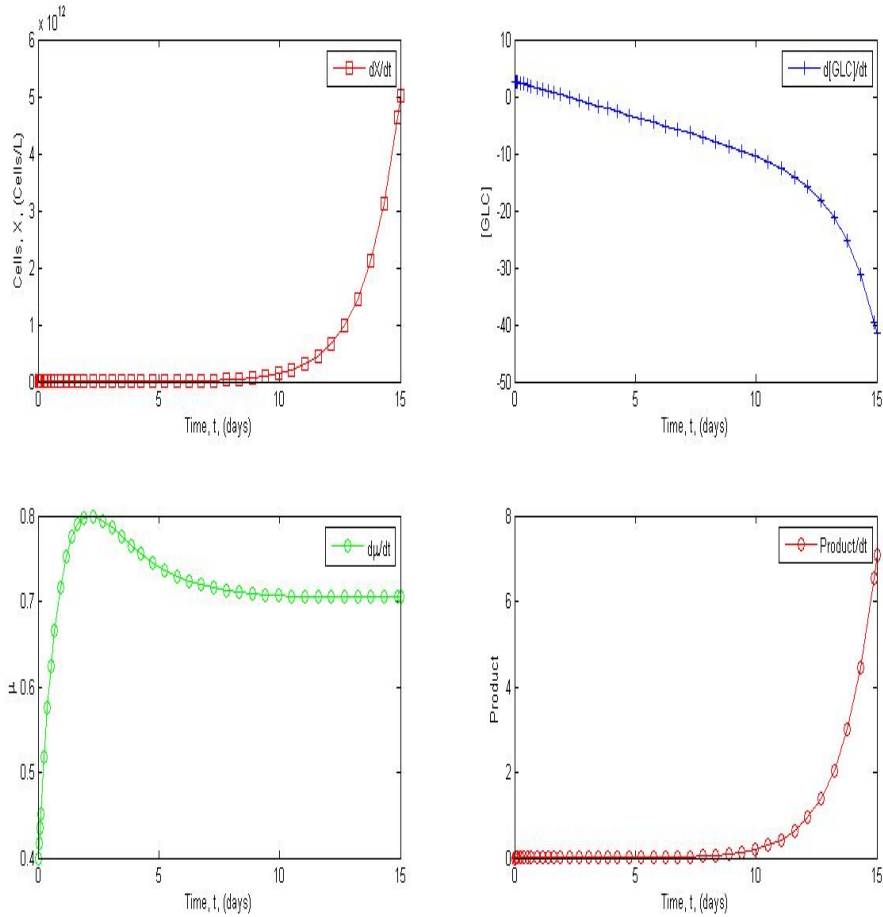


Figure 9: Graphs showing simulated prediction of response of cell growth, glucose consumption, growth rate, and product formation curve in a perfusion bioreactor.

The model generating the graphs in Figure 9, demonstrate that as expected cell growth and product formation increases with time, substrate consumption increases resulting in decrease in substrate concentration. The cell growth rate is

stabilized around day 11. Similar to Deschênes *et al.* (2006), for simplicity, cell death parameter is ignored, this multivariable approach demonstrates that the cell death parameter cannot be completely disregarded. Equations from Fenge and Lüllau (2006) were used to generate the graphs in Figure 9. The code used to generate these graphs is presented in Appendix 1, which uses parameters from both Deschênes *et al.* (2006) and Fenge and Lüllau (2006).

## CHAPTER 5

### CONCLUSION

The goal of a mathematical model is to understand cell growth in a bioreactor so that the protein-drug production from the cells could be maximized. Of course, the resulting efficiency in drug production has a direct and positive impact on its bottom-line and competitiveness – especially if competing against potential biosimilar manufacturer. A mathematical and computer model that is applicable across cell lines and to different types of bioreactors would be highly sought after in the biopharmaceutical industry because it will simulate drug-manufacturing process with limited need for expensive experiments because such a model must still be validated against experimental data. However, the need and cost of those experiments would be greatly reduced due to this model.

#### 5.1. Future direction

Further additions to mathematical and computer model of cell growth in a bioreactor might include using equations of state (e.g. NRTL, SAFT, PC-SAFT, UNIFAC or UNIQUAC) to model solubility of inorganic salts and amino acids in a background medium that more closely mimics the actual fermentation medium in the bioreactor. Those models might also use Kirkwood equation  $\left(\ln \frac{S_p}{S_0}\right) = K_i I - K_s I$  (Harrison *et al.*, 2003) to model solubility of protein in the broth and also

incorporate broth characteristics like viscosity (Lapasin *et al.*, 1996) and tank geometry, hydrodynamics and bubble dynamics.

As can be seen from the foregoing thesis, these are just a few factors that can be considered in this type of a mathematical and computer model. A mathematical model that considers more of these parameters is correspondingly more accurate in predicting actual cell behavior under actual process conditions.

From a computer-programming standpoint, such a model would be highly complex especially since that model must be applicable across cell lines so that it appeals to a majority biotechnology companies. Additionally, such mathematical models can employ neural network and artificial intelligence programming that “learn” from past runs (Karim *et al.*, 1997; Acuña *et al.*, 1998; Nagy, 2007). Neural networks can be “trained” to anticipate parameter changes needed for scaling the model – further reducing the costs associated with funding pilot scale laboratories. Such programming would greatly reduce the need for expensive experiments.

## 5.2. Continuation of the Current Work

One of the major challenges of this research has been limiting the scope of the model to make it more manageable. Some equations are available in published literature and some can be derived from laboratory scale experiments. However, incorporating those equations in a computer program requires intimate programming knowledge, which was not the focus of this research project.

Focus of this thesis was to demonstrate how to develop a mathematical model that biopharmaceutical companies can use for maintaining CO<sub>2</sub> levels and a steady state pH in a mammalian cell culture perfusion bioreactor. This thesis analyzes many of the parameters that can have a significant impact on such cell cultures. Mathematical and computer model developed in this thesis is based on the published equations and published laboratory research. Although this model has generated expected results, they must be verified against data from actual laboratory assays. As mentioned in earlier, programming charge and mass balance equations for all the ions present in the cell culture medium is outside the scope of this research. Similarly, the impact of ionic strength must be taken into account as discussed in Nagy (2007) and Goudar *et al.* (2011). For a more complete model that predicts pH and models how perturbations in one of the parameters affects values of other parameters as a function of time, such programming is essential.

Future work that concentrates on these aspects of the model will certainly produce a mathematical and computer model that would be highly sought after in the biopharmaceutical industry because such a model would further reduce costs and advance the understanding of mammalian cell cultures.



## APPENDIX 1

This Appendix contains the MATLAB® Code used to solve the equations developed in this thesis. The mathematical equations are run via graphical user interface (GUI) developed using MathWorks GUIDE tool.

Following is the code for the GUI:

```
function varargout = AGABioreactorGUI(varargin)
% AGABIOREACTORGUI M-file for AGABioreactorGUI.fig
%   AGABIOREACTORGUI, by itself, creates a new AGABIOREACTORGUI or raises
the existing
%   singleton*.
%
%   H = AGABIOREACTORGUI returns the handle to a new AGABIOREACTORGUI or
the handle to
%   the existing singleton*.
%
%   AGABIOREACTORGUI('CALLBACK',hObject,eventData,handles,...) calls the local
%   function named CALLBACK in AGABIOREACTORGUI.M with the given input
arguments.
%
%   AGABIOREACTORGUI('Property','Value',...) creates a new AGABIOREACTORGUI
or raises the
%   existing singleton*. Starting from the left, property value pairs are
%   applied to the GUI before AGABioreactorGUI_OpeningFunction gets called. An
%   unrecognized property name or invalid value makes property application
%   stop. All inputs are passed to AGABioreactorGUI_OpeningFcn via varargin.
%
%   *See GUI Options on GUIDE's Tools menu. Choose "GUI allows only one
%   instance to run (singleton)".
%
% See also: GUIDE, GUIDATA, GUIHANDLES

% Edit the above text to modify the response to help AGABioreactorGUI

% Last Modified by GUIDE v2.5 09-Jun-2011 13:12:11

% Begin initialization code - DO NOT EDIT
```

```

gui_Singleton = 0;
gui_State = struct('gui_Name',    mfilename, ...
                  'gui_Singleton', gui_Singleton, ...
                  'gui_OpeningFcn', @AGABioreactorGUI_OpeningFcn, ...
                  'gui_OutputFcn', @AGABioreactorGUI_OutputFcn, ...
                  'gui_LayoutFcn', [], ...
                  'gui_Callback', []);
if nargin && ischar(varargin{1})
    gui_State.gui_Callback = str2func(varargin{1});
end

if nargout
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end
% End initialization code - DO NOT EDIT

% --- Executes just before AGABioreactorGUI is made visible.
function AGABioreactorGUI_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to AGABioreactorGUI (see VARARGIN)

% Choose default command line output for AGABioreactorGUI
handles.output = hObject;

% Update handles structure
guidata(hObject, handles);

% UIWAIT makes AGABioreactorGUI wait for user response (see UIRESUME)
% uiwait(handles.BioreactorGUI);

% --- Outputs from this function are returned to the command line.
function varargout = AGABioreactorGUI_OutputFcn(hObject, eventdata, handles)
% varargout  cell array for returning output args (see VARARGOUT);
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```

```

% Get default command line output from handles structure
varargout{1} = handles.output;

%%
=====
====
% Bioreactor Parameters
%
=====
====
%% Number of Baffles
function txtBaffles_Callback(hObject, eventdata, handles)
% hObject   handle to txtBaffles (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtBaffles as text
%        str2double(get(hObject,'String')) returns contents of txtBaffles as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errorDlg('Enter a numerical value for number of baffles in the bioreactor.','Error');
    set(handles.txtBaffles,'Value',0);
end
if val <= 0 || val >= 5
    errorDlg('Number of baffles must be between 0 and 5','Error');
    set(handles.txtBaffles,'Value',0);
end
handles.txtBaffles = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtBaffles_CreateFcn(hObject, eventdata, handles)
% hObject   handle to txtBaffles (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end
end

```

```

%% Tank Diameter
function txtTankDiameter_Callback(hObject, eventdata, handles)
% hObject handle to txtTankDiameter (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtTankDiameter as text
% str2double(get(hObject,'String')) returns contents of txtTankDiameter as a
double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errordlg('Enter a numerical value for Tank Diameter (m).','Error');
    set(handles.txtTankDiameter,'Value',1);
end
handles.txtTankDiameter = val; % store in "data" structure as a field (add to
existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtTankDiameter_CreateFcn(hObject, eventdata, handles)
% hObject handle to txtTankDiameter (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Liquid Height
function txtLiqHeight_Callback(hObject, eventdata, handles)
% hObject handle to txtLiqHeight (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtLiqHeight as text
% str2double(get(hObject,'String')) returns contents of txtLiqHeight as a double

```

```

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errorlg('Enter a numerical value for height of liquid in tank (m).','Error');
    set(handles.txtLiqHeight,'Value',3);
end
handles.txtLiqHeight = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtLiqHeight_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtLiqHeight (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%    See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Tank Height

function txtTankHeight_Callback(hObject, eventdata, handles)
% hObject    handle to txtTankHeight (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtTankHeight as text
%    str2double(get(hObject,'String')) returns contents of txtTankHeight as a
double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errorlg('Enter a numerical value for height of tank (m).','Error');
    set(handles.txtTankHeight,'Value',4);
end
handles.txtTankHeight = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtTankHeight_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtTankHeight (see GCBO)

```

```

% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Tank Liquid Volume

function txtLiqVol_Callback(hObject, eventdata, handles)
% hObject handle to txtLiqVol (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtLiqVol as text
% str2double(get(hObject,'String')) returns contents of txtLiqVol as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errordlg('Enter a numerical value for liquid volume in tank (L).','Error');
    set(handles.txtLiqVol,'Value',3000);
end
handles.txtLiqVol = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtLiqVol_CreateFcn(hObject, eventdata, handles)
% hObject handle to txtLiqVol (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Total Tank Volume

```

```

function txtTotVol_Callback(hObject, eventdata, handles)
% hObject handle to txtTotVol (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtTotVol as text
% str2double(get(hObject,'String')) returns contents of txtTotVol as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errordlg('Enter a numerical value for total tank volume (L).','Error');
    set(handles.txtTotVol,'Value',4000);
end
handles.txtTotVol = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtTotVol_CreateFcn(hObject, eventdata, handles)
% hObject handle to txtTotVol (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Impeller Type/Combination

% --- Executes on selection change in popImpeller.
function popImpeller_Callback(hObject, eventdata, handles)
% hObject handle to popImpeller (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: contents = get(hObject,'String') returns popImpeller contents as cell array
% contents{get(hObject,'Value')} returns selected item from popImpeller

Impeller_Sel = get(hObject,'Value'); % Get the value entered by the user
Impeller_List = get(hObject,'String');

```

```

Selected_Impeller = Impeller_List{Impeller_Sel}; %Convert from cell array to string
handles.popImpeller = Selected_Impeller;
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function popImpeller_CreateFcn(hObject, eventdata, handles)
% hObject    handle to popImpeller (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

```

```

% Hint: popupmenu controls usually have a white background on Windows.
%    See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

```

```

%%
=====
====
% Broth Parameters
%
=====
====
%% Broth Rheology

```

```

% --- Executes on selection change in popRheology.
function popRheology_Callback(hObject, eventdata, handles)
% hObject    handle to popRheology (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```

```

% Hints: contents = get(hObject,'String') returns popRheology contents as cell array
%    contents{get(hObject,'Value')} returns selected item from popRheology

```

```

Rheo_Sel = get(hObject,'Value'); % Get the value entered by the user
Rheo_List = get(hObject,'String');
Selected_Rheo = Rheo_List{Rheo_Sel}; %Convert from cell array to string
handles.popRheology = Selected_Rheo;
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function popRheology_CreateFcn(hObject, eventdata, handles)

```



```

% hObject handle to popRheology (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: popupmenu controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Cell Growth Kinetics Equation

% --- Executes on selection change in popKineticsEqn.
function popKineticsEqn_Callback(hObject, eventdata, handles)
% hObject handle to popKineticsEqn (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: contents = get(hObject,'String') returns popKineticsEqn contents as cell
array
% contents{get(hObject,'Value')} returns selected item from popKineticsEqn

Eqn_Sel = get(hObject,'Value'); % Get the value entered by the user
Eqn_List = get(hObject,'String');
Selected_Eqn = Eqn_List{Eqn_Sel}; %Convert from cell array to string
handles.popKineticsEqn = Selected_Eqn;
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function popKineticsEqn_CreateFcn(hObject, eventdata, handles)
% hObject handle to popKineticsEqn (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: popupmenu controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

```

```

%%
=====
====
% ODE Solver
%
=====
====
% --- Executes on selection change in popODE.
function popODE_Callback(hObject, eventdata, handles)
% hObject    handle to popODE (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: contents = get(hObject,'String') returns popODE contents as cell array
%        contents{get(hObject,'Value')} returns selected item from popODE

Ode_Sel = get(hObject,'Value'); % Get the value entered by the user
Ode_List = get(hObject,'String');
Selected_Ode = Ode_List{Ode_Sel}; %Convert from cell array to string
handles.popODE = Selected_Ode;
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function popODE_CreateFcn(hObject, eventdata, handles)
% hObject    handle to popODE (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: popupmenu controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%%
=====
====
% Initial Gas Sparge Rate
%
=====
====
%% CO2 Sparge Rate

```

```

function txtCO2SpargeValue_Callback(hObject, eventdata, handles)
% hObject   handle to txtCO2SpargeValue (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtCO2SpargeValue as text
%   str2double(get(hObject,'String')) returns contents of txtCO2SpargeValue as a
double

CO2Value = get(handles.txtCO2SpargeValue,'String');
CO2Value = str2num(CO2Value);
if (isempty(CO2Value) || CO2Value < 0 || CO2Value > 200)
    set(handles.sldCO2Sparge,'Value',0);
    set(handles.txtCO2SpargeValue,'String','0');
    errorDlg('Enter a numerical value between 0 and 200.','Error');
else
    set(handles.sldCO2Sparge,'Value',CO2Value);
end

% --- Executes during object creation, after setting all properties.
function txtCO2SpargeValue_CreateFcn(hObject, eventdata, handles)
% hObject   handle to txtCO2SpargeValue (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%   See ISPC and COMPUTER.

if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

% --- Executes on slider movement.
function sldCO2Sparge_Callback(hObject, eventdata, handles)
% hObject   handle to sldCO2Sparge (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'Value') returns position of slider
%   get(hObject,'Min') and get(hObject,'Max') to determine range of slider

CO2Value = get(handles.sldCO2Sparge,'value');

```

```

set(handles.txtCO2SpargeValue,'string',int2str(CO2Value))
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function sldCO2Sparge_CreateFcn(hObject, eventdata, handles)
% hObject    handle to sldCO2Sparge (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: slider controls usually have a light gray background.
if isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor',[.9 .9 .9]);
end

%% O2 Sparge Rate
function txtO2SpargeValue_Callback(hObject, eventdata, handles)
% hObject    handle to txtO2SpargeValue (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtO2SpargeValue as text
%        str2double(get(hObject,'String')) returns contents of txtO2SpargeValue as a
double

O2Value = get(handles.txtO2SpargeValue,'String');
O2Value = str2num(O2Value);
if (isempty(O2Value) || O2Value < 0 || O2Value > 200)
    set(handles.sldO2Sparge,'Value',0);
    set(handles.txtO2SpargeValue,'String','0');
    errorDlg('Enter a numerical value between 0 and 200.','Error');
else
    set(handles.sldO2Sparge,'Value',O2Value);
end

% --- Executes during object creation, after setting all properties.
function txtO2SpargeValue_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtO2SpargeValue (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.

```

```

% See ISPC and COMPUTER.

if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

% --- Executes on slider movement.
function sldO2Sparge_Callback(hObject, eventdata, handles)
% hObject handle to sldO2Sparge (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'Value') returns position of slider
% get(hObject,'Min') and get(hObject,'Max') to determine range of slider

O2Value = get(handles.sldO2Sparge,'value');
set(handles.txtO2SpargeValue,'string',int2str(O2Value))
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function sldO2Sparge_CreateFcn(hObject, eventdata, handles)
% hObject handle to sldO2Sparge (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: slider controls usually have a light gray background.
if isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor',[.9 .9 .9]);
end
%%
=====
====
% Initial pH
%
=====
====
function txtpH_Callback(hObject, eventdata, handles)
% hObject handle to txtpH (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

```

```

% Hints: get(hObject,'String') returns contents of txtpH as text
%   str2double(get(hObject,'String')) returns contents of txtpH as a double
pHval = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(pHval) || isempty(pHval)
    errorlg('Enter a numerical value for pH.','Error');
    set(handles.txtpH,'Value',7.2);
end
if pHval < 0 || pHval > 14
    errorlg('pH must be between 0 and 14.','Error');
    set(handles.txtpH,'Value',7.2);
end
handles.txtpH = pHval; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtpH_CreateFcn(hObject, eventdata, handles)
% hObject   handle to txtpH (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   empty - handles not created until after all CreateFcns called

```

```

% Hint: edit controls usually have a white background on Windows.

```

```

%   See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end
%%

```

```

=====

```

```

====

```

```

% Process Parameters

```

```

%

```

```

=====

```

```

====

```

```

%% Recycle Ratio

```

```

function txtRecycleRatio_Callback(hObject, eventdata, handles)
% hObject   handle to txtRecycleRatio (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles   structure with handles and user data (see GUIDATA)

```

```

% Hints: get(hObject,'String') returns contents of txtRecycleRatio as text
%   str2double(get(hObject,'String')) returns contents of txtRecycleRatio as a
double

```

```

val = str2double(get(hObject,'String'));
if isnan(val) || isempty(val) || val < 0
    errorlg('Enter a positive numerical value for Recycle Ratio (Alpha).','Error');
set(handles.txtRecycleRatio,'Value',0.6);
end
handles.txtRecycleRatio = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtRecycleRatio_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtRecycleRatio (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

```

```

% Hint: edit controls usually have a white background on Windows.
%    See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

```

%% Cell Concentration Factor

```

function txtCellConcFactor_Callback(hObject, eventdata, handles)
% hObject    handle to txtCellConcFactor (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```

```

% Hints: get(hObject,'String') returns contents of txtCellConcFactor as text
%    str2double(get(hObject,'String')) returns contents of txtCellConcFactor as a
double

```

```

CellFactor = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(CellFactor) || isempty(CellFactor) || CellFactor < 0
    errorlg('Enter a numerical value for Cell Concentration Factor (c).','Error');
set(handles.txtCellConcFactor,'Value',2);
end
handles.txtCellConcFactor = CellFactor; % store in "data" structure as a field (add to
existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtCellConcFactor_CreateFcn(hObject, eventdata, handles)

```

```

% hObject  handle to txtCellConcFactor (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%   See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Feed Flow Rate Into Tank

function txtFeedIn_Callback(hObject, eventdata, handles)
% hObject  handle to txtFeedIn (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtFeedIn as text
%   str2double(get(hObject,'String')) returns contents of txtFeedIn as a double

FeedIn = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(FeedIn) || isempty(FeedIn) || FeedIn < 0
    errordlg('Enter a numerical value for Feed Flow Rate into the Tank (L/hr).','Error');
    set(handles.txtFeedIn,'Value',20);
end
handles.txtFeedIn = FeedIn; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtFeedIn_CreateFcn(hObject, eventdata, handles)
% hObject  handle to txtFeedIn (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%   See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

```



```
%% Perfusion Rate
```

```
function txtFeedOut_Callback(hObject, eventdata, handles)
% hObject handle to txtFeedOut (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtFeedOut as text
% str2double(get(hObject,'String')) returns contents of txtFeedOut as a double

valFeedOut = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(valFeedOut) || isempty(valFeedOut) || valFeedOut < 0
    error('Enter a numerical value for Perfusion Rate (L/h).','Error');
    set(handles.txtFeedOut,'Value',2);
end
handles.txtFeedOut = valFeedOut; % store in "data" structure as a field (add to
existing)
guidata(hObject, handles);
```

```
% --- Executes during object creation, after setting all properties.
```

```
function txtFeedOut_CreateFcn(hObject, eventdata, handles)
% hObject handle to txtFeedOut (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called
```

```
% Hint: edit controls usually have a white background on Windows.
```

```
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end
```

```
%% Saturation Constant
```

```
function txtSatConstant_Callback(hObject, eventdata, handles)
% hObject handle to txtSatConstant (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
```

```
% Hints: get(hObject,'String') returns contents of txtSatConstant as text
% str2double(get(hObject,'String')) returns contents of txtSatConstant as a
double
```

```

SatCon = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(SatCon) || isempty(SatCon)
    errorlg('Enter a numerical value for Saturation Constant (Ks).','Error');
    set(handles.txtSatConstant,'Value',0.05);
end
handles.txtSatConstant = SatCon; % store in "data" structure as a field (add to
existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtSatConstant_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtSatConstant (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

```

```

% Hint: edit controls usually have a white background on Windows.
%    See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

```

```

%% Maximum Specific Growth Rate

```

```

function txtMuMax_Callback(hObject, eventdata, handles)
% hObject    handle to txtMuMax (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```

```

% Hints: get(hObject,'String') returns contents of txtMuMax as text
%    str2double(get(hObject,'String')) returns contents of txtMuMax as a double
val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errorlg('Enter a numerical value for Maximum Specific Growth Rate (1/h).','Error');
    set(handles.txtMuMax,'Value',2.3);
end
handles.txtMuMax = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtMuMax_CreateFcn(hObject, eventdata, handles)

```

```

% hObject handle to txtMuMax (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Broth Temperature

function txtTemp_Callback(hObject, eventdata, handles)
% hObject handle to txtTemp (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtTemp as text
% str2double(get(hObject,'String')) returns contents of txtTemp as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val)
    errordlg('Enter a numerical value for Temperature (C).','Error');
    set(handles.txtTemp,'Value',37);
end
if val < 35 || val > 38
    errordlg('Temperature must be between 35 C and 38 C','Error');
    set(handles.txtTemp,'Value',37);
end
handles.txtTemp = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtTemp_CreateFcn(hObject, eventdata, handles)
% hObject handle to txtTemp (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
% See ISPC and COMPUTER.

```

```

if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Simulation Duration (Time)

function txtTime_Callback(hObject, eventdata, handles)
% hObject  handle to txtTime (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtTime as text
%       str2double(get(hObject,'String')) returns contents of txtTime as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val) || val <= 0
    errordlg('Enter a numerical value for Time (h).','Error');
    set(handles.txtTime,'Value',72);
end
handles.txtTime = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtTime_CreateFcn(hObject, eventdata, handles)
% hObject  handle to txtTime (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%       See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Cell Concentration in Tank

function txtCellinTank_Callback(hObject, eventdata, handles)
% hObject  handle to txtCellinTank (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles  structure with handles and user data (see GUIDATA)

```

```

% Hints: get(hObject,'String') returns contents of txtCellinTank as text
%     str2double(get(hObject,'String')) returns contents of txtCellinTank as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val) || val < 0
    errorDlg('Enter a numerical value for Cell Concentration in Tank (g/L).','Error');
    set(handles.txtCellinTank,'Value',10);
end
handles.txtCellinTank = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

% --- Executes during object creation, after setting all properties.
function txtCellinTank_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtCellinTank (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%     See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%% Cell Concentration in Feed

function txtCellinFeed_Callback(hObject, eventdata, handles)
% hObject    handle to txtCellinFeed (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Hints: get(hObject,'String') returns contents of txtCellinFeed as text
%     str2double(get(hObject,'String')) returns contents of txtCellinFeed as a double

val = str2double(get(hObject,'String')); % Get the value entered by the user
if isnan(val) || isempty(val) || val < 0
    errorDlg('Enter a numerical value for Cell Concentration in Feed (g/L).','Error');
    set(handles.txtCellinFeed,'Value',5);
end
handles.txtCellinFeed = val; % store in "data" structure as a field (add to existing)
guidata(hObject, handles);

```

```

% --- Executes during object creation, after setting all properties.
function txtCellinFeed_CreateFcn(hObject, eventdata, handles)
% hObject    handle to txtCellinFeed (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    empty - handles not created until after all CreateFcns called

% Hint: edit controls usually have a white background on Windows.
%   See ISPC and COMPUTER.
if ispc && isequal(get(hObject,'BackgroundColor'),
get(0,'defaultUicontrolBackgroundColor'))
    set(hObject,'BackgroundColor','white');
end

%%
=====
====
% Push Buttons
%
=====
====
%% Reset Button
%--- Executes on button press in btnReset.
function btnReset_Callback(hObject, eventdata, handles)
% hObject    handle to btnReset (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
selection = ...
questdlg('Are you sure you want to RESET Bioreactor Simulator?', ...
'Reset Bioreactor Simulator', ...
'Yes', 'No', 'Yes');
if strcmp(selection, 'No')
    return;
else
    closeGUI = handles.BioreactorGUI;
    guiPosition = get(handles.BioreactorGUI,'Position');
    guiName = get(handles.BioreactorGUI,'Name');
    eval(guiName)
    close(closeGUI);
    set(gcf,'Position',guiPosition);
end;

%% Close Button
% --- Executes on button press in btnClose.

```

```

function btnClose_Callback(hObject, eventdata, handles)
% hObject    handle to btnClose (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
selection = questdlg('Close Bioreactor Simulator? ', ...
    'Close Bioreactor Simulator', ...
    'Yes', 'No', 'Yes');
if strcmp(selection, 'No')
    return;
else
    close(gcf);
end;

%% Run Model Button
% --- Executes on button press in btnRunModel.
function btnRunModel_Callback(hObject, eventdata, handles)
% hObject    handle to btnRunModel (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

guidata(hObject, handles);
AGACellRecycle(handles.popKineticsEqn, handles.txtCO2SpargeValue, ...
    handles.txtO2SpargeValue, handles.txtpH, handles.txtRecycleRatio, ...
    handles.txtCellConcFactor, handles.txtFeedIn, handles.txtFeedOut, ...
    handles.txtSatConstant, handles.txtMuMax, handles.txtTemp, ...
    handles.txtTime, handles.txtCellinTank, handles.txtCellinFeed);
AGACO2Balance(handles.txtTankDiameter, handles.txtLiqHeight, ...
    handles.txtTankHeight, handles.txtCO2SpargeValue);

% List of Files:
% AGABioreactorGUI.m and AGABioreactor.fig: GUI program
% AGACellGrowth.m: Original Master file with all pertinent sections
% AGACellRecycle.m: Currently takes in variables from AGABioreactorGUI
% AGAContoisXS.m Contois cell growth equation
% AGAMonodXS.m: Monod cell growth equation
% AGATessierXS.m: Tessier cell growth equation
% AGAIonicStrength.m: Calculates ionic strength in the medium
% AGAO2Conc.m Calculates oxygen concentration in the medium
% --- Executes on key press with focus on btnRunModel and no controls selected.
function btnRunModel_KeyPressFcn(hObject, eventdata, handles)
% hObject    handle to btnRunModel (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```

```

%% Help Button
%--- Executes on button press in btnHelp.
function btnHelp_Callback(hObject, eventdata, handles)
% hObject    handle to btnHelp (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

HelpText{1} = 'TYPICAL VALUES TO INPUT FOR INITIAL VALUES';
HelpText{2} = '';
HelpText{3} = 'Maximum Specific Growth Rate, MuMax (1/hour): 0.4';
HelpText{4} = 'Concentration Factor, c (no units): 1';
HelpText{5} = 'Recycle Ratio, Alpha (no units): 1';
HelpText{6} = 'Saturation Constant Ks (g/L): 200';
HelpText{7} = 'Bioreactor Volume, V (L): 3000';
HelpText{8} = 'Temperature, T (C): 36.2';
HelpText{9} = 'Time, t (hours): 72';
helpdlg(HelpText, 'Help');

% Yield coefficient Y: 0.3
% Pump flow rate in L/hour: 0.4
% Concentration of the feed nutrient in mg/L: 20000
% How many time steps do you want?, >1000 recommended: 2000
% Total time period you would like to simulate in hours: 30
% Initial cell conc in the reactor in mg per L: 10000
% Initial residual substrate conc in the reactor in mg per L: 100
% Initial specific growth rate, i.e. dilution rate at equilibrium in 1/h:
% 0.35

%%
=====
====
%GUI Menu Items on GUI
%
=====
====
%% File
function mnuFile_Callback(hObject, eventdata, handles)
% hObject    handle to mnuFile (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

```



```

%% Exit
function mnuExit_Callback(hObject, eventdata, handles)
% hObject handle to mnuExit (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles structure with handles and user data (see GUIDATA)
close(gcf);

% --- Executes during object creation, after setting all properties.
function BioreactorGUI_CreateFcn(hObject, eventdata, handles)
% hObject handle to BioreactorGUI (see GCBO)
% eventdata reserved - to be defined in a future version of MATLAB
% handles empty - handles not created until after all CreateFcns called

```

The GUI calls two files: AGACellRecycle.m and AGACO2Balance.m. Following is the code for AGACellRecycle.m:

```

function AGACellRecycle(KineticsEqn, CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed)
% Calls function file for the user selected kinetics equation
% to solve the desired equation for Chemostat with Cell Recycle

% Cell Growth Kinetic Equations (From Shuler/Kargi)

switch KineticsEqn
case 'Contois'
    AGAContoisXS(CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed);
case 'Monod'
    AGAMonodXS(CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed);
case 'Tessier'
    AGATessierXS(CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed);
end
AGAIonicStrength(pH, Temp);

```

Following is the code for AGACO2Balance.m:

```
function AGACO2Balance(TankDiameter, LiqHeight, TankHeight, CO2SpargeValue)
%% Carbon Dioxide Equations
% Souces of CO2:
% 1. Cell respiration, CER;
% 2. CO2 Sparge;
% 3. Bicarbonate addition
% Sinks of CO2
% 1. Reactions in broths
% 2. Cell Intake
% 3. CO2 escape from headspace
%% CPR From Goudar, Piret and Konstantinov 2011
sCPR = 6.2037e-17; % [mol/cell-sec] Specific Carbon Dioxide Production Rate
%% KLaCO2 (From Hill 2006)
TStar = (T-27.5)/7.432; % [T in Celcius] Dimensionless Temperature
QStar = (Q-1.1)/0.5351; % [Q in L/min] Dimensionless Gas Flow Rate
RPMStar = (RPM-375)/133.8; % Dimensionless RPM
KLaCO2 = 33.9 + 7.96 * TStar + 15.7 * QStar + 18.8 * RPMStar + ...
        6.46 *(QStar^2) + 8.25 * TStar * QStar;
%% CO2 Concentration % From Dixon et al.
CO2aq = (10^(1.39))*(CO2SpargeValue + CER);

%% HCO3 Concentration % From Dixon et al.
b = 0.1;
pK0a1 = 6.305;
pKa1 = pK0a1 - fl - b*IS;
HCO3 = 10^(pH -pKa1 + log10(CO2aq));

H = 10^(-pH);
K1 = 10^(-6.30); % From Goudar, Piret, Konstantinov 2011
K2 = 10^(-10.23); % From Goudar, Piret, Konstantinov 2011
pctH2CO3 = ((H^2)*100)/((H^2)+H*K1+K1*K2); %From Goudar, Piret, Konstantinov
2011
pctHCO3 = (H*K1*100)/((H^2)+H*K1+K1*K2); %From Goudar, Piret, Konstantinov
2011
pctCO3 = (K1*K2*100)/((H^2)+H*K1+K1*K2); %From Goudar, Piret, Konstantinov
2011
%% From Zupke and Green
kLaCO2 = .89*kLaO2;
% Headspace volume (L)
TankVol = ((pi*((TankDiameter/2)^2)*TankHeight)*1000); % [L]
```

```

LiqVol = ((pi*((TankDiameter/2)^2)*LiqHeight)*1000); % [L]
HeadSpaceVol = TankVol - LiqVol; % [L]
% Headspace gas composition
HeadSpaceCO2In = (1/HeadSpaceVol)*((mfCO2Overlay*GasFlowOverlay)+...
    mfCO2Sparge*AvgSpargeFlow);
HeadSpaceCO2Out = (1/HeadSpaceVol)*(mfCO2 * (GasFlowOverlay +
    AvgSpargeFlow));
HeadSpaceCO2Surf = ((R*T*LiqVol*KiSurf)/(TopPressure * HeadSpaceVol))

```

AGACellRecycle.m calls the following 4 files:

1. AGAContoisXS.m

```

function [D, X, S] = AGAContoisXS(CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed)

% Cell Growth Kinetic Equations (From Shuler/Kargi)
% MuMax = 2.3;
% CellConcFactor = 2;
% RecycleRatio = .6;
% SatConstant = .05;
%N = 61;
%D = linspace(0, 6, N); % (initial values for) Rate of Dilution
D = 0:.1:5.9;
Yxs = 1; % (g/g) Yield Coefficient
So = 1; % Initial Substrate Concentration
A = 1 + RecycleRatio - (RecycleRatio * CellConcFactor);
for k = 1:length(D)
X(k) = ((Yxs * So) / A)*((MuMax - (D(k) * A))/...
    (MuMax - (D(k) * A) + Yxs * D(k) * SatConstant));
S(k) = (D(k) * SatConstant * X(k)*A) / (MuMax - (D(k) * A));
end

z(:,1) = D;
z(:,2) = X;
z(:,3) = S;
disp(z);
figure;
plot(D,S,D,X),xlabel('D'), ylabel('S and X');

```

## 2. AGAMonodXS.m

```
function AGAMonodXS(CO2SpargeValue, O2SpargeValue,...
    pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...
    SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed)
MuMax = 2.3;
CellConcFactor = 2;
RecycleRatio = .6;
SatConstant = .05;
Yxs = 1;
% MuMax = 1;
% CellConcFactor = 2;
% RecycleRatio = .5;
% SatConstant = .01;
% Yxs = 0.5; % (g/g) Yield Coefficient
BrothVolume = (pi*((TankDiameter/2)^2)*LiqHeight)*1000;
D = FeedIn/BrothVolume;
% D = 0:.1:5.5; % (hr-1) (initial values for) Rate of Dilution
So = 1; % Initial Substrate Concentration
% Cell Growth Kinetic Equations (From Shuler/Kargi)
A = 1 + RecycleRatio - (RecycleRatio * CellConcFactor);
for k = 1:length(D)
    Mu(k) = D(k) * A; % Specific Growth Rate
    S(k) = (Mu(k) * SatConstant) / (MuMax - Mu(k));
    X(k) = (Yxs/A) * (So - S(k));
    X2(k) = A*X(k);
    Xv(k) = 0;
end
z(:,1) = D;
z(:,2) = X;
z(:,3) = S;
z(:,4) = X2;
disp(z);
figure;
plot(D,X,'-xr',D,S,'-sg',D,X2,'-b'),xlabel('D'), ylabel('X, X2 and S');
```

## 3. AGATessierXS.m

```
function AGATessierXS(CO2SpargeValue, O2SpargeValue,...
```

```
pH, RecycleRatio, CellConcFactor, FeedIn, FeedOut,...  
SatConstant, MuMax, Temp, Time, CellinTank, CellinFeed)
```

```
% MuMax = 2.3;  
% CellConcFactor = 2;  
% RecycleRatio = .6;  
% SatConstant = .05;
```

```
% Cell Growth Kinetic Equations (From Shuler/Kargi)
```

```
N = 61;  
D = linspace(0, 6, N); % (hr-1) (initial values for) Rate of Dilution  
Yxs = 1; % (g/g) Yield Coefficient  
So = 1; % Initial Substrate Concentration  
A = 1 + RecycleRatio - (RecycleRatio .* CellConcFactor);  
p = 50;  
S = ((A .* D .* SatConstant) .* (MuMax .* ((A .* D) - 1))) .^ (1/p);  
X = (Yxs .* (So - (((A .* D .* SatConstant) .* (MuMax .* ((A .* D) - 1))) .^ (1/p)))) ./  
A;
```

```
z(:,1) = D;  
z(:,2) = X;  
z(:,3) = S;  
disp(z);  
figure;  
plot(D,S,D,X),xlabel('D'), ylabel('S and X');
```

#### 4. AGAlonicStrength.m

```
function [fI, IS, Hili] = AGAlonicStrength(pH, Temp)
```

```
pH = 7.4;  
Temp = 37;  
%% Inorganic salts concentration in IMDM Fermentation Media (from Burgener  
and Butler)  
%Initial Concentrations  
ionCa = 1.49e-3; %[M] Calcium ion  
ionK = 4.44075; %[M] Potassium ion  
ionMg = 8.14e-4; %[M] Magnesium ion  
ionNa = 0.115496; %[M] Sodium ion
```

ionCl = 8.501e-2; %[M] Chlorine ion  
ionCO3 = 1e-3; %[M] Initial Carbonate in media  
ionHCO3 = 3.6e-2; %[M] Initial Bicarbonate in media  
%ionNO3 = 7.5e-7; Too low -- not considered  
ionH2PO4 = 9.06e-4; %[M] Hydrogen Phosphate ion  
%ionSeO3 = 6.5e-8; Too low -- not considered  
ionSO4 = 8.14e-4; %[M] Sulphate ion  
ionH = 10<sup>(-pH)</sup>; % [M] Hydronium ion  
ionOH = 10<sup>(pH-14)</sup>; % [M] Hydroxyl ion

%% Amino Acid concentration in IMDM Fermentation Media (from Burgener and Butler)

% ionAla = 2.81e-4; %[M] L-Alanine  
% ionGly = 3.99e-4; %[M] L-Glycine  
% ionMet = 2.01e-4; %[M] L-Methionine  
% ionPro = 3.48e-4; %[M] L-Proline  
% ionSer = 4.00e-4; %[M] L-Serine  
% ionVal = 8.03e-4; %[M] L-Valine  
% ionHEPES = 2.5e-2; %[M] HEPES Buffer

% DGlc = 2.5e-2; %[M] Initial D-Glucose  
%% Activity coefficients (from Murray 2004)

actCa = 0.40;  
actK = 0.76;  
actMg = 0.45;  
actNa = 0.77;  
actCl = 0.76;  
actCO3 = 0.39;  
actHCO3 = 0.77;  
actH2PO4 = 0.77;  
actSO4 = 0.36;

%

%% H Parameter for Effect of Salts on Gas Solubility

% from Nagy and remaining from Blanch and Clark 358

% hCa = -0.303;  
% hK = -0.587;  
% hMg = -0.314;  
% hNa = -0.550;  
% hCl = 0.844;  
% hCO3 = 0.485;  
% %hHCO3  
% hH2PO4 = 0.997;  
% hSO4 = 0.460;  
% hH = -0.774;

```

% hOH      = 0.941;

%% Calculate Ionic Strength
ionTotal = [ionCa ionK ionMg ionNa ionCl ionCO3 ionHCO3 ionH2PO4 ionSO4];
ionCharge = [2 1 2 1 -1 -2 -1 -1 -2];
activity = [actCa actK actMg actNa actCl actCO3 actHCO3 actH2PO4 actSO4];
I = 0.5 .* ionTotal .* (ionCharge.^2);
Hili = 0;
fi = 0;
IS = 0;
    for k = 1:length(I)
        Hili = Hili + (I(k) .* activity(k));
        IS = IS + I(k);
    end
fi = (((IS^0.5)/(1+(IS^0.5)))-0.21)*((298/(Temp + 273.16))^(2/3));

```

Using stiff solver ODE15s, the following file, AGABioreactorEquations.m, solves some of the equations presented in sections 3.3.1.:

```

function AGABioreactorEquations

% Bioreactor Constants
alpha = 0.6; % Ratio
C      = 2; % Ratio
F0     = 200; % [L/hr]
V      = 3000; % [L]

% Biomass Constants
AMM0   = 0; % [mol/L]
GLC0   = 25e-3; % [mol/L]
GLN0   = 4e-3; % [mol/L]
LAC0   = 0; % [mol/L]
KexGLC = 1e-2; % [mol/L]
KIAMM  = 15e-3; % [mol/L]
KGLC   = 7.5e-4; % [mol/L]
KGLN   = 7.5e-5; % [mol/L]
KILAC  = 9e-2; % [mol/L]
KdAMM  = 15e-3; % [mol/L]
mGLC   = 2e-15; % [mol/(L.cell.hr)]

```

```

MuMax = 0.065; % [hr-1]
QexGLC = 2e-13; % [mol/(L.cell.hr)]
YAGLN = 0.7; % [mol/mol]
YLGLC = 2; % [mol/mol]
YXGLC = 2.37e8; % [cell/mol]
YXGLN = 8e8; % [cells/mol]
X0 = 300000; % [cells/L]

% Set up Differential Equations
[T, Y] = ode15s(@Biomass, [0 300], [AMM0; GLC0; GLN0; LAC0; X0;...
    0.05; 0.9e-13; 1.09e-13; 0.93-13; 1.17e-13]);
plot(T,Y(:,1),'-sr',T,Y(:,2),'+b')
disp(Y)
%plot(T,Y(:,1),'-sr')

% y(1) = AMM Ammonia concentration
% y(2) = GLC Glucose concentration
% y(3) = GLN Glutamine concentration
% y(4) = LAC Lactic Acid concentration
% y(5) = Xv Viable cell mass
% y(6) = Mu Specific Growth Rate
% y(7) = QAMM Specific Ammonia Production Rate
% y(8) = QGLC Specific Glucose Consumption Rate
% y(9) = QGLN Specific Glutamine Consumption Rate
% y(10) = QLAC Specific Lactate Production Rate
% y(11) =

% Nested Function
function dy = Biomass(t,y)

% Algebraic Equations
D = F0/V;

% Differential Equations
dy = zeros(10,1);
dy(1) = (y(7).*y(5)) + (KdAMM.*y(3)) - (D.*y(1));
dy(2) = (D.*GLC0) - (D.*y(2)) - (y(8).*y(5));
dy(3) = (D.*GLN0) - (D.*y(3)) - (y(9).*y(5));
dy(4) = (y(10).*y(5)) - (D.*y(4));
dy(5) = (D.*X0) + (alpha.*C.*D.*y(5)) - ((1+alpha).*D.*y(5))...
    + (y(6).*y(5));
dy(6) = MuMax.*(y(2)/(KGLC+y(2))).*(y(3)/(KGLN+y(3))).* ...

```



```

        (KIAMM./(KIAMM+y(1))).*(KILAC./(KILAC+y(4)));
dy(7) = YAGLN.*y(9);
dy(8) = (y(6) ./ YXGLC) + mGLC + (QexGLC .* (y(2) ./ (y(2) + KexGLC)));
dy(9) = y(6) ./ YXGLN;
dy(10) = YLGLC .* y(8);
end
end

```

## 5. function AGAPerfusion

```

% Constants
D    = 0.5; % Medium exchange rate
F0   = 5; % Substrate concentration in feed (L/day)
GLC0 = 2.7; % Initial substrate concentration (mol/L)
kGLC = 0.05; % Contois saturation constant
Mu0  = 0.4; % Initial specific cell growth rate (per day)
MuMax = 1e6; % Maximum specific cell growth rate (per day)
P0   = 0; % Initial product concentration (mol/L)
Pj   = 1e-7; % Product conc in feed out of cell separator (mol/L)
Sj   = 2.5; % Substrate conc in feed out of cell separator (mol/L)
X0   = 1e8; % Seeding concentration (cells/L)

% ODE Solve Statement
[T, Y] = ode15s(@Perfusion, [0 20], [X0; GLC0; Mu0; P0], []);

% Plot X v t
figure;
subplot(2,2,1);
plot(T,Y(:,1),'-sr')
xlabel('Time, t, (days)')
ylabel('Cells, X, (Cells/L)')
legend('dX/dt');

% Plot [GLC] v t
subplot(2,2,2);
plot(T,Y(:,2),'-+b')
xlabel('Time, t, (days)')
ylabel('[GLC]')
legend('d[GLC]/dt');

% Plot Mu v t
subplot(2,2,3);
plot(T,Y(:,3),'-og')

```

```

xlabel('Time, t, (days)')
ylabel('\mu')
legend('d\mu/dt');

% Plot P v t
subplot(2,2,4);
plot(T,Y(:,4),'-or')
xlabel('Time, t, (days)')
ylabel('Product')
legend('Product/dt');

% y(1) = dXdt Rate of Cell Growth
% y(2) = dGLCdt Rate of Change in Glucose Concentration
% y(3) = dMudt Rate of Change of Mu
% y(4) = dF0dt Rate of Change in Feed

% Nested Function
function dy = Perfusion(t,y)

dy = zeros(3,1);
dy(1) = y(3)*y(1);
dy(2) = -(3.6e-12*y(1)+D*(F0-Sj));
dy(3) = (MuMax*y(2))/((kGLC*y(1))+y(2));
dy(4) = ((1e-12)*y(1))-D*Pj;

end
end

```

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