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Investigation of a new effective bacterium for cellulosic degradation

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INVESTIGATION OF A NEW EFFECTIVE BACTERIUM FOR CELLULOSIC DEGRADATION

by

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A research project
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Declaration of Authorship

This DEGREE PROJECT entitled " Investigation of a New effective Bacterium for Cellulosic Degradation" has been prepared by the undersigned and all sources of assistance and information have been acknowledged and referenced.

This work is submitted to the Department of Civil Engineering in partial fulfillment of the requirement for the Degree of Master of Engineering in Civil Engineering.

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INVESTIGATION OF A NEW EFFECTIVE BACTERIUM FOR CELLULOSIC DEGRADATION

Weining Lin, Master of Engineering, 2008
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Abstract

Clostridium phytofermentans, a newly isolated mesophilic anaerobic bacterium from forest soil, has received considerable attention for its potential application in producing ethanol directly from cellulose. This microorganism produces ethanol, acetate, CO_2 and H_2 as major metabolites from cellulose. Potential applications of this research include the transformation of waste materials into valuable products, such as fuels and organic acids.

As an initial part of a multi-staged project, this study is to focus on the characterization of this microorganism growth and to verify the bacterium kinetics, including biomass growth, substrate utilization, and gas production. A series of batch fermentation experiments using cellulose substrate (GS-2C) was performed under the incubation temperature of 37°C. To investigate the effects of pH and substrate concentration (S_o) on growth, 12 trial experiments were conducted with various controlled pH values (7.0 to 8.5) and with various initial cellulose concentration settings (0.1 to 6.0 g/L). Our experimental results showed that the optimal growth condition for C. *phytofermentans* in batch culture was at pH = 8.4 and S_o = 6.0 g/L. Under such condition, the maximum growth rate of $0.37h^{-1}$ was observed. Comparing results with other celluloytic clostridium

studies, relatively high biomass growth rate using C. *phytofermentans* is confirmed by our experiments.

Mathematical models, using a combination modelling approach with the logistic equation, Monod model, and Luedeking-Piret model, were developed for biomass growth, substrate degradation, and biogas production, respectively, based on our experiment results. This study demonstrated the determination of the four parameters (μ_{max}, K_s, Y, and S_{min}), which can describe satisfactorily growth or degradation phenomena, using the proposed integration modelling approach. The experiments conducted under wide range conditions, such as changing pH and S_o, not only provide insight into growth kinetics but also provide an opportunity to evaluate the performance of the mathematical models and understand their limitations. This leads to look for improvement or modification to the models.

It is foreseen that the findings in this study will enhance the overall understanding of the kinetics of growth and substrate utilization and product formation of this bacterium, and provide important information on the design of the bench-scale anaerobic bioreactor for future studies.

Acknowledgments

I would like to express my sincere thanks to Dr. Grace Luk for her guidance and supervision during the research and experiments. Many thanks as well to the project's industrial sponsors: Mr. Jamie Bakos and Dr. Henry Miyamoto of Clean 16 Environmental Technologies Corp. for the opportunity to work on this exciting project. My appreciation also extends to Ms. Balinder Rai of Ontario Centres of Excellence for her support and coordination for the project. My sincere thanks to Dr. James Li for his advice during my graduate studies and his review and comments on this project report.

I have to acknowledge that the completion of this successful research project is a result of team effort. Special thanks to my team mates, Benjamin Percy, Mina Mirzajani, Mandana Ehsanipour, Robin Luong, and Bonnie Wilkinson at Ryerson University for all of your time and invaluable advice.

I would also like to thank my husband, William, my daughter and son, Bethany and Russell, my parents, and Mr. Norman Macleod, a retired professor of Ryerson University for their support, encouragement and understanding.

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μ	specific growth rate (rate of growth per unit of biomass), time^{-1}
μ_{max}	maximum specific growth rate (at a concentration of the growth-limiting substrate or above saturation), time^{-1}
K_s	saturation constant (equal to the limiting substrate concentration at one-half the maximum growth rate), mass/unit volume
X	biomass concentration, mass/unit volume
X_o	initial biomass concentration, mass/unit volume
X_{max}	maximum attainable microbial biomass concentration, mass/unit volume
S	concentration of growth-limiting substrate in solution, mass/unit volume
S_0	initial substrate concentration, mass/unit volume
S_{min}	substrate threshold concentration, mass/unit volume
γ	growth-associated substrate degradation coefficient, mass/mass
δ	non-growth-associated substrate degradation coefficient, mass/mass * time
Y	growth yield by substrate degradation, mass/mass
P	product formation, mass / unit volume
λ	product formation -associated substrate degradation coefficient, mass/mass
α	growth-associated product formation coefficient, mass/mass
β	non–growth-associated product formation coefficient, mass/mass * time

1 Introduction

1.1 Background

Every year, millions of tonnes of organic waste are generated from municipal (green bin waste and biosolids), commercial (food and beverage production), and agricultural residues (crop residues such as straw and corn stover). This potentially valuable material is often underutilized and left to decompose (i.e. in landfills) and contributes to the release of greenhouse gases such as methane and nitrous oxide. Appropriate integrated processes must be developed to create value-added products from these organic waste streams in order to reduce the generation of greenhouse gases and potential sources of water contamination.

With scientific proof of global warming and the inevitable depletion of the world's petroleum supply, significant attention on renewable fuel sources has been given since they can be successfully used for energy use. For example, ethanol is used today as an alternative fuel, a fuel extender, an oxygenate, and an octane enhancer. The majority of ethanol is used in gasoline mixtures as either E10 (10 percent ethanol in gasoline) or E85 (85 percent ethanol in gasoline) mixtures. The Government of Canada has proposed the Ethanol Expansion Program to set a target to have 35 percent of all gasoline in Canada containing a blend of 10-percent ethanol by 2010 (Government of Canada, 2005). From just over 10 million gallons of production in 1979 (Himmel and Sheehan, 2000), the U.S. fuel ethanol industry has grown to more than 4 billion gallons of annual production

capacity in 2004 (Kalogo et al., 2007), and expected to reach over 7.5 billion gallons before 2012 (Yang et al., 2007).

Currently ethanol is mainly produced by formulating a solution of yeast fermentable sugars from commodities such as corn, wheat, and sugar-cane. Some of the deficiencies with current ethanol production are cost (ethanol production can be more expensive than gasoline), lower energy value (the ethanol contains 20 to 30 percent less energy on a volume per volume basis than gasoline), and production requires large amounts of water, fertilizer, transportation, and hauling (Luk, 2007).

Recent food price crisis signifies an urgent need for alternatives of biofuel. World rice prices soared by as much as 30% in one day in early 2008. A surge in demand for biofuel has resulted in a sharp decline in agricultural land planted for food crops. According to National Post, dated on April 2, 2008, about 16% of U.S. agricultural land formerly planted with soybeans and wheat is now growing corn for biofuel. For the first time in history, there is a clear link between the price of fuel and the price of food. Ethanol produced from cellulosic feedstocks such as woods, grasses, and organic fractions of municipal solid waste is emerging as an attractive option for biofuel. This is because of developments in conversion technology, lower feedstock costs and higher potential for fossil fuel displacement, and reduction in greenhouse gas emissions compared to starch-ethanol.

Cellulose is the most abundant biomass available on earth. Municipal organic solid wastes such as paper, wood, and yard waste contain various kinds of organic substances, among

which cellulose is the most prominent and form about 60% of the dry weight of a typical municipal solid waste stream (Kalogo et. al., 2007; Noike et al., 1985). Unlike starch, cellulose is rather difficult to degrade biologically. There are currently two methods of producing cellulosic ethanol: (1) using enzymes to break down the cellulose, and then using yeasts to ferment this to ethanol, and (2) using cellulolytic thermophilic bacteria to digest the cellulose. Primarily due to the current poor conversion efficiency of the biomass into alcohol, the cost of producing cellulosic ethanol is currently in the range of two to five times the cost ethanol production from corn and wheat. A report presented to the United States Senate in June 2006 identified the cost of producing cellulosic ethanol at US$0.59/litre. At that price it would cost about $120 to substitute a barrel of oil, taking into account the lower energy content of ethanol. By contrast starch and wheat derived ethanol is cost competitive with oil at approximately $65 per barrel (Luk, 2007). It is now clear that advanced biotechnologies must be used to reduce the cost of cellulase activity delivered to the cellulosic ethanol process.

A research project has been approved by Ontario Centres of Excellence with support of Clean 16 Environmental Technologies Corporation. The proposed research project will confirm that various sources of biomass (such as "green bin" waste and agricultural residues) can be pre-processed to change the characteristics of the biomass so that the material can be fermented into alcohols and converted into other useful products. The research will lead to the development of a low-cost method utilizing waste biomass to produce ethanol in a more sustainable way than current practices. The research project is phased into the follow five-stage projects.

Stage 1. feasibility study;

Stage 2. characterization of the growth of *Clostridium phytofermentans;*

Stage 3. production of ethanol and acetate in batch-culture system;

Stage 4. production of ethanol and acetate in continuous-culture system; and

Stage 5. design of a bench-scale anaerobic bioreactor.

The feasibility study (Stage 1) was completed in Spring 2007. It confirms that household organic waste pre-treated with a thermal screw is amenable to anaerobic digestion and produces some H_2 and ethanol even without inoculation. The bacterium *Clostridium phytofermentans* is capable of digesting household organic waste and producing H_2 (Luk et al., 2007).

The investigation (referred to as "the study" hereafter) presented in this report is Stage 2 of the research project to characterize the growth and verify effectiveness of the bacterium *Clostridium phytofermentans,* a newly isolated mesophilic anaerobic bacterium by Warnick et al. in 2002. The following basic facts are reported by Joint Genome Institute (2007).

> *C. phytofermentans* is of particular interest for the production of high concentrations of ethanol during cellulose fermentation. Two to four times more ethanol than acetate are formed (on a molar basis), suggesting that *C. phytofermentans* possesses unusual fermentation pathways. Hydrogen production approaches expected maximum amounts based on the amounts of non-gaseous products formed.

1.2 Study Objectives

C. phytofermentans has received considerable attention for its potential application in producing ethanol directly from cellulose. However, the available literature on *C. phytofermentans* is very limited, as it was recently characterized. The ultimate goal of our research project is to reach an understanding of the appropriate parameters that will enable *C. phytofermentans* to efficiently digest the organic waste in a single stage process to produce ethanol and hydrogen. Therefore, the study presented in this report will first verify the bacterium *C. phytofermentans* kinetics of biomass growth, substrate utilization, and bioproduct formation and identify the bacterium optimal growth conditions. The specific objectives of the study can be summarized as follows:

- Conduct a thorough literature review on cellulosic degradation to develop our experimental plan and mathematical modeling approach;
- Carry out a series of experiments under various conditions of pH and initial cellulose concentrations to examine the growth kinetics and identify the optimal growth conditions of the bacterium *C. phytofermentans*; and
- Develop mathematical models for the bacterium growth kinetics to have a better quantitative understanding of the kinetics of the process, which is important in predicting the performance of a system and reducing the amount of experimental work necessary to design or optimize a process.

It is foreseen that the results obtained in this study will enhance the overall understanding of the kinetics of growth and substrate utilization and product formation of *C.*

phytofermentans, and provide important information on the design of the bench-scale anaerobic bioreactor for future studies

1.3 Project Team

The project team is composed by the following three parties, as shown in Figure 1-1.

- Interact research program facilitator, Ontario Centres of Excellence;
- Industrial partner and sponsor, Clean 16 Environmental Technologies Corp.; and
- Academic partner, Ryerson University.

The study is led by Dr. G. Luk, a professor and the program director of Graduate Studies at the Department of Civil Engineering at Ryerson University. Clean 16 Environmental Technologies Corp. brings the resources of seasoned veterans in clean technology development and implementation and provides the funding for this research project. Ontario Centres of Excellence builds industry and academic relationships and provides guidance and coordination for the parties in the interact programs.

Under Dr. Luk's guidance and supervision, six Master's students at Ryerson University, Benjamin Percy, Mina Mirzajani, Mandana Ehsanipour, Robin Luong, Bonnie Wilkinson and I were working on the project. Benjamin worked on the feasibility study (Stage 1) for his undergraduate project. He is the most experienced person among the academic research team members. In addition to the experimental works, Benjamin developed experimental plans and prepared sampling procedures. Most of the team members participated in all the tasks in the study with different degree of involvement, including literature review, carrying

out experiments, analysing experimental data, and model development. I was involved intensively in the literature review, review experimental data, mathematical model development and project reporting.

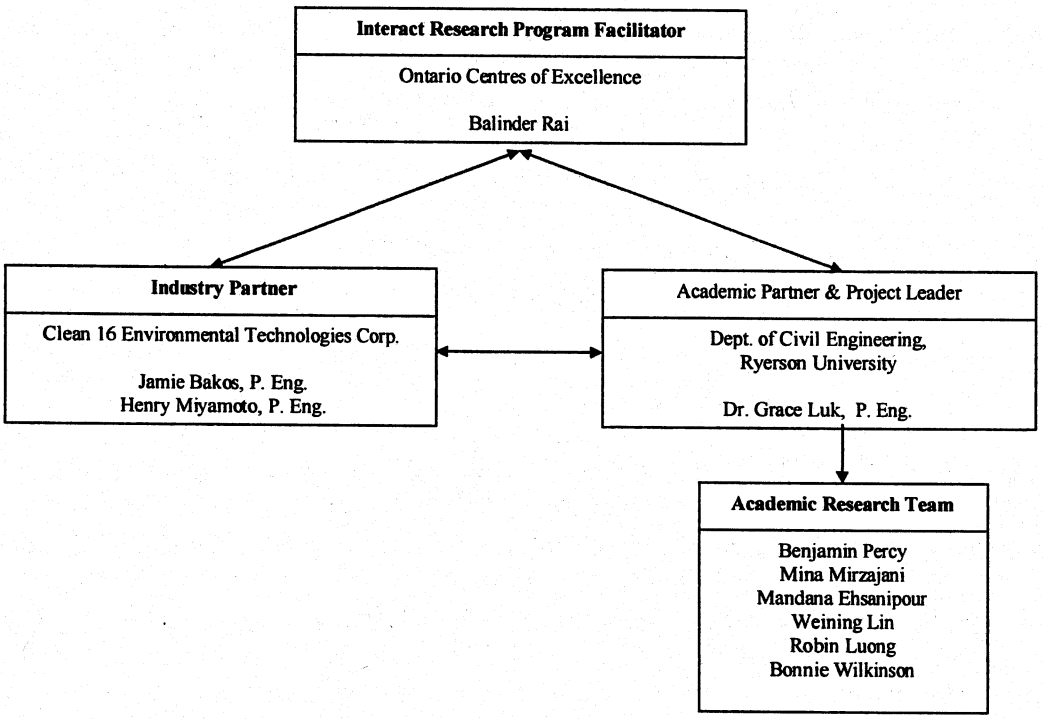

Figure 1-1 Project team

2 Literature Review

Based on the purpose of this study, the literature review focused on the 1) clostridium bacteria, 2) growth kinetic modelling, 3) factors affecting the growth kinetics, 4) experiment methods, and 5) analytical techniques used in the characterization of biomass growth. Although the investigation of ethanol production was not included in the experiment of the current study, literature review on 6) biomass ethanol production was carried out to prepare for the proposed research project.

2.1 Clostridium Bacteria

The main objective of the literature review on clostridium bacteria is to select a clostridium species grown on cellulose substrate so that the ethanol can be effectively produced by decomposing municipal organic wastes. As a result of this review, the type of substrate and the species of clostridium bacteria are determined for our experiments.

2.1.1 Cellulose as A Substrate

Organic wastes from municipal solid waste contain various kinds of organic substances, among which cellulose is the most prominent, and form about 60% of the dry weight of a typical municipal solid waste stream (Kalogo et. al., 2007; Noike et al., 1985). Cellulose is the most abundant and inexpensive biomass available. Because the potential supply of cellulosic biomass is far greater than of food crops and competing uses for biomass are limited, bioethanol should be able to make a major impact on transportation fuel markets. Therefore, studying the growth kinetics of clostridium bacterium that is grown on cellulosic

substrates will be beneficial to address both solid waste management and bioenergy production issues concurrently.

Cellulose is a linear insoluble biopolymer composed of the repeated union of β-D-glucopyranose linked by β-1,4 glycosidic bonds, as shown in Figure 2-1(a). Consequently, and in contrast to other glucan polymers such as starch or callose, the repeating structural unit in cellulose is not glucose (glucanohydrolases) but the disaccharide cellobiose (cellobiohydrolase). With a degree of polymerisation ranging from 2 to 7, the β-1,4 glucose oligomers, also called cellodextrins or cello- oligosaccharides, are water soluble (Pereira et al., 1988). In cellulose, the glucan chain can reach a length of more than 25,000 glucose residues (Brown et al., 1996). The association of cellulose macromolecules leads to the formation of a microfibril containing 15–45 chains in a regular crystalline arrangement, as shown in Figure 2-1(b). At the microscopic scale, the association of these microfibrils formed a cellulose fibril also called macrofibril or fibre at the macroscopic scale (Ljungdahl and Eriksson, 1985). Moreover, cellulose fibres contain various types of irregularities such as twists or voids, which increase their total surface area (Desvaux, 2005).

Despite its low density, cellulose is the most prominent, resistant and stable natural-organic compound known; consequently, it tends to accumulate in the environment (Bayer and Lamed, 1992). According to the estimation in the study by Cox et al. (2000), the net primary production of biomass in terrestrial ecosystems would be of 60 milliard tonnes of carbon per year and about half of this carbon would be fixed under the form of cellulose (Desvaux, 2005).

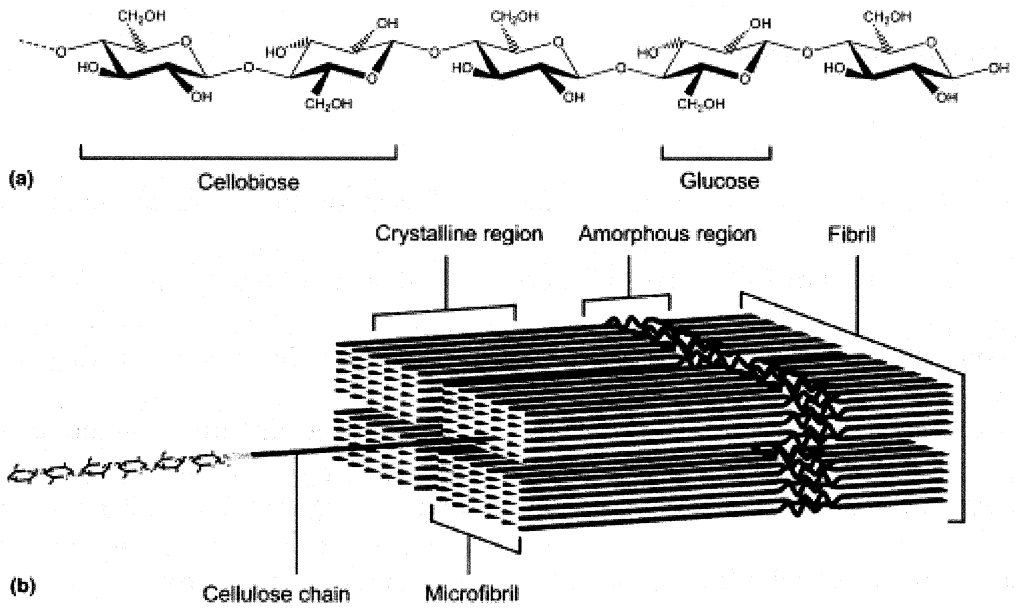

Figure 2-1 Schematic representation of structure of (a) cellulose. (b) a cellulose fibril (Desvaux, 2005)

2.1.2 Cellulose Degradation

Cellulose almost never occurs alone in nature but it is usually associated with other plant substances. This association may affect its natural degradation. Vast quantities of cellulose are degraded by cellulose-fermenting microorganisms in anaerobic environments (Leschine,1995). Anaerobic activity starts close to the surface in most environments, such as in soils, composts and aquatic environments, indicating that aerobic conditions normally prevail only in a thin crust at the atmospheric boundary. In the environment, cellulose is essentially degraded by microorganisms where the final products of the conversion are H_2O and CO_2 in aerobic conditions and also methane CH_4 in anaerobiosis (Ljungdahl and Eriksson, 1985; Wolin and Miller, 1987).

In considering processing of cellulosic biomass and organism development for consolidated bioprocessing, researches on organisms producing reduced metabolic products via an effectively anaerobic metabolism have been focused because this is responsive to the needs, constraints, and opportunities associated with microbial conversion of cellulosic feedstocks (Lynd et al., 2002).

Similar to the wastewater processes, the first step in the cellulose degradation is using cellulolytic microbes to produce enzymes that depolymerise cellulose into soluble sugar units, such as cellobiose, cellodextrins, and some glucose. Under anaerobic conditions, soluble organics are decomposed to intermediate end products, such as organic acids (e.g. acetate, propionate, butyrate) and alcohols, along with the production of CO_2 and water, as expressed in Anaerobic Reaction (1).

Anaerobic Reaction (1): Organics $\rightarrow$ intermediates + CO_2 + H_2O + energy

If proper environmental conditions exist to prevent excess acidity from the production of organic acid intermediates, populations of acid–splitting, methane-forming bacteria will develop and use the organic acids as substrate, as shown in Anaerobic Reaction (2) (Viessman and Hammer, 2005).

Anaerobic Reaction (2): Organic acid intermediates $\rightarrow$ CH_4 + CO_2 + energy

Very little H_2 escapes into the atmosphere in the process since it is immediately consumed by methanogens or homoacetogens. Methanogens use H_2 to reduce CO_2 to CH_4, and homoacetogens use H_2 to reduce CO_2 to acetate. Some methanogenic species use acetate produced by fermenters or by homoacetogens through the acetoclastic cleavage to CH_4 and CO_2 (Jones et al., 1987; Leschine and Canale-Parola 1984). Syntrophic bacteria play a key role in the conversion of cellulose to CH_4 and CO_2. These organisms ferment fatty acids such as propionate and butyrate, or alcohols, and produce acetate, CO_2, and H_2. They grow only in the presence of H_2-consuming organisms through interspecies H_2 transfer. Syntrophic bacteria grow very slowly, and thus the fermentation of fatty acids is usually the rate-limiting step in the anaerobic decomposition of cellulose (Miller, 1991; Wolin and Miller, 1986). In the cellulose degradation processes, lactate, succinate, and ethanol are also produced by fermentative bacteria but usually do not accumulate (Leschine, 1995). Through the combined activities of several major physiological groups of microbes, cellulose is completely dissimilated to CO_2 and CH_4. Thus, as a source of CO_2 and CH_4, the anaerobic decomposition of cellulose plays a major role in carbon cycling on the planet (Leschine and Canale-Parola 1989; Ljungdahl and Eriksson, 1985).

In summary, cellulose degradation under anaerobic conditions is the combination of biological processes of decomposition of raw organic matter to soluble organic intermediates and the gasification of the intermediates to CO_2 and CH_4. This process is also diagrammatically presented in Figure 2-2.

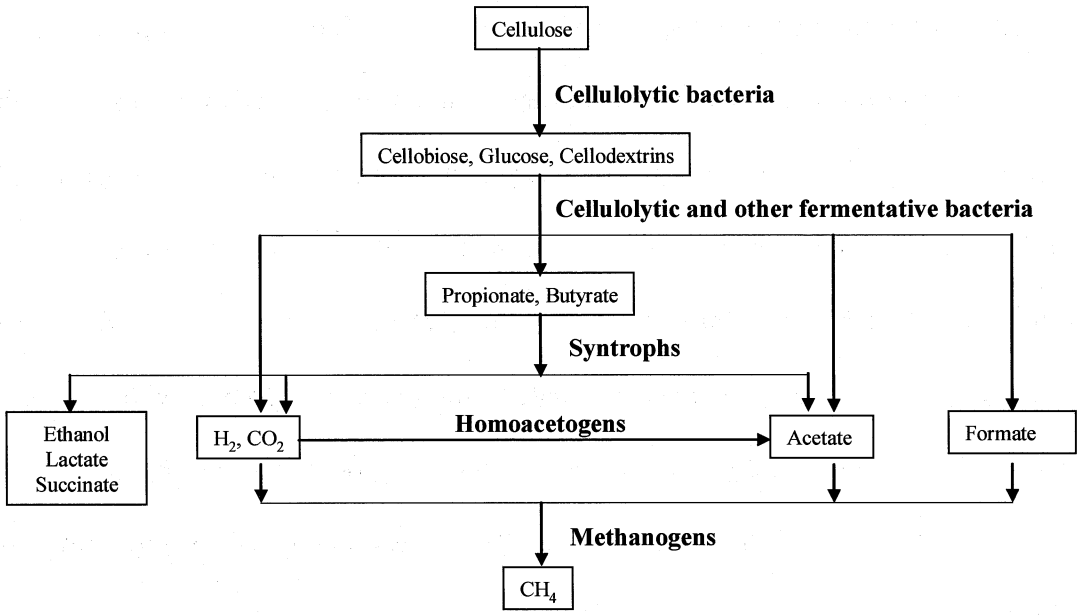

Figure 2-2 Diagrammatic representation of anaerobic cellulose degradation (modified Leschine, 1995)

2.1.3 Clostridium Bacteria

Study of cellulose degradation has been an area of active research for more 50 years. The focus of this research has frequently shifted. With the energy crises in the 1970s, the focus of research on cellulose biodegradation shifted to developing systems and procedures to use cellulose and other abundant plant polymers as a source of fuels and chemicals that could serve as a potential replacement for fossil hydrocarbons (Leschine, 1995).

Among the more than 600 cellulase genes known (Schwarz, 2001), there is a distinct difference in cellulolytic strategy between the aerobic and anaerobic groups. Generally, only a few species are actively cellulolytic. The distribution of cellulolytic capability among organisms differing in oxygen relationship, temperature, and salt tolerance is a

testament to the wide availability of cellulose across natural habitats (Lynd et al., 2002). Among cellulolytic microorganisms, bacteria of the class Clostridia occupy a place of choice. These bacteria (i) are everywhere in cellulosic anaerobic environments (Leschine, 1995), (ii) digest cellulose very efficiently via an extracellular enzymatic complex called the cellulosome (Schwarz, 2001), (iii) can convert cellulose into a large variety of metabolites notably ethanol (Mitchell, 1998), and (iv) can therefore be used in direct microbial conversion process, i.e. in consolidated bioprocessing (Lynd, 1996). However, the recursive interest in cellulose utilisation for bioenergetic prospects, notably for the production of H_2, CH_4 or biofuel, requires a better understanding of the physiology and metabolism of these bacteria (Desvaux, 2005).

After a preliminary review, based on our study purpose a detailed literature review was focused on the following three cellulolytic bacteria.

Clostridium thermocellum is the best characterized thermophilic anaerobic bacterium capable of the complete degradation of cellulose (Ljungdahl et al., 1981). Viljoen, Fred and Peterson (1926) first described *C. thermocellum* after isolating it from horse manure in 1926. However, it took about 25 years until a pure culture was obtained (McBee, 1950). The difficulty in isolating it from thermophilic glycolytic strains is because *C. thermocellum* is widespread in nature and its habitat is organic material in decomposition.

C. thermocellum grows in complete anaerobiosis and in the thermophilic temperature range. The optimum temperature for growth is 60-64°C (Krieg and Holt, 1984) and the

optimum pH ranges from 6.1 to 7.5 (Freier et al., 1988). The microorganism grows slowly. When grown on cellulose, the shortest doubling time reported is 7 hours (Wiegel and Ljungdahl, 1986; Freier et al., 1988; and Maugeri and Goma, 1988). In a set of batch studies, stationary phase growth was reached after 11 days of fermentation (Maugeri and Goma, 1988).

C. thermocellum degrades several forms of cellulose at different rates. Purified or treated cellulose is degraded at a higher rate than the unprocessed polymer in microcrystalline form. Besides cellulose, this microorganism can also degrade hemicellulose, cellobiose, and xylose oligomers. Sugars, such as glucose, fructose, and xylose, are degraded after adaptation of the culture. The enzymes that degrade the monomeric sugars are induced only after a long adaptation time. The main products of cellulolytic fermentation with *C. thermocellum* are glucose, cellobiose, lactic acid, acetic acid, formic acid, ethanol, CO_2, and H_2 (Freier et al., 1988).

One of the research groups actively working with *C. thermocellum* is leading by Lynd and Zhang. Their researches have been on going since 1980s. In the early stage of their research, limited by the availability of molecular techniques, the central body of thought in microbial physiology was occupied with phenomena such as the rate of cell growth and substrate utilization, the overall stoichiometry of substrate utilization and product formation, cell yields and the thermodynamic efficiency of cell synthesis, substrate utilization for cell maintenance, synthesis of key catabolic enzymes in response to cultivation conditions (e.g., substrate availability and growth rate), celllysis and death, and

the extent of metabolic coupling. In their recent researches, the focus of physiological studies is largely at the molecular level to expend knowledge of cellulose enzymes (ie. the molecular details of cellulose hydrolysis) and understand interactions among cellulase components. This includes a better understanding of synergistic interactions for an increasing number of noncomplexed cellulase systems, as well as a better and broader understanding of the structure and composition of cellulosomes (Lynd et al., 2002).

Although much of the work on cellulose degradation has involved thermophiles such as *C. thermocellum,* these bacteria probably play a minor role in cellulose decomposition in most natural environments because they require unusually high temperatures (greater than 50°C) for growth and cellulose fermentation (Leschine, 1995). Cellulose degradation can occur in common natural environments by employing mesophilic cellulose fermenting microorganisms.

Clostridium cellulolyticum is a mesophilic bacterium isolated from decayed grass and capable of degrading crystalline cellulose. Researches have demonstrated that this mesophilic cellulolytic bacterium had an optimum growth temperature of 34°C with a minimum and maximal growth temperature of 25 and 45°C respectively (Petitdemange et al., 1984). In addition to cellulose, this bacterium can grow on xylan, soluble cellodextrins (from cellobiose to cellohexaose), glucose, xylose and weakly on some other sugars found in the hemicelluloses such as arabinose, fructose, galactose, mannose and ribose. A variety of carbohydrates is fermented by this mesophilic anaerobe. The major fermentation

products from cellulose are carbon dioxide, hydrogen, ethanol, acetate, lactate, and formate (Petitdemange et al., 1984).

Petitdemange and his colleagues, Desvaux and Guedon, have been actively investigating *C. cellulolyticum* since 1980s. In their recent review on *C. cellulolyticum,* it is suggested that contrary to some assumptions of the enzymatic paradigm (Lynd et al., 2002), their investigations clearly indicate that cellulose depolymerisation is not the limiting step of microbial cellulose digestion by *C. celluloyticum.* Improvement of cellulolysis by *C. cellulolyticum* must primarily focus on bacterial metabolism rather than catalytic activity of cellulosome (Desvaux, 2005).

Clostridium phytofermentans, insolated by T. Warnick and S. Leschine in 2002, is an anaerobic ethanol- and hydrogen-producing mesophilic cellulolytic bacterium from forest soil that is capable of fermenting all major carbohydrate components of biomass. Cellulose, pectin, starch, and xylan are rapidly degraded and fermented with ethanol, acetate, CO_2 and H_2 formed as major metabolic products. The optimum temperature for growth is 35–37°C. Phylogenetically, *C. phytofermentans* is a member of Cluster XIVa of the low-G+C-content Gram-positive bacteria, only distantly related to *Clostridium thermocellum*, a cellulose-fermenting microbe with draft genome sequence determined by the U.S. Department of Energy Joint Genome Institute (2007).

C. phytofermentans is of particular interest for the production of high concentrations of ethanol during cellulose fermentation. It produces two to four times more ethanol than acetate (on a molar basis), whereas most other cellulolytic clostridia produce roughly equal

amounts of these fermentation products or less ethanol than acetate. These results suggest that pathways for fermentation product formation in *C. phytofermentans* may differ from those typically found in cellulolytic clostridia. Hydrogen production approaches expected maximum amounts based on the amounts of non-gaseous products formed. Furthermore, *C. phytofermentans* lacks detectable restriction endonucleesase activities common in other cellulolytic clostridia. This observation, along with results of other experiments, suggests that *C. phytofermentans* is amenable to genetic manipulation (Joint Genome Institute, 2007). The capability of *C. phytofermentans* remaining active even at high concentrations of carbohydrates is very attractive for the consolidated bioprocessing of cellulosic production, at which *C. phytofermentans* can ferment the raw cellulosic material into a fuel directly.

The characteristics of cellulose degradation and ethanol production exist commonly in the mesophilic clostridium species of *C. cellulolyticum* and *C. phytofermentans.* In a most recent research by Ren et al. (2007), six mesophilic species of Clostridium, including *C. cellulolyticum, C. phytofermentans* and other four C. strains, were selected to characterize the cellulolytic and hydrogen-producing activities. The results presented in Table 2.1 are obtained from the standardized batch experiments using three substrates, MN301 cellulose, Avicel and cellobios. It can be seen that *C. cellulolyticum* produces higher H_2 than *C. phytofermentans* by 12% to 48%, while *C. phytofermentans* produces higher ethanol than *C. cellulolyticum* by 29% to 40%. Among the six Clostridium species *C. phytofermentans* produces the highest ratios of ethanol to acetic acid, which represent approximately 2 to 2.5

times higher than *C. cellulolyticum.* Ethanol to acetic ratios of 0.58 and 1.03 were reported in batch cultures of *C. phytofermentans* depending on the substrate.

Table 2.1 Molar-based metabolites variation and carbon and electron recovery (Ren et al., 2007)

Clostridium species	Substrate	Electron recovery (fraction)	Carbon recovery (fraction)	Degraded substrate	H_2	Biomass	CO_2‡	Acetone	Ethanol	*n*-Propanol	Acetate	Formate	Butyrate
Cl. acetobutylicum	MN301	ND†	ND	ND	<4	<1·2	ND	ND	ND	ND	ND	ND	ND
	Avicel	ND	ND	ND	<4	<1·2	ND	ND	ND	ND	ND	ND	ND
	Cellobiose	0·89	0·89	23·1	52·3	9·5	26·8	<0·2	1·7	<0·2	14·2	<0·2	6·4
Cl. cellulolyticum	MN301	0·82	0·82	18·3	31·0	6·1	19·8	<0·2	4·1	0·8	17·2	<0·2	<0·1
	Avicel	0·82	0·80	17·4	27·4	6·2	17·9	<0·2	5·2	0·6	14·5	<0·2	<0·1
	Cellobiose	0·80	0·77	22·3	40·6	6·2	24·8	<0·2	8·2	<0·2	18·6	0·2	<0·1
Cl. cellobioparum	MN301	0·72	0·76	12·8	18·6	4·2	15·0	<0·2	2·6	<0·2	8·9	<0·2	0·8
	Avicel	0·70	0·73	13·9	15·8	4·1	15·4	<0·2	4·8	<0·2	7·8	<0·2	1·0
	Cellobiose	0·81	0·78	21·8	42·3	6·7	26·0	<0·2	7·8	<0·2	15·9	<0·2	0·6
Cl. celerecrescens	MN301	0·74	0·74	16·7	25·1	5·7	17·2	0·3	5·0	<0·2	8·5	<0·2	1·5
	Avicel	0·77	0·79	15·6	19·9	4·9	17·4	0·2	5·7	<0·2	7·2	<0·2	2·6
	Cellobiose	0·77	0·76	23·7	38·0	5·5	26·9	0·7	7·9	<0·2	16·6	<0·2	2·0
Cl. populeti	MN301	0·76	0·82	18·4	30·0	6·1	21·4	<0·2	<0·2	<0·2	6·5	<0·2	7·8
	Avicel	0·74	0·82	18·1	26·2	6·1	22·1	<0·2	<0·2	<0·2	3·3	<0·2	9·1
	Cellobiose	0·79	0·83	23·8	45·3	7·5	27·8	<0·2	<0·2	<0·2	16·5	<0·2	6·7
Cl. phytofermentans	MN301	0·83	0·83	13·9	19·3	4·9	14·7	<0·2	6·1	<0·2	10·6	1·2	<0·1
	Avicel	0·73	0·72	14·1	14·2	4·0	12·9	<0·2	7·3	<0·2	8·0	1·1	<0·1
	Cellobiose	0·80	0·77	22·6	35·6	5·9	27·1	<0·2	13·6	<0·2	13·2	<0·2	<0·1

Note: unless noted, the units for all data are mM.
†ND, Not determined.
‡CO_2 is the sum of headspace CO_2 and dissolved CO_2 (all species).

Desvaux et al. (2000) performed an investigation of cellulose degradation by *C. cellulolyticum* in a bioreactor with pH control of the batch culture in MN301 cellulose medium. A series of fermentation experiments was conduced with various initial cellulose concentrations of 5.6, 14.8, 24.1, 41.4, 78.4, 116.07, and 179.6 mM (in glucose equivalents). Depending on cellulose concentration, the carbon flow distribution was affected, showing the high flexibility of the metabolism. With less than 41.4 mM, acetate, ethanol, H_2, and CO_2 were the main end products of the fermentation and cellulose degradation reached more than 85% in 5 days. The electron flow from the glycolysis was balanced by the production of H_2 and ethanol, the latter increasing with increasing initial cellulose concentration. From 41.4 to 179.6 mM, the percentage of cellulose degradation

declined; most of the cellulase activity remained on the cellulose fibres, the maximum cell density levelled off, and the carbon flow was reoriented from ethanol to acetate.

This result of the degree of cellulose degradation decreasing with increasing substrate concentration contrasts with the result obtained using *C. phytofermentans* by Leschine and Warnick (2007). In one of their experiments, *C. phytofermentans* was grown in culture tubes anaerobically in GS-2 cellulose medium at an initial pH of 7.5 under an atmosphere of N_2. The initial *C. phytofermentans* concentration was about 0.8 - 1.1 x 10^7 cells/mL and the temperature of incubation was 30^0C. Three initial cellulose concentrations of 37, 74 and 148 mM (in glucose equivalents) were examined. The result shows the concentration of ethanol, acetate, formate, and lactate upon completion of cellulose decomposition as a function of initial cellulose concentration. (1) At an initial cellulose concentration of 37 mM, the concentrations of lactate, acetate, and ethanol, were 4 mM, 20 mM, and 59 mM, respectively. Formate was not detectable at this initial concentration. (2) At an initial cellulose concentration of 74 mM, the concentrations of lactate, formate, acetate, and ethanol, were 7 mM, 10 mM, 20 mM, and 123 mM, respectively; and (3) at a concentration of 148 mM, the concentrations of lactate, formate, acetate, and ethanol, were 10 mM, 17 mM, 20 mM, and 160 mM, respectively. This result shows that high concentrations of cellulose do not inhibit the action of *C. phytofermentans* , since the concentration of ethanol increases with increasing initial concentration of cellulose. It is also notable that when using *C. phytofermentans* the acetate levels do not significantly increase with increasing initial concentration of cellulose, which can be advantageous because more of the cellulose goes into making the more economically valuable ethanol. This is in sharp contrast to other

cellulolytic bacteria, which produce less ethanol than acetate (on a molar basis) and ethanol to acetate ratios decrease with increasing initial cellulose concentrations (for example, see Desvaux et al. above).

The results of ethanol and acetate using *C. cellulolyticum and C. phytofermentans* by Desvaux et al. (2000) and Leschine and Warnick (2007) respectively are shown in Figure 2-3. It suggests that the pathways for fermentation product formation in *C. phytofermentans* may differ from those typically found in cellulolytic clostridia by its ability to utilize starch.

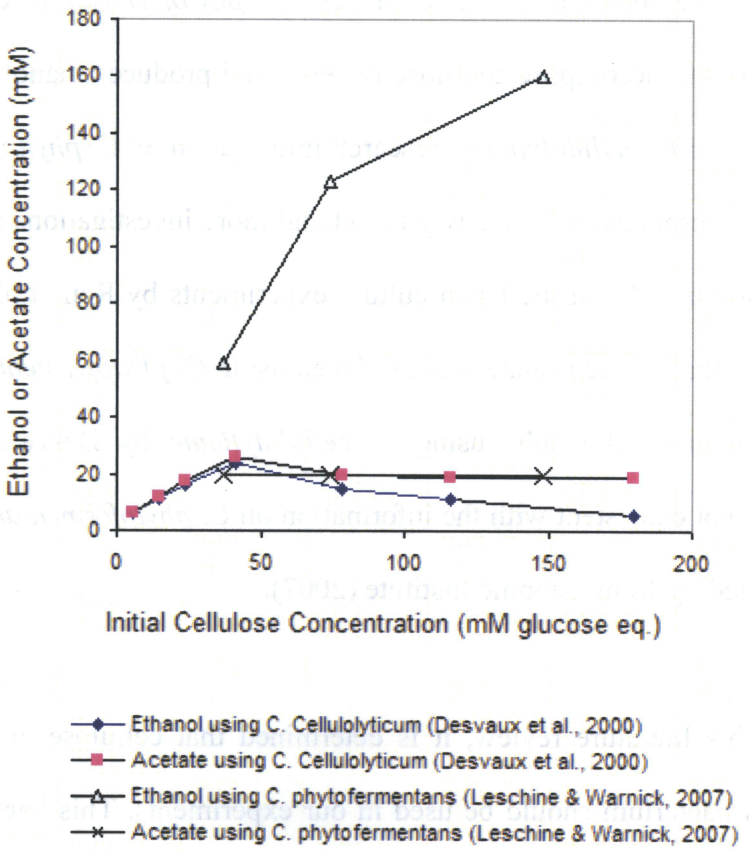

Figure 2-3 Cellulose fermentation products of ethanol and acetate by *C. cellulolyticum* and *C. phytofermentans*

Some of the investigation results on kinetics and ethanol fermentation using *C. cellulolyticum and C. phytofermentans* are presented in Table 2.2. It should be noted that there was not enough detailed explanations on how these parameters were obtained or calculated, except for Desveaux *et al.* (2000). He noted that "*the values were the maximum ones encountered during the entire incubation time and were not calculated over the same period of time*". Most data in the table were not obtained under identical conditions with respect to apparatus, substrate, and analytical methods; therefore, caution is appropriate when making comparative observations. Nevertheless, these data provide a quantitatively comparable growth characterization and ethanol-producing capabilities of these strains.

Based on the above–mentioned characteristics, *C. phytofermentans* appears to be an effective bacterium to decompose cellulose material and produce ethanol. However, unlike *C. thermocellum* and *C. cellulolyticum,* research information on *C. phytofermentans* is very limited up to the present since it is newly found and more investigations and confirmations are required. For example, in the batch culture experiments by Ren et al. (2007), roughly equal amount of ethanol and acetate was produced using *C. phytofermentans.* This is very close to the experimental results using *C. cellulolyticum* by Desveaux *et al.* (2000). However, this is not consistent with the information on *C. phytofermentans* provided in the basic facts reported by Joint Genome Institute (2007).

As a result of this literature review, it is determined that cellulose, a substrate, and *C. phytofermentans* bacterium should be used in our experiments. This bacterium is an ideal digester for several reasons, according to Warnick et al. (2002):

1. It is cellulolytic, meaning that it can directly hydrolyze cellulose and does not require assistance from other bacteria to break down cellulose.
2. Ethanol, acetate, CO_2 and H_2 are the major metabolites of the celluose fermentation using this bacterium, which produces higher ratios of ethanol to acetic acid than other cellulolytic clostridia.
3. High concentrations of cellulose do not inhibit the action of *C. phytofermentans* .
4. It is easy to achieve mesophilic temperature conditions without excessive cost.

Table 2.2 Kinetics and ethanol fermentation of mesophilic clostridium bacteria

Mesophilic clostridium species	Growth condition	Temper-ature (^{0}C)	pH	Substrate	Initial Substrate conc. in mM or (g/L)	μ_{max} (h^{-1})	Ethanol (mM)	Acetate (mM)	Ratio of ethanol : acetate (molar-based)	Cellulose degradation	Reference
C. cellulolyticum	Batch	34	7.2	MN301	5.6 (0.9)	0.057	6	6.5	0.92 : 1	91% after 5 days	Desvaux et al. (2000)
					14.8 (2.4)		11	12	0.92 : 1	93% after 5 days	
					24.1 (3.9)		16.5	17.5	0.94 : 1	90% after 5 days	
					41.4 (6.7)		24	26	0.93 : 1	85% after 5 days	
					78.4 (12.7)		14.5	20	0.73 : 1	52% after 5 days	
					116.07 (18.9)		11	19	058 : 1	30% after 5 days	
					179.6 (29.1)		5.6	19	0.3 :1	21% after 5 days	
	Continuous	34	7.2	MN301	11.5-166.7 (1.9 – 27)	0.083	1.73-4.15	3.5-16.04	0.49-0.26 : 1	32.3% - 8.3%	Desvaux et al. (2001), Lynd et al. (2002)
	Batch	35	6.5	MN301	32.7 (5)	0.021	4.1	17.2	0.24 : 1	56% after 14 days	Ren et al. (2007)
				Avicel	37.8 (5)	0.038	5.2	14.5	0.36 : 1	46% after 14 days	
C. phytofermentans	Batch	35	6.5	MN301	(5)	(a)	6.1	10.6	0.58 : 1	(a)	Ren et al. (2007)
				Avicel	(5)		7.3	8.0	0.91 : 1		
	(a)	30	7.5	GS-2	37	(a)	59	20	2.95 :1	(a)	Leschine and Warnick (2007)
					74		123	20	6:1		
					148		160	20	8:1		

Note: (a) information not available from the reference

2.2 Kinetic Modeling of Biomass Growth and Production Formation

One of our goals in this study is to develop mathematical models for the bacterium *C. phytofermentans* growth kinetics under different environmental conditions. In this section, several popular models and model selection are discussed and appropriate models are selected for our study.

Biomass growth kinetic modeling is to use a set of equations which describes the mathematical relation between the specific growth rate (μ) of biomass and the substrate concentration (S) and is an essential tool in all fields of microbiology, physiology, genetics, ecology, or biotechnology. According to Kovarova and Egli (1998), the principles and definitions of growth kinetics are frequently presented as if they were firmly established in the 1940s by Monod and during the following "golden age" in the 1950s and 1960s. This state of affairs is probably the consequence of stagnation in this area during the past three decades, in which the interest of many researchers was attracted by rapidly developing areas such as molecular genetics or the biochemistry of the degradation of xenobiotics. However, it might also be the consequence of certain frustration from the many attempts that had been made to obtain coherent experimental data.

Kinetic models are normally divided into two classes: structured and unstructured one. The development of structured (mechanistic) models for quantifying microbial growth kinetics is still limited because the mechanism of cell growth is very complex and is not yet completely understood (Kovarova and Egli, 1998). Therefore, most of the proposed growth models are unstructured and empirical, in which microorganisms are usually considered to

be a component or reactant in the system. This approach is the most frequently employed for modeling microbial systems because they are simple, but are good enough for technical purposes since they provide a framework helpful in identifying types of information required to develop a more complete understanding of microbial cellulose utilization at both the conceptual and quantitative levels. Depending on the purpose at hand, either a relatively simple or complex model may be sufficient (Chen et al., 2001; Hu et al., 2002; Lynd et al., 2002; Fujikawa et al., 2004; Mu et al., 2006; and Kong et al., 2006).

2.2.1 Cell Growth and Substrate Degradation

The two most widely accepted un-structural or empirical biomass growth kinetic models are the Logistic equations and the Monod model. An overview of these two models is presented as follow.

2.2.1.1 Logistic-type kinetics

Biomass Growth

The biomass growth of a given cell culture is presented with the well known ordinary differential form of the logistic equation:

$$\frac{dX}{dt} = \mu X \qquad [2.1]$$

where

μ = specific growth rate (rate of growth per unit of biomass), time^{-1}

X = concentration of biomass, mass/unit volume

Equation [2.1] thus implies that X increases with time regardless of substrate availability. However, the real growth kinetics is governed by a hyperbolic relationship, and there is a limit to the maximum attainable biomass concentration due to the interspecies competition and the influence of limiting factors on the growth rate. Taking all these factors into account, the logistic equation [2.2] was introduced (Mulchandani and Luong, 1988; Sakanoue, 2007).

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_{\max}}\right) \qquad [2.2]$$

where the new introduced symbol is:

X_{max} = the maximum attainable microbial biomass concentration, mass/unit volume

Equation [2.2] is indeed a famous one in population theory introduced by Verhulst (1838), Pearl and Reed (1920) independently. Verhulst-Pearl equation has been utilized as a fundamental growth model in ecological studies because of its mathematical simplicity and simple biological definition.

Integration of Equation [2.2] gives the following equation for microbial concentration:

$$X = \frac{X_0 e^{\mu t}}{[1 - (X_0 / X_{\max})(1 - e^{\mu t})]} \qquad [2.3]$$

Using $X_o = X_{(t=0)}$, the initial biomass concentration, Equation [2.3] gives a sigmoid variation of X as a function of time (t) which may represent both an exponential and a stationary phase (Weiss and Oills, 1980; Kong et al, 2006).

Rearrangement of Equation [2.3] yields the logarithmic form as follows:

$$\ln\frac{X}{X_{\max}-X} = \mu t - \ln\left(\frac{X_{\max}}{X_0}-1\right) \qquad [2.4]$$

The value of X_{max} can be obtained from the experimental data. Plotting the left side of Equation [2.4] against time (t) will produce a line of slope μ and y-intercept equal to $\ln\left(\frac{X}{X_{\max}-X_0}-1\right)$, from which the initial viable inoculum size, X_0, can be calculated. (Mu et al., 2006 and King et al., 2006).

Once X_o and μ are obtained, $X(t)$ can be determined by Eq. [2.3].

Substrate Utilization

A substrate is used to form cell material and metabolic activity as well as the maintenance of cells. In a recent kinetic model study by Kong et al. (2006), a logistic Luedeking-Piret-like model, in which the substrate used for product formation is assumed to be negligible, is used to describe the link between growth and substrate utilization, as shown in [2.5].

$$-\frac{dS}{dt} = \gamma \frac{dX}{dt} + \delta X \qquad [2.5]$$

where the new introduced symbols are:

S = concentration of growth-limiting substrate in solution, mass/unit volume

γ = growth-associated substrate degradation coefficient, mass/mass

δ = non-growth-associated substrate degradation coefficient, mass/mass *time

The two parameters, γ and δ, indicating the dependence of substrate degradation on biomass growth and biomass concentration, vary with degradation process. The benefit of this model is that δ can be calculated using the following equation at the stationary phase when dX/dt =0, and $X = X_{max}$:

$$\delta = -\frac{(dS/dt)}{X_{max}} \qquad [2.6]$$

To obtain γ, Equation [2.5] is rearranged to [2.7]:

$$-dS = \gamma\, dX + \delta \int X(t)dt \qquad [2.7]$$

Substrate concentration can be calculated from the integration of [2.7] and yield the following equation.

$$S = S_0 - \gamma\, C(t) - \delta\, D(t) \qquad [2.8]$$

where $S = S_0$ at $t = 0$, and

$$C(t) = X_o \left[\frac{e^{\mu_{max} t}}{1 - \left(\frac{X_o}{X_{max}} \right)(1 - e^{\mu_{max} t})} - 1 \right] \quad [2.9]$$

$$D(t) = \frac{X_{max}}{\mu_{max}} \ln \left[1 - \frac{X_0}{X_{max}} \left(1 - e^{\mu_{max} t} \right) \right] \quad [2.10]$$

Plotting $[S_0 - S - \delta D(t)]$ against *C(t)*, a line with a slope of γ can be obtained.

In the study by Tam and Finn (1977), substrate used for product formation is added to Equation [2.5] and shown as follows:

$$-\frac{dS}{dt} = \gamma \frac{dX}{dt} + \lambda \frac{dP}{dt} + \delta X \quad [2.11]$$

where the new introduced symbols are:

P = product formation, mass / unit volume

λ = product formation -associated substrate degradation coefficient, mass/mass

In addition to Equation [2.5], Equation [2.11] signifies that substrate concentration may continue to diminish due to product formation and to maintenance even after biomass concentration reaches stationary phase.

2.2.1.2 Monod-type kinetics

Biomass Growth

During the last half century, the concepts in microbial growth kinetics have been dominated by the relatively simple empirical model proposed by Monod (1942). By laboratory experimentation, Monod studied the growth bacteria in batch cultures. He found that growth was a function of both the concentration of microorganisms and the concentration of the growth-limiting substrate (Viessman and Hammer, 2005). The mathematical relationship proposed by Monod between the residual concentration of the growth-limiting substrate and the specific growth rate of biomass is the hyperbolic equation [2.12].

$$\mu = \frac{\mu_{\max} S}{K_S + S} \qquad [2.12]$$

where the new introduced symbols are:

μ_{max} = maximum specific growth rate (at a concentration of the growth-limiting substrate or above saturation), time^{-1}

K_s = saturation constant (equal to the limiting substrate concentration at one-half the maximum growth rate), mass/unit volume

Substituting the specific growth-rate relationship in Equation [2.12] for the proportionality constant in Equation [2.1], the relationship of biomass growth with the concentration of

microorganisms (X) and the concentration of the growth-limiting substrate (S) is explicitly expressed in Equation [2.13].

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{K_S + S} \qquad [2.13]$$

The original Monod equation does not consider the fact that cells may need substrate even when they do not grow. Various ways have been proposed to account for the substrate threshold value. For example, Equation[2.14] is a modification of the Monod model in which the threshold concentration is subtracted from the actual substrate concentration (Giraldo-Gomez et al., 1992; Frame and Hu, 1991a, b; Kovarova et al., 1996).

$$\mu = \mu_{max} \frac{S - S_{min}}{K_S + S - S_{min}} \qquad [2.14]$$

where the new introduced symbol is:

S_{min} = substrate threshold concentration, mass/unit volume

This model implies that growth is nil when S equals S_{min}, and growth is maximum when S is large. For intermediate concentrations, however, this model leads to a shift with respect to the Monod model, that is similar parameters K_S and μ_{max} predict different growth rates at a specific substrate concentration. Inversely, a fit of both models to the same dataset would yield different values for these parameters (Ribes et al., 2004). As pointed out by Kovarova et al. (1998), such thresholds should not be observed in closed systems like batch cultures

(Tros et al. 1996), because the maintenance requirement of cells implies continued utilization until all available substrate is exhausted.

Substrate Utilization

The link between growth and substrate utilization has already been made by Monod, who linearly related the growth yield (Y), defined as the incremental increase in biomass resulting from metabolism of an incremental amount of substrate (Viessman and Hammer, 2005). Growth yield can be expressed in derivative form as

$$-\frac{dX}{dt} = Y\frac{dS}{dt} \qquad [2.15]$$

where the new introduced symbol is:

Y = growth yield, mass/mass

The growth yield in a batch culture is the biomass increase during the exponential and declining growth phase (X_{max} - X_0) relative to the substrate used ($S_0 - S_{min}$) (Viessman and Hammer, 2005) . Therefore,

$$X_{max} - X_0 = Y(S_0 - S_{min}) \qquad [2.16]$$

Substituting Equation [2.15] into [2.13] results in an equation that defines the rate of substrate utilization in a solution in which the biomass rate is limited by the low concentration of substrate as shown in Equation [2.17].

$$-\frac{dS}{dt} = \frac{\mu_{max} XS}{Y(K_S + S)} \qquad [2.17]$$

2.2.1.3 A brief discussion on microbial growth models

Comparing the Monod model and the logistic equation models, some studies (Mulchandani and Luong, 1987; Lejeune et al., 1998; Mu et al., 2006; and Kong et al., 2006) suggested that the logistic model performs satisfactorily in the representation of microbial growth. On the other hand, some researchers (Noike et al., 1985; Huang and Chou, 1990; Velkovska et al., 1997; and Neill and Gignoux, 2006) attempt to examine the growth and growth–linked biodegradation kinetics as suggested in the Monod model. An excellent overview on growth modelling which describes quantitatively the substrate utilization or growth-associated product formation is given by Bailey and Ollis (1986).

The Monod model differs from the logistic growth models in the way that it introduces the concept of a growth-controlling substrate. He related the growth rate to the concentration of a single growth-controlling substrate via two "organism constants", the maximum specific growth rate (μ_{max}), and the substrate saturation constant (K_s) (Kovarova and Egli, 1998). Whereas the interpretation of μ_{max} as the maximum specific growth rate is straightforward, K_s represents the substrate concentration required to achieve 50% of the maximum growth rate. As suggested in the study by Mu et al. (2006), the application of this value could be used for adjusting the most appropriate substrate concentration in the feed. For most applications, it has turned out that growth or degradation phenomena can be described satisfactorily (usually based on a visual and not a statistical judgment) with four

parameters, the two kinetic parameters, μ_{max} and K_s, and the two stoichiometric parameters, Y and S_{min} (Kovarova and Egli, 1998).

However, because of the difficulties in obtaining accurate substrate concentrations, researchers use logistic-type kinetic model, which is substrate independent, to simulate growth. Mu et al. (2006) suggest that although the predictive power of the logistic model may be limited since it does not involve a substrate term, it is a fair approximation of the microbial growth curve for the batch H_2 production experiments in their study. The initial substrate concentrations and the inoculation volume were constant values in such experiments.

Various modified logistic models were developed for growth kinetics, for example, the Gompertz (1825) model and the Richards (1959) model. Dalgaard and Koutsoumanis (2001) compared four logistic models in their study and found that the Richards model estimated the maximum growth rate μ_{max} values more precisely than other models in the comparison and enabled lag times to be determined from individual absorbance growth curves. Limitations of these models were revealed due to the wide range of growth conditions studies. In the recent study by Sakanoue (2007), he proposed an extended logistic model for growth of single-species populations, which particularly highlights two types of relationship between resources availability and population size. One is the resource supply to a population, and another is the population demand for resources because populations consume resources for constructing and maintaining their size.

Through our literature review it was found that only few papers on mathematical growth kinetics modelling with insoluble substrate (Noike et al., 1985; Huang and Chou, 1990; and Velkovska et al., 1997). It is also very common that researchers modify the original logistic equation and Monod model to fit better with their experimental data.

2.2.2 Product Formation

The logistic model has often been found to express the relationship between lactic acid bacterial specific growth rate and biomass concentration much better (Mercier *et al.* 1992). Many models such as the Luedeking–Piret model (Luedeking and Piret, 1959) and modifications of it (Rogers et al., 1978) have been proposed as kinetic models for lactic acid production. The Luedeking–Piret model originally proposed for describing the lactic acid production by Lactobacillus delbrucckii (Luedeking and Piret, 1959) allows a correlation between the biomass and product concentration as shown in Equation [2.18].

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad [2.18]$$

where the new introduced symbols are:

α = growth-associated product formation coefficient, mass/mass

β = non–growth-associated product formation coefficient, mass/mass * time

Similar to the parameters γ and δ in the Luedeking–Piret model, Equation [2.5], used for substrate utilization estimation, the product formation coefficients, α and β, vary with fermentation conditions. They indicate the dependence of product formation on biomass

growth and biomass concentration. The benefit of this model is that β can be calculated using the following equation at the stationary phase when dX/dt =0, and $X = X_{max}$:

$$\beta = \frac{(dP / dt)}{X_{\max}} \qquad [2.19]$$

The rearrangement of Equation [2.18] gives an expression of product formation as a function of time:

$$dP = \alpha\, dX + \beta \int X(t)dt \qquad [2.20]$$

Product formation can be calculated from the integration of [2.20] and yield the following equation.

$$P = \alpha A(t) + \beta B(t) \qquad [2.21]$$

where $A(t)$ and $B(t)$ are:

$$A(t) = X_o \left[\frac{e^{\mu_{\max} t}}{1 - \left(\frac{X_o}{X_{\max}} \right)(1 - e^{\mu_{\max} t})} - 1 \right] \qquad [2.22]$$

$$B(t) = \frac{X_{\max}}{\mu_{\max}} \ln \left[1 - \frac{X_0}{X_{\max}} \left(1 - e^{\mu_{\max} t} \right) \right] \qquad [2.23]$$

Plotting [P- $\beta B(t)$] in Eq. [2.21] against $A(t)$, the slope of the straight line gives the growth associated product formation constant, α. The estimated values of α and β could be used to predict the productivity in the bioreactor design (Mu et al., 2006). Once the parameters of α, β, $A(t)$ and $B(t)$ are calculated, $P(t)$ can be determined by Eq. [2.21].

Table 2.3 provides a summary of the kinetic models reviewed in this study for biomass growth, substrate utilization, and product formation.

2.2.3 Model Selection

Base on the literature review on kinetic models the following models are selected for our study.

1. **Logistic-type modelling for biomass growth**

   The logistic-type model for growth is substrate independent and only requires the input of biomass concentration (X) and the maximum biomass concentration (X_{max}). These data are relatively easy to be obtained from experiments with confidence on the data accuracy. The model outcome provides biomass concentration at any time $X(t)$ and the maximum growth rate μ_{max}. Successful modelling applications on fermentation process were reported in some of the recent studies, such as Kong et al., (2006) and Mu et al. (2006). Although glucose and sucrose are substrates in their experiments, we examine the application of logistic modelling approach for insoluble substrate in this study.

2. Luedeking-Piret model for product formation

It has been well tested by several fermentation studies that Luedeking-Piret model can represent the kinetics of product formation satisfactorily. The input to the model includes biomass concentration (X), the maximum biomass concentration (X_{max}), and product formation (P). All these data are relatively easy to be obtained from experiments with confidence. The model outcome provides product formation at any time $P(t)$. X_0 and μ, which can be obtained from the growth model, are required in calculation of $A(t)$ and $B(t)$ in Eqs. [2.22] and [2.23].

3. Monod model for substrate utilization

Monod model represents the biological process by relating the biomass growth to the substrate degradation. It requires the input of biomass concentration (X) and the substrate concentration (S), which commonly reliable measurements of S are not available. However, when reliable growth data are available, we can estimate the substrate concentration function $S(t)$ using Monod model with reasonable assumptions. For example, when the growth reaches stationary state, the minimum substrate concentration (S_{min}) can be assumed to be zero (Viessman and Hammer, 2005), and the growth yield (Y) in the Monod's substrate utilization model can be calculated using Eq.[2.16]. Assumptions can also be made based on available experimental data or data from other studies. Moreover, when reliable data are available for both biomass and product formation, S_{min} can be calculated with higher confidence since the biomass growth and product formation are primarily caused by the substrate decomposition. Generally, for the same numbers of unknowns, more

defined solutions can be obtained in the growth phase when more constraints are needed to meet. The outcome from the Monod-type substrate utilization model not only provides substrate concentration at any time *S(t)* but also allows to determine K_s, which can be used in adjusting the most appropriate substrate concentration in the feed (Mu et al, 2006).

The detailed modelling approach and development are discussed in Section 4.

Table 2.3 Summary of Kinetic Models

Logistic-type kinetic model	Monod-type kinetic model
Biomass growth	
$\frac{dX}{dt} = \mu X\left(1-\frac{X}{X_{\max}}\right)$ **[2.2]**	$\frac{dX}{dt} = \frac{\mu_{\max} XS}{K_S + S}$ **[2.13]**
Kinetic parameters :	
μ	$\mu_{max,}, K_s$
Input to model:	
X, X_{max}	X, S
Output from model:	
dX/dt	dX/dt
Substrate utilization	
$-\frac{dS}{dt} = \gamma\frac{dX}{dt} + \delta X$ **[2.5]**	$-\frac{dS}{dt} = \frac{\mu_{\max} XS}{Y(K_S + S)}$ **[2.17]**
where	where
$\delta = -\frac{(dS/dt)}{X_{\max}}$ **[2.6]**	$Y = \frac{X_{\max} - X_0}{S_0 - S_{\min}}$ **[2.16]**
$S = S_0 - \gamma C(t) - \delta D(t)$ **[2.8]**	
$C(t) = X_o\left[\frac{e^{\mu_{\max} t}}{1-\left(\frac{X_o}{X_{\max}}\right)(1-e^{\mu_{\max} t})} - 1\right]$ **[2.9]**	
$D(t) = \frac{X_{\max}}{\mu_{\max}}\ln\left[1-\frac{X_0}{X_{\max}}\left(1-e^{\mu_{\max} t}\right)\right]$ **[2.10]**	
Kinetic parameters :	
μ, γ, δ	$\mu_{max,} K_{s,} Y$
Input to model:	
X, X_{max}, S	X, S
Output from model:	
dS/dt	dS/dt

Table 2.2 Summary of Kinetic Models (con't.)

Luedeking-Piret product formation model

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad \textbf{[2.18]}$$

where

$$\beta = \frac{(dP/dt)}{X_{\max}} \quad (\text{at } X = X_{max},\ dX/dt = 0) \qquad \textbf{[2.19]}$$

$$P = \alpha\, A(t) + \beta\, B(t) \qquad \textbf{[2.21]}$$

$$A(t) = X_o \left[\frac{e^{\mu_{\max} t}}{1 - \left(\frac{X_o}{X_{\max}}\right)(1 - e^{\mu_{\max} t})} - 1 \right] \qquad \textbf{[2.22]}$$

$$B(t) = \frac{X_{\max}}{\mu_{\max}} \ln\left[1 - \frac{X_0}{X_{\max}} \left(1 - e^{\mu_{\max} t}\right) \right] \qquad \textbf{[2.23]}$$

Kinetic parameters : μ, α, β

Input to model: X, X_{max}, P

Output from model: dP/dt

2.3 Factors Affecting Growth

For anaerobic cellulolytic clostridia, the most important factors affect the growth of microorganism are temperature, pH, availability of nutrients (ie. concentration of substrate), and type of substrate. In addition, Huang and Chou (1990) emphasized also the effects of substrate particle size, organic loading, and cell concentration on the rates of cell growth and substrate utilization. In the section, the effects of temperature, pH, and concentration of substrate are discussed.

2.3.1 Effect of Temperature

In a microbial process, cellulose utilization is subject to physical and chemical conditions in the environment. The effects of temperature are particularly dramatic. A comparison of maximum growth rate across cellulolytic species reveals a strong dependence on growth temperature. Figure 2-4 exhibits a general trend of increasing growth rates on crystalline cellulose as a function of temperature. Maximum growth rates of mesophilic cellulolytic clostridia were observed at temperatures between 28°C and 39°C (Lynd et al. (2002). For *C. phytofermentans* growth, the optimum temperature is 35°C to 37°C with the upper and lower limits between 5°C and 45°C (Warnick et a., 2002).

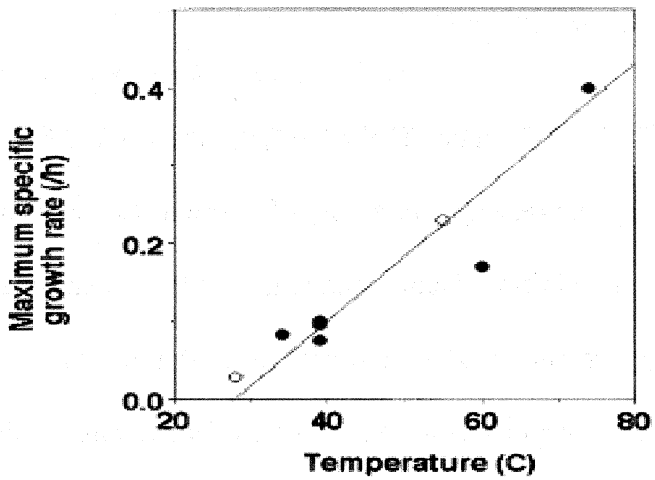

Figure 2-4 Relationship between growth temperature and μ_{max} (Lynd et al., 2002)

for aerobic (open circles) and anaerobic (solid circles) microorganisms grown on crystalline cellulose (r^2 = 0.90)

2.3.2 Effect of pH

Effects of pH on cellulose utilization have also been noted in laboratory cultures. Anaerobic cellulolytic bacteria, like most fermentative microbes, grow within a fairly narrow pH range between 6 and 7.5. In some habitats, pH fluctuations permit cellulose hydrolysis to occur at pH values below those supporting growth of the cellulolytic population (Lynd et al., 2002). For example, substantial cellulose hydrolysis can occur by ruminal bacteria at pH below 6.0, once the bacteria have adhered to cellulose, synthesized a glycocalyx, and initiated bacterial growth at a higher pH (Mourino et al., 2001).

Nevertheless, it is reported (Leschine and Warnick, 2007) that *C. phytofermentans* cells grow and ferment under a wide range of temperatures and pH ranges between 5.5 and 9.5. The pH of the fermentation medium may not need to be adjusted during fermentation.

2.3.3 Effect of Substrate Concentration

As presented in Section 2.1.3, the effect of initial substrate concentration on the cellulose degradation and fermentation product formation has been investigated by Desvaux et al. (2000) and Leschine and Warnick (2007). When cellulose concentration was above about 40 mM (in glucose equivalents), contrasting results were obtained from these two studies. The degree of cellulose degradation decreases and less amount of ethanol produces with increasing substrate concentration by *C. cellulolyticum.* Similar observation was obtained in the study of fermentation of cellulose substrates in continuous culture by *C. thermocellum* (Lynd et al., 1989). It was reported that lower conversion was obtained at higher feed substrate concentrations. However, increased concentration of ethanol was reported with increasing initial concentration of cellulose by *C. phytofermentans.* This indicates that high concentrations of cellulose do not inhibit the action of *C. phytofermentans* (Leschine and Warnick, 2007).

Based on the review in this section and the objective of this study, we decide that our experiments for *C. phytofermentans* growing on cellulose will be conducted for various pH values and various initial cellulose concentrations (S_0) at a constant temperature of 37°C, which is the optimum temperature for growth observed in the study by Warnick et al. (2002).

2.4 Experimental Methods

The literature review presented in this section assists in developing a experimental plan in our study and to determine the experimental methods, in terms of selection of culture systems, growth media, and inoculant maintenance methods.

2.4.1 Culture System

Microbial growth kinetics of suspended cells have been investigated in the laboratory in batch, continuous-culture, or fed-batch systems.

In **batch-culture** experiments, either the consumption of the growth-controlling substrate or the increase in biomass concentration was monitored as a function of time. Short-term batch culture growth experiments provide direct information on the dependence of growth rates on external nutrient concentrations (Tilman and Kilham, 1976).

Continuous culture experiments are used to establish the relationship between internal nutrients and steady state growth rates (Tilman and Kilham, 1976). In such experiments, an equilibrium concentration of the growth-controlling substrate is established independently of culture density and time. This allows the culture to grow at the set dilution rate by maintaining stable environmental growth conditions and hence the same physiological state. Therefore, in an ideal continuous culture, more precise, reproducible, and statistically relevant data can be collected than those obtained from batch cultures (Kovarova and Egli, 1998).

Continuous culture methods, which are particularly useful for studying growth and fermentation kinetics, are difficult to apply when cellulose or any other insoluble compound is the substrate for growth. Maintenance of relatively homogeneous suspensions of the insoluble substrate in nutrient reservoirs, fermentation vessels, and lines used to add nutrients and remove culture contents is a major problem. Therefore, an anaerobic continuous culture apparatus was designed which permits gas collection, continuous addition of nutrients with an insoluble substrate, and withdrawal of culture contents at a variety of retention times, ie. inverse of dilution rate (Pavlostathis et al., 1988).

At the initial phase of the multi-staged project, to focus on the characterization of *C. phytofermentans* for growth batch culture method is selected.

2.4.2 Defined Growth Media

As discussed in 2.1, *C. phytofermentans* is selected to be the bacteria growing on cellulose substarte in our experiments. American Type Culture Collection (ATCC) recommends using a chopped meat broth growing medium for *C. phytofermentans*. This type of medium with its insoluble components would not produce meaningful results for turbidity measurements. Therefore, other media must be used.

Warnick et al. (2002) used growth medium GS-2C with cellulose as the carbon source. Medium GS-2C, used for enrichment, isolation and routine cultivation of strain ISDg^T, was derived from GS-2 and contained the following (g/L): ball-milled cellulose (Leschine & Canale-Parola, 1983), 6.0; yeast extract, 6.0; urea, 2.1; K_2HPO_4, 2.9; KH_2PO_4, 1.5; MOPS, 10.0; trisodium citrate dihydrate, 3.0; cysteine hydrochloride, 2.0; resazurin, 0.001; and the

pH was adjusted to 7.0. More importantly, growth measurements with turbidity were made with the bacteria in MI media. Medium MI, which was used in growth and characterization studies, was a modified version of GS-2 that contained no yeast extract or urea but was supplemented with the following (g/L) as growth factors : tryptone (Difco), 2.0; adenine, 0.02; cytosine, 0.05; guanosine, 0.02; thymine, 0.05; uracil, 0.04. After autoclaving, 10 ml sterile vitamin solution was added per litre medium. Substrate added to MI medium varied as required. For different pH trials, the medium MOPS was replaced with MES, TAPS or CHES as needed according to the ATCC protocols.

2.4.3 Maintaining Inoculant Culture

Many experiments maintain a population of viable bacteria by using a chemostat culture in a continually stirred bioreactor at constant temperature and pH. Substrate input concentration and volume, as well as wasting, are continuously controlled to maintain a static bacterial culture.

However, if facilities are not available there are other ways to produce and maintain inoculant for experiments. Examples of these 'manual' techniques are given in the following:

1. "The culture was stored at 4°C in refrigerator and repeatedly subcultured once per month in CMS growing medium. All fermentation experiments were conducted by taking actively growing 5% (v/v) inoculum and incubating for 5 days at 37 ± 2°C (Ravinder, 2001)."

2. "Stock cultures of *C. thermocellum* were maintained in test tubes through biweekly transfer of 1 ml of culture into fresh CM3 growth medium. Since this strain did not utilize glucose, contamination checks were performed in CM3 that contained glucose rather than cellulose. Turbidity and gas production after 72 hour were taken as evidence of contamination (Weimer, 1977)."

3. "Experimental cultures were prepared by inoculating approximately 10^6 *C. thermocellum* cells (total count, equivalent to 0.2 to 0.5 ml from an exponential-phase culture) into 10 ml of reduced CM3 (Weimer, 1977)."

4. "Stock culture of *C. populeti* was maintained at 35°C by weekly transfer (2% v/v) into 10 ml of basal medium (Patel, 1988)."

5. "Inoculum for flask cultures and fermenter studies was grown in 100 ml on the BMY medium. The inoculum was grown for 24 h (35°C, stationary incubation), standardized to an A_{660} of 1.5 and inoculated into test media at 5% v/v (Patel, 1988)."

Based on the available laboratory facilities, inoculant culture maintenance is manually operated in our experiments similar to the one used by Weimer (1977).

2.5 Analytical Techniques

Cellulosic Ethanol Differential measurement of cell and cellulase concentrations is complicated because cellulose components or complexes are usually distributed among the culture broth, the cell surface, particulate biomass, and cellulose-enzyme-microbe complexes. In the frequent case where a significant fraction of the cellulase is retained on the cell surface, physical separation of cells and cellulase using described techniques is generally impractical. Because of these factors, **few** if any studies report quantitative data for the mass concentration of cells and cellulase accompanying microbial cellulose utilization. In this section, measuring and analysing methods for cell growth, substrate utilization, and biogas production are presented based on information obtained from literature review.

2.5.1 Measuring Cell Growth

Soluble Substrates

Mu *et al.* (2006) measured cell growth as dry cell weight by sampling, centrifuging, washing and drying at 80°C. Kong *et al.* (2006) measured cell growth as VSS according to APHA Standard Methods (1995). Desvaux *et al.* (2000) measured cell growth on cellobiose using absorbance measurements correlated to dry cell weight. Jud *et al.* (1997) used absorbance measurements correlated to Dry Cell Weight (DCW).

Biomass may be measured as DCW, by drying samples of known volume or mass in a 110°C oven for 8 hours (Cooney, 1981). Biomass may also be tracked using turbidity measurements from a spectrophotometer, where 1 absorbance unit at 600-700 nm

wavelength corresponds approximately 1.5 g/L DCW (Cooney, 1981). Relative calibration of the turbidity method may be using serial dilutions of a reference suspension. Absolute calibration of the turbidity method may be accomplished using measurements of DCW or total nitrogen (TKN) (Koch, 1981). Furthermore, photospectrometry and DCW calibration was used by Gerhard *et al.* (1993).

Insoluble Substrates

According to Lynd et al. (2002) measuring cell growth on cellulosic substrate requires a different approach than that used for soluble carbohydrate substrates. Because cellulosic substrates are insoluble, they will interfere with any turbidity or dry cell weight measurements, making these methods ineffective.

Desvaux *et al.*, (2000) estimated biomass grown on cellulose by bacterial protein measurement according to the method of Pavlostathis *et al.* (1988). A correlation is made between dry cell weight and protein content using bacteria grown on a soluble substrate such as cellobiose. It is assumed that the protein/dry cell weight ratio is constant regardless of substrate.

Estimation of protein. Protein was measured by a modification of the Bradford method (1976) as follows. Cell suspension (1 ml) was centrifuged at 16,000 g for 15 min and suspended in 1 ml of 1 N NaOH, placed in a boiling water bath for 10 min, and then cooled in an ice bath. The hydrolyzed samples were centrifuged, 50 to 100 µl of the supernatant was transferred to a test tube, and the 50 µl samples were

brought to 100 µl by the addition of 1 N NaOH. Coomassie brilliant blue G-250 (0.01%; 5 ml) was added and the contents of the tube were vigorously mixed. After a minimum of 10 min (but less than 1 hour), absorbance was measured at 595 nm. Bovine serum albumin in 1 N NaOH was used as the protein standard, and linearity was found up to 400 µg of protein per ml of sample.

Estimation of biomass. Cell dry weight was determined by centrifuging *R. albus* grown in batch culture with 1% cellobiose at 27,000 g for 20 min, discarding the supernatants, and washing the cell pellets with 0.9% NaCl solution. The pellets were dried at 105°C for 1 hour, equilibrated to room temperature in a desiccator, and weighed. The protein and carbohydrate contents of washed cells were also measured and found to be equal to 60 and 12% of the cell dry weight, respectively. The cell dry weight-protein correlation was assumed to be the same for cells grown on cellulose and was used to estimate the biomass of cultures grown on particulate cellulose."

2.5.2 Measuring Substrate Utilization

Soluble Substrates

Kong *et al.* (2006) tracked substrate consumption (glucose) using the DNS (dinitrosalicylic acid) method (Miller, 1959). Mu *et al.* (2006) measured sucrose utilization using the enthrone-sulfuric acid method (Koehler, 1952).

Measurements of glucose content may be accomplished using 'reducing sugar' methods, which involve titration with a compound which cause precipitation of the glucose and

render solution colourless (Cooney, 1981). Substrate quantities may also be defined in terms of COD measurements, as is often the case in wastewater applications (Degirmentas and Deveci, 2004). VSS has also been used by Mu *et al.* (2006).

Insoluble Substrates

Saeman et al. (1944) developed the quantitative saccharification method for the hydrolysis of cellulosic materials to reduce sugar in nearly quantitative yields. The method involves the treatment of the material with 72% sulphuric acid for 45 minutes at 300°C followed by a secondary hydrolysis for 1 hour in a 15-pound autoclave or for 4.5 hours at the boiling point. This method was employed in the study by Zhang and Lynd (2005).

Desvaux *et al.* (2000) employed the method of Huang and Forsberg (1990) to measure cellulose concentrations. Cellulose was washed of other materials using methods described by Updegraff (1969) and then measured using the phenol-sulfuric acid method described by Dubois *et al.* (1956).

2.5.3 Measuring Biogas Production

The volume of produced biogas can be measured by glass syringe, gas chromatograph using a thermal conductivity detector, or water–replace equipment. From the literature review, it is found that gas chromatograph method has been widely used and gas composition can be analyzed as well by this method according to APHA Standard Methods (1995).

Owen et al. (1978) provided a detailed description on using glass syringes equipped with needles to perform gas-volume sampling and removal during incubation. The sample syringe is initially flushed with the CO_2:N_2 gas mixture and lubricated with deionized water. Readings are taken at the incubation temperature and the syringe is held horizontal for measurement. Volume determinations are made by allowing the syringe plunger to move (gently twirling to pro-vide freedom of movement) and equilibrate between the bottle and atmospheric pressures. Readings are verified by drawing the plunger past the equilibrium point and releasing; the plunger should return to the original equilibration volume.

Based on the literature review, the purpose of this study and the available laboratory facilities, the following measuring and analysing techniques are selected for the experiments.

- Bradford dye test cell growth method for cell growth on insoluble substrate;
- Updegraff method for insoluble substrate utilization; and
- Glass syringe for measuring biogas production.

2.6 Converting Biomass to Ethanol

Although the investigation of ethanol production was not included in the experiment of the current study, a preliminary literature review in this section provides an introductory understanding for the future study.

Ethanol technology can be reviewed in a simplified term, which only four basic steps involved as shown in Figure 2-5 (Himmel and Sheehan, 2000). Production of biomass results in the fixing of atmospheric carbon dioxide into organic carbon. Conversion of this biomass to a useable fermentation feedstock (typically some form of sugar) can be achieved using a variety of different process technologies. These processes for sugar production constitute the critical differences among all of the ethanol technology options. Using biocatalysts (microorganisms including yeast and bacteria) to ferment the sugars released from biomass to produce ethanol in a relatively dilute aqueous solution is probably the oldest form of biotechnology developed by humankind. This dilute solution can be processed to yield ethanol that meets fuel-grade specifications. Finally, the economics of biomass utilization demands that any unfermented residual material left over after ethanol production must be used, as well.

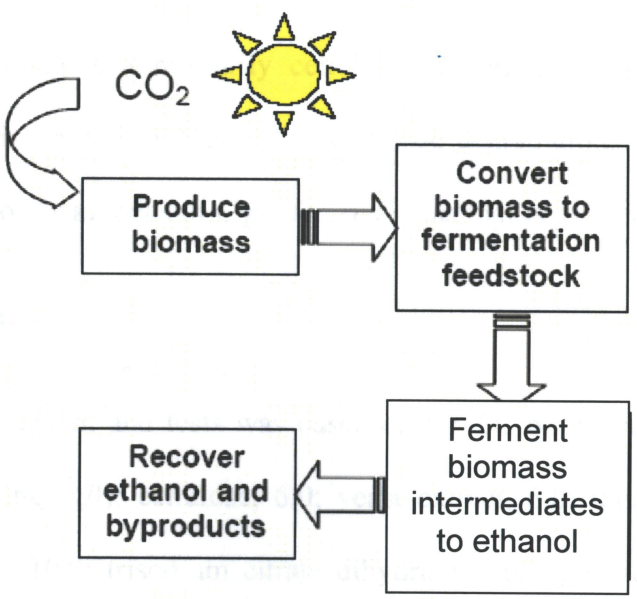

Figure 2-5 General scheme for converting biomass to ethanol (Himmel and Sheehan, 2000)

Keeping this simplistic description of biofuels technology in mind provides a clear and logical framework for various technology development strategies. Everything that has been done or could be done to improve production of bioethanol from biomass can be categorized in terms of sugar production or fermentation - with one exception. Researches and scientists are currently evaluating a longer term option for ethanol production that involves the replacement of sugars as the fermentation feedstock with simple single carbon intermediates that can be converted to ethanol.

3 Experimental Investigations

One of the objectives in this study is to carry out a series of experiments under various conditions of pH and initial cellulose concentrations so that we can examine the growth kinetics and identify the optimal growth conditions of *C. phytofermentans.* This section is to present the experimental plan, materials and methods used in our experiments.

3.1 Experimental Plan

Based on the discussions in the literature review and the current capacity of the laboratory facility, an experimental plan is established as shown in Table 3.1. The details of the materials, measuring methods and procedures are presented in the following section.

3.2 Materials and Methods

Municipal organic waste is a primarily cellulosic material, and reproduction of most cellulolytic bacteria is limited by the rate of cellulose degradation. Therefore, it is most useful to study the growth kinetics of *C phytofermentans* that is grown on cellulose.

3.2.1 Growth media

Growth media for inoculant and tests was based on GS-2C of Warnick *et al.* (2002) and contained the following (g/l): cellulose, 6.0; yeast extract, 6.0; urea, 2.1, K_2HPO_4, 2.9; KH_2PO_4, 1.5; MOPS, 10.0; trisodium citrate dihydrate, 3.0; cysteine hydrochloride, 2.0; resazurin, 0.001; and pH adjusted to 7.0. According to Warnick *et al.* (2002), other pH

values in growth media were obtained by using MES, TAPS or CHES in place of MOPS and adjusting proportions of K_2HPO_4 and KH_2PO_4 accordingly.

Table 3.1 Summary of Experimental Plan

Materials/ methods	Description
Substrate	GS-2C cellulose
Bacteria	*C. phytofermentans*
Culture system	Batch-culture
Culture equipment	500 ml flasks
Inoculant maintenance	In test tubes with weekly addition 1 ml actively growing culture into 9 ml of fresh growing medium
Temperature	37°C
pH	Testing at 7.0, 7.6, 8.0, 8.1, 8.2, 8.4, and 8.5 with S_0 set at 6.0 g of cellulose /L
Initial substrate concentration (g of cellulose /L)	Testing at 0.1, 0.5, 1.0, 4.0, and 6.0 with pH set at 8.5
Measuring methods • Cell growth • Substrate utilization • Biogas production	 • Bradford dye test • Updegraff method • Glass syringe
Experimental duration	At last 40 hours for all 12 experiments
Sampling	• Duplicated readings for each sampling and results averaged. • Samples are taking every four hours during the first 40 hours in each of the experiments.

3.2.2 Inoculant

Inoculant was maintained in test tubes by adding 1 ml actively growing culture into 9 ml of fresh growing medium weekly. Contamination checks were accomplished by addition 1 ml into 9 ml growth medium containing no cellulose and 3 g/L sucrose, which does not support the growth of *C. phytofermentans* (Warnick et al., 2002). Inoculant for test samples

was removed from these test tubes and added to samples during their exponential growth phase.

3.2.3 Experimental Culture

Experimental cultures was grown in 500 ml flasks with 5% v/v inoculant, filled to 75% flask volume, incubated at constant temperature 37°C and agitated continuously. Flasks were capped with rubber septum stoppers to ensure anaerobic condition and to allow for sampling and headspace overpressure relief.

3.2.4 Trials

To evaluate the impacts of the pH and initial cellulose concentration on the growth of *C. phytofermentans* under a constant temperature of 37°C, a total of 12 trails was divided into two groups. As shown in Table 3.2, the first group including seven trials (Trial ID: 1-7) is to test for various pH from 7.0 to 8.5 with initial substrate concentration at 6.0 g of cellulose / L, while the second group including five trials (Trial ID: 8-12) is tested for various initial substrate concentrations from 0.1 to 6.0 g/L at pH = 8.5. Trail 7 in the first group and Trial 12 in the second group run repeatedly with the same pH and the same initial substrate concentration.

For each trial, samples were taken at different time. To characterize the growth of *C. phytofermentans*, the kinetics during the exponential growth and declining growth phases are the primary interest in this study. Therefore, it was decided to take samples every four hours during the first 40 hours in each trial. After the first 40 hours, bigger step steps were used to monitor the biomass in the stationary and endogenous phases and biogas produced.

At each time step, duplicated samples were taken and results averaged.

Table 3.2 Summary of Experimental Conditions

Trial ID	Conditions			
	T (^{O}C)	pH	S_0 (g/L)	Duration of experiment (hour)
Variable initial pH:				
1	37.0	7.0	6.0	89
2	37.0	7.6	6.0	89
3	37.0	8.0	6.0	89
4	37.0	8.1	6.0	113
5	37.0	8.2	6.0	113
6	37.0	8.4	6.0	113
7	37.0	8.5	6.0	91
Variable initial substrate concentration:				
8	37.0	8.5	0.1	67
9	37.0	8.5	0.5	67
10	37.0	8.5	1.0	67
11	37.0	8.5	4.0	150
12	37.0	8.5	6.0	150

3.2.5 Cell Growth Measurement

Cell growth or biomass production was monitored using the Bradford dye test (Bradford, 1976). This method uses colour change to indicate protein content in a sample. The protein content is then correlated to dry cell weight from a standard curve developed in the lab.

Measuring the dry weight of protein is the simplest way to measure the weight of cells in a sample. There is a close correlation between the increase in protein weight and the cells growth. A standard curve should be established based on the standards solution in order to

measure the protein in any unknown sample. Bradford method was used to determine protein with spectrophotometer at the wavelength absorbance of 595nm.

3.2.5.1 Calibration curve

A calibration curve shown in Figure 3-1 was developed by changing protein concentrations. In our experiment, we used a standard concentration range of purified protein near the expected unknown concentration of our samples which measured by spectrophotometer at the absorbance of 595 nm. As shown in Figure 3-1(a), the calibration curve is perfectly represented by the polynomial equation [3.1] with $R^2 = 0.999$.

$$y = -0.1357\,x^2 + 0.8381\,x \qquad [3.1]$$

where

x = protein concentration , g/L

y = spectrophotometer absorbance of 595 nm.

As shown in Figure 3-1 (b), a linear equation [3.2] can also satisfactorily represent the calibration curve with $R^2 = 0.959$. For its simplicity, this was used in our experiments to determine the unknown protein concentrations based on the reading of spectrophotometer absorbance.

$$y = 0.6223\,x \qquad [3.2]$$

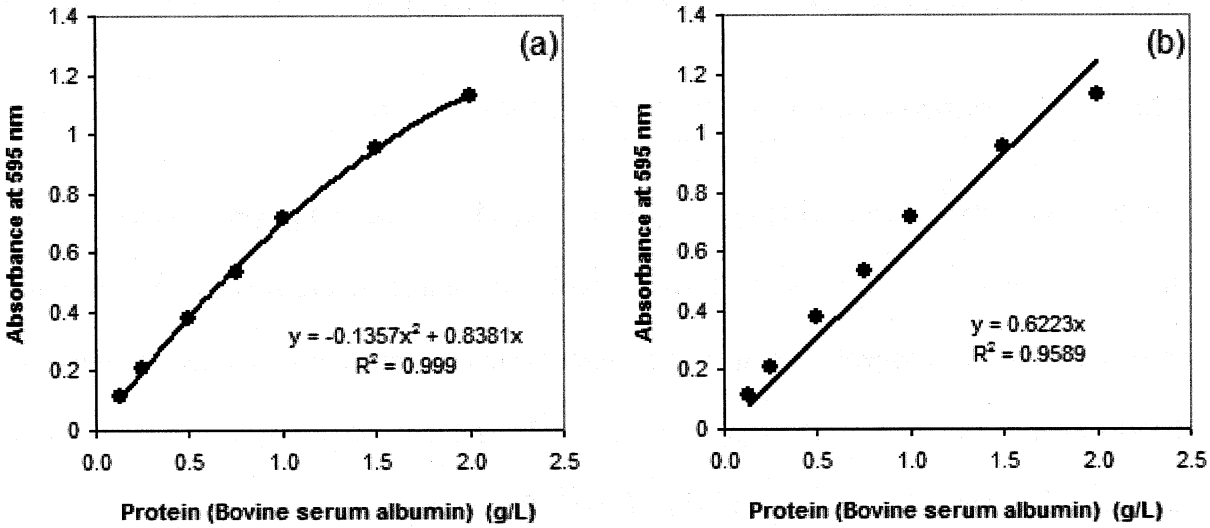

Figure 3-1 Bradford protein calibration

3.2.5.2 Bradford method for measuring protein

Bradford method is an assay that determines the protein using a reagent. The red form of the protein is converted to the blue form from upon binding of the dye to protein. The binding of the dye to protein was a very rapid process (about 2 minutes), however the protein-dye complex remains dispersed in the solution for an approximately 1 hour. Therefore, the measuring procedure did not need a critical timing. Protein measurement was made by centrifuging samples, hydrolyzing with NaOH, centrifuging again, adding a dye and measuring absorbance against a protein standard. The detailed descriptions on estimations of protein and biomass using this method are provided in Section 2.5.1.

3.2.5.3 Sample analysing procedure

Batch cultures were prepared at different pHs and conditions. After preparing cellulose cultures and inoculating, our samples were taken from the batch cultures every 4 hours

within the first 40 hours, and then bigger time steps were used. Before each sampling, the volume of produced gas in each batch culture was recorded.

Reagents:

Bradford dye reagent and DDW

Equipments:

Centrifuge; Hot water bath; Pipettes (10 and 5ml); 4.5 ml cuvettes and caps; 100 µl pipette and tips;1000 µl pipette and tips, ;Spectrophotometer.

Materials:

Glass syringes (2, 5, 20 and 50 ml); 10 ml plastic syringe; 1½" 22G sterile needles; 1½" 22G sterile needles; 5 ml plastic centrifuge tubes.

Sample analysis:

1) Remove 1 ml samples from refrigerator
2) Centrifuge at 3,200 rpm for 20 minutes
3) Remove 700 µl, being careful not to disturb the sediment solids
4) Add 1000 µl 1 N NaOH and mix
5) Boil in hot water bath with caps open for 10 minutes
6) Cool down the contents for about 15 minutes
7) Centrifuge at 3,200 rpm for 20 minutes

8) Carefully remove 60 µl supernatant and place in cuvette; for blank add 60 µl supernatant

9) Add 3 ml Bradford dye reagent

10) Cap and shake cuvette and wait 10 minutes

11) Measure absorbance at 595 nm

3.2.6 Product Measurement

The volume of produced biogas in each sampling interval was measured by using glass syringes and data was recorded every 4 hours. However, the produced gas should be identified upon further studies and the use of analytical instruments like gas chromatograph.

3.2.7 Substrate Utilization Measurement

Substrate utilization was measured using the methods of Updegraff (1969) and Dubois *et al.* (1956) as described by Huang and Forsberg (1990). The sample was washed of other non-cellulosic material with acetic-nitric acid reagent, centrifugation and water. The residual cellulose was then broken down using concentrated sulfuric acid. The absorbance of this solution was measured and compared to a standard curve relating glucose to absorbance.

3.2.7.1 Sample analysing procedure

Reagents:

Acetic-nitric reagent (150 ml 80% acetic acid + 15 ml nitric acid); phenol, sulfuric acid; and DDW.

Equipments:

Centrifuge; hot water bath; 10 ml and 5 ml pipettes; 100 ml volumetric flasks; glass COD tubes; 100 µl pipette and tips; 1000 µl pipette and tips; Spectrophotometer.

Washing off of non-cellulosic materials:

1) Remove 10 ml sample from refrigerator
2) Centrifuge 5 min at 3,200 rpm
3) Discard supernatant using 10 ml pipette and remove only as much as is possible without disturbing sediment
4) Add 3.0 ml acetic acid-nitric acid reagent and mix well
5) Place in boiling water bath with loosely screwed on caps for 30 minutes
6) Cool in water
7) Centrifuge 5 min at 3,200 rpm and discard supernatant
8) Add DDW as required to 10 ml and mix well
9) Centrifuge 5 min at 3,200 rpm and discard supernatant
10) Add DDW as required to 10 ml and mix well
11) Dilute to less than 50 mg/L cellulose

Sample Analysis:

1) Place amounts of diluted sample and DDW into tubes
2) Prepare blank tube and add 2 ml DDW only
3) Add 50 µl phenol
4) Add 5 ml H_2SO_4

5) Wait 10 minutes, tubes will become very hot

6) Cap and shake tubes

7) Wait 10 minutes

8) Measure absorbance at 490 nm

3.3 Experimental Results

To obtain the biomass concentration, duplicates of sample readings were taken at the same time step. The average of the duplicates was considered the representative measurement for that time step. Before taking the average data, each trial with all the raw data was plotted using the same X and Y scales. The purpose of this exercise is to visualize the trend within a trial, the trend of the trials for the same pH or the same initial cellulose concentration (S_0), and the trend of all the trials, and then further to determine if outliners exist. Although big differences (30-40%) were observed in some of the biomass data at the same time step in a trial, it was difficult to identify outliners from two readings, which followed reasonably the trend in the trial. The biogas production volume is the only parameter to be measured and recorded in this study for product formation. Only one reading was available at each time step since it was measured by glass syringes before taken samples for protein and cellulose concentration measurements.

The averaged biomass concentrations and the biogas volume obtained at each time step in the first 70 hours of the 12 trials are depicted in Figure 3-2 and the results for the entire experimental periods are summarised in Table 3.3. The detailed laboratory log sheets and raw measurements are presented in Appendix A.

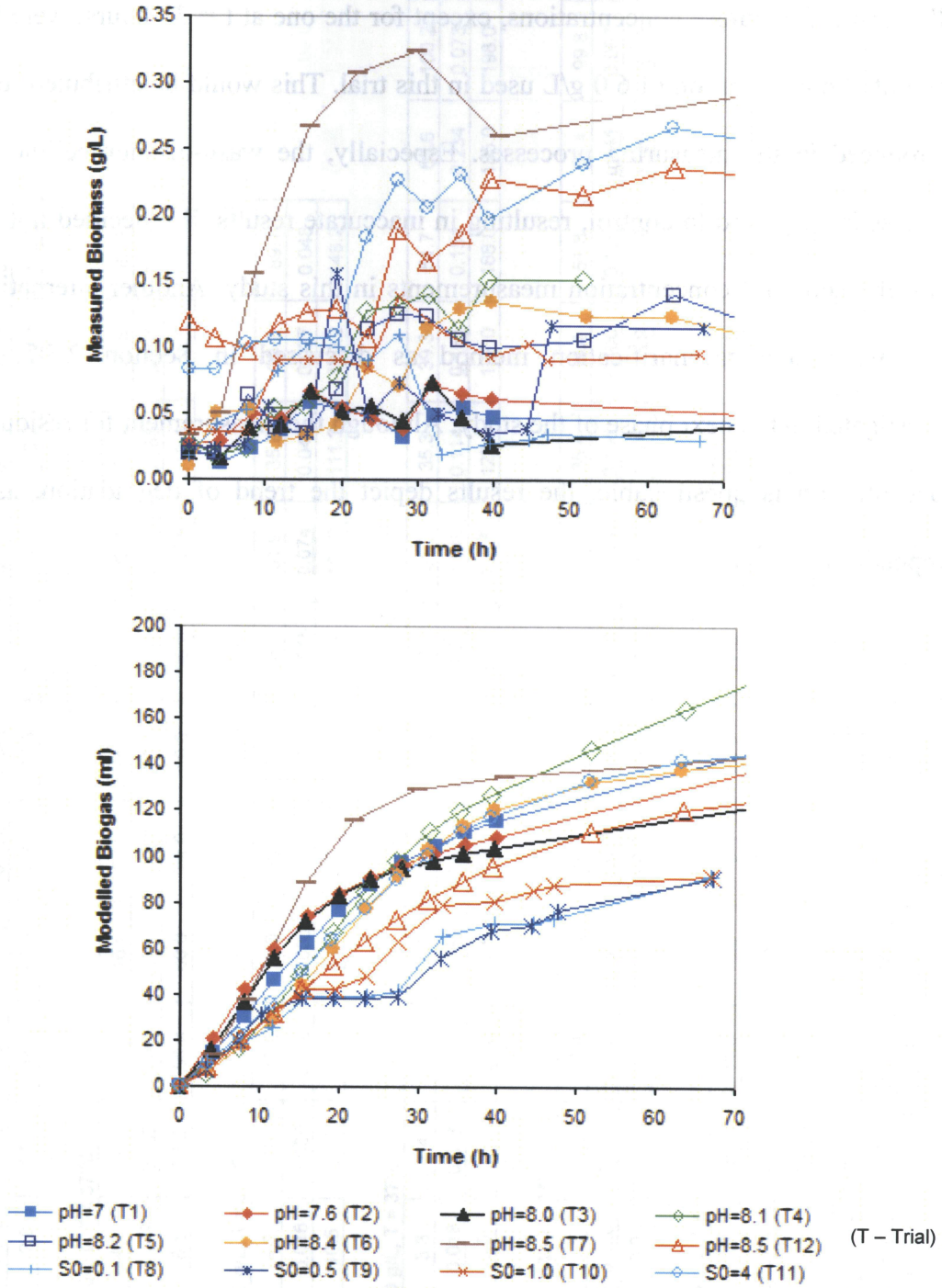

Figure 3-2 Measured biomass and biogas productions of 12 trials during the first 70 hours

Residual cellulose concentration data were only obtained for Trial 7. It was observed that all measured cellulose concentrations, except for the one at t = 70 hours, were higher than the initial concentration of 6.0 g/L used in this trial. This would be attributed to the errors introduced in the measuring processes. Especially, the wash-off non-cellulosic material process is very hard to control, resulting in inaccurate results. We decided not to continue with the cellulose concentration measurements in this study. Another alternative method, the quantitative saccharification method as discussed in Section 2.5.2, should be investigated in the next phase of the study. Although the measurement for residual cellulose concentration is questionable, the results depict the trend of degradation, as shown in Appendix B.

Table 3.3 Experimental data of biomass (X_e) and biogas production (P_e)

Trial 1: pH = 7.0, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	4.2	8.2	12.0	16.0	20.1	24.0	28.0	32.1	36.0	40.0	89.3
X_e (g/L)	0.025	0.012	0.023	0.037	**0.059**	0.052	0.049	0.032	0.048	0.054	0.048	0.041
P_e (ml)	0.0	12.0	17.5	45.5	63.5	89.5	97.5	100.9	104.4	106.2	110.5	153.5

Trial 2: pH = 7.6, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	4.2	8.2	12.0	16.0	20.0	24.0	28.0	32.0	35.9	39.8	89.2
X_e (g/L)	0.026	0.029	0.050	0.046	0.066	0.054	0.045	0.038	**0.073**	0.065	0.061	0.045
P_e (ml)	0.0	8.5	25.5	50.0	60.0	84.0	89.0	96.0	105.5	113.5	118.5	162.5

Trial 3: pH = 8.0, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	4.0	8.1	12.0	15.9	20.0	24.0	28.0	31.9	35.8	39.7	89.1
X_e (g/L)	0.021	0.016	0.039	0.042	0.066	0.051	0.055	0.045	**0.074**	0.047	0.027	0.048
P_e (ml)	0.0	10.5	25.7	52.7	64.7	77.7	82.7	90.2	100.2	111.2	120.2	148.2

Trial 4: pH = 8.1, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	3.3	7.4	11.2	15.2	19.3	23.3	27.3	31.5	35.3	39.3	51.7	63.6	100.2	111.3
X_e (g/L)	0.028	0.018	0.023	0.054	0.055	0.078	0.128	0.132	0.140	0.114	**0.151**	0.151	0.234	0.073	0.077
P_e (ml)	0.0	5.0	11.5	20.0	27.5	49.5	84.5	104.5	113.5	123.0	137.0	168.0	185.0	198.0	212.0

Trial 5: pH = 8.2, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	3.5	7.7	11.4	15.4	19.3	23.4	27.4	31.3	35.4	39.4	51.8	63.4	99.8	111.3
X_e (g/L)	0.020	0.020	0.064	0.040	0.035	0.068	0.114	0.124	0.124	0.106	0.100	0.106	**0.141**	0.067	0.058
P_e (ml)	0.0	8.0	11.0	15.1	21.1	40.1	76.1	94.1	100.1	105.6	113.9	136.9	154.9	202.9	212.9

Trial 6: pH = 8.4, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	3.6	8.0	11.6	15.5	19.4	23.6	27.5	31.3	35.6	39.7	52.0	63.2	99.5	111.3
X_e (g/L)	0.010	0.051	0.052	0.028	0.034	0.042	0.084	0.071	0.113	0.129	**0.134**	0.123	0.123	0.075	0.041
P_e (ml)	0.0	7.0	9.0	13.1	19.1	30.6	44.6	61.6	93.6	110.1	119.1	135.1	156.1	180.1	184.1

Trial 7: pH = 8.5, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	4.1	8.5	15.8	22.0	29.9	40.8	71.3	91.9
X_e (g/L)	0.033	0.050	0.155	0.267	0.306	**0.324**	0.260	0.290	0.177
P_e (ml)	0.0	19.0	52.5	102.0	119.0	127.0	132.0	140.0	140.0

Table 3.3 Experimental data of biomass (X_e) and biogas production (P_e) – (con't.)

Trial 8: pH = 8.5, S_0 = 0.1 g/L, T = 37°C

t (hour)	0.0	3.6	7.6	11.6	15.8	19.6	23.6	27.6	33.1	39.6	44.6	47.1	66.9
X_e (g/L)	0.017	0.041	0.053	0.082	0.103	0.100	0.091	**0.110**	0.019	0.028	0.031	0.035	0.031
P_e (ml)	0.0	10.0	18.5	25.5	39.0	39.0	39.0	41.2	65.2	71.2	71.2	73.6	91.6

Trial 9: pH = 8.5, S_0 = 0.5 g/L, T = 37°C

t (hour)	0.0	3.3	7.3	10.3	15.5	19.4	23.4	27.5	32.8	39.3	44.3	47.5	67.3
X_e (g/L)	0.024	0.023	0.027	0.056	0.035	**0.156**	0.048	0.074	0.051	0.035	0.039	0.117	0.116
P_e (ml)	0.0	7.0	20.0	31.0	38.0	38.0	38.0	38.7	55.7	67.7	70.2	76.2	91.2

Trial 10: pH = 8.5, S_0 = 1.0 g/L, T = 37°C

t (hour)	0.0	3.6	7.6	11.6	15.8	19.6	23.6	27.6	33.1	39.6	44.6	47.1	66.9
X_e (g/L)	0.038	0.037	0.036	0.090	0.091	0.091	0.085	**0.138**	0.112	0.093	0.103	0.091	0.096
P_e (ml)	0.0	7.0	19.5	32.0	42.0	42.0	48.0	63.1	79.1	80.1	85.1	87.3	91.3

Trial 11: pH = 8.5, S_0 = 4.0 g/L, T = 37°C

t (hour)	0.0	3.4	7.5	11.5	15.4	19.2	23.3	27.3	31.3	35.5	39.2	51.5	63.2	135.7	150.0
X_e (g/L)	0.083	0.083	0.102	0.106	0.105	0.108	0.183	0.226	0.206	0.230	0.197	0.239	**0.267**	0.200	0.192
P_e (ml)	0.0	15.0	20.5	24.4	29.4	32.9	40.9	72.9	92.9	106.4	107.9	145.9	165.9	186.9	186.9

Trial 12: pH = 8.5, S_0 = 6.0 g/L, T = 37°C

t (hour)	0.0	3.4	7.7	11.8	15.4	19.3	23.4	27.3	31.3	35.8	39.4	51.7	63.4	135.7	150.0
X_e (g/L)	0.120	0.107	0.097	0.118	0.126	0.129	0.106	0.189	0.166	0.186	0.228	0.215	**0.236**	0.197	0.170
P_e (ml)	0.0	6.0	10.5	10.5	16.0	18.8	21.3	47.3	71.3	83.8	88.6	110.6	155.6	184.6	184.6

Note: the measured maximum biomass concentration in each trial is indicated in bold and underline.

4 Development of Mathematical Kinetic Models

One of the objectives of this study is to develop kinetic model to assess its fitness to the batch cultivation of *C. phytofermentans* for the biomass growth. On one hand, its potential application in the production of cellulosic ethanol is important. On the other hand, a rational design and optimization of the modern bioprocessing requires a better quantitative understanding of the kinetics of the process. Our literature review indicates that such knowledge and understanding about *C. phytofermentans* are very limited. In this study, the experimental data from the 12 batch fermentations trials were examined with our developed models. The experimental data are very important for model developments and used as input data for model set up, model calibration and verification.

4.1 Modelling Approach

As discussed in Section 2.2.3, a combination of the logistic equation, the Monod model and the Luedeking-Piret equations will be used in this study to simulate the organism growth on cellulose, the substrate utilization, and the evolution of biogas. The meaning of combination here is reflected by the process that the output from the logistic model for growth becomes the input to other two models: the Monod model for substrate utilization and the Luedeking-Piret equation for product formation. This modelling approach is schematically presented in Figure 4-1. Detailed descriptions on the modelling are provided as follows.

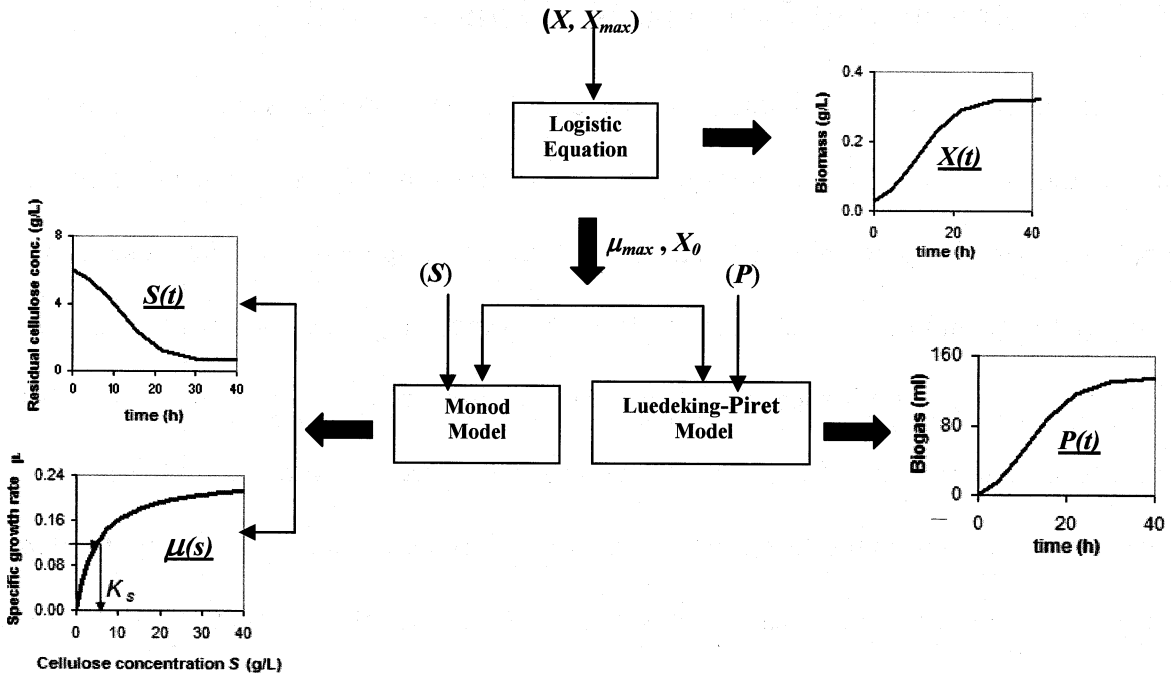

Figure 4-1 Modelling approach

(X, X_{max}, S, P are the required input parameters to the models as indicated)

4.2 Biomass Growth Kinetics

The logistic model was selected for modelling the biomass growth because it requires only growth measurements. Other models, including the Monod equation, require accurate measurements of substrate kinetics, which could not be achieved in this study. The input data required in the logistic modelling for biomass growth are the measured maximum biomass (X_{max}) and the measured biomass concentration (X) at different times.

Consider Trial 4 as an illustrative case of the biomass modelling process. Protein measurements were converted to biomass concentrations. The maximum biomass density obtained from the experiment, X_{max}, in this case was 0.151 g/L. Plotting the left side of

Equation [4.1] against time, as shown in Figure 4-2 yields a slope μ_{max} of 0.148 h^{-1} and an intercept of -2.2977.

$$\ln\frac{X}{X_{max}-X} = \mu t - \ln\left(\frac{X_{max}}{X_0}-1\right) \qquad [4.1]$$

This intercept corresponds to an initial biomass concentration X_o of 0.014 g/L. This was lower than the two sample measurements of 0.018 and 0.038 g/L with an averaged X_o = 0.028 g/L. As shown in Figure 4-2, in determining μ_{max} and X_o, all the biomass concentration measurements in the growth phase from t = 0 to t = 32 hours were used in the calculation.

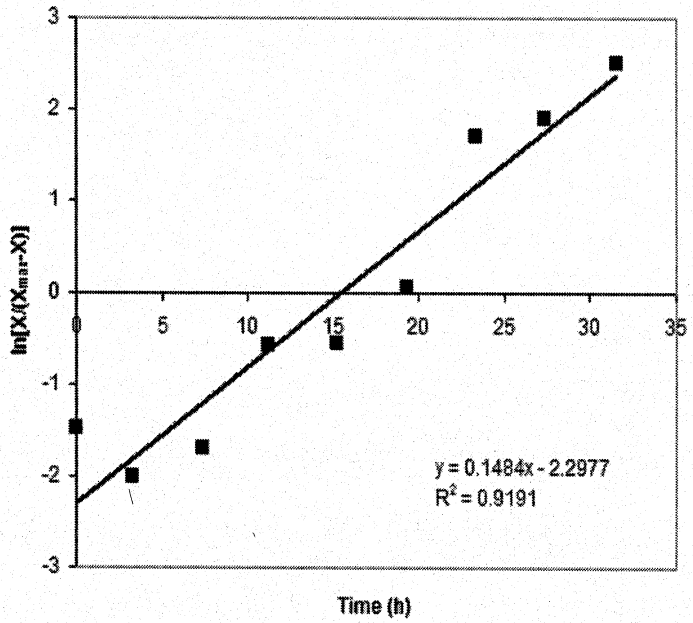

Figure 4-2 Evaluation of μ_{max} and X_o–Trials 4
(Trial 4 : 37°C, pH 8.1 and S_0 = 6.0 g/L cellulose, July 20 – 23, 2007)

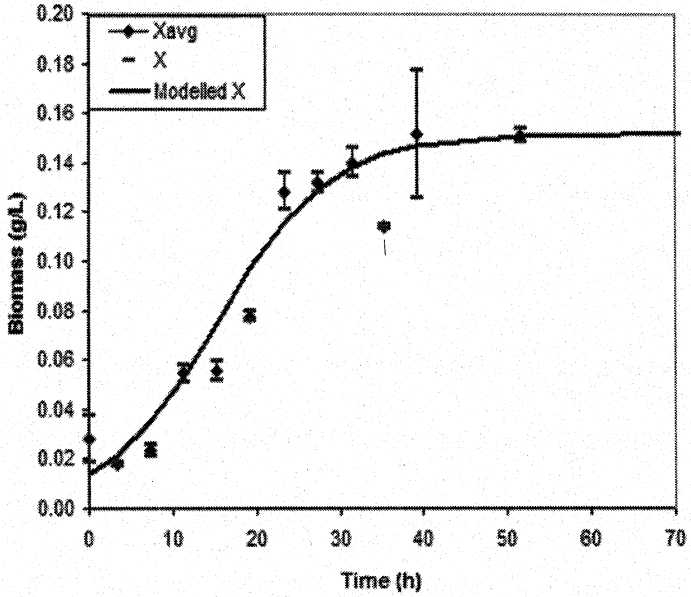

Figure 4-3 Measured and modelled biomass growth (R^2 = 0.93)- Trial 4
(Trial 4: 37°C, pH 8.1 and S_0 = 6.0 g/L cellulose, July 20 – 23, 2007)

Biomass concentrations are calculated using the logistic model Equation [4.2].

$$X = \frac{X_0 e^{\mu t}}{[1-(X_0 / X_{\max})(1-e^{\mu t})]} \qquad \textbf{[4.2]}$$

This gives the biomass growth model expressed in Equation [4.2.a] for Trial 4, which predicts the biomass concentration at any time during the growth phase. For example, at t = 40 hours, the predicted X is 0.147 g/L.

$$X = \frac{0.014 e^{0.148t}}{[1-(0.014/0.151)(1-e^{0.148t})]} = \frac{0.014 e^{0.148t}}{0.907+0.0927 e^{0.148t}} \qquad \textbf{[4.2.a]}$$

The modelled and the measured biomass growth are plotted in Figure 4-3. The correlation between the modeled and the measured data is evaluated using Pearson product moment correlation coefficient, R, a dimensionless index that reflects the extent of a linear relationship between two data sets. A reasonably good agreement with R^2 = 0.93 was obtained for Trial 4. Since the logistic model only models the growth and stationary phases of the biomass life cycle, the data used in the evaluation are within these two phases.

The detailed model calculations, results and the comparisons with measurements for all the trials are provided in Appendix A.

4.3 Biogas Production Kinetics

Biogas production modelling in this study was accomplished using the Luedeking-Piret model, as expressed in Equation [4.3].

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad [4.3]$$

The input data required for this model are the measured maximum biomass (X_{max}), the measured biomass concentration (X) and product formation (P) at different times. Again use Trial 4 as an example to illustrate the biogas modelling. Firstly, the non-growth associated product formation coefficient β was determined by Equation [4.4] using the gas production rate during the stationary phase and the maximum biomass concentration X_{max}.

$$\beta = \frac{(dP / dt)}{X_{max}} \qquad [4.4]$$

For Trial 4, when $X = X_{max} = 0.151$ g/L, $dP/dt = 1.43$ ml /h, and the resulted $\beta = 9.43$ /h. In order to find the growth rate-associated product formation coefficient α, it was necessary to use μ_{max} and X_o from the logistic model. The modelled μ_{max} and X_o are 0.148 / h and 0.014 g/L, respectively. The functions $A(t)$ and $B(t)$ were calculated according to Equations [4.5] and [4.6].

$$A(t) = X_o \left[\frac{e^{\mu_{max} t}}{1 - \left(\frac{X_o}{X_{max}} \right)(1 - e^{\mu_{max} t})} - 1 \right] \qquad [4.5]$$

$$B(t) = \frac{X_{max}}{\mu_{max}} \ln \left[1 - \frac{X_0}{X_{max}} \left(1 - e^{\mu_{max} t} \right) \right] \qquad [4.6]$$

The growth rate-associated product formation coefficient α (= 702.55) was obtained from the slope of a straight line in a plot of $A(t)$ verse $P - \beta B(t)$ in Figure 4-4. After obtaining coefficients α and β, the biogas production can be calculated by using Equation [4.7].

$$P = \alpha A(t) + \beta B(t) \qquad [4.7]$$

This gives the biogas production model expressed in Equation [4.7.a] for Trial 4, which predicts the biogas production at any time of the fermentation process. For example, at t = 40 hours, the predicted P is 128 ml.

$$P = 702.55\times 0.014\left[\frac{e^{0.148t}}{1-\left(\frac{0.014}{0.151}\right)(1-e^{0.148t})}-1\right]+9.43\times\frac{0.151}{0.148}\ln\left[1-\frac{0.014}{0.151}\left(1-e^{0.148t}\right)\right]$$

$$= \frac{9.836e^{0.148t}}{0.907+0.0927e^{0.148t}} + 9.621\ln\left(0.907+0.0927e^{0.148t}\right) - 9.836 \qquad \textbf{[4.7.a]}$$

The comparison of measured and modelled biogas volumes, with an R^2 value of 0.97, is presented in Figure 4-5. Similar to the statistics method used in the biomass growth kinetic modelling, R is a Pearson product moment correlation coefficient. The biogas data obtained in the whole fermentation experiment were used in the correlation evaluations.

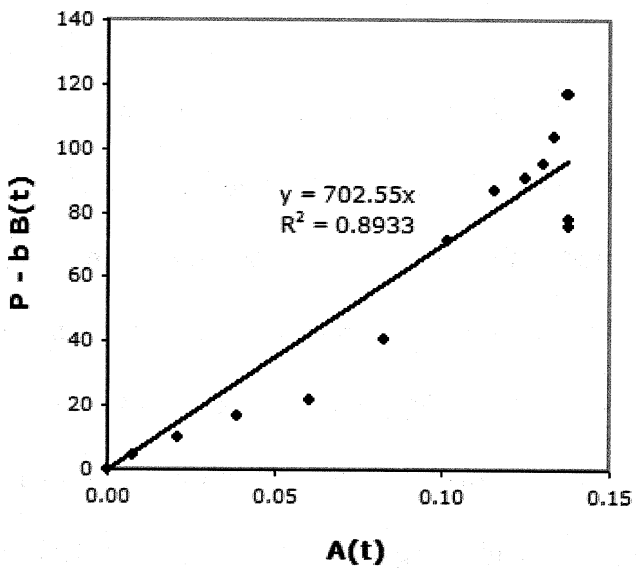

Figure 4-4 Evaluation of product formation coefficient α - Trial 4
(Trial 4 : 37°C, pH 8.1 and S_0 = 6.0 g/L cellulose, July 20 – 23, 2007)

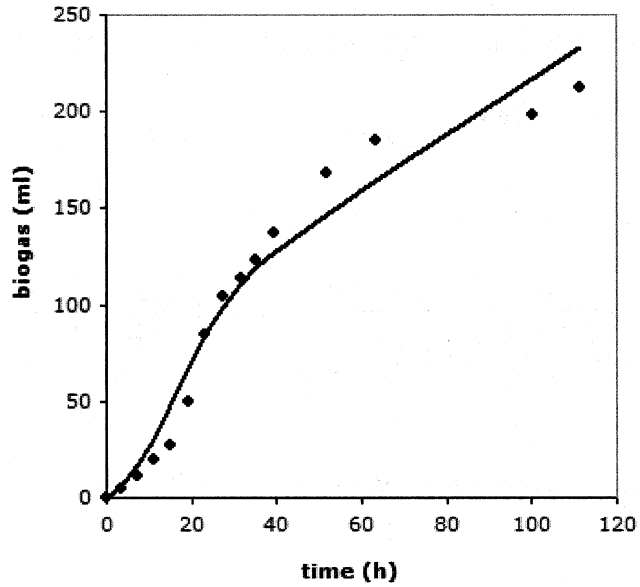

Figure 4-5 Measured and modelled biogas production (R^2 = 0.97) – Trial 4
(Trial 4: 37°C, pH 8.1 and S_0 = 6.0 g/L cellulose, July 20 – 23, 2007)

The detailed biogas model calculations, results and the comparisons with measurements for all the trials are provided in Appendix A.

4.4 Substrate Utilization Kinetics

The Monod model was developed to express the relationship between the residual concentration of the growth-limiting substrate and the specific growth rate of biomass. To apply the Monod model, the primary input data are the measured biomass and substrate concentrations at different times. Although accurate measurements of substrate kinetics could not be achieved in this study, the measurements for biomass were reasonable good and the modelled biomass growth in some of the trials, such as Trials 4, 7 and 8, were well represented by using the logistic equation with R^2 greater 0.93. The outcomes from these

models can be used in the Monod model to define the substrate kinetics. More importantly the K_s value can be determined by the Monod model.

4.4.1 Substrate Utilization Model

In this section, Trial 7 is used to demonstrate the development of substrate utilization model using Monod's approach, in spite of this trial is suggested for confirmation test as discussed earlier. The reasons to select this trial as an illustrative case are 1) the logistic model represented the growth with R^2=0.98, which means we have confidence on the μ_{max} and X_o determined by the model; and 2) this is the only trial we analysed the cellulose concentrations for the samples. As noted in Section 3.3, although the measurements for residual cellulose concentration are questionable, the results depict the trend of degradation.

The first step in this approach is using Equation [4.8] to determine the growth yield Y.

$$X_{max} - X_0 = Y(S_0 - S_{min}) \qquad [4.8]$$

where X_{max} = 0.324 g/L and X_0 = 0.029 g/L were obtained from the logistic model, and S_0 = 6.0 g/L was the initial cellulose concentration used in the trial. Since we did not have the measurements for S_{min}, it was initially assumed that S_{min} = 0 considering growth is limited by depletion of the substrate (Viessman and Hammer, 2005). Therefore, Y was calculated as 1 g/L cellulose producing 0.049 g/L biomass.

Then, use Equation [4.9] to calculate the substrate utilization rate, dS/dt, at each time step since dX/dt could be determined from the logistic model.

$$-\frac{dX}{dt} = Y\frac{dS}{dt} \qquad [4.9]$$

Once *dS/dt* was known, the time series of cellulose concentration *S(t)* was calculated by Equation [4.10].

$$S_2 = \frac{dS}{dt}(t_2 - t_1) + S_1 = -\frac{1}{Y}\frac{dX}{dt}(t_2 - t_1) + S_1 \,. \qquad [4.10]$$

Comparing the trend of cellulose degradation obtained from the measurements, *dS/dt* was calibrated by adjusting S_{min} value. Ultimately, S_{min} was a calibrated parameter in the model. It was determined S_{min}, the residual cellulose concentration after biomass reaching the maximum value X_{max}, was 10% of S_0, or 0.6 g/L. The growth yield Y was recalculated using S_{min} = 0.6 g/L and resulted as 1 g/L cellulose producing 0.055 g/L biomass. The results of the substrate utilization, and the biomass and biogas models with measurements are presented in Figure 4-6, illustrating the relationship between the cellulose degradation and biomass and biogas productions. It should be kept in mind that the measurements for cellulose concentration in the figure are not most accurate, due to the reasons described in Section 3.3. However, its trend of degradation is valuable for modelling calibration.

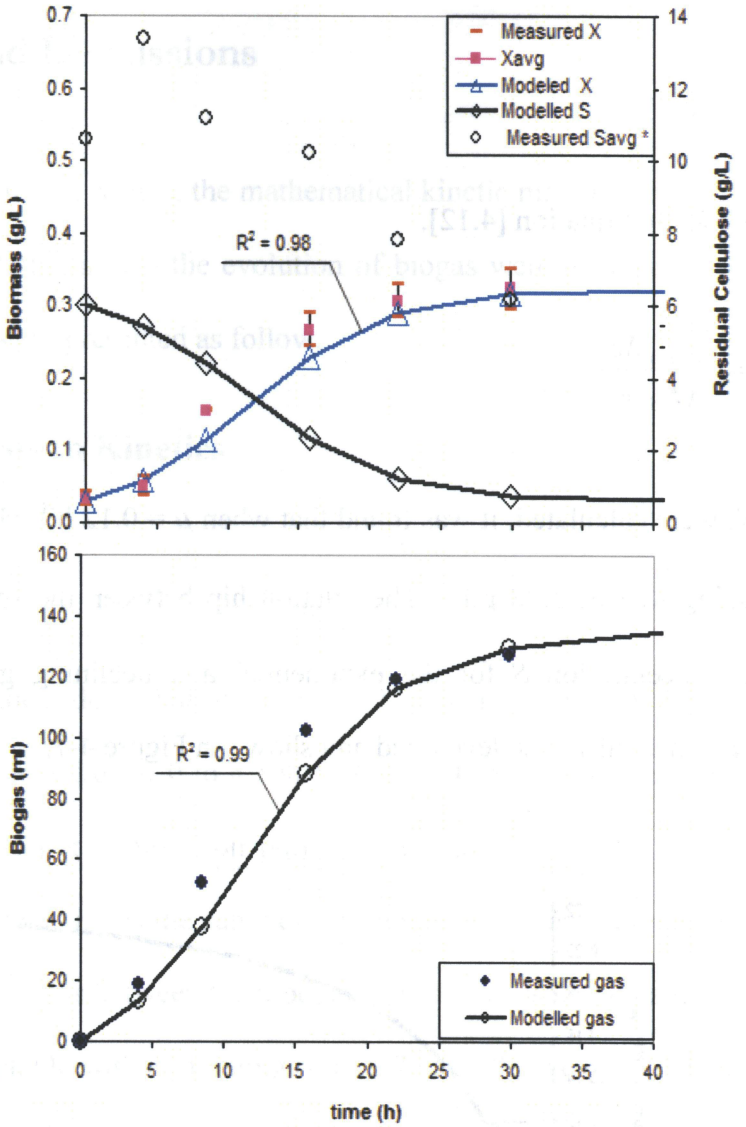

Figure 4-6 Growth and residual cellulose concentration for Trial 7

(* The cellulose measurements shown are not correct. However, the trend of the degradation is valuable for modelling calibration.)

4.4.2 Determination of K_s

In the Monod's model, in addition to $\mu_{max,}$ another kinetic parameter is $K_{s,}$ (which is a saturation constant) equal to the limiting substrate concentration at one-half the maximum growth rate. To obtain K_s, the first step was to calculate μ at different times using Equation [4.11].

$$\frac{dX}{dt} = \mu X \qquad [4.11]$$

Then, calculate K_s by Equation [4.12].

$$-\frac{dS}{dt} = \frac{\mu_{max} XS}{Y(K_S + S)} \qquad [4.12]$$

Once μ and K_s were calculated, it was found that when μ = 0.12 h^{-1} which was half of μ_{max}, the corresponding K_s was 5.08 g/L. The relationship between the specific growth rate μ and cellulose concentration S for the exponential and declining growth phases of *C. phytofermentans* in Trial 7 was developed and shown in Figure 4-7.

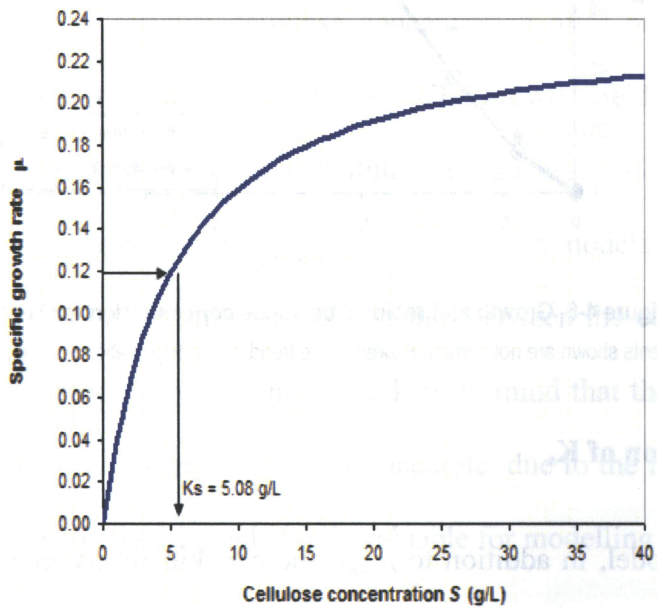

Figure 4-7 Specific growth rate μ *Vs.* cellulose concentration *S* of Trial 7

The detailed modelling calculation for substrate utilization and K_s for Trial 7 is provided in Appendix B.

5 Results and Discussions

Based on the experimental results, the mathematical kinetic models for (1) biomass growth, (2) substrate utilization, and (3) the evolution of biogas were developed. Discussions on the modelling results are presented as follow.

5.1 Biomass Growth Kinetics

5.1.1 Results

Using the logistic model described in Section 4.2, biomass growth of the seven trials with various initial pH values from 7.0 to 8.5 at S_0 = 6.0 g/L were modelled and the results are shown in Figure 5-1. For comparison purpose, the modelling result for Trial 12 is also included in this figure as it has the same experimental condition, in terms of pH and S_0, as in Trial 7. The correlations between the modeled and the measured data in the growth phase are reasonably acceptable with R^2 ranging from 0.73 to 0.98, except for Trials 2 (R^2=0.49). Figure 5-2 shows the measured biomass concentrations for the trials in this group.

The effects of varying the initial cellulose concentrations from 0.1 to 6.0 g/L when pH = 8.5 on the biomass growth were investigated. The modeled and measured results are shown in Figures 5-3 and 5-4, respectively. The R^2 for this group of trials is in the range of 0.77 to 0.98, except for Trial 9 with R^2=0.63.

Table 5.1 summaries the modelled and measured biomass growth parameters.

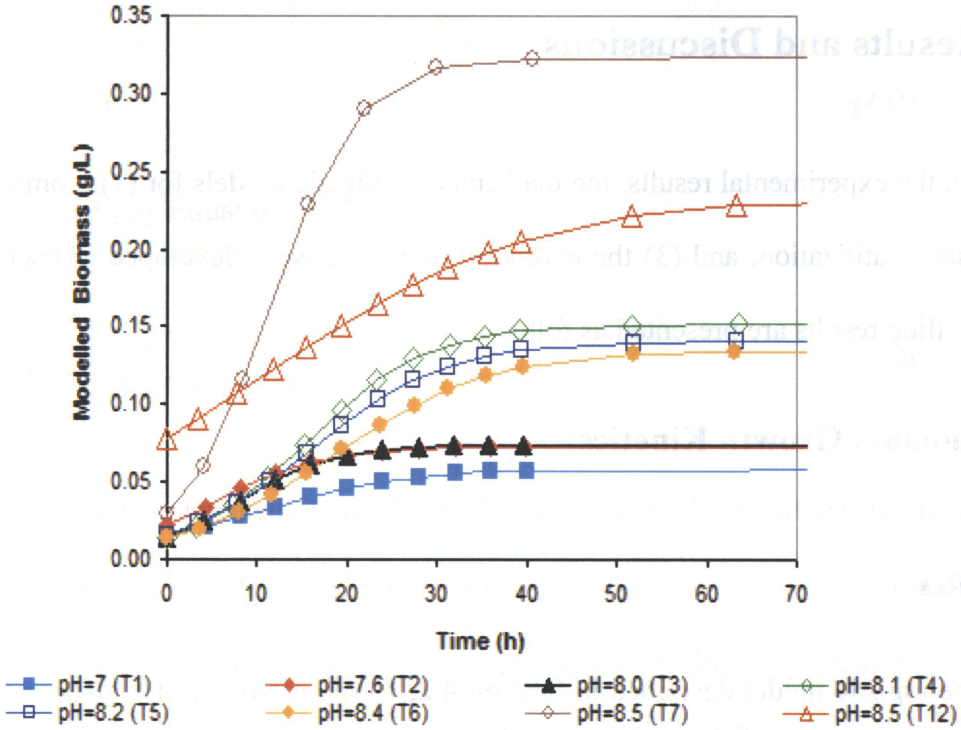

Figure 5-1 Modelled biomass growth for Trials 1-7 &12 (pH 7.0 – 8.5, S_0 = 6.0 g/L)

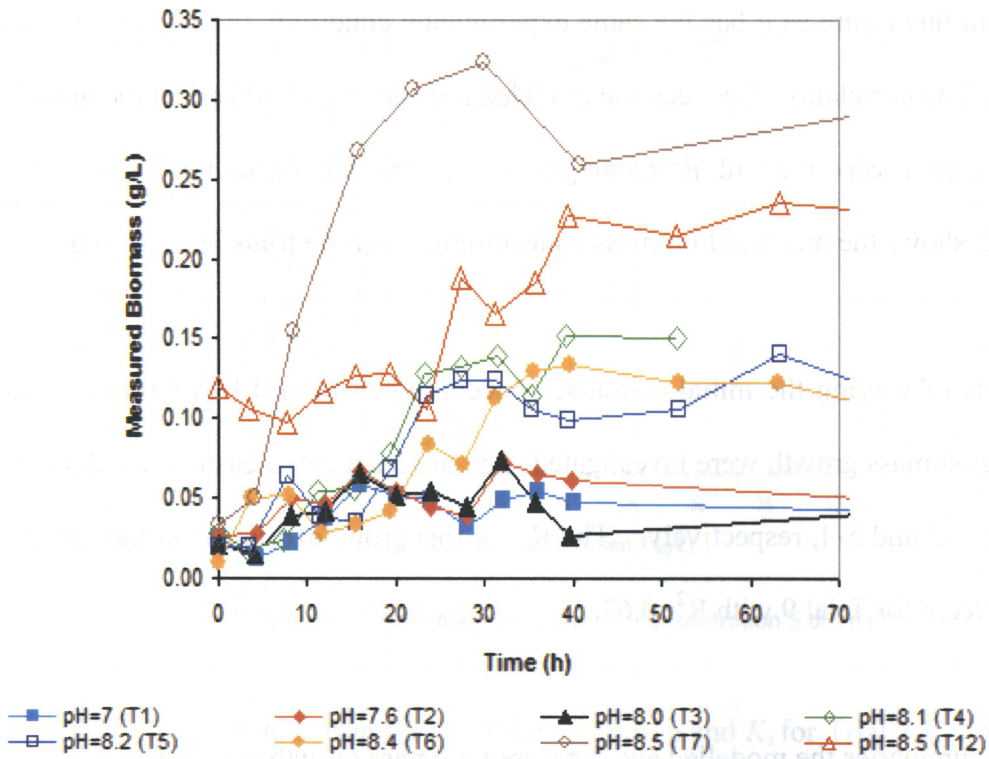

Figure 5-2 Measured biomass growth for Trials 1-7 &12 (pH 7.0 – 8.5, S_0 = 6.0 g/L)

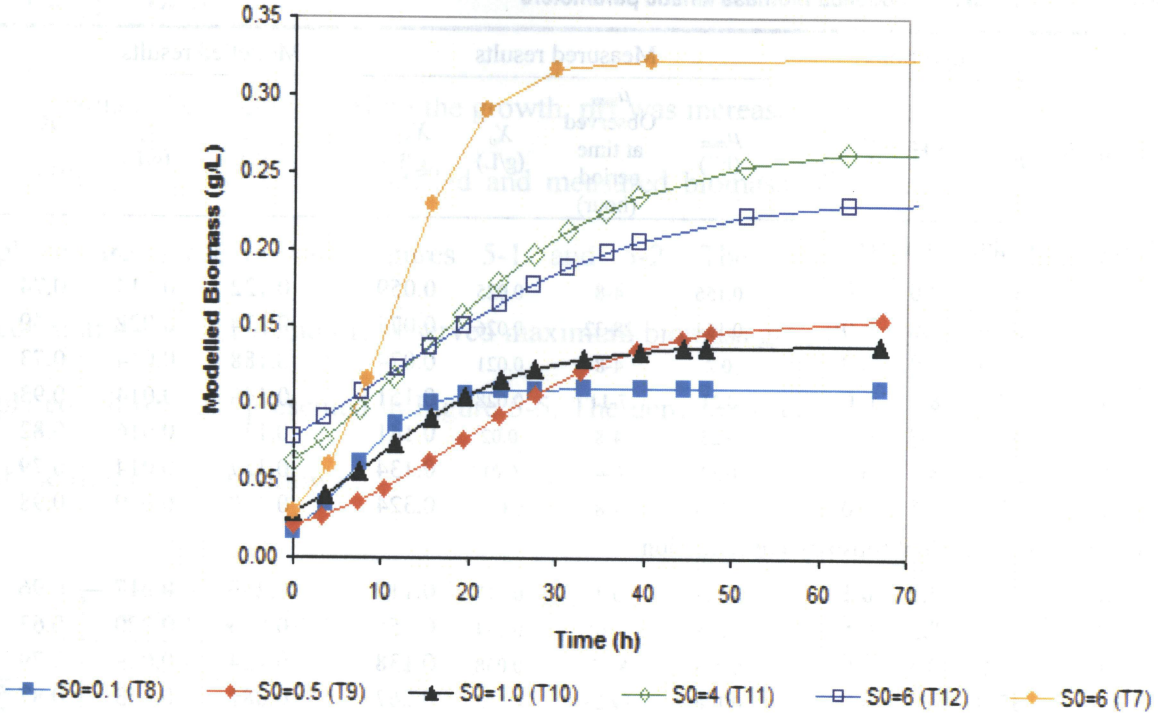

Figure 5-3 Modelled biomass growth for Trials 8-12 &7 (S_0 = 0.1– 6.0 g/L, pH=8.5)

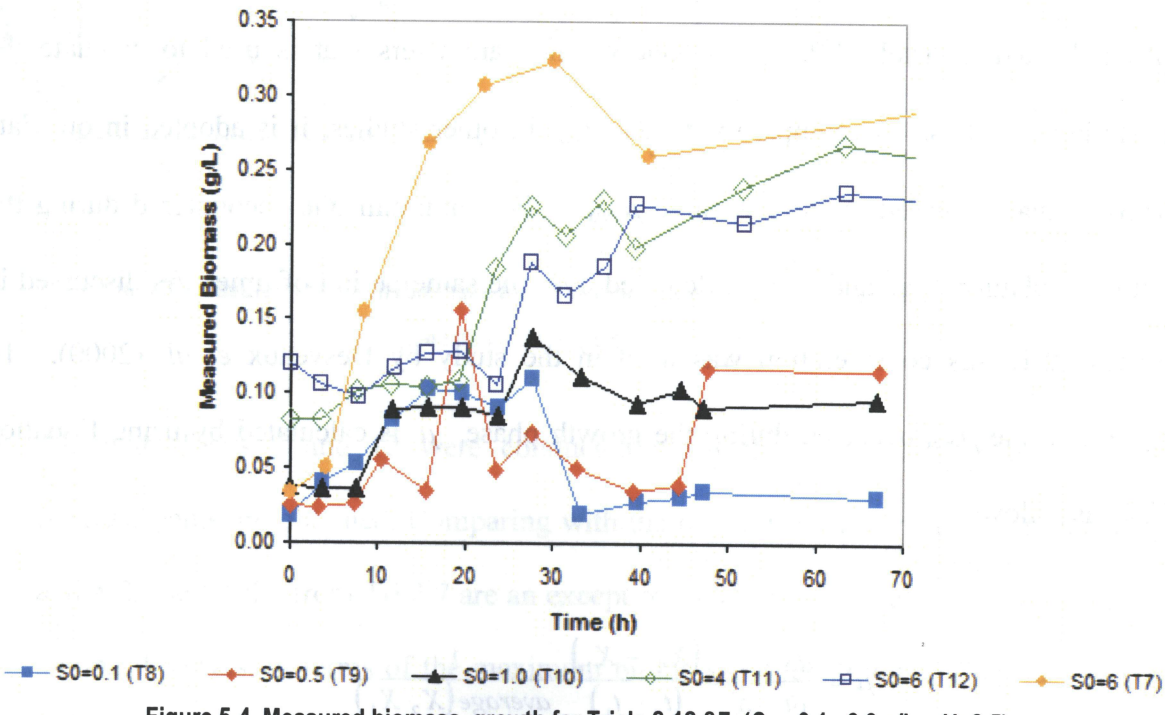

Figure 5-4 Measured biomass growth for Trials 8-12 &7 (S_0 = 0.1– 6.0 g/L, pH=8.5)

Table 5.1 Measured and modelled biomass kinetic parameters

Trial ID	Conditions			Measured results				Modelled results		R^2
	T (°C)	pH	S_0 (g/L)	μ_{max} (h^{-1})	μ_{max} Observed at time period (hour)	X_o (g/L)	X_{max} (g/L)	μ_{max} (h^{-1})	X_o (g/L)	
Group 1: variable initial pH										
1	37.0	7.0	6.0	0.156	4-8	0.025	0.059	0.122	0.014	0.74
2	37.0	7.6	6.0	0.154	28-32	0.026	0.073	0.174	0.022	0.49
3	37.0	8.0	6.0	0.2	4-8	0.021	0.074	0.188	0.014	0.73
4	37.0	8.1	6.0	0.21	7-11	0.028	0.151	0.148	0.014	0.93
5	37.0	8.2	6.0	0.25	4-8	0.02	0.141	0.132	0.016	0.82
6	37.0	8.4	6.0	0.37	0-4	0.01	0.134	0.117	0.014	0.79
7	37.0	8.5	6.0	0.235	4-8	0.033	0.324	0.240	0.029	0.98
Group 2: variable initial substrate concentration										
8	37.0	8.5	0.1	0.223	0-4	0.017	0.110	0.259	0.017	0.96
9	37.0	8.5	0.5	0.238	7-10	0.024	0.156	0.096	0.020	0.63
10	37.0	8.5	1.0	0.215	8-12	0.038	0.138	0.124	0.029	0.79
11	37.0	8.5	4.0	0.126	19-23	0.083	0.267	0.081	0.063	0.87
12	37.0	8.5	6.0	0.143	23-27	0.12	0.236	0.065	0.079	0.77

As indicated in Table 2.2, μ_{max} is one of the parameters that is used to evaluate the bacterium kinetics. To compare with the μ_{max} in other studies, it is adopted in our data analysis that observed or the measured μ_{max} is the maximum one encountered during the entire incubation time and is not calculated over the same period of time. As discussed in Section 2.1, this consideration was used in the study by Desveaux *et al.* (2000). To determine the μ_{max} occurred during the growth phase, μ is calculated by using Equation [2.1] as follows.

$$\mu = \frac{dX}{dt} \cdot \frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)} \cdot \frac{1}{average(X_2, X_1)}$$

5.1.2 pH Effect

To evaluate the effects of pH on the growth, pH was increased from 7.0 to 8.5 in Trials 1 to 7 with S_0 = 6.0 g/L. The modelled and measured biomass concentrations in the growth phase are presented in Figures 5-1 and 5-2. The measured maximum biomass concentrations (X_{max}) and the observed maximum biomass growth rates (μ_{max}) under various pH conditions are presented in Figure 5-5. The general observations and discussions from these figures are as follows.

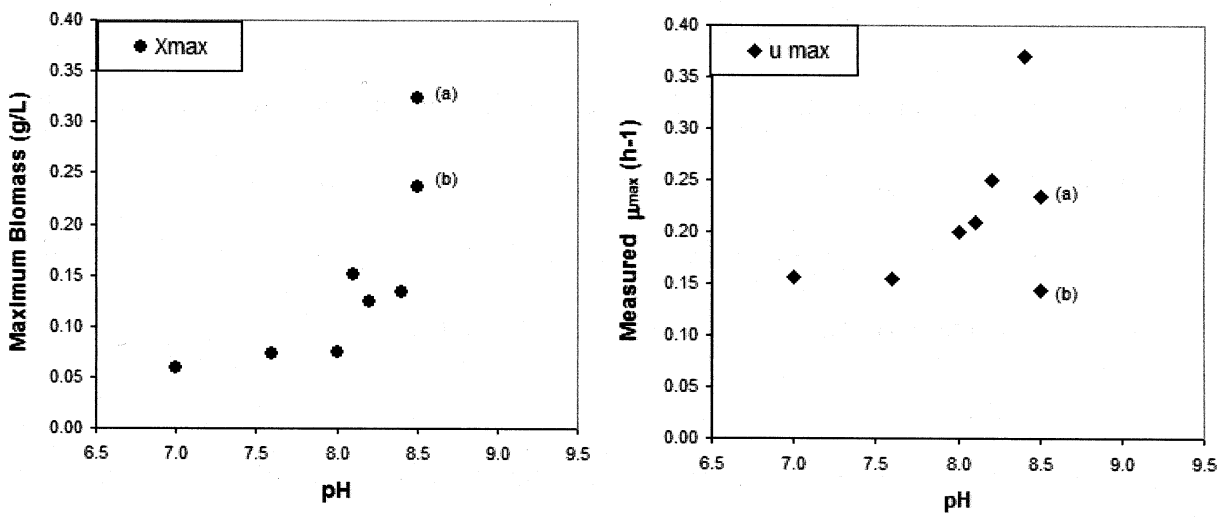

Figure 5-5 Measured maximum biomass and maximum growth rate at various pH when S_0=6.0 g/L

(a)data from Trial 7, (b) data from Trial 12

- Although Trials 7 and 12 were conducted under the same pH and S_0, different measurements are obtained. Comparing with the trends from other trials in Figures 5-1 and 5-2, the results from Trial 7 are an exception and represent the best growth kinetics among the trials, in terms of the maximum biomass concentration and the fast growth rate in the entire growth phase. However, confirmation for this trial is required in the next phase of the study.

- Figure 5-5 demonstrates that the optimal growth condition is at pH=8.4 and S_0=6.0g/L. The highest μ_{max} value is 0.37 h^{-1}. The maximum biomass growth rate μ_{max} is with an exponential-type increases towards pH=8.4,after that it drops rapidly at pH =8.5.

- The maximum biomass concentration X_{max} shows a wide variability depending on the initial pH value. This dependence is also reflected in Figure 5-5 with an exponential-type increase in X_{max} towards pH 8.5. The X_{max} under pH=8.5 is the highest among the experiments in this group.

- As shown in Figure 5-1 and Table 5-1, the modelled maximum growth rate μ_{max}, which occurs during the growth phase, is closely grouped in the range from 0.117 to 0.188 h^{-1} for all the trials, except for Trials 7 and 12. This shows that there is relatively little deviation in the maximum growth rate with changes in pH.

- As pH increases, the time reaches the maximum biomass concentration is longer. For example, the maximum biomass concentration occurs approximately at 30 hours when pH between 7 and 8, while this occurs around 40–60 hours when pH between 8.1 and 8.5.

5.1.3 Substrate Concentration (S_0) Effect

To evaluate the effects of initial substrate concentration on the growth, S_0 was increased from 0.1 to 6.0 g of cellulose per litre in Trials 8 to 12 with pH = 8.5, which was found the

high growth condition in the first group of experiments, in terms of the maximum biomass quantity generated. The modelled and measured biomass concentrations in the growth phase are presented in Figures 5-3 and 5-4. The measured X_{max} and the observed μ_{max} under various initial substrate concentration conditions are presented in Figure 5-6. The general observations and discussions from these figures are as follows.

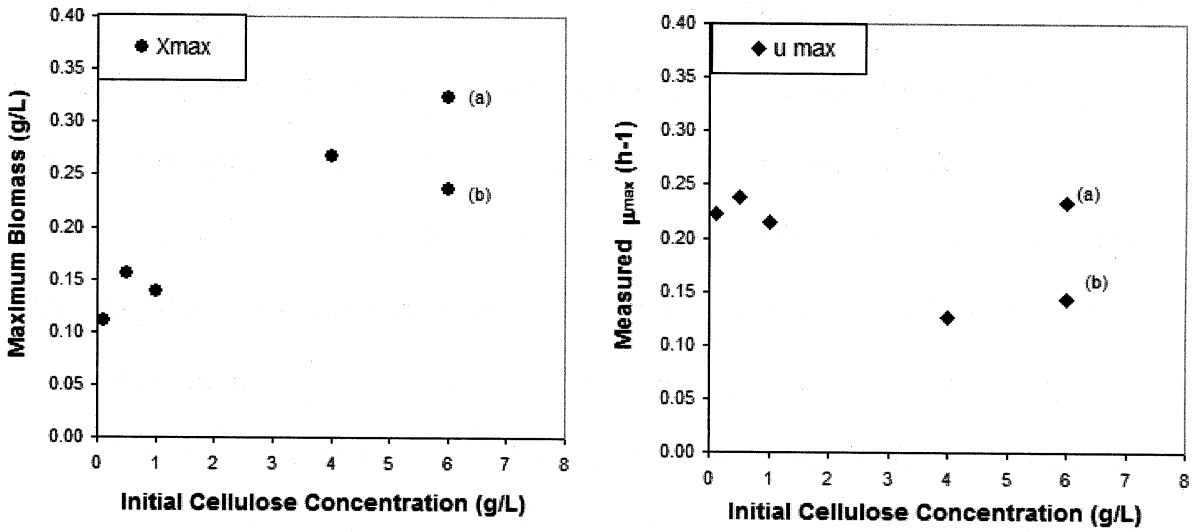

Figure 5-6 Measured maximum biomass and maximum growth rate under various S_0 when pH=8.5

(a)data from Trial 7, (b) data from Trial 12

- The variation of initial cellulose concentration affected the growth rates and maximum concentration of *C. phytofermentans.* To compare the trends among the trials in this group, it is more reasonable to use Trial 12 than Trial 7 as the former follows the trend of the trials. Notably from the modelling results in Table 5.1, the lowest μ_{max} was 0.065 h^{-1} when the cellulose concentration was at 6.0 g/L (Trial 12), while the highest μ_{max} was 0.259 h^{-1} when the cellulose concentration was at 0.1 g/L cellulose (Trial 8). Similar results were obtained from the observed μ_{max} as shown in Table 5.1 and Figure

5-6. The decreasing in μ_{max} is an indication of slower substrate degradation, and so is fermentation product formation. This may suggest that increased growth efficiency is achieved in a substrate limited environment, and would have implications on the organic loading rate of an anaerobic digestion process using *C. phytofermentans*. This supports the suggestion for confirmation experiments since such observation is not consistent with the results reported by Leschine and Warnick (2007). According to Leschine and Warnick (2007), this should not be the case when *C. phytofermentans* is employed. They reported that high concentrations of cellulose do not inhibit the action of *C. phytofermentans,* since the concentration of ethanol increases with increasing initial concentration of cellulose. On the other hand, the degree of cellulose degradation decreases and less amount of ethanol produces with increasing substrate concentrations by *C. cellulolyticum* and *C. thermocellum* were reported in the studies by Desvaux et al. (2000 and 2001) and Lynd et al.(1989), respectively.

- Similar to the results in other cellulolytic clostridia studies, high X_{max} were observed with high cellulose concentrations, and lower X_{max} values at lower cellulose concentrations. For example, a X_{max} of 0.236 g/L at 6.0 g/l cellulose and a X_{max} of 0.110 g/L at 0.1 g/L cellulose. More biomass is produced with increases S_0. A linear increasing relationship between S_0 and X_{max} is observed when S_0 in the range of 0.1 to 4.0 g/L, as shown in Figure 5-6, in which X_{max} is increased by 33 mg/L as S_0 increasing 1 g/L of cellulose. This observation is consistent with the results in the series of batch experiments using *C. cellulolyticum* by Desvaux et al. (2000). It was reported in their study that there is a steep linear relationship between S_0 and X_{max} when S_0 in the range

of 0.9 to 6.4 g/L, in which X_{max} was increased by 110 mg/L as S_0 increasing by 1 g/L of cellulose. However, it became a very flat linear relationship when S_0 from 6.4 to 29.1 g/L, in which X_{max} was only increased by 1.8 mg/L as S_0 increasing 1 g/L of cellulose.

- Again, a confirmation trial for pH=8.5 and S_0 = 6.0 g/L used in Trials 7 and 12, is required in the furture study. Although the trend of Trial 12 compares reasonably well with those in other trials, it is questionable that its maximum biomass concentration is lower than the one in Trial 11, in which S_0= 4.0 g/L was used. According to Lynd et al. (1989) and Desvaux et al. (2000), although the rate of growth would be slow down after S_0 reaching a certain limit, biomass increases as S_0 increasing. Therefore, confirmation for both Trials 11 and 12 is also required from this point of view.

- As S_0 increases, the time reaches the maximum biomass concentration is longer. When S_0 is less than 1 g/L, it takes slightly less than 30 hours to reach the maximum biomass concentrations. When S_0 are 4 and 6.0 g/L, it takes 40 to 60 hours to reach the maximum biomass concentrations.

5.1.4 Trade off between pH and S_0

By plotting the results from the trials testing the effects of pH and S_0 on growth in Figure 5-7, it can be seen that there is no equivalent effect by adjusting S_0 from 0.1 to 6.0 g/L when pH=8.5 to reach highest μ_{max} of 0.37 h^{-1} obtained at pH = 8.4 and S_0 = 6.0 g/L. However, there is an alternative to have μ_{max} to be at approximately 0.165 h^{-1} either by controlling S_0 = 6.0 g/L with pH =7.6 or S_0 = 2.72 g/L with pH=8.5. Sometimes, this kind

of alternative analysis is required for various reasons such as cost benefit, technology feasibility, and environmental impacts.

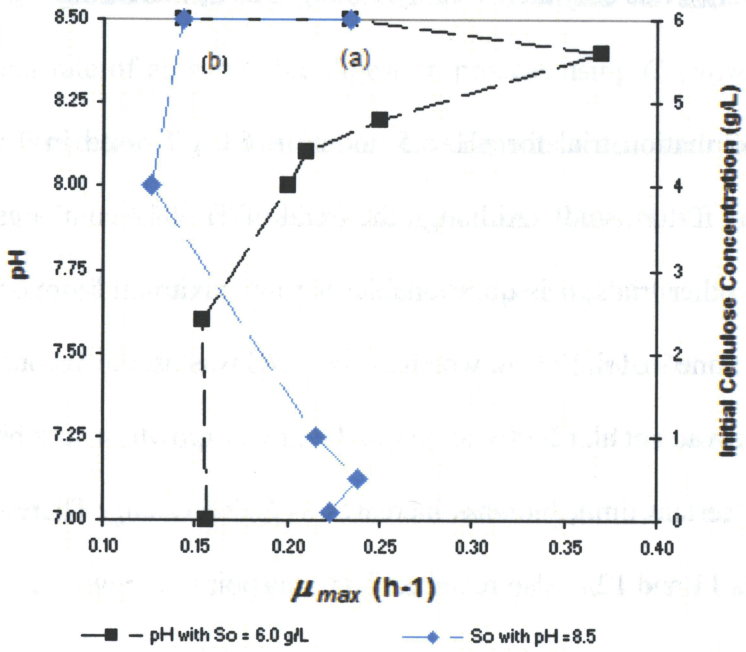

Figure 5-7 Observed μ_{max} Vs. pH and *So* of 12 Trials
(a)data from Trial 7, (b) data from Trial 12

5.1.5 Measured and Modelled μ_{max}

The measured and the modeled maximum biomass growth rates of μ_{max}, are different, as shown in Tables 5.1 and Figure 5-8. This is due to the difference in the calculation of this parameter. The μ_{max} from the logistic model describes the growth rate in the entire growth phase, while the measured μ_{max} is only the maximum one encountered during the growth phase. It can been seen from Figure 5-4 that when S_0 is 4.0 to 6.0 g/L with pH=8.5, there is a lag time, approximately 15- 20 hours, for *C. phytofermentans* to start the exponential growth phase. On the other hand, when pH is lower than 8 with S_0 = 6.0 g/L , such lag

does not occur and the measured and modelled maximum growth rates are about the same as shown in Figure 5-8.

When lag is included in the μ_{max} calculation, low μ_{max} will be resulted. Logistic model is used to simulate the entire growth phases from t = 0 to the beginning of the stationary phase. A review of the successful logistic modelling applications, such as King et al. (2006) and Mu et al. (2006), reveals that time lag is not observed in their experiments using soluble substrates. Our modelling results also confirm that higher R^2 values, greater than 0.93, were obtained when there was no such lag occurred in these trials.

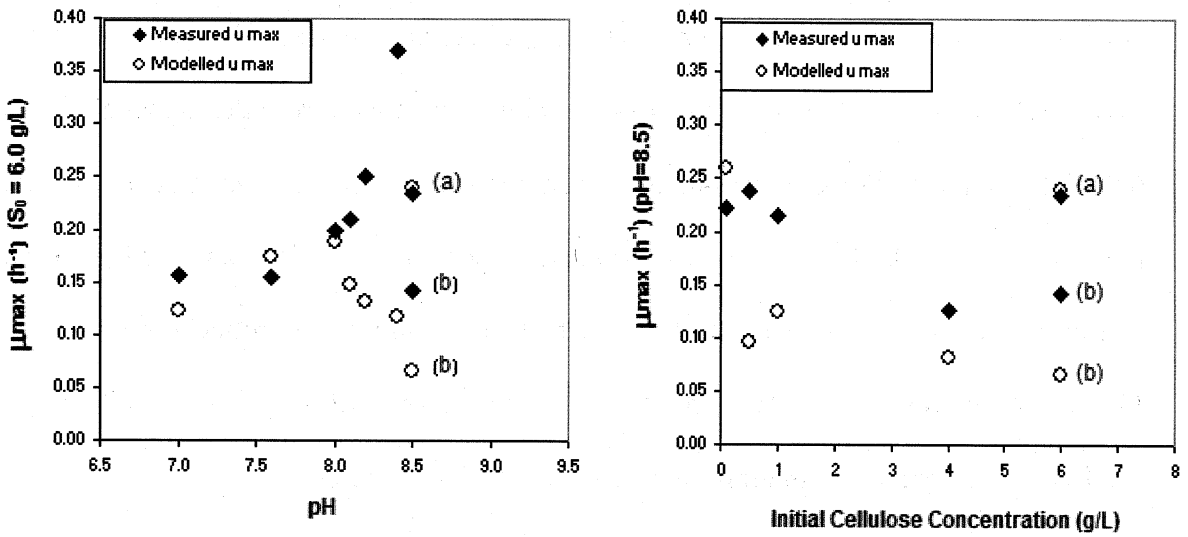

Figure 5-8 Comparison of modelled and measured μ_{max}

(a)data from Trial 7, (b) data from Trial 12

5.2 Biogas Production Kinetics

Using the Luedeking-Piret model described in Section 4.3, biogas production in the seven trials with various initial pH values from 7.0 to 8.5 at S_0 = 6.0 g/L were modelled and the results are shown in Figure 5-9. The correlations between the modeled and the measured

data are strongly in agreement with R^2 ranging from 0.94 to 0.99. Figure 5-10 shows the measured biogas volumes obtained from the trials in this group. During the first 25 hours, more biogas was generated from the trials with lower pH. By t =70 hours, the measured biogas generated from this group is in the range of 140 to 160 ml, except for Trial 4 (pH=8.1) which produced 187 ml biogas.

The effects of varying the initial cellulose concentrations from 0.1 to 6.0 g/L when pH = 8.5 on the biogas production were investigated. The modeled and measured results are shown in Figures 5-11 and 5-12, respectively. Again, very good correlations between the modeled and measured results are obtained with R^2 ranging from 0.93 to 0.96. Higher volume of gas production and higher rate of production occurred when there was a high initial cellulose concentration. The measured biogas generated from the higher S_0 (ie. 4 and 6.0 g/L) is about 45% more than the ones from the lower S_0 (ie. 0.1, 0.5 and 1.0 g/L). This may be associated with the higher population densities in trials using high cellulose concentrations. However, it is questionable that the accumulated produced biogas in Trial 11 (S_0 = 4.0 g/L) is constantly higher than the ones in Trial 12 in spite of less S_0 is used in Trial 11. Therefore, confirmation for Trials 11 and 12 is required.

Table 5.2 summaries the modelled and measured biogas production parameters. For illustrative purpose, the measured and modelled accumulative biogas volumes at 67 hours are presented in the table, which is the longest common duration for all the 12 trials. It was noted that Trial 4 with pH=8.1 and S_0 = 6.0 g/L produced the highest biogas volume among our experiments.

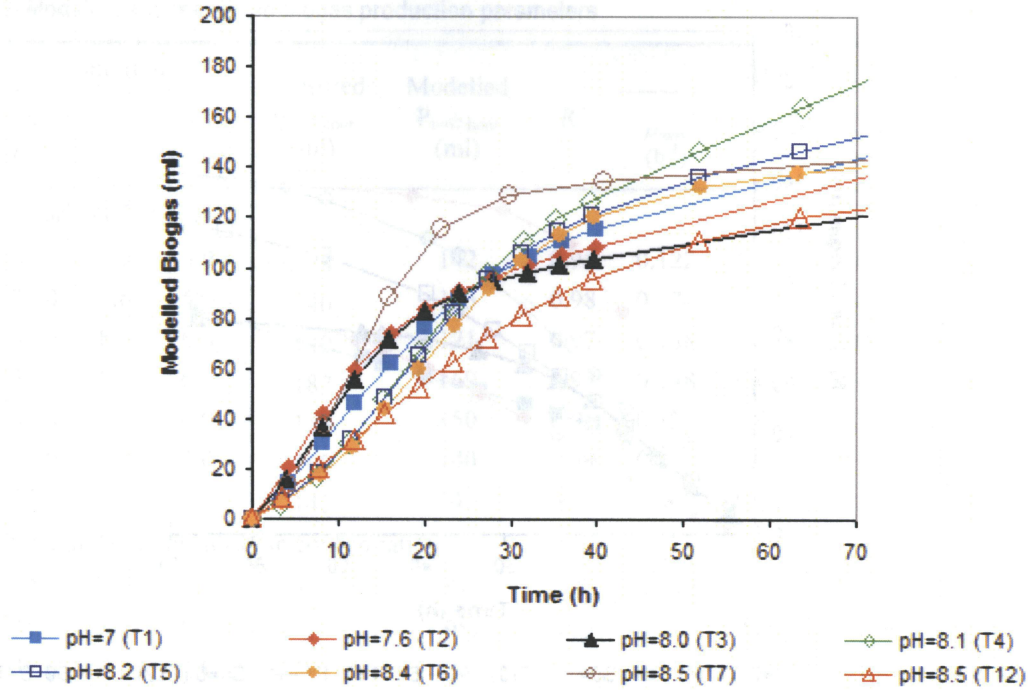

Figure 5-9 Modelled biogas production for Trials 1-7 &12 (pH 7.0 – 8.5, S_0 = 6.0 g/L)

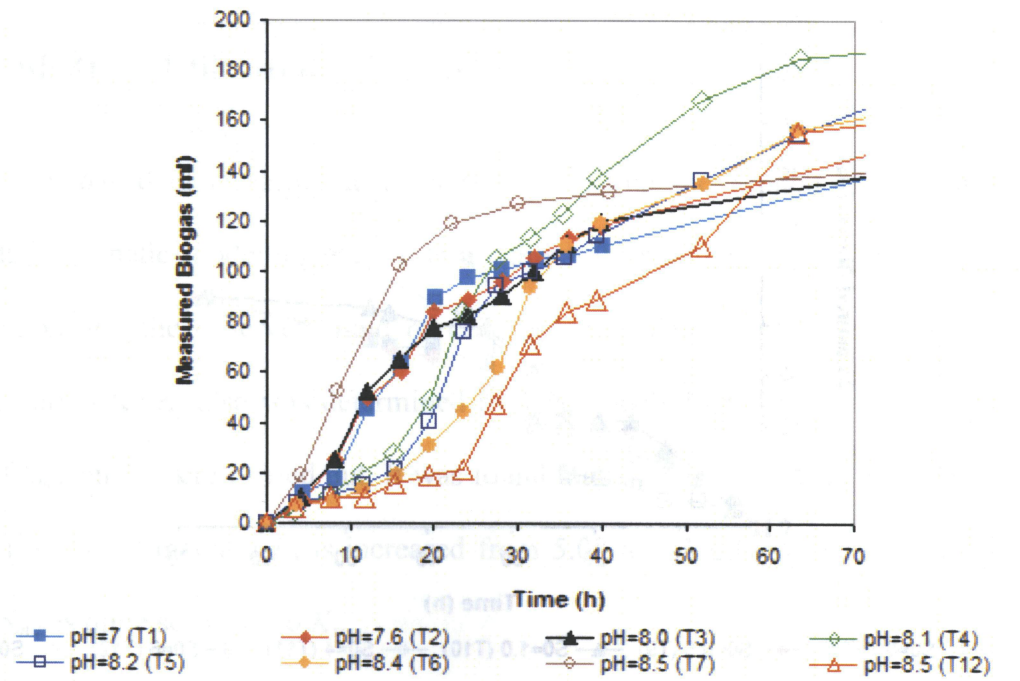

Figure 5-10 Measured biogas production for Trials 1-7 &12 (pH 7.0 – 8.5, S_0 = 6.0 g/L)

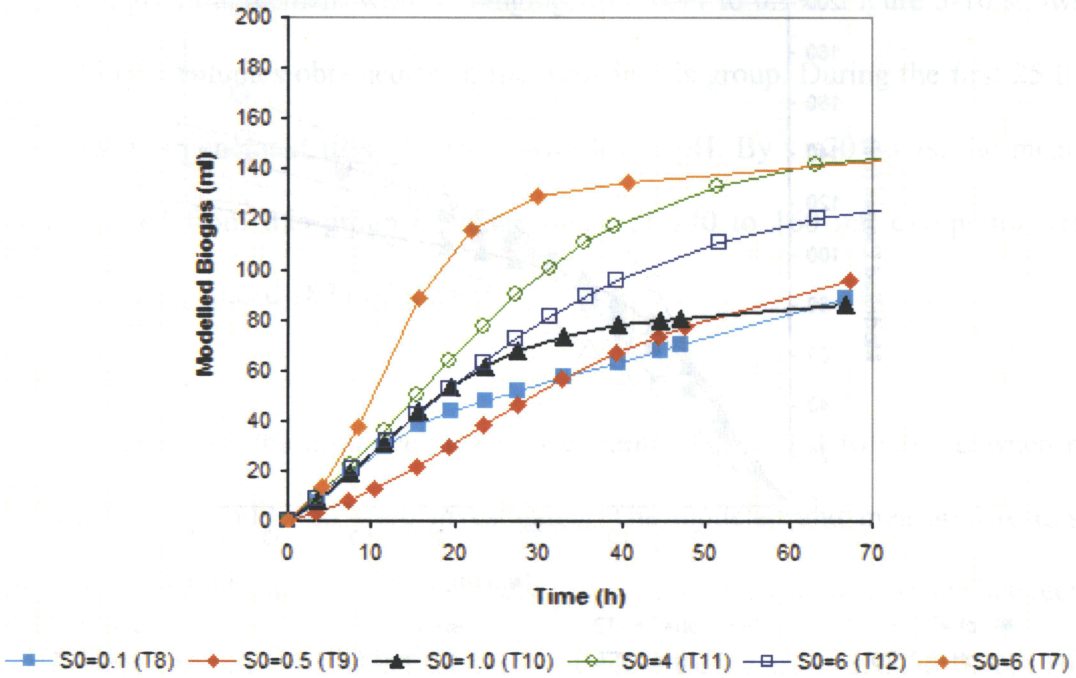

Figure 5-11 Modelled biogas production for Trials 8-12 &7 (S_0 = 0.1– 6.0 g/L, pH=8.5)

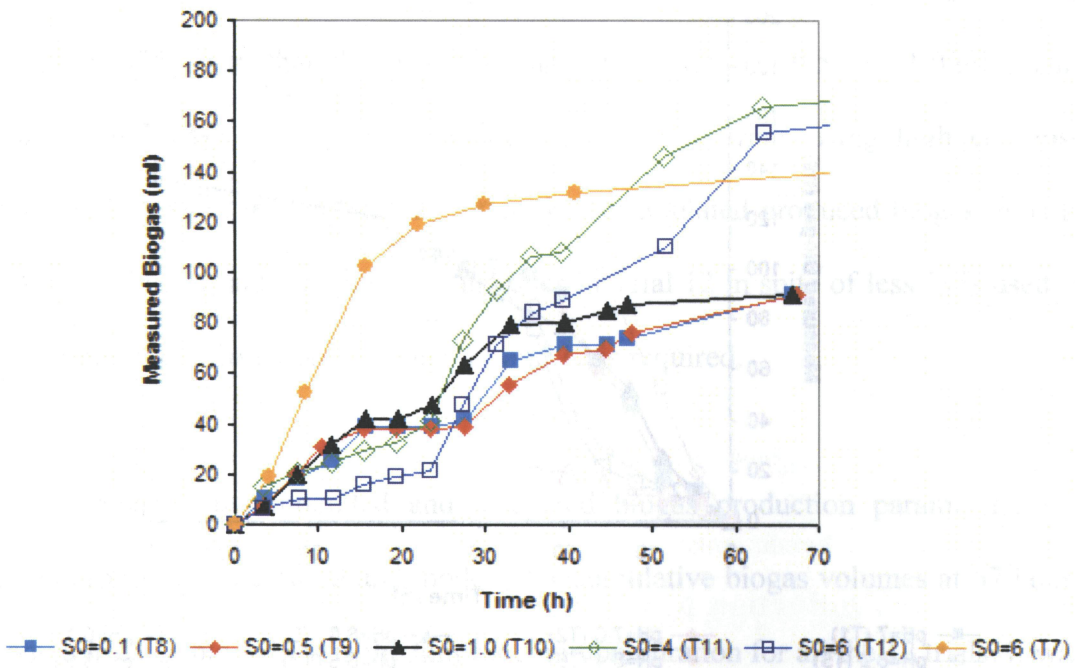

Figure 5-12 Measured biogas production for Trials 8-12 &7 (S_0 = 0.1– 6.0 g/L, pH=8.5)

Table 5.2 Modelled and measured biogas production parameters

Trial ID	Conditions			Measured $P_{t=67\ hour}$ (ml)	Modelled $P_{t=67\ hour}$ (ml)	R^2	Modelling parameters				
	T (°C)	pH	S_0 (g/L)				μ_{max} (h^{-1})	X_o (g/L)	X_{max} (g/L)	α	β
Group 1: variable initial pH											
1	37.0	7.0	6.0	138	142	0.98	0.122	0.014	0.059	2100	14.8
2	37.0	7.6	6.0	140	133	0.98	0.174	0.022	0.073	1567	12.3
3	37.0	8.0	6.0	140	121	0.97	0.188	0.014	0.074	1469	7.6
4	37.0	8.1	6.0	187	169	0.97	0.148	0.014	0.151	703	9.43
5	37.0	8.2	6.0	158	150	0.97	0.132	0.016	0.141	849	6.2
6	37.0	8.4	6.0	160	140	0.94	0.117	0.014	0.134	1027	2.5
7	37.0	8.5	6.0	140	142	0.99	0.240	0.029	0.324	435	0.8
Group 2: variable initial substrate concentration											
8	37.0	8.5	0.1	92	89	0.96	0.259	0.017	0.110	359	8.4
9	37.0	8.5	0.5	91	95	0.95	0.096	0.020	0.156	452	4.9
10	37.0	8.5	1.0	91	86	0.96	0.124	0.029	0.138	688	1.5
11	37.0	8.5	4.0	170	144	0.94	0.081	0.063	0.267	645	1.1
12	37.0	8.5	6.0	160	123	0.93	0.065	0.079	0.236	677	1.7

5.3 Substrate Utilization Kinetics

This study has demonstrated the advantages of using the Monod model for substrate degradation kinetic modelling. S_{min} was a calibrating parameter based on the reasonable assumption and the observed trend of cellulose degradation. Another important growth kinetic parameter K_s also was determined by this modelling approach. Further, sensitivity tests of S_{min} on K_s were carried out. It was found that an increase of S_{min} from 10% of S_0 to 30% of S_0 would increase K_s is increased from 5.08 to 5.3 g/L for the test trial (Trial 7). Thus, S_{min} is quite sensitive to K_s.

6 Conclusions & Recommendations

6.1 Conclusions

Twelve trials of experiments were conducted with incubation temperature at 37°C under various controlled pH values (7.0 to 8.5) and various initial cellulose concentration settings (0.1 to 6.0 g/L). The experiment results indicate that there are dependencies of biomass growth and biogas production on both pH and initial substrate concentration. Specifically, there is an indication that an increase in biomass growth is resulted from higher pH values, with the maximum biomass growth rate towards a pH of 8.4. On the other hand, with higher initial substrate concentration more biomass can be produced, but not the maximum growth rate. Our experimental results showed that the optimal growth condition for C. *phytofermentans* in batch culture was at pH = 8.4 and S_o = 6.0 g/L. Under such condition, the maximum growth rate of 0.37h^{-1} was observed. Comparing the μ_{max} from Desveaux *et al.* (2000), the rates of μ_{max} using *C. phytofermentans* in this study are in the range of 0.126 to 0.37h^{-1}, which is about two to seven times higher than the one (0.057 h^{-1}) using *C. cellulolyticum.* This confirms that *C. phytofermentans* is a relatively fast growth cellulosic bacterium.

Mathematical models, using a combination modelling approach with the logistic equation, Monod model, and Luedeking-Piret model, were developed for biomass growth, substrate degradation, and biogas production based on our experiment results. This study demonstrated the determination of the four parameters (μ_{max}, K_s, Y, and S_{min}), which can

describe satisfactorily growth or degradation phenomena, using the proposed integration modelling approach. The application of Luedeking-Piret model to simulate product formation in our experiments shows that there is a strong correlation between the modeled and the measured data for all the trials. All correlation coefficients (R^2) were in the range of 0.93 to 0.99. This suggests that Luedeking-Piret model was able to describe the product formation by *C. phytofermentans* in this study.

The results from this study will very much narrow down the investigations of the growth characteristics of *C. phytofermentans* in future studies. Although the optimum growth condition was identified in this study, additional experiments to confirm the findings are required due to the limitations of the available measurements and inconsistency in some of the results in this study. Fermentation is a very complex dynamic process and is often very difficult to clarify what actually happens in a particular fermentation (e.g. the experimental data from Trials 7 and 12). The discrepancy in the experimental results could perhaps attribute to 1) limitation of the models, 2) cell apoptosis and environmental conditions that changed during cultivation, and 3) the human errors in the carrying out experiments and taking measurements. We learn that experiments conducted under wide range conditions, such as changing pH and S_o, not only provide insight into growth kinetics but also provide an opportunity to evaluate the performance of the mathematical models and understand their limitations. This leads to look for improvement or modification to the models in the future study.

6.2 Recommendations for Future Study

- Although optimal growth condition has been identified as pH=8.4, S_o =6.0 g/L at 37°C, it is suggested that additional experiments with pH raging from 8.2 to 8.5 with high S_0 input at 4.0 – 6.0 g/L should be conducted before conducting experiments with other pH and S_0. This is to address the issues of the limitations of the available measurements and inconsistency in some of the results in this study.
- Base on the observations from this study, the growth phase using *C. phytofermentans* may be longer than 40 hours. It was observed in Desvaux et al. (2000) experiments, the cellulose hydrolysis by *C. cellulolyticum* increased up to 50 to 70 hours. Since *C. phytofermentans* grow slower than *C. cellulolyticum* (Ren et al. 2007), the close monitoring period should be increased to longer than 70 hours in our future studies.
- The compositions of the produced gas need to be identified. Thus, use of analytical instruments, such as gas chromatograph, is required in future studies.
- In our experiments, as shown in Figure 5-6, the μ_{max} tends to decrease as S_o increase although confirmation is required for Trials 7 and 12. The decreasing in μ_{max} is an indication of slower substrate degradation, and so is fermentation product formation. It is also reported in the studies by Lynd et al. (1989) and Desvaux et al. (2000) that the degree of cellulose degradation, hence ethanol to acetate ratios, decrease with increasing cellulose concentrations. This results contrast with the results reported by Leschine and Warnick (2007) that high concentrations of cellulose do not inhibit the action of *C. phytofermentans* , since the concentration

of ethanol increases with increasing initial concentration of cellulose while the acetate levels do not significantly increase.

- It was claimed that two to four times more ethanol than acetate are formed, whereas most other cellulolytic clostridia produce roughly equal amounts of these fermentation products or less ethanol than acetate (Joint Genome Institute, 2007 and Leschine and Warnick, (2007). However, the ethanol to acetic ratios of 0.58 and 1.03 were reported in batch cultures of *C. phytofermentans* depending on the substrate grown on (Ren et al., 2007). Identification and quantification of ethanol and acetic acid formations will be conducted in the next phase of our study to verify this issue.
- The incubation temperature was at 37°C in this study. Test temperature effects on *C. phytofermentans* growth should be conducted in future.
- Since the situation of a microorganism under natural conditions is most probably somewhere between the closed batch culture and the open continuous-culture system, further investigations with chemostats should allow a better understanding of the *C. phytofermentans* behaviour in its ecological niche with its natural insoluble substrate, the cellulose (Desvaux et al., 2000). Experiments under continuous culture will be undertaken to predict what would be in the natural conditions.
- Inaccurate measurement results of residual cellulose concentrations were obtained in this study using Updegraff (1969) method. Alternative method(s) should be investigated .

- Each step during the experiment has impacts on the experimental results. Thus, consistence in sample handling, testing, and analyzing is very important.
- Computer programs of the three model integration can verify the assumptions used in the modelling, such as S_{min}, since modeling results from logistic model and Luedeking-Piret model have reasonable good R^2 values, especially, those resulted from Luedeking-Piret model.
- Based on the results from confirmation runs, modification to logistic model may be required.
- Quantify ethanol yield is important in our future studies since one of the major determining factors for the potential application of cellulosic ethanol is the cost. Before we can answer the question of the amount of ethanol generated from a given amount of municipal source-separated organics (SSO) wastes, we need to conduct experiments on the amounts of ethanol produced from certain amounts of cellulose to identify the optimal condition of high ethanol yield.
- For future application, seasonal changes in the characteristics of municipal SSO wastes need to be studied. The differences in the SSO characteristics or parameters may have impacts on the effectiveness of the cellulosic fermentation and production formation.

6.3 Improvement on Laboratory Facility

Experience gained from this and the feasibility study in Stage 1 (Luk et al., 2007) on *C. phytofermentans* has confirmed the effectiveness of the use of our controlled experimental approach. However, some of the analytical works was hampered because of the

inadequacies of our laboratory facilities. The following items would be of great asset to speed up with accuracy the testing and sampling procedures:

1) Manifold: with a series of valves and luer lock needles to minimize exposing samples to oxygen
2) Serum Vials with Septa and Crimpers: to replace Erlenmeyer flask for better seal
3) pHController: continuously and automatically adjust the pH level through out the bacterial growth process
4) High Speed Micro-Centrifuge: up to 24 samples with max. speed at 14000rpm. Currently our centrifuge can only accommodate 8 samples with max. speed at 3600 rpm
5) Water Bath with Vial Racks: for easy control of temperature and higher capacity

Depending on the course of our project, these items will be required to carry out future studies:

1) Reagents for HPLC (High Performance Liquid Chromatography);
2) GC Columns: for hydrogen gas;
3) Glassware: separate glassware for different tests to avoid contamination;
4) Anaerobic Bio-Reactor; and
5) Chemostats.

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Appendixes

Appendix A: Biomass and Biogas Experimental Data and Modelling Results

Trial ID: 1 37°C, pH 7.0, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken	sample taken	elapsed time	analysis	A_{sss}	protein	Measured X	Measured Xavg	Xerror	μ (1)	ln (Xf(X$_m$-X))	Modeled X	(5)
	[d/m/y]	[hh:mm]	[hour]	[d/m/y]		[g/l]	[g/l]	[g/l]	[g/L]	[hour^{-1}]		[g/L]	
4.1.a	26/07/2007	15:40	0.0	30/07/2007	0.029	0.016	0.029	0.025	0.004		-0.337	0.014	
4.1.b				30/07/2007	0.021	0.011	0.020						
4.2.a	26/07/2007	19:50	4.2	30/07/2007	0.009	0.005	0.009	0.012	0.003	-0.162	-1.355	0.020	
4.2.b				30/07/2007	0.016	0.009	0.015						
4.3.a	26/07/2007	23:50	8.2	30/07/2007	0.027	0.015	0.027	0.023	0.004	**0.156**	-0.444	0.027	
4.3.b				30/07/2007	0.020	0.011	0.019						
4.4.a	27/07/2007	3:40	12.0	30/07/2007	0.042	0.024	0.042	0.037	0.005	0.125	0.553	0.034	0.738
4.4.b				30/07/2007	0.033	0.018	0.033						
4.5.a	27/07/2007	7:40	16.0	30/07/2007	0.068	0.039	0.070	**0.059**	0.011	0.112	#DIV/0!	0.041	
4.5.b				30/07/2007	0.047	0.027	0.047						
4.6.a	27/07/2007	11:45	20.1	30/07/2007	0.053	0.030	0.054	0.052	0.002	-0.029	2.060	0.046	
4.6.b				30/07/2007	0.050	0.028	0.051						
4.7.a	27/07/2007	15:40	24.0	30/07/2007	0.050	0.028	0.051	0.049	0.002	-0.016	1.601	0.050	
4.7.b				30/07/2007	0.047	0.027	0.047						
4.8.a	27/07/2007	19:40	28.0	30/07/2007	0.031	0.017	0.031	0.032	0.001	-0.107	0.151	0.053	
4.8.b				30/07/2007	0.033	0.018	0.033						
4.9.a	27/07/2007	23:45	32.1	30/07/2007	0.054	0.031	0.055	0.048	0.006	0.103	1.542	0.055	
4.9.b				30/07/2007	0.042	0.024	0.042						
4.10.a	28/07/2007	3:40	36.0	30/07/2007	0.050	0.028	0.051	0.054	0.003	0.027	2.371	0.057	
4.10.b				30/07/2007	0.056	0.032	0.057						
4.11.a	28/07/2007	7:40	40.0	30/07/2007	0.050	0.028	0.051	0.048	0.003	-0.029	1.477	0.057	
4.11.b				30/07/2007	0.045	0.025	0.045						
4.12.a	30/07/2007	9:00	89.3	30/07/2007	0.044	0.025	0.044	0.041	0.003	-0.003	0.833	0.059	
4.12.b				30/07/2007	0.038	0.021	0.038						

X_{max}	0.059	(2)
X_o	0.014	(3)
μ_{max}	0.122	(4)

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ (X_2, X_1)}$$

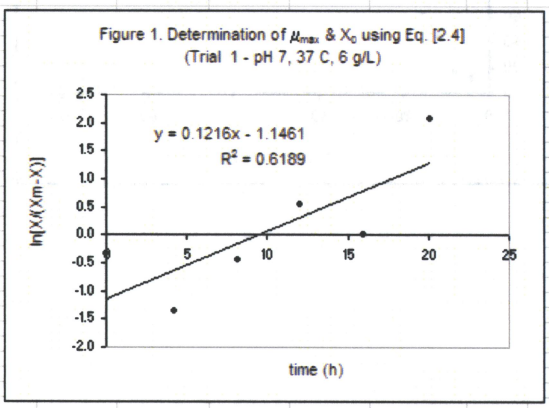
(2) X_{max} obtained from the measured maximum average data during the growth phase

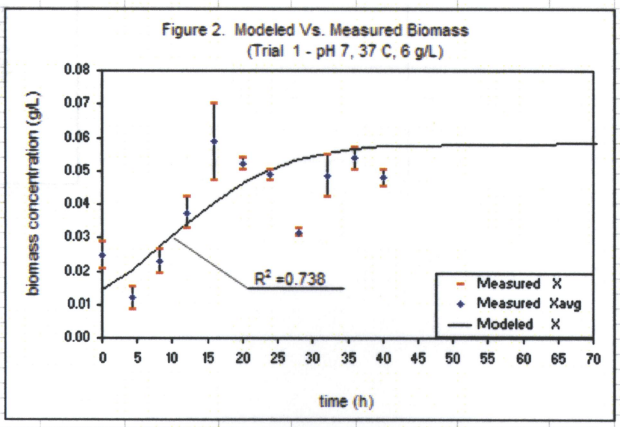
(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 1 37°C, pH 7.0, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2 (3)
			Eq.[2.22]	Eq.[2.23]		Eq.[2.21]	
	Measured P	Measured dP/dt	A(t)	B(t)	P - β*B(t)	Modelled P	
	(ml)	(ml/h)				(ml)	
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
4.2	12.0	2.9	0.006	0.072	10.9	14.1	
8.2	17.5	1.4	0.013	0.167	15.0	29.9	
12.0	45.5	7.4	0.020	0.284	41.3	45.9	
16.0	63.5	4.5	0.026	0.433	57.1	62.0	
20.1	89.5	6.3	0.032	0.612	80.4	76.5	0.978
24.0	97.5	2.1	0.036	0.801	85.6	87.8	
28.0	100.9	0.9	0.039	1.008	85.9	97.1	
32.1	104.4	0.9	0.041	1.231	86.1	104.7	
36.0	106.2	0.5	0.042	1.450	84.7	110.6	
40.0	110.5	1.1	0.043	1.678	85.6	115.7	
89.3	153.5	0.9	0.045	4.566	85.8	161.4	

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

β	14.8	(1)
α	2099.8	(2)

Figure 1. Determination of α using Eq. [2.21]
(Trial 1 - pH 7, 37 C, 6 g/L)

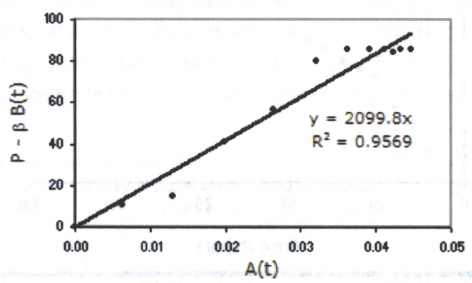
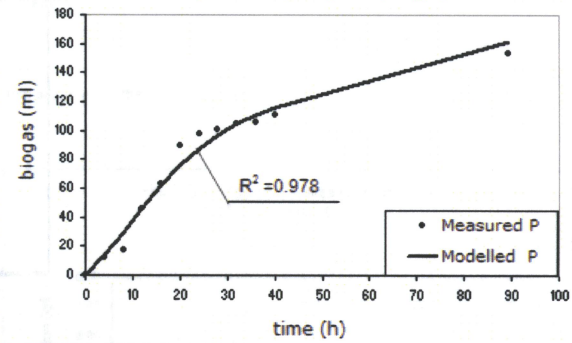


Figure 2. Modeled Vs. Measured Biogas
(Trial 1 - pH 7, 37 C, 6 g/L)

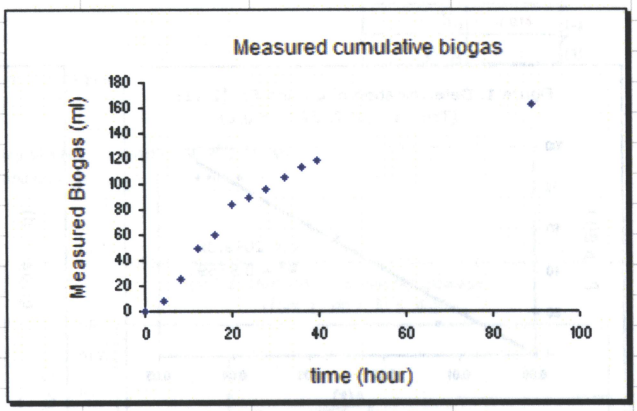


Trial #2

Trial ID: 2
Inoculum ID: 3.4
Description: 37°C, pH 7.6, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	26/07/2007	15:55	0.0	0.0	0.0
2	26/07/2007	20:05	4.2	8.5	8.5
3	26/07/2007	0:05	8.2	17.0	25.5
4	27/07/2007	3:55	12.0	24.5	50.0
5	27/07/2007	7:55	16.0	10.0	60.0
6	27/07/2007	11:55	20.0	24.0	84.0
7	27/07/2007	15:55	24.0	5.0	89.0
8	27/07/2007	19:55	28.0	7.0	96.0
9	27/07/2007	23:55	32.0	9.5	105.5
10	28/07/2007	3:50	35.9	8.0	113.5
11	28/07/2007	7:45	39.8	5.0	118.5
12	30/07/2007	9:10	89.2	44.0	162.5



Trial ID: 2 37°C, pH 7.6, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken	sample taken	elapsed time	analysis	A_{sss}	protein	Measured X	Measured Xavg	Xerror	μ (1)	ln (X/(X$_m$-X))	Modeled X	(5)
	[d/m/y]	[hh:mm]	[hour]	[d/m/y]		[g/l]	[g/l]	[g/l]	[g/L]	[hour^{-1}]		[g/L]	
5.1.a	26/07/2007	15:55	0.0	30/07/2007	0.031	0.017	0.031	0.026	0.005		-0.581	0.022	
5.1.b				30/07/2007	0.022	0.012	0.021						
5.2.a	26/07/2007	20:05	4.2	30/07/2007	0.028	0.015	0.028	0.029	0.001	0.022	-0.433	0.034	
5.2.b				30/07/2007	0.030	0.017	0.030						
5.3.a	26/07/2007	00:05	8.2	30/07/2007	0.059	0.034	0.060	0.050	0.010	0.137	0.801	0.046	
5.3.b				30/07/2007	0.040	0.022	0.040						
5.4.a	27/07/2007	03:55	12.0	30/07/2007	0.037	0.021	0.037	0.046	0.010	-0.020	0.572	0.056	
5.4.b				30/07/2007	0.055	0.031	0.056						
5.5.a	27/07/2007	07:55	16.0	30/07/2007	0.064	0.037	0.066	0.066	0.001	0.089	2.378	0.063	0.484
5.5.b				30/07/2007	0.065	0.038	0.067						
5.6.a	27/07/2007	11:55	20.0	30/07/2007	0.058	0.033	0.059	0.054	0.005	-0.050	1.094	0.068	
5.6.b				30/07/2007	0.049	0.028	0.049						
5.7.a	27/07/2007	15:55	24.0	30/07/2007	0.047	0.027	0.047	0.045	0.002	-0.046	0.503	0.070	
5.7.b				30/07/2007	0.043	0.024	0.043						
5.8.a	27/07/2007	19:55	28.0	30/07/2007	0.042	0.024	0.042	0.038	0.004	-0.041	0.116	0.071	
5.8.b				30/07/2007	0.035	0.019	0.035						
5.9.a	27/07/2007	23:55	32.0	30/07/2007	0.073	0.043	0.076	**0.073**	0.003	**0.154**	#DIV/0!	0.072	
5.9.b				30/07/2007	0.067	0.039	0.069						
5.10.a	28/07/2007	03:50	35.9	30/07/2007	0.061	0.035	0.063	0.065	0.003	-0.027	2.196	0.072	
5.10.b				30/07/2007	0.066	0.038	0.068						
5.11.a	28/07/2007	07:45	39.8	30/07/2007	0.063	0.036	0.065	0.061	0.004	-0.018	1.652	0.072	
5.11.b				30/07/2007	0.056	0.032	0.057						
5.12.a	30/07/2007	09:10	89.2	30/07/2007	0.058	0.033	0.059	0.045	0.014	-0.006	0.485	0.073	
5.12.b				30/07/2007	0.031	0.017	0.031						

X_{max}	**0.073**	(2)
X_o	**0.022**	(3)
μ_{max}	0.174	(4)

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ (X_2, X_1)}$$

(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

Figure 1. Determination of μ_{max} & X_o using Eq. [2.4]
(Trial 2 - pH 7.6, 37 C, 6 g/L)

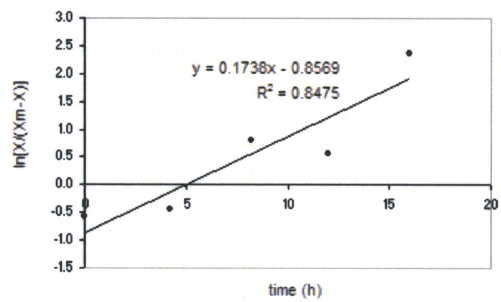
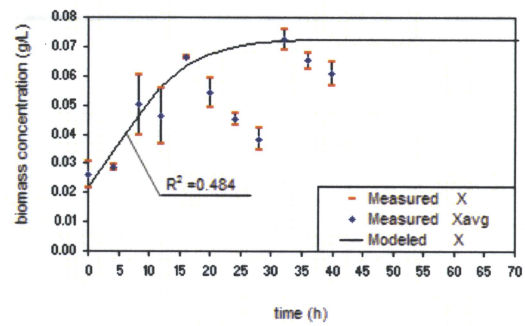


Figure 2. Modeled Vs. Measured Biomass
(Trial 2 - pH 7.6, 37 C, 6 g/L)



Trial ID: 2 37°C, pH 7.6, 6.0 g/L cellulose

Biogas (P) measurement & modelling

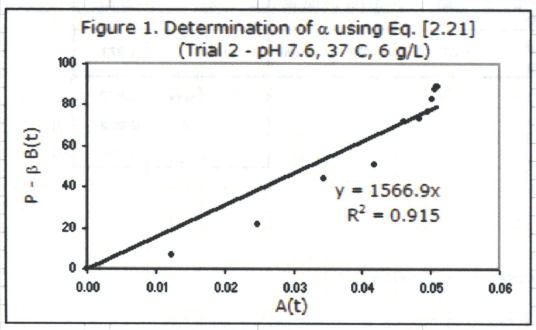
time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	(3)
0	0.0	0.0	0.000	0.000	0.0	0.0	
4.2	8.5	2.0	0.012	0.116	7.1	20.8	
8.2	25.5	4.3	0.025	0.277	22.1	42.1	
12	50.0	6.4	0.035	0.472	44.2	59.9	
16	60.0	2.5	0.042	0.712	51.3	74.1	
20	84.0	6.0	0.046	0.975	72.0	84.0	0.979
24	89.0	1.3	0.048	1.251	73.6	91.2	
28	96.0	1.8	0.050	1.533	77.2	96.6	
32	105.5	2.4	0.050	1.820	83.2	101.1	
35.9	113.5	2.1	0.051	2.101	87.7	105.1	
39.8	118.5	1.3	0.051	2.383	89.2	108.8	
89.2	162.5	0.9	0.051	5.965	89.3	153.0	

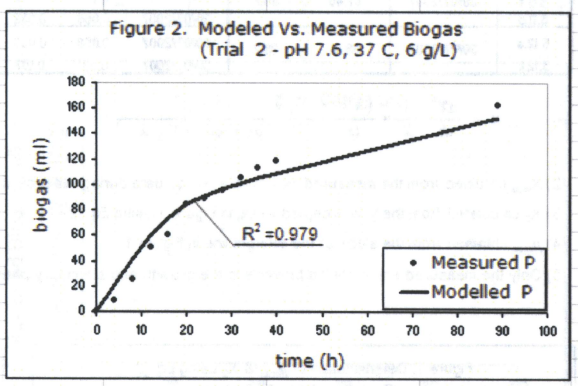
(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

β	12.3	(1)
α	1566.9	(2)



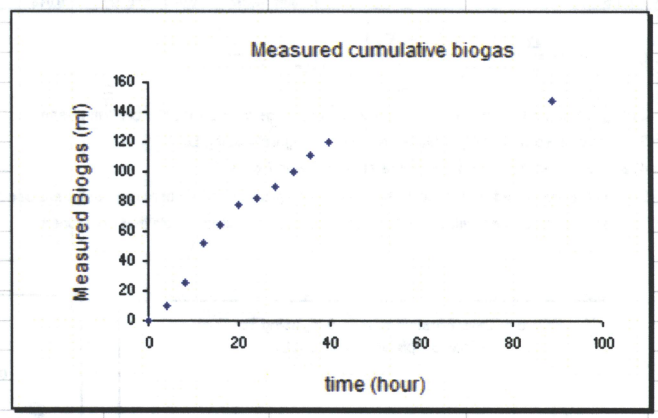


Trial #3

Trial ID: 3
Inoculum ID: 3.4
Description: 37°C, pH 8.0, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	26/07/2007	16:15	0.0	0.0	0.0
2	26/07/2007	20:15	4.0	10.5	10.5
3	27/07/2007	0:20	8.1	15.2	25.7
4	27/07/2007	4:15	12.0	27.0	52.7
5	27/07/2007	8:10	15.9	12.0	64.7
6	27/07/2007	12:15	20.0	13.0	77.7
7	27/07/2007	16:15	24.0	5.0	82.7
8	27/07/2007	20:15	28.0	7.5	90.2
9	28/07/2007	0:10	31.9	10.0	100.2
10	28/07/2007	4:00	35.8	11.0	111.2
11	28/07/2007	7:55	39.7	9.0	120.2
12	30/07/2007	9:20	89.1	28.0	148.2



Trial ID: 3 37°C, pH 8.0, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{sss}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_\infty$-X))	Modeled X [g/L]	(5)
6.1.a	26/07/2007	16:15	0.0	30/07/2007	0.022	0.012	0.021	0.021	0.001		-0.933	0.014	
6.1.b				30/07/2007	0.021	0.011	0.020						
6.2.a	26/07/2007	20:15	4.0	30/07/2007	0.018	0.010	0.017	0.016	0.001	-0.060	-1.254	0.025	
6.2.b				30/07/2007	0.016	0.009	0.015						
6.3.a	27/07/2007	00:20	8.1	30/07/2007	0.037	0.021	0.037	0.039	0.003	**0.200**	0.124	0.038	
6.3.b				30/07/2007	0.042	0.024	0.042						
6.4.a	27/07/2007	04:15	12.0	30/07/2007	0.037	0.021	0.037	0.042	0.005	0.013	0.239	0.051	
6.4.b				30/07/2007	0.046	0.026	0.046						
6.6.a	27/07/2007	08:10	15.9	30/07/2007	0.069	0.040	0.071	0.066	0.006	0.116	2.061	0.061	0.733
6.6.b				30/07/2007	0.059	0.034	0.060						
6.6.a	27/07/2007	12:15	20.0	30/07/2007	0.053	0.030	0.054	0.051	0.003	-0.062	0.791	0.067	
6.6.b				30/07/2007	0.048	0.027	0.048						
6.7.a	27/07/2007	16:15	24.0	30/07/2007	0.056	0.032	0.057	0.055	0.002	0.018	1.041	0.071	
6.7.b				30/07/2007	0.052	0.030	0.053						
6.8.a	27/07/2007	20:15	28.0	30/07/2007	0.053	0.030	0.054	0.045	0.008	-0.048	0.447	0.073	
6.8.b				30/07/2007	0.037	0.021	0.037						
6.9.a	28/07/2007	00:10	31.9	30/07/2007	0.076	0.045	0.079	**0.074**	0.005	0.124	--	0.073	
6.9.b				30/07/2007	0.067	0.039	0.069						
6.10.a	28/07/2007	04:00	35.8	30/07/2007	0.047	0.027	0.047	0.047	0.001	-0.116	0.534	0.074	
6.10.b				30/07/2007	0.046	0.026	0.046						
6.11.a	28/07/2007	07:55	39.7	30/07/2007	0.020	0.011	0.019	0.027	0.008	-0.137	-0.554	0.074	
6.11.b				30/07/2007	0.035	0.019	0.035						
6.12.a	30/07/2007	09:20	89.1	30/07/2007	0.048	0.027	0.048	0.048	0.000	0.011	0.627	0.074	
6.12.b				30/07/2007	0.048	0.027	0.048						

X_{max}	**0.074**	(2)
X_o	0.014	(3)
μ_{max}	**0.198**	(4)

(1) $$\mu = \frac{dX}{dt}\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ (X_2, X_1)}$$

(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.
The maximum measured value in the growth phase is indicated in bold and underlined.

Figure 1. Determination of μ_{max} & X_o using Eq. [2.4]
(Trial 3 - pH 8.0, 37 C, 6 g/L)

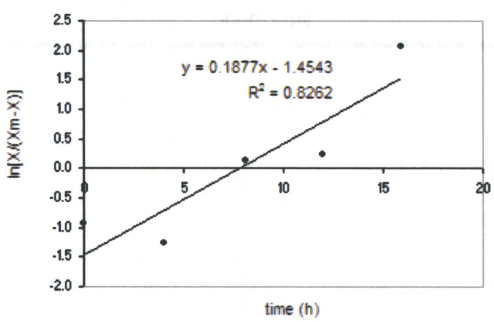
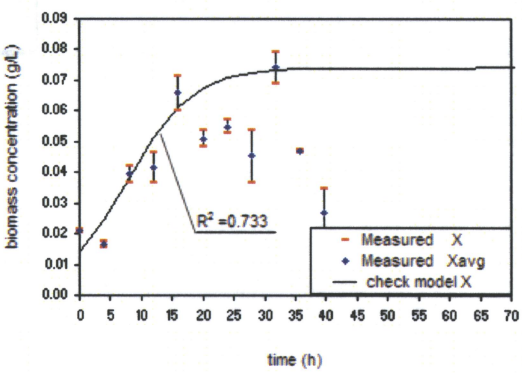


Figure 2. Modeled Vs. Measured Biomass
(Trial 3 - pH 8.0, 37 C, 6 g/L)



Trial ID: 2 37°C, pH 7.6, 6.0 g/L cellulose

Biogas (P) measurement & modelling

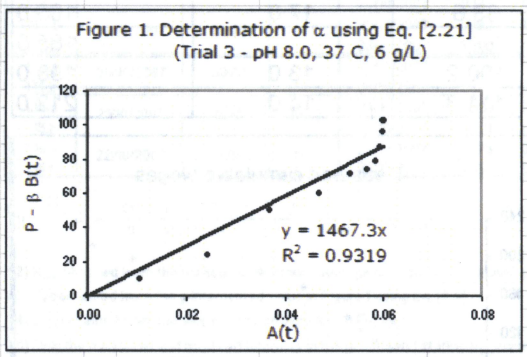
time (h)	Measurment		Model				R^2
	Measured P (ml)	Measured dP/dt (ml/h)	Eq.[2.22] A(t)	Eq.[2.23] B(t)	P - β*B(t)	Eq.[2.21] Modelled P (ml)	(3)
0.0	0.0	0.00	0.000	0.000	0.000	0.000	
4.0	10.5	2.63	0.011	0.076	9.920	16.013	
8.1	25.7	3.71	0.024	0.204	24.139	37.195	
12.0	52.7	6.92	0.037	0.380	49.802	57.383	
15.9	64.7	3.08	0.047	0.600	60.122	73.492	
20.0	77.7	3.17	0.053	0.864	71.101	84.973	0.969
24.0	82.7	1.25	0.057	1.142	73.983	92.099	
28.0	90.2	1.88	0.059	1.429	79.289	96.837	
31.9	100.2	2.56	0.059	1.714	87.113	100.237	
35.8	111.2	2.82	0.060	2.002	95.919	103.029	
39.7	120.2	2.31	0.060	2.290	102.717	105.522	
89.1	148.2	**0.57**	0.060	5.957	102.724	133.784	

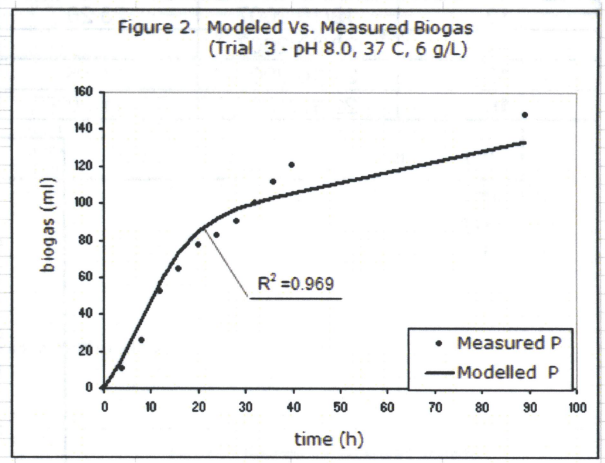
(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

β	**7.6**	(1)
α	**1467.3**	(2)



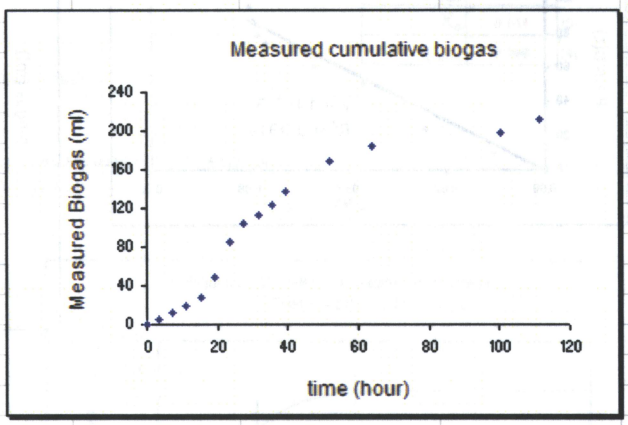


Trial #4

Trial ID: 4
Inoculum ID: 3.4
Description: 37°C, pH 8.1, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	17/10/2007	7:45	0.0	0.0	0.0
2	17/10/2007	11:05	3.3	5.0	5.0
3	17/10/2007	15:08	7.4	6.5	11.5
4	17/10/2007	19:00	11.2	8.5	20.0
5	17/10/2007	23:00	15.2	7.5	27.5
6	18/10/2007	3:00	19.3	22.0	49.5
7	18/10/2007	7:00	23.3	35.0	84.5
8	18/10/2007	11:00	27.3	20.0	104.5
9	18/10/2007	15:10	31.5	9.0	113.5
10	18/10/2007	19:00	35.3	9.5	123.0
11	18/10/2007	23:00	39.3	14.0	137.0
12	19/10/2007	11:24	51.7	31.0	168.0
13	19/10/2007	23:20	63.6	17.0	185.0
14	20/10/2007				185.0
15	21/10/2007	11:55	100.2	13.0	198.0
16	22/10/2007	11:00	111.3	14.0	212.0



Trial ID: 4 37°C, pH 8.1, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken	sample taken	elapsed time	analysis	A_{sss}	protein	Measured X	Measured Xavg	Xerror	μ (1)	ln (X/(X$_m$-X))	Modeled X	(5)
	[d/m/y]	[hh:mm]	[hour]	[d/m/y]		[g/l]	[g/l]	[g/l]	[g/L]	[hour^{-1}]		[g/L]	
1.1.a	17/10/2007	07:45	0.0	23/10/2007	0.038	0.021	0.038	0.028	0.010		-1.477	0.014	
1.1.b				23/10/2007	0.019	0.010	0.018						
1.2.a	17/10/2007	11:05	3.3	23/10/2007	0.018	0.010	0.017	0.018	0.000	-0.134	-2.006	0.021	
1.2.b				23/10/2007	0.019	0.010	0.018						
1.3.a	17/10/2007	15:08	7.4	23/10/2007	0.022	0.012	0.021	0.023	0.002	0.065	-1.696	0.035	
1.3.b				23/10/2007	0.026	0.014	0.025						
1.4.a	17/10/2007	19:00	11.2	23/10/2007	0.057	0.033	0.058	0.054	0.004	**0.209**	-0.581	0.052	
1.4.b				23/10/2007	0.050	0.028	0.051						
1.1.a	17/10/2007	23:00	15.2	23/10/2007	0.058	0.033	0.059	0.055	0.004	0.005	-0.550	0.074	
1.1.b				23/10/2007	0.051	0.029	0.052						
1.1.a	18/10/2007	03:00	19.3	23/10/2007	0.076	0.045	0.079	0.078	0.002	0.081	0.050	0.097	
1.1.b				23/10/2007	0.073	0.043	0.076						0.934
1.7.a	18/10/2007	07:00	23.3	23/10/2007	0.111	0.068	0.121	0.128	0.007	0.123	1.707	0.115	
1.7.b				23/10/2007	0.123	0.076	0.136						
1.8.a	18/10/2007	11:00	27.3	23/10/2007	0.123	0.076	0.136	0.132	0.004	0.007	1.910	0.129	
1.8.b				23/10/2007	0.117	0.072	0.128						
1.9.a	18/10/2007	15:10	31.5	23/10/2007	0.122	0.075	0.134	0.140	0.006	0.014	2.514	0.139	
1.9.b				23/10/2007	0.131	0.082	0.146						
1.10.a	18/10/2007	19:00	35.3	23/10/2007	0.105	0.064	0.113	0.114	0.001	-0.054	1.114	0.144	
1.10.b				23/10/2007	0.106	0.064	0.115						
1.11.a	18/10/2007	23:00	39.3	23/10/2007	0.155	0.099	0.177	**0.151**	0.026	0.070	#DIV/0!	0.147	
1.11.b				23/10/2007	0.115	0.071	0.126						
1.12.a	19/10/2007	11:24	51.7	23/10/2007	0.137	0.086	0.154	0.151	0.003	0.000	5.709	0.151	
1.12.b				23/10/2007	0.133	0.083	0.148						
1.13.a	19/10/2007	23:20	63.6	23/10/2007	0.192	0.128	0.229	0.234	0.006	0.036	#NUM!	0.151	
1.13.b				23/10/2007	0.200	0.135	0.240						
1.14.a	20/10/2007	00:00		23/10/2007									
1.14.b				23/10/2007									
1.15.a	21/10/2007	11:55	100.2	23/10/2007	0.069	0.040	0.071	0.073	0.001	-0.029	-0.085	0.151	
1.15.b				23/10/2007	0.071	0.041	0.074						
1.16.a	22/10/2007	11:00	111.3	23/10/2007	0.079	0.046	0.083	0.077	0.006	0.005	0.021	0.151	
1.16.b				23/10/2007	0.068	0.039	0.070						

X_{max}	**0.151**	(2)
X_0	**0.014**	(3)
μ_{max}	**0.148**	(4)

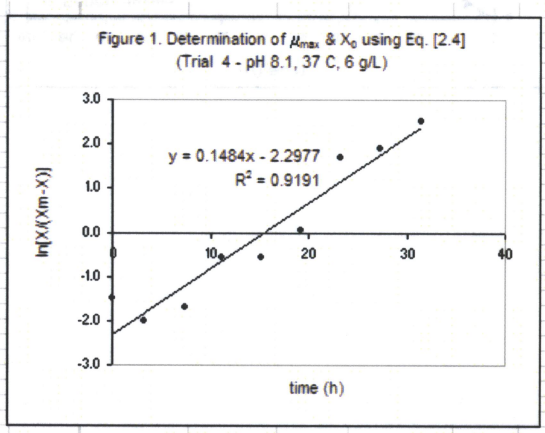
(1) $$\mu = \frac{dX}{dt}\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\frac{1}{average\ (X_2, X_1)}$$

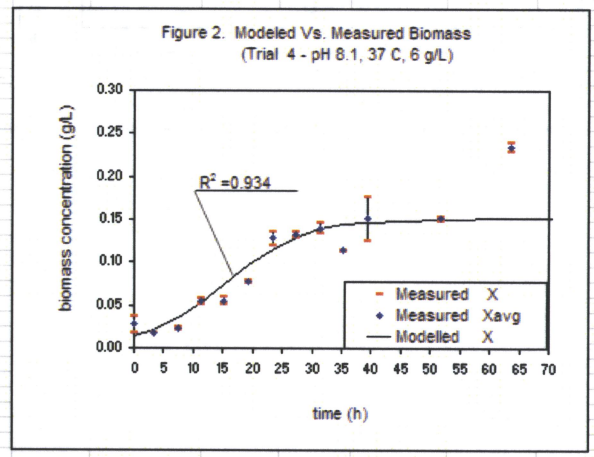
(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_0 calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation. The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 4 37°C, pH 8.1, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2
	Measured P (ml)	Measured dP/dt (ml/h)	Eq.[2.22] A(t)	Eq.[2.23] B(t)	P - β*B(t)	Eq.[2.21] Modelled P (ml)	(3)
0.0	0.000	0.000	0.000	0.000	0.000	0.000	
3.3	5.000	1.515	0.008	0.057	4.460	5.814	
7.4	11.500	1.585	0.021	0.171	9.886	16.534	
11.2	20.000	2.237	0.039	0.336	16.831	30.289	
15.2	27.500	1.875	0.060	0.588	21.950	47.911	
19.3	49.500	5.366	0.083	0.939	40.643	67.012	
23.3	84.500	8.750	0.101	1.364	71.632	84.155	
27.3	104.500	5.000	0.115	1.855	87.006	98.475	0.966
31.5	113.500	2.143	0.125	2.419	90.689	110.460	
35.3	123.000	2.500	0.130	2.956	95.120	119.233	
39.3	137.000	3.500	0.133	3.539	103.625	127.052	
51.7	168.000	2.500	0.137	5.392	117.143	147.062	
63.6	185.000	1.429	0.138	7.191	117.180	164.432	
100.2	198.000	0.355	0.138	12.734	77.902	216.795	
111.3	212.000	1.261	0.138	14.415	76.045	232.652	
						β	**9.431** (1)
						α	702.550 (2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 4 - pH 8.1, 37 C, 6 g/L)

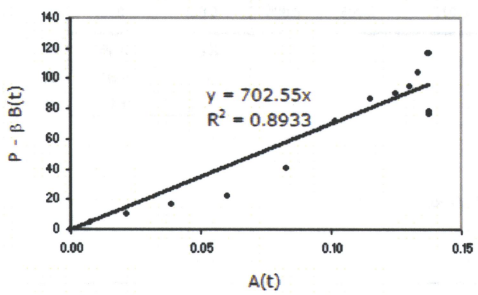
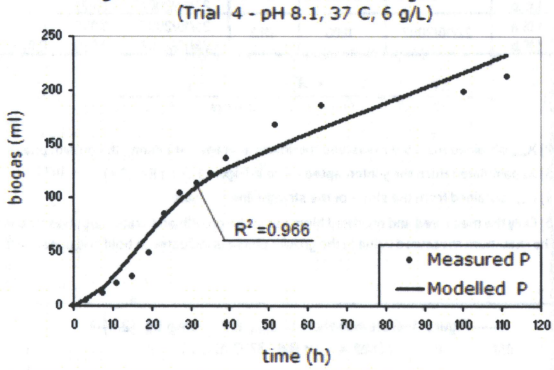


Figure 2. Modeled Vs. Measured Biogas
(Trial 4 - pH 8.1, 37 C, 6 g/L)

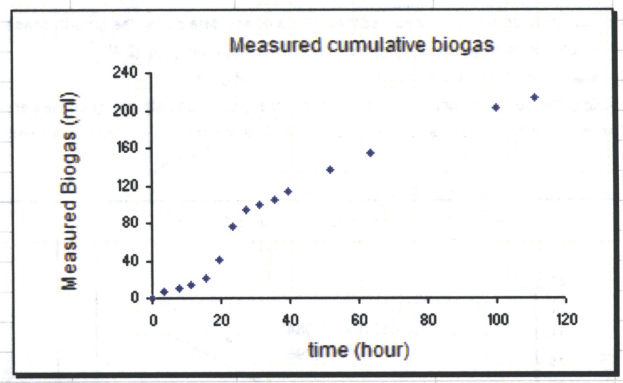


Trial #5

Trial ID: 5
Inoculum ID: 3.4
Description: 37°C, pH 8.2, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	17/10/2007	7:45	0.0	0.0	0.0
2	17/10/2007	11:15	3.5	8.0	8.0
3	17/10/2007	15:30	7.7	3.0	11.0
4	17/10/2007	19:11	11.4	4.1	15.1
5	17/10/2007	23:10	15.4	6.0	21.1
6	18/10/2007	3:05	19.3	19.0	40.1
7	18/10/2007	7:10	23.4	36.0	76.1
8	18/10/2007	11:10	27.4	18.0	94.1
9	18/10/2007	15:05	31.3	6.0	100.1
10	18/10/2007	19:10	35.4	5.5	105.6
11	18/10/2007	23:10	39.4	8.3	113.9
12	19/10/2007	11:36	51.8	23.0	136.9
13	19/10/2007	23:10	63.4	18.0	154.9
14	20/10/2007				154.9
15	21/10/2007	11:35	99.8	48.0	202.9
16	22/10/2007	11:05	111.3	10.0	212.9



Trial ID: 5 37°C, pH 8.2, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{535}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_m$-X))	Modeled X [g/L]	(5)
1.1.a	17/10/2007	07:45	0.0	23/10/2007	0.016	0.009	0.015	0.020	0.004		-1.800	0.016	
1.1.b				23/10/2007	0.025	0.014	0.024						
1.2.a	17/10/2007	11:15	3.5	23/10/2007	0.025	0.014	0.024	0.020	0.004	0.000	-1.800	0.023	
1.2.b				23/10/2007	0.016	0.009	0.015						
1.3.a	17/10/2007	15:30	7.7	23/10/2007	0.071	0.041	0.074	0.064	0.010	**0.249**	-0.192	0.036	
1.3.b				23/10/2007	0.053	0.030	0.054						
1.4.a	17/10/2007	19:11	11.4	23/10/2007	0.050	0.028	0.051	0.040	0.011	-0.127	-0.941	0.050	
1.4.b				23/10/2007	0.029	0.016	0.029						
1.1.a	17/10/2007	23:10	15.4	23/10/2007	0.038	0.021	0.038	0.035	0.003	-0.032	-1.117	0.068	
1.1.b				23/10/2007	0.032	0.018	0.032						
1.1.a	18/10/2007	03:05	19.3	23/10/2007	0.071	0.041	0.074	0.068	0.006	0.166	-0.067	0.086	
1.1.b				23/10/2007	0.061	0.035	0.063						0.818
1.7.a	18/10/2007	07:10	23.4	23/10/2007	0.105	0.064	0.113	0.114	0.001	0.123	1.447	0.103	
1.7.b				23/10/2007	0.106	0.064	0.115						
1.8.a	18/10/2007	11:10	27.4	23/10/2007	0.111	0.068	0.121	0.124	0.004	0.022	2.026	0.116	
1.8.b				23/10/2007	0.117	0.072	0.128						
1.9.a	18/10/2007	15:05	31.3	23/10/2007	0.119	0.073	0.131	0.124	0.007	-0.001	1.985	0.125	
1.9.b				23/10/2007	0.108	0.066	0.117						
1.10.a	18/10/2007	19:10	35.4	23/10/2007	0.098	0.059	0.105	0.106	0.001	-0.037	1.119	0.131	
1.10.b				23/10/2007	0.100	0.060	0.107						
1.11.a	18/10/2007	23:10	39.4	23/10/2007	0.095	0.057	0.101	0.100	0.002	-0.016	0.883	0.135	
1.11.b				23/10/2007	0.092	0.055	0.098						
1.12.a	19/10/2007	11:36	51.8	23/10/2007	0.094	0.056	0.100	0.106	0.005	0.005	1.097	0.140	
1.12.b				23/10/2007	0.103	0.062	0.111						
1.13.a	19/10/2007	23:10	63.4	23/10/2007	0.116	0.071	0.127	**0.141**	0.014	0.025	#DIV/0!	0.141	
1.13.b				23/10/2007	0.138	0.087	0.155						
1.14.a	20/10/2007	00:00		23/10/2007									
1.14.b				23/10/2007									
1.15.a	21/10/2007	11:35	99.8	23/10/2007	0.068	0.039	0.070	0.067	0.003	-0.020	-0.099	0.141	
1.15.b				23/10/2007	0.062	0.036	0.064						
1.16.a	22/10/2007	11:05	111.3	23/10/2007	0.049	0.028	0.049	0.058	0.008	-0.013	-0.367	0.141	
1.16.b				23/10/2007	0.064	0.037	0.066						

(1) $$\mu = \frac{dX}{dt}\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\frac{1}{average\ (X_2, X_1)}$$

X_{max}	0.141	(2)
X_o	0.016	(3)
μ_{max}	0.132	(4)

(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.
The maximum measured value in the growth phase is indicated in bold and underlined.

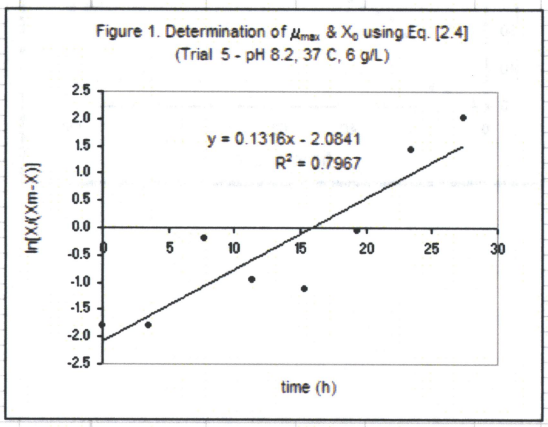


Figure 1. Determination of μ_{max} & X_o using Eq. [2.4]
(Trial 5 - pH 8.2, 37 C, 6 g/L)

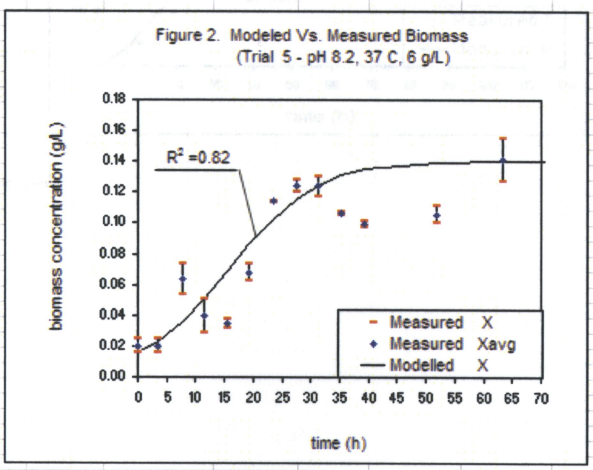


Figure 2. Modeled Vs. Measured Biomass
(Trial 5 - pH 8.2, 37 C, 6 g/L)

Trial ID: 5 37°C, pH 8.2, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	(3)
0	0.0	0.0	0.000	0.000	0.0	0.0	
3.5	8.0	2.3	0.008	0.067	7.6	6.9	
7.7	11.0	0.7	0.020	0.190	9.8	18.5	
11.4	15.1	1.1	0.035	0.349	12.9	31.8	
15.4	21.1	1.5	0.053	0.586	17.5	48.5	
19.3	40.1	4.9	0.071	0.888	34.6	65.5	
23.4	76.1	8.8	0.087	1.277	68.2	82.0	
27.4	94.1	4.5	0.100	1.715	83.5	95.6	0.973
31.3	100.1	1.5	0.109	2.185	86.6	106.1	
35.4	105.6	1.3	0.115	2.709	88.9	114.7	
39.4	113.9	2.1	0.119	3.242	93.9	121.3	
51.8	136.9	1.9	0.124	4.951	106.3	135.9	
63.4	154.9	1.6	0.125	6.578	114.3	146.8	
99.8	202.9	1.3	0.125	11.704	130.7	178.7	
111.3	212.9	0.9	0.125	13.324	130.7	188.7	

β	6.2	(1)
α	849.4	(2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 5 - pH 8.2, 37 C, 6 g/L)

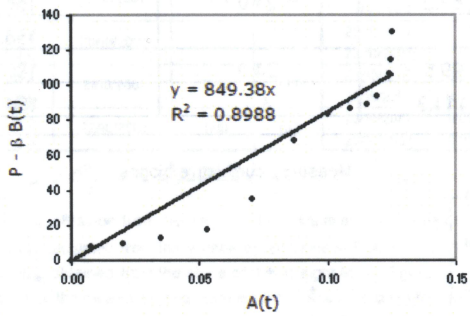
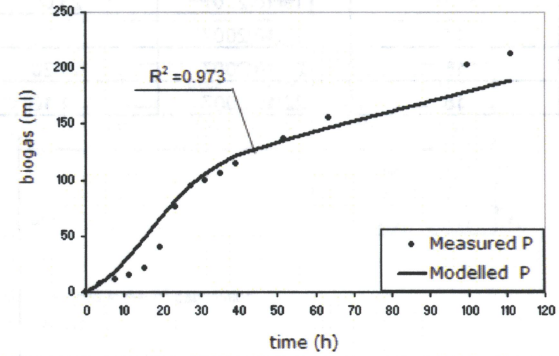


Figure 2. Modeled Vs. Measured Biogas
(Trial 5- pH 8.2, 37 C, 6 g/L)

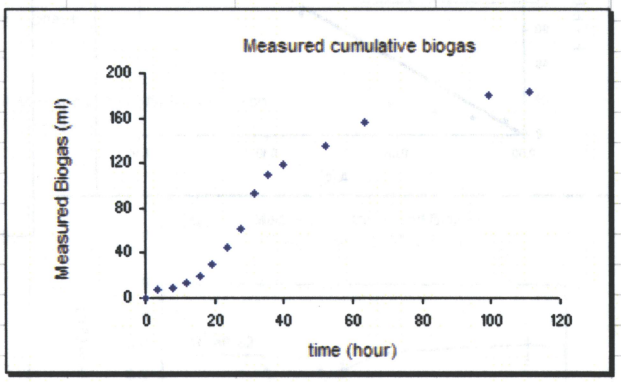


Trial #6

Trial ID: 6
Inoculum ID: 3.4
Description: 37°C, pH 8.4, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	17/10/2007	7:45	0.0	0.0	0.0
2	17/10/2007	11:20	3.6	7.0	7.0
3	17/10/2007	15:45	8.0	2.0	9.0
4	17/10/2007	19:22	11.6	4.1	13.1
5	17/10/2007	23:15	15.5	6.0	19.1
6	18/10/2007	3:10	19.4	11.5	30.6
7	18/10/2007	7:21	23.6	14.0	44.6
8	18/10/2007	11:15	27.5	17.0	61.6
9	18/10/2007	15:05	31.3	32.0	93.6
10	18/10/2007	19:22	35.6	16.5	110.1
11	18/10/2007	23:25	39.7	9.0	119.1
12	19/10/2007	11:46	52.0	16.0	135.1
13	19/10/2007	23:00	63.2	21.0	156.1
14	20/10/2007				156.1
15	21/10/2007	11:20	99.5	24.0	180.1
16	22/10/2007	11:10	111.3	4.0	184.1



Trial ID: 6 37°C, pH 8.4, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{535}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_m$-X))	Modeled X [g/L]	(5)
1.1.a	17/10/2007	07:45	0.0	23/10/2007	0.014	0.008	0.014	0.010	0.003		-2.510	0.014	
1.1.b				23/10/2007	0.007	0.004	0.007						
1.2.a	17/10/2007	11:20	3.6	23/10/2007	0.047	0.027	0.047	0.051	0.003	**0.370**	-0.506	0.020	
1.2.b				23/10/2007	0.053	0.030	0.054						
1.3.a	17/10/2007	15:45	8.0	23/10/2007	0.060	0.034	0.061	0.052	0.009	0.007	-0.453	0.030	
1.3.b				23/10/2007	0.043	0.024	0.043						
1.4.a	17/10/2007	19:22	11.6	23/10/2007	0.036	0.020	0.036	0.028	0.008	-0.167	-1.331	0.041	
1.4.b				23/10/2007	0.021	0.011	0.020						
1.1.a	17/10/2007	23:15	15.5	23/10/2007	0.040	0.022	0.040	0.034	0.006	0.047	-1.094	0.055	
1.1.b				23/10/2007	0.028	0.015	0.028						
1.1.a	18/10/2007	03:10	19.4	23/10/2007	0.047	0.027	0.047	0.042	0.005	0.056	-0.787	0.070	0.785
1.1.b				23/10/2007	0.037	0.021	0.037						
1.7.a	18/10/2007	07:21	23.6	23/10/2007	0.094	0.056	0.100	0.084	0.016	0.159	0.515	0.086	
1.7.b				23/10/2007	0.066	0.038	0.068						
1.8.a	18/10/2007	11:15	27.5	23/10/2007	0.069	0.040	0.071	0.071	0.001	-0.044	0.108	0.099	
1.8.b				23/10/2007	0.068	0.039	0.070						
1.9.a	18/10/2007	15:05	31.3	23/10/2007	0.106	0.064	0.115	0.113	0.001	0.122	1.686	0.110	
1.9.b				23/10/2007	0.104	0.063	0.112						
1.10.a	18/10/2007	19:22	35.6	23/10/2007	0.116	0.071	0.127	0.129	0.002	0.031	3.250	0.118	
1.10.b				23/10/2007	0.120	0.074	0.132						
1.11.a	18/10/2007	23:25	39.7	23/10/2007	0.102	0.078	0.139	**0.134**	0.005	0.009	#DIV/0!	0.124	
1.11.b				23/10/2007	0.096	0.073	0.129						
1.12.a	19/10/2007	11:46	52.0	23/10/2007	0.126	0.074	0.132	0.123	0.009	-0.007	2.402	0.132	
1.12.b				23/10/2007	0.118	0.064	0.115						
1.13.a	19/10/2007	23:00	63.2	23/10/2007	0.120	0.074	0.132	0.123	0.009	0.000	2.402	0.134	
1.13.b				23/10/2007	0.106	0.064	0.115						
1.14.a	20/10/2007			23/10/2007						0.016			
1.14.b				23/10/2007									
1.15.a	21/10/2007	11:20	99.5	23/10/2007	0.071	0.041	0.074	0.075	0.002	0.010	0.243	0.134	
1.15.b				23/10/2007	0.074	0.043	0.077						
1.16.a	22/10/2007	11:10	111.3	23/10/2007	0.051	0.029	0.052	0.041	0.011	-0.050	-0.820	0.134	
1.16.b				23/10/2007	0.031	0.017	0.031						

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average}\frac{1}{(X_2, X_1)}$$

X_{max}	0.134	(2)
X_o	0.014	(3)
μ_{max}	0.117	(4)

(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.

Figure 1. Determination of μ_{max} & X_o using Eq. [2.4]
(Trial 6 - pH 8.4, 37 C, 6 g/L)

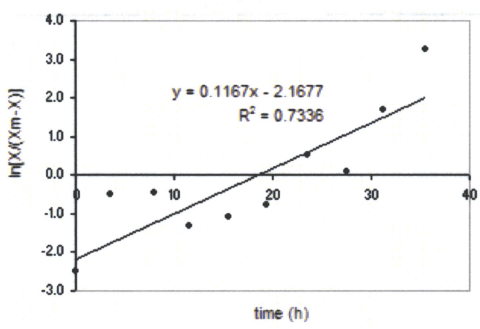
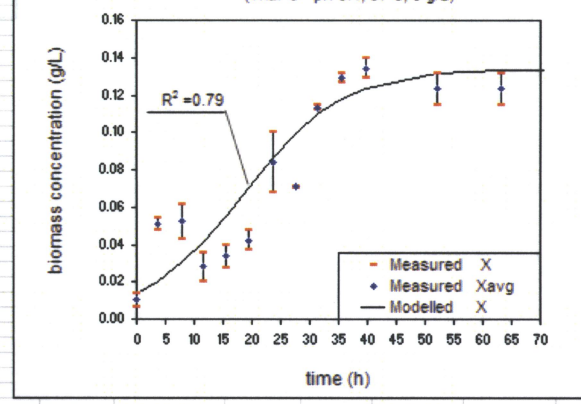


Figure 2. Modeled Vs. Measured Biomass
(Trial 6 - pH 8.4, 37 C, 6 g/L)



Trial ID: 6 37ºC, pH 8.4, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2 (3)
	Measured P (ml)	Measured dP/dt (ml/h)	Eq.[2.22] A(t)	Eq.[2.23] B(t)	P - β*B(t)	Eq.[2.21] Modelled P (ml)	
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.6	7.0	1.9	0.006	0.060	6.8	6.5	
8.0	9.0	0.5	0.017	0.170	8.6	17.4	
11.6	13.1	1.1	0.027	0.298	12.3	29.0	
15.5	19.1	1.5	0.041	0.486	17.9	43.8	
19.4	30.6	2.9	0.057	0.731	28.8	60.0	
23.6	44.6	3.3	0.073	1.060	41.9	77.2	
27.5	61.6	4.4	0.086	1.424	58.0	91.5	0.935
31.3	93.6	8.4	0.096	1.821	89.0	103.0	
35.6	110.1	3.8	0.104	2.312	104.3	113.1	
39.7	119.1	2.2	0.110	2.810	112.0	120.2	
52.0	135.1	1.3	0.118	4.392	124.0	132.2	
63.2	156.1	1.9	0.120	5.881	141.3	138.0	
99.5	180.1	0.7	0.121	10.755	153.0	151.0	
111.3	184.1	0.3	0.121	12.342	153.0	155.0	

β	2.5	(1)
α	1027.100	(2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 6 - pH 8.4, 37 C, 6 g/L)

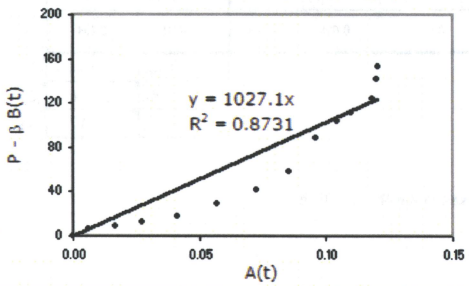
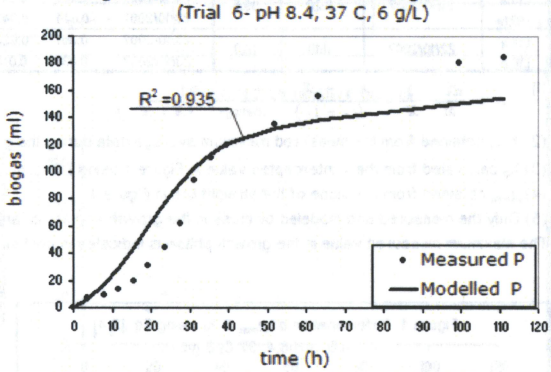


Figure 2. Modeled Vs. Measured Biogas
(Trial 6- pH 8.4, 37 C, 6 g/L)

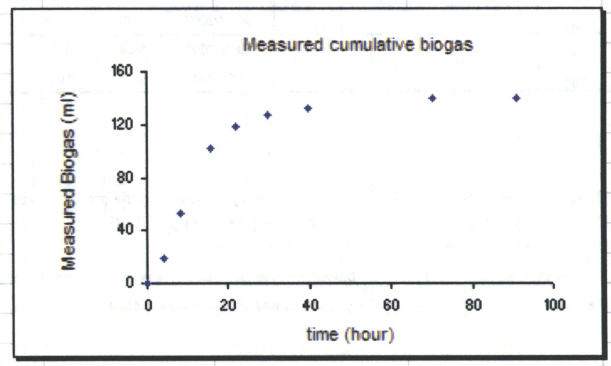


Trial #7

Trial ID: 7
Inoculum ID: 2.2
Description: 37°C, pH 8.5, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	7/5/2007	14:35	0.0	0	0
2	7/5/2007	18:40	4.1	19	19
3	7/5/2007	23:03	8.5	33.5	52.5
4	7/6/2007	6:22	15.8	49.5	102
5	7/6/2007	12:35	22.0	17	119
6	7/6/2007	20:30	29.9	8	127
7	7/7/2007	6:25	39.8	5	132
8	7/8/2007	12:55	70.3	8	140
9	7/9/2007	9:30	90.9	0	140



Trial ID: 7 37°C, pH 8.5, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{sss}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_\infty$-X))	Modeled X [g/L]	(5)
7.1.a	05/07/2007	14:35	0.0	30/07/2007	0.023	0.023	0.041	0.033	0.008		-2.167	0.029	
7.1.b				30/07/2007	0.014	0.014	0.025						
7.2.a	05/07/2007	18:40	4.1	30/07/2007	0.020	0.020	0.036	0.050	0.014	0.097	-1.705	0.060	
7.2.b				30/07/2007	0.035	0.036	0.064						
7.3.a	05/07/2007	23:03	8.5	30/07/2007	0.082	0.086	0.153	0.155	0.002	**0.235**	-0.080	0.115	
7.3.b				30/07/2007	0.084	0.088	0.157						
7.4.a	06/07/2007	06:22	15.8	30/07/2007	0.149	0.163	0.290	0.267	0.023	0.072	1.556	0.229	0.978
7.4.b				30/07/2007	0.127	0.137	0.244						
7.5.a	06/07/2007	12:35	22.0	30/07/2007	0.146	0.159	0.284	0.306	0.022	0.022	2.878	0.290	
7.5.b				30/07/2007	0.167	0.185	0.329						
7.6.a	06/07/2007	20:30	29.9	30/07/2007	0.177	0.197	0.351	**0.324**	0.027	0.007	--	0.316	
7.6.b				30/07/2007	0.152	0.166	0.297						
7.7.a	07/07/2007	06:25	39.8	30/07/2007	0.128	0.138	0.246	0.260	0.014	-0.022	1.404	0.323	
7.7.b				30/07/2007	0.141	0.153	0.273						
7.8.a	08/07/2007	12:55	70.3	30/07/2007	0.180	0.200	0.357	0.290	0.067	0.004	2.167	0.324	
7.8.b				30/07/2007	0.117	0.125	0.224						
7.9.a	09/07/2007	09:30	90.9	30/07/2007	0.098	0.104	0.185	0.177	0.008	-0.024	0.190	0.324	
7.9.b				30/07/2007	0.090	0.095	0.169						

X_{max}	0.324	(2)
X_o	0.029	(3)
μ_{max}	0.239	(4)

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ (X_2, X_1)}$$

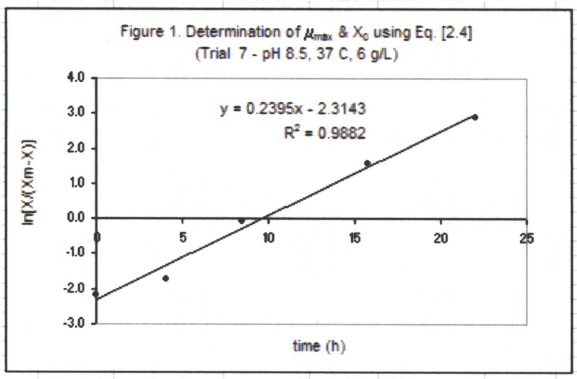
(2) X_{max} obtained from the measured maximum average data during the growth phase

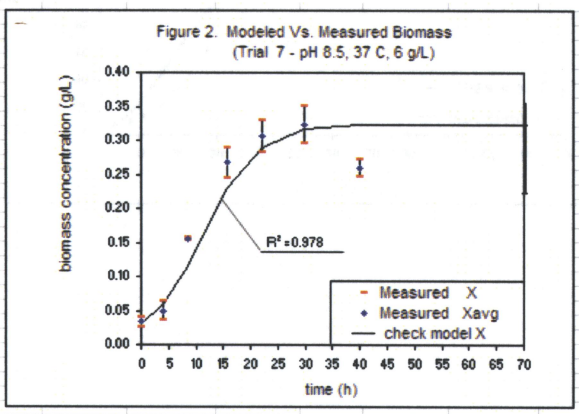
(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 7 37°C, pH 8.5, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2 (3)
	Measured P (ml)	Measured dP/dt (ml/h)	Eq.[2.22] A(t)	Eq.[2.23] B(t)	P - β*B(t)	Eq.[2.21] Modelled P (ml)	
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
4.1	19.0	4.7	0.031	0.175	18.9	13.4	
8.5	52.5	7.6	0.086	0.548	52.1	37.7	
15.8	102.0	6.8	0.200	1.812	100.6	88.4	
22.0	119.0	2.7	0.261	3.448	116.2	116.1	0.985
29.9	127.0	1.0	0.287	5.870	122.3	129.5	
39.8	132.0	0.5	0.293	9.046	124.8	134.9	
70.3	140.0	0.3	0.295	18.910	124.9	143.3	
90.9	140.0	0.0	0.295	25.569	119.5	148.7	
						β	0.8 (1)
						α	434.65 (2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 7 - pH 8.5, 37 C, 6 g/L)

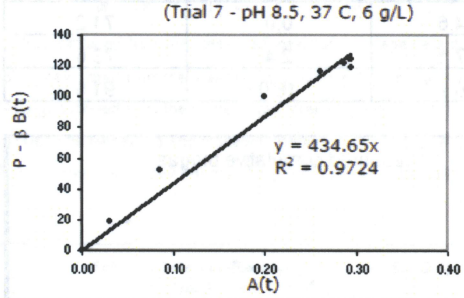
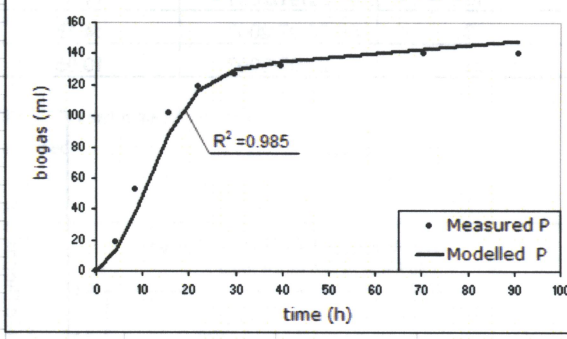


Figure 2. Modeled Vs. Measured Biogas
(Trial 7- pH 8.5, 37 C, 6 g/L)

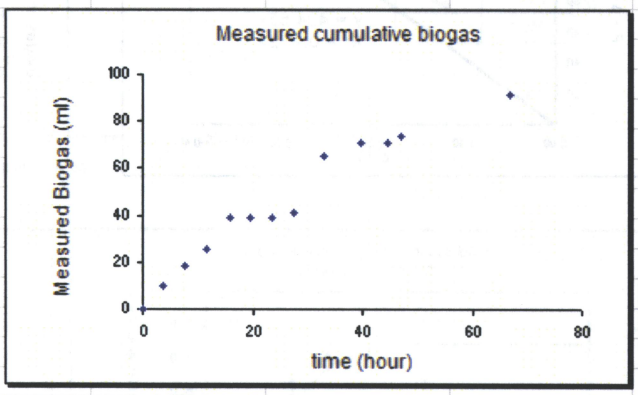


Trial #8

Trial ID: 8
Inoculum ID: 3.3.1
Description: 37°C, pH 8.5, 0.1 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	20/07/2007	14:30	0.0	0.0	0.0
2	20/07/2007	18:05	3.6	10.0	10.0
3	20/07/2007	22:05	7.6	8.5	18.5
4	21/07/2007	1:05	11.6	7.0	25.5
5	21/07/2007	6:15	15.8	13.5	39.0
6	21/07/2007	10:05	19.6	0.0	39.0
7	21/07/2007	14:05	23.6	0.0	39.0
8	21/07/2007	18:05	27.6	2.2	41.2
9	21/07/2007	23:35	33.1	24.0	65.2
10	22/07/2007	6:05	39.6	6.0	71.2
11	22/07/2007	11:05	44.6	0.0	71.2
12	22/07/2007	14:15	47.1	2.4	73.6
13	23/07/2007	10:05	66.9	18.0	91.6



Trial ID: 8 37°C, pH 8.5, 0.1 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis					model		R^2
										Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{sss}	protein [g/l]	Measured X [g/l]	μ (1) [hour^{-1}]	ln (X/(X$_o$-X))	Modeled X [g/L]	(5)
19.1	20/07/2007	14:30	0.0	07/23/07	0.018	0.010	0.017		-1.665	0.017	
19.2	20/07/2007	18:05	3.6	07/23/07	0.041	0.023	0.041	**0.223**	-0.519	0.035	
19.3	20/07/2007	22:05	7.6	07/23/07	0.052	0.030	0.053	0.063	-0.081	0.062	
19.4	21/07/2007	1:05	11.6	07/23/07	0.078	0.046	0.082	0.108	1.062	0.086	0.959
19.5	21/07/2007	6:15	15.8	07/23/07	0.096	0.058	0.103	0.054	2.659	0.101	
19.6	21/07/2007	10:05	19.6	07/23/07	0.094	0.056	0.100	-0.006	2.350	0.106	
19.7	21/07/2007	14:05	23.6	07/23/07	0.086	0.051	0.091	-0.025	1.567	0.108	
19.8	21/07/2007	18:05	27.6	07/23/07	0.102	0.062	**0.110**	0.047	--	0.109	
19.9	21/07/2007	23:35	33.1	07/23/07	0.020	0.011	0.019	-0.254	-1.535	0.110	
19.11	22/07/2007	6:05	39.6	07/23/07	0.028	0.015	0.028	0.053	-1.095	0.110	
19.11	22/07/2007	11:05	44.6	07/23/07	0.031	0.017	0.031	0.021	-0.951	0.110	
19.12	22/07/2007	14:15	47.1	07/23/07	0.035	0.019	0.035	0.051	-0.772	0.110	
19.13	23/07/2007	10:05	66.9	07/23/07	0.031	0.017	0.031	-0.006	-0.951	0.110	

(1) $\mu = \frac{dX}{dt}\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\frac{1}{average\ (X_2, X_1)}$

X_{max}	0.110	(2)
X_o	0.017	(3)
μ_{max}	0.259	(4)

(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.

Figure 1. Determination of μ_{max} & X_o using Eq. [2.4]
(Trial 8 - pH 8.5, 37 C, 0.1 g/L)

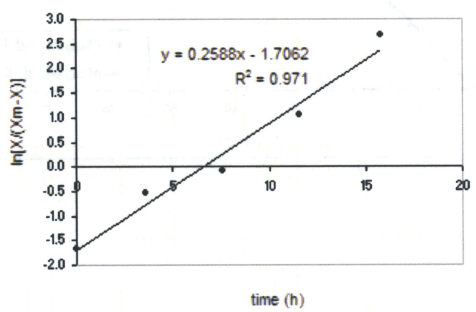
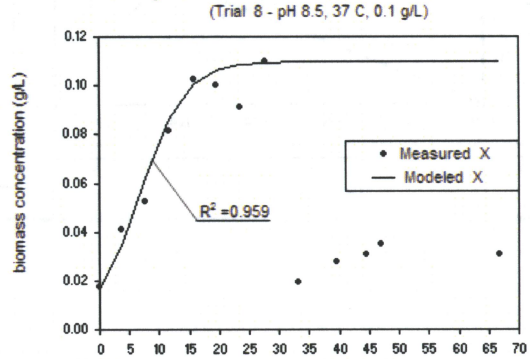


Figure 2. Modeled Vs. Measured Biomass
(Trial 8 - pH 8.5, 37 C, 0.1 g/L)



Trial ID: 8 37°C, pH 8.5, 0.1 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	(3)
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.6	10.0	2.8	0.018	0.090	9.2	7.1	
7.6	18.5	2.1	0.045	0.282	16.1	18.6	
11.6	25.5	1.8	0.069	0.582	20.6	29.8	
15.8	39.0	3.2	0.084	0.978	30.8	38.2	
19.6	39.0	0.0	0.089	1.372	27.5	43.6	
23.6	39.0	0.0	0.092	1.802	23.9	48.0	0.958
27.6	41.2	0.6	0.092	2.238	22.4	52.0	
33.1	65.2	4.4	0.093	2.840	41.3	57.2	
39.6	71.2	0.9	0.093	3.554	41.3	63.2	
44.6	71.2	0.0	0.093	4.103	36.7	67.8	
47.1	73.6	1.0	0.093	4.377	36.8	70.1	
66.9	91.6	0.9	0.093	6.551	36.5	88.4	
						β	8.4 (1)
						α	358.85 (2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 8 - pH 8.5, 37 C, 0.1 g/L)

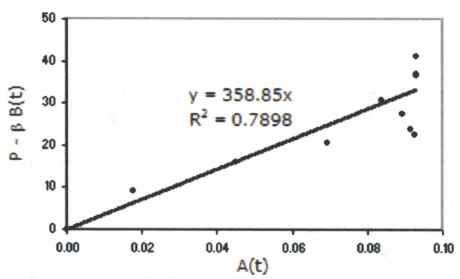
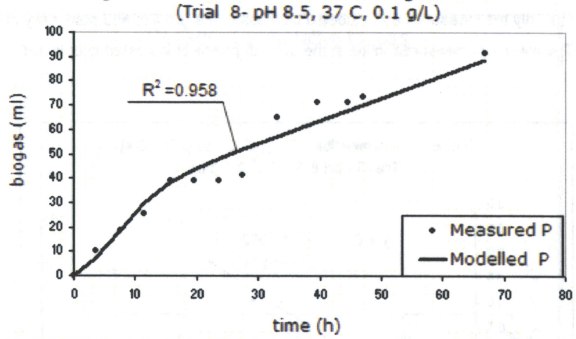


Figure 2. Modeled Vs. Measured Biogas
(Trial 8- pH 8.5, 37 C, 0.1 g/L)



Trial #9

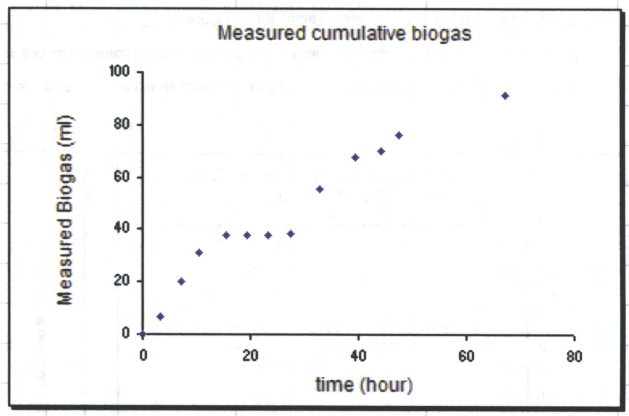
Trial ID: 9

Inoculum ID: 3.3.2

Description: 37°C, pH 8.5, 0.5 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	20/07/2007	14:50	0.0	0.0	0.0
2	20/07/2007	18:10	3.3	7.0	7.0
3	20/07/2007	22:10	7.3	13.0	20.0
4	21/07/2007	1:10	10.3	11.0	31.0
5	21/07/2007	6:20	15.5	7.0	38.0
6	21/07/2007	10:15	19.4	0.0	38.0
7	21/07/2007	14:15	23.4	0.0	38.0
8	21/07/2007	18:20	27.5	0.7	38.7
9	21/07/2007	23:40	32.8	17.0	55.7
10	22/07/2007	6:10	39.3	12	67.7
11	22/07/2007	11:10	44.3	2.5	70.2
12	22/07/2007	14:20	47.5	6	76.2
13	23/07/2007	10:10	67.3	15	91.2



Trial ID: 9 37°C, pH 8.5, 0.5 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis					model		R^2
										Eq. [2.3]	
sample ID	sample taken	sample taken	elapsed time	analysis	A_{sss}	protein	Measured X	μ (1)	ln (X/(X$_m$-X))	Modeled X	(5)
	[d/m/y]	[hh:mm]	[hour]	[d/m/y]		[g/l]	[g/l]	[hour^{-1}]		[g/L]	
20.1	20/07/2007	14:50	0.0	07/23/07	0.025	0.014	0.024		-1.682	0.020	
20.2	20/07/2007	18:10	3.3	07/23/07	0.024	0.013	0.023	-0.013	-1.732	0.027	
20.3	20/07/2007	22:10	7.3	07/23/07	0.027	0.015	0.027	0.030	-1.587	0.036	0.632
20.4	21/07/2007	1:10	10.3	07/23/07	0.055	0.031	0.056	**0.238**	-0.583	0.045	
20.5	21/07/2007	6:20	15.5	07/23/07	0.035	0.019	0.035	-0.090	-1.252	0.062	
20.6	21/07/2007	10:15	19.4	07/23/07	0.139	0.088	**0.156**	0.326	--	0.077	
20.7	21/07/2007	14:15	23.4	07/23/07	0.048	0.027	0.048	-0.263	-0.800	0.092	
20.8	21/07/2007	18:20	27.5	07/23/07	0.071	0.041	0.074	0.101	-0.113	0.106	
20.9	21/07/2007	23:40	32.8	07/23/07	0.050	0.028	0.051	-0.070	-0.737	0.122	
20.10	22/07/2007	6:10	39.3	07/23/07	0.035	0.019	0.035	-0.057	-1.252	0.135	
20.11	22/07/2007	11:10	44.3	07/23/07	0.039	0.022	0.039	0.023	-1.104	0.143	
20.12	22/07/2007	14:20	47.5	07/23/07	0.108	0.066	0.117	0.313	1.098	0.146	
20.13	23/07/2007	10:10	67.3	07/23/07	0.107	0.065	0.116	-0.001	1.057	0.155	

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average(X_2, X_1)}$$

X_{max}	0.156	(2)
X_o	0.020	(3)
μ_{max}	0.096	(4)

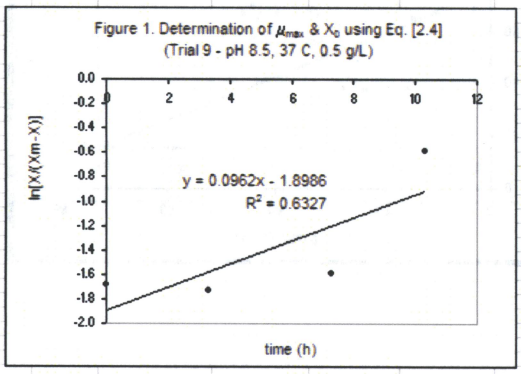
(2) X_{max} obtained from the measured maximum average data during the growth phase

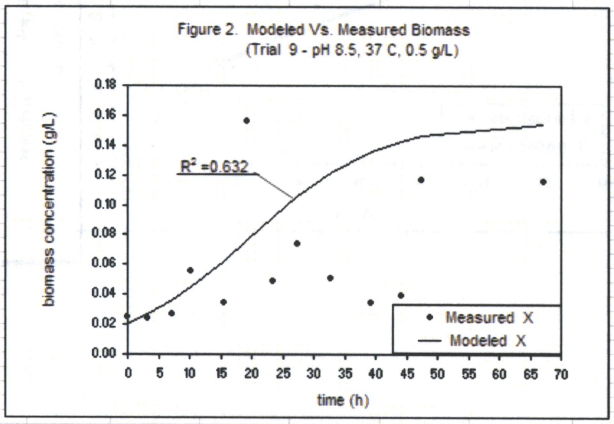
(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 9 37ºC, pH 8.5, 0.5 g/L cellulose

Biogas (P) measurement & modelling

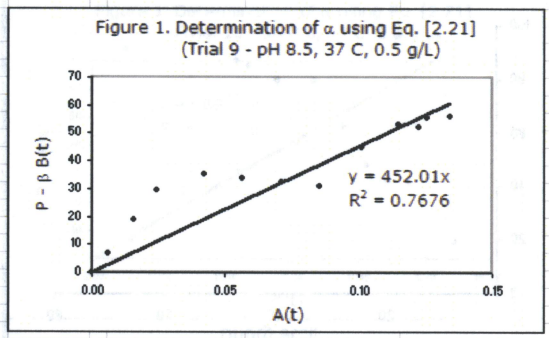
time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	(3)
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.3	7.0	2.1	0.006	0.077	6.6	3.2	
7.3	20.0	3.3	0.016	0.202	19.0	8.2	
10.3	31.0	3.7	0.025	0.324	29.4	12.7	
15.5	38.0	1.3	0.042	0.601	35.1	21.9	
19.4	38.0	0.0	0.056	0.872	33.8	29.7	
23.4	38.0	0.0	0.071	1.209	32.1	38.1	0.945
27.5	38.7	0.2	0.086	1.615	30.9	46.5	
32.8	55.7	3.2	0.101	2.219	44.9	56.5	
39.3	67.7	1.8	0.115	3.058	52.9	66.9	
44.3	70.2	0.5	0.122	3.754	52.0	73.5	
47.5	76.2	1.9	0.126	4.216	55.7	77.3	
67.3	91.2	0.8	0.134	7.216	56.2	95.7	

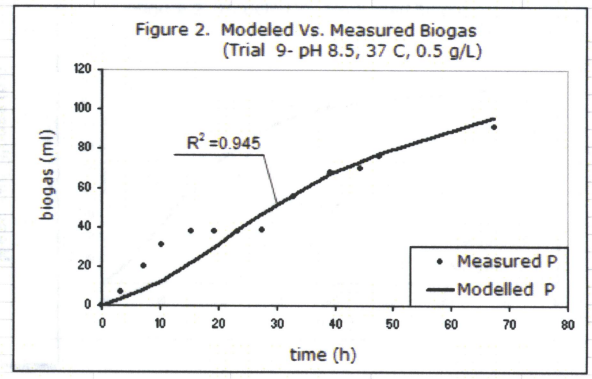
β	**4.9**	(1)
α	452.01	(2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.



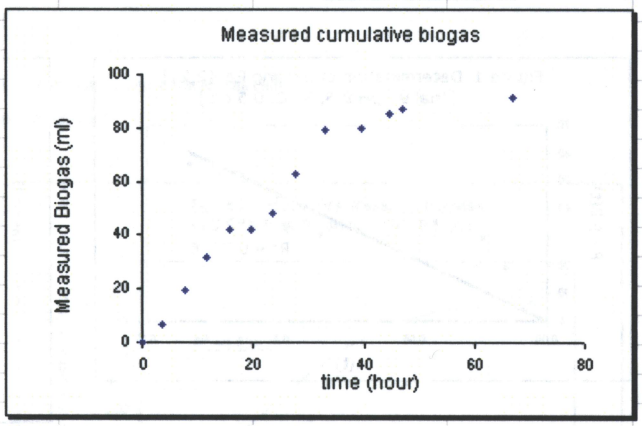


Trial #10

Trial ID: 10
Inoculum ID: 3.3.2
Description: 37°C, pH 8.5, 1.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	20/07/2007	15:05	0.0	0.0	0.0
2	20/07/2007	18:15	3.6	7.0	7.0
3	20/07/2007	22:15	7.6	12.5	19.5
4	21/07/2007	1:15	11.6	12.5	32.0
5	21/07/2007	6:25	15.8	10.0	42.0
6	21/07/2007	10:25	19.6	0.0	42.0
7	21/07/2007	14:20	23.6	6.0	48.0
8	21/07/2007	18:30	27.6	15.1	63.1
9	21/07/2007	23:45	33.1	16.0	79.1
10	22/07/2007	6:15	39.6	1.0	80.1
11	22/07/2007	11:15	44.6	5.0	85.1
12	22/07/2007	14:25	47.1	2.2	87.3
13	22/07/2007	10:15	66.9	4.0	91.3



Trial ID: 10 37°C, pH 8.5, 1.0 g/L cellulose

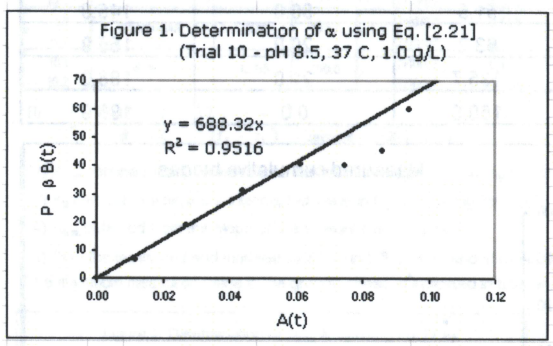
Biogas (P) measurement & modelling

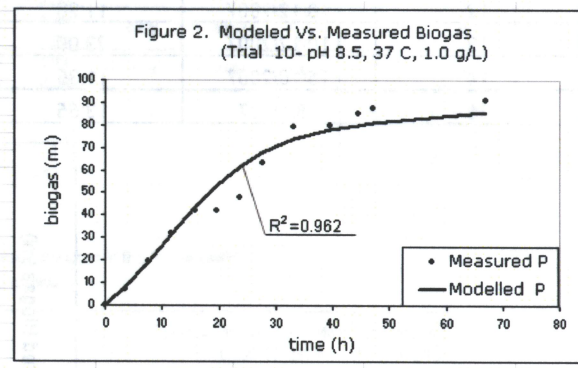
time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.6	7.0	1.9	0.012	0.126	6.8	8.2	
7.6	19.5	3.1	0.027	0.319	19.0	19.2	
11.6	32.0	3.1	0.044	0.579	31.2	31.3	
15.8	42.0	2.4	0.061	0.925	40.6	43.6	
19.6	42.0	0.0	0.075	1.296	40.1	53.5	
23.6	48.0	1.5	0.086	1.736	45.5	61.7	0.962
27.6	63.1	3.8	0.094	2.214	59.9	67.9	
33.1	79.1	2.9	0.101	2.913	74.8	73.8	
39.6	80.1	0.2	0.105	3.775	74.6	78.0	
44.6	85.1	1.0	0.107	4.452	78.6	80.1	
47.1	87.3	0.9	0.107	4.794	80.3	81.0	
66.9	91.3	0.2	0.109	7.519	80.3	85.9	
						β	1.5 (1)
						α	688.32 (2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

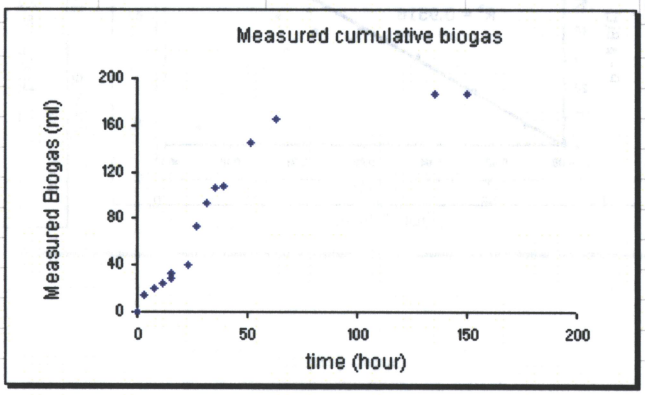




Trial #11

Trial ID: 11 **Log Sheet**
Inoculum ID: 3.4
Description: 37°C, pH 8.5, 4.0 g/L cellulose

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	10/10/2007	7:55	0.0	0.0	0.0
2	10/10/2007	11:20	3.4	15.0	15.0
3	10/10/2007	15:25	7.5	5.5	20.5
4	10/10/2007	19:25	11.5	3.9	24.4
5	10/10/2007	23:18	15.4	5.0	29.4
6	10/11/2007	3:08	15.2	3.5	32.9
7	10/11/2007	7:10	23.3	8.0	40.9
8	10/11/2007	11:10	27.3	32.0	72.9
9	10/11/2007	15:10	31.3	20.0	92.9
10	10/11/2007	19:20	35.5	13.5	106.4
11	10/11/2007	23:05	39.2	1.5	107.9
12	10/12/2007	11:20	51.5	38.0	145.9
13	10/12/2007	23:05	63.2	20.0	165.9
14	15/10/2007	11:35	135.7	21.0	186.9
15	16/10/07	13:55	150.0	0.0	186.9



Trial ID: 11 37°C, pH 8.5, 4.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{sss}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_o$-X))	Modeled X [g/L]	(5)
1.1	10/10/07	7:55	0.0	17/10/07	0	0.047	0.083	0.083	0.000		-0.799	0.063	
1.2				17/10/07	0	0.047	0.083						
2.1	10/10/07	11:20	3.4	17/10/07	0	0.047	0.083	0.083	0.000	0.000	-0.799	0.077	
2.2				17/10/07	0	0.047	0.083						
3.1	10/10/07	15:25	7.5	17/10/07	0.113	0.059	0.105	0.102	0.002	0.051	-0.479	0.096	
3.2				17/10/07	0.090	0.056	0.100						
4.1	10/10/07	19:25	11.5	17/10/07	0.118	0.059	0.106	0.106	0.000	0.008	-0.424	0.117	
4.2				17/10/07	0.118	0.059	0.106						
5.1	10/10/07	23:18	15.4	17/10/07	0.105	0.058	0.103	0.105	0.002	-0.001	-0.432	0.137	
5.2				17/10/07	0.126	0.060	0.107						
6.1	10/11/07	3:08	19.2	17/10/07	0.128	0.061	0.108	0.108	0.000	0.008	-0.385	0.158	
6.2				17/10/07	0.131	0.061	0.108						
7.1	10/11/07	7:10	23.3	17/10/07	0.325	0.091	0.162	0.183	0.022	**0.126**	0.785	0.178	0.866
7.2				17/10/07	0.440	0.115	0.205						
8.1	10/11/07	11:10	27.3	17/10/07	0.510	0.133	0.237	0.226	0.010	0.052	1.715	0.196	
8.2				17/10/07	0.465	0.121	0.216						
9.1	10/11/07	15:10	31.3	17/10/07	0.463	0.121	0.215	0.206	0.009	-0.023	1.217	0.212	
9.2				17/10/07	0.421	0.111	0.197						
10.1	10/11/07	19:20	35.5	17/10/07	0.455	0.119	0.211	0.230	0.019	0.026	1.829	0.225	
10.2				17/10/07	0.534	0.140	0.249						
11.1	10/11/07	23:05	39.2	17/10/07	0.399	0.106	0.188	0.197	0.009	-0.041	1.041	0.235	
11.2				17/10/07	0.443	0.116	0.206						
12.1	10/12/07	11:20	51.5	17/10/07	0.518	0.135	0.241	0.239	0.002	0.015	2.133	0.254	
12.2				17/10/07	0.510	0.133	0.237						
13.1	10/12/07	23:05	63.2	17/10/07	0.597	0.159	0.283	**0.267**	0.016	0.010	#DIV/0!	0.262	
13.2				17/10/07	0.538	0.141	0.251						
14.1	15/10/2007	11:35	135.7	17/10/07	0.425	0.112	0.199	0.200	0.002	-0.004	1.101	0.267	
14.2				17/10/07	0.433	0.113	0.202						
15.1	16/10/07	13:55	150.0	17/10/07	0.400	0.106	0.189	0.192	0.004	-0.003	0.946	0.267	
15.2				17/10/07	0.418	0.110	0.196						

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ (X_2, X_1)}$$

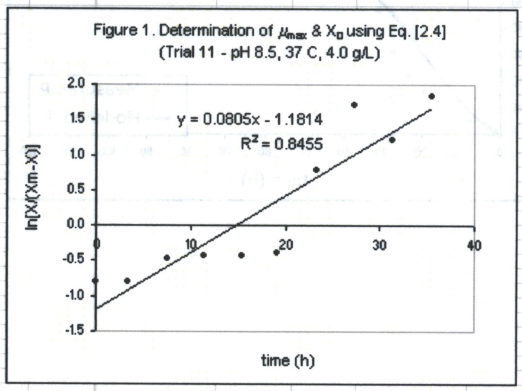
X_{max}	0.267	(2)
X_o	0.063	(3)
μ_{max}	0.081	(4)

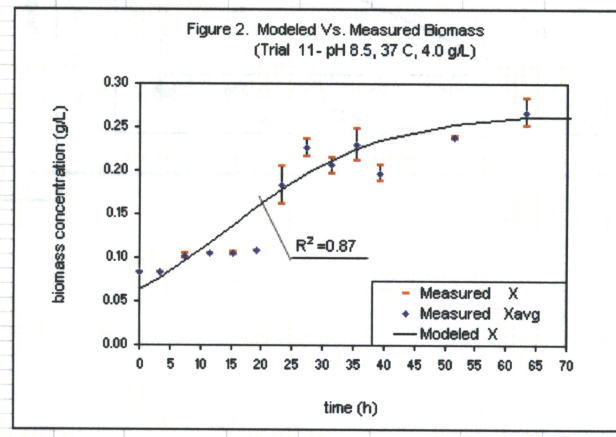
(2) X_{max} obtained from the measured maximum average data during the growth phase

(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.
The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 11 37°C, pH 8.5, 4.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2
	Measured P (ml)	Measured dP/dt (ml/h)	Eq.[2.22] A(t)	Eq.[2.23] B(t)	P - β*B(t)	Eq.[2.21] Modelled P (ml)	@
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.4	15.0	4.4	0.014	0.237	14.7	9.3	
7.5	20.5	1.3	0.033	0.590	19.9	22.1	
11.5	24.4	1.0	0.054	1.015	23.3	35.9	
15.4	29.4	1.3	0.075	1.510	27.8	49.9	
19.2	32.9	0.9	0.095	2.071	30.7	63.5	
23.3	40.9	2.0	0.115	2.759	37.9	77.5	
27.3	72.9	8.0	0.133	3.509	69.1	89.9	0.940
31.3	92.9	5.0	0.149	4.325	88.2	100.7	
35.5	106.4	3.2	0.162	5.243	100.7	110.4	
39.2	107.9	0.4	0.172	6.093	101.3	117.5	
51.5	145.9	3.1	0.191	9.114	136.0	133.3	
63.2	165.9	1.7	0.199	12.138	152.7	141.6	
135.7	186.9	0.3	0.204	31.433	152.8	165.9	
150.0	186.9	0.0	0.204	35.252	148.7	170.1	
						β	**1.1** (1)
						α	645.24 (2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 11 - pH 8.5, 37 C, 4.0 g/L)

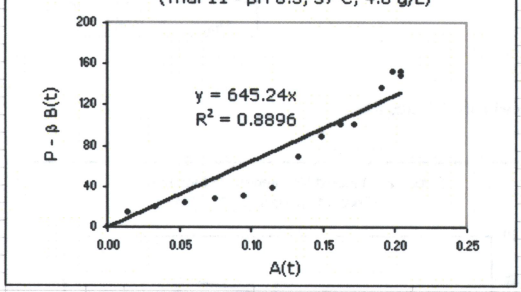
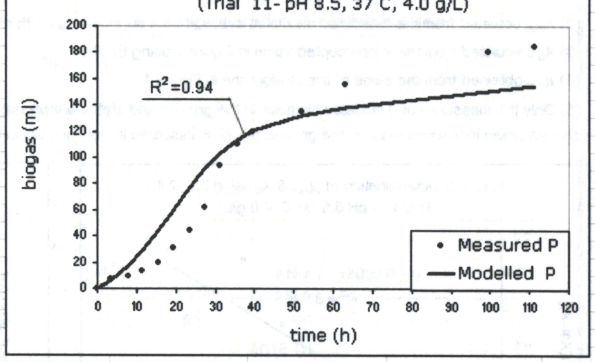


Figure 2. Modeled Vs. Measured Biogas
(Trial 11- pH 8.5, 37 C, 4.0 g/L)



Trial #12

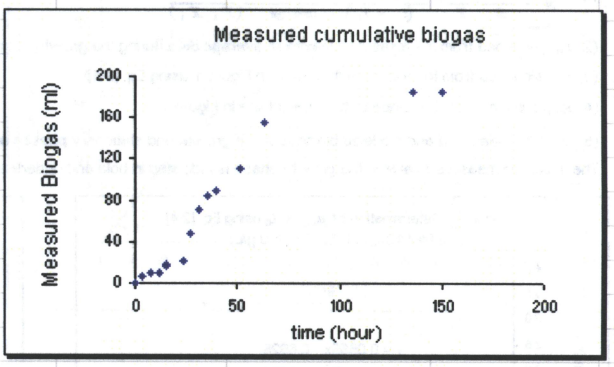
Trial ID: 12

Inoculum ID: 3.4

Description: 37°C, pH 8.5, 6.0 g/L cellulose

Log Sheet

sequence [#]	date [dd/mm/yyyy]	time [hh:mm]	time [hour]	measured biogas [ml]	cumulative biogas [ml]
1	10/10/2007	7:58	0.0	0.0	0.0
2	10/10/2007	11:25	3.4	6.0	6.0
3	10/10/2007	15:40	7.7	4.5	10.5
4	10/10/2007	19:45	11.8	0.0	10.5
5	10/10/2007	23:23	15.4	5.5	16.0
6	10/11/2007	3:14	15.3	2.8	18.8
7	10/11/2007	7:20	23.4	2.5	21.3
8	10/11/2007	11:15	27.3	26.0	47.3
9	10/11/2007	15:15	31.3	24.0	71.3
10	10/11/2007	19:40	35.8	12.5	83.8
11	10/11/2007	23:20	39.4	4.8	88.6
12	10/12/2007	11:40	51.7	22.0	110.6
13	10/12/2007	23:20	63.4	45.0	155.6
14	15/10/2007	11:40	135.7	29.0	184.6
15	16/10/07	14:00	150.0	0.0	184.6



Trial ID: 12 37°C, pH 8.5, 6.0 g/L cellulose

Biomass (X) analysis & modelling

sample				analysis							model		R^2
												Eq. [2.3]	
sample ID	sample taken [d/m/y]	sample taken [hh:mm]	elapsed time [hour]	analysis [d/m/y]	A_{535}	protein [g/l]	Measured X [g/l]	Measured Xavg [g/l]	Xerror [g/L]	μ (1) [hour^{-1}]	ln (X/(X$_o$-X))	Modeled X [g/L]	(5)
1.1	10/10/07	7:58	0.0	17/10/07	0.190	0.069	0.123	0.120	0.002		0.033	0.079	
1.2				17/10/07	0.170	0.066	0.118						
2.1	10/10/07	11:25	3.4	17/10/07	0.139	0.062	0.110	0.107	0.004	-0.035	-0.196	0.091	
2.2				17/10/07	0.105	0.058	0.103						
3.1	10/10/07	15:40	7.7	17/10/07	0.071	0.054	0.096	0.097	0.001	-0.022	-0.361	0.107	
3.2				17/10/07	0.082	0.055	0.098						
4.1	10/10/07	19:45	11.8	17/10/07	0.159	0.065	0.115	0.118	0.003	0.047	-0.008	0.123	
4.2				17/10/07	0.181	0.068	0.120						
5.1	10/10/07	23:23	15.4	17/10/07	0.245	0.077	0.137	0.126	0.011	0.020	0.139	0.137	
5.2				17/10/07	0.161	0.065	0.115						
6.1	10/11/07	3:14	19.3	17/10/07	0.212	0.072	0.128	0.129	0.001	0.005	0.183	0.151	
6.2				17/10/07	0.217	0.073	0.130						
7.1	10/11/07	7:20	23.4	17/10/07	0.107	0.058	0.103	0.106	0.003	-0.047	-0.200	0.165	0.768
7.2				17/10/07	0.135	0.061	0.109						
8.1	10/11/07	11:15	27.3	17/10/07	0.400	0.106	0.189	0.189	0.000	**0.143**	1.377	0.177	
8.2				17/10/07	0.399	0.106	0.188						
9.1	10/11/07	15:15	31.3	17/10/07	0.332	0.092	0.164	0.166	0.002	-0.032	0.854	0.188	
9.2				17/10/07	0.341	0.094	0.167						
10.1	10/11/07	19:40	35.8	17/10/07	0.403	0.107	0.190	0.186	0.004	0.026	1.309	0.198	
10.2				17/10/07	0.382	0.102	0.182						
11.1	10/11/07	23:20	39.4	17/10/07	0.471	0.123	0.219	0.228	0.009	0.056	3.315	0.205	
11.2				17/10/07	0.511	0.133	0.237						
12.1	10/12/07	11:40	51.7	17/10/07	0.451	0.118	0.210	0.215	0.006	-0.005	2.330	0.221	
12.2				17/10/07	0.476	0.124	0.221						
13.1	10/12/07	23:20	63.4	17/10/07	0.493	0.128	0.229	**0.236**	0.008	0.008	#DIV/0!	0.229	
13.2				17/10/07	0.524	0.137	0.244						
14.1	15/10/2007	11:40	135.7	17/10/07	0.406	0.107	0.191	0.197	0.006	-0.003	1.609	0.236	
14.2				17/10/07	0.434	0.114	0.202						
15.1	16/10/07	14:00	150.0	17/10/07	0.350	0.096	0.170	0.170	0.001	-0.010	0.936	0.236	
15.2				17/10/07	0.346	0.095	0.169						

(1) $$\mu = \frac{dX}{dt}\cdot\frac{1}{X} = \frac{(X_2 - X_1)}{(t_2 - t_1)}\cdot\frac{1}{average\ \ (X_2, X_1)}$$

X_{max}	0.236	(2)
X_o	0.079	(3)
μ_{max}	0.065	(4)

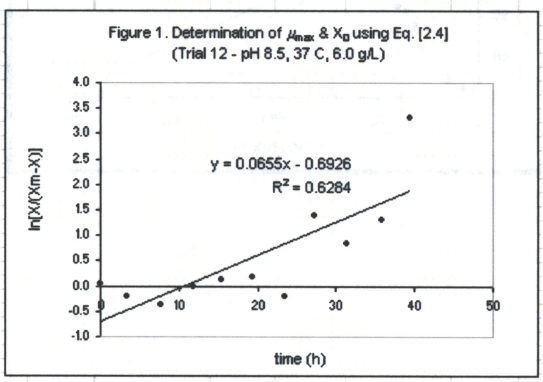
(2) X_{max} obtained from the measured maximum average data during the growth phase

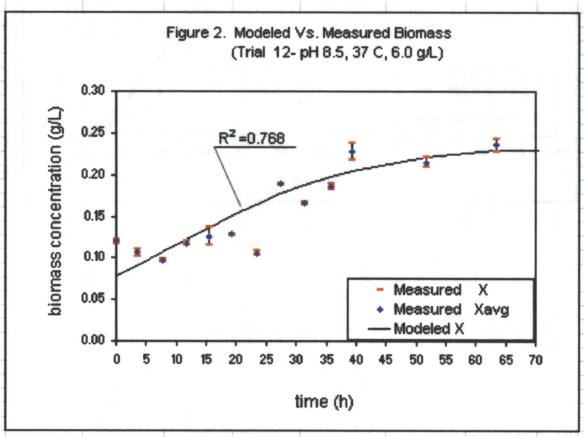
(3) X_o calculated from the y-intercepted value in Figure 1 using Eq. [2.4]

(4) μ_{max} obtained from the slope of the straight line in Figure 1

(5) Only the measured and modeled biomass in the growth and stationary phases are used in the R^2 calculation.

The maximum measured value in the growth phase is indicated in bold and underlined.





Trial ID: 12 37°C, pH 8.5, 6.0 g/L cellulose

Biogas (P) measurement & modelling

time (h)	Measurment		Model				R^2
			Eq.[2.22]	Eq.[2.23]	P - β*B(t)	Eq.[2.21]	
	Measured P (ml)	Measured dP/dt (ml/h)	A(t)	B(t)		Modelled P (ml)	
0.0	0.0	0.0	0.000	0.000	0.0	0.0	
3.4	6.0	1.8	0.012	0.288	5.5	8.7	
7.7	10.5	1.0	0.028	0.713	9.3	20.3	
11.8	10.5	0.0	0.044	1.184	8.5	31.8	
15.4	16.0	1.5	0.058	1.651	13.2	41.9	
19.3	18.8	0.7	0.072	2.212	15.0	52.6	
23.4	21.3	0.6	0.086	2.860	16.4	63.2	
27.3	47.3	6.7	0.098	3.527	41.3	72.5	0.925
31.3	71.3	6.0	0.109	4.257	64.1	81.1	
35.8	83.8	2.8	0.119	5.126	75.1	89.5	
39.4	88.6	1.3	0.126	5.853	78.7	95.5	
51.7	110.6	1.8	0.142	8.485	96.2	110.8	
63.4	155.6	3.8	0.150	11.124	136.7	120.6	
135.7	184.6	0.4	0.157	28.089	136.9	154.2	
150.0	184.6	0.0	0.157	31.466	131.2	160.0	

β	1.7	(1)
α	676.860	(2)

(1) β calculated by Eq. [2.19]

(2) α obtained from the slope of the straight line in Figure 1

(3) All the measured and modeled biogas in the entire fermentation process are used in the R^2 calculation.

Figure 1. Determination of α using Eq. [2.21]
(Trial 12 - pH 8.5, 37 C, 6.0 g/L)

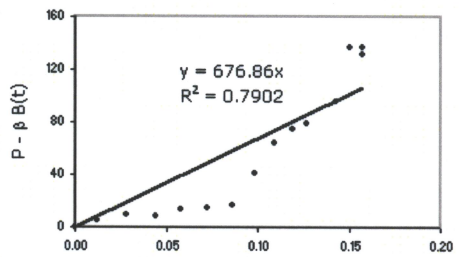
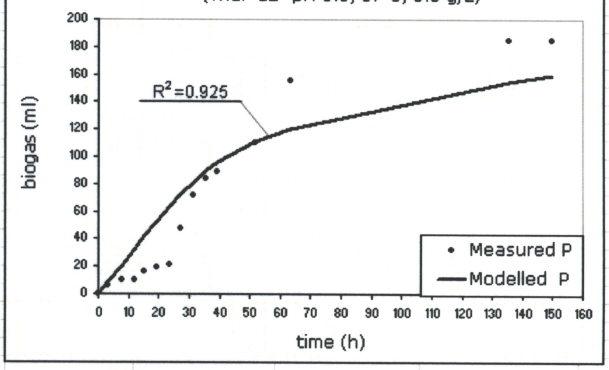


Figure 2. Modeled Vs. Measured Biogas
(Trial 12- pH 8.5, 37 C, 6.0 g/L)



Appendix B: Cellulose Experimental Data and Modelling Results

Trial #7:

Cellulose concentration (S) analysis & modelling

Trial ID: 7, 37°C, pH 8.5, 6.0 g/L cellulose

sample ID	sample		analysis					
	sample taken [d/m/y h:mm]	elapsed time [h]	analysis [d/m/y h:mm]	dilution factor	A_{490}	cellulose (S) [g/l]	S_{avg} [g/l]	S error [g/l]
7.1.a	5/7/2007 14:35	0.0	11/7/2007 11:00	200	1.134	9.072	10.572	1.500
7.1.b	5/7/2007 14:35	0.0	11/7/2007 11:00	200	1.509	12.072		
7.2.a	5/7/2007 18:40	4.1	11/7/2007 11:00	200	1.795	14.360	13.376	0.984
7.2.b	5/7/2007 18:40	4.1	11/7/2007 11:00	200	1.549	12.392		
7.3.a	5/7/2007 23:03	8.5	11/7/2007 11:00	200	1.386	11.088	11.200	0.112
7.3.b	5/7/2007 23:03	8.5	11/7/2007 11:00	200	1.414	11.312		
7.4.a	6/7/2007 6:22	15.8	11/7/2007 11:00	200	1.325	10.600	10.236	0.364
7.4.b	6/7/2007 6:22	15.8	11/7/2007 11:00	200	1.234	9.872		
7.5.a	6/7/2007 12:35	22.0	12/7/2007 15:00	500	0.419	8.380	7.820	0.560
7.5.b	6/7/2007 12:35	22.0	12/7/2007 15:00	500	0.363	7.260		
7.6.a	6/7/2007 20:30	29.9	12/7/2007 15:00	500	0.296	5.920	6.180	0.260
7.6.b	6/7/2007 20:30	29.9	12/7/2007 15:00	500	0.322	6.440		
7.7.a	7/7/2007 6:25	39.8	12/7/2007 15:00	500	--	--	8.700	
7.7.b	7/7/2007 6:25	39.8	12/7/2007 15:00	500	0.435	8.700		
7.8.a	8/7/2007 12:55	70.3	12/7/2007 15:00	500	0.222	4.440	4.170	0.270
7.8.b	8/7/2007 12:55	70.3	12/7/2007 15:00	500	0.195	3.900		

model							
			Eq. [2.16]	Eq.[2.15]		Eq. [2.17]	Eq. [2.1]
t (hour)	Modeled X (g/L)	dX/dt (g/L /hour)	Y	dS/dt (g/L /hour)	Modelled S (g/L)	K_s (g/L)	μ (h^{-1})
0	0.029				6		
4.1	0.060	0.007	0.055	0.137	5.440	5.00	0.1250
8.5	0.115	0.013	0.055	0.230	4.430	5.31	0.1092
15.8	0.229	0.016	0.055	0.287	2.335	5.86	0.0684
22	0.290	0.010	0.055	0.179	1.222	7.46	0.0338
29.9	0.316	0.003	0.055	0.061	0.736	15.92	0.0106
39.8	0.323	0.001	0.055	0.012	0.619	73.22	0.0020
70.3	0.324	0.000	0.055	0.001	0.600	1401.52	0.0001

Modeled	μ_{max} (h^{-1})	0.24	
	S_0 (g/L)	6	
Calibrateed parameter:	S_{min} (g/L)	10% (% of S_0)	=0.6

By interpolating μ = 0.12 h^{-1} (ie. 50% of μ_{max}) in the shaded area, it was found that K_s = 5.075 g/L. The relationship between the specific growth rate μ and the cellulose concentration S was developed using Eq. [2.1], as shown in Figure 2.

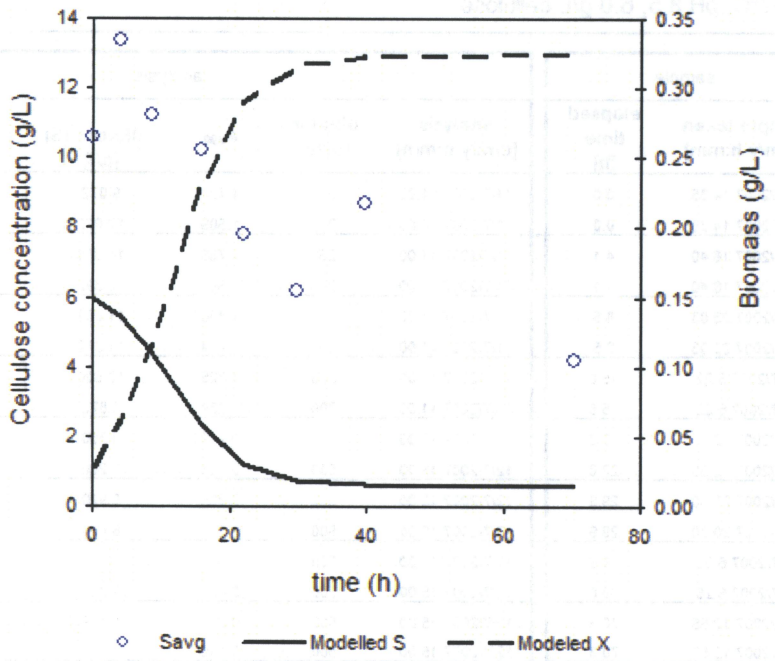


Figure 1. Modelled cellulose degradation and biomass growth

(The cellulose measurements shown are not correct. However, the trend of the degradation is valuable for modelling calibration.)

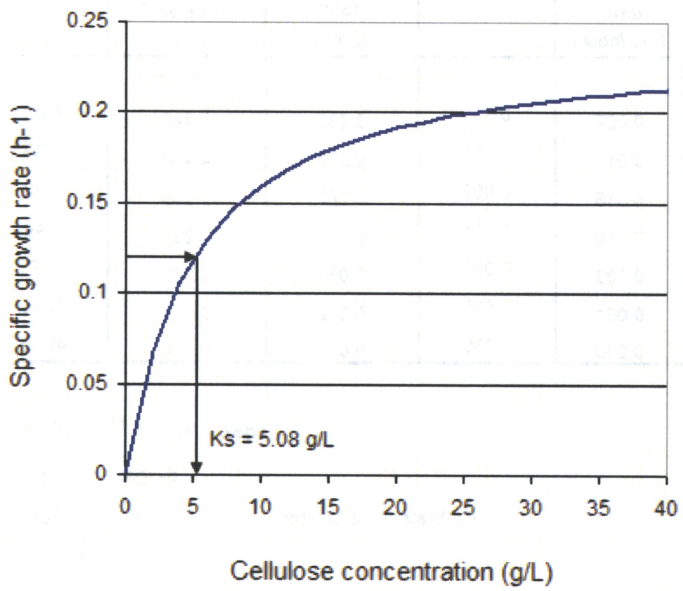


Figure 2. Modelled Specific growth rate μ Vs. cellulose concentration S