OPTIMIZATION AND MODELING OF LACTIC ACID PRODUCTION FROM PINEAPPLE WASTE

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FINAL REPORT

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I declare that this thesis entitled "*Optimization and Modeling of Lactic Acid Production from Pineapple Waste*" is the result of our own research except as cited in the references.

Signature : ……..

Name : Dr Roslina Rashid

Date : April 2008

To my beloved parents and friends

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ABSTRACT

Despite a great deal of research work on lactic acid fermentation in the past, the production of lactic acid from pineapple waste fermentation using immobilized cells has yet to be investigated. In this study lactic acid was produced from liquid pineapple waste fermentation by *Lactobacillus delbrueckii* entrapped in calcium alginate gel using batch fermentation systems. Lactic acid production by *Lactobacillus delbrueckii* was evaluated under immobilized cell fermentation conditions. The factors considered in the experimental design include pH, temperature, concentration of sodium alginate, cultivate size and bead diameter. The substrate concentration used throughout the experiment is 31.3 g/L. The glucose concentration and product formation were analyzed using high performance liquid chromatography (HPLC) and the cell numbers were determined by plate counting method. The experiment results revealed that the bead diameter the most important factor influencing production of lactic acid followed by Na-alginate concentration, pH and temperature. Maximum production, 30.27 g/L of lactic acid is obtained when using 2.0 %w/v sodium alginate concentration of bead diameter 1.0 mm at an initial pH of 6.5 at 37^{o}C and 5 g of cultivate, thus reflecting the optimum conditions. Kinetics of the immobilized fermentation was analyzed based on batch growth model in terms of specific growth rate, yield constant or substrate utilization and rate of product formation. Results indicate an average μ_{max} in the region of 0.09033 h^{-1} obtained at optimum conditions. For 2 liter fermentation, the Na-alginate immobilized cells produced 0.606g/L lactic acid/g/L glucose. The μ_{net} calculated was 0.033 $hour^{-1}$. Multilayer Perceptron (MLP) network was used in this study to predict the relationship between cell number and glucose concentration, between cell number and lactic acid concentration and between glucose concentration and lactic acid concentration at various temperatures using. It is found that the performance of MLP model is greatly influenced by the data sets used. The optimum structures of the MLP models are 1-8-1, 1-6-1 and 1-10-1 and the optimum transfer functions for hidden and output layer are Logsig and Tansig.

ABSTRAK

Berikutan dengan persaingan hebat kerja-kerja penyelidikan ke atas fermentasi asid laktik yang lalu, penghasilan asid laktik daripada fermentasi sisa nenas menggunakan sel tersekatgerak masih belum dikaji. Di dalam kajian ini, asid laktik dihasilkan daripada fermentasi sisa cecair nenas oleh organisma homofermentatif, *Lactobacillus delbrueckii* yang disekatgerak di dalam kalsium alginat menggunakan sistem fermentasi kelompok. Penghasilan asid laktik oleh *Lactobacillus delbrueckii* dikaji di dalam keadaan fermentasi immobilisasi sel. Faktor-faktor yang diambil kira di dalam rekabentuk eksperimen adalah pH, suhu, kepekatan Na-alginat, saiz kultur dan diameter manik. Kepekatan substrat yang digunakan sepanjang eksperimen ialah 31.3 g/L. Kepekatan glukosa dan hasil produk dianalisis menggunakan kromatografi cecair berprestasi tinggi (HPLC) dan bilangan sel ditentukan melalui kaedah kiraan plat. Hasil penyelidikan jelas menunjukkan diameter manik merupakan faktor utama mempengaruhi penghasilan asid laktik, diikuti dengan kepekatan Na-alginat, pH dan suhu. Kepekatan asid laktik yang maksimum ialah 30.27 g/L diperolehi apabila menggunakan kepekatan Na-alginat 2.0%, manik berdiameter 1.0 mm, pada suhu 37^{o}C, pH 6.5 dan 5 g kultur, lantas mengambarkan keadaan optimum. Kinetik bagi fermentasi immobilisasi telah dianalisis berdasarkan model pertumbuhan kelompok terhadap kadar pertumbuhan spesifik, penggunaan substrat dan kadar hasil produk. Hasil penyelidikan jelas menunjukkan kadar purata pertumbuhan spesifik adalah dalam lingkungan 0.09033 h^{-1} dicapai pada suhu 37^{o}C dan pH 6.5. Kajian ini memfokuskan ramalan hubungkait antara bilangan sel dan kepekatan glukosa, antara bilangan sel dan kepekatan asid laktik dan juga antara kepekatan glukosa dan asid laktik pada pelbagai suhu menggunakan Multilayer Perceptron (MLP). Melalui kajian ini, telah diketahui bahawa prestasi sesuatu model MLP adalah sangat dipengaruhi oleh set data yang digunakan. Struktur model yang optimum ialah 1-8-1, 1-6-1 dan 1-10-1. Manakala fungsi angkutan yang paling sesuai digunakan pada lapisan terlindung dan lapisan keluaran ialah Logsig dan Tansig.

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NOMENCLATURE

X	Cell concentration (g/L)
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
t	Fermentation time (h)
X_o	Initial cell concentration (g/L)
S	Substrate concentration (g/L)
P	Lactic acid concentration (g/L)

Ks	Saturation constant (g/L)
m	Coefficient of maintenance (g glucose/ h.g cell)
$Y_{x/s}$	Cell yield on the utilized substrate (g cell/g glucose)
$Y_{p/s}$	Product yield on the utilized substrate (g lactic acid/g glucose)
α	Growth associated constant for product formation
β	Non-growth associated constant for product formation (h^{-1})

LIST OF ABBREVIATIONS

ATCC	American type culture collection, Rockville, Marryland, USA
DSMZ	Deutcdche Summlung von Mikrorganismen und Zelkultuuren
HPLC	High performance liquid chromatography
KPUM	Kementerian Perusahaan Utama Malaysia
LAB	Lactic acid bacteria
MRS	De Man, Rogosa and Sharpe
UV	Ultra violet

PLA	Polylactic acid
ADM	Archer Daniels Midland
AHA	Alpha hydroxy acid
PET	Polyethylene terephthalate
PCM	Pineapple cannery of Malaysian
FFD	Full factorial design
ATP	Adenosibne-5-triphosphate
DNS	3,5-dinitrosalicilioc acid
DOE	Design of experiment
ANOVA	Analysis of variance
RI	Reflective index

LIST OF APPENDICES

IDENTIFICATION OF IMPORTANT FACTORS THAT INFLUENCE THE PRODUCTION OF LACTIC ACID FERMENTATION BY IMMOBILIZED *LACTOBACILLUS DELBRUECKII* USING WASTE AS SUBSTRATE

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CHAPTER 1

RESEARCH BACKGROUND

1.1 Introduction

Environmental pollution by waste generated from economic activities such as chemical, petrochemical, agricultural and food industries are common problems faced by the world nowadays. Pineapple canning industry is one of the many food industries producing large quantities of solid and liquid waste. Due to the stringent environmental regulations regarding waste disposal, the industry have to provide proper treatment. If these waste discharges to the environment are left untreated they could cause a serious environmental problem.

There is a potential for food processing waste such as pineapple waste to be used as raw material, or for conversion into useful and higher value added products. The pineapple waste can also be used as food or feed after biological treatment. About 30% of the pineapple is turned into waste during the canning operation. These wastes contain high content of carbohydrate that can be utilized for the production of organic acid. Based on the physio-chemical properties of the pineapple waste can be potentially used as carbon sources for production of lactic acid by microbial systems (Kroyer, 1991).

Lactic acid is considered as a very important chemical compound with significant applications in pharmaceutical, chemical industry and especially in the food industry. Worldwide demand for lactic acid is growing at a rate of

approximately 12-15% a year. Lactic acid production from agricultural crops such as wheat, corn and beet has recently received much attention because of the increasing demands for polylactic acid, which is used in biodegradable plastics (Akerberg and Zacchi, 2000). The production of such biodegradable polymer can replace non-degradable plastics and thus solve the environmental pollution problem. The increasing use of chemical synthesis plastics, which takes about hundred years to degrade, has cause environmental deterioration, with these waste plastic clogging landfills, strangling wildlife and littering beaches. The production of PLA will increase if new economic production routes are developed to increase annual lactic acid consumption (Datta and Tsai, 1995).

World demand for lactic acid is currently estimated at $150 million (100 000 tons). An annual growth of 8.6% of the lactic acid market is expected between 2000 and 2003. About 50% of the market is in food and beverage applications, which is a mature and stable market. For polylactic acid, the potential market is expected to reach about 160 000 tons in 2003 and 390 000 tons in 2008 (Bogaert and Coszach, 2000). This type of fermentation could nevertheless be important because the carbon sources are waste product that would otherwise be difficult and expensive to discard, rather than agricultural crops that could be put to other uses in the production of human food and animal feed.

Lactic acid can be produced by microbial fermentation or by chemical synthesis but in recent years fermentation process has become more industrially successful because of the increasing demand for naturally produced lactic acid. Lactic acid producing microorganisms are proprietary (Holten, 1971). However only homofermentative organism are of industrial importance for lactic acid manufacture. It is believed that most of the strains used in the industry belong to genus *Lactobacillus*, which usually produce one of the two kind isomers, L(+) or D(-), or a racemic mixture of both. However, ideal fermentation cultures need to produce exclusively L(+)lactic acid from an economic substrate (Buchta, 1983).

Currently, lactic acid production through free cell fermentation provides about 50% of the world supply, but productivity is very low in conventional batch processes. However employing cell immobilization method that provides high density can increase the productivity. Immobilization cell is one of the most attractive methods in maintaining high cell concentration in the bioreactor (Chang, 1996). Immobilized cell systems offer the advantages of high volumetric productivity than batch fermentation system, the possibility of continuous operation and higher stability (Goksungur and Guvenc, 1999). The immobilized preparation can then be reused either in batch or in a continuous system and hence diminished the cost of the process. For immobilized cell system, for instance, dilution rates, which far exceed the growth rate of the cells, can be used without risk for cell washout, as it would occur in the comparable free cell system. Immobilized cells exhibit many advantages over free cells, such as relative ease of product separation, reuse of biocatalysts, high volumetric productivity, improved process control and reduces susceptibility of cell contamination (Goksungur and Guvenc, 1999).

Entrapment in Ca-alginate is the most widely used procedure for lactic acid bacteria immobilization. Stenroos *et al*. (1982), immobilized *Lactobacillus delbrueckii*, Boyaval and Goulet (1988), immobilized *Lactobacillus helveticus*, Kurosawa *et al*. (1988), co-immobilized *Lactobacillus lactis* and *Aspergillus awamori*, Guoqiang *et al*. (1991), immobilized *Lactobacillus Casei*, Roukas and Kotzekidou (1991), co immobilized *Lactobacillus casei* and *Lactobacillus lactis*, Abdel Naby *et al*. (1992), immobilized *Lactobacillus lactis* and Kanwar *et al*. (1995), immobilized *Sporolactobacillus cellulosolvens* in Ca-alginate gel for the production of lactic acid.

In this study, calcium alginate was used for immobilization of bacteria *Lactobacillus delbrueckii*. In order to carry out the lactic acid production from pineapple waste using the immobilized *Lactobacillus delbrueckii* process successfully, many important factors have to be considered. The factors such as pH, temperature, calcium alginate concentration, inoculum size and beads diameter have to be studied systematically.

1.2 Research Problem

Pineapple canning industries are located in tropical regions such as Malaysia, Thailand and Indonesia producing large quantities of solid and liquid waste. However if waste can be transformed into valuable products such as organic acid, this would heighten the profits and competitiveness of the industry. For instance the pineapple waste produced from the pineapple canning industries can be used as a substrate for organic acid production such as lactic acid. Therefore the use of pineapple waste for lactic acid production may be an option for utilizing low value waste material in producing commercial products while solving the environmental problems.

Lactic acid is one such product that has numerous applications in chemical compound pharmaceutical, cosmetic, technical and especially in food industry. New application such as biodegradable plastic made from poly (lactic) acid, have the potential to greatly expand the market for lactic acid if more economical processes could be developed (Wang, 1995). In order to commercialize polylactic plastic production, it is necessary to explore a reliable, less expensive substrate, optimize the bioconversion conditions to produce lactic acid in large quantities economically.

Given the low productivity of batch processes for lactic acid production, recent research has focused on increasing the cell concentration in the reactor cell immobilization. The use of cell in free solution is wasteful, although not necessarily uneconomic. Immobilization cell is one of the most attractive methods in maintaining high cell concentration in the bioreactor (Chang, 1996). Considerable interest has been focused on the development of fermentation processes utilizing carbohydrates derived from inexpensive pineapple waste material. Studies on lactic acid production by immobilized organism are focused on using pineapple waste as substrate containing glucose as carbon source.

1.3 Objective and Scope

The physical and chemical characteristics of pineapple waste produced from canning process will vary according to the process obtained as well as areas, season of pineapple fruit generated. Therefore, characterization of the waste is important and has to be carried out in order to determine the physical and chemical composition such as sugar content, which influence the fermentation process. Hence, the first objective of this study is determine the sugar content such as glucose, sucrose, fructose and organic acid such as citric acid and malic acid and macro elements.

The objective of this study is also to produce high lactic acid from pineapple waste using immobilized *lactobacillus delbrueckii*. A batch process for immobilized cell fermentation and lactic acid production is developed. The immobilized cell of *lactobacillus delbrueckii* was investigated using entrapment method, where the cell is mixed with sodium alginate, an acidic polysaccharide and the mixture is dropped into a solution of calcium chloride. In this research work, the influential of factors such as pH, temperature, sodium alginate concentration, substrate concentration, bead diameter and temperature on production of lactic acid using immobilized technique is also investigated. The significant factors, the optimum immobilized condition and relationship between factors and response viable will be determined using the two-level full factorial design.

A special interest will be focused on applying the local substrate such as pineapple waste, which is rich in nutrients, and its potential to be used as a carbon sources for lactic acid fermentation. Previous experiments showed that liquid pineapple waste containing 30.86 g/l of total sugar was successfully fermented to lactic acid using *Lactobacillus delbrueckii* with up to 86% sugar conversion (Busairi, 2002). However the production of the lactic acid was performed in free solution batch process, which resulted in low yields. Since cell immobilization is one of the attractive methods in maintaining high and stable cell concentration, an attempt is made in this study to use the cell immobilization fermentation method to produce lactic acid using pineapple waste as a substrate.

Finally, kinetics parameters of the fermentation process such specific growth rate, cell yield, saturation constant, product yield, growth associated and non-growth associated constant for product formation were also evaluated to describe the simultaneous cell growth, substrate consumption and lactic acid production.

1.4 Outline of the Thesis

The thesis is basically divided into six chapters. The research background, research objectives and scope are outlined in Chapter I. A comprehensive literature review had been carried out prior to any experimental work. Literature review was conducted in providing state of the art background to the research project and these were discussed in detail in chapter II. Chapter III provides preliminarily studies for pineapple waste characterization and comparison between free cell and immobilized cell fermentation. In this chapter, most of the physical and chemical properties of the pineapple waste together with its contents are listed. Determination of significant factors using two-level full factorial design was discussed in chapter IV. In Chapter IV, the significant factors affecting the fermentation process were investigated using the full factorial design. It involves evaluate of mathematical models to describe predicting lactic acid production. The optimization module of the DESIGN-EXPERT software was utilized to search for optimal solution. The research outcomes for parametric study of lactic acid fermentation using immobilized *Lactobacillus delbrueckii* and kinetic study of bacterial growth, substrate utilization and lactic acid production are presented in chapter V. Parameters such as pH, temperature, Na-alginate concentration and bead diameter were studied in details. Finally, Chapter VI concludes the outcome of research project and highlights some recommendations for future studies. The schematic diagram summarizing the overall experimental approach is shown in Figure 1.1.

CHAPTER 2

LITERATURE REVIEW

This chapter briefly reviews the background of lactic acid production, immobilization cell, pineapple industry and bacterial fermentation. Immobilized living cell systems are used for the production of lactic acid. More than half of the total consumption of lactic acid is produced traditionally in simple batch fermentation in low productivity. Generally the primary objective of whole cell immobilization is to increase the extent of reaction or the volumetric productivity of the process over more traditional methods of applying microbial process.

2.1 Lactic Acid

2.1.1 Historical Background

Lactic acid (2-hydroxypropionic acid, $C_3H_6O_3$) is an organic hydroxyl acid whose occurrence in nature is widespread. It was discovered and isolated in 1780 by Swedish Chemist Carl Wilhem Scheele in sour milk (Datta and Tsai, 1995). It was the first organic acid to be commercially produced by fermentation, with production beginning in 1881 (Ruter, 1975 and Severson, 1998). It is present in many foods both naturally or as a product of microbial fermentation. It is also a principal metabolic intermediate in most living organisms from anaerobic prokaryotes to humans.

In 1839, Fremy performed lactic acid fermentation of several carbohydrates, such as sugar, milk sugar, mannite, starch and dextrin. A discovery that was then confirmed by Gay-Lussac. In 1840, Louradour prepared lactic acid by fermentation of whey and converted it into iron lactate by dissolution of metallic iron in it. Other fermentation experiments were performed by many different scientists to produce lactic acid from cane sugar beyond 1847 (Holten, 1971).

Blondeau discovered lactic acid as a fermentation product in 1847. Originally, the lactic acid of fermentation and that found in muscle tissue were regarded as identical. Liebig, who in 1947 re-examined meat extract, suspected that the two acids might not be identical. He asked Engelhardt to carry out an examination of the salts of the two acids. Engelhardt confirmed Liebig's thought that the contents of water of crystallization and the solubility of the salts of the two lactic acids differed and thus the acids were different (Holten, 1971).

Welceneus, in 1873, proved they have the same structure, but different physical properties. It was also investigated by Pasteur as one of this first microbiological yeast cultures of distilleries, it was not until the year 1877 that lactic acid bacteria were isolated in pure cultures when Lister isolated *Streptococcus lactis*. During this same period, Delbruck was endeavoring to find out the most favorable temperature for lactic acid fermentation in distilleries. He concluded that relatively high temperature favored high yields of lactic acid (Holten, 1971).

In the USA until 1963, lactic acid was produced solely by fermentation, when Sterling Chemicals, Inc., started producing lactic acid by a chemical process using petroleum by products, supplying nearly half the American demand for lactic acid. In 1996, Sterling abandoned the lactic acid business, leaving lactic acid production again exclusively to fermentation companies (Severson, 1998). In the early 1990s, Ecological Chemical Products (EcoChem), a joint venture of E.I du Pont Nemours & Co., and Con Agra produced only 1 to 2 million pounds of lactic acid by fermentation of whey permeate. In 1993, the current leader in basic chemical fermentation, Archer Daniels Midland (ADM), entered the lactic acid business and produced, in a facility designed for 40 million pound per year, 10 million pounds of

lactic acid from corn sugar. With a potential market for lactic acid in polymer production, the demand for lactic acid may reach as high as 2000 million and above per year (Severson, 1998).

2.1.2 Physical and Chemical Properties

Pure anhydrous lactic acid is a white crystalline solid with a low melting point of 53^{o}C and appears generally in form of more or less concentrated aqueous solution, as syrupy liquid. It also can be a colorless to yellow liquid after melting or it dissolved in water. Lactic acid is considered as a stable substance and it is a combustible substance as well. Lactic acid is compatible with strong oxidizing agents. Normally lactic acid is observed as a clear to slightly yellowish liquid, typically supplied to formulators in an 88 to 92% concentration. Lactic acid normally appears in diluted or concentrated aqueous solution.

Lactic acid is colorless, sour in taste, odorless and soluble in all proportions in water, alcohol and ether but insoluble in chloroform as shown in Table 2.1. It is a weak acid with low volatility (Casida, 1964). In solutions with roughly 20% or more lactic acid, self-estrification occurs because of the hydroxyl and carboxyl functional groups and it may form a cyclic dimmer (lactide) or more linear polymers. Lactic acid is very corrosive; therefore corrosion resistance material such as high molybdate stainless steel, ceramic, porcelain or glass lined vessel (Paturau, 1982) must be used for its production. The presence of hydroxyl and carboxyl two functional groups permits a wide variety of chemical reactions for lactic acid. The primary classes of these reactions are oxidation, reduction, condensation and substitutions.

Table 2.1: Characteristics of Lactic Acid (Martin, 1996)

Property	Characteristics
Optical activity	Exists as L(+), D(-) and recemic mixture
Crystallization	Forms crystals when highly pure
Color	None or yellowish
Odor	None
Solubility	Soluble in all proportions with water Insoluble in chloroform, carbon disulphide
Miscibility	Miscible with water, alcohol, glycerol and furfural
Hygroscopicity	Hygroscopic
Volatility	Low
Self-esterification	In solutions of > 20%
Reactivity	Versatile; e.g. as organic acid or alcohol

Lactic acid is the simplest hydroxy acid having an asymmetric carbon atom and it therefore exists in a racemic form and in two optically active form with opposite rotations of polarized light L(+) and D(-)lactic acid as shown in Figure 2.1. The optically active form of lactic acid is simply an equimolecular mixture of both and may be denoted as DL-lactic acid or racemic mixture. The optical composition does not affect many of the physical properties with important exception of the melting point of the crystalline acid. Table 2.2 shows a summary of lactic acid physical and thermodynamic properties.

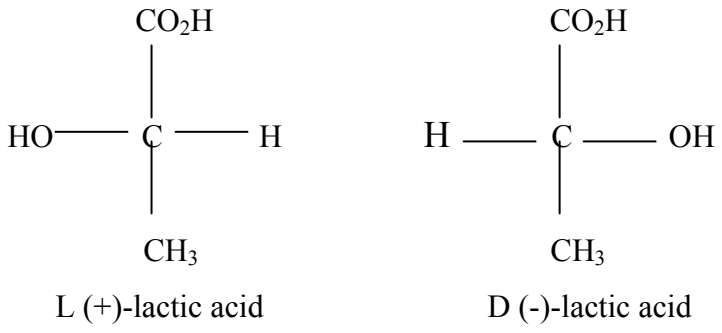

Figure 2.1: The isomer forms of Lactic acid

Table 2.2: Physical and thermodynamic properties of lactic acid (Holten, 1971)

Property	**Value**	**Isomer**
Molecular weight	90.08	D, L, DL
Melting Point, ^{o}C	52.8 53.0 16.8	D L DL
Boiling point (at 0.5mmHg), ^{o}C (at 14mmHg), ^{o}C	82.0 122.0	DL DL
Dissociation constant (K_a at 25^{o}C)	3.83 3.79	D L
Heat of combustion (ΔH_c), cal/kg	3615	DL
Specific heat (C_p at 20^{o}C), J/mol.^{o}C	190	DL
Specific rotation (22^{o}C, D line)	+2.6	L

Holten (1971) reported that the solubility properties of the isomers are also different. The D(-) isomer is soluble in water, alcohol and acetone, ethyl ether and glycerol and is practically insoluble in chloroform. The recemic mixture is soluble in water, alcohol and furfural. It is practically insoluble in chloroform and acetic acid.

Densities of aqueous solution of various lactic acid concentrations has shown that the density increased almost linearly with concentration and decreased almost linearly with temperature. The viscosity of lactic acid solution increased rapidly with the concentration and decreased rapidly with increasing temperature.

2.1.3 Application of Lactic Acid

Lactic acid is sold in food, pharmaceutical and technical grades. Since the lactic acid has gained increasing importance and has been used in a great variety of applications, its salt, ester and many derivatives have been developed. The uses of lactic acid can be broken down by grade and by lactic acid derivatives. Some of the important applications of lactic acid are detailed below.

2.1.3.1 Pharmaceutical

Lactic acid is used in pharmaceutical industry as a very important ingredient. Pharmaceutical and food industries show presence for the L(+)lactic acid because the D(-) isomer is not metabolized by the human body. Lactic acid and its salts have been mentioned for various medical uses. They provide the energy and volume for blood besides regulation of pH. Calcium, sodium, ferrous and other salt of lactic acid are used in pharmaceutical industry in various formulations find use for their anti tumor activity. Lactic acid finds medical applications as an intermediate for pharmaceutical manufacture, for adjusting the pH of preparations and in tropical wart medications (Vickroy, 1991).

Biodegradable plastic made of poly (lactic acid) is used for suture that do not need to be removed surgically and has been evaluated for use as a biodegradable implant for the repair of fractures and other injuries. These applications can be divided into:

- Medical/ pharmaceutical
    - Bone implants
    - Sutures
    - Ca-lactate in calcium tablets
    - Co-polymers in controlled drug release
    - Sodium lactate in dialysis solutions
    -
- Skin and hair care (cosmetics industry)
    - Lactic acid (skin renewal process)
    - Sodium and ammonium-lactate (skin moisturizer)
    - Hair conditioner

The calcium salts of lactic acid are produced in a granular and powdered form. Calcium lactate trihydrate is used in pharmaceuticals primarily as a dietary calcium source and also as a blood coagulant for use in the treatment of hemorrhages and to inhibit bleeding during dental operations. Sodium lactate is used in the production of some antibiotics and to buffer pharmaceutical preparations.

Natural L (+) lactic acid is used in many applications in cosmetics. Lactic acid is an alpha hydroxy acid (AHA) and is found in the skin. It is used as a skin-rejuvenating agent, pH regulator. It is a common ingredient in moisturizers, skin whiteners and anti acne preparation. Since L (+) Lactic acid is naturally present in the skin, lactic acid and sodium lactate are extensively used as moisturizing agents in many skin care products. Lactic acid is also used as a pH-regulator. It is one of the most effective AHAs and has the lowest irritation potential. Lactates are regarded as skin whitening agents that have been shown to produce a synergistic effect when combined with other skin whitening agents (Vickroy, 1991).

2.1.3.2 Food Industry

Lactic acid occurs naturally in many food products. Its has been in use as an acidulant, preservative and pH regulator for quite some time. Some of the important applications of lactic acid in the food industry are detailed below. There are many properties of lactic acid, which make it a very versatile ingredient in the food industry. It has a pronounced preservative action, and it regulates the microflora. It has been found to very effective against certain type of microorganisms. Some times a combination of lactic acid and acetic acid is used as it has a greater bactericidal activity. Because it occurs naturally in many food stuffs, it does not introduce a foreign element into the food. The salts are very soluble, and this gives the possibility of partial replacing the acid in buffering the acid in buffering systems (Vickroy, 1991).

Lactic acid is non-toxic and is deemed “Generally Recognized As Safe” (GRAS) as a general-purpose food additive in the USA. The same status is accorded in many other countries too. The calcium salt of lactic acid, calcium lactate, has greater solubility than the corresponding salt of citric acid. In such products, where turbidity caused by calcium salts is a problem, the use of lactic acid gives products, which are clear. L(+) Lactic acid is the natural lactic acid found in biological systems and hence its use as an acidulant does not introduce a foreign element into the body. Lactic acid are widely used in food industry such as confectionery as acidulant, beverages industries as natural flavoring, a preservatives for fermented vegetable and meat, and also an vital element for producing dairy’s product.

Direct acidification with lactic acid in dairy products such as cottage cheese is preferred to fermentation as the risks of failure and contamination can be avoided. The processing time also can be saved. Lactic acid is also used as an acidulant in dairy products like cheese and yogurt powder. The production of processed cheese can be greatly simplified if a sufficient amount of lactic acid is added to the freshly drained cheese curd to lower the pH to 4.8-5.2, then the curd can be processed without further curing, to adjust acidity and improved flavor, texture and stability.

2.1.3.3 Technical

The technical uses for lactic acid comprise a relatively small portion of the world’s production. These applications can be divided into:

- Electronics
  - Lactate esters in solvents photo resist formulations
  - Solder flux remover
- Cleaning

- Replacing ozone-depleting solvents
  - Degreasing/ cleaning of metal surfaces

- Coating and ink
  - Cataphoretic electro-deposition coating (acid)
  - Solvent for coating and ink (ester)

- Polylactic acid (PLA)

In the United State, Europe and Japan, several companies are actively pursuing development and commercialization of polylactic acid products. PLA polymers can be synthesized from various monomers. Low molecular weight polymers are obtained by step-growth polymerization of lactic acid. Whereas high molecular weight polymers are synthesized by ring-opening polymerization of lactide as shown in Figure 2.2. Lactide is composed of two lactic acid units linked to form a diester cyclic monomer. Step growth polymerization of optically pure L-lactic acid (or pure D-lactic acid) and ring opining polymerization of optically pure L-lactide (or pure D-lactide) should lead to the same chain growth.

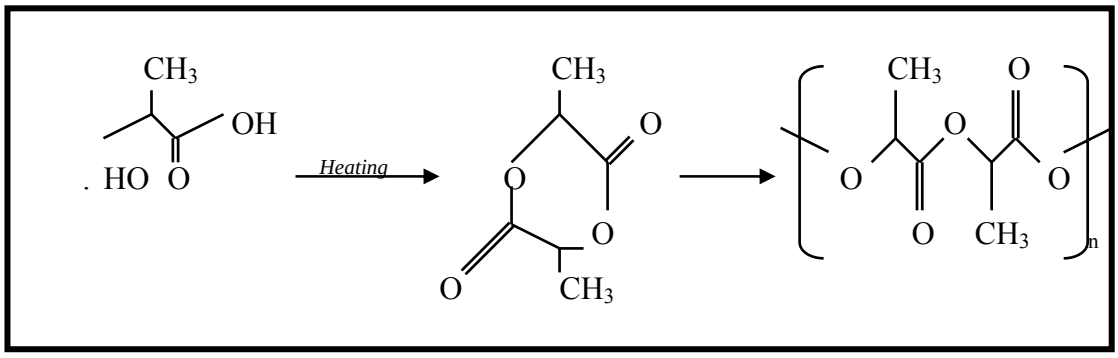

Figure 2.2: Synthesis of PLA using ring-opening polymerization

Actually dramatic differences in main chain structures are observed as soon as one deals with stereocopolymers of L-and D-lactic acid repeating units. The step growth polymerization of mixtures of L- and D-lactic acid leads to poly (D,L-lactic acid) with a random distribution of the L- and D-lactyl units, whereas ring opening polymerization of the lactide dimmers lead to non-random distribution because chains grow through a pair addition mechanism (Cassanas *et al*., 1998). The difference in the crystallinity of poly (D, L-lactic acid) and poly (L-lactic acid) has important practical ramifications. Since poly (D, L-lactic acid) is an amorphous

polymer; it is usually considered for applications such as drug delivery where it is important to have homogenous dispersion of the active species within a monophasic matrix. On the other hand, the semi crystalline poly (L-lactic acid) is preferred in applications where high mechanical strength and toughness is required (i.e. sutures and orthopedic devices).

PLA polymers offer a broad balance of functional performance that makes them suitable for a wide variety of market applications. They are expected to compete with hydrocarbon-based thermoplastics on a cost or performance basis. It also exhibits a tensile strength and modulus comparable to some thermoplastics. Like PET (polyethylene terephthalate), these polymers resist grease and oil and offer good flavor and odor barrier. PLA polymers also provide for heat stability at lower temperature than polyolefin sealant resin. The polymer can be processed by most melt fabrication techniques including thermoforming, sheet and film extrusion, blown film processing, fiber spinning and injection molding.

This material biodegrades completely to carbon dioxide and water when composted in municipal or industrial facilities, unlike traditional degradable plastics that need ultraviolet radiation to degrade. PLA needs only water and thus will degrade in the landfills. Biodegradation of PLA proceeds by a two-step process. Initially hydrolysis produces progressive chain length reduction by what is essentially an ester interchange process. This reaction is catalyzed by heat and pH. There are no bacteria involved in this phase of the process. When the chain length is reduced, producing very low molecular weight fragments, naturally occurring bacteria digest residues and liberate carbon dioxide and water (Lunt, 1996).

2.1.4 Production Technology

Lactic acid is a naturally occurring organic acid that can be produced by fermentation and chemical synthesis. However, it is more commonly produced from renewable resources via fermentation process. In fermentation processes, bacteria or

other microorganism produce lactic acid as they metabolize carbon-containing (e.g. carbohydrate) raw material.

2.1.4.1 Synthetic Methods

The synthetic manufacture of lactic acid on a commercial scale began around 1963 in Japan and United States (Holten, 1971). Chemical synthesis of lactic acid produces a racemic lactic acid mixture. Lactonitrile produced by combining of hydrogen cynide and acetaldehyde in liquid phase reaction at atmospheric pressure as shown in Figure 2.3. The crude lactonitrile is recovered and purified by distillation and is then hydrolyzed into lactic acid using either concentrated sulfuric or hydrochloric acid, producing an ammonium salts as a by-product. This crude preparation is esterified with methanol to produce methyl lactate. Methyl lactate is recovered, purified by distillation and then hydrolyzed under acidic conditions to produce a purified lactic acid, which is further concentrated and packaged. The sequence of the reactions is demonstrated as the follows:

$$HCN + CH_3CHO \longrightarrow CH_3CH(OH)CN$$

$$CH_3CH(OH)CN + 2H_2O + HCl \longrightarrow CH_3CH(OH)CO_2H + NH_4Cl$$

There are other routes for chemically synthesizing of lactic acid, for example: oxidation of propylene glycol; reaction of acetaldehyde with carbon monoxide and water at elevated temperatures and pressure; hydrolysis of chloropropionic acid and nitric acid oxidation of propylene. However, none of these processes are commercialized (Datta and Tsai, 1995). Due to the growing demand for lactic acid for biodegradable thermoplastics, there is a need for pure chiral forms, D- or L- lactic acid. Chemical synthesis produces a racemic mixture of lactic acid, D and L isomeric forms.

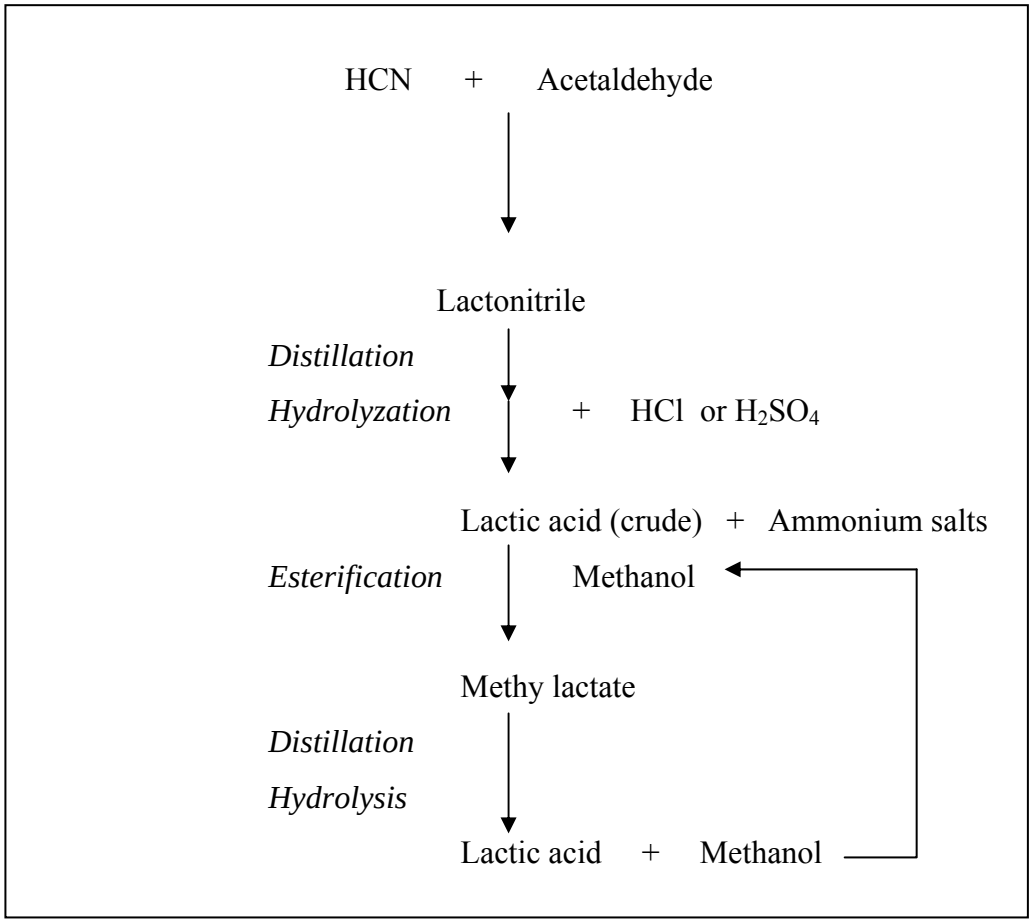

Figure 2.3: Chemical synthesis of lactic acid (Datta and Tsai, 1995)

2.2 Fermentation Processes

Fermentation processes are characterized by biological degradation of substrate (glucose) by a population of microorganism (biomass) into metabolites such as ethanol, citric acid and lactic acid (Maher *et al*., 1995). Lactic acid is produced from mono or disaccharide via the Embden Mayerhof glycolysis. Under anaerobic condition, the pyruvic acid produced is reduced to lactic acid by the enzyme lactic dehydrogenase.

2.2.1 Fermentation through Lactic Acid Bacteria

Lactic acid bacteria are a group of Gram-positive bacteria, non-respiring, non-spore forming, cocci or rods, anaerobic bacteria that excrete lactic acid as the main fermentation product into the medium if supplied with suitable carbohydrate. Lactic acid bacteria have been traditionally defined by the formation of lactic acid as a sole or main end product from carbohydrate metabolism (Holzapfel and Wood, 1995). Historically, bacteria from the genera *Lactobacillus*, *Leuconostoc*, *Bifidobacteria*, *Pediococcus* and *Streptococcus* are the main species involved. Several more have been identified but play minor role in lactic fermentations (Harvey, 1984).

There are two types of fermentation for these lactic acid bacteria, homofermentative and heterofermentative. Homofermentative lactic acid bacteria produce lactic acid as a sole end product; heterofermentative lactic acid bacteria produce other product such as acetic acid, ethanol as well as lactic acid the end product. The fermentation type and products of lactic acid as the end products of lactic acid bacteria have been summarized in Table 2.3.

Homolactic fermentation

The fermentation of 1 mole of glucose yields two moles of lactic acid;

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH$$

Glucose lactic acid

Heterolactic fermentation

The fermentation of 1 mole of glucose yields 1 mole each of lactic acid, ethanol and carbon dioxide;

$$C_6H_{12}O_6 \longrightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Glucose lactic acid + ethanol + carbon dioxide

Only the homofermentative lactic acid bacteria are of industrial importance for lactic acid manufacture. Homofermentative L(+)lactic acid producers are required if the lactic acid produced will be used as a feedstock for manufacture of 100% biodegradable plastics and or as a physiological active food additive. All species of *Streptococcus* produce L(+)lactic acid as the main end product when growing rapidly under conditions of carbohydrate excess, however in most cases, *Streptococcus* requires complex culture media, which often contain expensive meat extracts, peptone and blood or serum. Also under glucose limiting conditions and at low dilution rates in continuous culture, other end products including formate, acetic acid and ethanol are produced by Streptococcus.

Next to the *Pediococcus* and lastly the homofermenters of the *Lactobacillus* species, which produce the most acid, follow the heterofermentative species of *Lactobacillus*, which produce intermediate amounts of acid. Homofermenters, convert sugars primarily to lactic acid, while heterofermenters produce about 50% lactic acid plus 25 % acetic acid and ethyl alcohol and 25% carbon dioxide. These other compounds are important as they impart particular tastes and aromas to the final product (Vickroy, 1991).

Table 2.3: The fermentation types and products of lactic acid bacteria(Kandler, 1983)

Genus	Fermentation type	Main product	Isomer
Leuconostoc	heterofermentative	lactic acid (1) acetic acid (1) CO_2 (1)	D(-)
Bifidobacteria	heterofermentative	lactic acid (1) acetic acid (1.5)	L(+)
Lactobacillus	heterofermentative (pentose substrate)	lactic acid (1) acetic acid (1)	L(+), D(-) and DL
Lactobacillus	homofermentative	lactic acid (2)	L(+), D(-) And DL
Pediococcus	homofermentative	lactic acid (2)	DL, L(+)
Streptococcus	homofermentative	lactic acid (2)	L(+)

1) The number of moles of the product when one mole of dextrose (glucose) is fermented

2.2.2 Fermentation via Lactobacillus Bacteria

There are numerous species of bacteria and fungi that are capable to producing relatively large amount of lactic acid from carbohydrates (Atkinson and Mavituna, 1991). However in industrial fermentation the use of various species of *Lactobacillus* is preferred because of their higher conversion, yield and rate of metabolism (Mercier *et al*., 1992).

Lactobacillus is more suited to grow in plant extracts (Crueger, 1984). They are often found in carbohydrate containing substrates such as plants and materials of plant origin (Hammes and Whiley, 1993). It is believed that homofermentative *Lactobacillus* cultures are the most important commercial species for lactic acid production by fermentation (Vickroy, 1985). *Lactobacillus* cultures produce either L(+) or D(-)lactic acid or DL mixture. The species producing L(+)-lactic acid from cellulosic substrate have the most potential for future uses. In general, the desirable characteristics of potential industrial Lactobacillus cultures are the ability to rapidly and completely convert cheap substrate to L(+)-lactic acid with a minimum amount of nitrogenous substance supplement. Several bacterial strains (*Lactobacillus rhamnosus*, *L. casei and L. delbrueckii*) can be used in fermentation. *Lactobacillus delbrueckii* as in Figure 2.4 are used more commonly than the fungus by virtue of the bacteria's high rates of production and high conversion efficiency. The major and secondary products for this bacteria strain are shown in Table 2.4

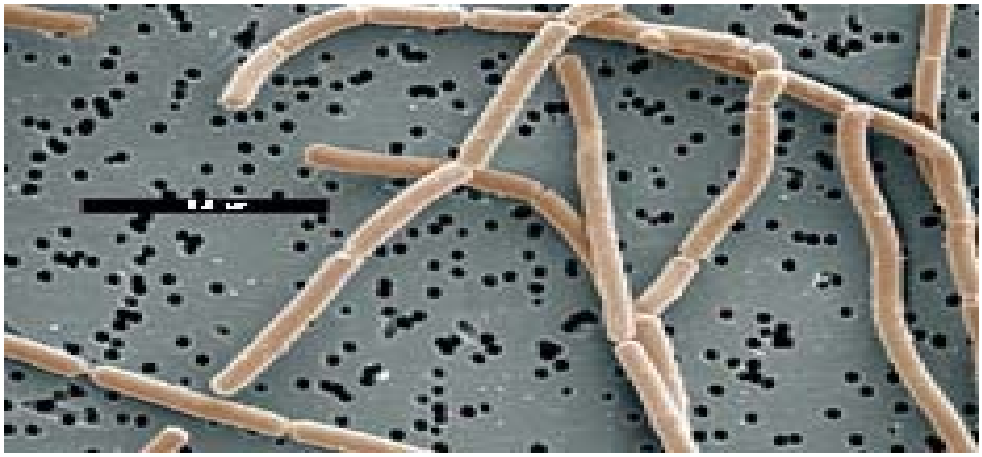

Figure 2.4: *Lactobacillus delbrueckii*

Table 2.4: Major and secondary products of *Lactobacillus* (L.) species (Martin, 1996)

Species	Substrate	Major product	Secondary product
L. bulgaricus	Lactose	D(-)Lactic acid	Acetaldehyde, Acetone, Diacetyle, Ethanol
L. helveticus	Lactose	DL-Lactic acid	Acetaldehyde, Acetic acid, Acetone, Diacetyle, Ethanol
L. lactis	Lactose	D(-)Lactic acid	Acetaldehyde, Acetone, Diacetyle, Ethanol
L. acidophilus	Glucose	DL-Lactic acid	Acetaldehyde, Ethanol
L. casei	Lactose	L(+) lactic acid	Acetic acid, Ethanol
L. delbrueckii	Glucose	L(+) lactic acid	-

Additional by-products may include glycerol, formate, pyruvate, succinate and minnitol. Only the homofermentative organisms are of industrial importance for the lactic acid manufacture, which grow optimally at temperatures around 37^{o}C and at a pH of 5-6.5. As shown in Table 2.5 and 2.6, several species have been identified that produce predominantly one isomer.

Table 2.5: Lactic acid isomer produced by *Lactobacillus* species

L(+)lactic acid producer	D(-)lactic acid producer	DL-lactic acid
L. rhamnosus	*L. coryniformis*	*L. acidophilus*
L. amylophilus	*L.bulgaricus*	*L. helveticus*
L. bavaricus	*L. jensenii*	
L. casei	*L. lactis*	
L.maltaromicus		
L. delbrueckii		

The selection of an organism depends primarily on the carbohydrate to be fermented. Lactose is fermented by *L. bulgaricus*, *L. casei* or *S. lactis* while glucose is fermented by *L. delbrueckii* and *L. leichmannii*. Xylose is fermented by *L. pentoaceticus*.

2.2.3 Fermentation Operating Condition and Parameters

Lactic acid fermentation has been studied since 1935 using different types of microorganism and fermentation operation conditions such as pH, carbon source, temperature, inoculum size, initial substrate conditions and nitrogen source (Hofvendal and Hagerdal, 1997). A batch process in which the conditions undergo a continuous change as a result of consumption of nutrients, multiplication of cells and accumulation of products, etc normally carries out the lactic acid fermentation. The culture condition vary from the strain which grow efficiently with good acid production on one carbon source will frequently not do so on another (Hofvendahl, and Hagerdal, 1999). Several parameters and operating condition effect the optimal production of lactic acid which include:

2.2.3.1 Microbial strain

Selection of the production strains is one of the most important parameters of successful production. First, strain development in the lactic acid industry does not only aim at high yields and productivities but also at the ability to transform cheap raw materials and to utilize substrates with constituents that maybe harmful to the production strain. Strain selection for these complex properties has generally been accomplished empirically.

A large number of bacteria have the ability to produce lactic acid. Strains of *Lactobacillus* were compared with regard to the fermentation of various sugars. Strain giving the highest lactic acid concentration and yield usually also showed the highest productivity. On lactose, including whey and milk, *Str. thermophilus* was in most studies superior to *Lactobacillus delbrueckii spp. bulgaricus* and *L. lactis*. In wheat flour hydrolysate *L. lactis* showed the highest productivity, whereas *Lb. delbrueckii spp. delbrueckii* resulted in the highest lactic acid concentration and yield. Generally the temperature used was adjusted to the optimum for each organism (Hofvendahl and Hagerdal, 1999).

2.2.3.2 Carbon sources

A number of different substrates have been used to fermentative production of lactic acid by lactic acid bacteria. A wide variety of carbon source is capable of producing lactic acid, including molasses, fruits waste, glucose, sucrose, fructose and lactose. If these substrates contain high level of metal ions they must be removed prior to production. The purest product is obtained when a pure sugar is fermented, resulting in lower purification costs. However, this is economically unfavorable, because pure sugars are expensive and lactic acid is a cheap product.

2.2.3.3 Effect of temperature

Temperature is one of the most important environment factors that effect lactic acid production. Various researchers have studied the effect of temperature on the lactic acid production and they found the optimal temperature between 41-45^{o}C (Hofvendahl and Hagerdal, 2000). Lactic acid bacteria can be classified as thermophilic or mesophilic. *Lactobacillus delbrueckii* is mesophilic bacteria, which grow at 17-50^{o}C and have optimum growth between 20-40 ^{o}C (Buchta, 1983). The yield increased with each increase at temperature level of fermentation (30 to 40^{o}C).

The lactic acid production begins to decrease when the temperature is above 45^{o}C. The highest yield at 79.8% was achieved at temperature of 40^{o}C (Busairi, 2002).

Goksungur and Guvenc (1997) reported that the optimal temperature is at 45^{o}C and this might be due to the different substrates used in the lactic acid fermentation. Maximum yield obtained at 45 ^{o} C in 53.61 g/l of lactic acid or 76.59% yield similarly when the temperature was increased to above 45^{o}C, the lactic acid production or yield decreased rapidly to 25.14 g/l lactic acid or 35.30% yield.

2.2.3.4 Effect of pH

There are various ways to control pH of the fermentation process. It can be set at the beginning and then left to decrease due to the acid production. In cases, when the pH is controlled, base titration can be carried out. The fermentation pH is set either at the beginning or then left to decrease due to acid production, or it is controlled by base titration, or by extraction, adsorption or electrodialysis of lactic acid. Various researchers studied the effect of pH on lactic acid production and found that the optimum pH for lactic acid production is between 5-7 (Hofvendahl and Hagerdal, 1999 and Goksungur and Guvenc, 1997). Goksungur and Guvenc, (1997), found that the effect of pH on lactic acid production is important and the optimal pH was 6.0 with lactic acid production found to be 54.97 g/l and the yield value 79%.

When the controlled pH was increased to 6.5, lactic acid production and yield value was reduced to 21.88 g/l and 31.25% respectively (Busairi, 2002). Busairi (2002) also reported that lower production rate of 11.59 g/l or 16.55% yield was obtained with lower pH of 5.5. In all cases, titration to a constant pH resulted in higher or equal lactic acid concentration, yield and productivity in comparison with no pH control.

2.2.3.5 Nitrogen sources

The medium composition has been investigated from many aspects, including the addition of various concentrations of nutrient. The lactic acid bacteria require substrates with high nitrogen content and have a particular demand for B vitamins. The nutrients are added in the form of malt sprout, corn steep liquor, and yeast extract. Lactic acid production increases with the concentration of the supplement especially yeast extract. The highest production rate was found with addition of 5-15 g/l yeast extract (Lund, 1992). Lactic acid increases with the increasing concentration of N_2.

The addition of nutrients and higher nutrient concentrations generally had a positive effect on the lactic acid production. MRS medium, which contains yeast extract, peptone and meat extract was superior to yeast extract, which in turn was better than malt extract. This reflects the complex nutrient demands of lactic acid bacteria, being fastidious because of limited biosynthesis capacity. Yeast extract alone at high concentration gave higher lactic acid production than yeast extract and peptone in low amounts, but the opposite resulted when the concentration of yeast extract was kept constant and peptone was added.

2.2.3.6 Fermentation mode

Lactic acid is most commonly produced in the batch mode but numerous examples of continuous culture exist as well as some fed batch and semi continuous/ repeated batch fermentations. When comparing batch and continuous fermentation modes, the former gave higher lactic acid concentration and yield in most of the studies. This is mainly due to that all substrate is used in the batch mode, whereas a residual concentration remains in the continuous one.

On the other hand, the continuous mode generally resulted in higher productivities. The major reason is probably that the continuous cultures were run at a high dilution rate, where the advantages over the batch mode are most pronounced. Varying the dilution rates in continuous culture affects both the substrate and nutrient concentrations. However the effects on the yield and productivities were inconclusive. Fed batch, semi continuous and repeated batch mode gave higher yields than the batch mode (Hofvendahl and Hagerdal, 1997).

In this section, the types of microorganism and the range of operation conditions used will be described briefly in order to provide the background for the present study which will be helpful in selecting the appropriate microorganism and operational conditions for lactic acid fermentation of pineapple waste.

2.2.3.6.1 Batch Fermentation

The basic fermentation process is batch. The culture is grown in a series of inoculums vessels and then transferred to the production fermentor. The inoculum size is usually 5-10% of the liquid volume in the fermentor. The fermentation is typically controlled at 35-45^{o}C and at pH 5-6.5 by the addition of the suitable base, such as ammonium hydroxide. At a pH of 5.0, Venkatesh (1997) attained a lactic acid concentration of 62 g/L in 6 days of simultaneous fermentation using *T.reesei* and *L. bulgaricus*. However, at a pH of 4.2, the lactic acid concentration dropped down to 18 g/l at the end of 6 days. Product concentrations of lactic acid have been reported as high as 115 g/L in 11 hours on whey permeate and yeast extract medium with *Lactobacilli bulgaricus* (Mehaia and Cheryan, 1987). At pH 5-6.5, for enzyme thinning corn starch, concentrations greater than 150 g/L in 30 hours have been reported with *Lactobacillus amylovorus* (Cheng *et al*., 1991). The molar conversion of carbohydrates was 94-95% for the two examples. Benthin and Villadsen (1995) produced optically pure D(-)lactic acid by fermentation of lactose with *L. bulgaricus*. The product was purified by crystallization as magnesium d-lactate followed by extraction with butanol. The overall yield of D(-)lactic acid was 72% and the purity was more than 99%.

The major limitation of the batch fermentation process is that both the presence of the lactic acid in the fermentation and the associated drop in pH, reduce the cells ability to secrete lactic acid. Adding a basic solution such as $CaCO_3$ will precipitate the Ca-lactate and prevent the pH drop, however, this precipitate has to be dissolved using another acid such as sulfuric acid. While this process is not technically difficult, it is expensive on a large scale and consumes large quantities of other chemicals. Instead, removing the produced lactic acid during the fermentation process can eliminate both of these events.

2.2.3.6.2 Continuous Fermentation

Continuous fermentation may be conducted to obtain fermentation products as a laboratory tool in the study of the physiology, metabolism or genetics of microorganisms or to produce microorganisms efficiently (Holten, 1971). It is characterized by the inflow of fresh nutrient medium into the culture vessel and the outflow at the same rate of the medium modified by the metabolic activity of the organisms together with part of the grown organisms. The concentration of all components, cells, substrates and products is identical in the whole cultivation volume and therefore in the out flowing fluid as well.

This type of fermentation can also be in a multi-stage process. The application of the multi-stage continuous system becomes necessary when we are concerned with the formation of certain products, with the chemical transformation of complex molecules by cells that are in a certain physiological state or with the stabilization of a certain enzymatic system (Ricica, 1996). The efficiencies and advantages of continuous process over the batch processes; stability, ease of control and increase in the productivity, make the continuous process more attractive for the industry than a simple batch process. Nevertheless, continuous charge of the nutrients and substrate may lead to substantial losses that will add to the cost of the final product.

Goksungur and Guvenc (1997) conducted a comparative study on batch and continuous fermentation of pretreated beet molasses using *L. delbrueckii*. The batch study was performed with temperature control at 45^{o}C and pH control at 6.0, the resulting lactic acid volumetric productivity was 4.83 g/dm^{3}h. On the other hand, a maximum lactic acid volumetric productivity of 11.2 g/dm^{3}h was obtained in the continuous experiment at a dilution rate of 0.5 h^{-1}. Ohleyer *et al*. (1985) compared the growth and lactic acid production of *L. delbrueckii* using glucose and lactose as carbon source. A continuous-flow stirred tank fermentor was couple with a cross flow filtration unit to permit operation at high cell concentration.

The lactic acid production was found to depend on the choice of carbon substrate. At steady state, yeast extract requirements for lactic acid production were lower when glucose was used as a substrate than with the lactose fermentation. Consequently, more growth factors were needed for lactose fermentation than for the glucose.

Several modifications have been done on the basic continuous process to increase the volumetric productivity such as the coupling of the fermentation unit with electrodialysis unit, ion-exchange unit, extraction unit or adsorption unit.

2.1.4 Substrate of Lactic Acid Production via Fermentation

Several carbohydrate materials have been used for the commercial production of lactic acid by fermentation. Refined sucrose from cane and beet sugar, followed by dextrose and maltose from hydrolyzed starch, have been the most commonly used substrates since the 50's (Vickroy, 1985). However, sugar and starch also have food and feed value and their sources are limited. Several raw materials or by-products have been evaluated as potential inexpensive substrates for lactic acid production.

The raw materials for the fermentation process consist of carbohydrates and nutrients for growth of the cells. For large-scale fermentation, the carbohydrates have primarily been lactose from whey or hydrolyzed corn syrup. The latter is predominantly glucose with some higher saccharides. A large number of carbohydrates materials have been used, tested or proposed for the manufacture of lactic acid by fermentation. Table 2.7 summarizes the substrates for lactic acid fermentation.

Table 2.7: Summary of the substrates for lactic acid fermentation (Martin, 1996)

Principal substrate	source
Lactose	Casein whey Cheese whey Sweet whey
Glucose	Corn
Sucrose	Molasses Cane sugar Beet sugar
Other	Potatoes Cellulose Sorghum extract

It is useful to compare feedstock based on the following desirable qualities:

1. Low cost
2. Low levels of contaminants
3. Fast fermentation rate
4. High lactic acid yield
5. Little or no by-product formation
6. Ability to be fermented with little or no pretreatment
7. Year- round availability

Crude feedstock has been avoided because high levels of extraneous materials can cause separation problems in the recovery stages. Use of pentose sugars results in the production of acetic acid that will incur extra process equipment for separation. Sucrose from cane and beet sugar, whey containing lactose and maltose and dextrose from hydrolyzed starch are presently used commercially. Since the 50's, potato, molasses and cheese whey have been studied as substrate for lactic acid production (Monteagudo, 1993). The results showed that cheese whey is a good inexpensive substrate for lactic acid production. However, the amount of whey supply is limited.

2.3 Pineapple Industry

2.3.1 Pineapple Industries in Malaysia

Pineapple is one of the principal canned fruits; most canned pineapple is produced in Asia, which are Thailand, Philippines and Indonesia; these countries export 77500 tons of canned pineapple annually (Numajiri *et al*., 2002). In Malaysia, the pineapple industry is the oldest agro-based export-oriented industry dating back to 1888. Though relatively small compared to palm oil and rubber, the industry also plays important role in the country's socio-economic development of Malaysia, particularly in Johore. The three registered canneries situated in Johore currently produce all the Malaysian canned pineapple (KPUM, 1990).

Although pineapple can be grown all over the country, the planting of pineapple for canning purpose is presently confined to the peat soil area in the state of Johore, which is the only major producer of Malaysian canned pineapple. In other states such as Selangor, Perak, Kelantan, Terengganu, Negeri Sembilan and Sarawak, pineapples are planted specifically for domestic fresh consumption (KPUM, 1990).

In view of the good market opportunities for canned pineapple in the world, there is prospect for Malaysia to step up its pineapple production. Likewise, the industry will have to take the necessary steps to increase production and export of canned pineapple to compete in growing world market. The structure of the pineapple planting will be further improved whereby estate planting will be extended and encouraged to achieve higher production yield as well as greater competitiveness. With the production of better quality fruits, recovery in processing will improve which will contribute towards improving Malaysia's competitiveness in the world market (KPUM, 1990).

2.3.2 Nutritive Aspects of Pineapple

The edible portion of most type of fruit contains 75-95% water. Fruits are low in protein but in general, contain substantial carbohydrates. The latter may include various proportions of glucose, fructose, sucrose and starch according to the type of fruit and its maturity. The main acids in fruits are citric, tartaric and malic acids. The total acidity often decreases during ripening and storage. The pH of fruits is usually from to 2.5 to 4.5. Other constituents of fruits include cellulose and woody fibers, mineral salts, pectin, gums, tannins and pigments (Young, 1986).

As in other fruits of this group, sucrose is the major sugar present in pineapples. Citric acid is the predominant acid with malic and oxalic acids also present. Acetic acid, furfural, formaldehyde and acetone were the major volatile constituents contain in canned pineapple juice (Shewfelt, 1986).

Krueger *et al*. (1992) have been reported that major constituents of fresh pineapple juice are glucose, fructose, sucrose, citric acid, malic acid and mineral potassium. The dominant sugar was sucrose; the glucose and fructose levels were similar to each other with fructose slightly higher than glucose. The compositions of sugar depend on the geographical origins and varying degrees of ripeness.

2.3.3 Pineapple Waste

2.3.3.1 Pineapple Canning Industry

The fresh pineapple referred here is strictly of the canning varieties that are delivered to registered pineapple canneries. It is of paramount importance for the industry to receive a continuous supply of fruit to the canneries. The two canneries draw their supplies of fresh fruits mainly from their own estates (KPUM, 190). The Pineapple Cannery of Malaysian (PCM) receives its supply of fresh fruits both from is own estates and the small growners sector. The production levels at 150,000 metric tonnes over the ten years. Only in 1991 where production reached its highest level, the quality of canned pineapple production depends very much on the fresh pineapple supply. The major producers of canned pineapple are Thailand, Philippine, Indonesia and Kenya which are together contribute to more than 80% of total world canned pineapple production of 1997 shown in Figure 2.5.

When the fresh fruits arrived in the canning factory, the fruits will be graded into several sizes according to the fruit diameter. Then fruit will be peeled, core removed, sliced, sorted and canned. All the peeled skin, unwanted fruits or the core will be sent to the crush machine for crushing. After crushing, the solid waste will be sent to cattle feeding while the liquid waste is send to the storage for fermentation process.

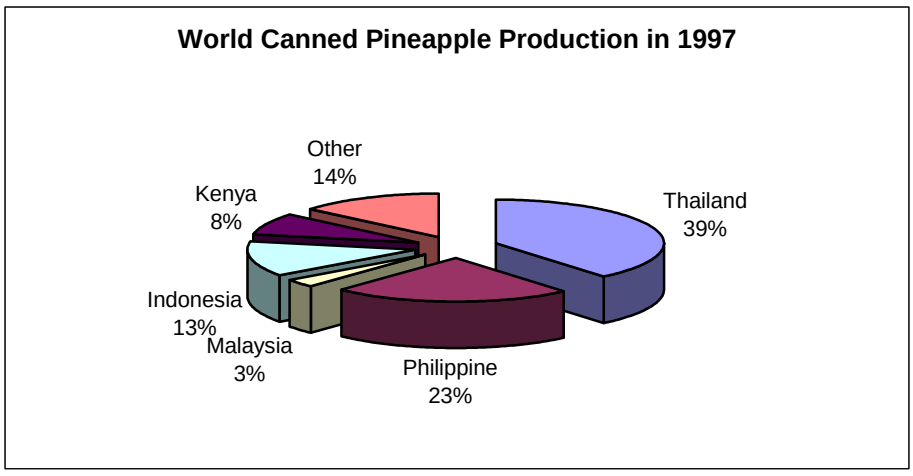

Figure 2.5: Pineapple canning industry

2.3.3.2 Pineapple Waste Characteristics

The waste generated by fruits processing are primarily solid in the form of peels, stems, pits, culls and organic matter in suspension. The first stage in the optimization of waste reduction is to identify and characterized the waste (solid and liquid) produced. Each particular food industry generates specific type and amount of wastes. The fruits and vegetables industry generates much more solid waste than the dairy industry. The characteristics of the waste load of various fruit processing industry, which indicate the problem of suspended organic matter in the wastewater. The magnitude of the problem is only apparent when the volume of the waste produced is considered (Moon and Woodroof, 1986). The characteristics of liquid waste from pineapple processing are given in Table 2.8

Table 2.8: The Characteristics of liquid waste (Sasaki et al., 1991)

Composition	Liquid waste	
	Before sterilization	After sterilization
COD (g/l)	100.8	103.7
Total sugar (g/l)	100.0	100.9
Reducing sugar (g/l)	39.20	41.20
Dextran (g/l)	1.50	1.50
Raffinose (g/l)	2.60	1.50
Sucrose (g/l)	40.1	40.1
Glucose (g/l)	23.6	23.6
Galactose (g/l)	1.70	2.10
Fructose (g/l)	14.0	15.6
Soluble protein (g/l)	0.90	-

The compositions vary considerably depending on the season, area and canning process. The waste contains mainly sucrose and fructose while dextrin, raffinose and galactose exist as minor components. The moisture content of solid waste was found to be range 87.50-92.80%; the difference of moisture content in the sample might be due to various geographical origins and also the varying degree of

ripeness. The nitrogen total content in wastes are 0.95% and ash content at range 3.90-10.60%. Although the waste contains very little nitrogen, soluble protein and trace elements such as Mg, Mn, Na, and K, these concentrations are adequate for lactic acid bacteria growth.

2.4 Cell Immobilization

2.4.1 Principles of Immobilized Cell Technology

Whole cell immobilization is defined as the localization of intact cells to a defined region of space with the preservation of catalytic activity (Karel *et al*., 1985). An immobilized cell system is described by Abbott (1978) to be any system in which microbial cells are confined within a bioreactor, thus permitting their reuse.

In nature the immobilization whole cells is widespread and plays an important role in microbial ecology. Whole cell immobilization occurs to some extent in all microbial-based industrial processes as well, including those for water and wastewater treatment. Because enzymes and cells have similar requirements for maintaining activity, developments in immobilization techniques for enzymes have been applied to whole cells. This review includes descriptions of the classifications for immobilized cell systems, and the physical, chemical and biological characteristics of these systems.

Generally the primary objective of whole cell immobilization is to increase the extent of reaction or the volumetric productivity of the process over more traditional methods of applying microbial processes. Confinement of cells to surfaces or particles reduces or eliminates the need for the separation of cells from the product stream. Another objective might to be minimize start-up time by growing the required biomass in a nutrient-rich growth medium (Tampion, 1987)

In choosing a biocatalyst process, the effort to produce the catalyst and the ability to maintain the activity and specificity of the catalyst must be considered for each process. Immobilized cell processes often are compared with those for free cells and immobilized enzymes. If a biocatalyst is difficult or expensive to produce, it must have a longer working lifetime in order to be competitive with more easily produced options.

Immobilized cell technology has been successfully employed for various types of fermentation processes using lactic acid bacteria. Traditional fermented dairy products (yogurt, cheese and cream) as well as starters and metabolites can be produced with a higher productivity than free cell bioreactors (Champagne *et al*., 1994; Norton & Vullemard, 1994). In addition, immobilized cell technology allows to stabilize the activity of bioreactors in successive or continuous operations, increasing bacteriophage resistance and plasmid stability and decreasing inhibition by antibiotics or salts (Champagne *et al*., 1994). Therefore, in order to be a more desirable alternative, immobilized cells must have a significantly longer working lifetime than free cell systems.

2.4.2 Cell immobilization Methods

Immobilized cell systems may be classifies according to the physical mechanism of immobilization. There are different techniques to obtain an immobilized cell preparation. Immobilization cell should be carried out under mild conditions in order to maintain the activity of the cells. Methods for immobilization of microbial cells include physical entrapment within porous matrix, encapsulation, adsorption or attachment to a pre-formed carrier and cross-linking. Figure 2.6 illustrates basic immobilization techniques (Tampion, 1987).

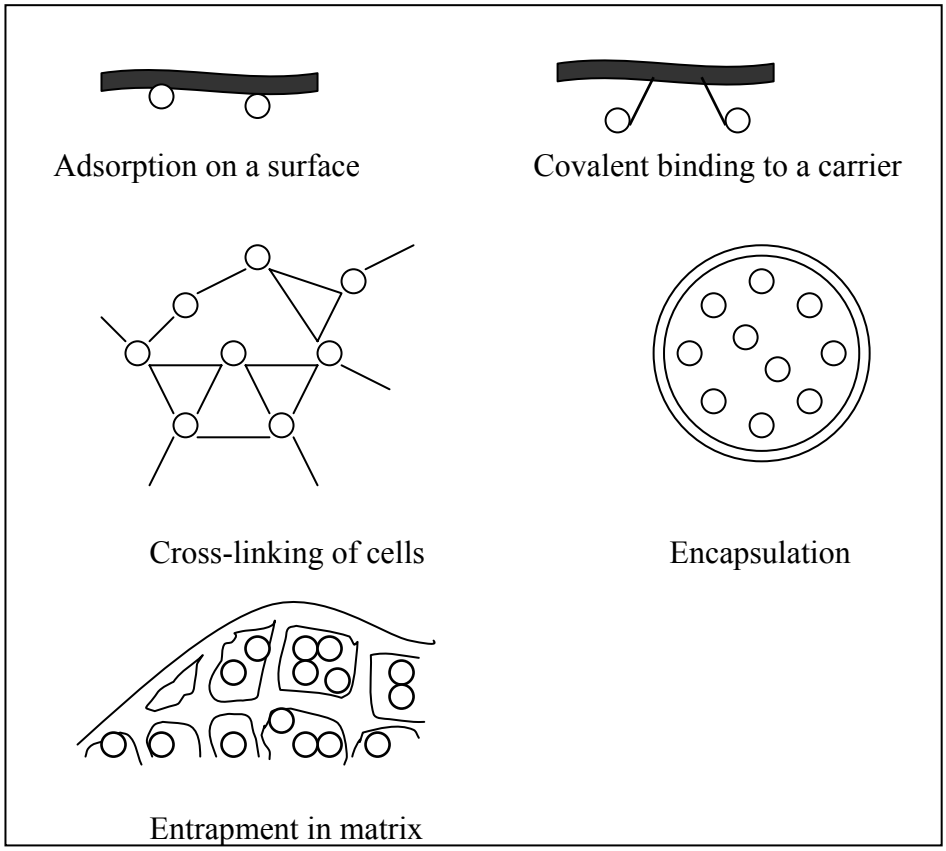

Figure 2.6: The immobilization cell methods

These categories are commonly used in immobilized enzyme technology. However due to the completely different size and environmental parameters of the cell, the relative importance of these methods is considerably different. The criteria imposed for cell immobilization technique usually determine the nature of the application.

2.4.2.1 Adsorption Method

Adsorption involves the reversible attachment of biomass to a solid support mainly by electrostatic, ionic and hydrogen bonding interactions. Because it is known that yeast cells have a net negative surface charge, a positively charged support will be most appropriate for immobilization (Bickerstaff, 1997). There are two main types of whole cell adsorptive immobilization carriers: (a) carrier that allow adsorption only onto external surfaces because pore sizes are too small to

allow microorganisms to penetrate inside, and (b) carriers with large enough pores to allow adsorption onto internal surfaces (O'Reilly and Scott, 1995).

Biomass loading is generally lower in adsorbed cell systems than those obtainable in gel entrapment matrices, but mass transfer may be more rapid. Adsorptive matrices do not have the additional gel diffusion barrier between the cells and bulk fermentation medium. Another advantage to using adsorption matrices is the regenerability of the support. The application for this method has been used widely in waste water treatment, ethanol production and cell mass production with fritted glass, activated carbon, porous glass, wood chips, controlled pore glass and modified cellulose used as solid support.

The strength of cell attachment to an adsorption carrier depends on both cell and matrix type. Since there is no barrier between cells and surrounding medium, these immobilization matrices may have significant cell leakage. This is not appropriate for processes requiring a cell-free effluent. Environmental ionic strength, pH, temperature, along with physical stresses such as agitation and abrasion can induce cell desorption. Another limitation of adsorption cell carrier is the possibility of non-specific binding of charged materials within the fermentation medium (Bickerstaff, 1997).

2.4.2.2 Cross-Linking Method (Aggregation of Cells by Flocculation)

Studies on this method are rather few and this method is not suitable for immobilization of microbial cells in a living state. Self-aggregated or flocculated cells also can be regarded as immobilized cells because their large size provides similar advantages as immobilization by other methods. While molds will from pellets naturally, some bacteria or yeast cells require flocculation. The formation of cell aggregates by flocculation shown in Figure 2.6 is the most simple and least expensive immobilization method, but the least predictable.

Tampion (1987) define flocculation as 'the formation of an open agglomeration that relies upon molecules acting as bridges between separate particles'. The natural flocculating ability of yeast cells may be exploited (Paiva *et al*., 1996) or cross-linkers may be added to bolster the process of aggregation for cells that do not do so naturally. The control of cell aggregation is important to maximize bioreactor efficiency. Factors which influence the natural flocculation characteristics of brewer's yeast strains include the genetic make-up of the strain, the cell wall structure and surface charge, the growth phase, incubation temperature, medium pH, cation composition of the medium and other wort components (Paiva *et al*., 1996).

Weak flocculation activity will result in slow cell sedimentation rates, which could cause cells to be washed out of the bioreactor with the fermentation medium and result in a low cell concentration in the bioreactor with insufficient fermentation rates. On the other hand, larger flocs with a very high flocculation activity may result in low concentrations of active yeast cells due to the diffusion limitation of substrate to the cells inside the flocs (Kuriyama *et al*., 1993).

2.4.2.4 Encapsulation Method

Encapsulation is another method of cell entrapment. In this type of immobilization, cells are confined to a desired area in the fermenter using a membrane. The cells may be suspended in the liquid phase or the cells may be attached to the surface and or entrapped within the membrane matrix (Gekas, 1986). A barrier formed by the liquid-liquid interface between two immiscible fluids can also be used for immobilization (Karel *et al*., 1985). Cell retention behind a membrane barrier has not been widely used to immobilize yeast cells for the continuous production of beer, but there are several groups who have investigated the concept for continuous ethanol production (Mulder and Smolders, 1986). Kyung and Gerhardt (1984) investigated continuous ethanol production using *Saccharomyces cerevisiae* immobilized in a membrane-contained fermenter.

Microporous dialysis membrane provided a barrier, which retained the yeast cells in the fermenter and simultaneously allowed inhibitory fermentation products such as ethanol to be continuously removed in order to boost reactor productivity. The problem of membrane plugging must be overcome for this immobilization mode to become a practical industrial-scale method for continuous ethanol production in the future.

2.4.2.4 Entrapment Method

Entrapment is the most commonly used method of immobilizing both viable and non-viable cells. Due to several advantages this method is preferable for cell immobilization. The procedure is simple. Cells and polymer or monomers are mixed and upon gel formation the cell are encaged in a polymeric network (Chang, 1998).

The entrapment of immobilized cells within a porous polymeric matrix such as calcium alginate (Bejar *et al*., 1992 and Shindo *et al*., 1994) or Kappa-carrageenan (Norton and D'Amore, 1994 and Wang *et al*., 1995), along with some others (Gopal and Hammond, 1993; Okazaki *et al*., 1995), has been studied extensively. Polymeric beads are usually spherical with diameters raging from 0.3 to 3.0mm. Immobilizing yeast cells using entrapment is a relatively simple method and a high biomass concentration is facilitated. Margaritis *et al*., (1987) reported one of the first pilot scale gas-lift draft tube bioreactor systems, using immobilized yeast in calcium alginate beads to produce ethanol in repeated fed-batch operation.

Entrapment in calcium alginate gel is the most widely used procedure for lactic acid bacteria immobilization. Stenroos *et al*. (1982), immobilized *Lactobacillus delbrueckii*, Boyaval and Goulet (1988), immobilized *L. helveticus*, Kurosawa and Tanaka, (1990) coimmobilized *L. lactis* and *Aspergillus awamori*, Guoqiang *et al*., (1991) immobilized *L. casei*, Roukas and Kotzekidou (1998), coimmobilized *L. lactis* and *L caseis*, Abdel-Naby *et al*. (1992) immobilized *L. lactis*

and Kanwar *et al*. (1995) immobilized *Sporolactobacillus cellulosolvens* in calcium alginate gel for the production of lactic acid. Kanwar *et al*. (1995) produced lactic acid from cane molasses in continuous culture by both free and calcium alginate immobilized *Sporolactobacillus cellulosolvents*. Goksungur and Guvenc (1999) produced lactic acid from pretreated beet molasses by the homofermentative organism *L. delbrueckii* IFO 3202 entrapped in calcium alginate gel using batch, repeated batch and continuous fermentation systems. In batch fermentation studies successful results were obtained with 2.0-2.4mm diameter beads prepared from 2% sodium alginate solution. The highest effective yield (82.0%) and conversion yield (90.0%) were obtained from beet molasses concentrations of 52.1 and 78.2gdm^{-3} respectively.

Some researchers have moved away from entrapment matrices and are currently focusing on adsorption techniques for several reasons. At present, gel entrapment matrices are not produced economically on an industrial scale. Diffusion limitations due to the gel matrix and high biomass loadings can cause metabolite concentration gradients within the polymer beads. The concept of utilizing the different microenvironments within a gel entrapment matrix is being studied for wastewater treatment systems by Dos-Santos *et al*. (1996) who refer to the magic bead concept in which the nitrifying bacterium *Nitrosomonas europaea* and the denitrifier *Paracoccus denitrificans* are coimmobilized in double layer gel beads. It was found that oxygen (Kurosawa and Tanaka, 1990), due to limitation of its uptake and diffusion, rarely penetrates greater than a few hundred micrometers into the gel bead when it is the limiting substrate.

Another limitation of gel entrapment includes the loss of gel mechanical integrity, by dissolution or by breakdown due to abrasion, compression or internal gas accumulation (Gopal and Hammond, 1993). Researchers have treated alginate gel beads with stabilizing agents such as sodium meta-periodate and glutaraldehyde (Birnbaum *et al*., 1981) or Al^{3-} (Roca *et al*., 1995) to improve gel mechanical strength.

The method is gentle, because of the wide variety of polymeric material, which can be used. A system can usually be chosen that retains the cells in a viable state. The preparation exhibits decreased cell leakage. The preparation has high loading capacity. A variety of polymeric materials have been used, including synthetic and natural polymers.

a) Synthetic polymer

The following polymers are employed as the matrices for immobilization: polyacrylamide, polyvinylchloride, photo-crosslinkable resin and polyurethane. Among these matrices, polyacrylamide gel has been extensively used for immobilization of many kinds of microbial cells. Photo-crosslinkable resin, which has recently been developed, is suitable for immobilized living cell systems because the immobilization can be performed under mild conditions.

b) Natural polymers

The natural polymers used for the immobilization of cells are mainly polysaccharides such as calcium alginate, k-carrageenan and agar. Besides polysaccharides, collagen and gelatin also have been used for the immobilization. Since 1975, calcium alginate gel has been used for the immobilization of cells and enzymes. In 1979, Cheetham *et al*. found that this gel provided suitable matrix for the immobilization by entrapment of whole microbial cells, sub-cellular organelles and isolated enzymes. Then the gel has been extensively used for immobilization of microbial cells in a living state.

Recently, it was found that k-carrageenan is a very useful matrix for immobilization of microbial cells. K-carrageenan, which is composed of unit structure of β-D-galactose sulfate and 3,6-anhydro-α-D-galactose, is a readily available nontoxic polysaccharide isolated from seaweed and is widely used as a food additive. K-carrageenan easily becomes a gel under the following conditions. It becomes a gel by cooling as in the case of agar.

The major disadvantage of using alginate immobilization is the leakage of cells from cell division occurring within the individual beads. Cell leakage can be minimized either by increasing the alginate or calcium chloride concentrations in beads or by making the beads small. However, the increase of the alginate and calcium chloride concentration in the beads can decrease the substrate diffusion rate through the gel and may affect the viability of entrapped cells (Cheetham *et al*., 1979).

2.4.3 Application and Uses of Immobilized Cell

The first application of useful compounds by immobilized living cell system may be the quick vinegar fermentation process with the trickle-filter developed in the beginning of the last century. This vinegar process, a carrier-binding method had been mainly used for earlier studies on immobilized living cell. However, recently the entrapping method has gained popularity, since it was found that the yeast cells entrapped into gel grew in the gel matrix and formed a dense cell layer near the surface gels. Thus entrapping method has become extensively used for the immobilized living cell system (Harvey, 1984).

Immobilized living cells can be applied to various multistep enzyme reactions. Various compounds such as alcohols, organic acids, amino acids, antibiotics, steroids and enzymes have been produce using immobilized living cells

i) Production of alcohol

Various alcohols such as ethanol, butanol, isopropanol are produced from carbohydrates using immobilized whole cell systems. Among them, large-scale industrial ethanol production is already beyond the stage of pilot plant operation. However, its economic feasibility still depends on the oil market. A considerable amount of research has been carried out on ethanol production processes using

immobilized microorganisms as model systems for immobilized whole cells (Harvey, 1984).

ii) Production of organic acid

Organic acids are extensively used in the food and pharmaceutical industries and some of them are products of microbial processes. Industrial processes for the production of organic acids have been carried out using immobilized treated microbial cells as functional catalysts similarly to those used for the production of amino acids. Many studies on the production of organic acids by immobilized growing microbial cells have been performed. However, in cases of organic acid production using immobilized living cells, lactic acid has been investigated most extensively amongst various organic acids such as citric acid, gluconic acid, and acetic acid. This is because the cultivation of lactic acid bacteria is little affected by the oxygen concentration, which could often be a limiting factor of a production system using immobilized cell.

iii) Production of amino acids

Amino acids are widely used for medical purposes and as additives of foods, feeds and cosmetics. $_{L}$-Isomer of amino acids is mainly applied for these purposes, although $_{D}$-isomer is useful for the synthesis of antibiotics. Biosynthesis of $_{L}$-amino acids by microbial cells and optical resolution of chemically synthesized of $_{L}$-amino acids by microbial enzymes have been extensively investigated. Several processes have been successfully applied on industrial scale, in which immobilized treated microbial cells are employed to catalyze single enzymatic reactions.

iv) Continuous production of antibiotics

Production of antibiotics, which is one of the most important subjects in the field of biochemical engineering, has been carried out through microbial processes, enzymatic reactions, chemical synthesis or combinations of these methods.

Although about 150 antibiotics are commercially produced, microbial processes produce most of them. One of the most important subjects related to antibiotic production using immobilized living cells is a continuous stable production of non-growth associated secondary metabolites. Microbial processes mainly have been performed with batch-wise systems because antibiotics are synthesized after exponential growth of microbial cells, that is, antibiotics are non-growth associated secondary metabolites, and the producing activities of microorganisms are often unstable. It is, therefore, quite difficult to produce antibiotics continuously during the prolonged cultivation of microbial cells (Chang, 1998).

v) Transformation of steroid

Various microbial cells are able to catalyze the transformation of steroids. Stereo-specific hydroxylation of steroids has been investigated by using immobilized growing or living cells. Steroid hydroxylated at a desired position are useful raw materials with considerable commercial value for the production of pharmaceutical steroid hormones. Utilization of living or growing cells is supposed to be advantageous for the hydroxylation of steroids, which involves quite complex reactions including activation of molecular oxygen and continuous supply of reducing power.

vi) Production of enzymes

Microbial cells are the best sources supplying large quantities of useful enzymes at a low price and the production of extracellular enzymes such as carbohydrate-hydrolyzing enzymes and proteolytic enzymes has been mainly studies by using immobilized growing microbial cells.

2.4.4 Benefits and Advantages of Immobilized Cell

The immobilized preparation can then be reused either in batch or in a continuous system and hence diminished the cost of the process. Immobilization of microorganisms, enzymes, animal and plant cells in a variety of carriers has been investigated for utilization of the advantages of immobilized biocatalysts over the use of free cells in various biotechnological processes. This immobilized cell system is a new technique, which looks like the combined technique of both fermentation and conventional immobilized whole cell system.

Immobilized whole cell systems exhibit some advantages over presently accepted batch or continuous fermentations using free-cells. These advantages include (i) operation at high dilution rates without washout (the dilution rate can be varied independently of the growth rate of the cells), (ii) greater volumetric productivity as a result of higher cell density, (iii) tolerance to higher concentrations of substrate and products, without inhibition, (iv) relative ease of downstream processing, (v) use of simple and less expensive reactor configurations (Prasad and Mishra, 1995).

In particular, immobilized living cells offer general advantages such as ability to synthesize various useful chemicals using multi-enzyme reactions, and regeneration activity to prolong their catalytic life (Tanaka and Nakajima, 1990; Furusaki and Seki, 1992). In fermentation conditions, immobilized cell systems avoid washout of cells, ensure higher cell concentration in small volumes and provide easy product separation. Advantages of immobilized cell formulations for environmental and agricultural applications have been recently described by Cassidy *et al*. (1996). In general, immobilization methods, in addition to above-mentioned advantageous characteristic, provide an excellent protection of cells from adverse environmental effect.

The immobilization process changes the environmental, physiological and morphological characteristics of cells, along with the catalytic activity. Stability of productivity is higher because microbial cells are reproduced in gel during operation. The degree of retention of a particular activity normally present in free cells will depend on the immobilization technique and reaction conditions (Karel *et al*., 1985).

2.4.5 Factors Affecting Immobilized Cell

Several parameters and operating condition has been known to influence the optimal production of lactic acid, which includes:

(a) Sodium alginate concentration

Lactic acid production decreased due to lower diffusion efficiency of the beads when the Na-alginate concentration is above 2.0%. Goksungur and Guvenc (1999) found that the maximum lactic acid production of 5.93% with a yield of 5.93% with a yield of 75.8% were obtained with bead prepared from 2.0% sodium alginate at pH 6.0 and temperature 45^{o}C using beet molasses. Abdel Naby *et al*. (1991) investigated lactic acid production by calcium alginate immobilized *L. lactis* and determined the maximum lactic acid production with beads containing 3% ca-alginate. They obtained lower yields with beads made of 4 and 5% alginate due to diffusion problems.

(b) The bead diameter

The effect of bead diameter on lactic acid production was determined by Goksungur and Guvenc (1999) using gel beads containing 2.0% sodium alginate. Bead diameters in the range of 1.3mm to 3.2mm were used in their work. It was found that increase in bead diameter deceased lactic acid production. Highest lactic acid production of 5.91% was obtained with cells

entrapped in 2.0-2.4mm calcium alginate beads. Abdel Naby *et al*. (1992) obtained maximum lactic acid production with cell entrapped in 2.0-2.2mm Ca-alginate beads. They also showed that a gradual increase in bead diameter beyond 3.0mm resulted in a gradual decrease in lactic acid production.

(c) Substrate concentration

Maximum productivity of $4.74gdm^{-3}h^{-1}$ and mean volumetric productivity of $4.21gdm^{-3}h^{-1}$ were obtained at sucrose concentration of $78.2gdm^{-3}$, the corresponding yield value was 90.0% and effective yield value was 75.8%. When the initial sugar concentration exceeded $78.2gdm^{-3}$, yield values deceased due to inhibition produced by high sugar concentration (Goksungur and Guvenc, 1999). Substrate inhibition in lactic acid production was also reported by Mehaia and Cheryan (1987) for *L. bulgaricus* on lactose, Goncalves *et al*. (1991) for *L. delbrueckii* on glucose and Monteagudo *et al*. (1994) for *L. delbrueckii* on sucrose;

(d) Fermentation temperature

Increasing the fermentation temperature from 37 to $40^{o}C$, with immobilized cells, improved the lactic acid concentration by14%. Deceasing the temperature to $31^{o}C$ resulted is only below 13% of with the level of lactic acid achieved at $37^{o}C$ (Yan, 2001).

2.5 Lactic Acid Fermentation Models

The kinetic models play an important role in monitoring and predicting fermentation process. In batch fermentation the kinetic model provides information to predict the rate of cell mass of product generation, while continuous fermentation predicts the rate of product formation under given conditions (Russel, 1987).

The models contain kinetic of growth, substrate utilization and product formation. According to this view, the cell, growth models can be divided into unstructured and structured types. Most of the available mathematical models for lactic acid fermentation process are unstructured. Unstructured model are the simplest, they take the cell mass as a uniform quantity without internal dynamics whose reaction rate depends only upon the conditions in the liquid phase of the reactor. This model contains a small number of parameters which can easily be estimated on the basis of steady state experiments and open ended and can rather easily be extended to describe more complex systems (Roels, 1983).

2.5.1 Kinetics of Microbial Growth

Batch growth of a microorganism consists of the following phases: lag phase, transition phase, exponential or logarithmic phase, a second transition phase, stationary phase and death phase (Lewis and Young, 1995). The rate of microbial growth is given by equation 2.1.

$$\frac{dx}{dt} = \mu X \tag{2.1}$$

Where: X = the concentration of microbial biomass in gram/liter
μ = the specific growth rate in hours^{-1}
t = fermentation time in hours

During the exponential growth phase, the specific growth rate of the cells, μ, is constant and reaches its maximum, μ_{max} as seen in equation 2.2.

$$\frac{dx}{dt} = \mu_{max} X \tag{2.2}$$

The kinetic of microbial growth in lactic acid fermentation has been studied by Mercier and Yerushalmi (1991) and Norton and Vullemard (1994). They used the logistic model that express the relationship of the growth rate and two kinetic parameters such as the maximum specific growth (μ_{max}). The two parameters were estimated by non-linear regression using the least square methods.

$$\frac{dx}{dt} = \mu_{\max} X\left(\frac{1-X}{X_{\max}}\right) \tag{2.3}$$

Integration of equation (2.3), gives;

$$X_t = \frac{X_o X_m \exp(\mu_{\max} t)}{X_m - X_o + X_o \exp(\mu_{\max} t)} \tag{2.4}$$

An unstructured model, which is frequently used in the kinetics description of microbial growth, is the Monod equation. This model expresses that the specific growth rate of microorganism increase if the substrate concentration in the medium is increased, however the increase in specific growth rate becomes progressively less if the substrate concentration level is higher. The relationship between μ and the residual growth-limiting substrate is represented in the equation below:

$$\mu = \mu_m\left(\frac{S}{K_s + S}\right) \tag{2.5}$$

K_s is the substrate utilization constant numerically equal to substrate concentration when μ is half μ_{max} and is a measure of the affinity of the organism for its substrate. The kinetics of microbial growth by combining equation (2.1) with (2.5) was proposed by Hanson *et al*. (1972). This model is represented in the equation below:

$$\frac{dx}{dt} = \mu_{\max}\left(\frac{S}{K_s + S}\right)X \tag{2.6}$$

Similar model has been proposed by Suscovic *et al*. (1992) and they assumed that the death rate can not be neglected. The equation is as follows:

$$\frac{dx}{dt} = \mu_{\max}\left(\frac{S}{K_s + S}\right)X - K_d X \tag{2.7}$$

2.5.2 Kinetic Model of Substrate Utilization

The substrate utilization kinetics for lactic acid fermentation using *Lactobacillus delbrueckii* may be expressed by the equation proposed by Monteagudo *et al*. (1997) which considers both substrate consumption for maintenance and substrate conversion to biomass and product. The rate of substrate utilization is related stochiometrically to the rates of biomass and lactic acid formation. The substrate requirement to provide energy for maintenance is usually assumed to be first order with respect to biomass concentration, mX. The equation is express as the follows:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} + \frac{1}{Y_{P/S}}\frac{dP}{dt} + mX \tag{2.8}$$

The parameters of the biomass yield on the utilized substrate $Y_{x/s}$, the product yield on the utilized substrate ($Y_{p/s}$) and maintenance coefficient (m) were estimates by non-linear regression analysis. A similar model was used for the kinetics of substrate utilization in lactic acid fermentation using *Lactobacillus amylophilus* by Mercier and Yerushalmi (1991) and *Streptococcus cremoris* by Aborhey and Williamson (1977).

Yeh *et al*. (1991) have also proposed simpler models. They assumed that since the maintenance coefficient is much smaller than the specific growth rate, it can be omitted, thus only the substrate utilization for conversion of biomass and product is considered. The equation has the following form:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dx}{dt} + \frac{1}{Y_{P/S}}\frac{dP}{dt} \tag{2.9}$$

The simplest model has been proposed by Suscovic *et al*. (1992). They assumed that the substrate utilization only for conversion of biomass. By the combining of Monod equation to this model can be obtained the following equation:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\mu_{\max}\left(\frac{S}{K_s + S}\right)X \tag{2.10}$$

The parameters of biomass yield on the utilized substrate ($Y_{x/s}$) and saturation constant (K_s) can be estimated using linear regression analysis.

2.5.3 Kinetics of Lactic Acid Production

Lactic acid fermentation that was described by Luedeking and Piret (1959), Norton *et al*. (1994) reported that lactic acid production was strongly linked to biomass production. Basically three types of fermentation can be distinguished such as growth associated product formation, mixed growth associated product formation and non-growth associated product formation (Moser, 1983).

Many researchers used the mixed growth associated product formation for lactic acid production kinetics. This model was described by Luedeking and Piret (1959) and is represented below:

$$\frac{dP}{dt} = \alpha \frac{dx}{dt} + \beta X \tag{2.11}$$

Where dP / dt is the volumetric product formation rate, α is the growth associated product formation and β is the non growth associated product formation.

Mathematical modeling and estimation of kinetics parameters for lactic acid production using high-glucose, high fructose and high sucrose syrup by *L. delbrueckii* have been studied by Suscovic *et al*. (1992). The growth associated lactic acid production constant (α) and non growth associated product formation constant (β) were estimated by linear regression and obtained values of α always higher than β.

The kinetics model for lactic acid production on beet molasses using *L. delbrueckii* was proposed by Monteagudo *et al*. (1997). Using model given by Luedeking and Pilet (1959), it improved by the addition of a term indicating dependence of the rate of lactic acid production on inhibitor concentration the lactic acid. The model has the following form:

$$\frac{dP}{dt} = \left(\alpha \frac{dx}{dt} + \beta X \right) \left(1 - \frac{P}{P_{\max}} \right) \tag{2.12}$$

The parameters were estimated by non-linear regression analysis and similar results were also obtained as reported by previous researcher Suscovic *et al*. (1992).

CHAPTER 3

METHODOLOGY

3.1 Introduction

From the previous study, the optimal condition for the lactic acid production fermentation with immobilized *Lactobacillus delbreuckii* were found to be: bead diameter, 1.0mm, pH at 6.5 and temperature, 37^{o}C (Suzana, 2004). In this preliminary study on lactic acid fermentation using immobilized *lactobacillus delbreuckii*, the research comprises of various phases. The first stage of this study was involved the comparison the different between the classical entrapment method using *lactobacillus delbreuckii* entrapped in calcium alginate gels and innovative technique, PVA-sodium alginate beads method. Then, aiming at developing immobilized cell systems to be employed in the lactic production, we have taken into consideration an immobilization procedure which allows PVA-sodium alginate as immobilization matrix. For the final stage, attempts were made to exploit, food processing waste such as pineapple waste as a raw material and immobilized cell using airlift bioreactor for lactic acid fermentation. Figure 3.1 shows a schematic diagram summarizing the overall experimental approach.

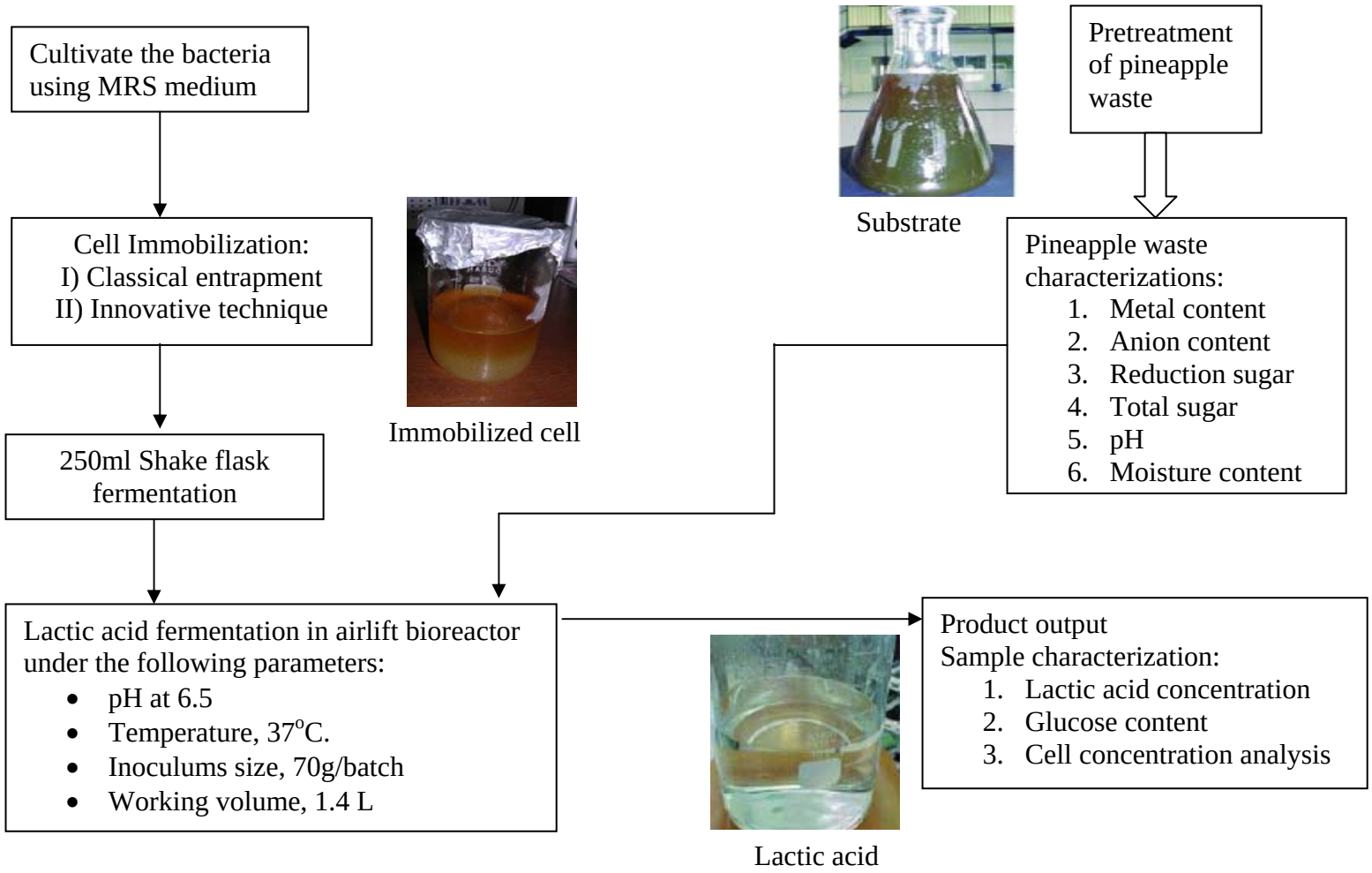

Figure 3.1 Schematic diagram summarizing the experimental methodology

3.2 Materials and Methods

3.2.1 Chemicals

Basically the chemicals that are required for the experiments in this study were divided into three categories: chemicals for immobilization, chemicals for pineapple waste characterization and fermentation (MRS medium and plate). All the chemicals used were of analytical grade and purchased from various suppliers. The Lactic acid standard used in this study was obtained from SIGMA (Code No.L-6402 and L-0625).

3.2.2 Strain

The microorganism used in this study was *Lactobacillus delbrueckii subsp. Debrueckii ATCC 9649*, a mesophilic homofermentative lactic acid bacterium. It was bought from DSMZ (Deutsche Sammlung von Mikroorganismen und Zelkultuuren GmbH) Germany.

3.2.3 Liquid Pineapple Waste Source

The liquid pineapple wastes used through out the experiments were obtained from the waste treatment plant of Malaysian Cannery of Sdn. Bhd. at Pekan Nenas, Pontian, Johor. The wastes were stored at –25^{o}C deep freezer pending fermentation and analysis.

3.2.4 Culture Media

The culture media used was MRS (deMan Rigosa Sharpe) medium, which suggested by DSMZ catalogue (1993). The compositions for 1 liter MRS medium are shown in Table 3.1

Table 3.1: Composition of MRS medium (1L)

Material	Composition(g)
$MgSO_4.7H_2O$	0.58
$MnSO_4$	0.25
Sodium acetate	2
K_2HPO_4	2
Diammonium citrate	5
Yeast extract	5
Meat extract	5
Peptone	10
Glucose	20
Tween-80	1ml

3.3 Experimental Methods

3.3.1 Preparation of Liquid Pineapple Waste

The liquid pineapple waste was boiled for 5 minutes resulting in flocculation of particulates and these settled rapidly upon cooling to room temperature. Then, the particulate was separated by centrifugation for 15 minutes at 5000 rpm. The clear

supernatant was filtered using Whatman no 54 filter paper under vacuum and was stored at –18^{o}C (Lazaro, 1989).

3.3.2 Inoculums Preparation

The culture in the petri dish was aseptically inoculated into a 250ml flask which contains 50ml MRS medium. The biological safety cabinet must be swabbed with disinfectant (alcohol) to reduce the risk of contamination and the work must be accomplished in minimum time to prevent exposure. The loop was flamed and allowed to cool before transfering the bacteria. The mouth of the fermentation mediums was flamed before and after adding the culture. The inoculating loop was re-flamed after completing. The flask was then incubated in the incubator shaker at 37^{o}C, 150 rpm for 24 hours (Sakamoto and Komagata, 1996).

3.3.3 Cell Immobilization (Classical Entrapment Method)

In the preparation of immobilized cell, *Lactobacillus delbrueckii* cells grown in a 25 cm^{3} MRS broth was mixed with an equal volume (1:1, v/v) of 2% Na-alginate solution. Then, the alginate-cell solution was dropped slowly into the 0.2 M $CaCl_2$ solution by a peristaltic pump. The alginate solidified upon contact with $CaCl_2$, forming beads, thus entrapping bacteria cells. The beads were allowed to harden for 30 minutes and were then washed with 0.85% NaCl solution to remove excess calcium ions and cells. Finally, the beads were stored at 4^{o}C until use. In order to improve the immobilization results, a ratio of $CaCl_2$ and NaCl of 1.1 was used in the solution preparation. The immobilization method is shown in figure 3.2.

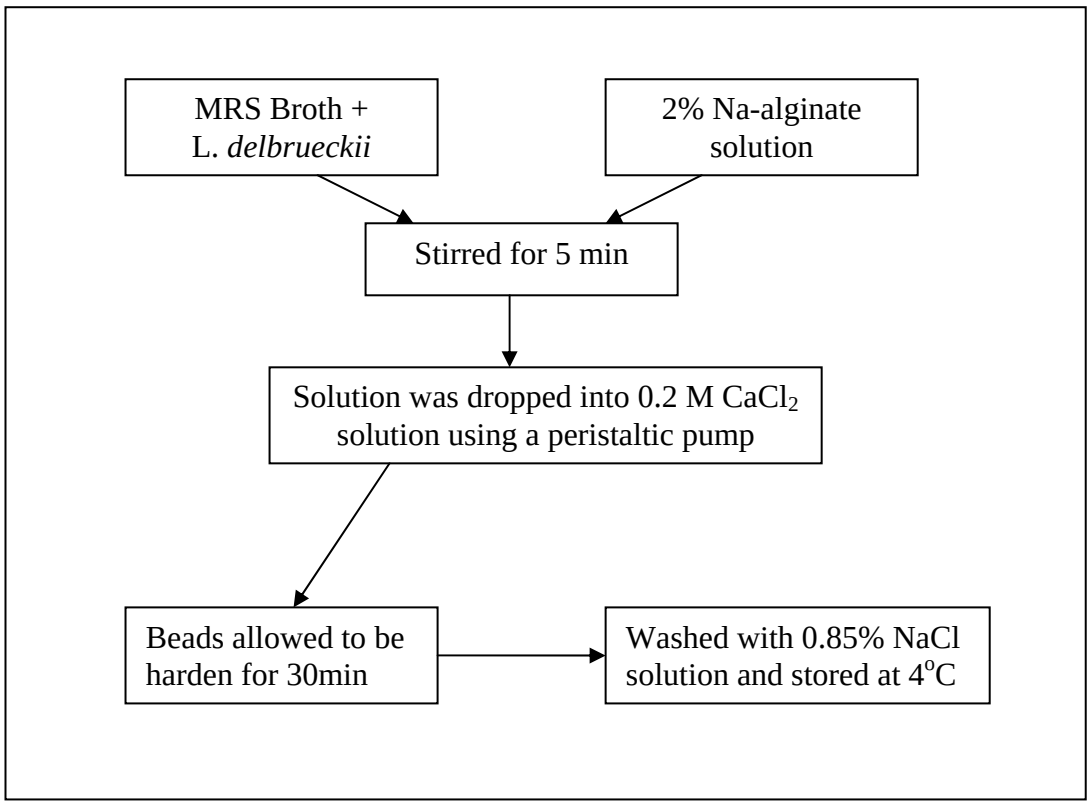

Figure 3.2 Preparation of Immobilized cell

3.3.4 Cell Immobilization (Innovative Entrapment Method)

This new and innovative entrapment method is the combination method from Long *et al.* (2003) and Szczesna-Antczak and Galas (2001). First, PVA (9% w/v) and sodium alginate (1% w/v) solution was mixed with an equal volume (1:1, v/v) of inoculums. The mixed solution was dropped gently into the solution containing 3% boric acid and 2% calcium chloride using a syringe to form beads. The forming beads were stirred for duration of 30 to 50 minutes. The beads were stored at 4 °C for 24 hours. After that, the PVA- alginate beads were stirred in sodium sulphate solution for half an hour. The innovative method could be viewed in figure 3.3.

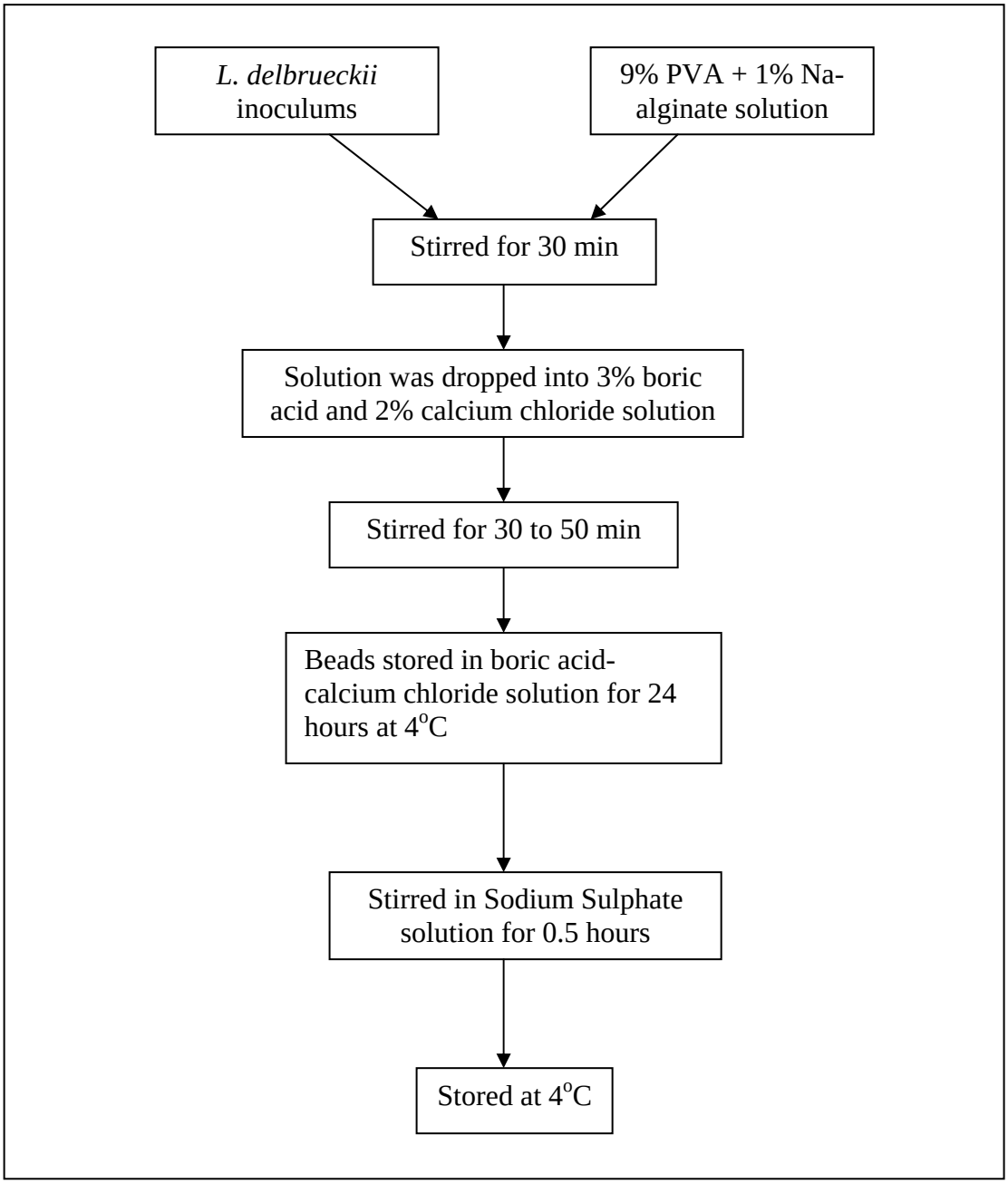

Figure 3.3 PVA-alginate beads method

3.3.5 Shake flask Fermentation

The shake flask fermentation was then incubated in the incubator shaker at 37°C, 150 rpm for 24 hours. The fermentation was performed by transferring 5g of

PVA- alginate beads to a 250ml Erlenmeyer flask containing 100ml of fermentation medium. The initial pH was adjusted to 6.5 and the flask was flushed with nitrogen gas and then sealed with stopper to create anaerobic condition. The samples were collected in the bacteria transfer chamber in order to maintain the anaerobic conditions and to prevent the contamination. The lactic acid and glucose concentration of collected samples were determined.

3.3.6 2 Liter Airlift Bioreactor Fermentation

For each experiment, 70g of Ca-alginate beads were transferred to the 2 liter airlift bioreactor (Culture Vessel M2, BBRAUN) with the complete monitoring and controlling system containing 1.4 liter fermentation medium. The temperature was maintained at 37°C and the pH was controlled at pH 6.8 during cultivation via a pH controller. The incubation was carried out for 72 hr. In order to maintain the anaerobic conditions, nitrogen gas will be supplied along the fermentation. The submerged fermentation in the airlift bioreactor is set up as shown in the figure 3.4.

Figure 3.4 Fermentation set up

3.4 Analytical Procedures

3.4.1 Liquid Pineapple Waste

3.4.1.1 Cation Contents and Anion Content

The cation contents and anion content liquid pineapple waste was analyzed according to Standard Methods for Examination of water and waste-water (American Public Health Association, 1995).

3.4.1.2 pH

An accurate and practical method for measuring pH involves the use of a pH meter. The pH meter is a potentiometer which measures the potential developed between a glass electrode and a reference electrode. To obtain accurate results the pH meter need to be calibrated before using. The calibration is normally performed using a standard pH meter with standard pH 4.00, 7.00 and 9.00 buffers. When using the pH meter, care must be taken to rinse the electrode carefully with the test solution and immersed in the solution to sufficient depth. The pH reading was taken after a minimum five minute.

3.4.1.3 Moisture Content

Moisture content measurement was carried out according to Malaysian Standard 1973. A sample of 5g is accurately weighed into a dish and dried in an air oven at 105±2°C for about 4 hours. The sample was then cooled in a desiccator and weighted. The process of drying, cooling and weighing was repeated after an hour until two consecutive weighs did not deviate by more than 1 milligram. The moisture content was calculated according to equation (3.1) below:

$$\text{Moisture content} = \left(\frac{w_1 - w_2}{w_1 - w} \right) \times 100 \qquad (3.1)$$

where:

w = weight empty dish (g)

w_1 = weight dish and sample before drying (g)

w_2 = weight dish and sample after drying (g)

3.4.1.4 Reducing Sugar

A dinitrosalicilioc acid (DNS) assay has been available since 1955 and is still useful for the quantitative determination of reduction sugar. Typically, the analysis involved a set of glucose standard ranging from 0.0 to 1.0 mg/ml (total sample volume 1ml). After that, 1.0 ml DNS reagent and 2 ml water was added to each tube (include sample tube) using pipettes. All the tubes were heated in boiling water bath for 5 minutes to allow the reaction between glucose and DNS to occur. Each volume was cooled and adjusted to 10 ml accurately with distilled water, using pipette or burette. The solution was mixed well and the absorbance of each solution was read at 540 nm. Then a standard curve could be drawn by this set of glucose standard. The concentration of sugar was determined by standard curve.

3.4.1.5 Total Sugar

Before the total sugar concentration could be measured. All non-reducing sugar (sucrose) is needed to be hydrolyzed to reducing sugars (glucose and fructose). This step could be achieved by pipetting adding 2.5 ml HCl 2M into 25.0 ml sample and boiling for 5 minutes. After the solution was cooled and neutralized with phenolphthalein containing 10% NaOH and is made up to 50ml.

3.4.2 Fermentation Product Analysis

3.4.2.1 Glucose and Lactic acid concentration

The glucose and lactic acid content were measured by Biochemistry analyzer, YSI 2000. 1.5-2.0ml of sample was filled into an appendorf tube. Then, samples were centrifuged at 5000rpm for 3 minutes. The supernatants were withdrawn using 25µl pipette and lactic acid and glucose test were performed. The 2700 SELECT allows immediate verification of formulation for intervention and reformulation, if necessary. Because the instrument is simple to use, extensive operator training is not required.

3.4.2.2 Cell Concentration

Since the cells were entrapped in Ca-alginate beads thereby beads need to squash in 10 ml of 0.3 M sodium citrate solution (adjusted to pH 5.0 with 1 M citric

acid) for 20 minute with continuous stirring at room temperature. In order to obtain better results, dilutions may be needed. The number of cell liberated from Ca-alginate beads was obtained by streaking dissolving beads on MRS agar plate and incubating them at 37^{o}C for 48 hours. When a plate count is performed, it is important that only limited number develop in the plate. When too many colonies are present, some cell are overcrowded and do not develop; these condition cause inaccuracies in the count. To ensure the accuracy, the original inoculums is diluted several times in a process called serial dilution.

CHAPTER 4

NEURAL NETWORK MODEL

A neural network used in this study is Multilayer Perceptron (MLP) that has one input layer, one hidden layer and one output layer. The input and output layer composed of one neuron each while the number of neurons in hidden layer varies for each case. There are three cases which are studied in this project. The cases are:

i. Relationship between cell number and lactic acid concentration

ii. Relationship between lactic acid concentration and glucose concentration

iii. Relationship between cell number and glucose concentration

Levenberg-Marquardt algorithm is adopted as the learning algorithm in this study for all cases. For networks that contain up to a few hundred weights, the Levenberg-Marquardt algorithm is known to have the fastest convergence and also has the ability to obtain lower mean square error than other algorithm in many cases (Demuth and Beale, 2005). Four sets of data are used for training and two sets for

validation of the model. The iteration bound is set to 2000 iterations for all cases. All data used in this study have been normalized as mentioned in chapter 3.

The number of neurons in hidden layer for each model varies and it is determined by trial and error. Trials have been done for each model by changing the number of hidden neurons in order to find the best structure. The structure featured in this report is the best structure found to represent the models.

4.1 Relationship between cell number and lactic acid concentration

In predicting the relationship between cell number and lactic acid concentration, there are three models (1a, 2a, 3a) that had been developed depending on different set of training and validation sets. Table 4.1 shows the structure of each model and the data sets used for training and validation of model.

Table 4.1 Structure and data sets utilized for model a

Model	Structure	Data set for training	Data set for validation
1a	1-8-1	27^{o}C, 37^{o}C, 40^{o}C & 50^{o}C	30^{o}C & 45^{o}C
2a	1-5-1	27^{o}C, 30^{o}C, 45^{o}C & 50^{o}C	37^{o}C & 40^{o}C
3a	1-7-1	27^{o}C, 30^{o}C, 37^{o}C & 40^{o}C	45^{o}C & 50^{o}C

The models uses log sigmoid as the transfer function for hidden layer and tan sigmoid for output layer. The mean square error (goal) was changed from the default

value of 0 to 0.01. This is to improve the generalization of the models built. The number of neurons in hidden layer which had been determined through trial and error differs for each model. Residual plot consists of error versus sample point where the error was calculated by subtracting simulated value with targeted (experimental) value. Generally, when comparing residual plots between all three models for training set, it can be concluded that it is unstructured for all plots. The error seems to be randomly scattered and range between ($-0.3 \leq$ error ≤ 0.3). Figure 4.1, figure 4.2 and figure 4.3 shows the residual plots for all three models built respectively.

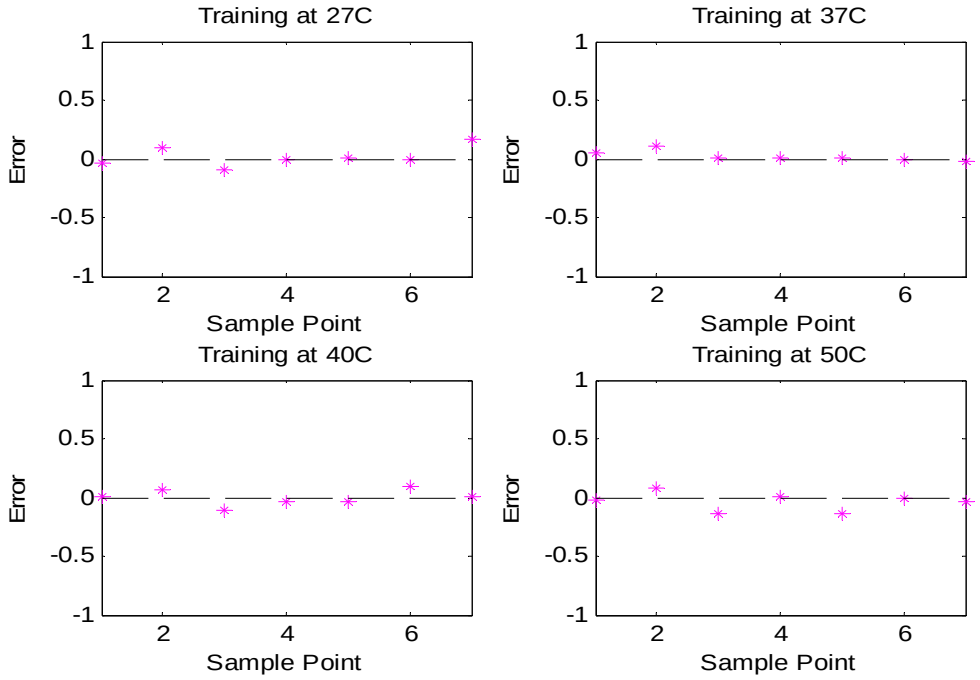

Figure 4.1 Residual plot for training sets model 1a

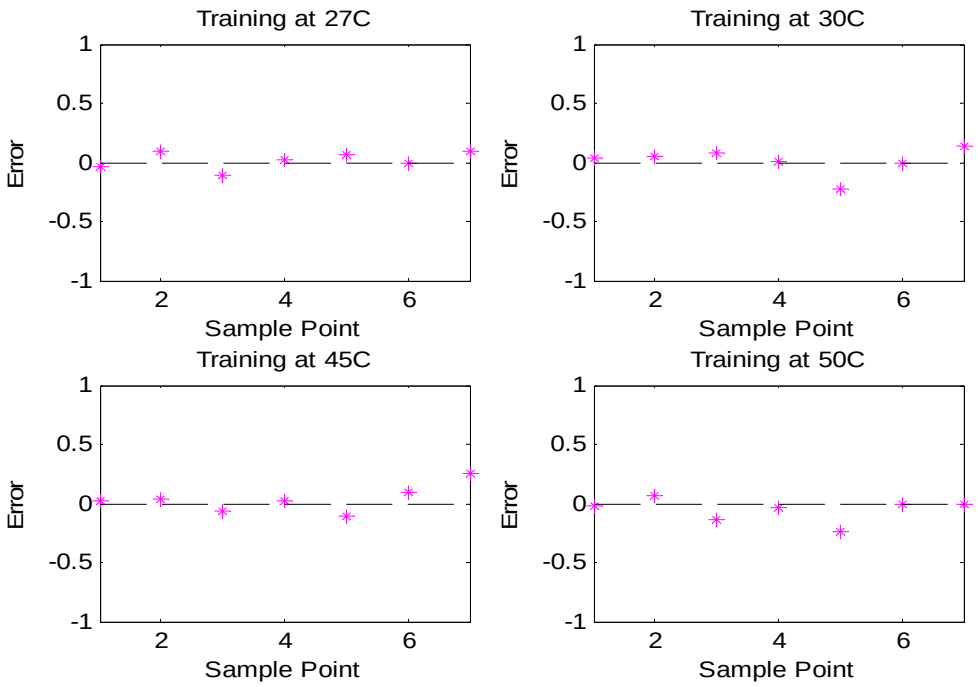

Figure 4.2 Residual plot for training sets model 2a

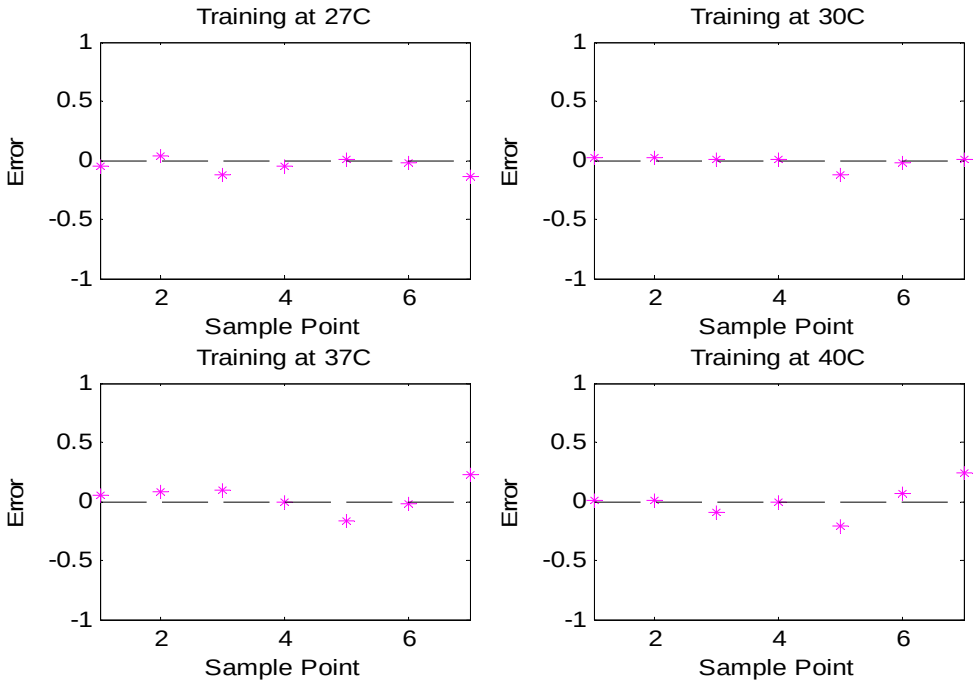

Figure 4.3 Residual plot for training sets model 3a

Validations of the models were done using two sets of data. Figure 4.4, figure 4.5 and figure 4.6 shows the residual plots for the test sets of each model. From these residual plots, the models can be assessed to see its generalization ability. The best model among the three models built is model 1a since it has the smallest range of error and this indicates the ability of the model to generalize well. The ability of model 1a to predict the output with less error compared to other models might be due to the sets of data used for training which covers the whole range of data in this process. Besides that, figure 4.5 and figure 4.6 also shows that certain sample points is predicted with large deviation from the actual value. This factor had caused the models to be considered unable to generalize well despite its good performance for predicting the output for training sets.

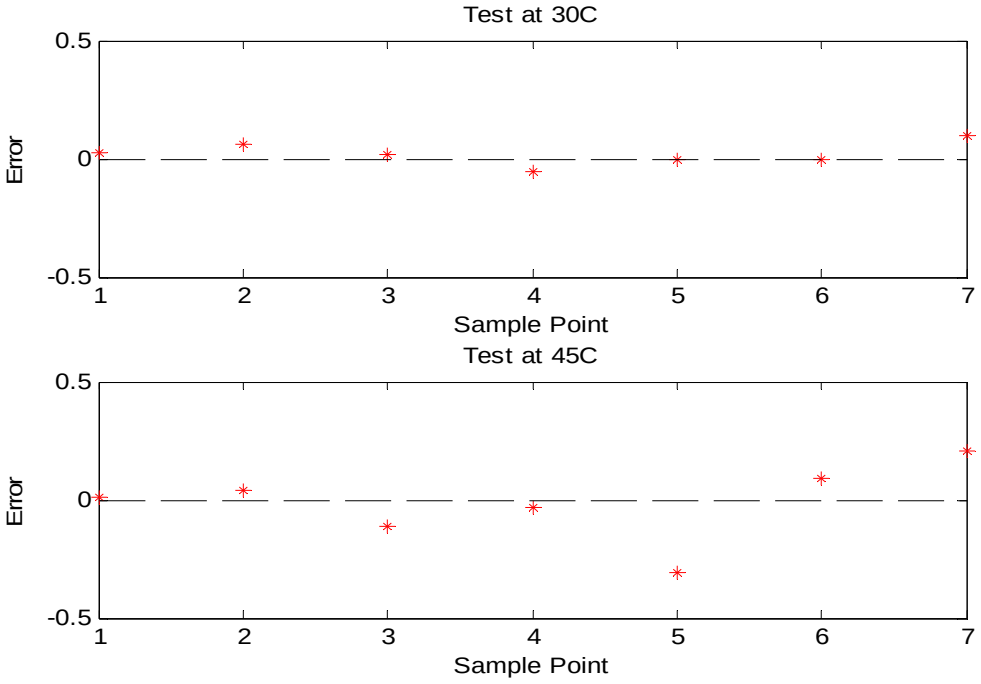

Figure 4.4 Residual plot for test sets model 1a

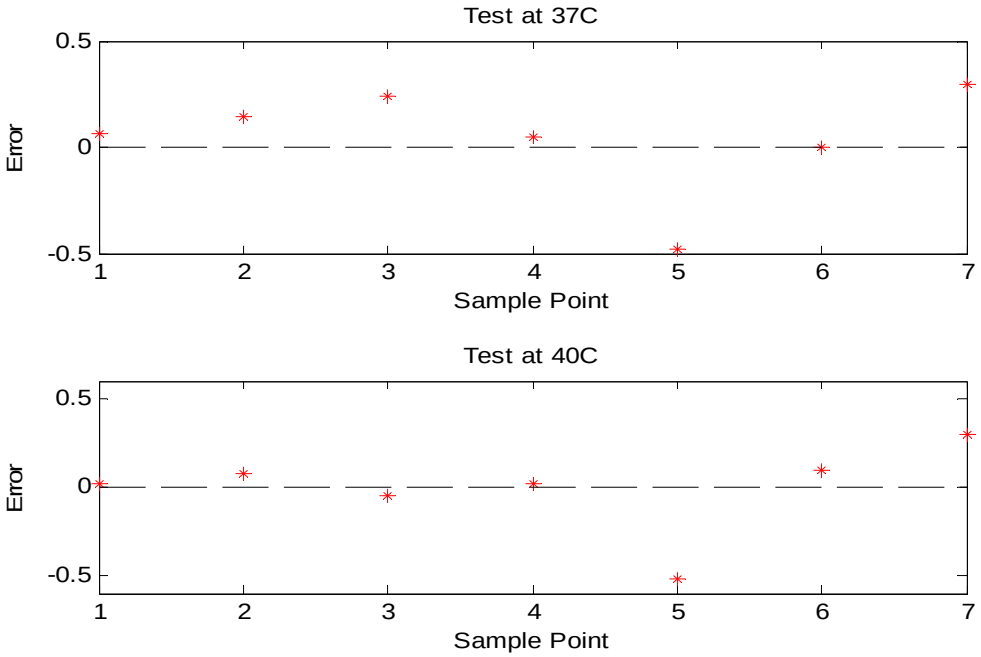

Figure 4.5 Residual plot for test sets model 2a

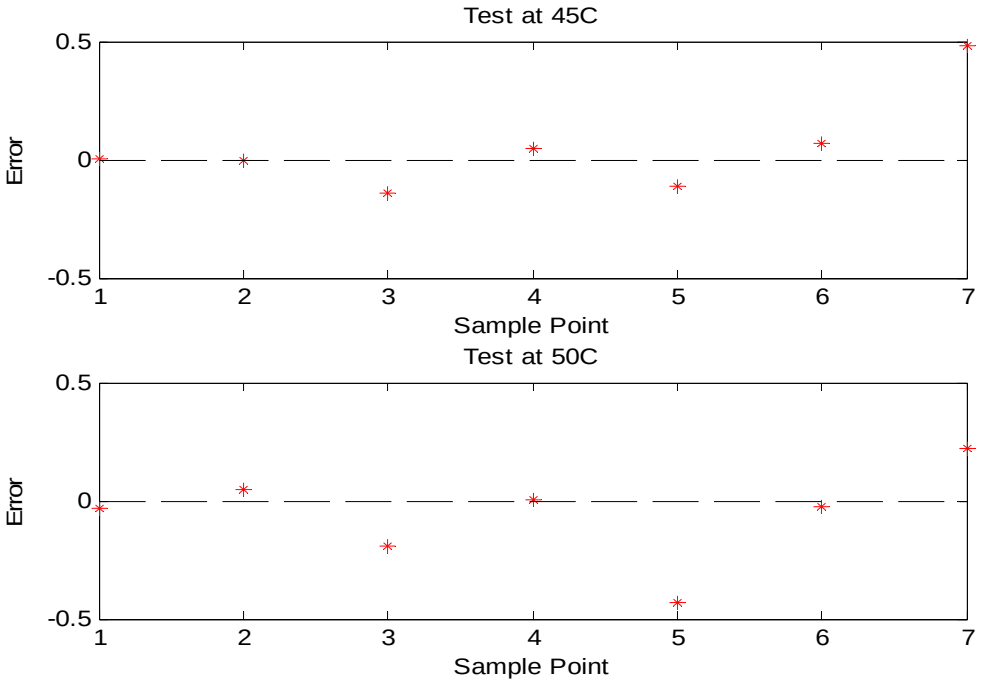

Figure 4.6 Residual plot for test sets model 3a

For a better view of comparison between the simulated and experimental (actual) result, the output in this case which is the cell number had been plotted against time for both actual value and simulated value. A good model should produce a plot with both simulated and experimental value located at the same spot. Figure 4.3 indicates the ability of model 1a to simulate the cell number with minimum deviation compared to model 2a and 3a.

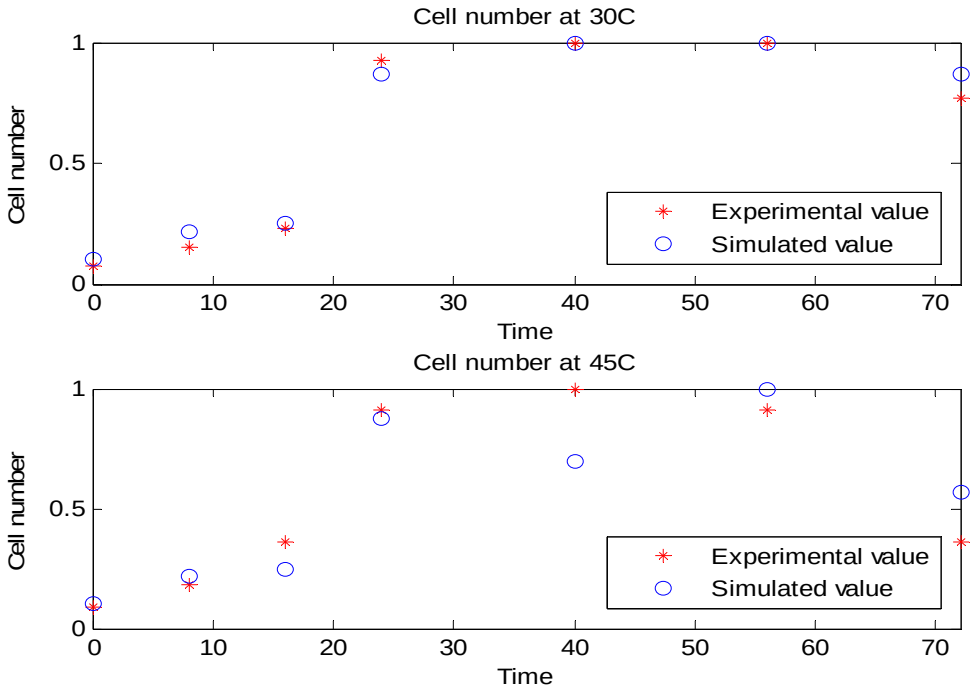

Figure 4.7 Graph cell number versus time for test set model 1a

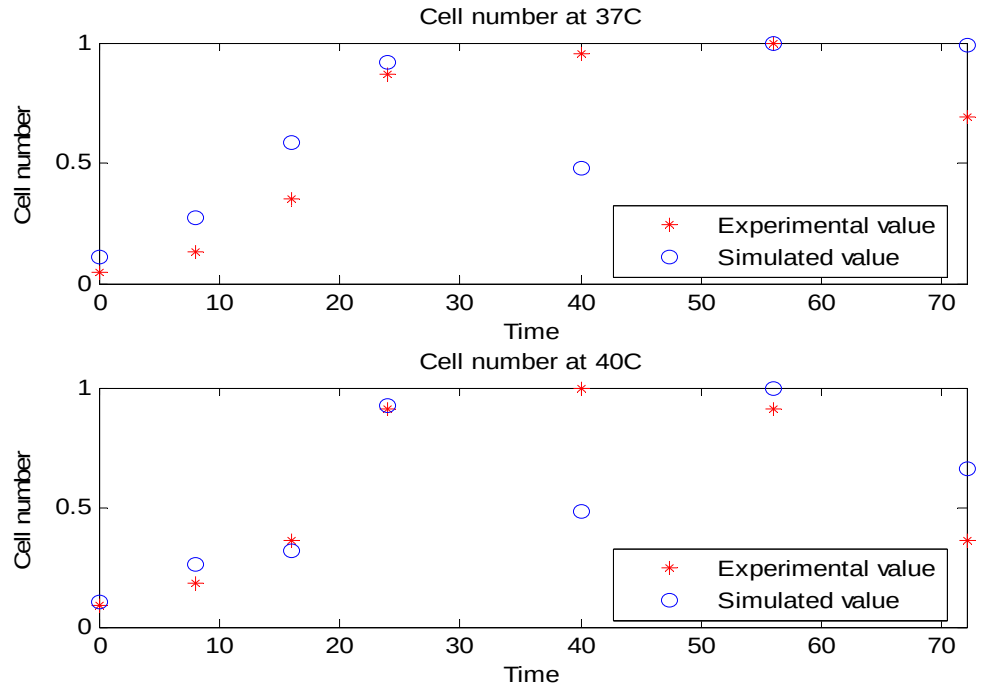

Figure 4.8 Graph cell number versus time for test set model 2a

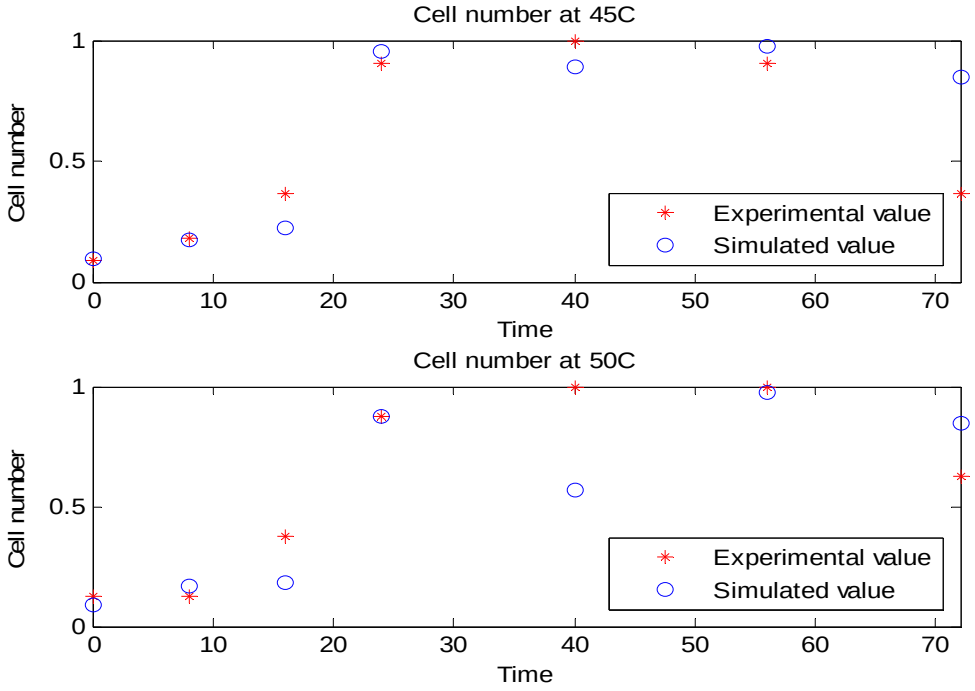

Figure 4.9 Graph cell number versus time for test set model 3a

4.2 Relationship between lactic acid concentration and glucose concentration

As in the previous case, the prediction of lactic acid concentration was also done in three models. Each model uses different data set for training and model validation. The sets of data used are shown in Table 4.2.

Table 4.2 Structure and data sets utilized for model b

Model	Structure	Data set for training	Data set for validation
1b	1-6-1	27^{o}C, 30^{o}C, 37^{o}C & 50^{o}C	40^{o}C & 45^{o}C
2b	1-7-1	27^{o}C, 37^{o}C, 40^{o}C & 50^{o}C	30^{o}C & 45^{o}C
3b	1-6-1	37^{o}C, 40^{o}C, 45^{o}C & 50^{o}C	27^{o}C & 30^{o}C

The transfer function used for hidden layer is tan sigmoid and for output layer is log sigmoid. In this study, it is found that the choice of transfer function affects the performance of the models built. Pure linear transfer function cannot be utilized in output layer of these models because the range of output produced is within -1 and 1. Whenever the output is a negative value, the error is very large and unacceptable. Therefore, the transfer functions suitable for use are only sigmoid function as it produces output within the range of zero and one. For these models, the mean square error (mse) was set to 0.01. The default value is zero. Based on this study, as the mean square error is set to larger values, the generalization seems to improve. Using the default value, the prediction is good for training sets but performs badly during validation process.

Figure 4.10, figure 4.11 and figure 4.12 shows the residual plots for training sets of all three models (1b,2b and 3b) respectively. The error produced for all three models is within the range of -0.5 and 0.5. For model 1b, the error for training set at 50^{o}C seems

to be scattered in a pattern and not randomly scattered as it should. Meanwhile, for model 2b, the error for training set 27^{o}C and 50^{o}C also showed some pattern. For model 3b, the error for 37^{o}C, 45^{o}C and 50^{o}C are not randomly scattered. This indicates that the model produces bias error which is not good because the model's simulation will tend to be influenced by the patterned error. This is proved through figure 4.13, figure 4.14 and figure 4.15 which show the residual plot for test sets of 1b, 2b and 3b respectively. The error for model 1b are scattered randomly while for model 3b, the error followed the same pattern as the residual plot for training sets. This indicates that the model is bias and tends to simulate and produce the same pattern of error.

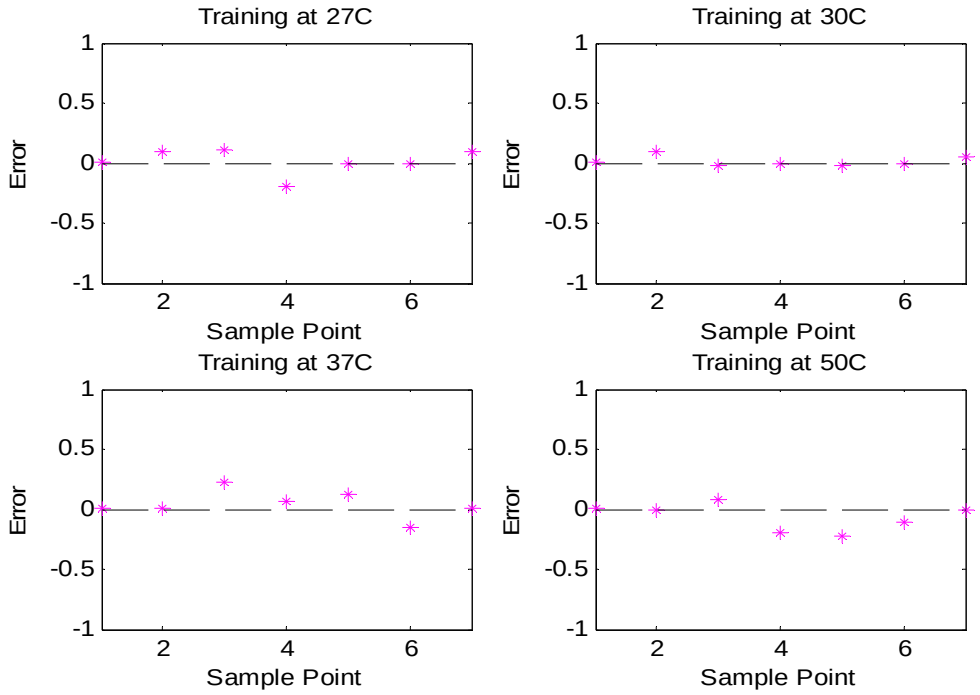

Figure 4.10 Residual plot for training sets model 1b

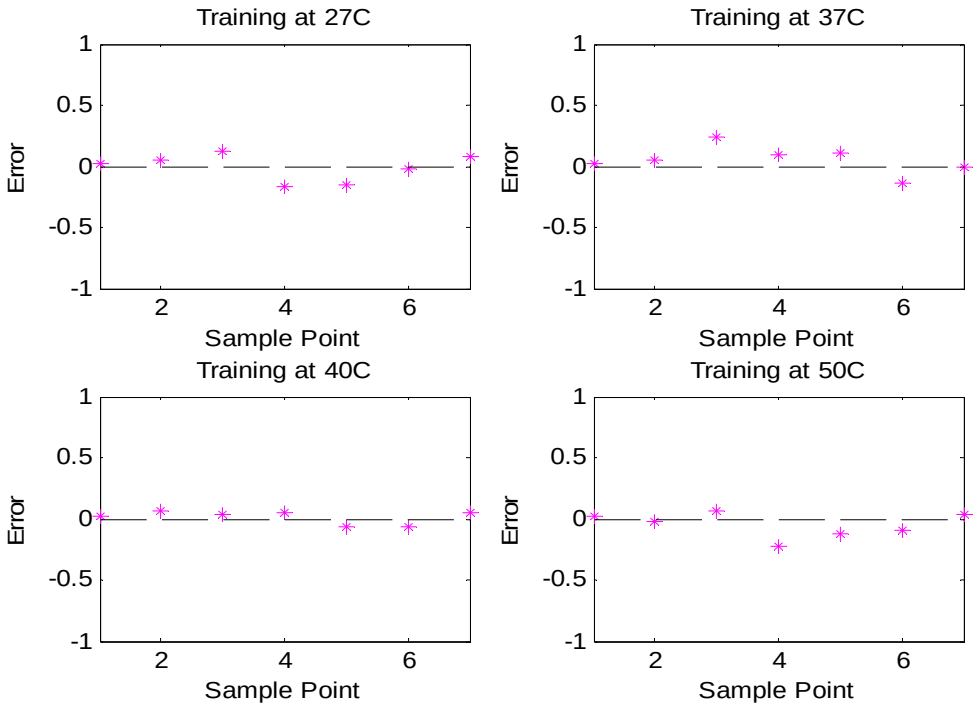

Figure 4.11 Residual plot for training sets model 2b

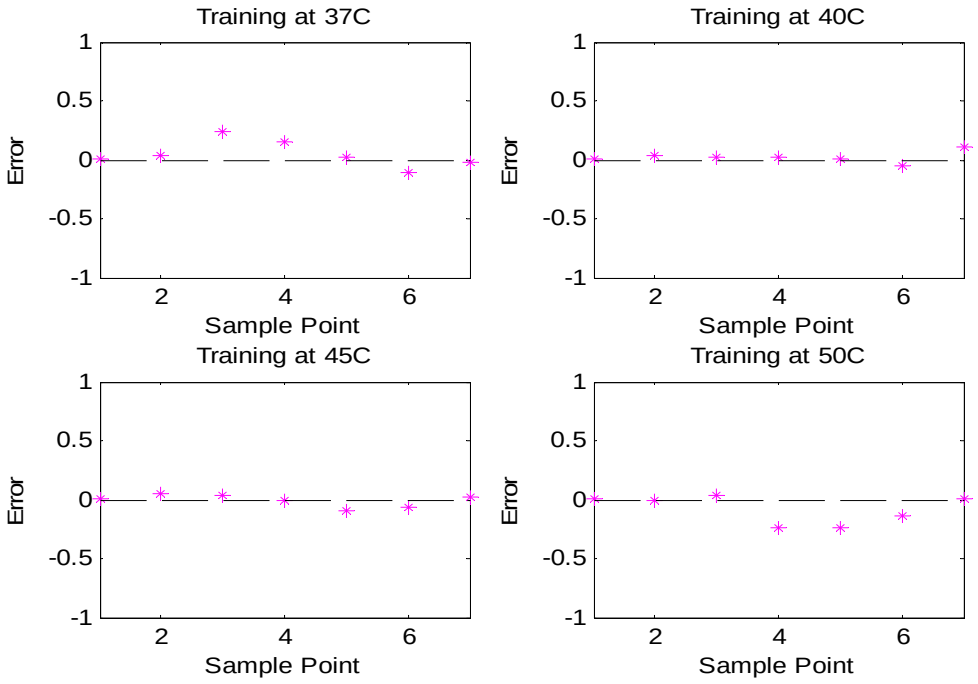

Figure 4.12 Residual plot for training sets model 3b

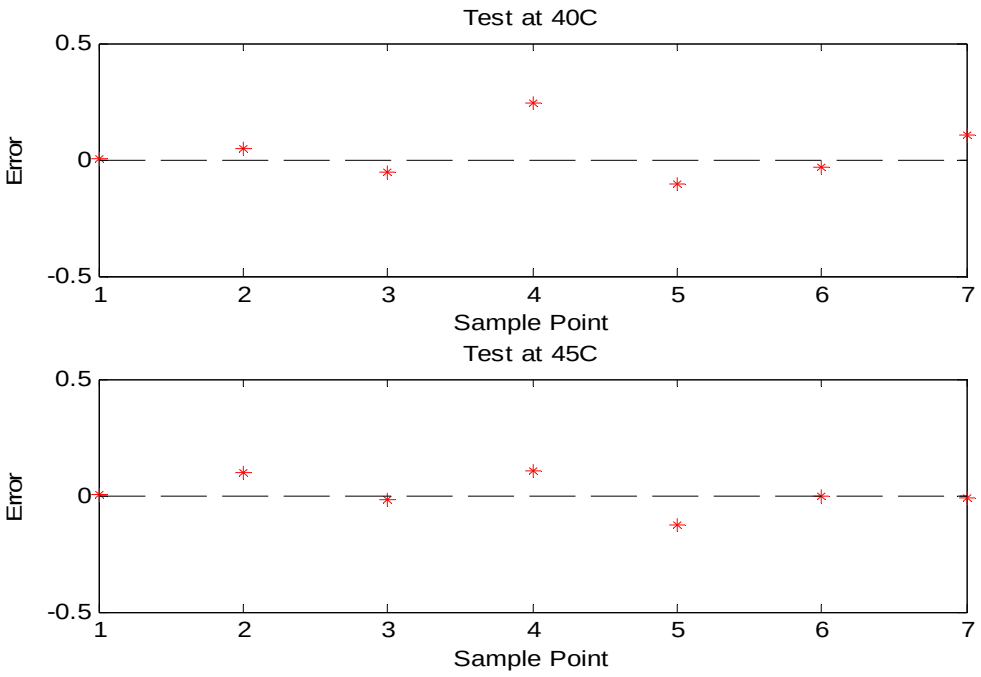

Figure 4.13 Residual plot for test sets model 1b

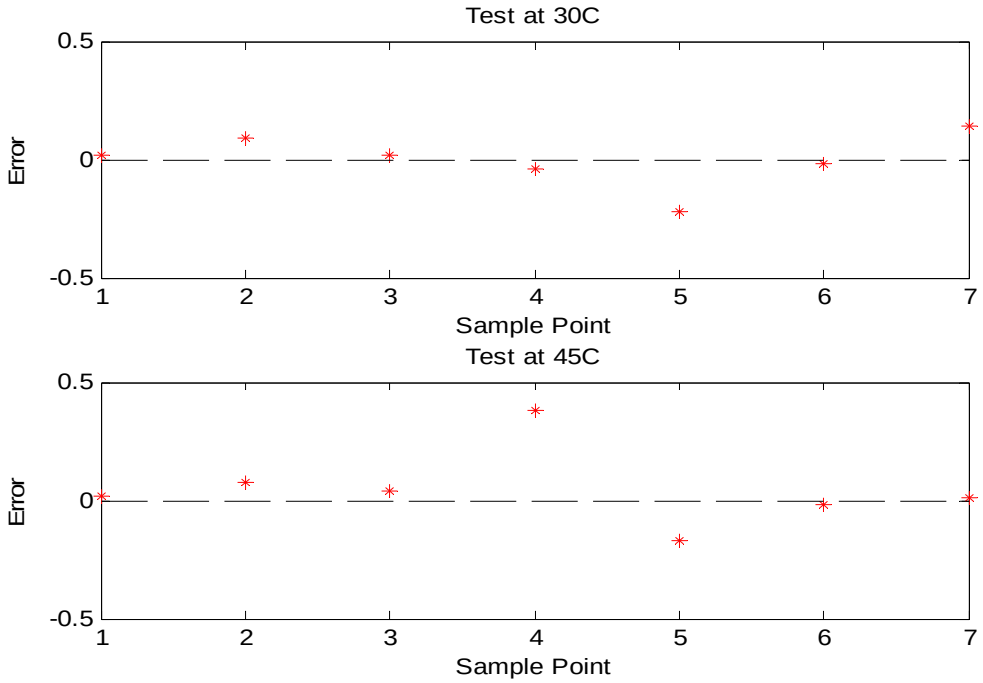

Figure 4.14 Residual plot for test sets model 2b

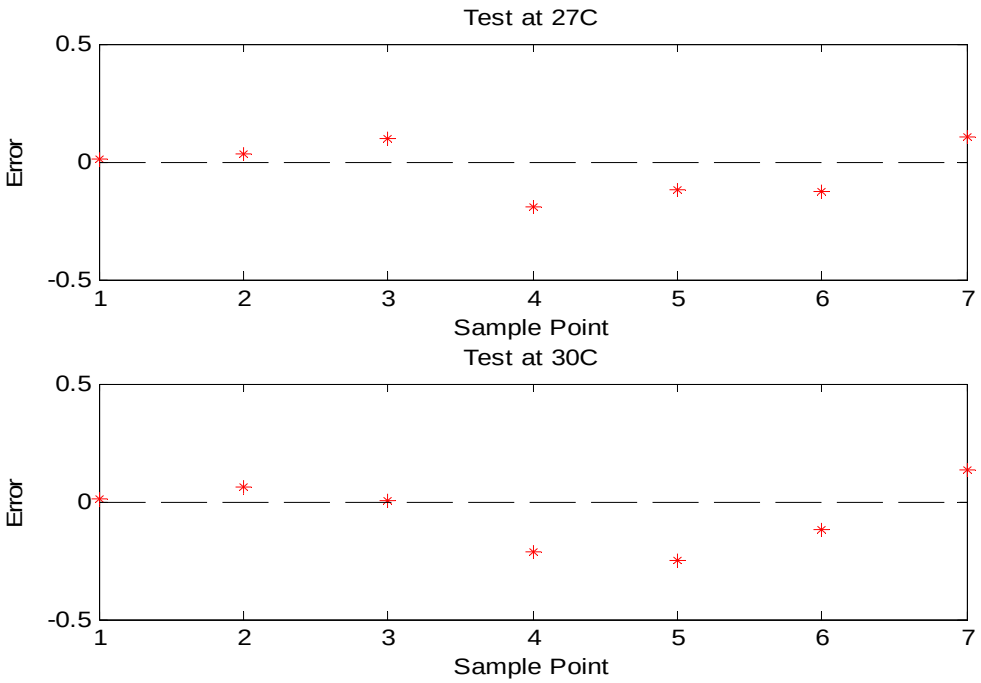

Figure 4.15 Residual plot for test sets model 3b

Figure 4.16 have shown the ability of model 1b to predict the lactic acid concentration with less error compared to other model. This might be due to the data sets used for training model 1b is sufficient to cover the data range of the lactic acid production process. Figure 4.17 and figure 4.18 indicate the comparison between simulated value and experimental value for model 2b and 3b respectively. Among three models developed, model 1b is chosen as the best model to represent the relationship between lactic acid concentration and glucose concentration.

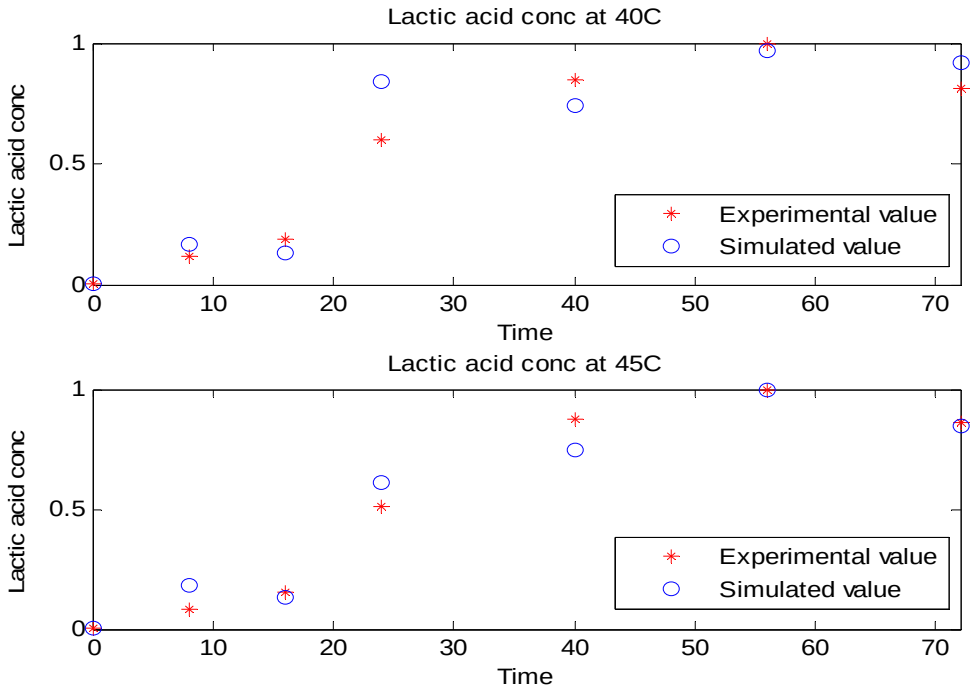

Figure 4.16 Graph of lactic acid concentration versus time for test set model 1b

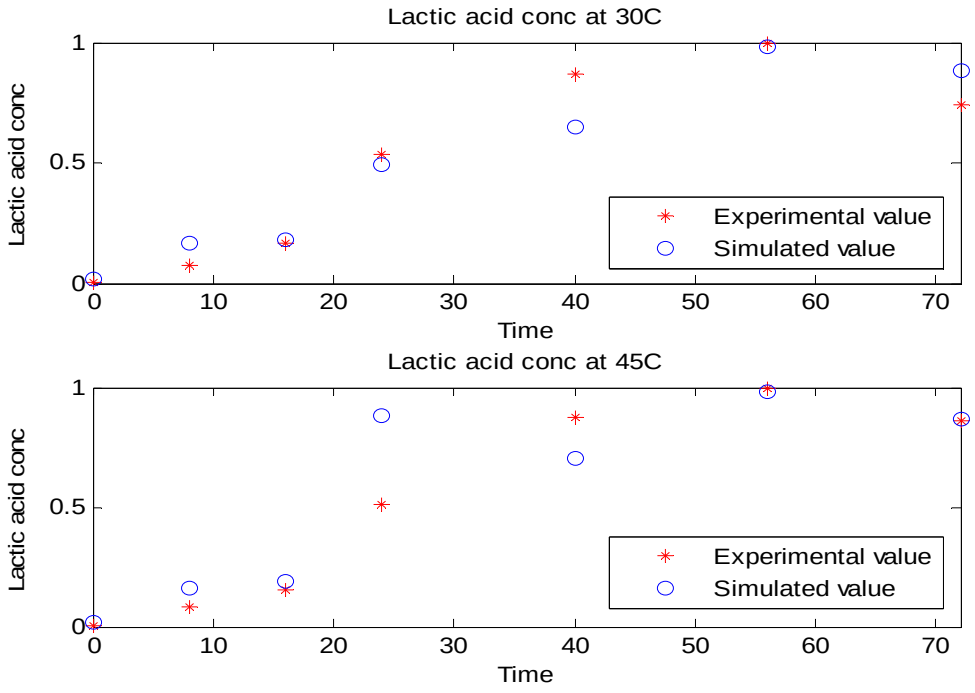

Figure 4.17 Graph of lactic acid concentration versus time for test set model 2b

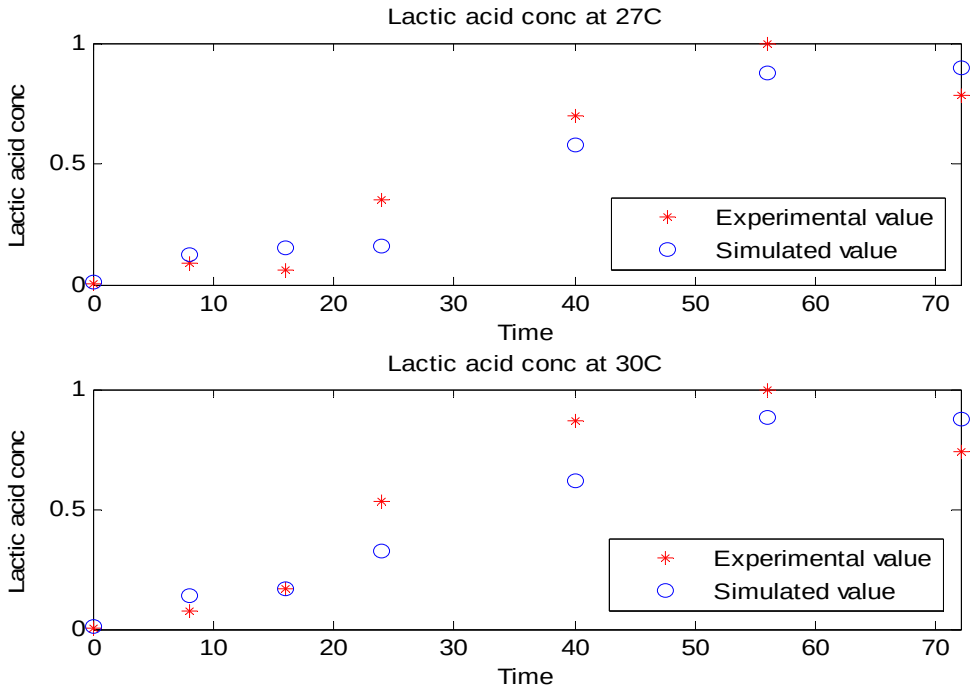

Figure 4.18 Graph of lactic acid concentration versus time for test set model 3b

4.3 Relationship between cell number and glucose concentration

The prediction of cell number from glucose concentration data was also done through three models in this study. Each model utilizes different sets of data for training and validation of model. The data sets used the structure for each model was shown in Table 4.3.

Table 4.3 Structure and data sets utilized for model c

Model	Structure	Data set for training	Data set for validation
1c	1-10-1	27°C, 37°C, 40°C & 50°C	30°C & 45°C
2c	1-2-1	27°C, 30°C, 37°C & 40°C	45°C & 50°C
3c	1-6-1	27°C, 30°C, 37°C & 50°C	40°C & 45°C

In order to predict the relationship between cell number and glucose concentration, three models were built as shown in Table 4.3. The mean square error was set to 0.015 for model 1c and 0.05 for both models 2c and 3c. For model 1c, the mean square error was set smaller because it tends to produce large errors when the mean square error was set to 0.05. The transfer function used for hidden layer is log sigmoid and for output layer is tan sigmoid. The reason why transfer function pure linear was not implemented because the output of the transfer function could be negative. A negative output will cause the error to large and unacceptable. Among the three cases that have been studied in this project, this case is the hardest to obtain a good and useable model. Based on the residual plots for training set (figure 4.20 and figure 4.21), model 2c and 3c exhibit a significant pattern in their residual plots. These clearly indicate that the models produce bias error when simulating. This factor had proved to influence the ability to simulate where when validation of model is done, the residual plot for the test sets exhibit similar behavior as the residual plots for training sets. This is shown through figure 4.23 and figure 4.24.

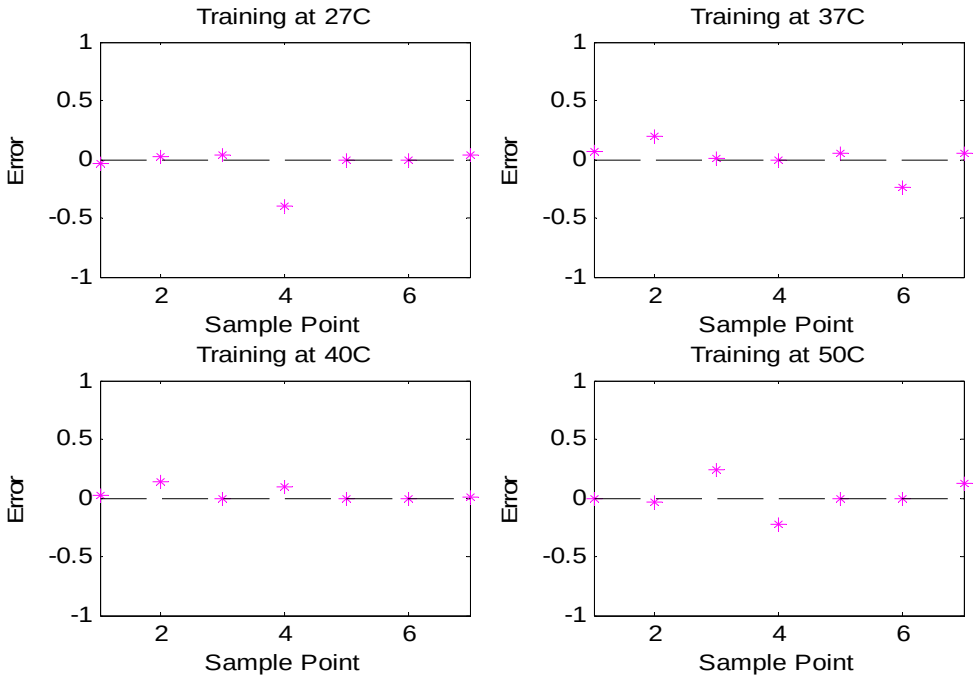

Figure 4.19 Residual plots for training set model 1c

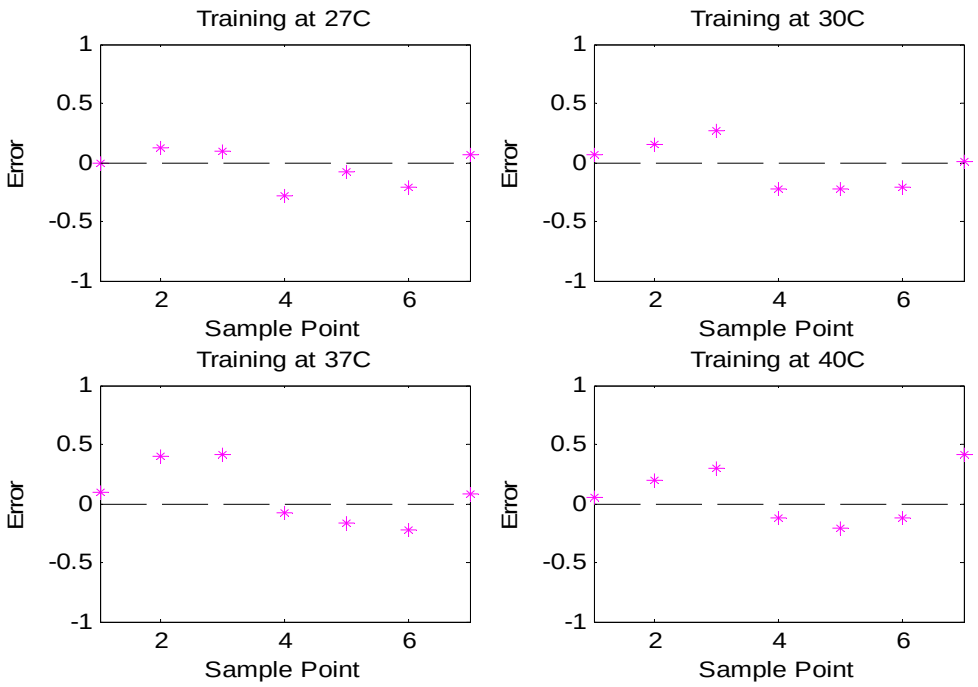

Figure 4.20 Residual plots for training set model 2c

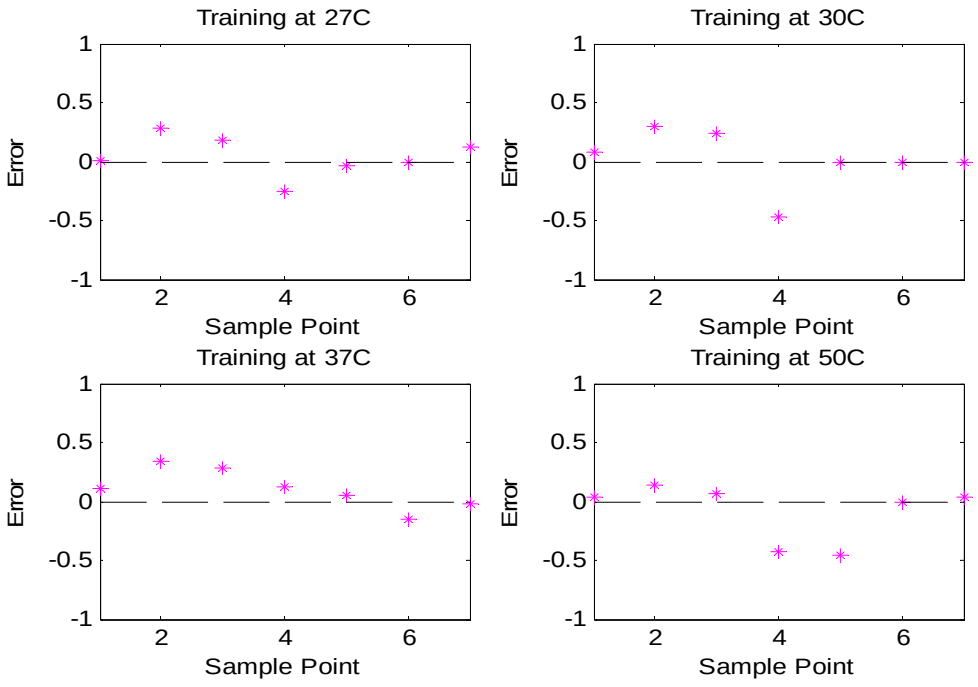

Figure 4.21 Residual plots for training set model 3c

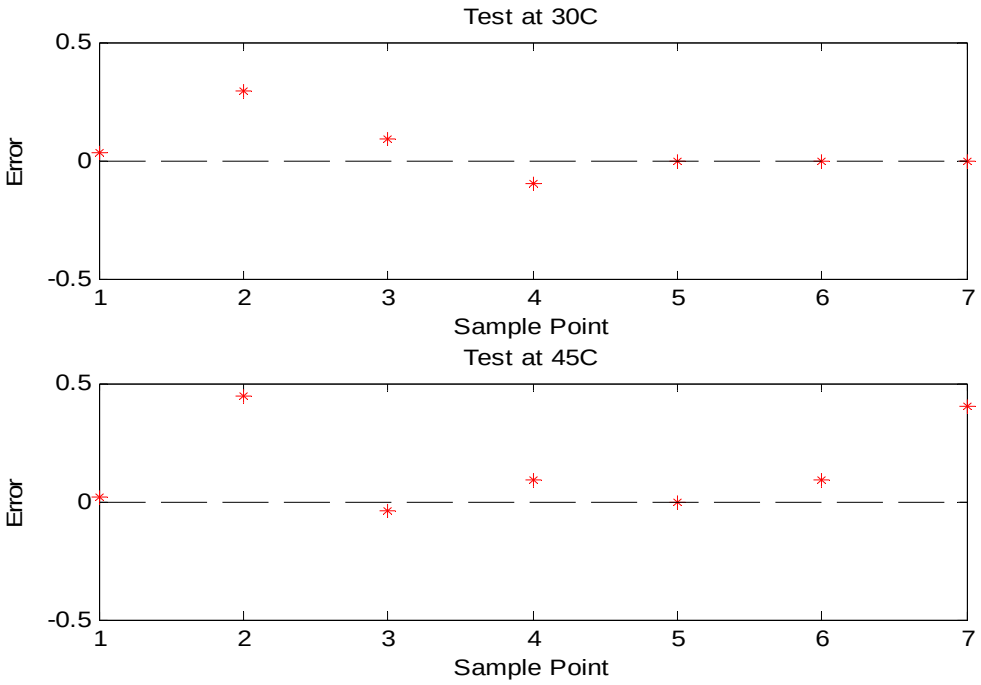

Figure 4.22 Residual plots for test sets model 1c

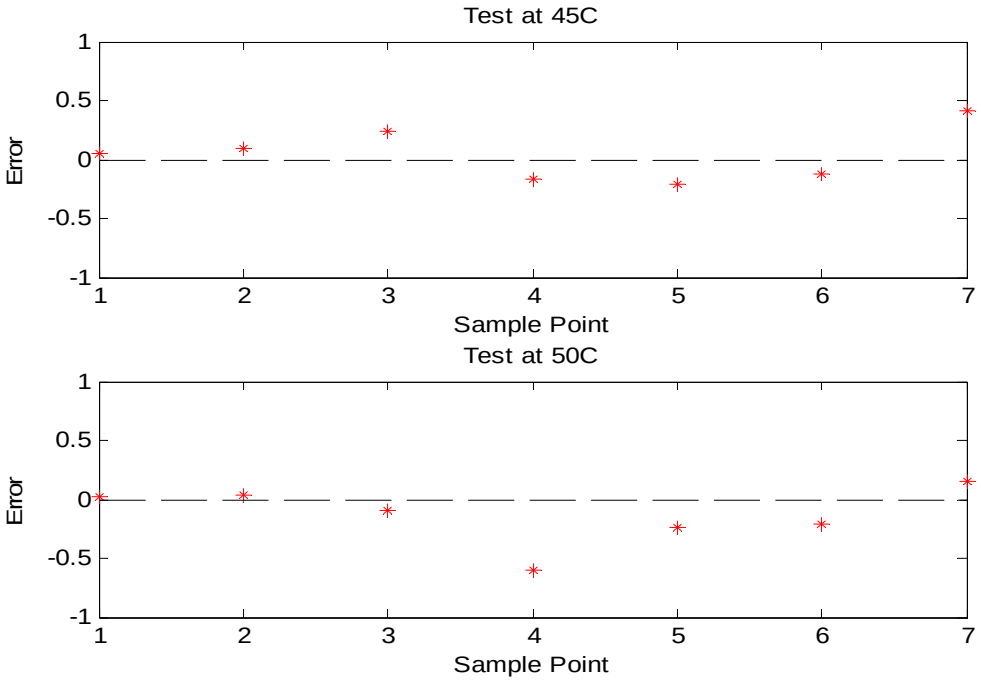

Figure 4.23 Residual plots for test sets model 2c

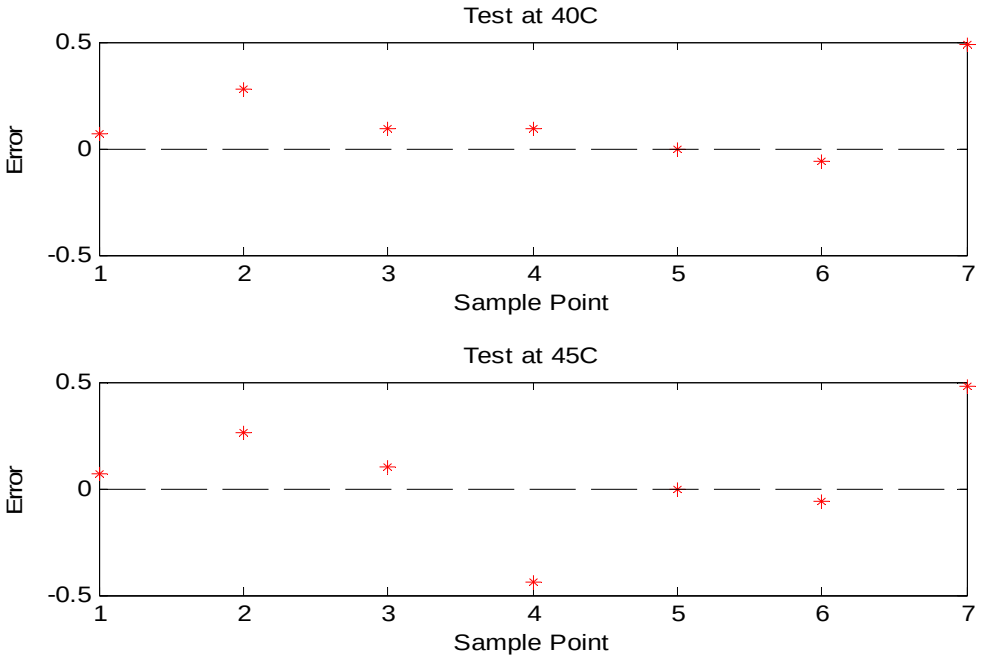

Figure 4.24 Residual plots for test sets model 3c

Figure 4.25, figure 4.26 and figure 4.27 shows the experimental and simulated value of cell concentration plotted against time to observe the ability of the models built to predict the cell number. By comparing the result from all three models built, it is concluded that model 1c is the best model among those three to predict cell number from glucose concentration. Except for the second data point for both set at 30^{o}C and 45^{o}C, all data have been predicted with high accuracy. The second data point turns to be predicted with large deviation might be due to the data which is not within the trained data. Model 2c and 3c clearly exhibit inaccuracy when simulating the error where the deviation is quite large. For model 2c, there is no data point which is predicted accurately meanwhile for model 3c, there is only one data for each test set is predicted accurately.

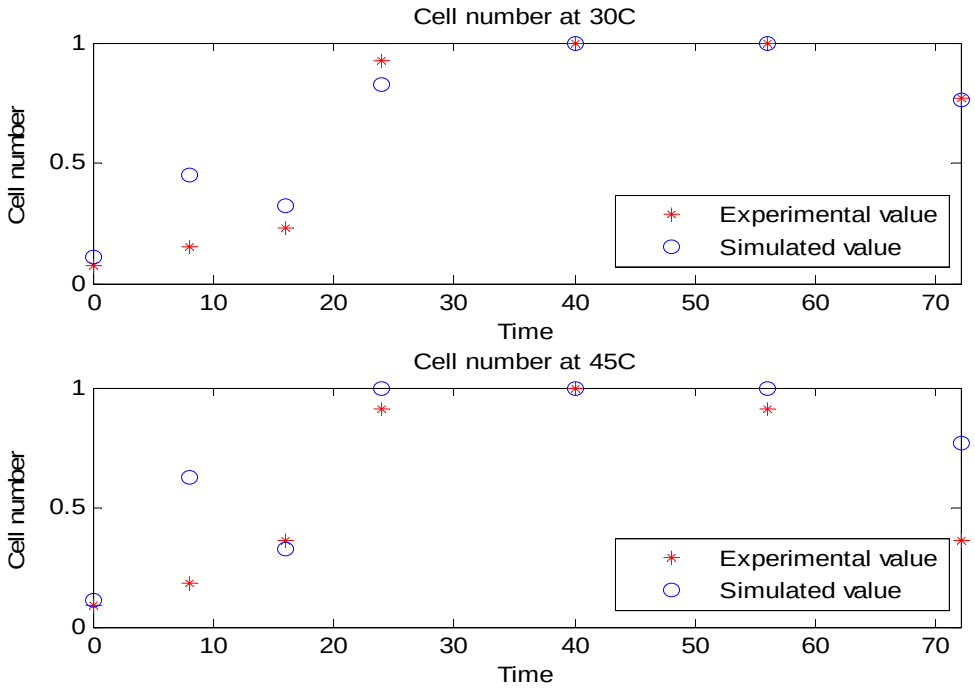

Figure 4.25 Graph cell number versus time for test set model 1c

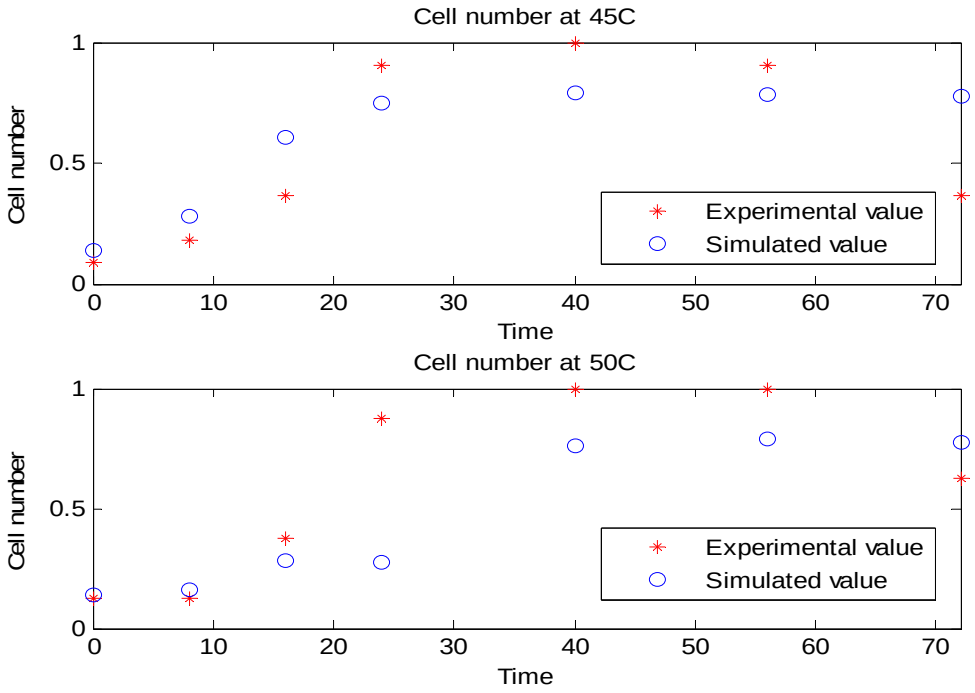

Figure 4.26 Graph cell number versus time for test set model 2c

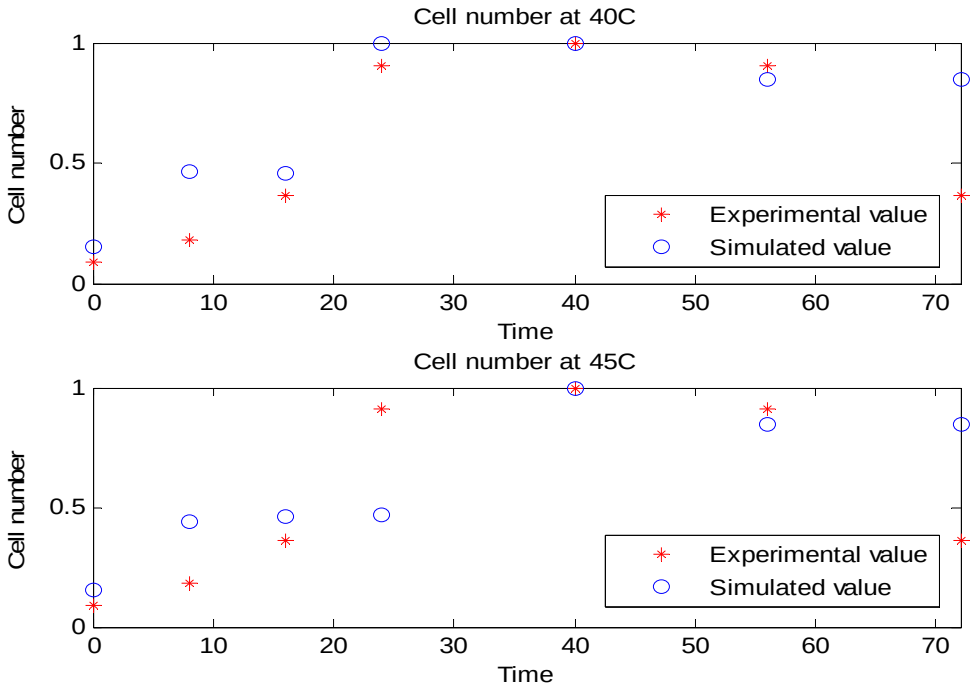

Figure 4.27 Graph cell number versus time for test set model 3c

CHAPTER 5

PARAMETRIC STUDY OF LACTIC ACID FERMENTATION

Based on the two level full factorial design experiments performed in the previous Chapter, it can conclusively said that temperature, initial pH, Na-alginate concentration and bead diameter are significant factors that will effect lactic acid production using immobilized cells. Thus in this Chapter, these factors were analyzed in detail.

5.1 Fermentation Conditions

The submerged fermentations were carried out in 250 ml Erlenmeyer flasks containing 100 ml of pineapple waste with 31.3 g/L of glucose concentration. Flushing the flasks to Nitrogen and sealing them with tight fitting rubber stoppers maintained anaerobic conditions. The fermentation flasks were placed in a controlled incubator shaker with an agitation rate of 150 rpm.

5.1.1 Effect of Temperature

The effect of temperature, fermentations were carried out at various temperatures of 27^{o}C, 30^{o}C, 37^{o}C, 40^{o}C, 45^{o}C and 50^{o}C for 72 hours. Initial pH of

the fermentation medium was 6.5, 2% w/v of Na-alginate and 5.0g beads with 1.0 mm bead diameter.

5.1.2 Effect of initial pH

The effect of initial pH was studied by conducting fermentation at various initial pH of 4.5, 5.5, 6.5, 7.5 and 8.5 with 0.2 M sodium hydroxide. These flasks were incubated at 37^{o}C, 5 g bead with 1.0mm bead diameter and 2.0 % w/v of Na-alginate concentration. Samples of the fermentation, which were intimately taken every 4 to 8 hours, are centrifuge to separate the biomass. The supernatant collected was sampled for lactic acid and residual sugar.

5.1.3 Effect of Na-alginate Concentration

The effect of Na-alginate concentration was investigated by conducting submerged fermentation at various Na-alginate concentrations of 1.0%, 2.0%, 4.0%, 6.0% and 8.0% for 72 hours. Initial pH of fermentation medium was 6.5, 5.0g bead with 1.0mm diameter size and incubated at 37^{o}C. Samples were collected daily to determined culture growth, lactic acid production and glucose consumption.

5.1.4 Effect of Bead Diameter

The effect of bead diameter on lactic acid production was determined using 1.0mm, 3.0mm and 5.0mm under static condition of fermentation at 37^{o}C, pH 6.5, 2.0% w/v of Na-alginate concentration, and 5.0g beads. The fermentation was conducted under static conditions for 72 hours. Samples were collected daily and analyzed for lactic acid concentration, glucose consumption and cell concentration.

5.2 Results

5.2.1 Effect of initial pH

Effects of initial pH were conducted in 250 ml Erlenmeyer flask with working volume of 100 ml at 37^{o}C using liquid pineapple waste containing 31.3 g/L of glucose concentration. The initial pH of the fermentation medium was controlled using 2.0M sodium hydroxide as pH control agent. The effect of initial pH was studied at five different initial pH values of 4.5, 5.5, 6.5, 7.5 and 8.5. The results of bacterial growth, glucose utilization and lactic acid production are shown in Figure 5.1-5.3.

The effect of initial pH on the cell growth of the immobilized *Lactobacillus delbrueckii* during the batch fermentation of liquid pineapple waste is illustrated in Figure 5.1. The observed lag period for initial pH 6.5 was only 8 hours, shorter compared to the other initial pH. The exponential growth rate at initial pH 6.5 is the fastest compared to the other initial pH values (showed by the steep gradient). The maximum concentration of cell or cell number was 7.3 x 10^6 cfu/ml at initial pH 6.5. At starting initial pH of 4.5 and 8.5, the bacteria exhibited a prolonged lag phase and bacteria did not grow as well as at higher initial pH value. As the initial pH is increased above 4.5, the cell growth is increased however until up to a certain limit. Beyond initial pH 6.5, its growth rate is decreased. Therefore, the optimal initial pH growth for the liquid pineapple waste fermentation using immobilized *Lactobacillus delbrueckii* was 6.5, which is similar to those reported by Goksungur and Guvenc (1987) by using beet molasses as a substrate.

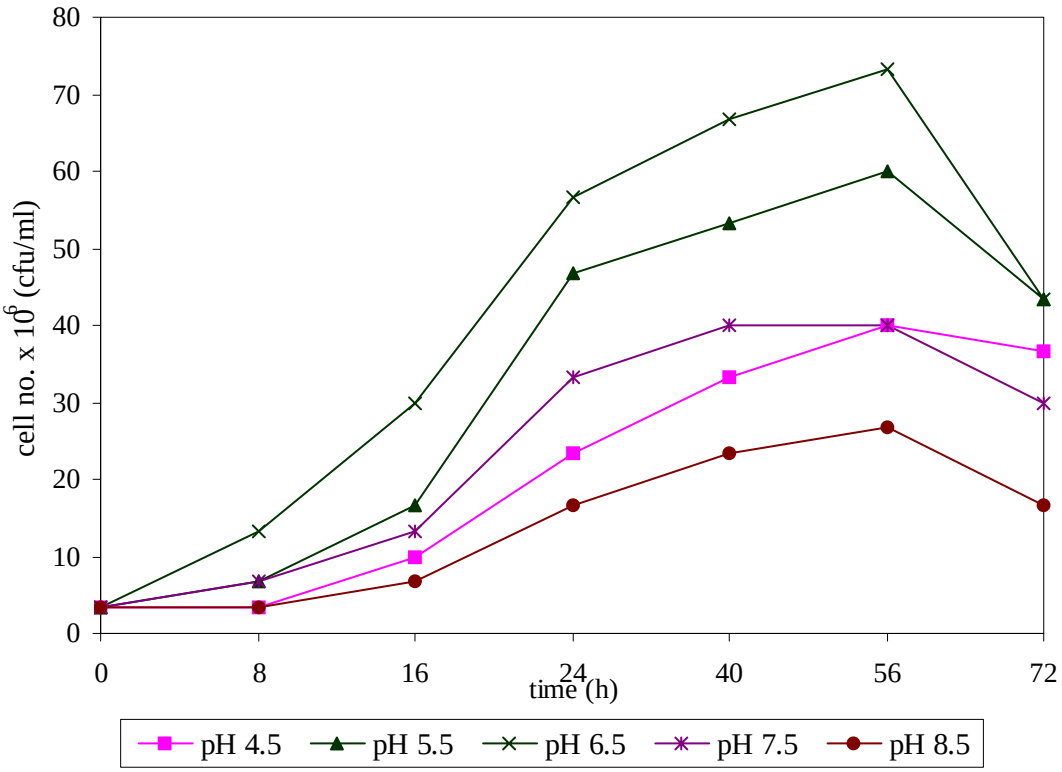

Figure 5.1: Effect of initial pH on cell concentration by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C. bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

Figure 5.2 shows the consumption pattern of the glucose during the fermentation process at five different initial pH. Initial concentration of glucose is 31.3 g/L respectively for all samples. For initial pH 6.5, there were 31.3 g/L and 0.35 g/L glucose at initial and after 72 hours of fermentation respectively. We found that as the initial pH would approach 8.5 there was little glucose consumption and therefore less lactic acid produced. It is possible that the higher initial pH brought too much stress on the organism metabolic abilities (Goksungur and Guvenc, 1999). The results show that at initial pH 6.5, cell started to utilize glucose earlier than others initial pH. Thus, initial environment of initial pH 6.5, encouraged the *Lactobacillus delbrueckii* to consume glucose rapidly contributing to the high cell concentration. When glucose concentration reduced rapidly, lactic acid achieved maximum level within that time as can be observed in Figure 5.3.

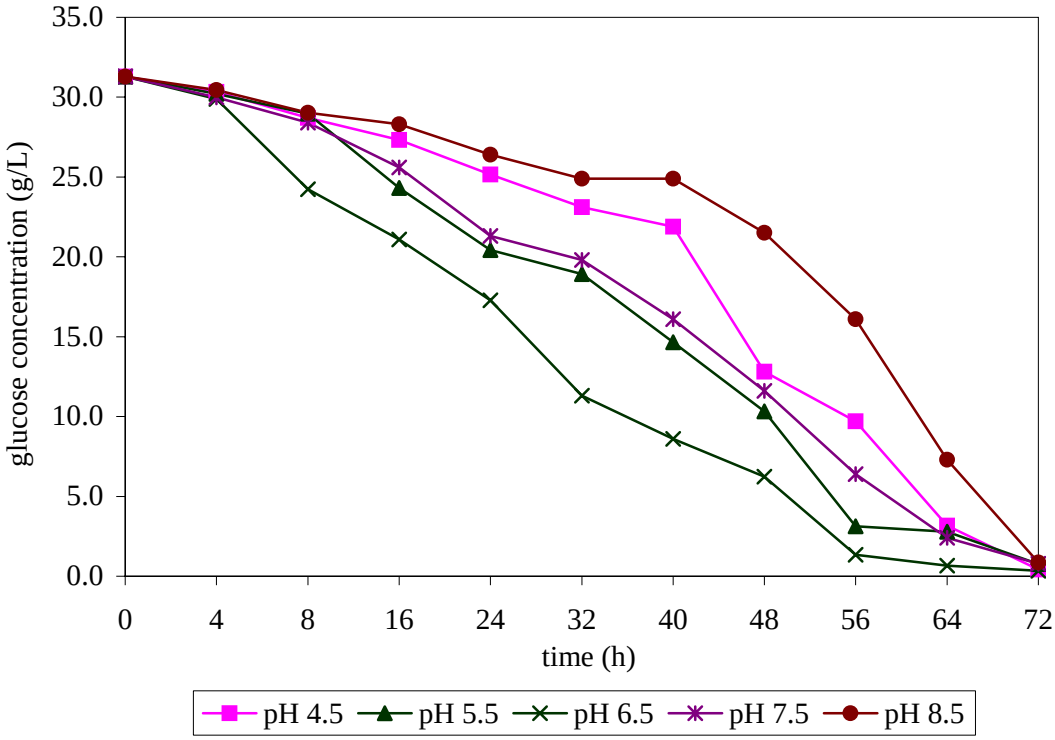

Figure 5.2: Effect of initial pH on glucose consumption by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C. bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

A similar trend is also observed for the production of lactic acid. Maximum lactic acid concentration is attained at initial pH 6.5 with a yield of 29.02 g/L and 92.7% as observed from Figure 5.3. Further increase in initial pH beyond 6.5 does not improve the lactic acid production. At initial pH 8.5, the lactic acid yield is the lowest at 20.31 g/L. The bacteria, *Lactobacillus delbrueckii* seems to grow well in a neutral environment with an initial pH in the region of 5.5 to 7.5, but best at initial pH 6.5. An environment, which is too acidic and alkaline, is not conducive for lactic acid production. These results seem to be in agreement those obtained by Goksungur and Guvenc (1997) where optimum initial pH of 6.5 is obtained using beet molasses.

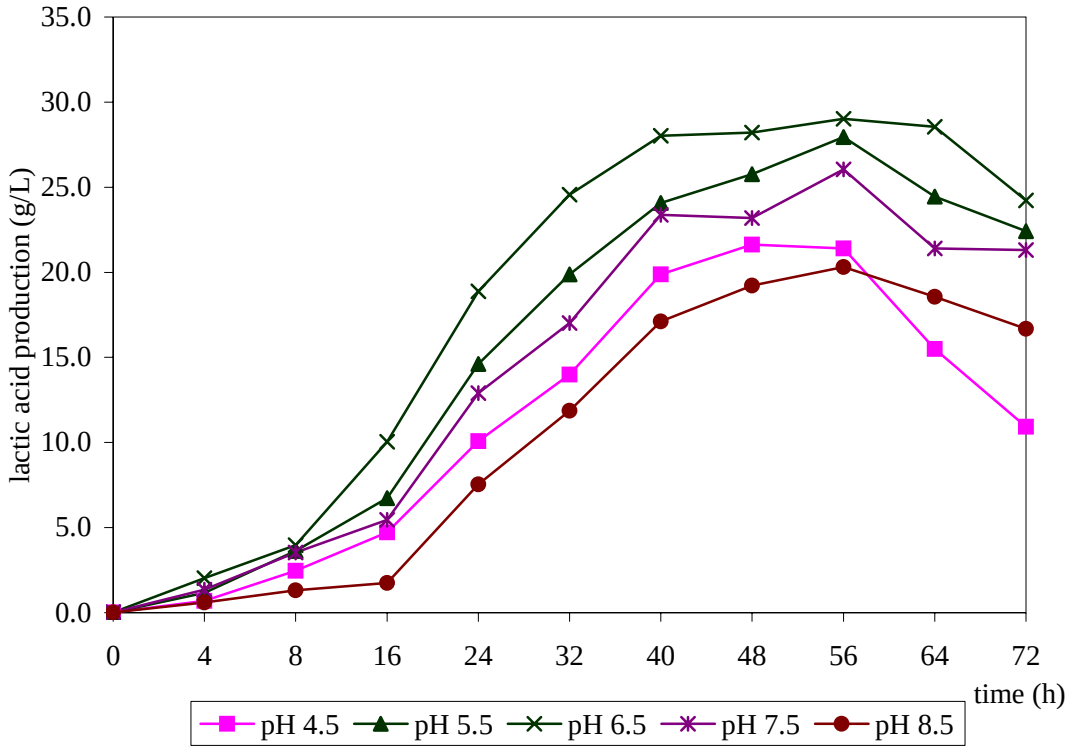

Figure 5.3: Effect of initial pH on lactic acid production by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C. bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

5.2.2 Effect of Temperature

Temperature is one of the important factors that affect the growth of microorganism. Most species have a characteristic range of temperature in which they can grow, but they do not grow at the same rate over the whole of temperature range. Microbial growth is governed by the rate of chemical reaction catalyzed by enzymes with the cell. Lactic acid bacteria are classified as thermophilic or mesophilic bacteria. The *Lactobacillus delbrueckii* is a mesophilic bacteria, which grows at 17 to 50^{o}C, and have optimum growth between 20 to 40^{o}C (Goksungur and Guvenc, 1999).

The influence of temperature on lactic acid fermentation was investigated between 27 to 50^{o}C using 31.3 g/L of glucose concentration at pH 6.5. The effect of temperature on bacterial growth or cell concentration by immobilized *Lactobacillus delbrueckii* in pineapple waste is shown in Figure 5.4. The lag phase of bacterial growth for 27, 30, 40, 45^{o}C and 50^{o}C was longer than for 37^{o}C. At 37^{o}C the lag phase is 8 hours. This longer lag phase was due to the bacteria needed to adapt with their environment. The maximum concentration of cell decreases when temperature increases. This might be due to the fact that at 45^{o}C the cells start to lose their activity (Yan, 2001). The culture grew well in the pineapple waste at 37^{o}C and 40^{o}C where the number of cell were 76.7 x 10^{6} cfu/ml and 63.3 x 10^{6} cfu/ml respectively at 56 hours of fermentation. Comparing the fermentations at 27^{o}C and 50^{o}C the cell grew more slowly from lag phase. This might be due to the inhibition effect by lactic acid production and depletion of nutrient concentration. The maximum concentration of number of cell obtained at 37^{o}C was 76.7 x 10^{6} cfu/ml respectively.

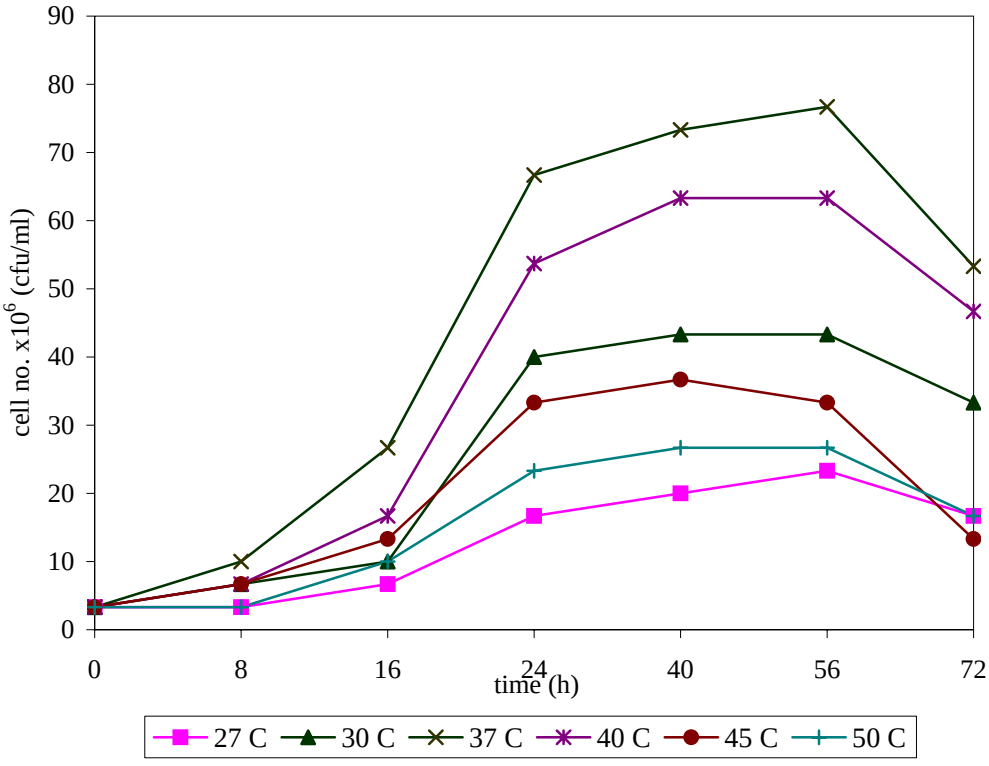

Figure 5.4: Effect of temperature on cell concentration by Ca-alginate immobilized *Lactobacillus delbrueckii* (initial pH=6.5, bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

Figure 5.5 shows the trends of glucose concentration during the fermentation process at various temperatures. Concentration of glucose for initial fermentation was 31.3 g/L. The results show that at 37°C, the cells start to utilize glucose earlier compared with other temperatures. Thus, at 37°C, the cell started to produced lactic acid faster than at the fermentation of 27, 30, 40, 45 and 50°C. When the glucose concentration was reduced rapidly, the lactic acid achieved maximum concentration.

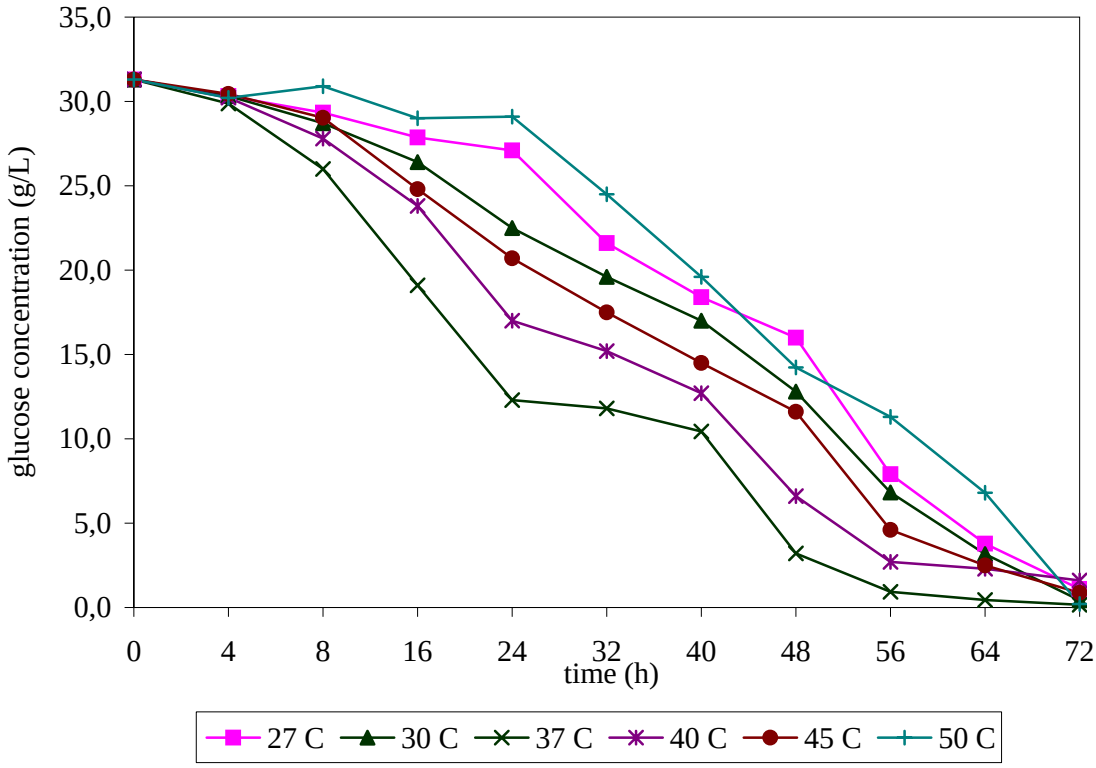

Figure 5.5: Effect of temperature on glucose consumption by Ca-alginate immobilized *Lactobacillus delbrueckii* (initial pH=6.5, bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

The effect of temperature on the lactic acid production is depicted in Figure 5.6. The highest lactic acid production was obtained at 37°C and the yield obtained were 28.73 g/L with the yield of 91.7%. When the temperature was increased to 45°C the lactic acid production reduced to 26.79 g/L or 85.6% yield. A further increased in temperature at 50°C results in a decrease of lactic acid production to 20.53 g/L or 65.6%.

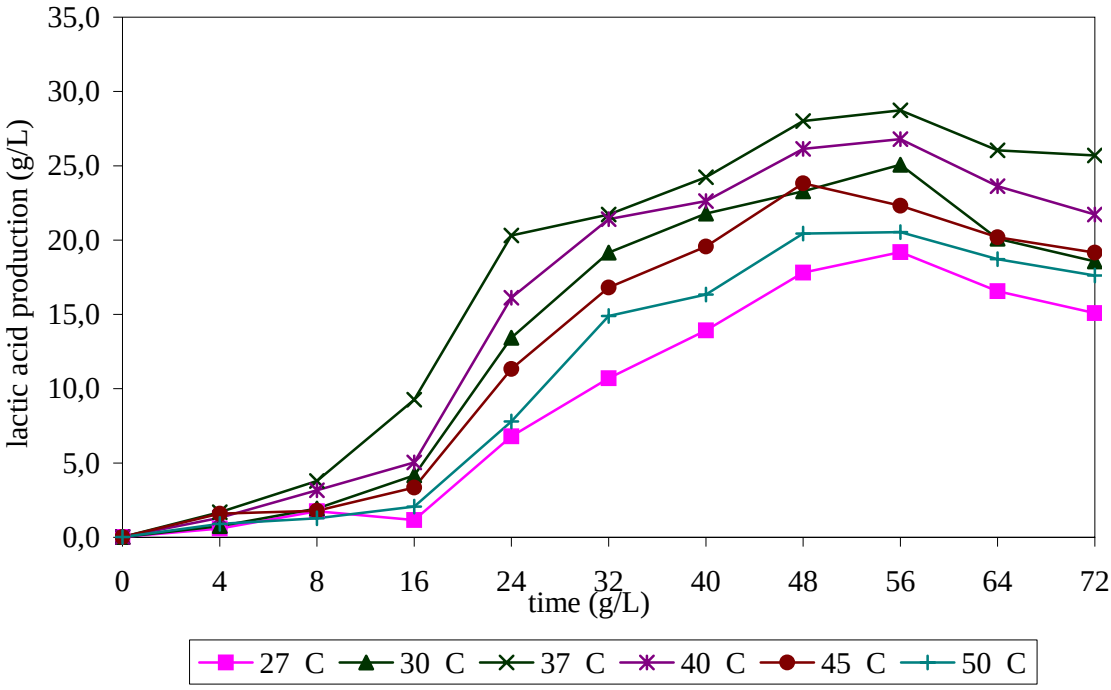

Figure 5.6: Effect of temperature on lactic acid production by Ca-alginate immobilized *Lactobacillus delbrueckii* (initial pH=6.5, bead diameter = 1.0 mm, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

The results indicate that the lactic acid production depends on microbial growth or cell concentration. *Lactobacillus delbrueckii* growth seem to be optimum at 37^{o}C promoting maximum cell concentration and this contributes to maximum lactic acid production. Increasing temperature to 50^{o}C does not promote cell growth, thus lactic acid production is decreased. These results are different to those reported by Goksungur and Guvenc (1997) who used beet molasses as the substrate for their lactic acid production. They obtained the highest yield at 45^{o}C and this might be due to the different substrate and strain used in lactic acid fermentation process.

5.2.3 Effect of Na-alginate Concentration

Lactic acid bacteria were immobilized in Ca-alginate beads prepared from different concentration of Na-alginate (1.0%, 2.0%, 4.0%, 6.0% and 8.0%) and their fermentation efficiency was investigated in liquid pineapple waste containing 31.3

g/L of glucose initially. Figure 5.7 shows the growth pattern for five concentrations of sodium alginate. The lag phase of bacterial growth for 1.0, 4.0, 6.0 and 8.0% Na-alginate concentration are longer; 24 hours compared to the 2.0% Na-alginate concentration, which is only 8 hours. Increasing the Na-alginate concentration above 2.0% only prolong the lag phase and the bacteria does not exhibit improved growth. The exponential growth can be seen in all the flasks accept for the 1.0% of Na-alginate's flask. 2.0% of Na-alginate concentration produces more cell number compared to other samples. The exponential phase begins after 8 hours and the cell grows gradually until 56 hours where the death phase begins. Thus, the presence of only 2.0% Na-alginate concentration in the calcium alginate beads creates the optimum condition for *Lactobacillus delbrueckii*. The result is similar to those reported by Goksungur and Guvenc (1999) using beet molasses as the substrate.

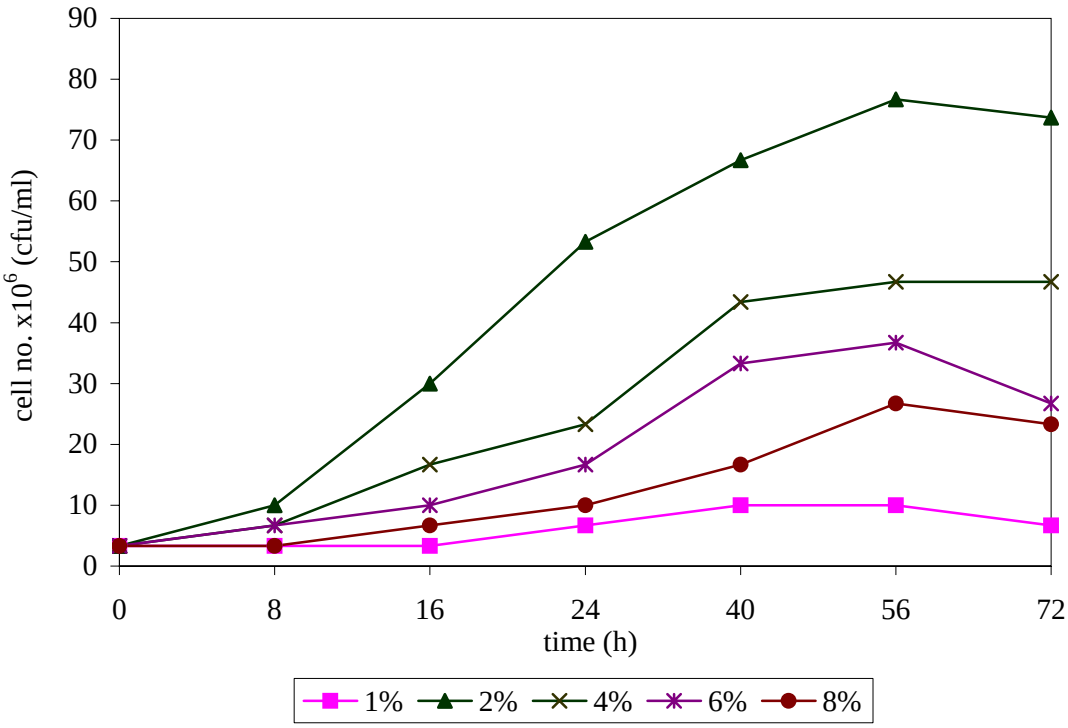

Figure 5.7: Effect of sodium alginate concentration on cell concentration by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C. bead diameter = 1.0 mm, cultivate size = 5.0 g, initial pH = 6.5 and substrate concentration = 31.3 g/L)

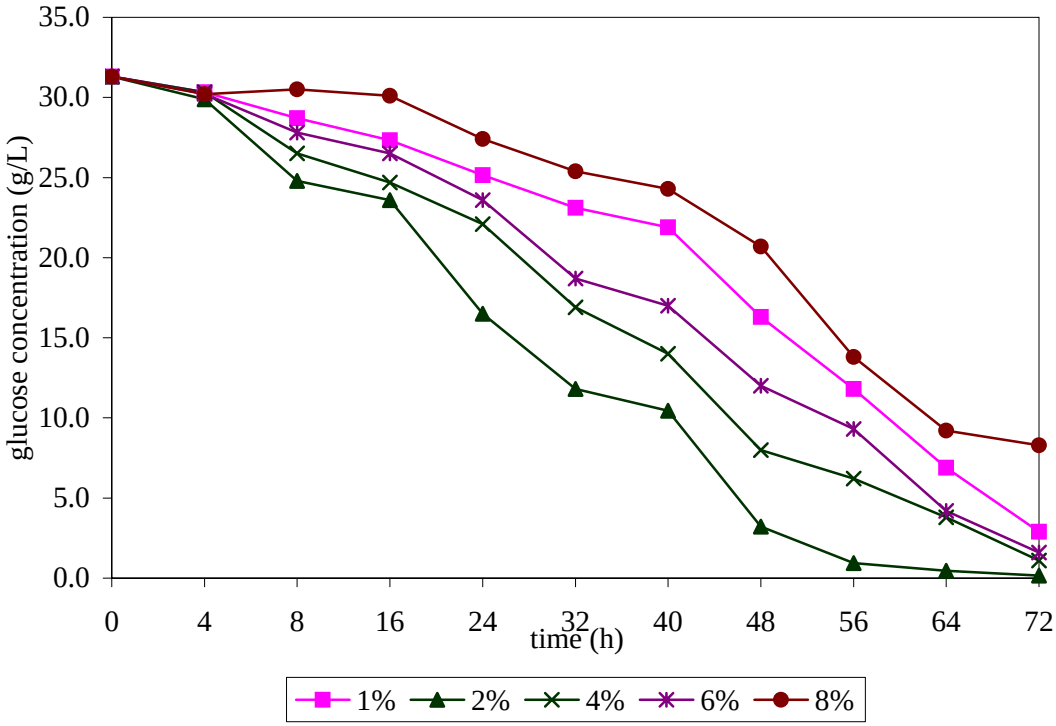

Figure 5.8: Effect of sodium alginate concentration on glucose consumption by Ca-alginate immobilized *Lactobacillus delbrueckii* (initial pH=6.5, bead diameter = 1.0 mm, cultivate size = 5.0 g, initial pH=6.5 and substrate concentration = 31.3 g/L)

Figure 5.8 shows the consumption pattern of the glucose during fermentation of the liquid pineapple waste. Initial concentration of glucose is 31.3 g/L respectively for all samples. Glucose was consumed completely for all concentration of sodium alginate. As seen in Figure 5.8, the 2.0% Na-alginate start to utilize glucose earlier than the other inoculates size. Glucose concentration reduced gradually after 56 hours and the concentration was 0.16 g/L after 72 hours. As we can saw 2.0% Na-alginate concentration sample utilized better than other concentration samples where the sugar were not completely utilized.

The effect of Na-alginate concentration on the lactic acid production is depicted in Figure 5.9. The highest lactic acid production is obtained for the 2.0% of Na-alginate concentration with a yield of 29.39 g/L and 93.8%. Increasing the Na-alginate concentration above 2.0%, lactic acid production decreased due to the lower diffusion efficiency of the beads.

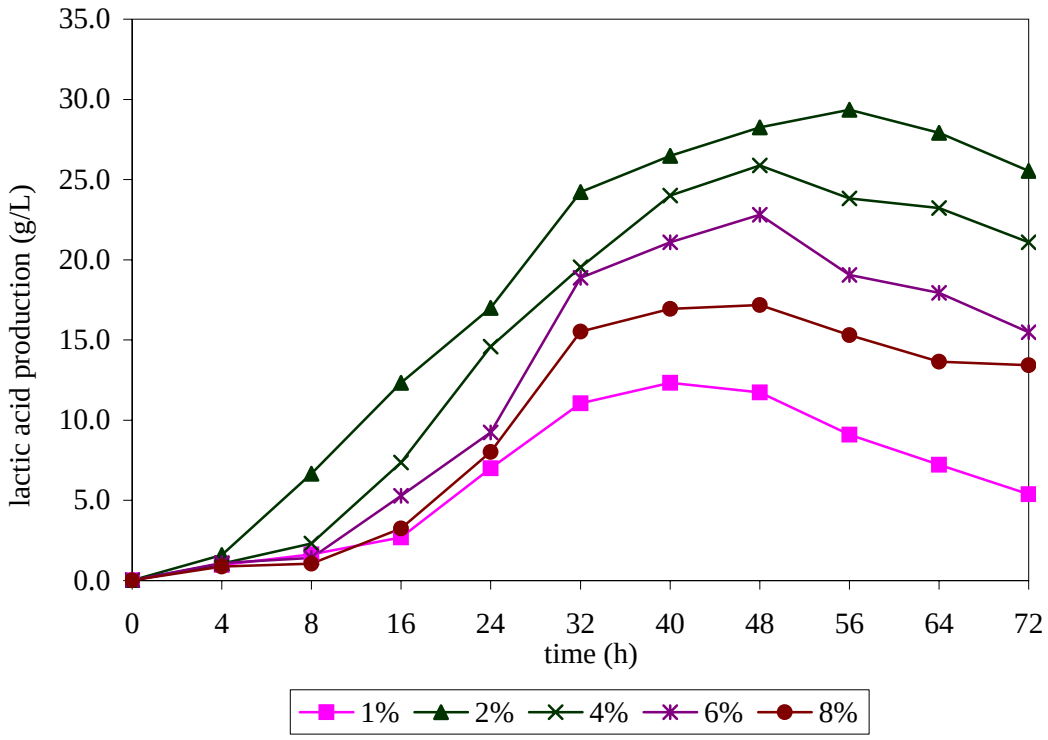

Figure 5.9: Effect of sodium alginate concentration on lactic acid production by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C. bead diameter = 1.0 mm, cultivate size = 5.0 g, initial pH=6.5 and substrate concentration = 31.3 g/L)

Beads prepared from 1.0% of Na-alginate concentration were much softer and most of the beads were disrupted in the medium at the end of fermentation. The 1.0% of Na-alginate concentration, the lactic acid yield is the lowest at 12.33g/L. Abdel Naby *et al*. (1992) investigated lactic acid production by Ca-alginate immobilized *L. lactis* and determined the maximum lactic acid production with beads containing 3 % Ca-alginate. They obtained lower yields with bead made of 4 and 5 % due to diffusion problem. Further decrease in the Na-alginate concentration below 2.0% and increase in Na-alginate beyond 2.0% does not improve the lactic acid production.

5.2.4 Effect of Bead Diameter

The effect of bead diameter (1.0 mm, 3.0 mm and 5.0 mm) on lactic acid production was determined using gel beads containing 2.0% Na-alginate. From the experimental design results, the bead diameter is the most significant factor effecting lactic acid production using immobilized *Lactobacillus delbrueckii* in pineapple waste medium. Figure 5.10 showed the growth pattern for three different sizes of bead diameter. The 1.0 mm bead produced more cell number (73.3 x 10^6 cfu/ml) compared to the 3 mm (50.0 x 10^6 cfu/ml) and 5 mm (26.7 x 10^6 cfu/ml) beads. The lag phase of bacterial growth for 3 mm and 5 mm are longer than 1mm bead diameter.

The 1.0mm bead diameter went into exponential phase growth at the 8^{th} hours until 24^{th} hours before the stationary phase started. The high cell growth promotes lactic acid production, which also started at about the same time. Different patterns were observed for the 3.0mm and 5.0mm beads, where the exponential growth started only after from 16^{th} hours. The numbers of cell produced were less compared to the 1.0mm bead. Thus, when the bead diameter is increased to 3.0mm, the bacteria grew even more slowly producing less lactic acid.

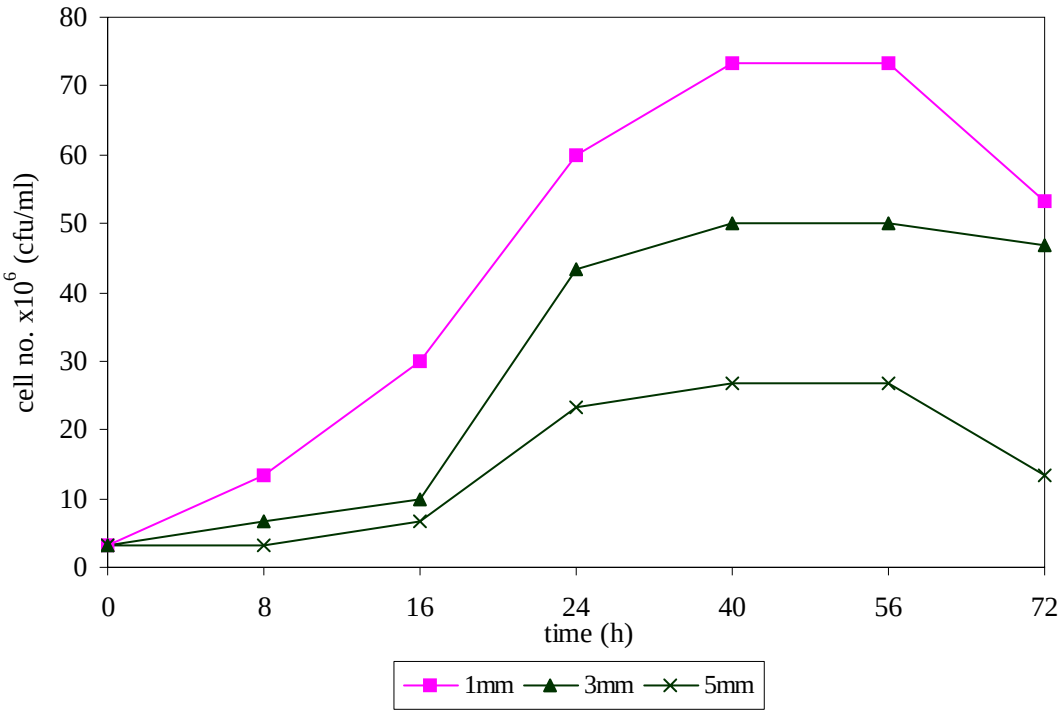

Figure 5.10: Effect of bead diameter on cell concentration by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C, initial pH =6.5, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

Figure 5.11 depicts that all glucose available in the pineapple waste was fully metabolized after 56 hour of fermentation for the 1mm bead. Glucose concentration reduced gradually after 56 hours and during that time lactic acid concentration was optimum. The results revealed that the cell entrapped in 1.0 mm bead start utilize glucose earlier than other beads. Glucose still can be detected at the 72nd hour of fermentation for the 5.0mm bead, which implies lower metabolic activity. The results show that sugar utilization decreases as bead diameter continues to increase. Goksungur and Guvenc (1999) had studied the effect of bead diameter on lactic acid production, cell concentration and sucrose utilization in beet molasses medium and found the optimum bead diameter for sucrose utilization which is the sole carbon in the medium is between 1.5 to 2.0 mm.

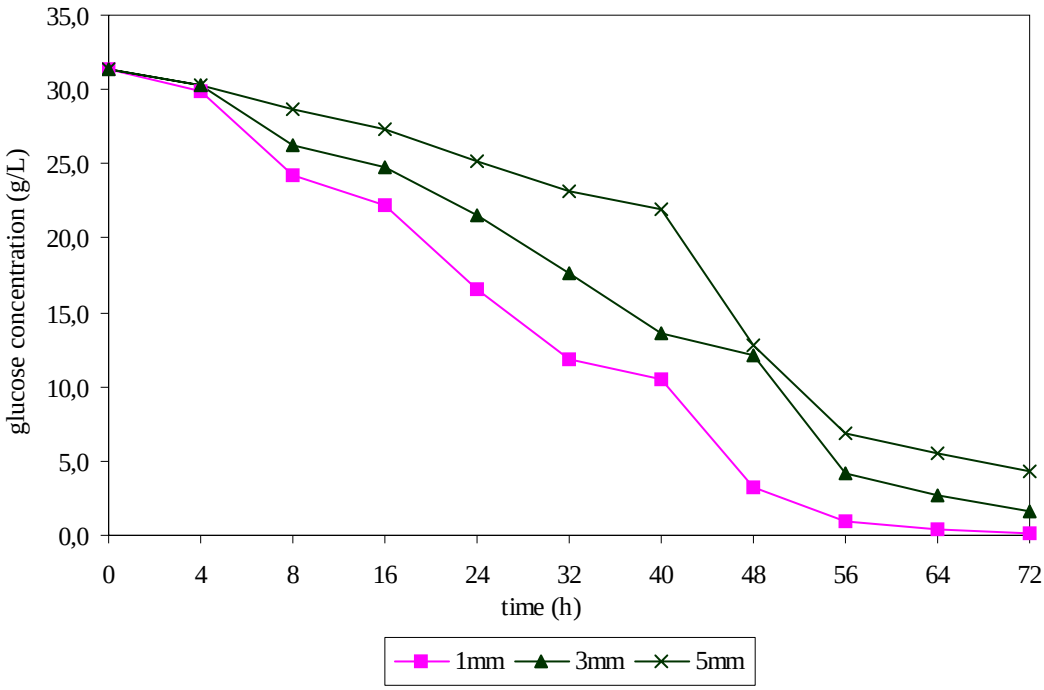

Figure 5.11: Effect of bead diameter on glucose consumption by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C, initial pH= 6.5, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

A similar trend is also observed for the production of lactic acid in Figure 5.12. Maximum lactic acid concentration is attained for the 1.0 mm bead diameter with a yield of 30.27g/L and 96.7%. Smaller diameter beads yields more lactic acid due to an increase in the surface-volume ratio. A further increase in the bead diameter to 5.0mm results in a decrease of lactic acid production to 17.65g/L or 50.7%. Abdel-Naby *et al*. (1992) had studied the effect of bead diameter for lactic acid production and found the optimum lactic acid yield was obtained using a 2.0 mm bead diameter. They also showed that lactic acid production increase as bead diameter continues to decrease.

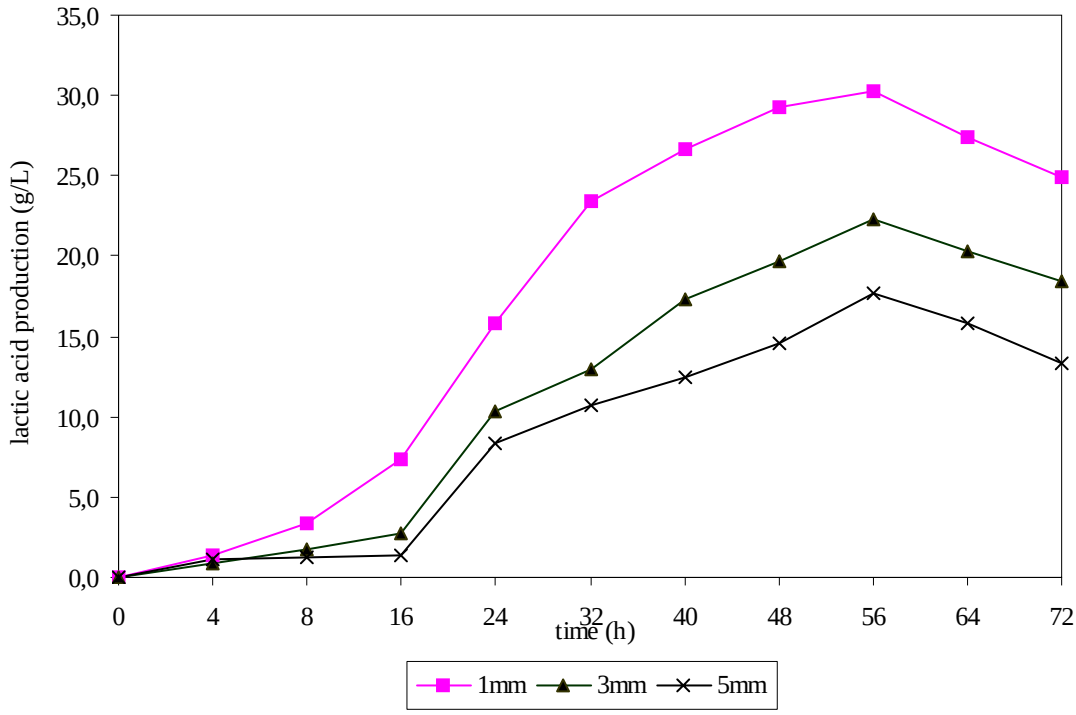

Figure 5.12: Effect of bead diameter on lactic acid production by Ca-alginate immobilized *Lactobacillus delbrueckii* (T=37^{o}C, initial pH=6.5, cultivate size = 5.0 g, 2.0% Na-alginate and substrate concentration = 31.3 g/L)

5.3 Kinetic Evaluation

Growth which characterized by increase in cell concentration or cell number occurs only where certain chemical and physical condition are satisfied such as acceptable temperature and pH as well as the availability of required nutrients. The kinetics of growth and product formation reflects the cell ability to respond to the environment and here in lies the rationale for a study of growth kinetics. Thus the effect of temperature and pH on kinetic parameters were determined and presented.

5.3.1 Effect of Temperature

Effect of temperature on kinetic parameters, μ_{max}, $Y_{x/s}$, $Y_{p/s}$, K_s, α and β were evaluated at 27, 30, 37, 40, 45 and 50^oC. The data obtained in kinetics of microbial growth on pineapple waste for different temperature are depicted in Table 5.1. The highest maximum specific growth value, μ_{max} was 0.09033 h^{-1} at 37^oC, at temperature 45^oC the value decreased to 0.036 h^{-1} and at 50^oC the μ_{max} become lower than other temperature. The effects of temperature on bacterial yield shows that at temperature at 37^oC, the optimum value of $Y_{x/s}$ was 0.0019g cell/g glucose. It is evident that the cell concentration is maximum at 37^oC. Microbial growth is governed by the rate of chemical reaction catalyzed by enzymes within the cell. The maximum concentration of cell decreased which temperature increasing. It might be due to above 40^oC, the enzymes started to lose their activity. Increasing temperature beyond 37^oC caused a decrease in cell yield. As seen in Table 5.1, at 37^oC, the lactic acid yield on sugar, $Y_{p/s}$ (0.8248 g lactic acid/g glucose) was higher.

Metabolic product formation can be similarly related to nutrient consumption. The highest value of α and β were 211.45 and 2.7721 h^{-1} were at 37^oC compared to other temperature. Furthermore the value for growth associated coefficient, α is higher than non-growth associated coefficient, β in all cases. This indicating that the production of lactic acid from liquid pineapple waste is mixed growth associated.

Table 5.1: Effect of temperature on kinetic parameters

Temperature	μ_{max} (h^{-1})	K_s (g/L)	α	β (h^{-1})	$Y_{x/s}$ (g/g)	$Y_{p/s}$ (g/g)
27^oC	0.03457	0.18947	45.164	24.284	0.00053	0.4990
30^oC	0.04215	0.38011	201.99	23.357	0.00116	0.6005
37^oC	0.09033	9.26565	211.45	2.7721	0.00192	0.8248
40^oC	0.08078	6.91703	170.50	1.2085	0.00175	0.7306
45^oC	0.03600	1.82498	131.970	14.485	0.00039	0.6285
50^oC	0.02794	0.21288	76.1950	20.502	0.00051	0.5660

5.3.2 Study on initial pH

Effect of pH on kinetic parameters, μ_{max}, $Y_{x/s}$, $Y_{p/s}$, K_s, α and β were evaluated at pH 4.5 to 8.5 and these values were revealed in Table 5.2. μ_{max}, for pH 5.5 was 0.04356 $h^{-1}$and this value is at pH 6.5 the μ_{max} had increased to 0.05401 h^{-1}. Thus the highest maximum specific growth value was at pH 6.5. Specific growth rate indicates the rate of biomass production, thus a μ_{max} value indicate that it is the best condition, therefore the best pH for cultivation of *Lactobacillus delbrueckii* to lactic acid production was at pH 6.5. At pH 6.5, the cell growth well and rapidly compared to other pH.

K_s, which is the Michaelis constant reflects the limitation substrate concentration at which the reaction rate is half its maximum value. The saturation constant, K_s was affected by pH. The K_s for pH 6.5 were 7.2214 g/L. If the pH was increased to pH 7.5, the K_s decreased and if the pH was from 5.5 to pH 4.5, the K_s also decreased from 1.5407g/L to 0.5739 g/L. Chassy and Thompson (1983) found a K_s value for lactose uptake in *Lactobacillus casei* to be 4.7g/L without discussing the uptake mechanisms of lactose. Metabolic product formation can be similarly related to nutrient consumption. Furthermore the product formation cannot occur without the presence of cell. Thus it is expected that growth and product formation will be coupled to growth and or cell concentration.

Effects of pH 4.5 to 6.5 on bacterial yield shows that the pH 6.5 gave the highest value of $Y_{x/s}$ which 0.0015 g cell/g glucose as given in Table 5.2. If the pH was increased to pH 8.5 the cell yield decreased to 0.0005 g cell/g glucose. This can be shown by the maximum specific growth rate obtained for pH 6.5. It was higher than pH 7.5 and pH 8.5. With pH 5.5 and pH 4.5, the cell yield was 0.0015 g and 0.0013 g cell/g glucose. If the maximum specific growth rate increases this indicates the rate of biomass production increases, therefore the glucose medium is the best for the cultivation of *Lactobacillus delbrueckii* to produce lactic acid.

Table 5.2: Effect of pH on kinetic parameters value

pH	μ_{max} (h^{-1})	K_s (g/L)	α	β (h^{-1})	$Y_{x/s}$ (g/g)	$Y_{p/s}$ (g/g)
4.5	0.02965	0.5739	172.93	17.846	0.0013	0.3530
5.5	0.04356	1.5407	213.13	13.007	0.0015	0.7338
6.5	0.05402	7.2214	233.78	4.359	0.0016	0.7822
7.5	0.04295	0.7801	203.69	15.321	0.0018	0.6978
8.5	0.02072	0.4951	122.7	29.389	0.0005	0.5474

5.4 Discussion

The effect of pH on optimum Lactic acid production is clearly revealed in Figure 5.13. The optimum pH for lactic acid fermentation using immobilized *Lactobacillus delbrueckii* ATCC 9646 is 6.5. Increasing pH beyond these value do not result in any increase of lactic acid yield. The bacteria, *Lactobacillus delbrueckii* seems to grow well in neutral environment with a pH in the region of 5.5 to 7.5, but best at pH 6.5. An environment, which is too acidic and alkaline, is not conducive for lactic acid production.

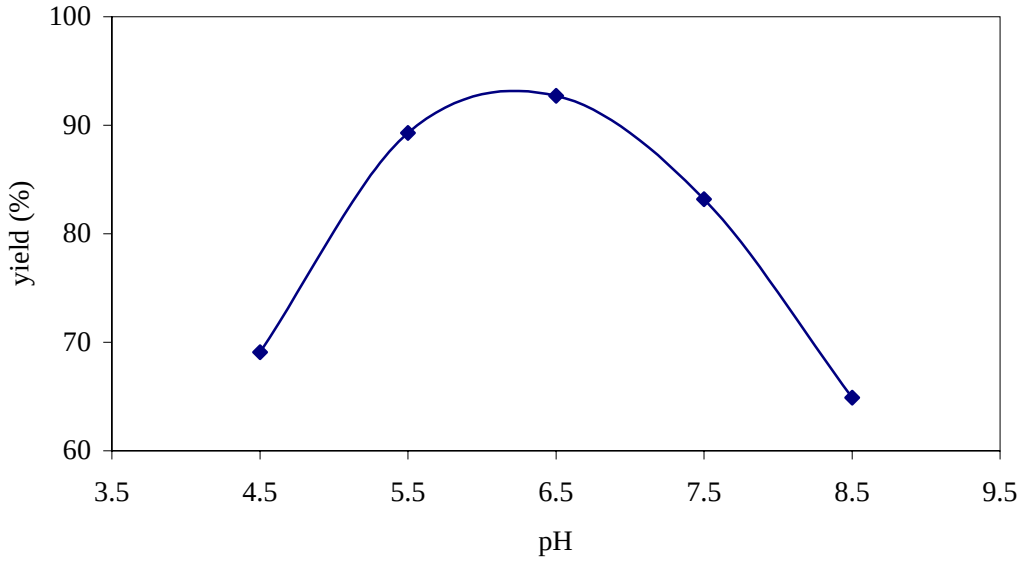

Figure 5.13: Effect of pH on Lactic acid production at time 56 hours.

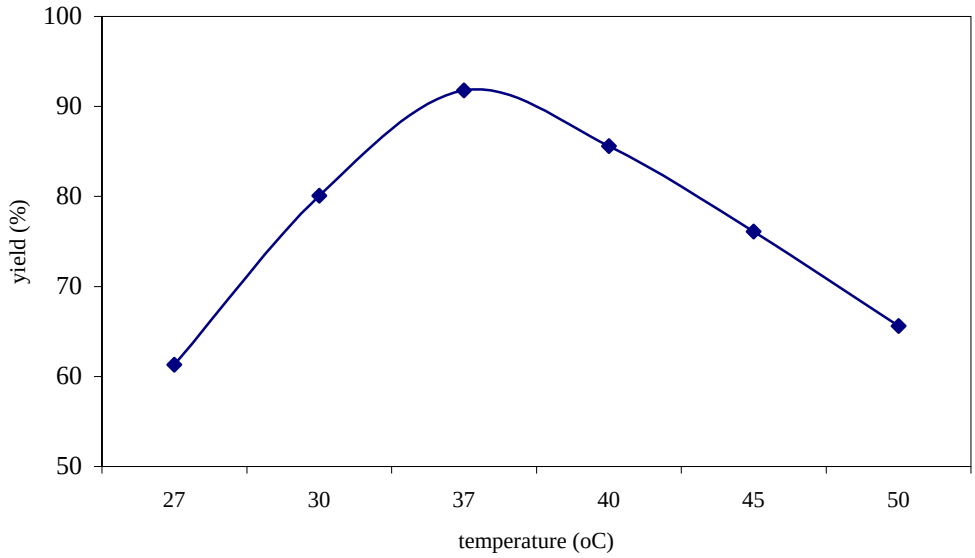

Figure 5.14: Effect of temperature on lactic acid yield at time 56 hours.

Effect of temperature on lactic acid production is clearly revealed in Figure 5.14. The optimum temperature for the fermentation of lactic acid using immobilized *Lactobacillus delbrueckii* ATCC 9646 is 37^{o}C respectively. Increasing temperature and beyond these values do not result in any increase of lactic acid production. The results indicate that the lactic acid production depend on microbial growth or cell concentration, as shown in Figure 5.4. *Lactobacillus delbrueckii* growth seems to be optimum at 37^{o}C promoting maximum cell concentration and this contributes to high lactic acid production. Increasing temperature to 50^{o}C does not promote cell growth, thus lactic acid production is reduced.

Figure 5.15 show the pattern of lactic acid production during the fermentation process at various Na-alginate concentrations. The results show the highest yield of lactic acid was obtained when 2.0% of Na-alginate concentration was used in lactic acid fermentation process. Increasing Na-alginate concentration beyond these value do not result in any increase of lactic acid yield. These results seems to be in agreement those obtained by Goksungur and Guvenc (1999) where optimum Na-alginate concentration is 2.0%.

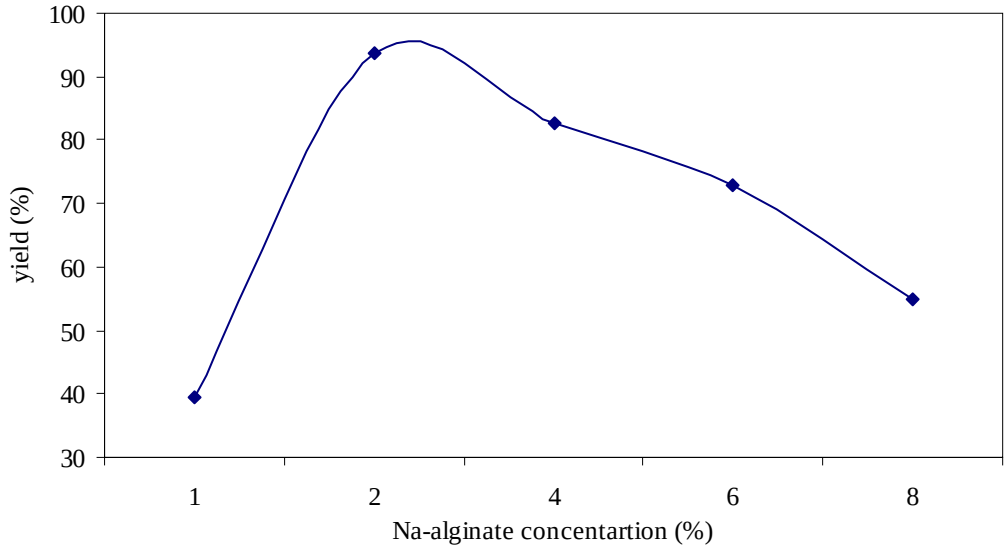

Figure 5.15: Effect of Na-alginate concentration on lactic acid yield at 56 hours.

Too low Na-alginate concentration results in very soft beads whilst increased Na-alginate to above 2.0% hardens the beads, thus causing diffusion problems to occur. At high Na-alginate concentration, the bacteria do not get enough nutrients (food) as the substrate has difficulty in diffusing through the beads. However when only 1.0% Na-alginate concentration is used, the beads which are too soft as mentioned earlier are easily broken since their mechanical strength are lower and the bacteria leaks out from the bead.

Effect of bead diameter on lactic acid yield is clearly revealed in Figure 5.16. The optimum bead diameter for the fermentation of lactic acid for cell entrapped in Ca-alginate is 1.0mm. Increasing bead diameter and beyond to 3.0mm and 5.0mm did not improve production value, which were 71.3% and 56.4%, respectively. While decreased bead diameter to 1.0mm, the lactic acid production increased to 96.7%.

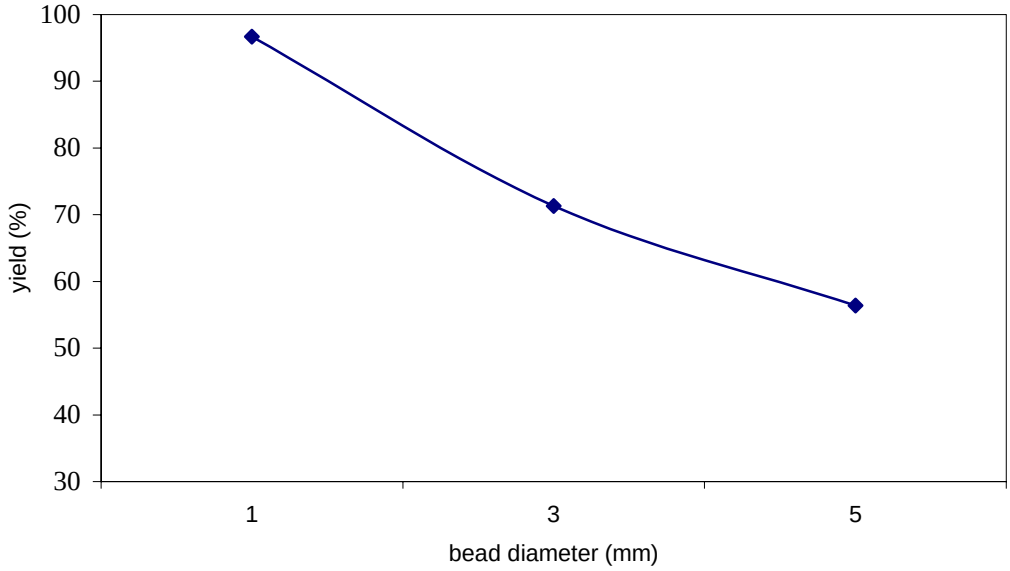

Figure 5.16: Effect of bead diameter on lactic acid yield at 56 hours

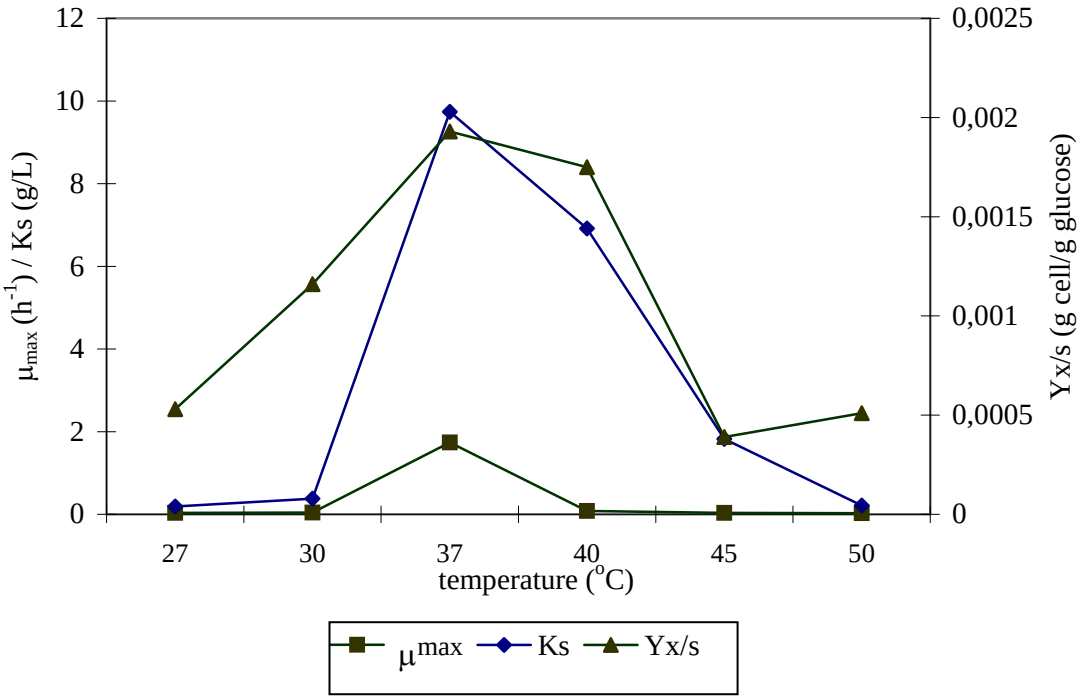

Figure 5.17: The relation between specific growth rate, Ks and yield of cell on total glucose at various temperatures

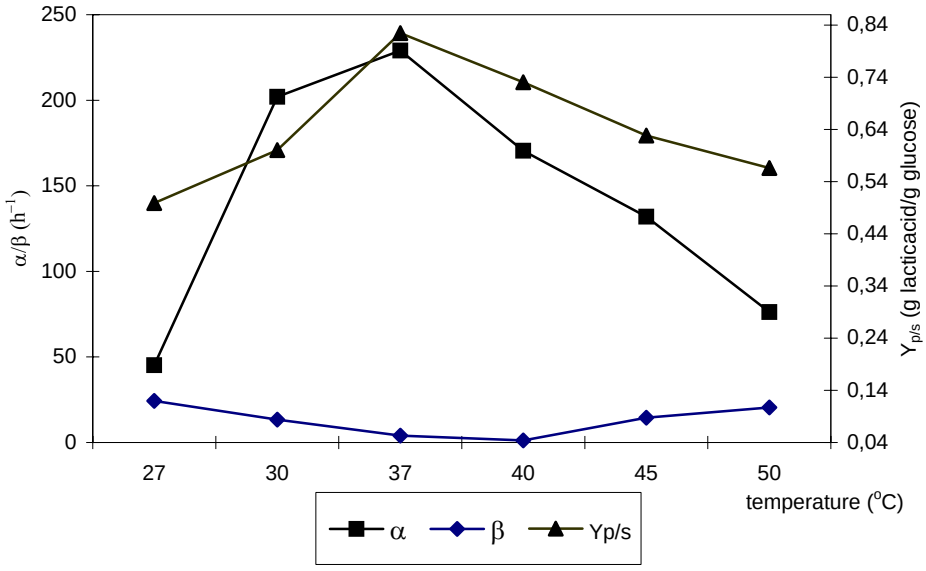

Figure 5.18: The relation between yield of product, growth associated and non-growth associated constant for product formation at various temperatures

The effects of temperature on bacterial yield shows that at temperature 37°C, the optimum value of $Y_{x/s}$ was 0.0019 g cell/ g glucose. The $Y_{x/s}$ obtained for 40 and 50°C were 0.0018 and 0.0005 g cell/g glucose respectively. The cell growth pattern and relation of cell concentration with fermentation temperature was observed. If the temperature was increased, the biomass yield decreased. This can be shown by the maximum specific growth rate. The maximum specific growth rate for *Lactobacillus delbrueckii* grown on glucose in this work was 0.09033h^{-1}. The value obtained for 37°C was higher than 40°C and 50°C. The following table displays the experimental data while the Figure 5.17 and 5.18 shows the graphical relation. The saturation constant, K_s was also affected by temperature and K_s obtained for 37°C was 9.2656 g/L. If the temperature was increased to 45°C, the K_s was decreased and if the temperature was decreased from 30°C to 27°C the K_s decreased from 0.38011 g/L to 0.1895 g/L.

As seen in Figure 5.18, at 37°C, the lactic acid yield on sugar, $Y_{p/s}$ (0.8248 g lactic acid/g glucose) was higher. It should be point out here that, the cell yield coefficients, $Y_{x/s}$ listed above may not reflect the exact amount of substrate that was converted into product, because the medium used in the anaerobic fermentation contained not only glucose, but also yeast extract and trypticase peptone. These

materials contain protein, vitamins and other nutrients that are preferred for cell growth *by L. delbrueckii.*

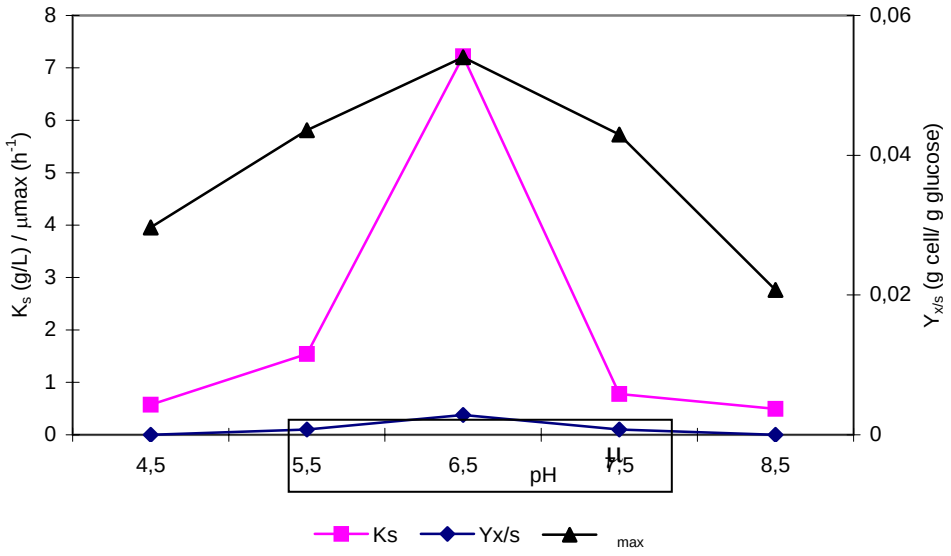

Figure 5.19: The relation between specific growth rate, Ks and yield of cell on total glucose at various pH

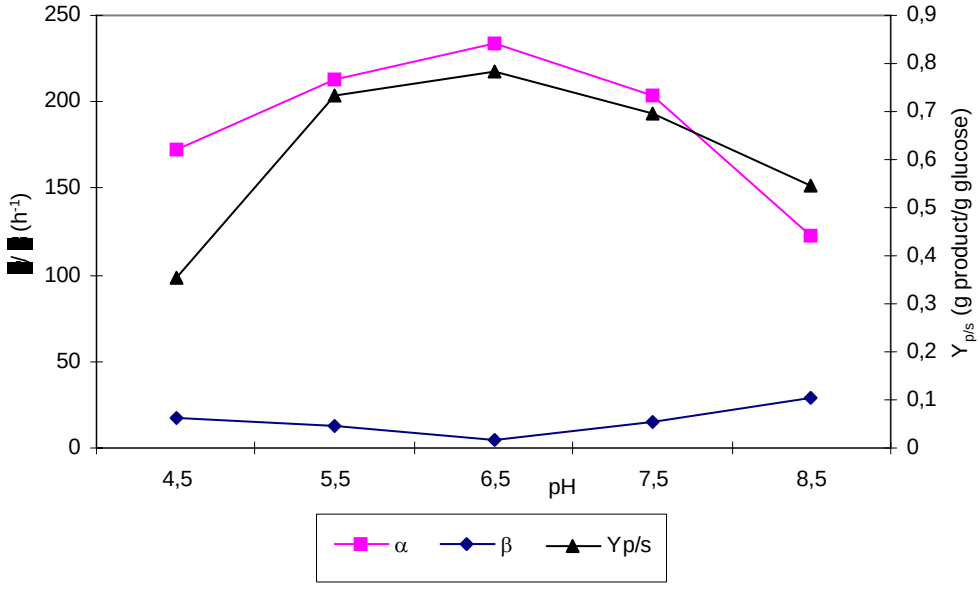

Figure 5.20: The relation between yield of product, growth associated and non-growth associated constant for product formation at various pH

The relationship between growth patterns, glucose utilization and product formation at various initial pH are shown in Figure 5.19 and 5.20 respectively. It was found that the maximum specific growth rate for initial pH 6.5 was higher than at pHs 5.5 and 7.5. This can be seen from the growth rate obtained at initial pH was 0.054 h^{-1}. As seen in Figure 5.19, at initial pH 6.5, the lactic acid yield on sugar, $Y_{p/s}$ (0.7822 g lactic acid/g glucose) was higher. If the initial pH was increased to 8.5, the biomass yield decreased to 0.0005 g cell/ g glucose). This can be shown by the maximum specific growth rate obtained for initial pH 6.5. Microbial growth is usually characterized by an increase in cell mass and cell number with the time. Mass doubling time may differ from cell doubling time because the cell mass can increase without an increase in cell number. The saturation constant, K_s was affected by the pH. The K_s for initial pH 6.5 was 7.221 g/L. If the initial pH was increased to 7.5 the K_s deceased and when the initial pH decreased from 5.5 to 4.5, the K_s also deceased from 1.541g/L to 0.574 g/L.

The value of growth associated constant for product formation, α and non-growth associated constant for product formation β depend on the initial pH value. The α and β values are affected by variable of initial pH with the highest α value at initial pH 6.5. Table 5.2 shows that the growth associated portion of lactic acid formation by immobilized *Lactobacillus delbrueckii* is favored by fermentation at initial pH in the range of initial pH 5.5 to pH 6.5. Luedeking and Piret (1959) have studied about lactic acid fermentation of glucose by *Lactobacillus delbrueckii*, which indicated that the product formation kinetics combined growth associated and non-growth associated. Luedeking and Piret found that constant α and β value in the model were strongly dependent on initial pH. In this work at initial pH 6.5, the α and β values obtained were 233.78 and 4.359 h^{-1} respectively. The $\beta < \alpha$ ($\alpha/\beta >$ 1.0) indicates that the growth associated portion is higher than the non-growth associated portion of lactic acid formation by *Lactobacillus delbrueckii*. These bacteria produce lactic acid proportionally to the concentration not depending on their growth phase.

5.5 Summary

The present study had been carried out extensively to study the effect of parameter such as temperature, bead diameter, Na-alginate concentration and pH of fermentation medium based on two level full factorial design experiment results. A mathematical model based on Monod equation was used to determine the kinetic of microbial growth, kinetic model of substrate utilization and kinetics of lactic acid production. The growth which characterized by increase in cell mass and or number occurs only where certain chemical and physical conditions are satisfied such as acceptable temperature and pH as well as the availability of required nutrients. The kinetics of growth and product formation reflects the cell ability to respond to the environment and have in lies the rationale for a study of growth kinetics.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

This final chapter is written to summarize all the results and discussion of the data presented in Chapter 3, 4 and 5. Recommendation for further study is also suggested for lactic acid fermentation using pineapple waste.

6.1 Conclusion

This study was carried out in order to utilize of liquid pineapple waste for the production of lactic acid. The first experimental steps were to evaluate the waste to ensure the availability of nutrients and trace elements needed to support the growth and consequently the production of lactic acid and comparison between free cell and immobilized cell fermentation. The best way to ferment sugar to produce lactic acid was by using immobilized cell fermentation. The results indicated that lactic acid production was improved when the culture was immobilized in calcium alginate. Preliminary results indicated that lactic acid produced using immobilized cell is higher compared to the free cell fermentation.

The second stage of the experiment was tailored to evaluate several parameters that were thought to influence the lactic acid production using liquid pineapple waste. A two-level full factorial design was used to determine the significant factors and the optimal condition of the process variable. These screening experiments have identified that pH, temperature, Na-alginate concentration and

bead diameter are the significant factors. The optimal values of tested variables were found to be: bead diameter, 1.0mm; Na-alginate concentration, 2.0%w/v; initial pH at 6.5, temperature, 37^{o}C and cultivate size, 5.0 g. The maximum of lactic acid yield predicted was 94.3%. Whist the cultivate size and other interaction effect are insignificant and thus can be neglected.

Since the screening experiments has identified the significant factors to be bead diameter, Na-alginate concentration, initial pH and temperature, further experiments were carried out to study in detail the correlation between lactic acid production and these factors. The regression analysis carried out on the third stage revealed that there is a fairly strong correlation between initial pH and lactic acid production, whereby as the initial pH is increased, the lactic acid production increase until the critical initial pH of 6.5 is reached. Beyond this initial pH, lactic acid production begins to decrease. A similar trend is observed for the temperature, where lactic acid production increased when the temperature is increased until a critical temperature of 37^{o}C. Beyond 37^{o}C, a reversal trend occurred. The lactic acid yield is also very affected by the Na-alginate concentration in the same manner. Increase in the Na-alginate concentration beyond 2.0%, resulted in a increase in lactic acid yield. For the bead size, increasing its diameter resulted in a lower lactic acid yield. Finally, the kinetic parameters were evaluated.

The data obtained during the parametric study were applied on the simple batch model (simplified unstructured kinetic model) in terms of specific growth rate, yield constant or substrate utilization and rate of product formation or production of lactic acid. Pineapple waste demonstrated the highest product formation rate of lactic acid with a specific growth rate of 0.09033h^{-1} at 37^{o}C. The value of growth associated constant for product formation, α and non-growth associated constant for product formation β is affected by process variables such as pH and temperature.

6.4 Recommendations for Further Study

The screening process, regression analysis and kinetic studies carried out up to this extent are considered as at the preliminary stage for further optimization of the fermentation process. Comparison can be made between the mathematical model and the experimental results. Nevertheless the right value of different parameters in the model must be known to avoid unnecessary effort in obtaining accurate values of less relevant parameters. Parameters sensitivity analysis can be conducted to obtain an insight into the influence of the parameters.

The 100 ml shake flasks fermentation carried out in this study are the first stage for the scale up process. The kinetic data evaluated and the optimum fermentation parameter obtained in this study provided the condition needed for the scale up. Scale-up involves maintaining these conditions no matter what the volume. If the conditions are the same and no mutation occur which might cause the growth kinetics or the metabolic products to change, the production rate per unit volume should be the same in large and small system. To evaluate the effect of scale up on the yield, fermentation process can be carried out in 3 litres fermentor with working volume of 1 litre. Biomass accumulation, sugar utilization and product formation shall be studied throughout the course of fermentation and the results shall be compared against those of 100 ml shake flask to determine the impact of the scale up.

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APPENDIX A

LIST OF CHEMICALS AND SUPPLIERS

A.1 List of Chemicals

Table A.1: Culture medium

Chemical	Chemical formula	Supplier
Agar powder	$C_2H_{18}O_9$	Fluka-Biochemika
D-(+)-Glucose	$C_6H_{12}O_6$	Sigma
Diammonium citrate	$C_6H_{14}N_2O_7$	Fluka
Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$	Fluka-Chemika
Manganese (II)sulfate-1-hydrate	$MnSO_4.H_2O$	Hamburg Chemical GmbH
Meat extract		Merck
Peptone		Merck
Potassium dihydrogen orthophasphate	K_2HPO_4	BDH-GPR
Sodium acetate	$C_2H_3NaO_2$	Fluka-Chemika
Tween-80		Fisher
Yeast extract		Fluka-Biochemika

Table A.2: General Chemicals

Chemical	Chemical formula	Supplier
D-(-)Fructose	$C_6H_{12}O_6$	Sigma
L(+)Lactic acid	$C_3H_6O_3$	Sigma
Calcium carbonate	$CaCO_3$	Merck
Calcium chloride anhydrous	$CaCl_2$	HmbG Chemical
Sodium chloride	NaCl	Merck
Sodium alginate		Fluks-Biochemika
Phenolphthalein		Sigma
Ammonia	NH_3	BDH
Ammonium molybdate	NH_3MoO	Merck
Sodium hydroxide	NaOH	Merck
Sodium citrate	$Na_3C_6H_5O_7.2H_2O$	Ajax Chemical
Methyl alcohol	CH_3OH	BDH
Hydrochloride acid	HCl	J.T.Baker
Acetonitrile	CH_3CN	Fluka
Phosphoric acid	HPO_3	Fluka

APPENDIX B

L(+)LACTIC ACID SPECIFICATION

B.1 L(+)Lactic acid specification

Table B.1: Specification for L(+)Lactic acid standard

SPECIFICATION	
L-(+)- Lactic Acid (Assayed by using HPLC)	> 98%
Molecular weight	90.08
Molecular formula	$C_3H_6O_6$
Residue on ignition	< 0.1%
Solubility (1 M in water, 20^{o}C)	Colorless
Insoluble matter	< 0.1%
D-(-)-Lactic Acid (assayed by using HPLC)	> 95%
Molecular weight	90.08
Molecular formula	$C_3H_6O_3$
Purity	96%

APPENDIX C

HPLC CHROMATOGRAMS

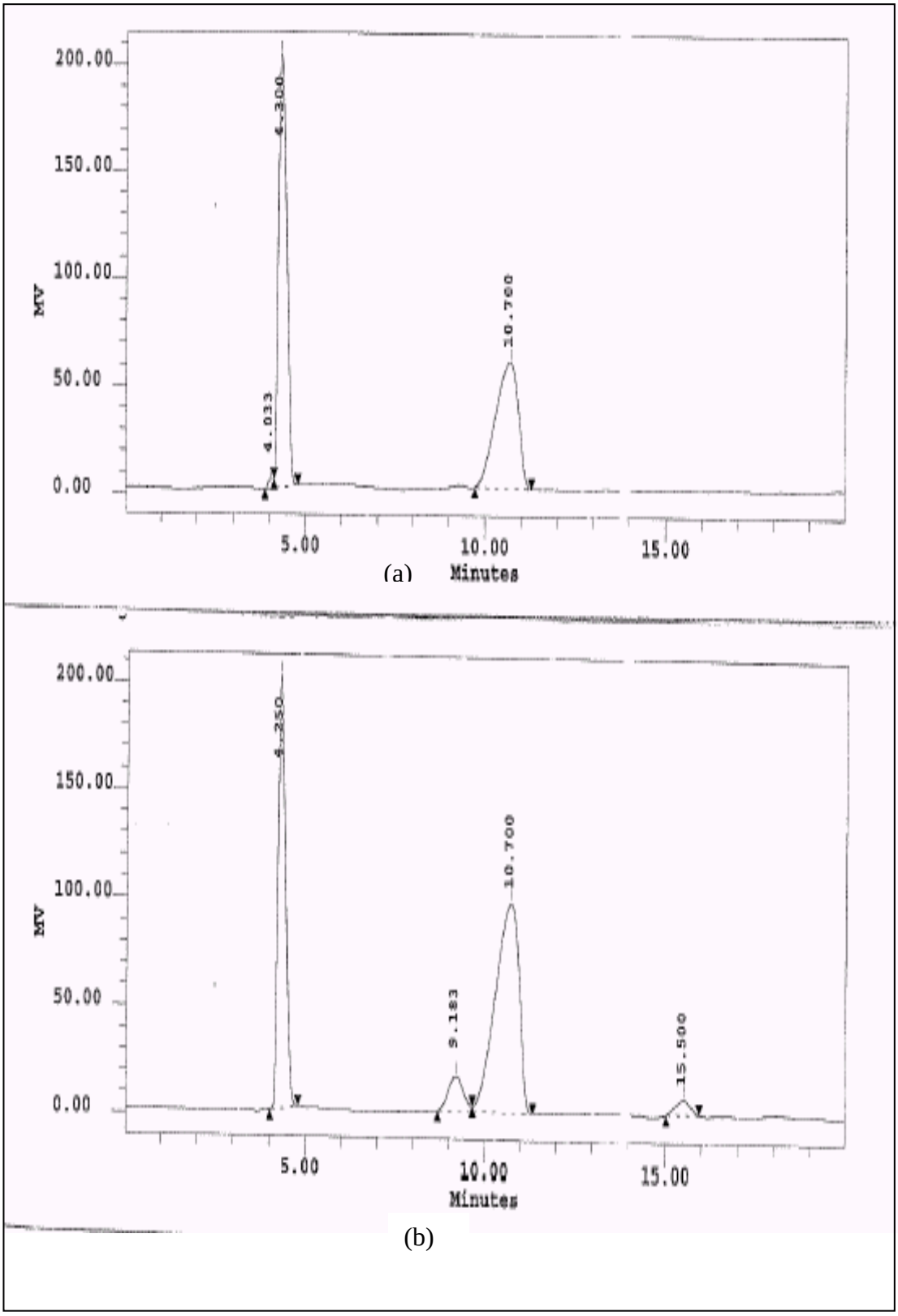


Figure C.1: Retention time for glucose at 10.700. (a) HPLC chromatography for standard glucose and (b) HPLC chromatography for pineapple waste

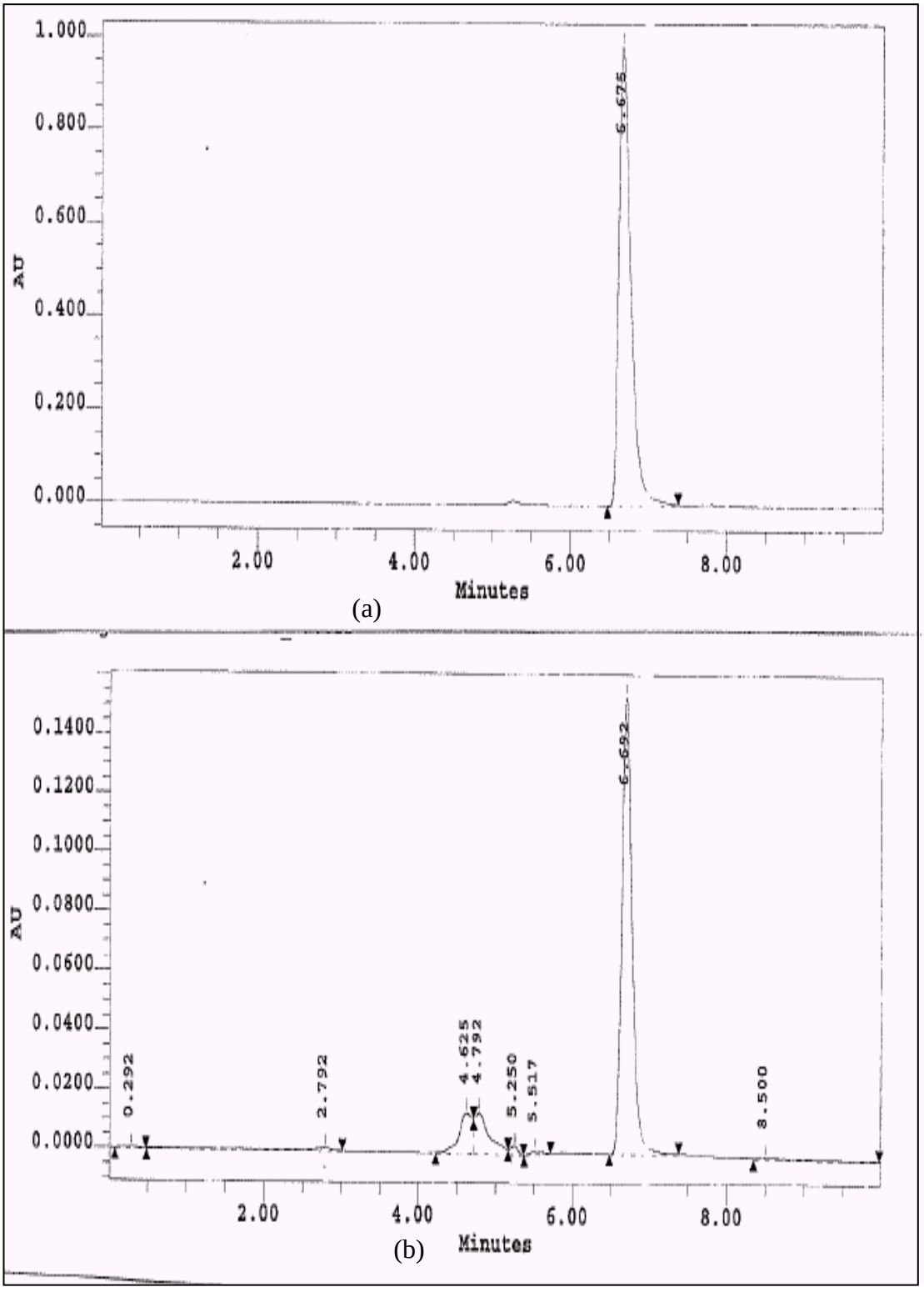


Figure C.2: Retention time for L(+)Lactic acid at 6.678. (a) HPLC chromatography for standard L(+)Lactic acid and (b) HPLC chromatography for pineapple waste

APPENDIX D

TWO LEVEL FULL FACTORIAL DESIGN

D.1 Experimental result for run 1

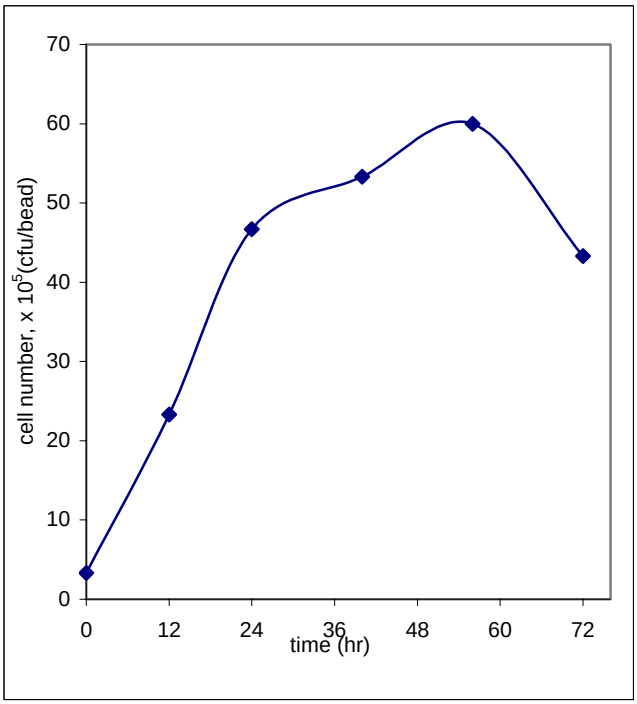


Figure D.1.1: Cell concentration for run 1

Table D.1.1: Data of cell concentration for run 1

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	23.3
24	46.7
40	53.3
56	60.0
72	43.3

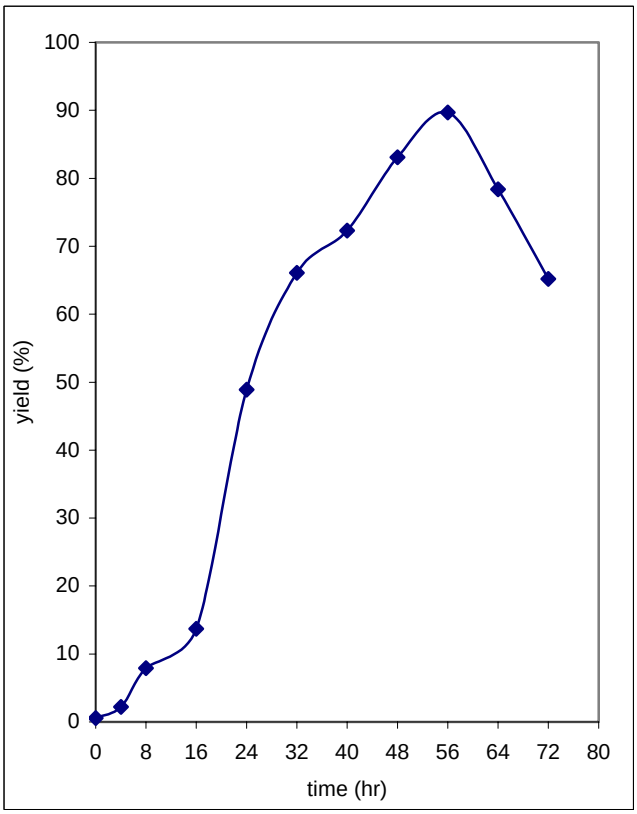


Figure D.1.2: Lactic acid production for run 1

Table D.1.2: Data of lactic acid production for run 1

Time (h)	Lactic acid production %
0	0.02
4	2.2
8	7.9
16	13.7
24	48.9
32	66.1
40	72.3
48	83.1
56	89.7
64	78.4
72	65.2

D.2 Experimental result for run 2

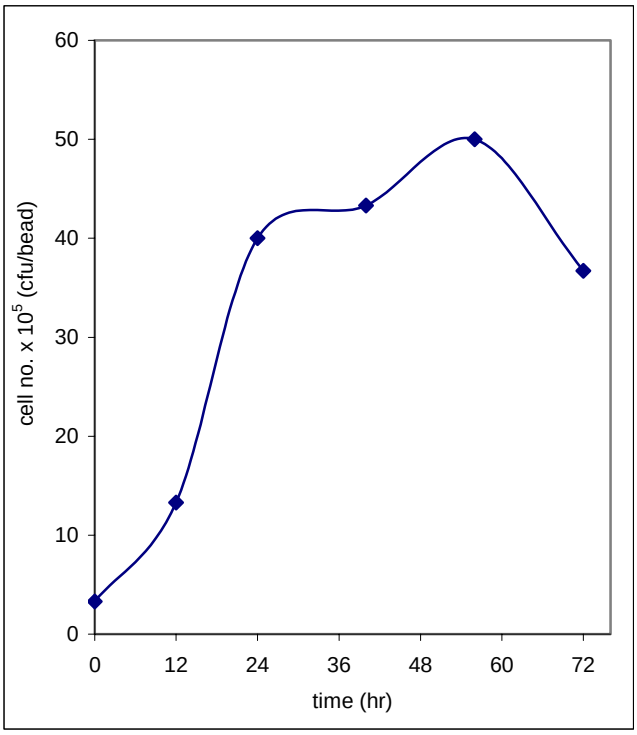


Figure D.2.1: Cell concentration for run 2

Table D.2.1: Data of cell concentration for run 2

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	13.3
24	40.0
40	43.3
56	50.0
72	36.7

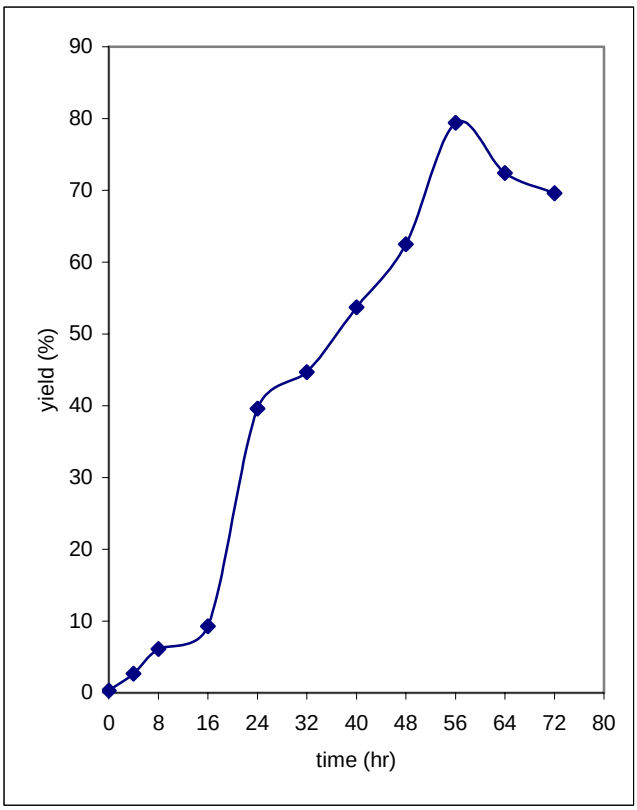


Figure D.2.2: Lactic acid production for run 2

Table D.2.2: Data of lactic acid production for run 2

Time (h)	Lactic acid production %
0	0.02
4	2.71
8	6.10
16	9.32
24	39.61
32	44.71
40	53.74
48	62.51
56	79.42
64	72.44
72	69.63

D.3 Experimental result for run 3

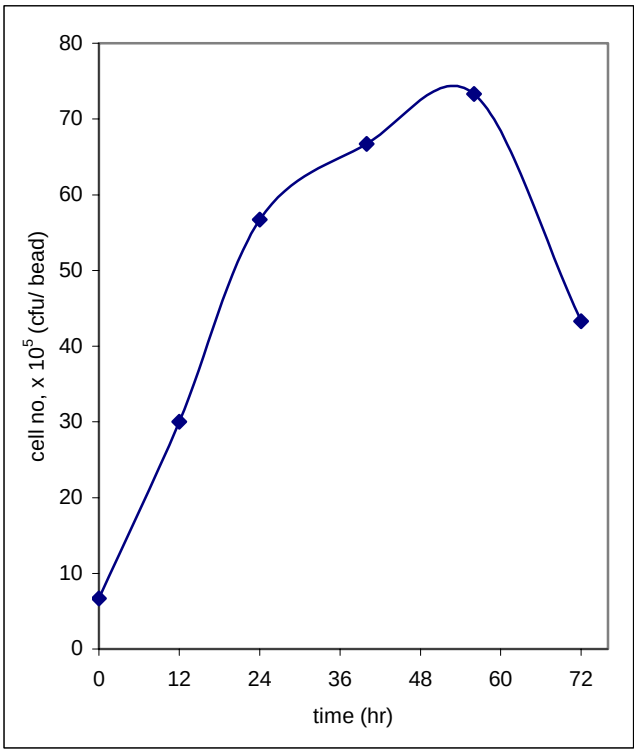


Figure D.3.1: Cell concentration for run 3

Table D.3.1: Data of cell concentration for run 3

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	30.0
24	56.7
40	66.7
56	73.3
72	43.3

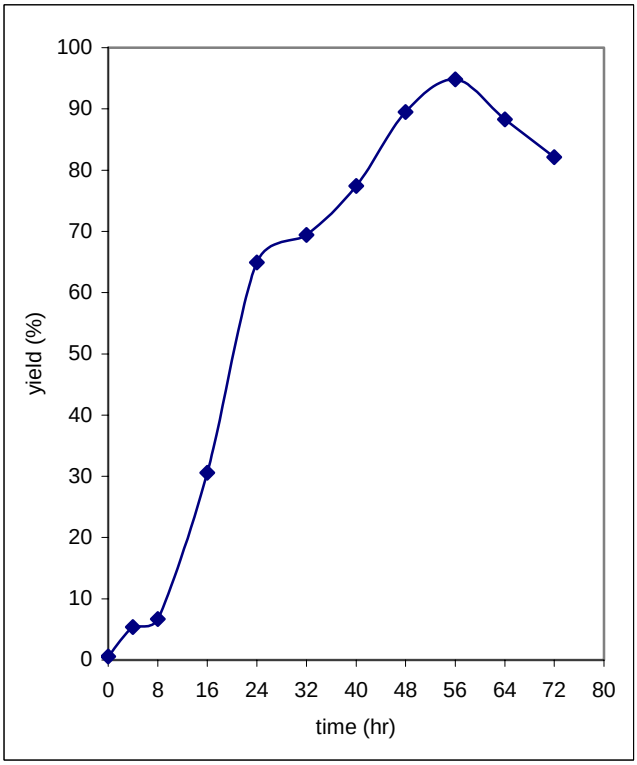


Figure D.3.2: Lactic acid production for run 3

Table D.3.2: Data of lactic acid production for run 3

Time (h)	Lactic acid production %
0	0.02
4	5.40
8	6.74
16	30.62
24	64.90
32	69.41
40	77.44
48	89.51
56	94.83
64	88.34
72	82.13

D.4 Experimental result for run 4

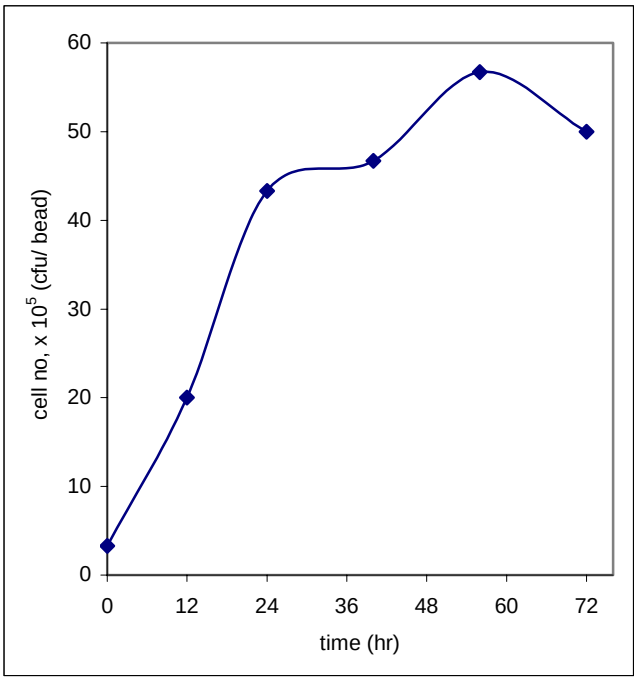


Figure D.4.1: Cell concentration for run 4

Table D.4.1: Data of cell concentration for run 4

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	20.0
24	43.3
40	46.7
56	56.7
72	50.0

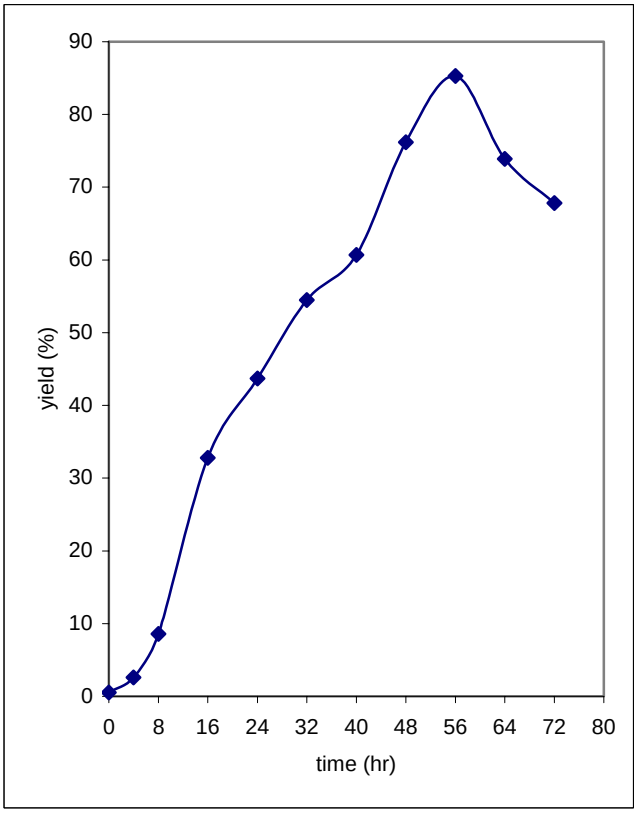


Figure D.4.2: Lactic acid production for run 4

Table D.4.2: Data of lactic acid production for run 4

Time (h)	Lactic acid production %
0	0.02
4	2.62
8	8.64
16	32.80
24	43.72
32	54.51
40	60.73
48	76.22
56	85.32
64	73.91
72	67.80

D.5 Experimental result for run 5

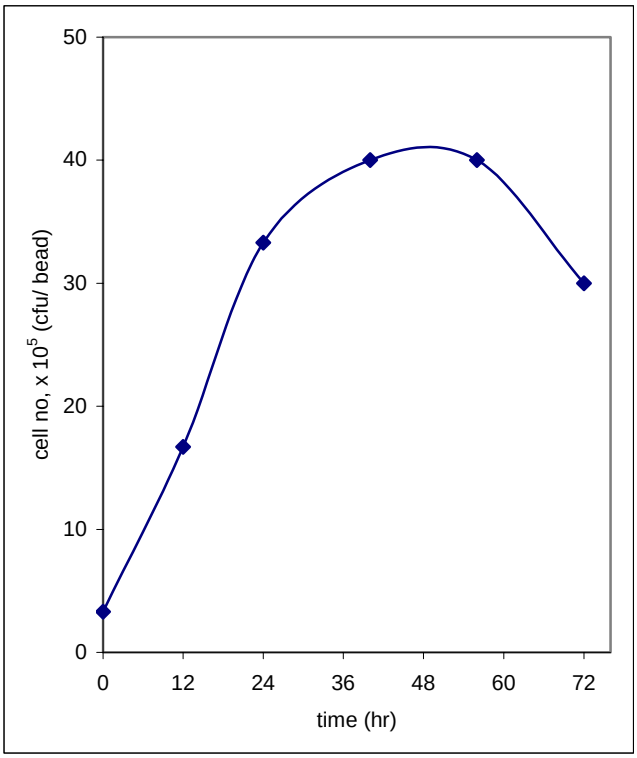


Figure D.5.1: Cell concentration for run 5

Table D.5.1: Data of cell concentration for run 5

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	16.7
24	33.3
40	40.0
56	40.0
72	30.0

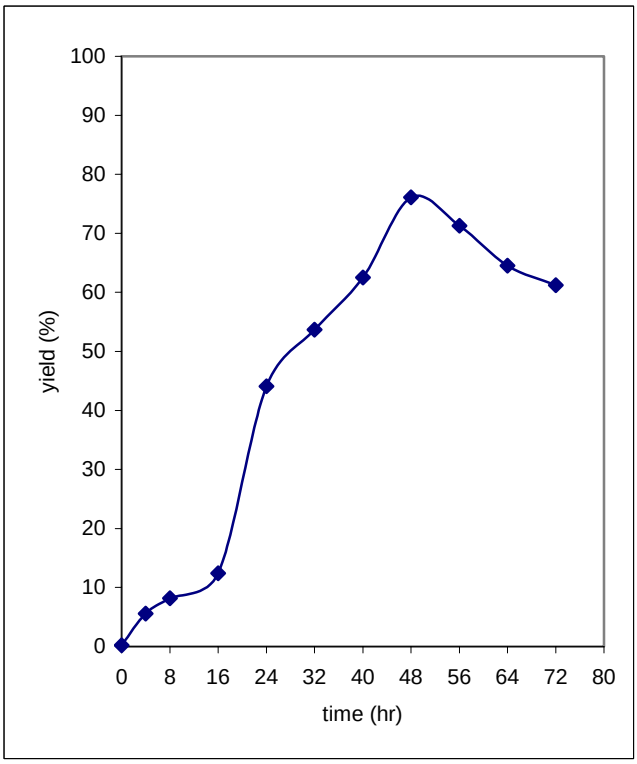


Figure D.5.2: Lactic acid production for run 1

Table D.5.2: Data of lactic acid production for run 5

Time (h)	Lactic acid production %
0	0.02
4	5.63
8	8.22
16	12.40
24	44.14
32	53.72
40	62.54
48	76.14
56	71.33
64	64.52
72	61.24

D.6 Experimental result for run 6

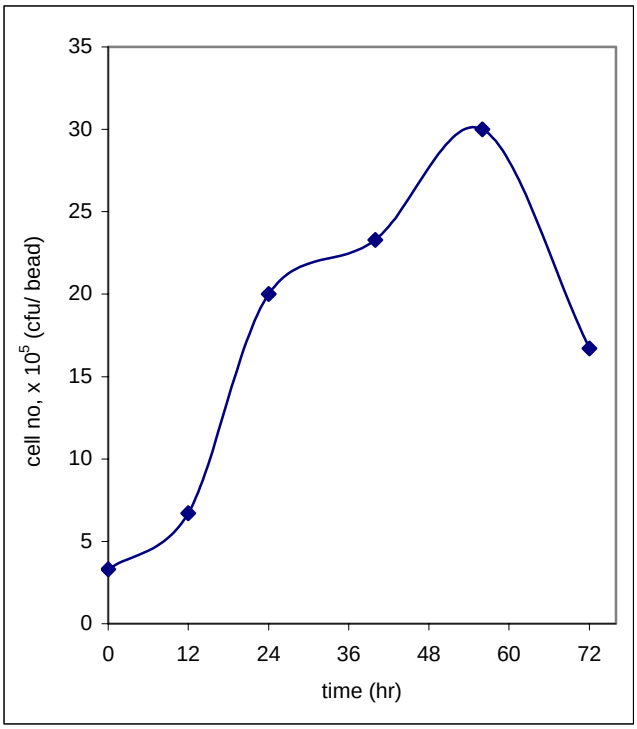


Figure D.6.1: Cell concentration for run 6

Table D.6.1: Data of cell concentration for run 6

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	20.0
40	23.3
56	30.0
72	16.7

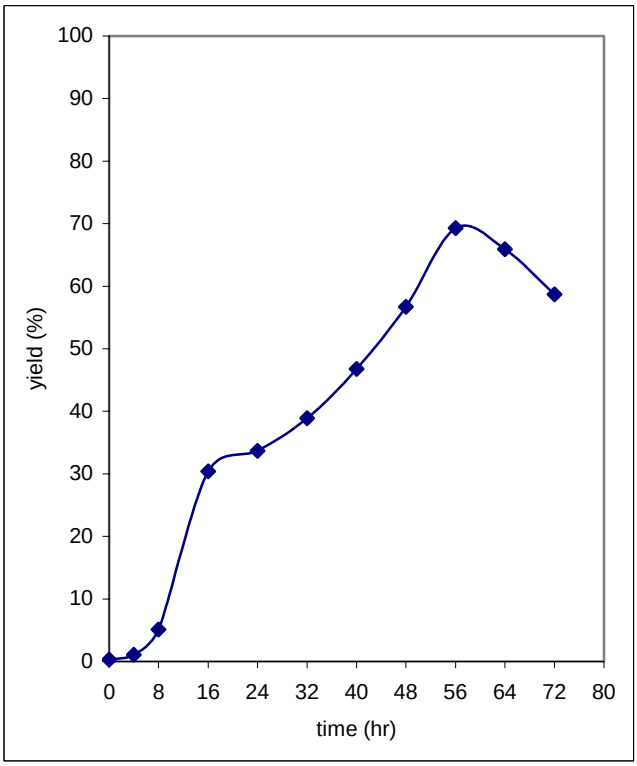


Figure D.6.2: Lactic acid production for run 6

Table D.6.2: Data of lactic acid production for run 6

Time (h)	Lactic acid production %
0	0.02
4	1.13
8	5.14
16	30.40
24	33.74
32	38.92
40	46.82
48	56.71
56	69.32
64	65.91
72	58.73

D.7 Experimental result for run 7

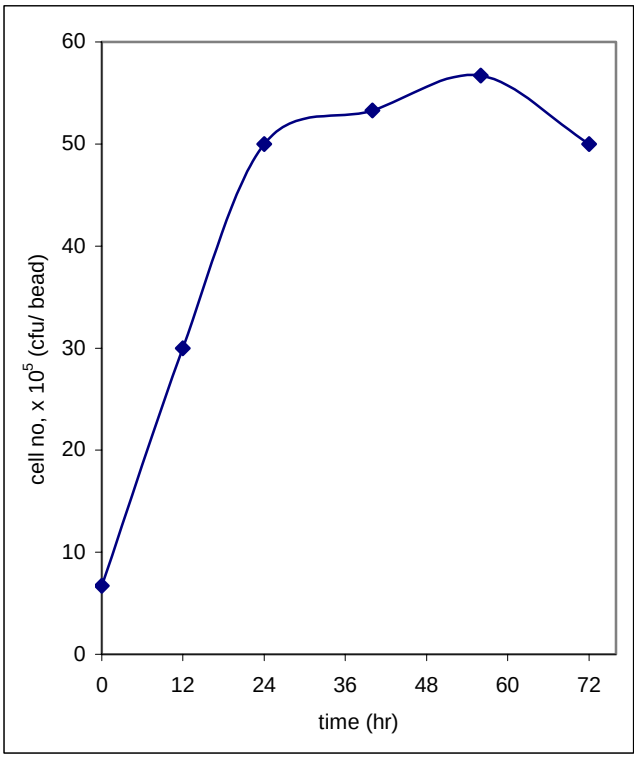


Figure D.7.1: Cell concentration for run 7

Table D.7.1: Data of cell concentration for run 7

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	30.0
24	50.0
40	53.3
56	56.7
72	50.0

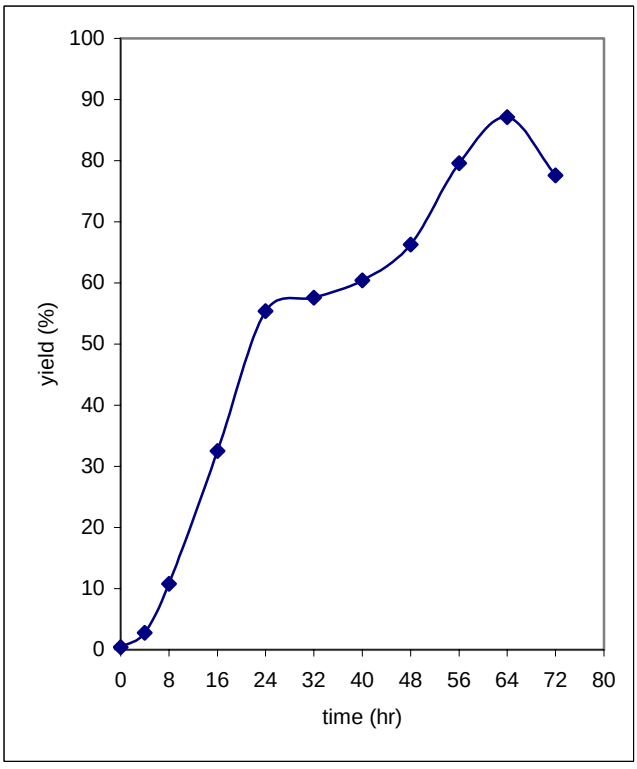


Figure D.7.2: Lactic acid production for run 7

Table D.7.2: Data of lactic acid production for run 7

Time (h)	Lactic acid production %
0	0.02
4	2.82
8	10.84
16	32.51
24	55.43
32	57.62
40	60.44
48	66.32
56	79.63
64	87.12
72	77.62

D.8 Experimental result for run 8

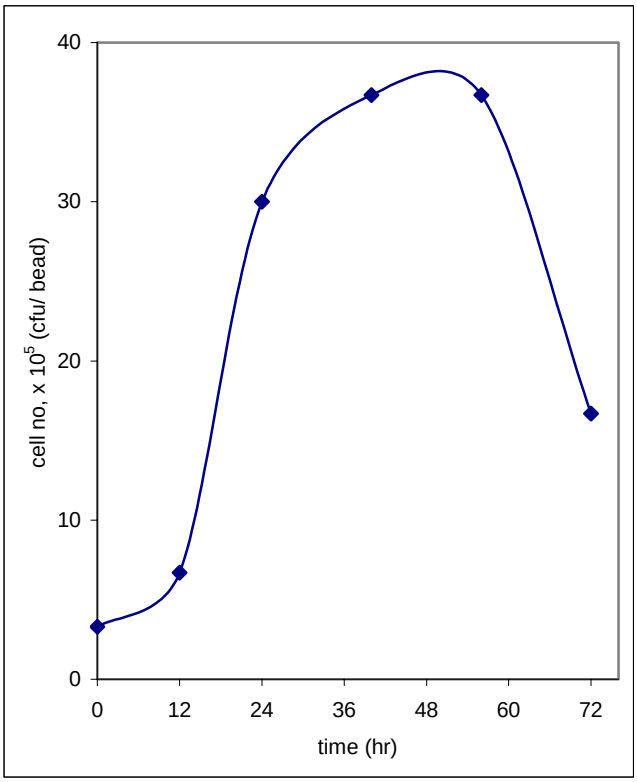


Figure D.8.1: Cell concentration for run 8

Table D.8.1: Data of cell concentration for run 8

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	30.0
40	36.7
56	36.7
72	16.7

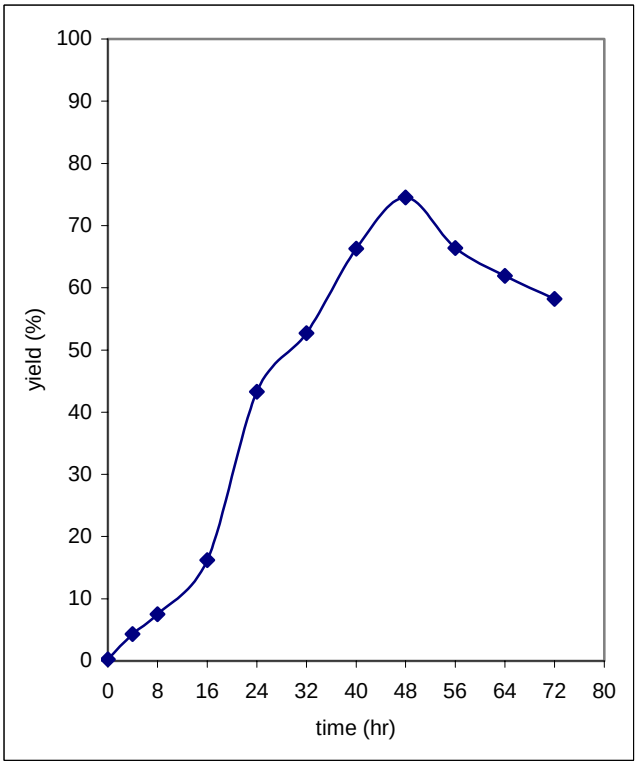


Figure D.8.2: Lactic acid production for run 8

Table D.8.2: Data of lactic acid production for run 8

Time (h)	Lactic acid production %
0	0.02
4	4.31
8	7.53
16	16.22
24	43.31
32	52.72
40	66.30
48	74.54
56	66.40
64	61.93
72	58.24

D.9 Experimental result for run 9

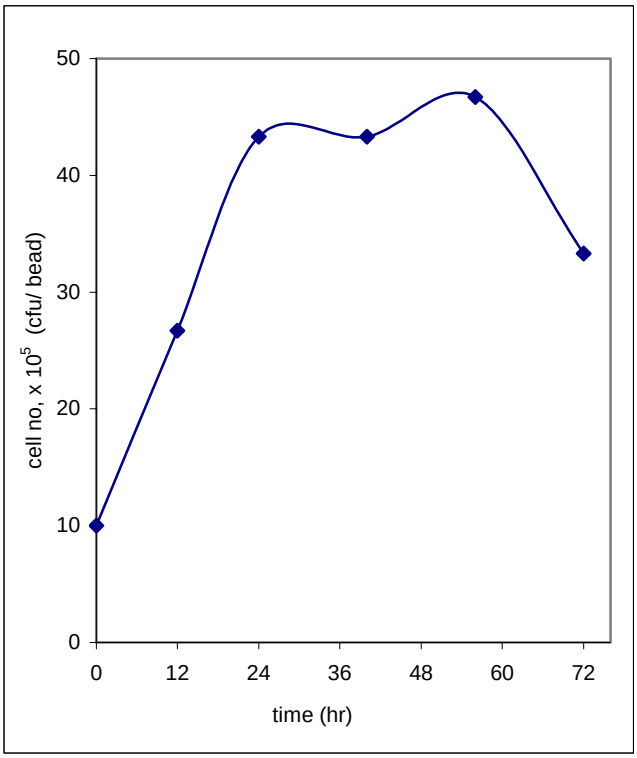


Figure D.9.1: Cell concentration for run 9

Table D.9.1: Data of cell concentration for run 9

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	10.0
12	26.7
24	43.3
40	43.3
56	46.7
72	33.3

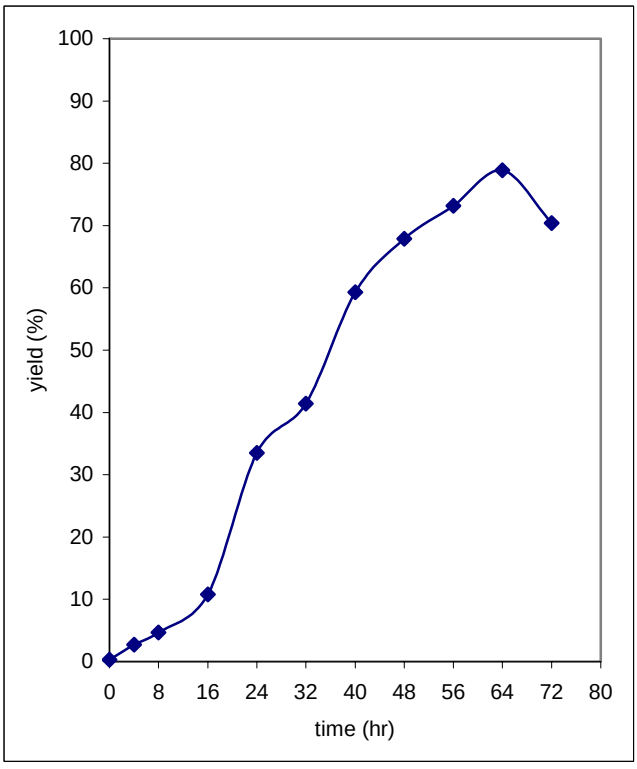


Figure D.9.2: Lactic acid production for run 9

Table D.9.2: Data of lactic acid production for run 9

Time (h)	Lactic acid production %
0	0.02
4	2.7
8	4.7
16	10.8
24	33.5
32	41.4
40	59.3
48	67.9
56	73.2
64	78.9
72	70.4

D.10 Experimental result for run 10

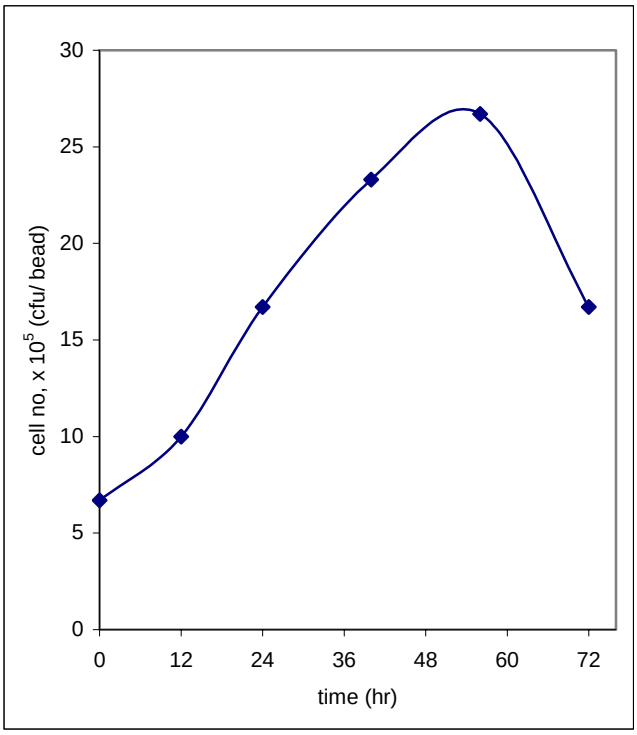


Figure D.10.1: Cell concentration for run 10

Table D.10.1: Data of cell concentration for run 10

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	16.7
40	23.3
56	26.7
72	16.7

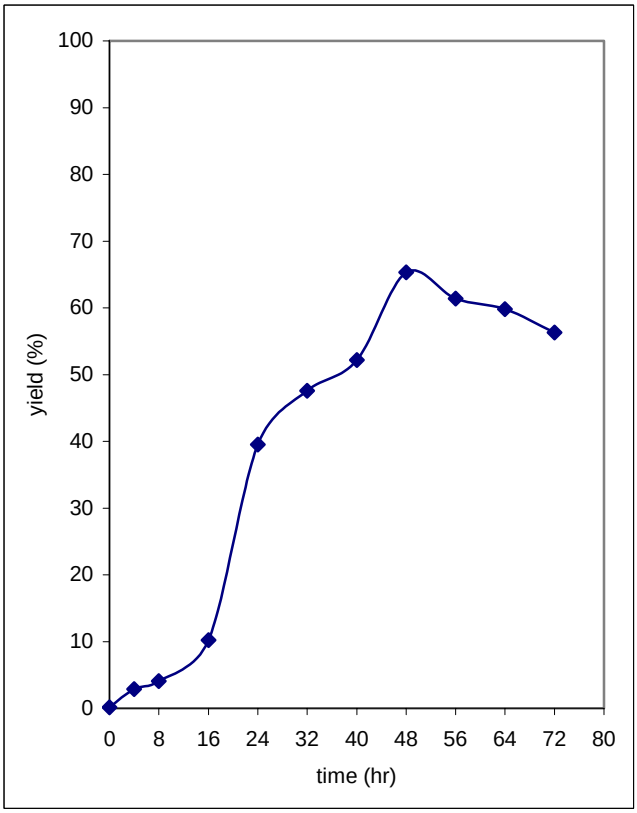


Figure D.10.2: Lactic acid production for run 10

Table D.10.2: Data of lactic acid production for run 10

Time (h)	Lactic acid production %
0	0.02
4	2.9
8	4.1
16	10.2
24	39.5
32	47.6
40	52.2
48	65.3
56	61.4
64	59.8
72	56.3

D.11 Experimental result for run 11

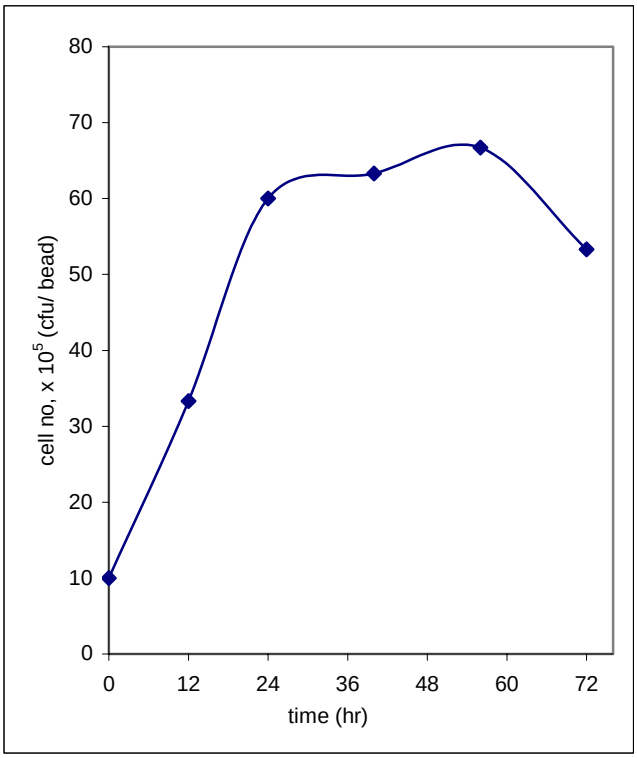


Figure D.11.1: Cell concentration for run 11

Table D.11.1: Data of cell concentration for run 11

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	10.0
12	33.3
24	60.3
40	63.3
56	66.7
72	53.3

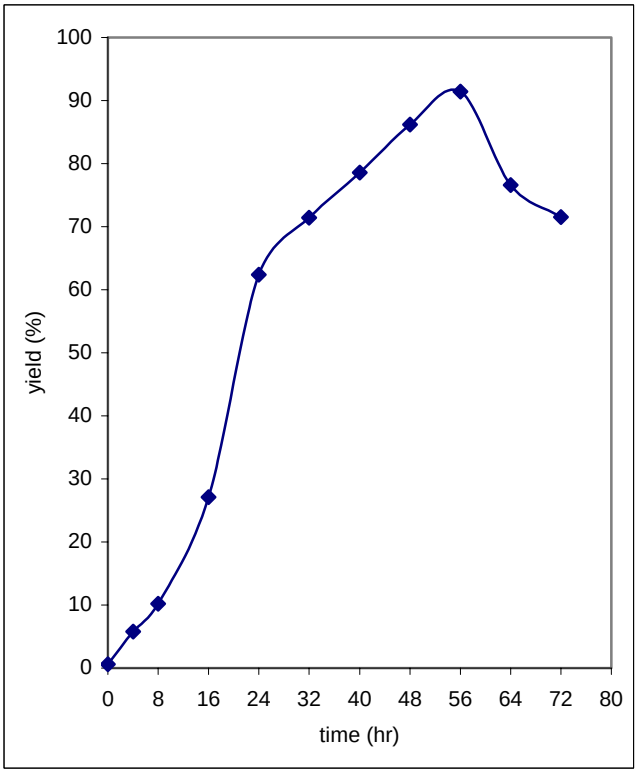


Figure D.11.2: Lactic acid production for run 11

Table D.11.2: Data of lactic acid production for run 11

Time (h)	Lactic acid production %
0	0.02
4	5.8
8	10.2
16	27.1
24	62.4
32	71.4
40	78.6
48	86.2
56	91.4
64	76.6
72	71.5

D.12 Experimental result for run 12

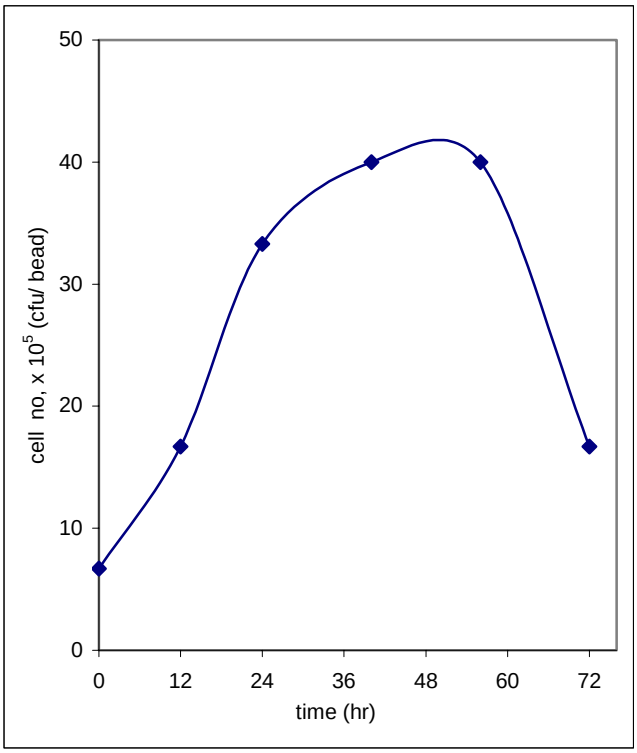


Figure D.12.1: Cell concentration for run 12

Table D.12.1: Data of cell concentration for run 12

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	16.7
24	33.3
40	40.0
56	40.0
72	16.7

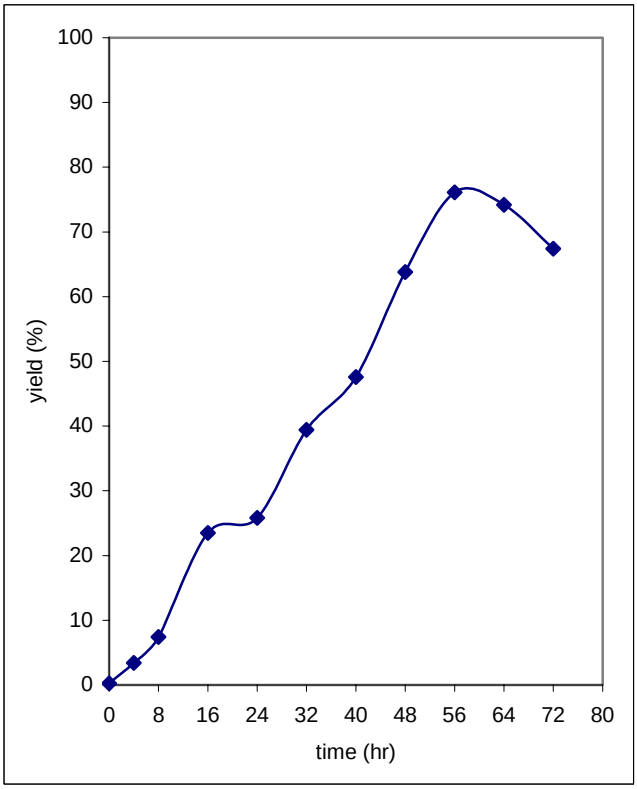


Figure D.12.2: Lactic acid production for run 12

Table D.12.2: Data of lactic acid production for run 12

Time (h)	Lactic acid production %
0	0.02
4	3.4
8	7.4
16	23.5
24	25.8
32	39.4
40	47.6
48	63.8
56	76.1
64	74.2
72	67.4

D.13 Experimental result for run 13

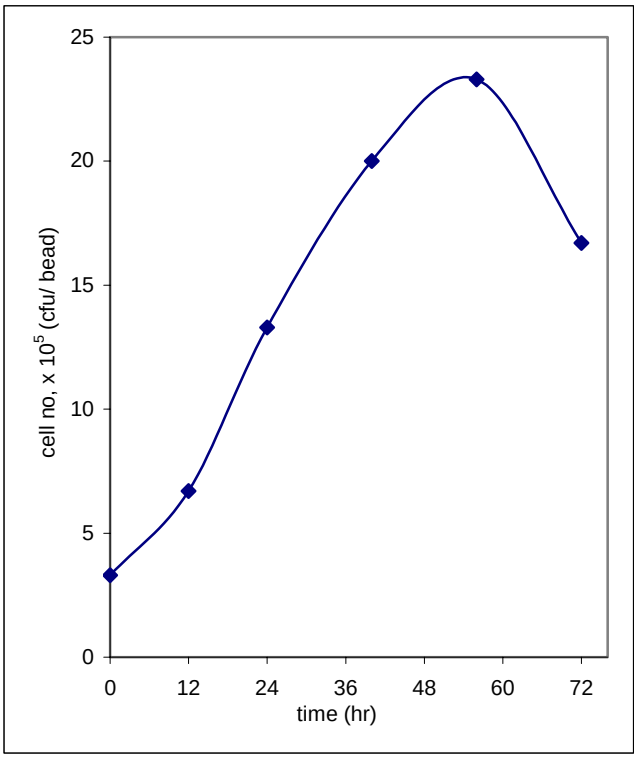


Figure D.13.1: Cell concentration for run 13

Table D.13.1: Data of cell concentration for run 13

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	13.3
40	20.0
56	23.3
72	16.7

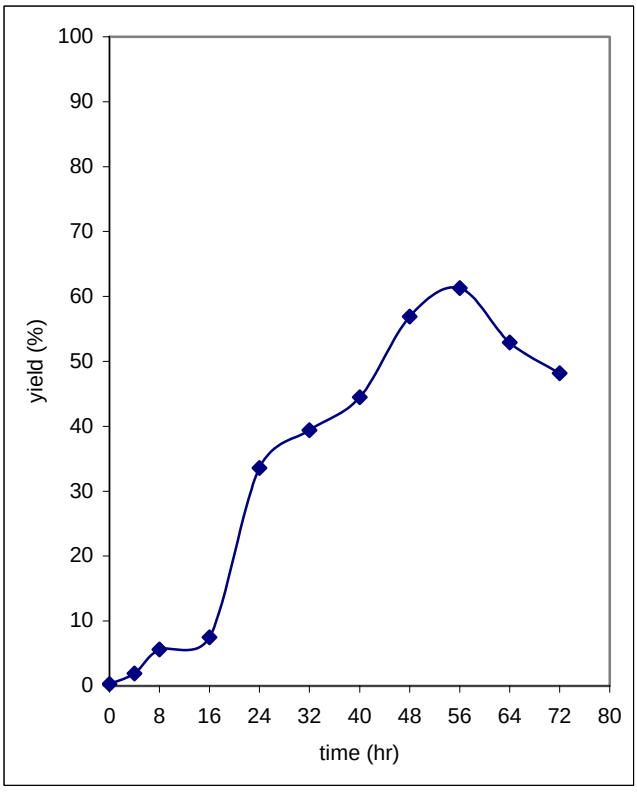


Figure D.13.2: Lactic acid production for run 13

Table D.13.2: Data of lactic acid production for run 13

Time (h)	Lactic acid production %
0	0.02
4	1.9
8	5.6
16	7.5
24	33.6
32	39.4
40	44.5
48	56.9
56	61.3
64	52.9
72	48.2

D.14 Experimental result for run 14

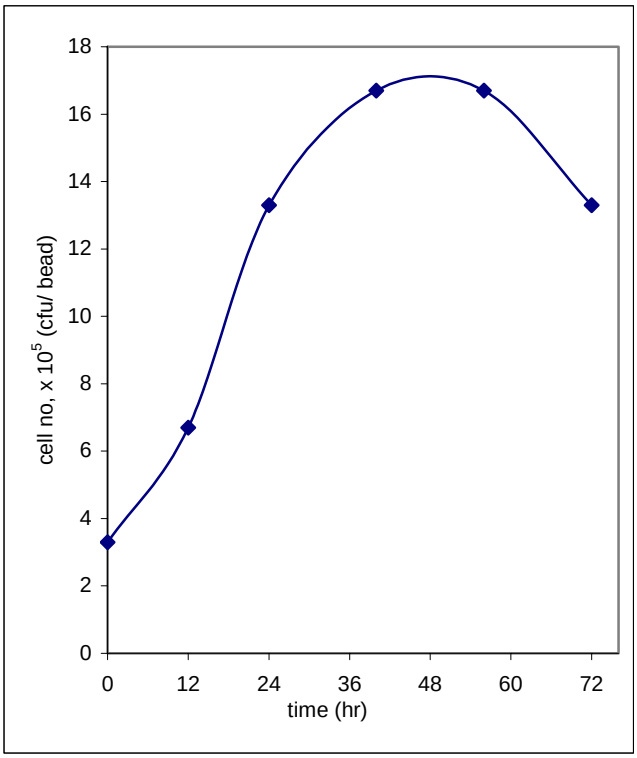


Figure D.14.1: Cell concentration for run 14

Table D.14.1: Data of cell concentration for run 14

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	13.3
40	16.7
56	16.7
72	13.3

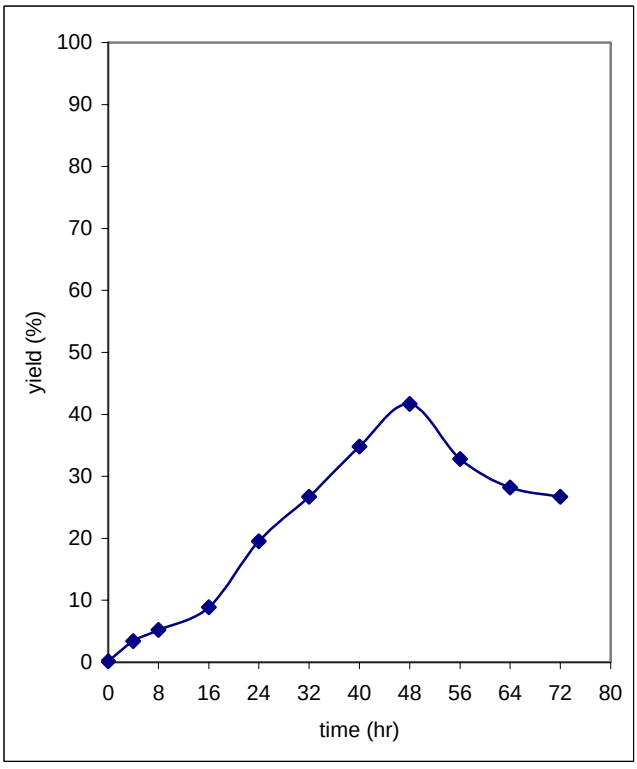


Figure D.14.2: Lactic acid production for run 14

Table D.14.2: Data of lactic acid production for run 14

Time (h)	Lactic acid production %
0	0.02
4	3.4
8	5.2
16	8.9
24	19.5
32	26.7
40	34.8
48	41.7
56	32.8
64	28.2
72	26.7

D.15 Experimental result for run 15

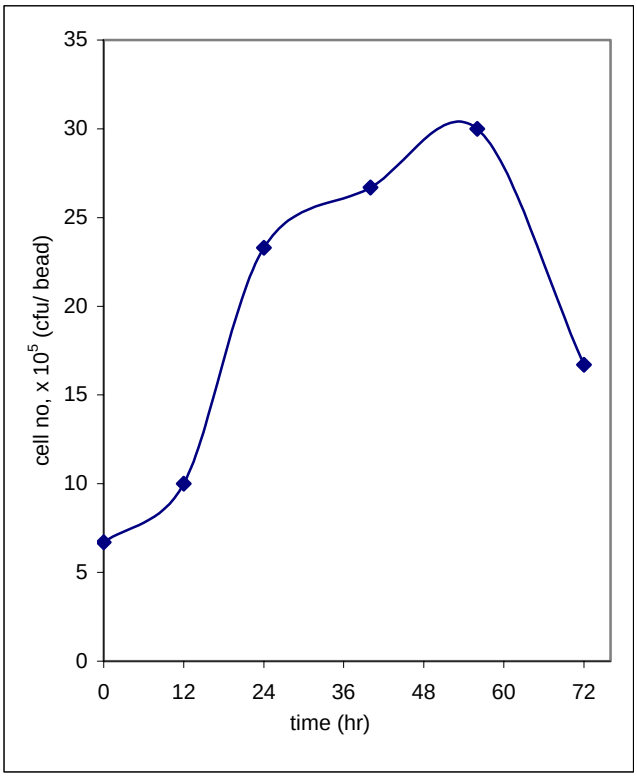


Figure D.15.1: Cell concentration for run 15

Table D.15.1: Data of cell concentration for run 15

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	23.3
40	26.7
56	30.0
72	16.7

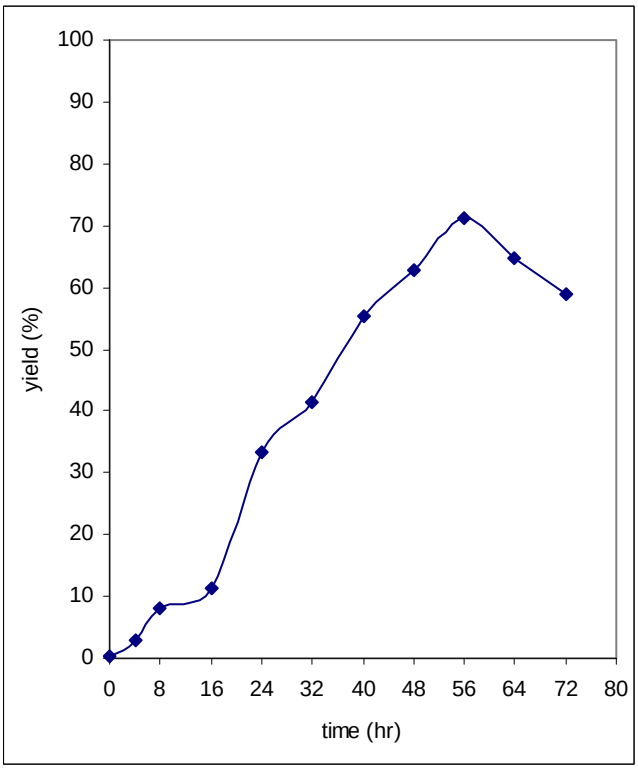


Figure D.15.2: Lactic acid production for run 15

Table D.15.2: Data of lactic acid production for run 15

Time (h)	Lactic acid production %
0	0.02
4	2.9
8	8.2
16	11.4
24	33.2
32	41.4
40	55.3
48	62.7
56	71.3
64	64.7
72	58.8

D.16 Experimental result for run 16

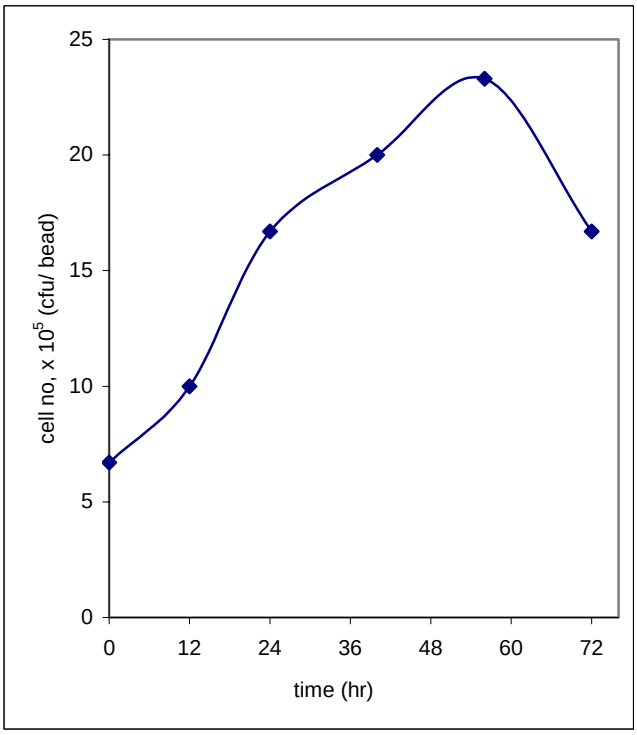


Figure D.16.1: Cell concentration for run 16

Table D.16.1: Data of cell concentration for run 16

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	16.7
40	20.0
56	23.3
72	16.7

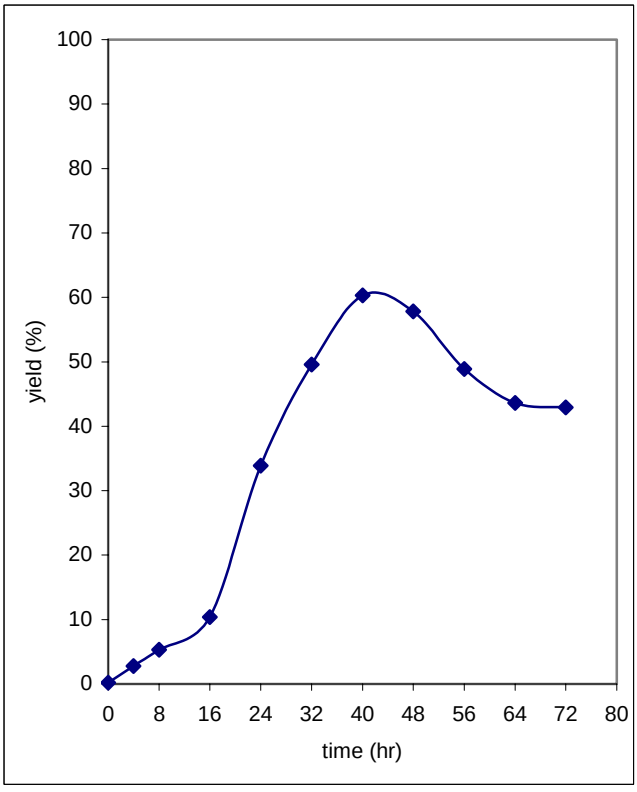


Figure D.16.2: Lactic acid production for run 16

Table D.16.2: Data of lactic acid production for run 16

Time (h)	Lactic acid production %
0	0.02
4	2.8
8	5.3
16	10.4
24	33.9
32	49.6
40	60.3
48	57.8
56	48.9
64	43.6
72	42.9

D.17 Experimental result for run 17

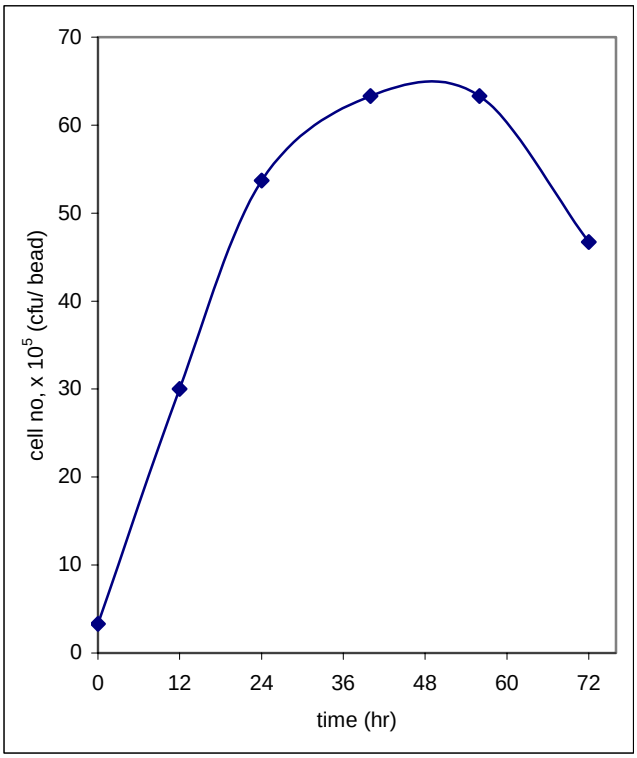


Figure D.17.1: Cell concentration for run 17

Table D.17.1: Data of cell concentration for run 17

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	30.0
24	53.7
40	63.3
56	63.3
72	46.7

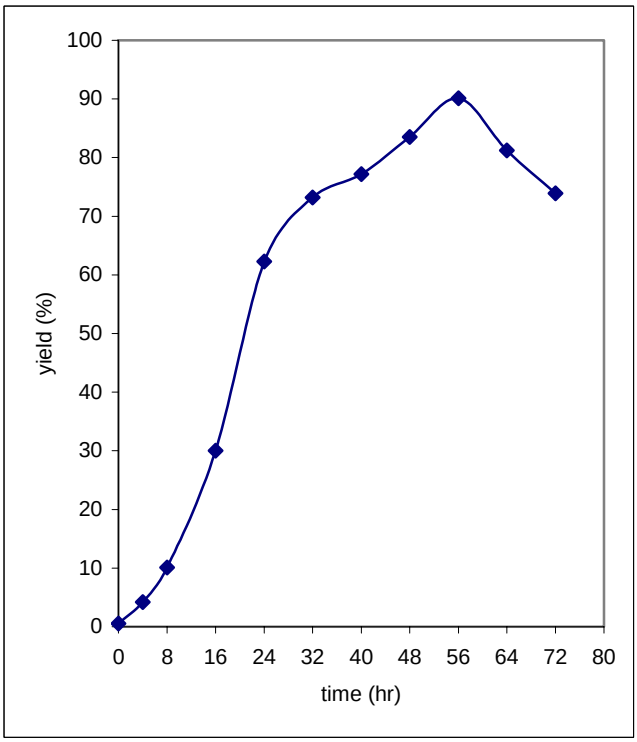


Figure D.17.2: Lactic acid production for run 17

Table D.17.2: Data of lactic acid production for run 17

Time (h)	Lactic acid production %
0	0.02
4	4.2
8	10.1
16	30.0
24	62.3
32	73.2
40	77.2
48	83.5
56	90.1
64	81.2
72	73.9

D.18 Experimental result for run 18

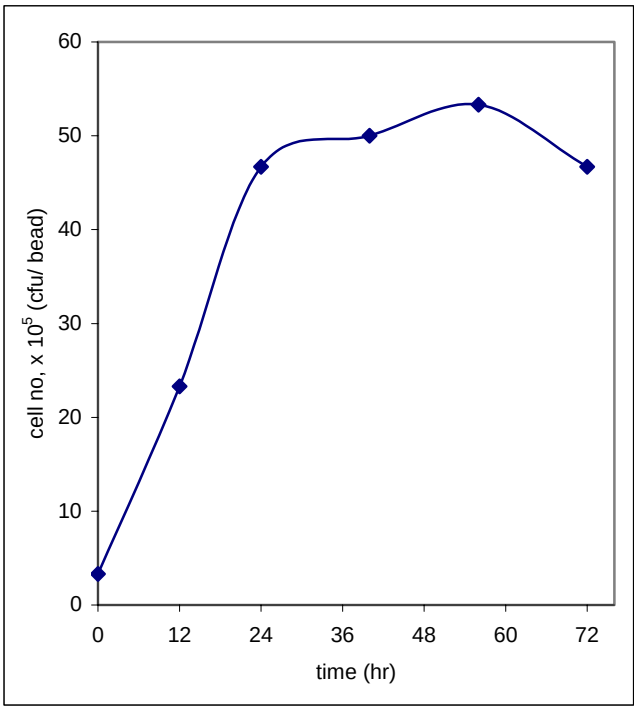


Figure D.18.1: Cell concentration for run 18

Table D.18.1: Data of cell concentration for run 18

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	23.3
24	46.7
40	50.0
56	53.3
72	46.7

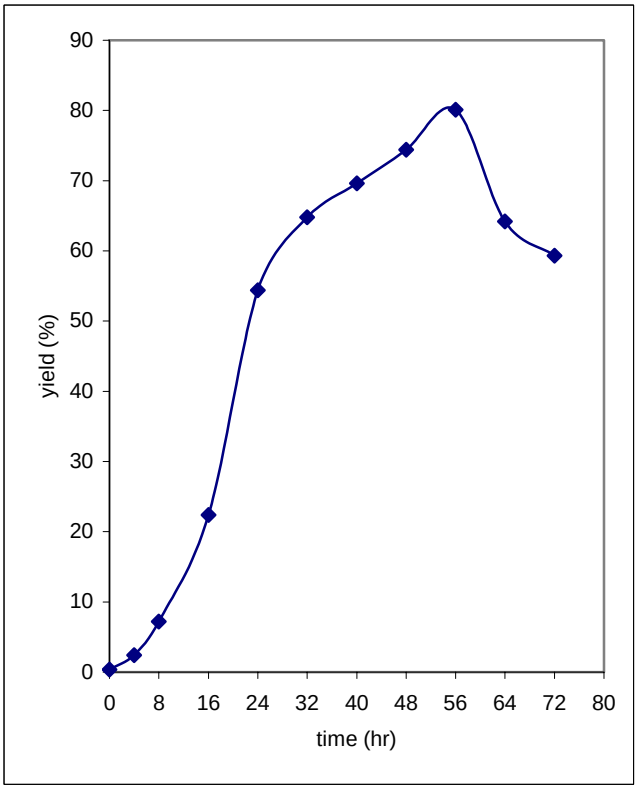


Figure D.18.2: Lactic acid production for run 18

Table D.18.2: Data of lactic acid production for run 18

Time (h)	Lactic acid production %
0	0.02
4	2.4
8	7.2
16	22.4
24	54.4
32	64.8
40	69.6
48	74.4
56	80.1
64	64.2
72	59.3

D.19 Experimental result for run 19

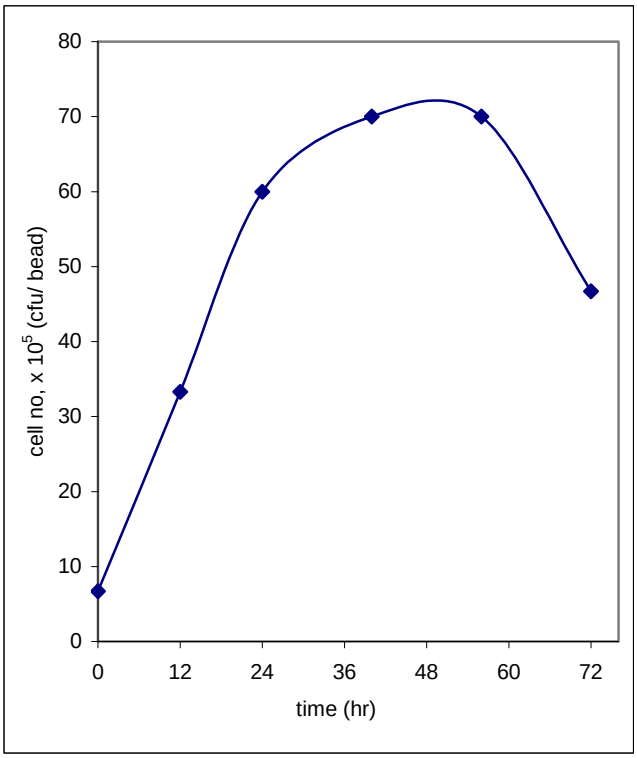


Figure D.19.1: Cell concentration for run 19

Table D.19.1: Data of cell concentration for run 19

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	33.3
24	60.0
40	70.0
56	70.0
72	46.7

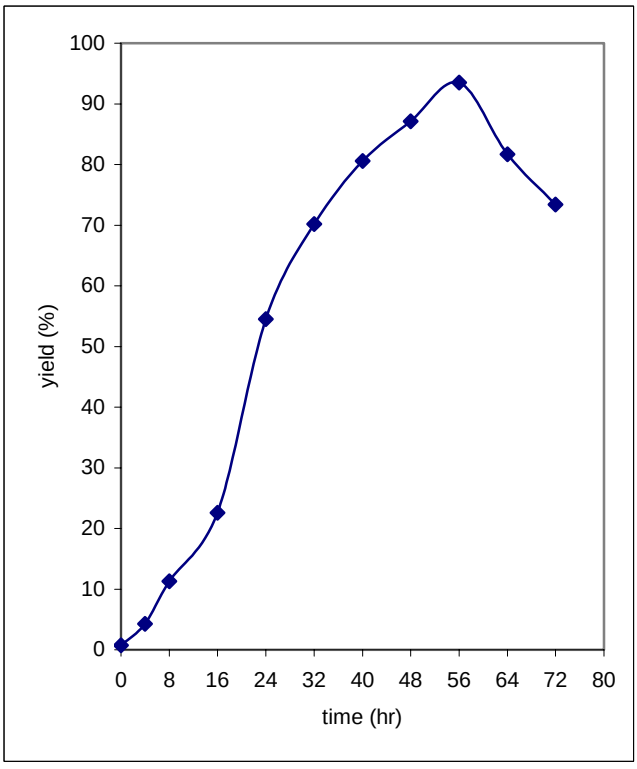


Figure D.19.2: Lactic acid production for run 19

Table D.19.2: Data of lactic acid production for run 19

Time (h)	Lactic acid production %
0	0.02
4	4.3
8	11.3
16	22.6
24	54.5
32	70.2
40	80.6
48	87.1
56	93.5
64	81.7
72	73.4

D.20 Experimental result for run 20

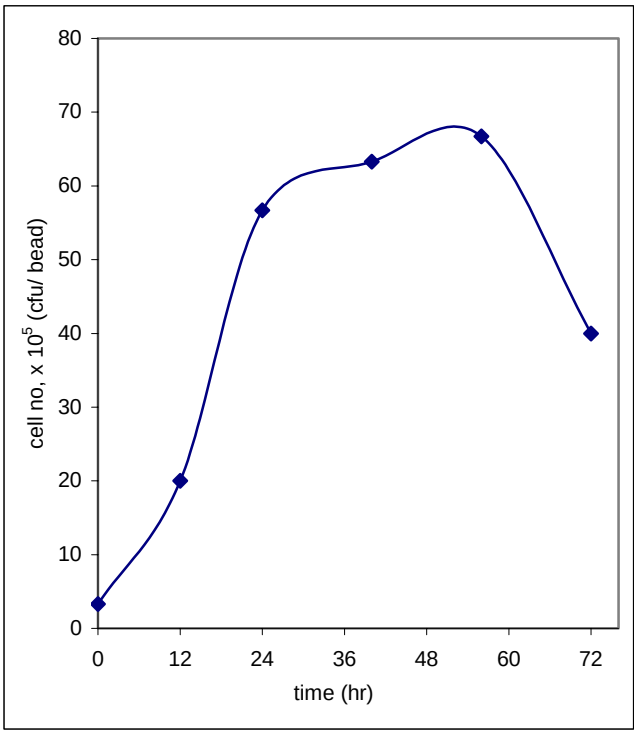


Figure D.20.1: Cell concentration for run 20

Table D.20.1: Data of cell concentration for run 20

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	20.0
24	56.7
40	63.3
56	66.7
72	40.0

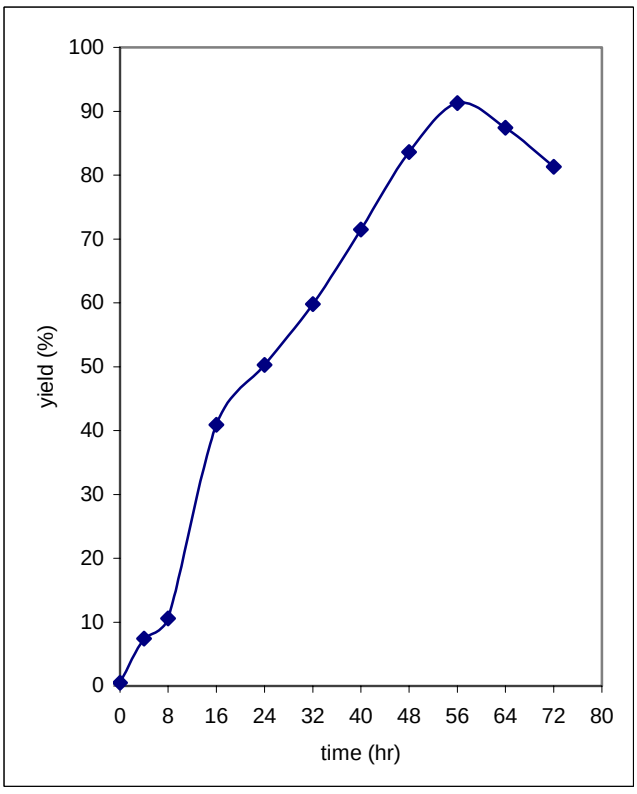


Figure D.20.2: Lactic acid production for run 20

Table D.20.2: Data of lactic acid production for run 20

Time (h)	Lactic acid production %
0	0.02
4	7.4
8	10.6
16	40.9
24	50.3
32	59.8
40	71.5
48	83.6
56	91.3
64	87.4
72	81.3

D.21 Experimental result for run 21

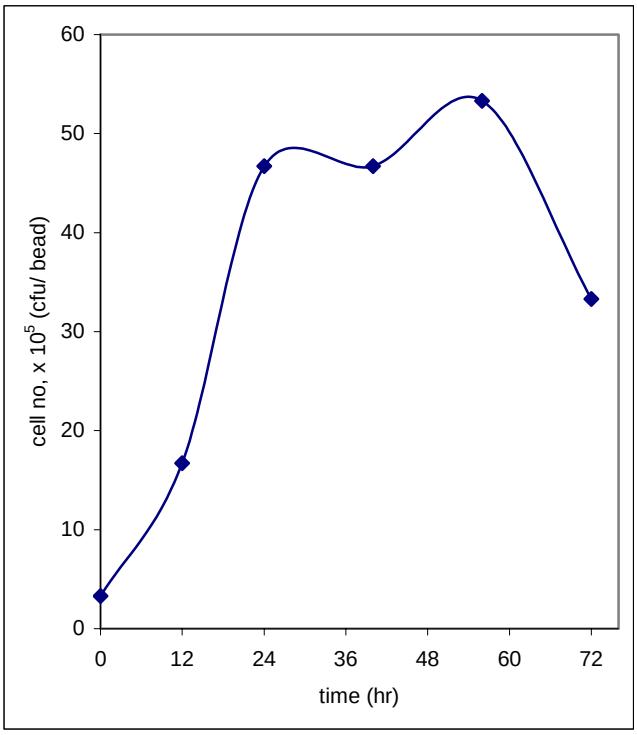


Figure D.21.1: Cell concentration for run 21

Table D.21.1: Data of cell concentration for run 21

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	16.7
24	46.7
40	46.7
56	53.3
72	33.3

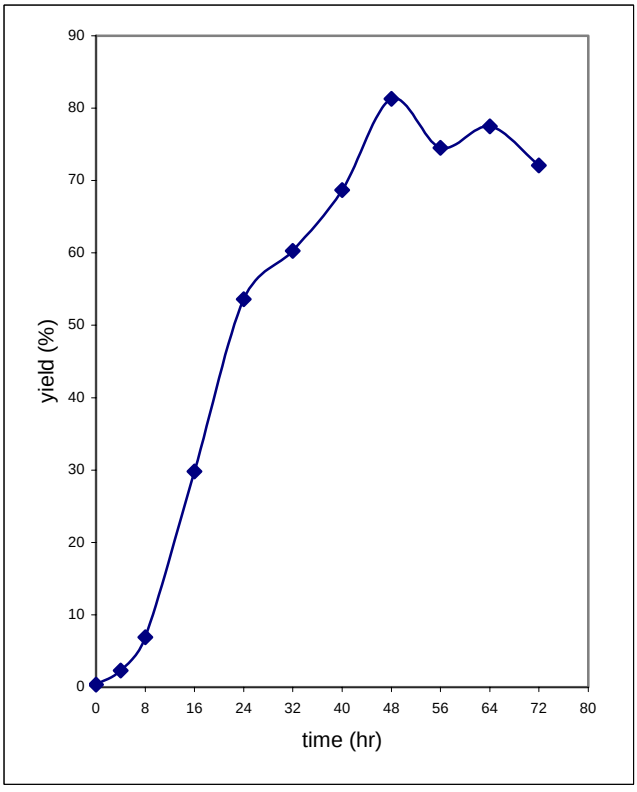


Figure D.21.2: Lactic acid production for run 21

Table D.21.2: Data of lactic acid production for run 21

Time (h)	Lactic acid production %
0	0.02
4	2.3
8	6.9
16	29.8
24	53.6
32	60.3
40	68.7
48	81.3
56	74.5
64	77.5
72	72.1

D.22 Experimental result for run 22

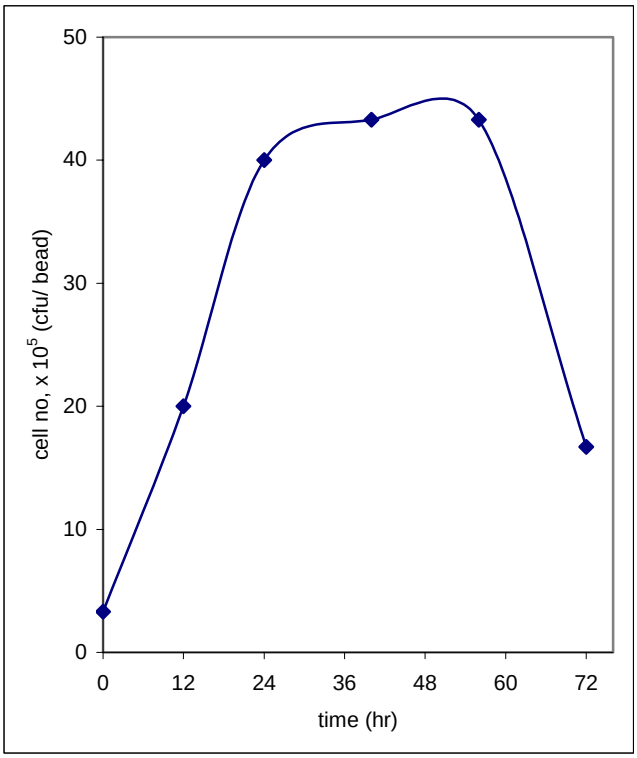


Figure D.22.1: Cell concentration for run 22

Table D.22.1: Data of cell concentration for run 22

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	20.0
24	40.0
40	43.3
56	43.3
72	16.7

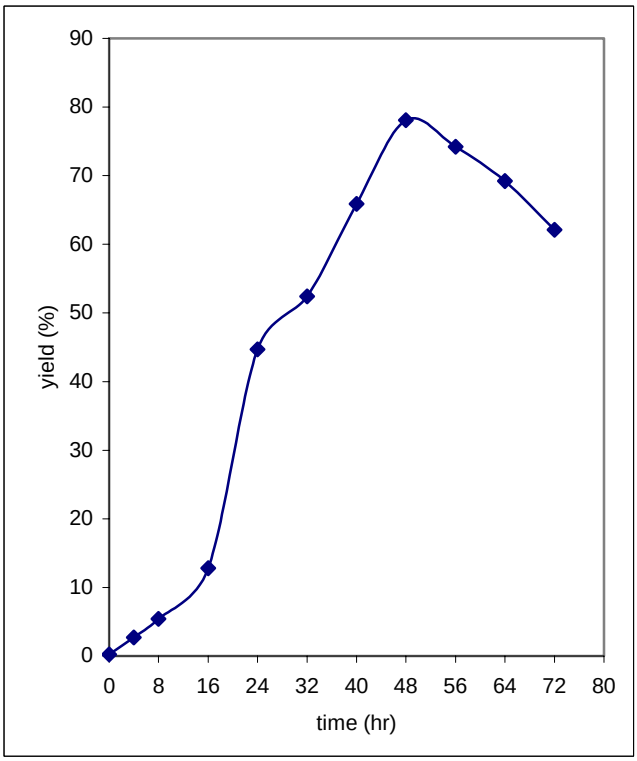


Figure D.22.2: Lactic acid production for run 22

Table D.22.2: Data of lactic acid production for run 22

Time (h)	Lactic acid production %
0	0.02
4	2.7
8	5.4
16	12.8
24	44.7
32	52.4
40	65.9
48	78.1
56	74.2
64	69.2
72	62.1

D.23 Experimental result for run 23

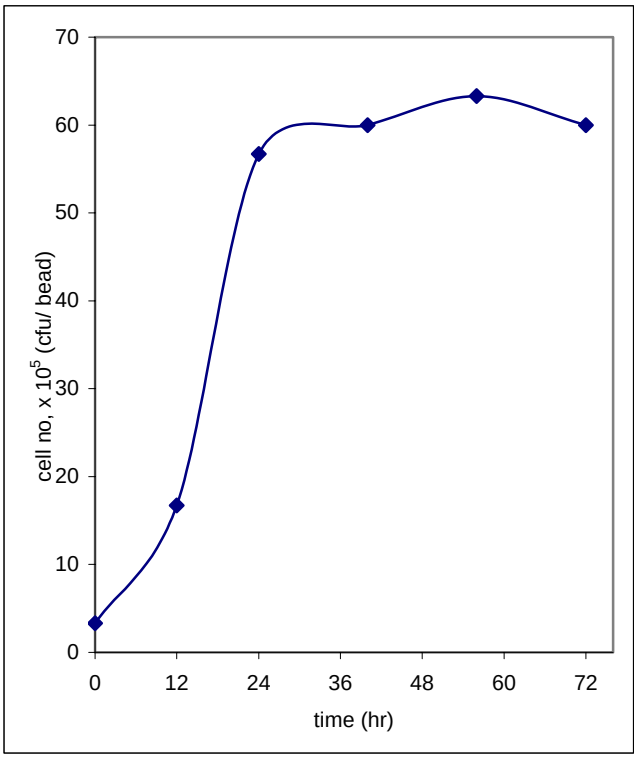


Figure D.23.1: Cell concentration for run 23

Table D.23.1: Data of cell concentration for run 23

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	20.0
24	40.0
40	43.3
56	43.3
72	16.7

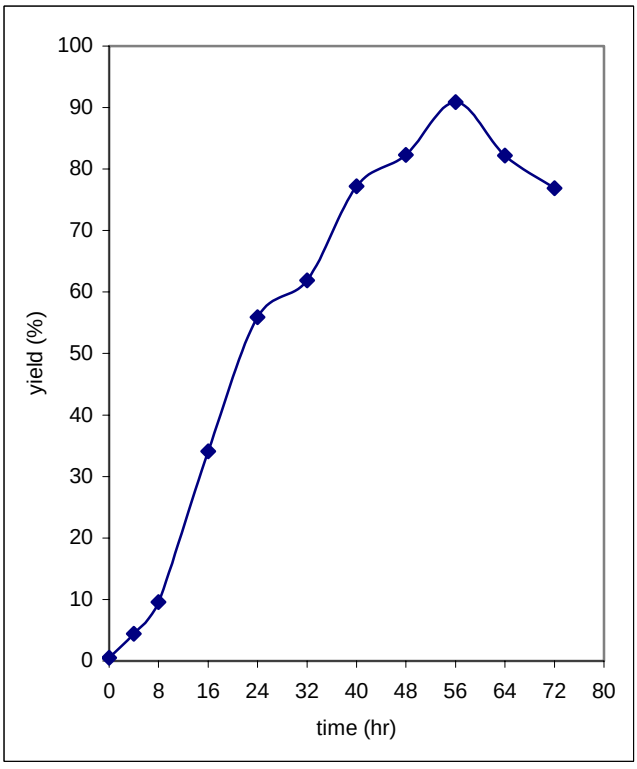


Figure D.23.2: Lactic acid production for run 23

Table D.23.2: Data of lactic acid production for run 23

Time (h)	Lactic acid production %
0	0.02
4	4.4
8	9.6
16	34.1
24	55.9
32	61.9
40	77.2
48	82.3
56	90.0
64	82.2
72	76.9

D.24 Experimental result for run 24

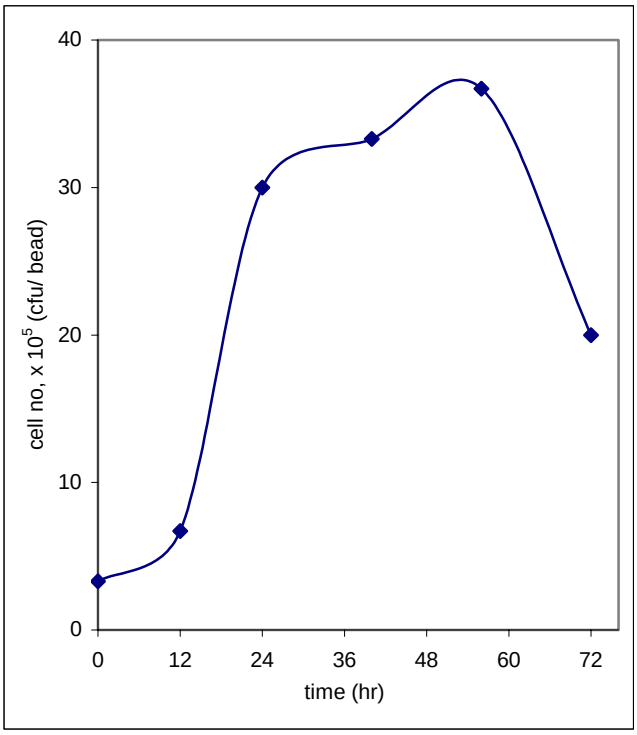


Figure D.24.1: Cell concentration for run 24

Table D.24.1: Data of cell concentration for run 24

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	30.0
40	33.3
56	36.7
72	20.0

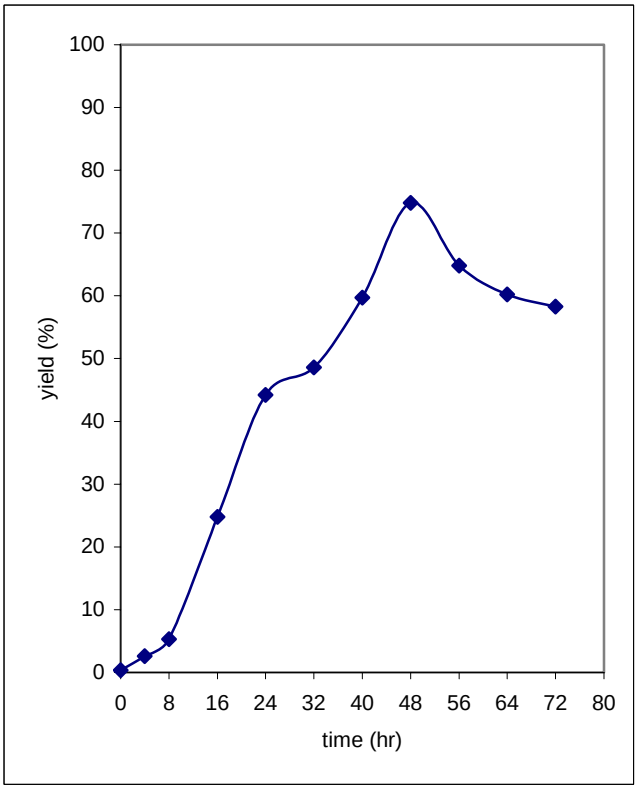


Figure D.24.2: Lactic acid production for run 24

Table D.24.2: Data of lactic acid production for run 24

Time (h)	Lactic acid production %
0	0.02
4	2.6
8	5.3
16	24.8
24	44.2
32	48.6
40	59.7
48	74.8
56	64.8
64	60.2
72	58.3

D.25 Experimental result for run 25

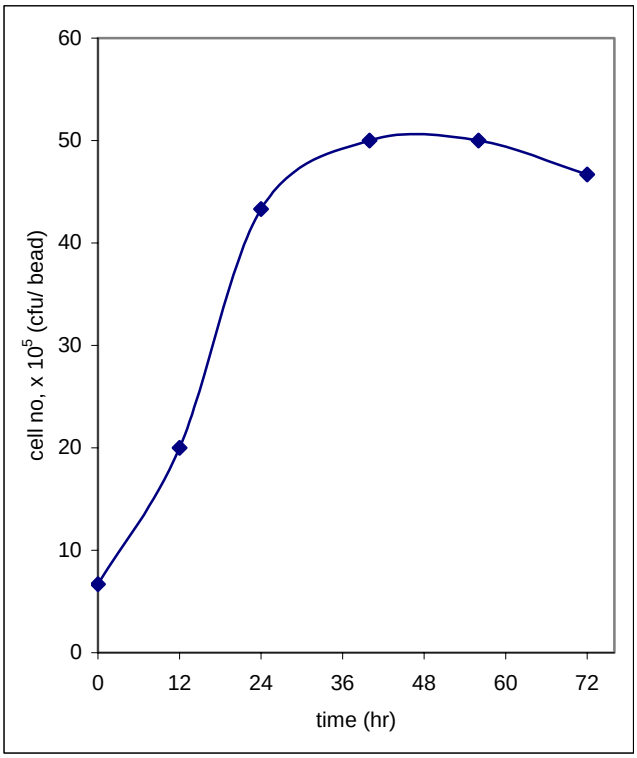


Figure D.25.1: Cell concentration for run 25

Table D.25.1: Data of cell concentration for run 25

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	20.0
24	43.3
40	50.0
56	50.0
72	46.7

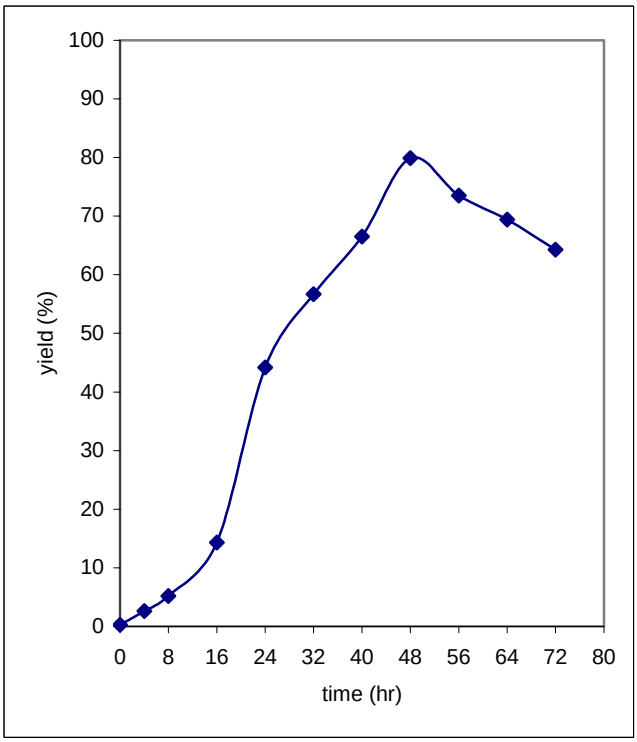


Figure D.25.2: Lactic acid production for run 25

Table D.25.2: Data of lactic acid production for run 25

Time (h)	Lactic acid production %
0	0.02
4	2.6
8	5.2
16	14.3
24	44.2
32	56.7
40	66.5
48	79.9
56	73.5
64	69.4
72	64.3

D.26 Experimental result for run 26

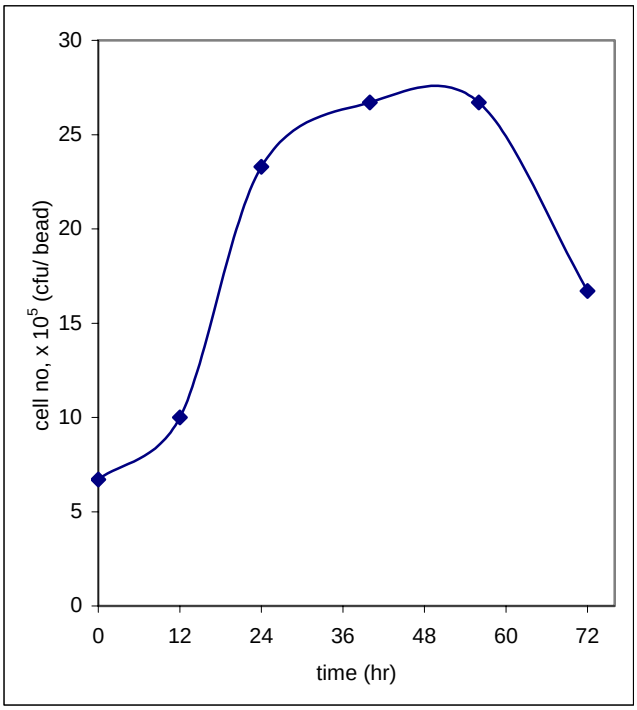
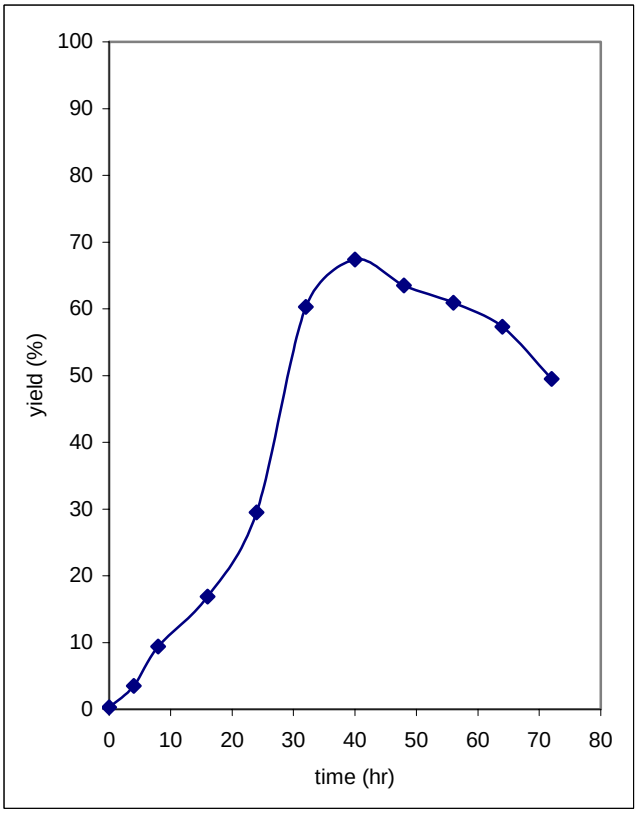


Figure D.26.1: Cell concentration for run 26

Table D.26.1: Data of cell concentration for run 26

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	23.3
40	26.7
56	26.7
72	16.7



igure D.26.2: Lactic acid production for run 26

Table D.26.2: Data of lactic acid production for run 26

Time (h)	Lactic acid production %
0	0.02
4	3.5
8	9.4
16	16.9
24	29/5
32	60.3
40	67.4
48	63.5
56	60.9
64	57.3
72	49.5

D.27 Experimental result for run 27

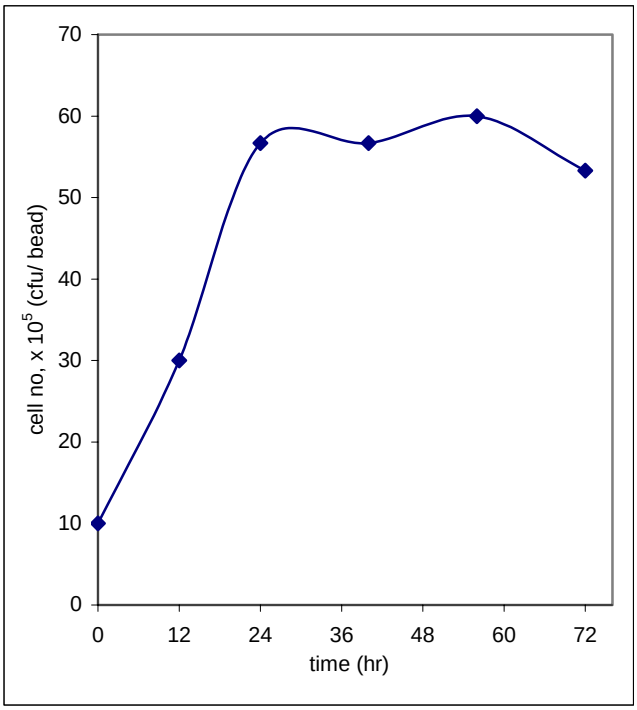


Figure D.27.1: Cell concentration for run 27

Table D.27.1: Data of cell concentration for run 27

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	10.0
12	30.0
24	56.7
40	56.7
56	60.0
72	53.3

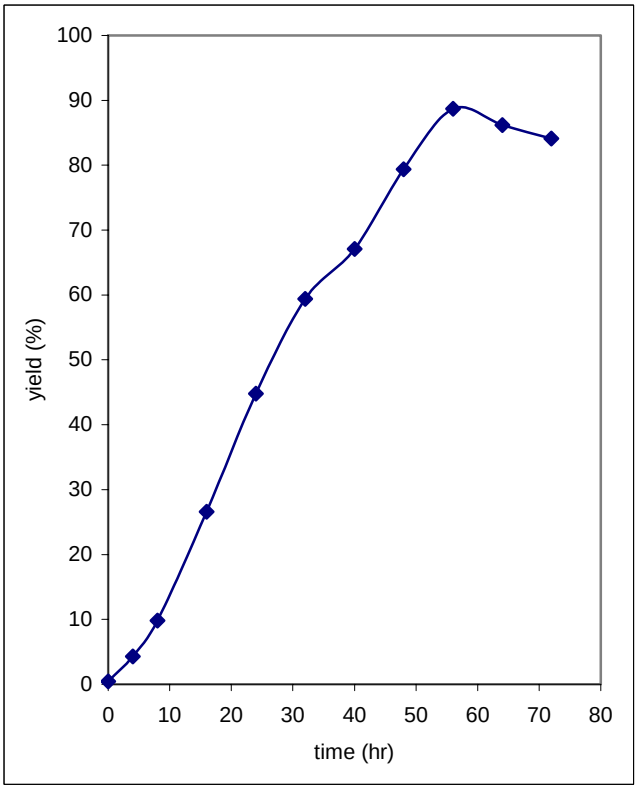


Figure D.27.2: Lactic acid production for run 27

Table D.27.2: Data of lactic acid production for run 27

Time (h)	Lactic acid production %
0	0.02
4	4.3
8	9.8
16	26.6
24	44.8
32	59.4
40	67.1
48	79.4
56	88.7
64	86.2
72	84.1

D.28 Experimental result for run 28

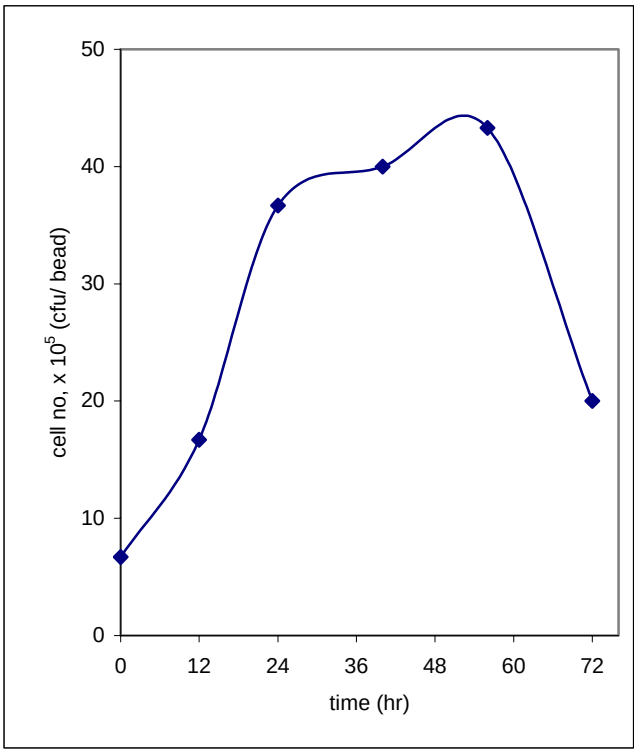


Figure D.28.1: Cell concentration for run 28

Table D.28.1: Data of cell concentration for run 28

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	16.7
24	36.7
40	40.0
56	43.3
72	20.0

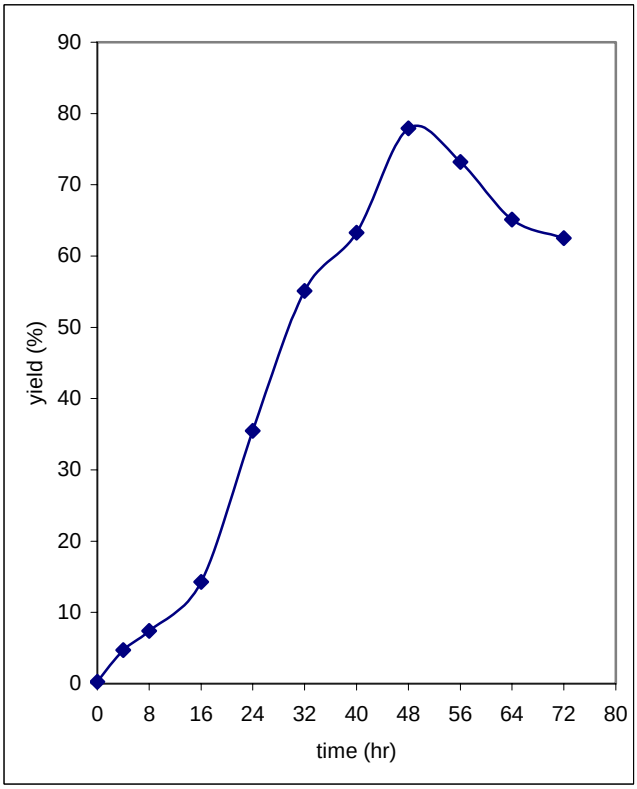


Figure D.28.2: Lactic acid production for run 28

Table D.28.2: Data of lactic acid production for run 28

Time (h)	Lactic acid production %
0	0.02
4	4.7
8	7.4
16	14.3
24	35.5
32	55.1
40	63.3
48	77.9
56	73.2
64	65.1
72	62.5

D.29 Experimental result for run 29

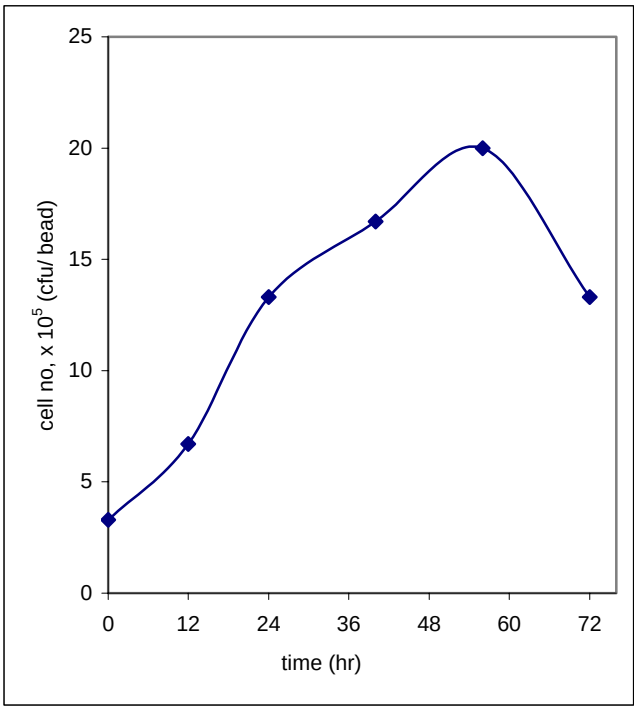


Figure D.29.1: Cell concentration for run 29

Table D.29.1: Data of cell concentration for run 29

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	3.3
12	6.7
24	13.3
40	16.7
56	20.0
72	13.3

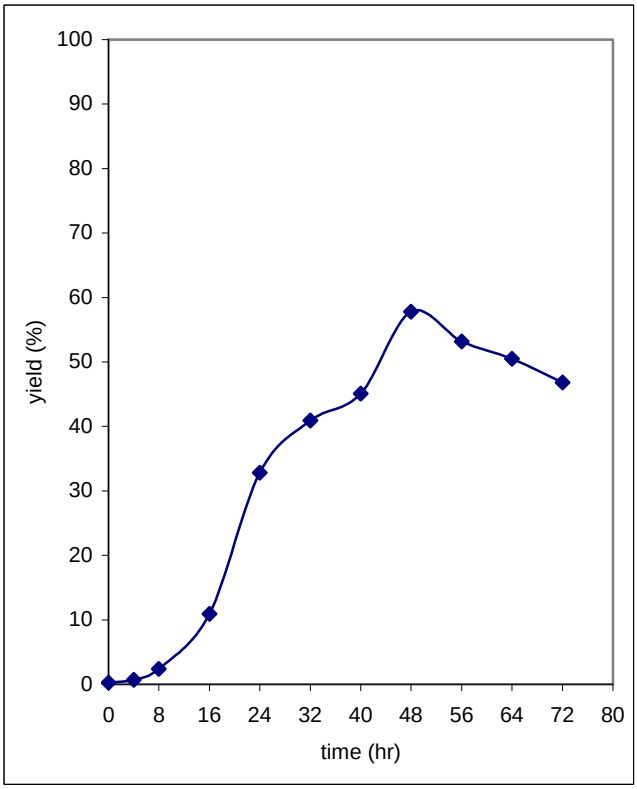


Figure D.29.2: Lactic acid production for run 29

Table D.29.2: Data of lactic acid production for run 29

Time (h)	Lactic acid production %
0	0.02
4	0.7
8	2.4
16	10.9
24	32.8
32	40.9
40	45.1
48	57.8
56	53.2
64	50.5
72	46.8

D.30 Experimental result for run 30

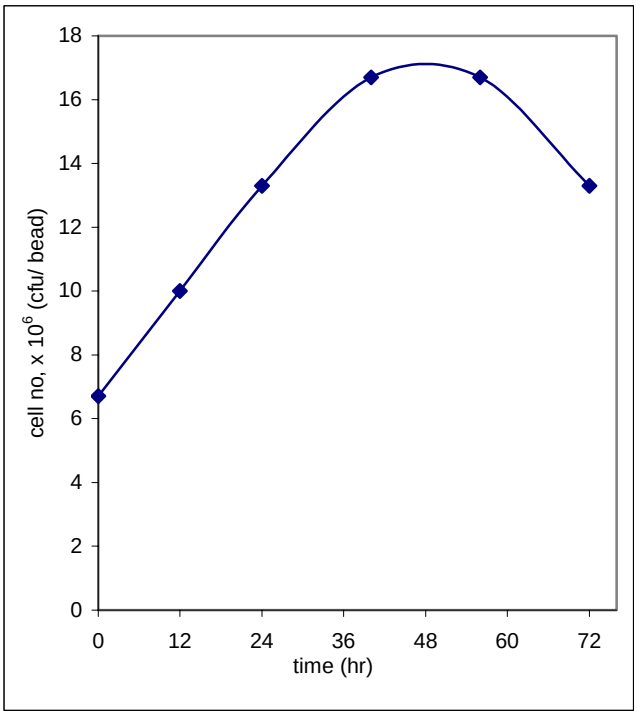


Figure D.30.1: Cell concentration for run 30

Table D.30.1: Data of cell concentration for run 30

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	13.3
40	16.7
56	16.7
72	13.3

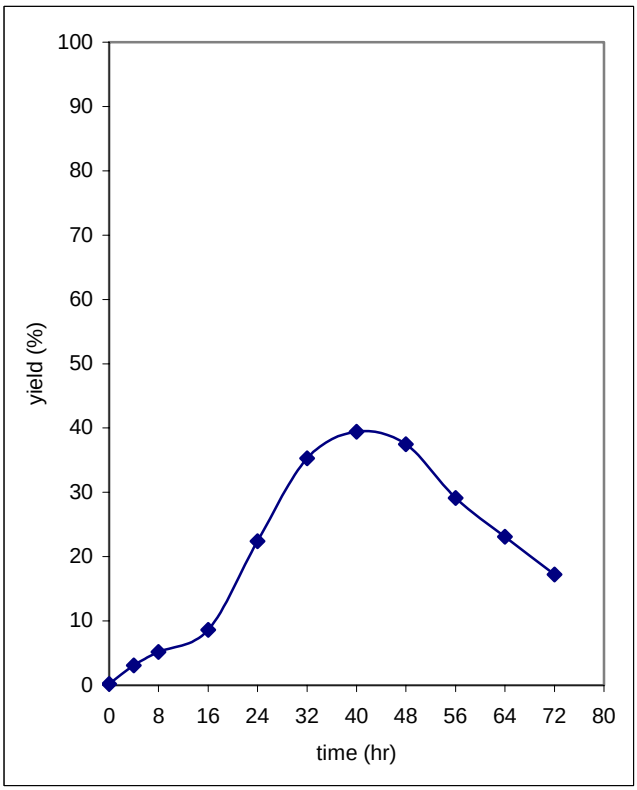


Figure D.30.2: Lactic acid production for run 30

Table D.30.2: Data of lactic acid production for run 30

Time (h)	Lactic acid production %
0	0.02
4	3.1
8	5.2
16	8.6
24	22.4
32	35.3
40	39.4
48	37.5
56	29.1
64	23.1
72	17.2

D.31 Experimental result for run 31

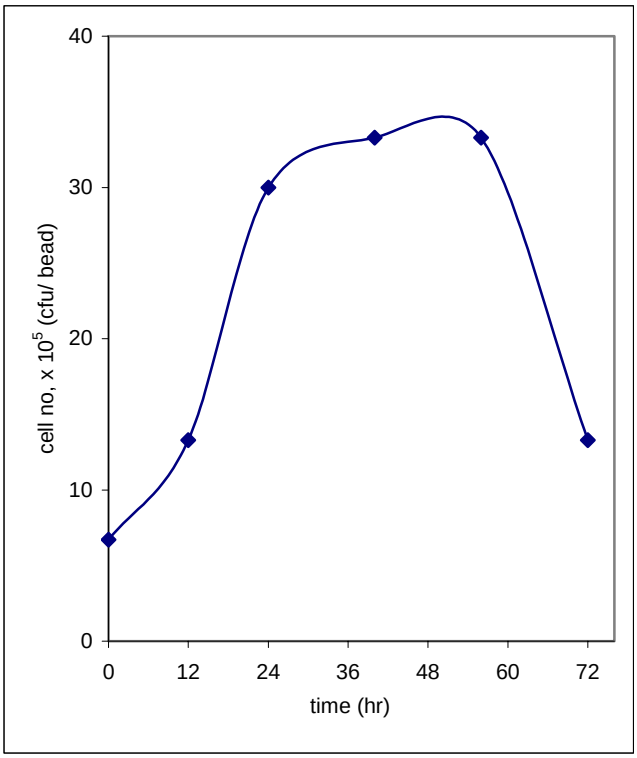


Figure D.31.1: Cell concentration for run 31

Table D.31.1: Data of cell concentration for run 31

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	13.3
24	30.0
40	33.3
56	33.3
72	13.3

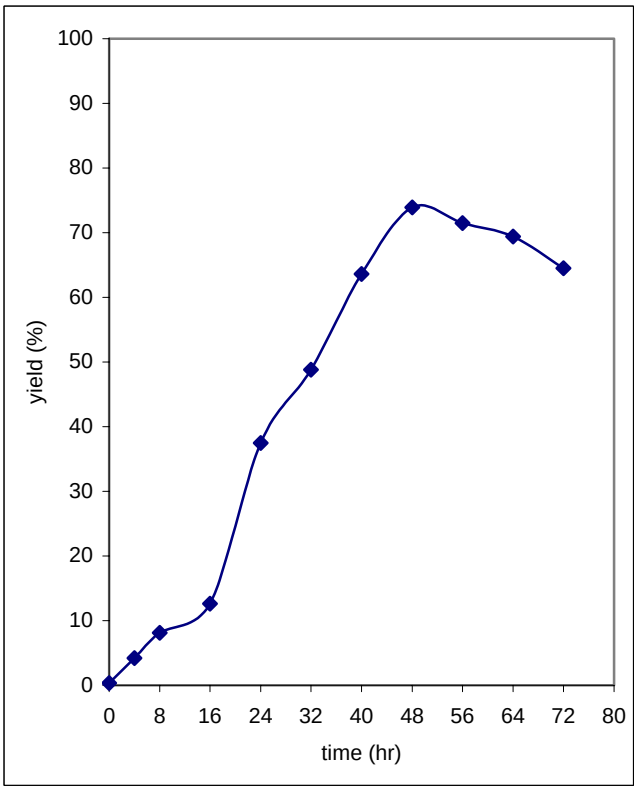


Figure D.31.2: Lactic acid production for run 31

Table D.31.2: Data of lactic acid production for run 31

Time (h)	Lactic acid production %
0	0.02
4	4.2
8	8.1
16	12.6
24	37.5
32	48.8
40	63.6
48	73.9
56	71.5
64	69.4
72	64.5

D.32 Experimental result for run 32

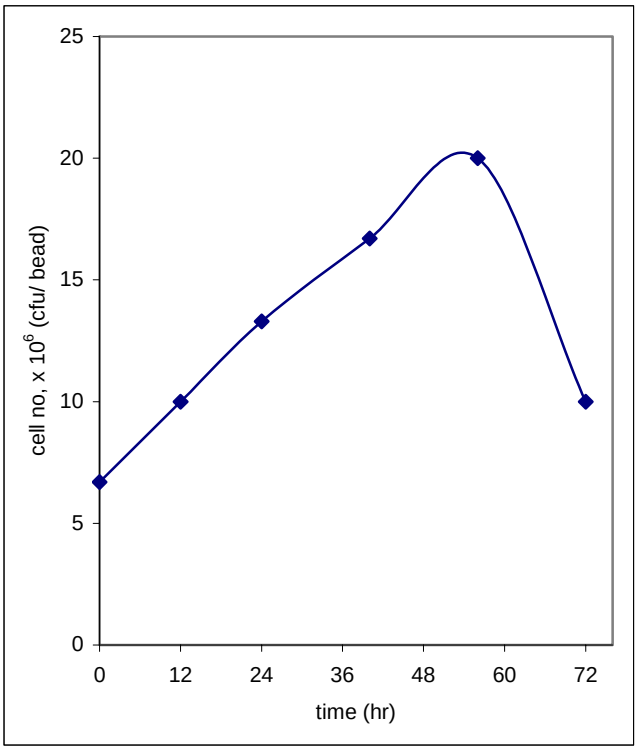


Figure D.32.1: Cell concentration for run 32

Table D.32.1: Data of cell concentration for run 32

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	10.0
24	13.3
40	16.7
56	20.0
72	10.0

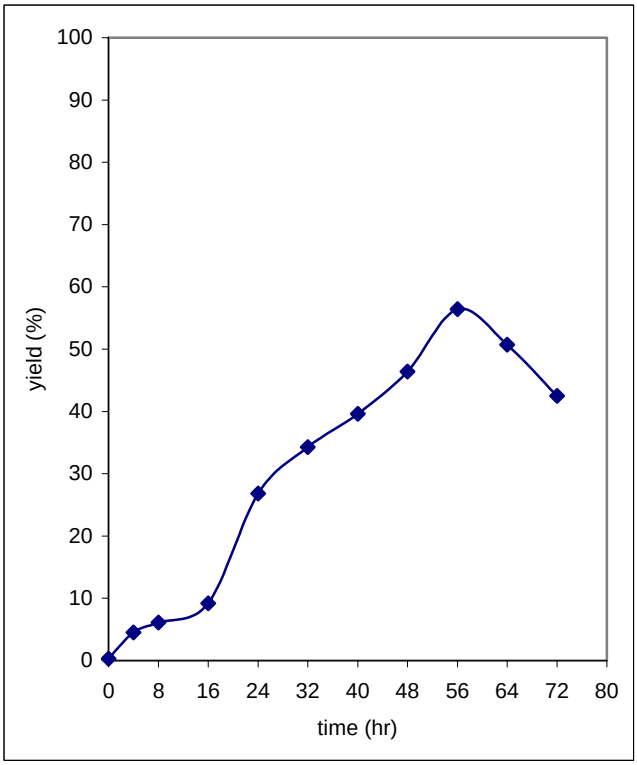


Figure D.32.2: Lactic acid production for run 32

Table D.32.2: Data of lactic acid production for run 32

Time (h)	Lactic acid production %
0	0.02
4	4.5
8	6.1
16	9.2
24	26.8
32	34.3
40	39.6
48	46.4
56	56.4
64	50.7
72	42.5

D.33 Experimental result for run 33

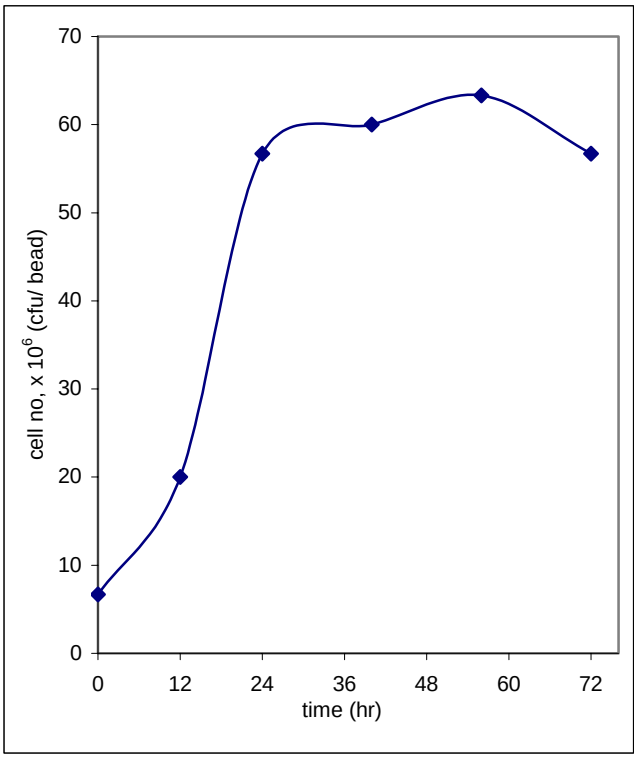


Figure D.33.1: Cell concentration for run 33

Table D.33.1: Data of cell concentration for run 33

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	20.0
24	56.7
40	60.0
56	63.3
72	56.7

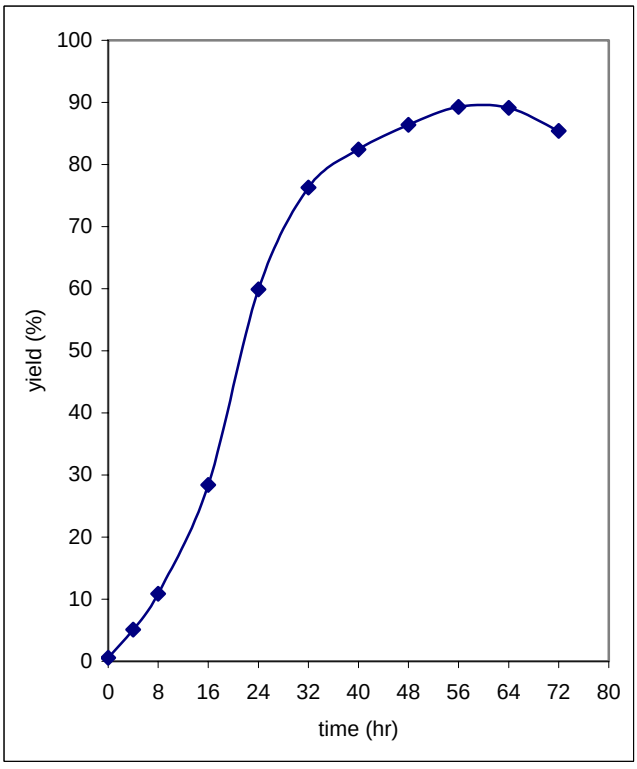


Figure D.33.2: Lactic acid production for run 33

Table D.33.2: Data of lactic acid production for run 33

Time (h)	Lactic acid production %
0	0.02
4	5.1
8	10.9
16	28.4
24	59.9
32	76.3
40	82.4
48	86.4
56	89.3
64	89.1
72	85.4

D.34 Experimental result for run 34

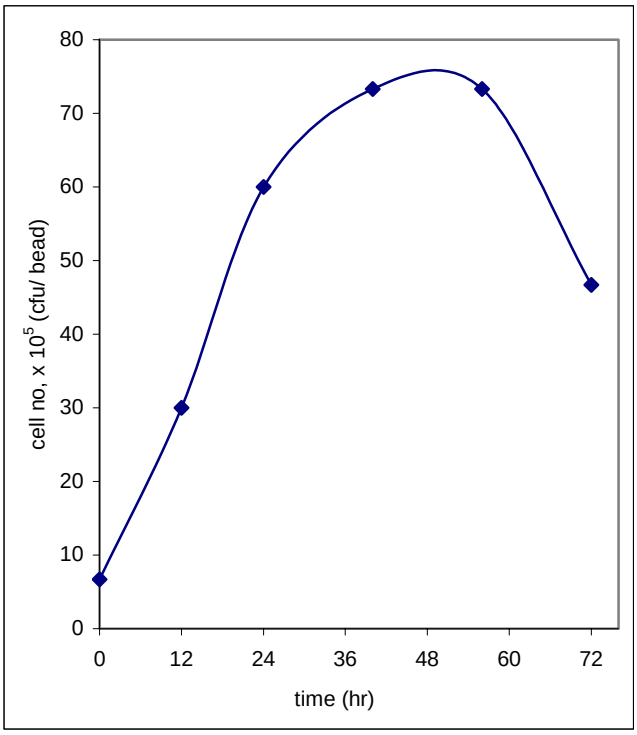


Figure D.34.1: Cell concentration for run 34

Table D.34.1: Data of cell concentration for run 34

Time (hr)	Cell number, x 10^5 (cfu/ml)
0	6.7
12	30.0
24	60.0
40	73.3
56	73.3
72	46.7

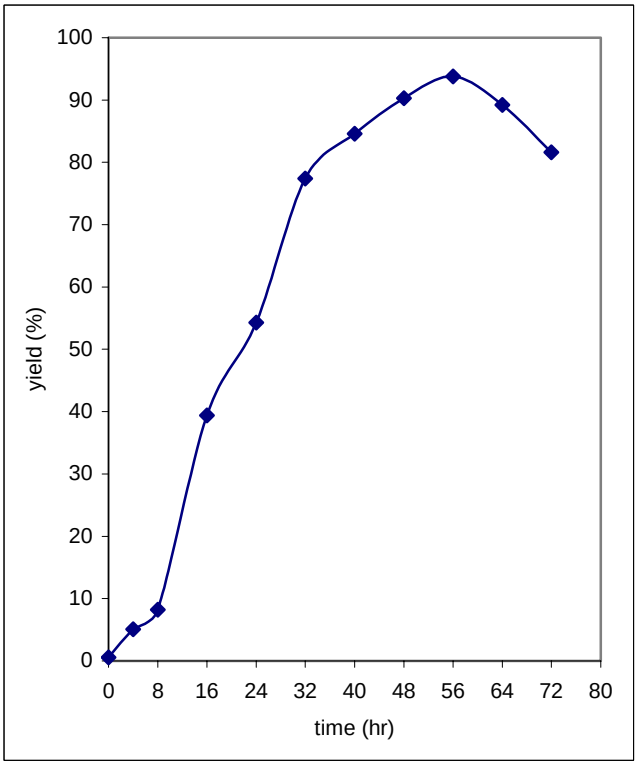


Figure D.34.2: Lactic acid production for run 34

Table D.34.2: Data of lactic acid production for run 34

Time (h)	Lactic acid production %
0	0.02
4	5.1
8	8.2
16	39.4
24	54.3
32	77.4
40	84.6
48	90.3
56	93.8
64	89.2
72	81.6

APPENDIX E

KINETIC MODELING AT OPTIMUM CONDITION

E.1 Kinetic evaluation at optimum condition (run 3)

Table E.1.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (hr)	X (g/l)	S (g/l)	$\frac{dx}{dt}$	μ	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.0804	31.3	0.0327	0.4067164	0.03195	2.45872
16	0.4668	28.7	0.02144	0.0459212	0.03484	21.7765
24	0.6804	22.36	0.01696	0.0249206	0.04472	40.1274
40	0.8004	5.39	0.0103	0.0128686	0.18553	77.7087
56	0.8796	1.02	0.00672	0.0076353	0.98039	130.971

Table E.1.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (hr)	P (g/l)	$\frac{dP}{dt}$	X g/L	μ	$\frac{dP/dt}{X}$
0	0.24	0.5158	0.0804	0.4067164	6.42E+00
16	9.33	0.6886	0.4668	0.0459212	1.48E+00
24	17.06	0.6598	0.6804	0.0249206	9.70E-01
40	25.23	0.3718	0.8004	0.0128686	4.65E-01
56	29.85	-0.2234	0.8796	0.0076353	-2.54E-01

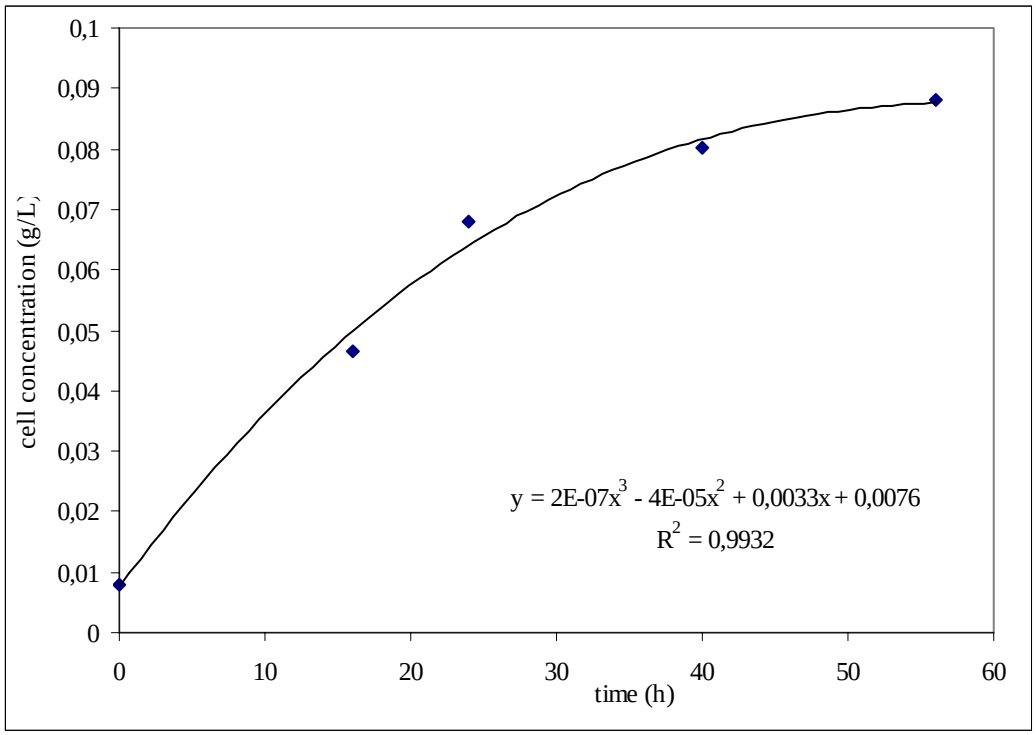


Figure E.1.1: Cell concentration versus fermentation time

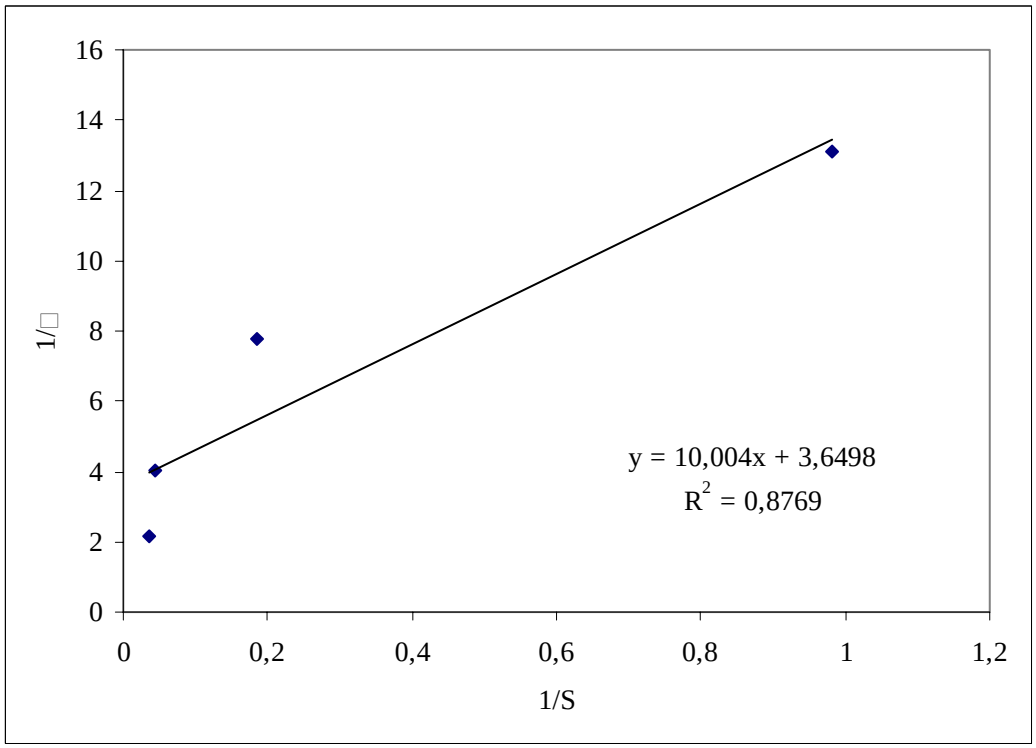


Figure E.1.2: Relationship between cell growth and substrate concentration

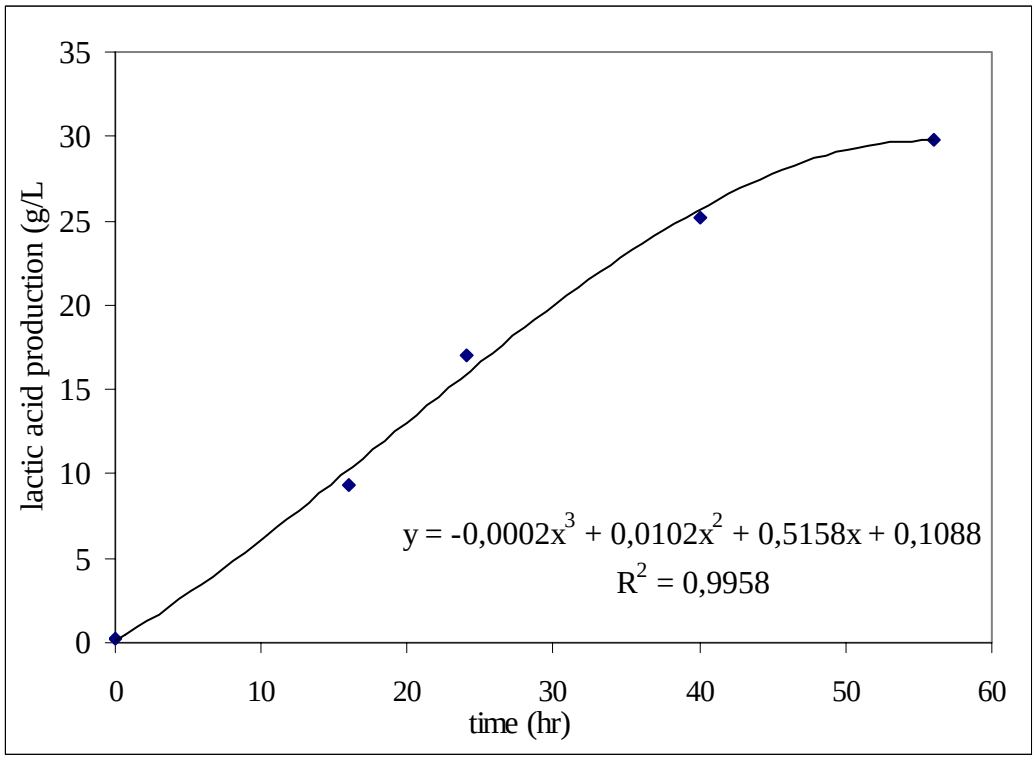


Figure E.1.3: Lactic acid production versus fermentation time

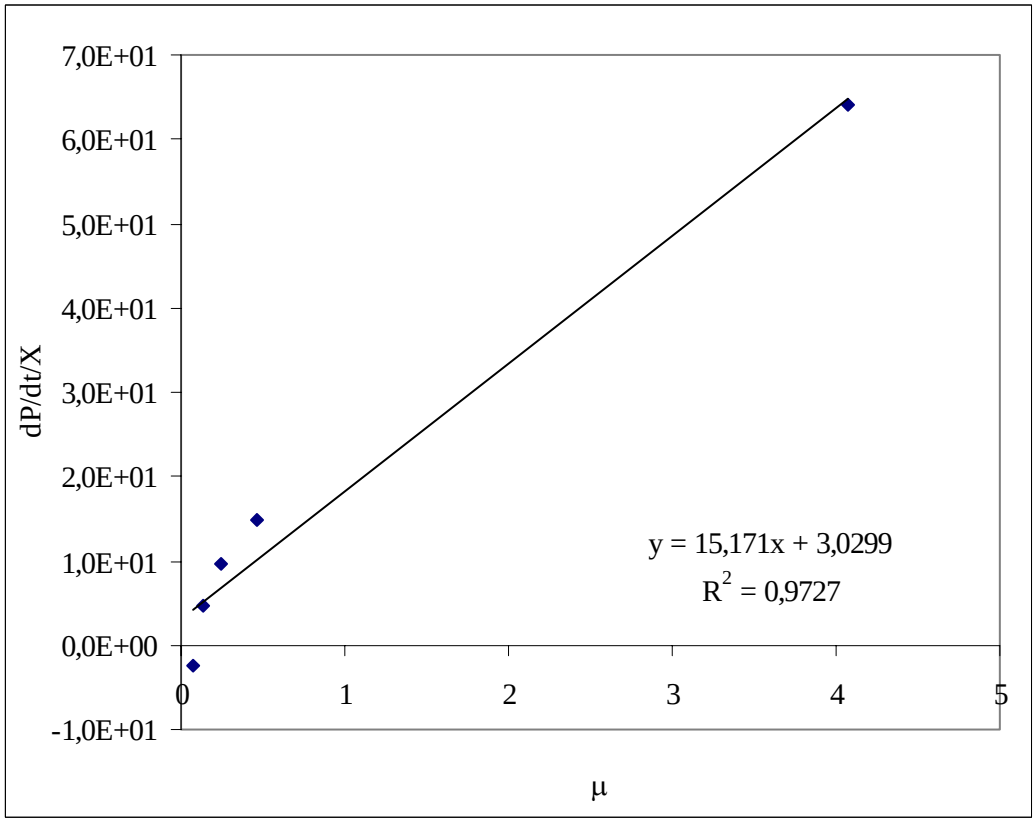


Figure E.1.4: Relationship growth rate with lactic acid production

APPENDIX F.1

FERMENTATION DATA (TEMPERATURE)

Table F.1.1: Effect of temperature on cell concentration

Fermentation time (hr)	Cell number, x 10^6 (cfu/L)					
	27°C	30°C	37°C	40°C	45°C	50°C
0	3.3	3.3	3.3	3.3	3.3	3.3
8	3.3	6.7	10.0	6.7	6.7	3.3
16	6.7	10.0	26.7	13.3	13.3	10.0
24	16.7	40.0	66.7	33.3	33.3	23.3
40	20.0	43.3	73.3	36.7	36.7	26.7
56	23.3	43.3	76.7	33.3	33.3	26.7
72	16.7	33.3	53.3	13.3	13.3	16.7

Table F.1.2: effect of temperature on sugar consumption

Fermentation time (hr)	Glucose concentration (g/L)					
	27°C	30°C	37°C	40°C	45°C	50°C
0	31.3	31.3	31.3	31.3	31.3	31.3
4	30.32	30.32	29.88	30.23	30.45	30.21
8	29.34	28.71	26.00	27.80	29.03	30.90
16	27.87	26.40	19.10	23.80	24.80	29.00
24	27.10	22.50	12.30	17.00	20.70	29.10
32	21.60	19.60	11.80	15.20	17.50	24.50
40	18.40	17.00	10.44	12.70	14.50	19.60
48	16.00	12.80	3.21	6.60	11.60	14.23
56	7.90	6.82	0.93	2.70	4.60	11.30
64	3.79	3.17	0.45	2.30	2.50	6.80
72	1.10	0.43	0.16	1.60	0.87	0.21

Table F.1.3: Effect of temperature on lactic acid production

Fermentation time (hr)	Lactic acid concentration (g/L)					
	27°C	30°C	37°C	40°C	45°C	50°C
0	0.02	0.02	0.02	0.02	0.02	0.02
4	0.59	0.75	1.69	1.31	1.60	0.91
8	1.75	1.94	3.79	3.16	1.78	1.28
16	1.16	4.16	9.26	5.04	3.35	2.07
24	6.79	13.43	20.31	16.12	11.33	7.79
32	10.70	19.16	21.72	21.41	16.81	14.90
40	13.93	21.78	24.23	22.63	19.56	16.34
48	17.81	23.29	28.01	26.14	23.82	20.44
56	19.19	25.07	28.73	26.79	22.32	20.53
64	16.56	20.09	26.04	23.63	20.19	18.72
72	15.09	18.56	25.70	21.72	19.16	17.62

APPENDIX F.2

FERMENTATION DATA (pH)

Table F.2.1: Effect of pH on cell concentration

	Cell number, x 10^6 (cfu/L)				
	pH 4.5	pH 5.5	pH 6.5	pH 7.5	pH 8.5
0	3.3	3.3	3.3	3.3	3.3
8	3.3	6.7	13.3	6.7	3.3
16	10.0	16.7	30.0	13.3	6.7
24	23.3	46.7	56.7	33.3	16.7
40	33.3	53.3	66.7	40.0	23.3
56	40.0	60.0	73.3	40.0	26.7
72	36.7	43.3	43.3	30.0	16.7

Table F.2.2: Effect of pH on glucose consumption

Fermentation time (hr)	Glucose concentration (g/L)				
	pH 4.5	pH 5.5	pH 6.5	pH 7.5	pH 8.5
0	31.3	31.3	31.3	31.3	31.3
4	30.32	30.21	29.89	29.98	30.45
8	28.71	28.96	24.23	28.40	29.03
16	27.32	24.32	21.09	25.60	28.30
24	25.16	20.43	17.28	21.30	26.40
32	23.11	18.92	11.31	19.80	24.90
40	21.89	14.65	8.60	16.10	24.90
48	12.80	10.33	6.24	11.60	21.50
56	9.70	3.12	1.34	6.40	16.10
64	3.17	2.78	0.67	2.40	7.30
72	0.43	0.77	0.35	0.78	0.87

Table F.2.3: Effect of pH on lactic acid production

Fermentation time (hr)	Lactic acid concentration (g/L)				
	pH 4.5	pH 5.5	pH 6.5	pH 7.5	pH 8.5
0	0.02	0.02	0.02	0.02	0.02
4	0.69	1.16	2.03	1.35	0.59
8	2.47	3.63	3.98	3.54	1.31
16	4.73	6.73	10.05	5.45	1.75
24	10.08	14.62	18.87	12.90	7.54
32	13.99	19.88	24.57	17.03	11.86
40	19.88	24.07	28.01	23.38	17.12
48	21.63	25.76	28.20	23.19	19.22
56	21.41	27.95	29.02	26.04	20.31
64	15.49	24.45	28.55	21.41	18.56
72	10.92	22.41	24.23	21.32	16.68

APPENDIX F.3

FERMENTATION DATA (Na-ALGINATE CONCENTRATION)

Table F.3.1: Effect of temperature on cell concentration

Fermentation time (hr)	Cell number, x 10^6 (cfu/L)				
	1.0%	2.0%	4.0%	6.0%	8.0%
0	3.3	3.3	3.3	3.3	3.3
8	3.3	10.0	6.7	6.7	3.3
16	3.3	30.0	16.7	10.0	6.7
24	6.7	53.3	23.3	16.7	10.0
40	10.0	66.7	43.4	33.3	16.7
56	10.0	76.7	46.7	36.7	26.7
72	6.7	73.7	46.7	26.7	23.3

Table F.3.2: Effect of Na-alginate concentration on glucose consumption

Fermentation time (hr)	Glucose concentration (g/L)				
	1.0%	2.0%	4.0%	6.0%	8.0%
0	31.30	31.30	31.30	31.30	31.30
4	30.32	29.88	30.32	30.23	30.21
8	28.71	24.80	26.50	27.80	30.50
16	27.32	23.60	24.70	26.50	30.10
24	25.16	16.50	22.10	23.60	27.40
32	23.11	11.80	16.90	18.70	25.40
40	21.89	10.44	14.00	17.00	24.30
48	16.30	3.21	7.99	12.00	20.70
56	11.80	0.93	6.21	9.31	13.80
64	6.90	0.45	3.79	4.20	9.20
72	2.89	0.16	1.10	1.60	8.30

Table F.3.3: Effect of Na-alginate concentration on lactic acid production

Fermentation time (hr)	Lactic acid concentration (g/L)				
	1.0%	2.0%	4.0%	6.0%	8.0%
0	0.02	0.02	0.02	0.02	0.02
4	0.97	1.60	1.06	1.10	0.88
8	1.63	6.67	2.32	1.41	1.06
16	2.69	12.33	7.36	5.29	3.26
24	7.01	17.00	14.59	9.23	8.01
32	11.05	24.23	19.53	18.87	15.52
40	12.33	26.48	24.01	21.10	16.93
48	11.74	28.26	25.89	22.82	17.18
56	9.11	29.36	23.82	19.06	15.31
64	7.23	27.92	23.22	17.93	13.65
72	5.38	25.54	21.10	15.49	13.43

APPENDIX F.4

FERMENTATION DATA (BEAD DIAMETER)

Table F.4.1: effect of bead diameter on cell concentration

Fermentation time (hr)	Cell number, x 10^6 (cfu/ml)		
	1.0mm	3.0mm	5.0mm
0	3.3	3.3	3.3
8	13.3	6.7	3.3
16	30.0	10.0	6.7
24	60.0	43.3	23.3
40	73.3	50.0	26.7
56	73.3	50.0	26.7
72	53.3	46.7	13.3

Table F.4.2: Effect of bead diameter on glucose consumption

Fermentation time (hr)	Glucose concentration (g/L)		
	1.0mm	3.0mm	5.0mm
0	31.30	31.30	31.30
4	29.88	30.23	30.32
8	24.20	26.30	28.71
16	22.20	24.80	27.32
24	16.50	21.50	25.16
32	11.80	17.60	23.11
40	10.40	13.60	21.89
48	3.21	12.10	12.80
56	0.93	4.20	6.82
64	0.45	2.70	5.50
72	0.16	1.60	4.34

Table F.4.3: Effect of bead diameter on lactic acid production

Fermentation time (hr)	Lactic acid concentration (g/L)		
	1.0mm	3.0mm	5.0mm
0	0.02	0.02	0.02
4	1.38	0.91	1.10
8	3.35	1.75	1.28
16	7.29	2.79	1.41
24	15.87	10.39	8.39
32	23.41	12.96	10.74
40	26.70	17.31	12.39
48	29.27	19.63	14.52
56	30.27	22.32	17.65
64	27.42	20.25	15.87
72	24.91	18.40	13.30

APPENDIX G.1

KINETIC PARAMETERS (TEMPERATURE AT $27^{O}C$)

Table G.1.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0005	0.126263	0.0319489	7.92
8	0.00396	29.34	0.000514	0.129899	0.0340832	7.69828927
16	0.00804	27.87	0.000529	0.065771	0.0358809	15.204236
24	0.02004	27.1	0.000543	0.027106	0.0369004	36.892489
40	0.024	18.4	0.000572	0.023833	0.0543478	41.958042
56	0.02796	7.9	0.000601	0.021488	0.1265823	46.5379494
72	0.02004	1.1	0.00063	0.031417	0.9090909	31.8297332

Table G.1.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.0512	0.00396	0.126263	12.929293
8	1.75	0.1712	0.00396	0.129899	43.232323
16	1.16	0.2912	0.00804	0.065771	36.218905
24	6.79	0.4112	0.02004	0.027106	20.518962
40	13.93	0.6512	0.024	0.023833	27.133333
56	19.19	0.8912	0.02796	0.021488	31.874106
72	15.09	1.1312	0.02004	0.031417	56.447106

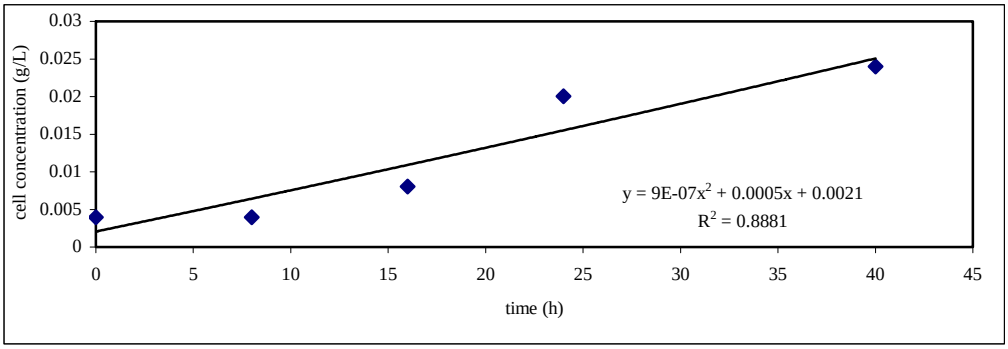


Figure G.1.1: Cell concentration versus fermentation time

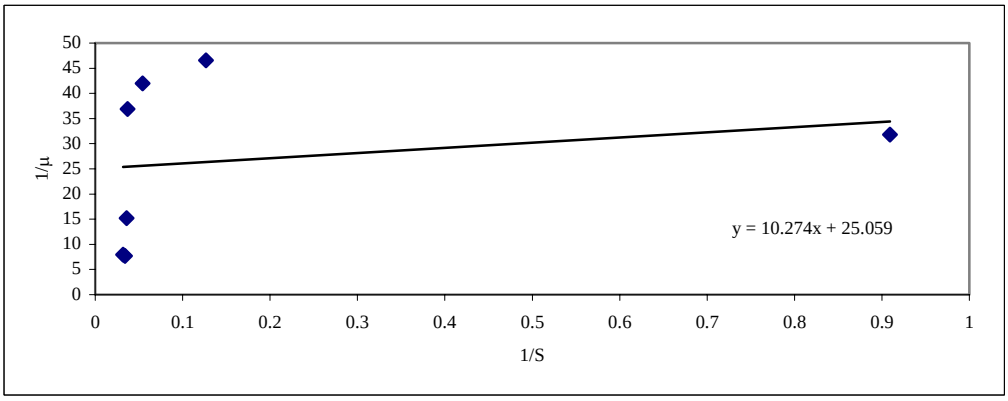


Figure G.1.2: Relationship between cell growth and substrate concentration

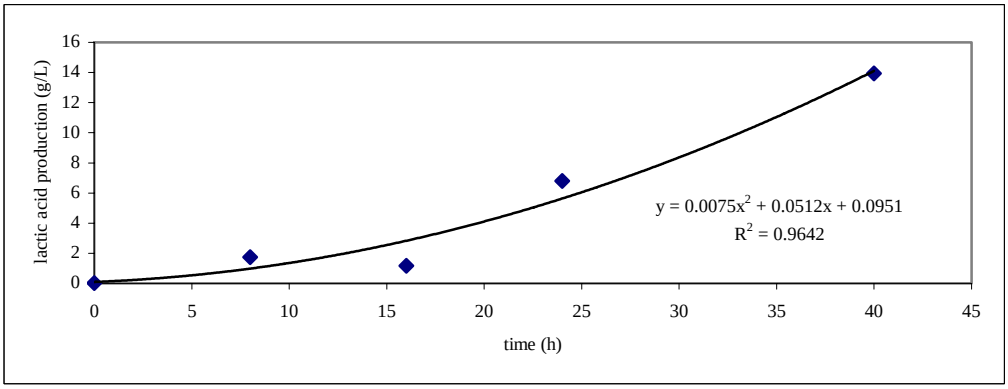


Figure G.1.3: Lactic acid production versus fermentation time

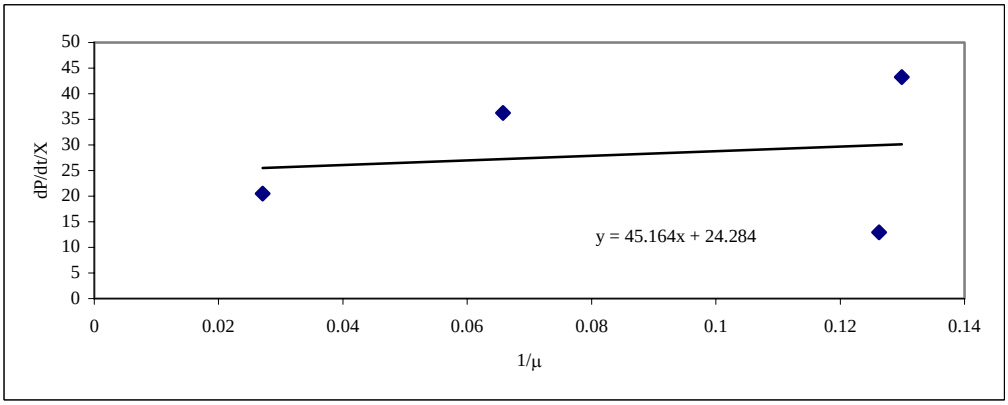


Figure G.1.4: Relationship growth rate with lactic acid production

APPENDIX G.2

KINETIC PARAMETERS (TEMPERATURE AT $30^{O}C$)

Table G.2.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0015	0.378788	0.0319489	2.64
8	0.00804	28.71	0.001436	0.178607	0.0348311	5.5988858
16	0.012	26.4	0.001372	0.114333	0.0378788	8.7463557
24	0.048	22.5	0.001308	0.02725	0.0444444	36.697248
40	0.0519	17	0.00118	0.022736	0.0588235	43.983051
56	0.0519	6.82	0.001052	0.02027	0.1466276	49.334601
72	0.03996	0.43	0.000924	0.023123	2.3255814	43.246753

Table G.2.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.3484	0.00396	0.378788	87.979798
16	4.16	0.5244	0.012	0.111667	43.7
24	13.43	0.6124	0.048	0.02625	12.758333
40	21.78	0.7884	0.0519	0.021195	15.190751
56	25.07	0.9644	0.0519	0.018112	18.581888
72	18.56	1.1404	0.03996	0.01952	28.538539

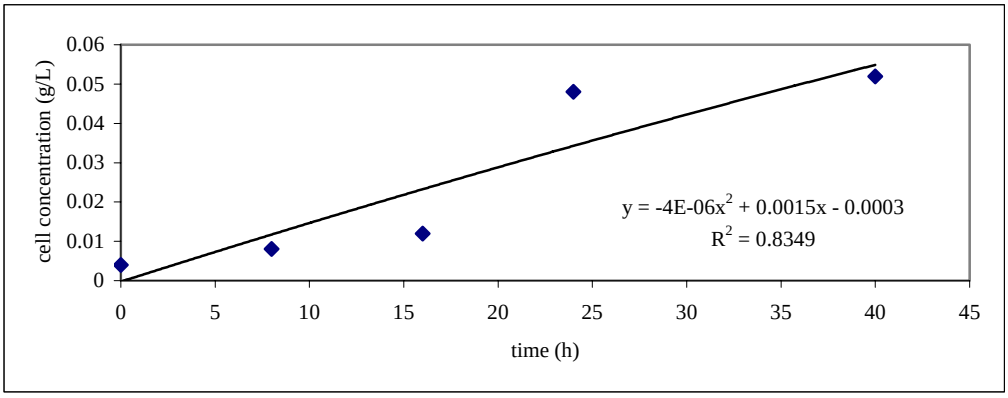


Figure G.2.1: Cell concentration versus fermentation time

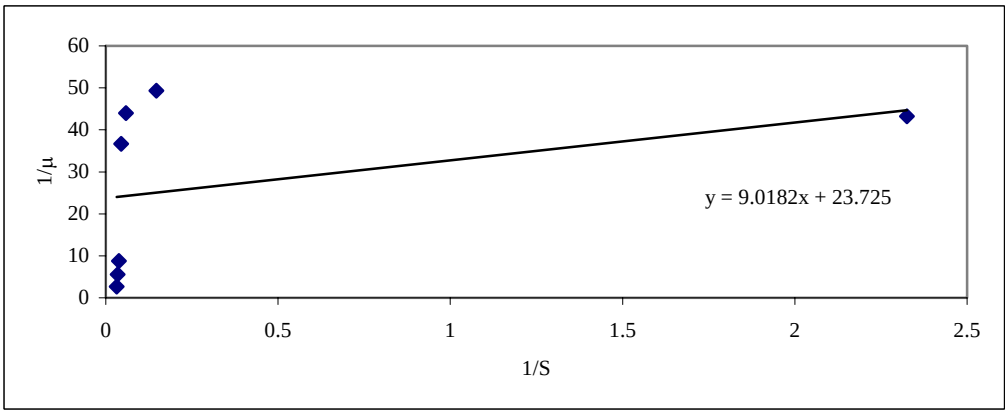


Figure G.2.2: Relationship between cell growth and substrate concentration

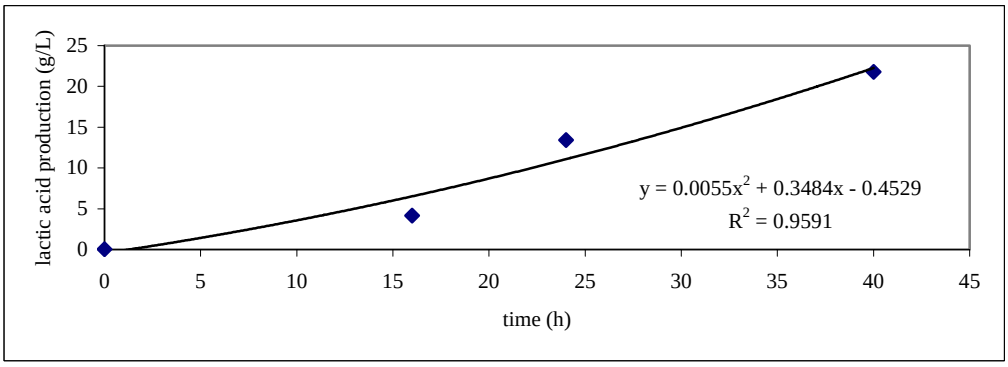


Figure G.2.3: Lactic acid production versus fermentation time

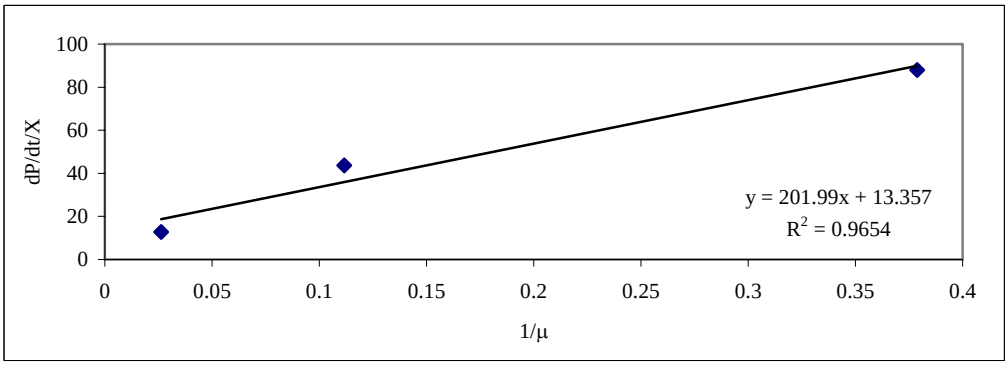


Figure G.2.4: Relationship growth rate with lactic acid production

APPENDIX G.3

KINETIC PARAMETERS (TEMPERATURE AT $37^{O}C$)

Table G.3.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0036	0.909091	0.0319489	1.1
16	0.03204	19.1	0.00264	0.082397	0.052356	12.13636
24	0.08004	12.3	0.00216	0.026987	0.0813008	37.05556
40	0.08796	10.44	0.0012	0.013643	0.0957854	73.3
56	0.09204	0.93	0.00024	0.002608	1.0752688	383.5
72	0.06396	0.16	-0.00072	-0.011257	6.25	-88.83333

Table G.3.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.7716	0.00396	0.909091	194.8485
16	9.26	0.7153	0.03204	0.082397	22.32459
24	20.31	0.6353	0.08004	0.026987	7.937031
40	24.23	0.3716	0.08796	0.013643	4.224648
56	28.73	-0.0303	0.09204	0.002608	-0.329422
72	25.7	-0.5705	0.06396	-0.011257	-8.919325

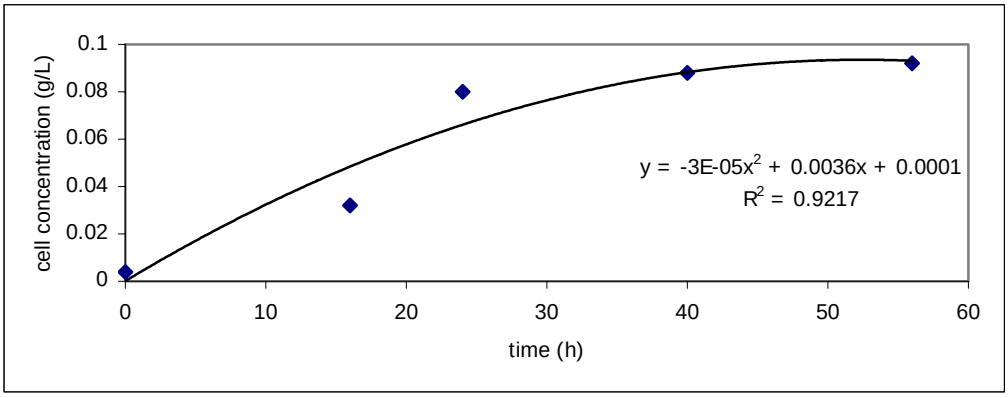


Figure G.3.1: Cell concentration versus fermentation time

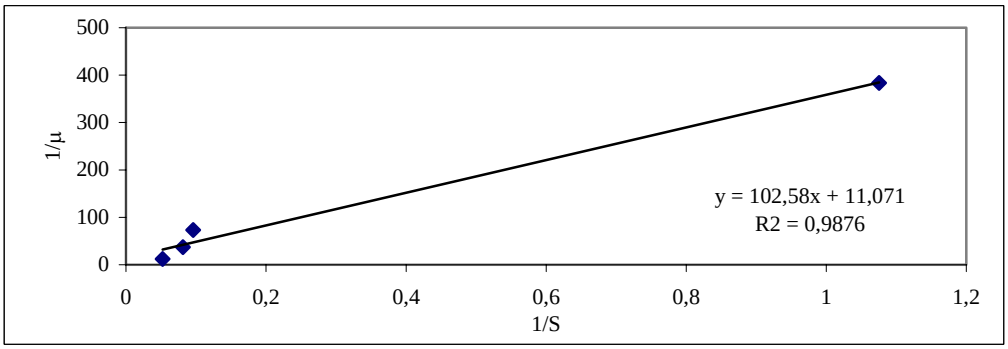


Figure G.3.2: Relationship between cell growth and substrate concentration

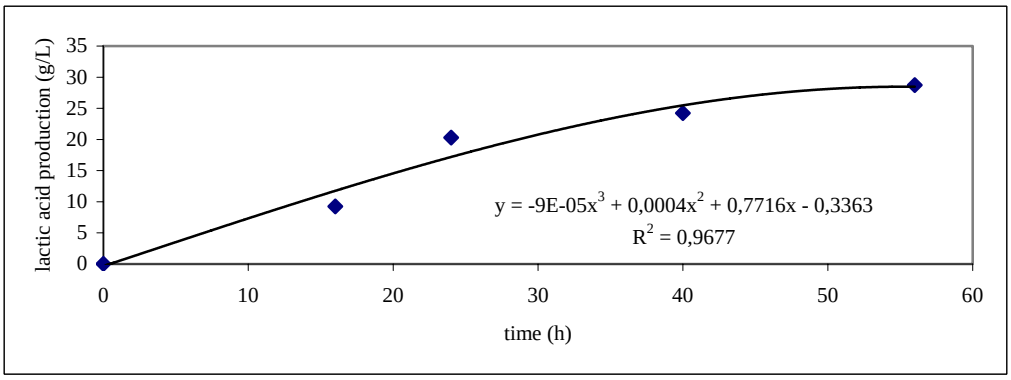


Figure G.3.3: Lactic acid production versus fermentation time

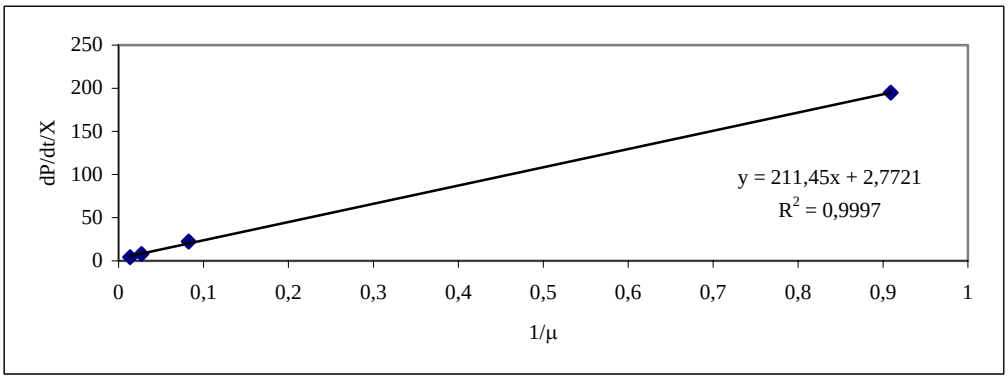


Figure G.3.4: Relationship growth rate with lactic acid production

APPENDIX G.4

KINETIC PARAMETERS (TEMPERATURE AT $40^{O}C$)

Table G.4.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0021	0.530303	0.031949	1.88571429
8	0.00804	27.8	0.002068	0.257214	0.035971	3.88781431
16	0.02004	23.8	0.002036	0.101597	0.042017	9.84282908
24	0.06396	17	0.002004	0.031332	0.058824	31.9161677
40	0.07596	12.7	0.00194	0.02554	0.07874	39.1546392
56	0.07596	2.7	0.001876	0.024697	0.37037	40.4904051
72	0.05604	1.6	0.001812	0.032334	0.625	30.9271523

Table G.4.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.6995	0.00396	0.555556	176.6414
16	5.04	0.5939	0.03204	0.101796	18.5362
24	16.12	0.5411	0.08004	0.030644	6.76037
40	22.63	0.4355	0.08796	0.023697	4.951114
56	26.79	0.3299	0.09204	0.02159	3.584311
72	21.72	0.2243	0.06396	0.02641	3.506879

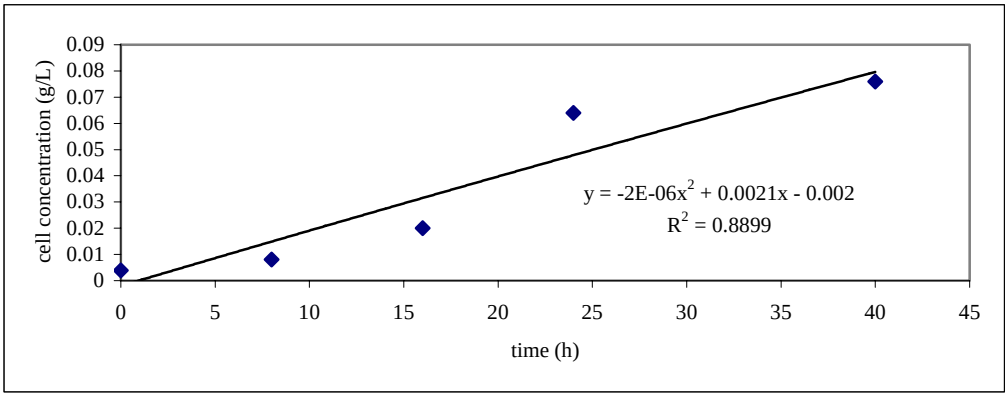


Figure G.4.1: Cell concentration versus fermentation time

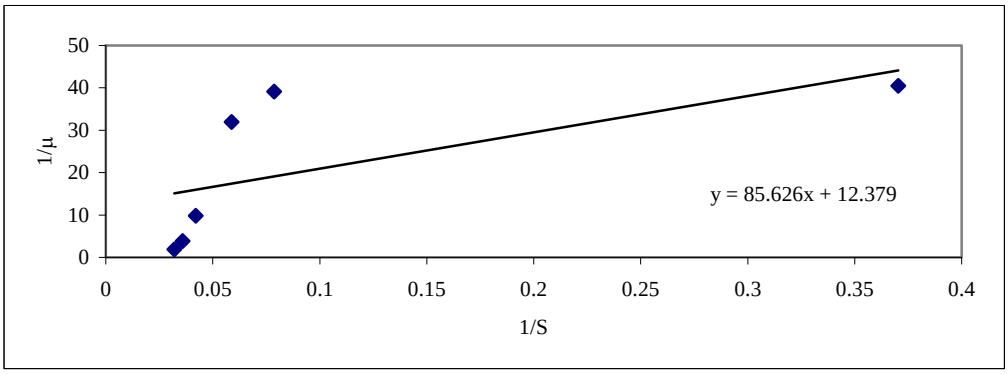


Figure G.4.2: Relationship between cell growth and substrate concentration

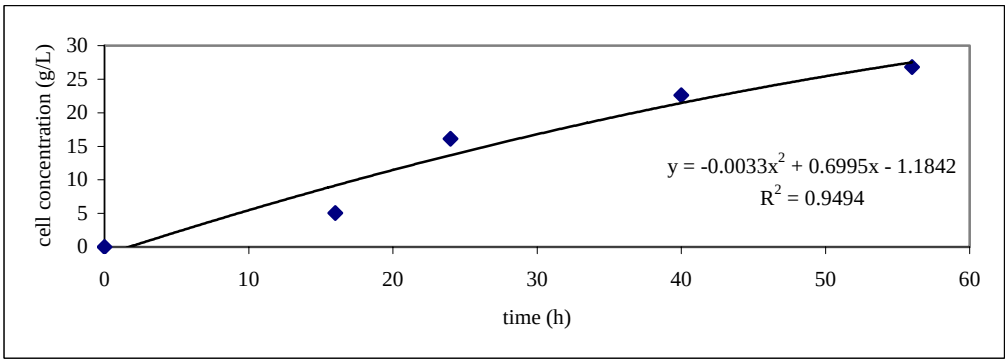


Figure G.4.3: Lactic acid production versus fermentation time

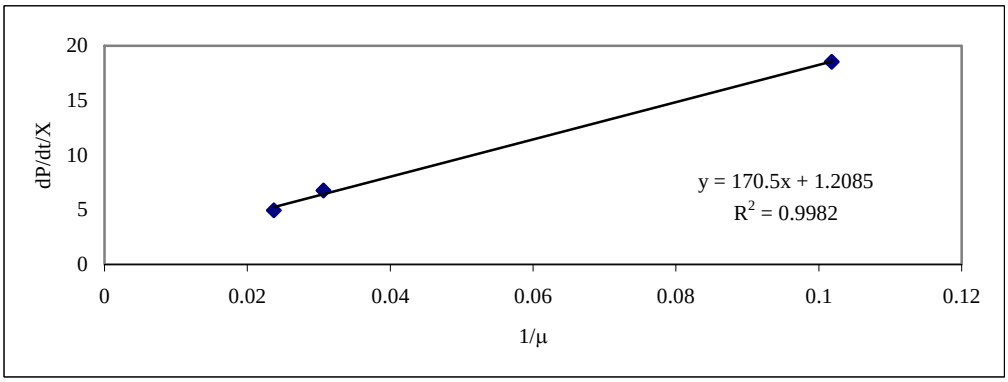


Figure G.4.4: Relationship growth rate with lactic acid production

APPENDIX G.5

KINETIC PARAMETERS (TEMPERATURE AT $45^{O}C$)

Table G.5.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0015	0.378788	0.031949	2.64
8	0.00804	29.03	0.001356	0.168657	0.034447	5.929204
16	0.01595	24.8	0.001212	0.075987	0.040323	13.16007
24	0.03996	20.7	0.001068	0.026727	0.048309	37.41573
40	0.04404	14.5	0.00078	0.017711	0.068966	56.46154
56	0.03996	4.6	0.000492	0.012312	0.217391	81.21951
72	0.01596	0.87	0.000204	0.012782	1.149425	78.23529

Table G.5.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.2529	0.00396	0.378788	63.86364
16	3.35	0.4577	0.01595	0.073981	28.69592
24	11.33	0.5601	0.03996	0.025526	14.01652
40	19.56	0.7649	0.04404	0.015895	17.3683
56	22.32	0.9697	0.03996	0.00951	24.26677
72	19.16	1.1745	0.01596	0.003759	73.59023

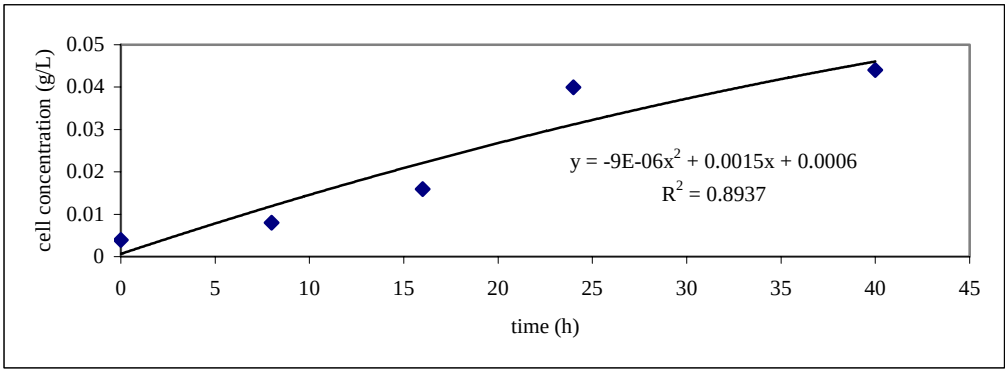


Figure G.5.1: Cell concentration versus fermentation time

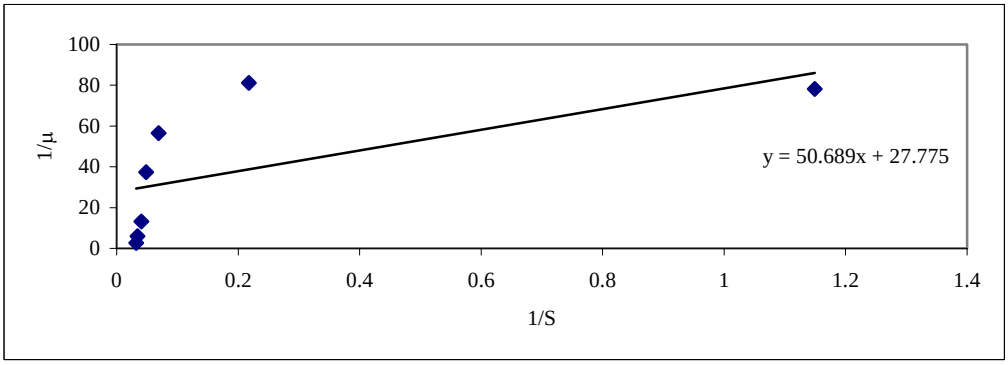


Figure G.5.2: Relationship between cell growth and substrate concentration

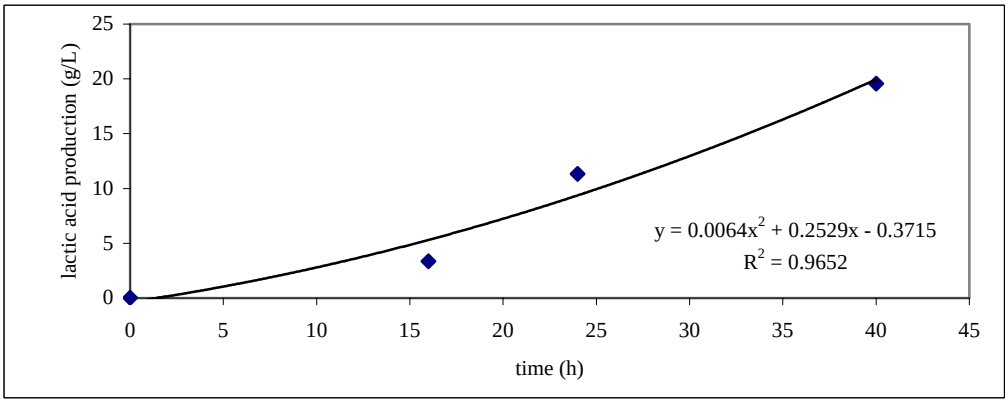


Figure G.5.3: Lactic acid production versus fermentation time

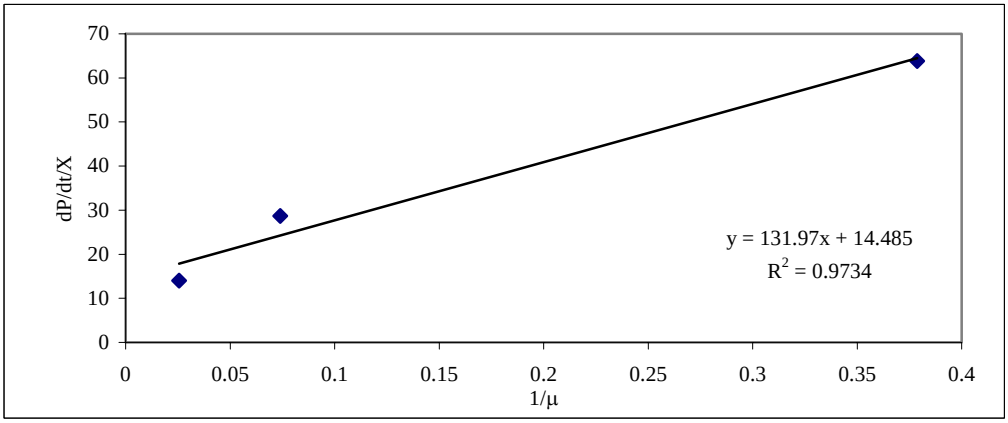


Figure G.5.4: Relationship growth rate with lactic acid production

APPENDIX G.6

KINETIC PARAMETERS (TEMPERATURE AT 50^{O}C)

Table G.6.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.001	0.252525	0.031949	3.96
16	0.012	29	0.00084	0.07	0.034483	14.28571
24	0.02796	29.1	0.00076	0.027182	0.034364	36.78947
40	0.03204	19.6	0.0006	0.018727	0.05102	53.4
56	0.03204	11.3	0.00044	0.013733	0.088496	72.81818
72	0.02004	0.21	0.00028	0.013972	4.761905	71.57143

Table G.6.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.0819	0.00396	0.227273	20.68182
8	1.28	0.2163	0.00396	0.215152	54.62121
16	2.07	0.3507	0.012	0.067	29.225
24	7.79	0.4851	0.02796	0.027039	17.34979
40	16.34	0.7539	0.03204	0.020599	23.52996
56	20.53	1.0227	0.03204	0.017603	31.91948
72	17.62	1.2915	0.02004	0.023353	64.44611

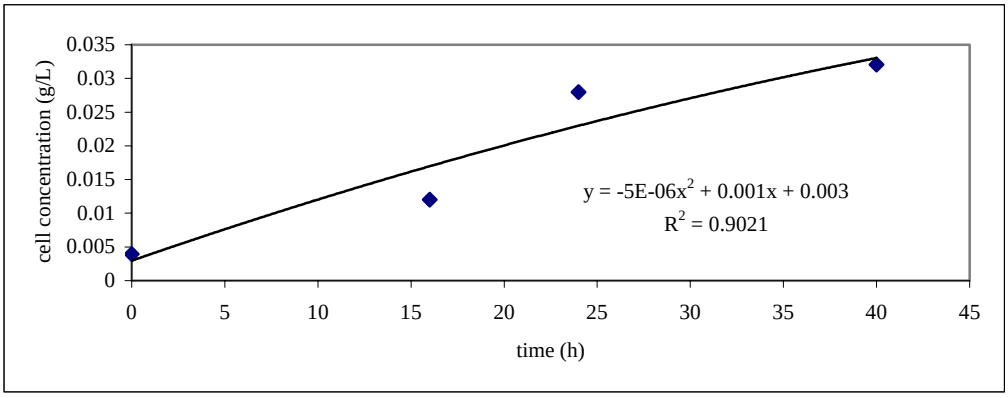


Figure G.6.1: Cell concentration versus fermentation time

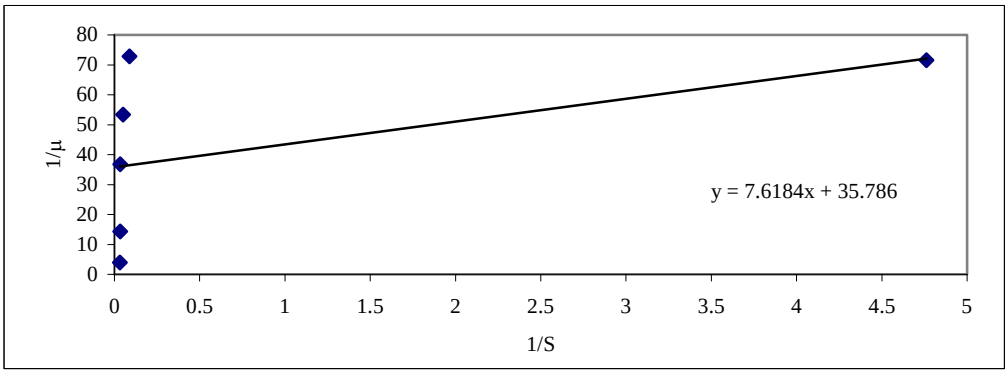


Figure G.6.2: Relationship between cell growth and substrate concentration

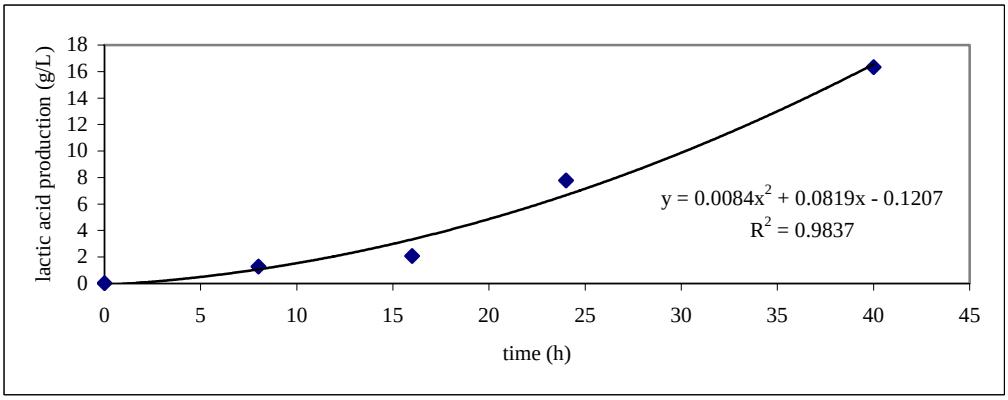


Figure G.6.3: Lactic acid production versus fermentation time

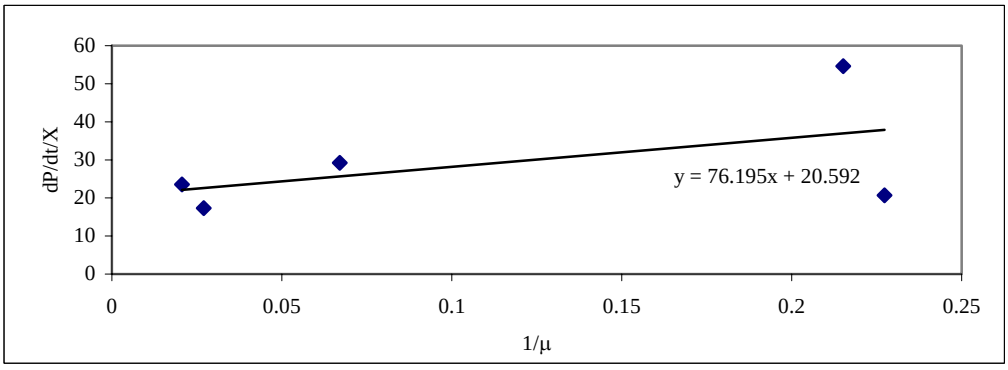


Figure G.6.4: Relationship growth rate with lactic acid production

APPENDIX H.1

KINETIC PARAMETERS (pH 4.5)

Table H.1.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.001	0.252525	0.031949	3.96
16	0.012	27.32	0.000904	0.075333	0.036603	13.2743363
24	0.02796	25.16	0.000856	0.030615	0.039746	32.6635514
40	0.03996	21.89	0.00076	0.019019	0.045683	52.5789474
56	0.048	9.7	0.000664	0.013833	0.103093	72.2891566
72	0.04404	0.43	0.000568	0.012897	2.325581	77.5352113

Table H.1.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.238	0.00396	0.252525	60.10101
16	4.73	0.4492	0.012	0.075333	37.43333
24	10.08	0.5548	0.02796	0.030615	19.84263
40	19.88	0.766	0.03996	0.019019	19.16917
56	21.41	0.9772	0.048	0.013833	20.35833

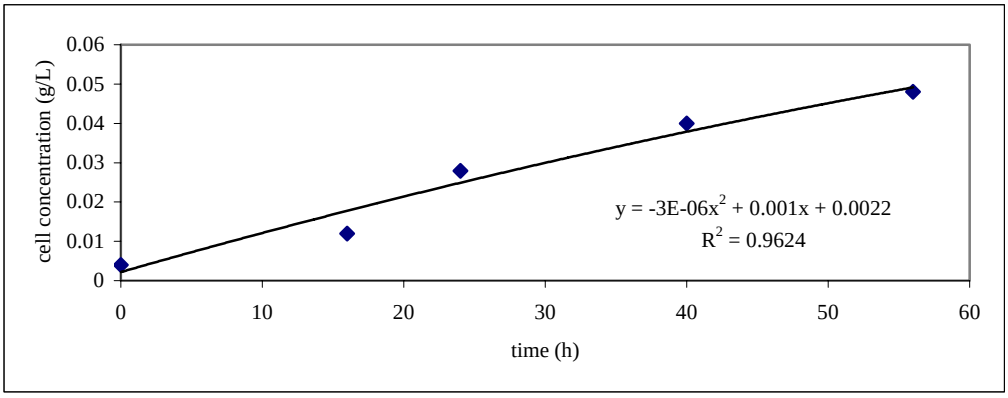


Figure H.1.1: Cell concentration versus fermentation time

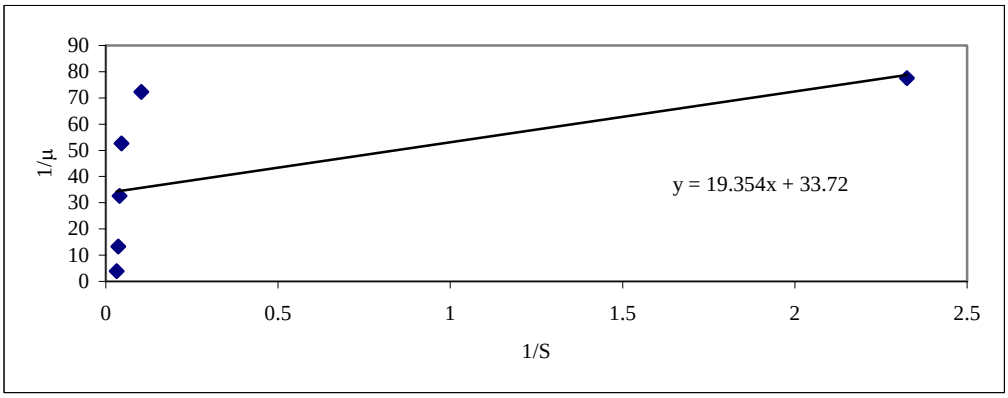


Figure H.1.2: Relationship between cell growth and substrate concentration

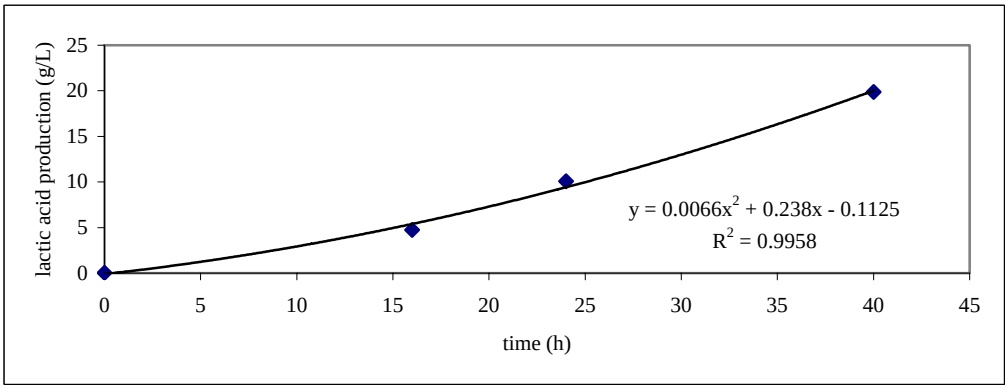


Figure H.1.3: Lactic acid production versus fermentation time

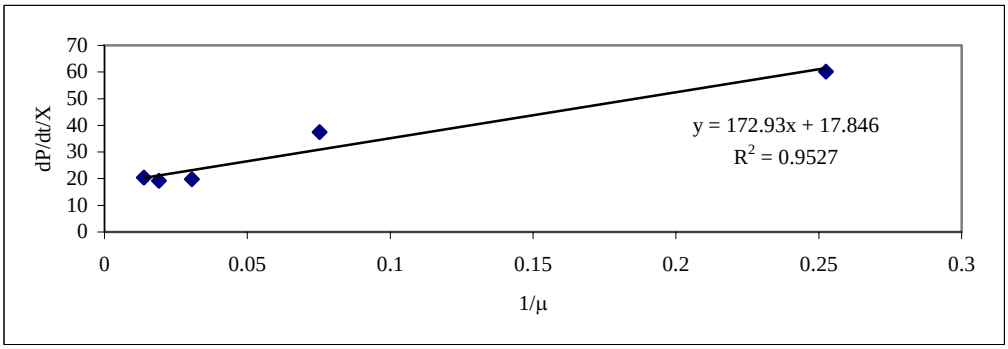


Figure H.1.4: Relationship growth rate with lactic acid production

APPENDIX H.2

KINETIC PARAMETERS (pH 5.5)

Table H.2.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.002	0.505051	0.031949	1.98
8	0.00804	28.96	0.001872	0.232836	0.03453	4.2948718
16	0.02004	24.32	0.001744	0.087026	0.041118	11.490826
24	0.05604	20.43	0.001616	0.028837	0.048948	34.678218
40	0.06396	14.65	0.00136	0.021263	0.068259	47.029412
56	0.072	3.12	0.001104	0.015333	0.320513	65.217391
72	0.05192	0.77	0.000848	0.016333	1.298701	61.226415

Table H.2.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.4731	0.00396	0.505051	119.4697
8	3.63	0.5307	0.00804	0.232836	66.00746
16	6.73	0.5883	0.02004	0.087026	29.35629
24	14.62	0.6459	0.05604	0.028837	11.5257
40	24.07	0.7611	0.06396	0.021263	11.89962
56	27.95	0.8763	0.072	0.015333	12.17083

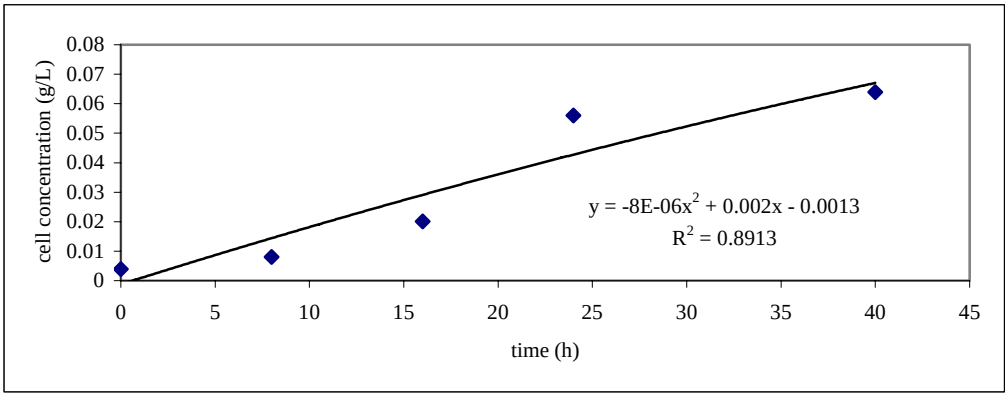


Figure H.2.1: Cell concentration versus fermentation time

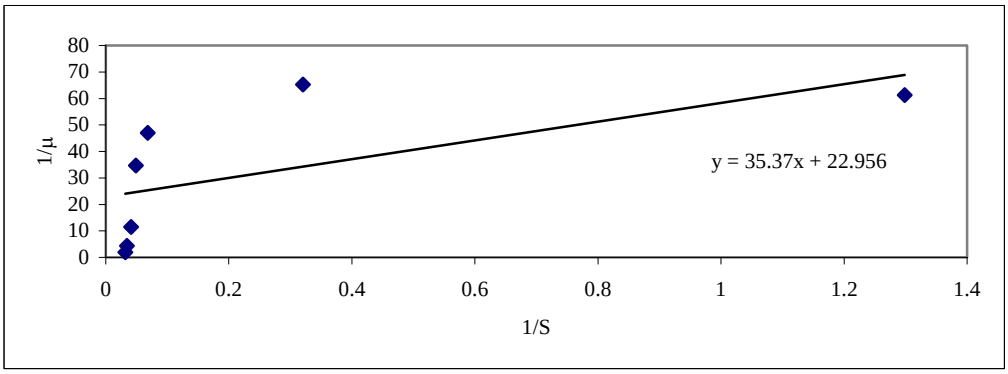


Figure H.2.2: Relationship between cell growth and substrate concentration

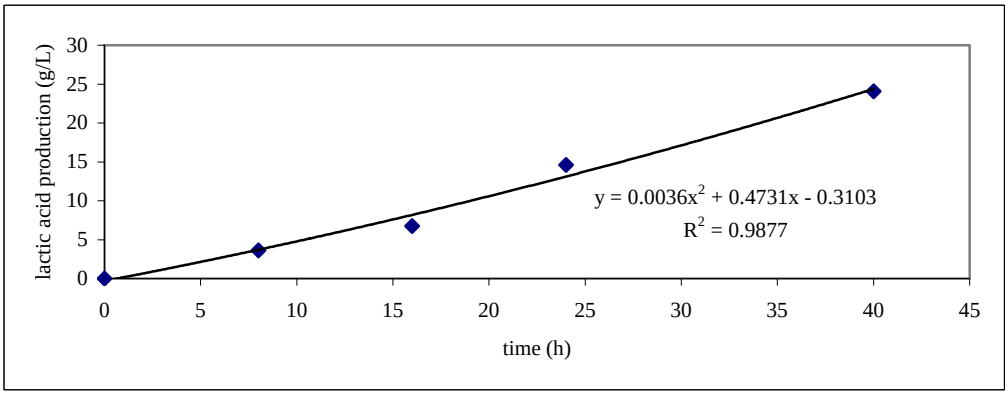


Figure H.2.3: Lactic acid production versus fermentation time

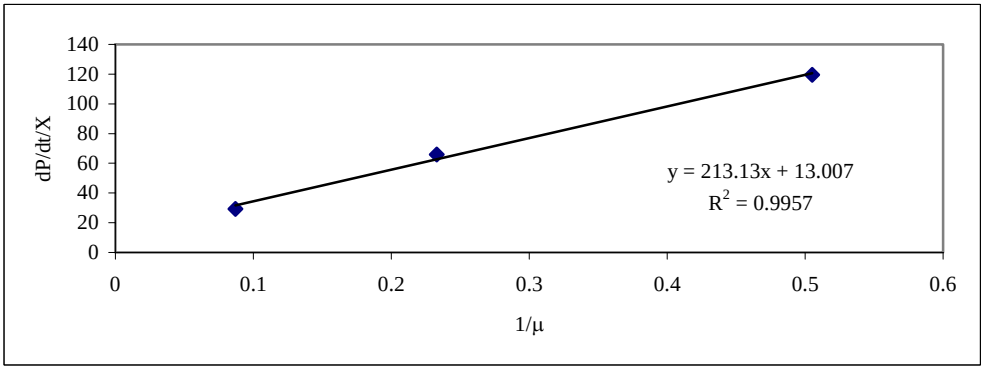


Figure H.2.4: Relationship growth rate with lactic acid production

APPENDIX H.3

KINETIC PARAMETERS (pH 6.5)

Table H.3.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.003	0.757576	0.031949	1.32
8	0.01596	24.23	0.00268	0.16792	0.041271	5.9552239
16	0.036	21.09	0.00236	0.065556	0.047416	15.254237
24	0.06804	17.28	0.00204	0.029982	0.05787	33.352941
40	0.08004	8.6	0.0014	0.017491	0.116279	57.171429
56	0.08796	1.34	0.00076	0.00864	0.746269	115.73684
72	0.05196	0.35	0.00012	0.002309	2.857143	433

Table H.3.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.7616	0.00396	0.757576	192.3232
16	10.05	0.7232	0.036	0.065556	20.08889
24	18.87	0.704	0.06804	0.029982	10.34685
40	28.01	0.6656	0.08004	0.017491	8.315842
56	29.02	0.6272	0.08796	0.00864	7.130514

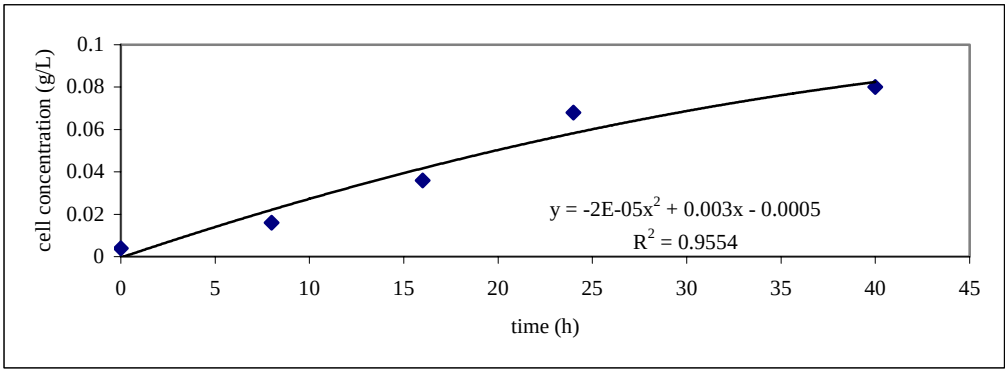


Figure H.3.1: Cell concentration versus fermentation time

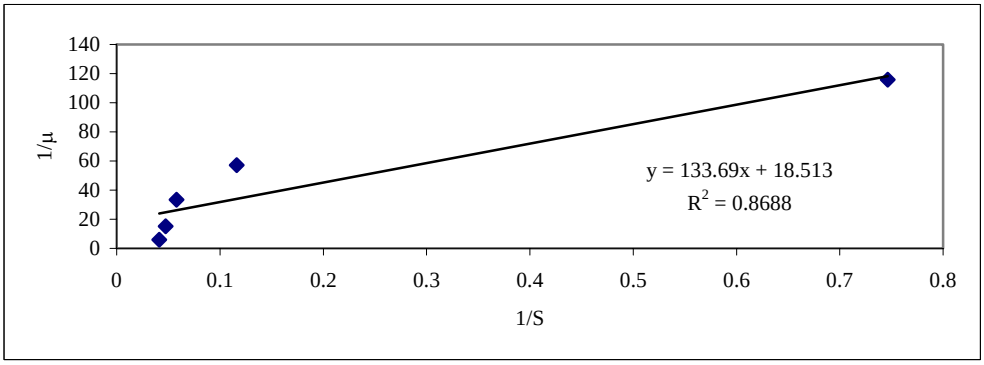


Figure H.3.2: Relationship between cell growth and substrate concentration

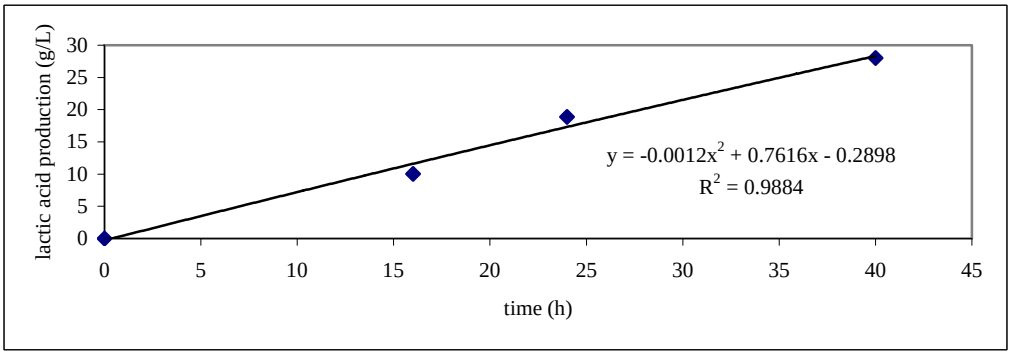


Figure H.3.3: Lactic acid production versus fermentation time

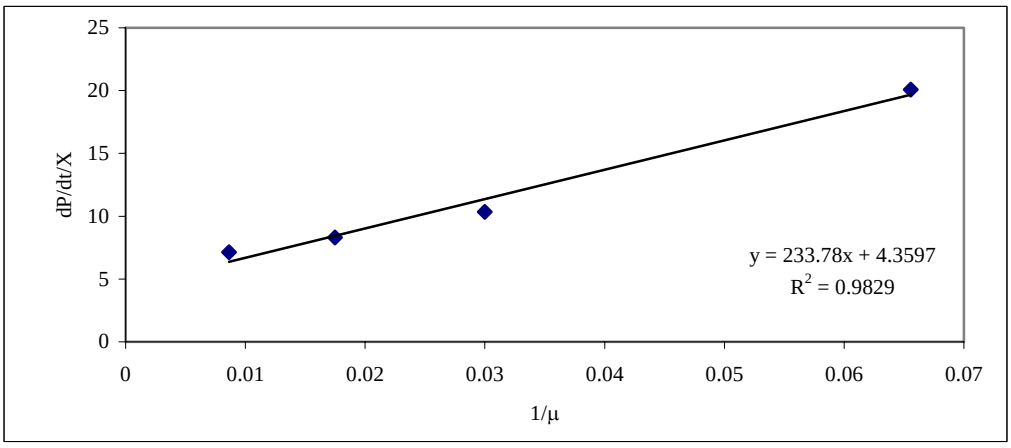


Figure H.3.4: Relationship growth rate with lactic acid production

APPENDIX H.4

KINETIC PARAMETERS (pH 7.5)

Table H.4.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0014	0.353535	0.031949	2.828571
8	0.00804	28.4	0.001336	0.166169	0.035211	6.017964
16	0.01596	25.6	0.001272	0.079699	0.039063	12.54717
24	0.03996	21.3	0.001208	0.03023	0.046948	33.07947
40	0.048	16.1	0.00108	0.0225	0.062112	44.44444
56	0.048	6.4	0.000952	0.019833	0.15625	50.42017
72	0.036	0.78	0.000824	0.022889	1.282051	43.68932

Table H.4.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.3357	0.00396	0.353535	84.77273
8	3.54	0.4381	0.00804	0.166169	54.49005
16	5.45	0.5405	0.01596	0.079699	33.86591
24	12.9	0.6429	0.03996	0.03023	16.08859
40	23.38	0.8477	0.048	0.0225	17.66042
56	26.04	1.0525	0.048	0.019833	21.92708

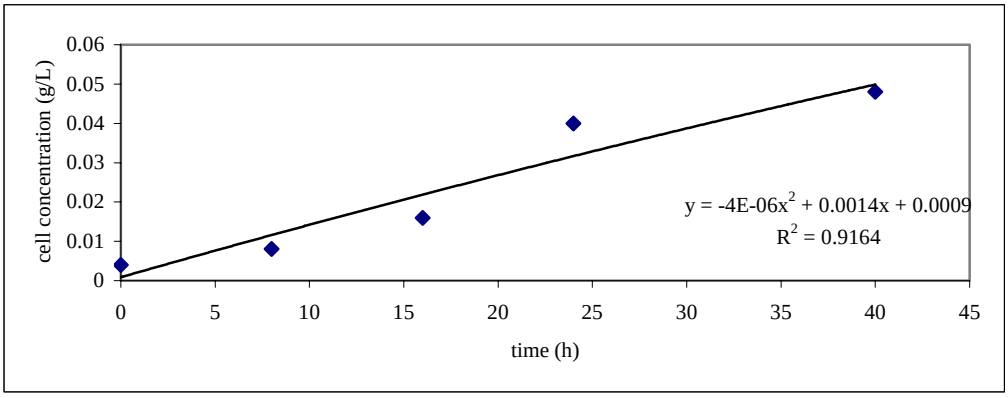


Figure H.4.1: Cell concentration versus fermentation time

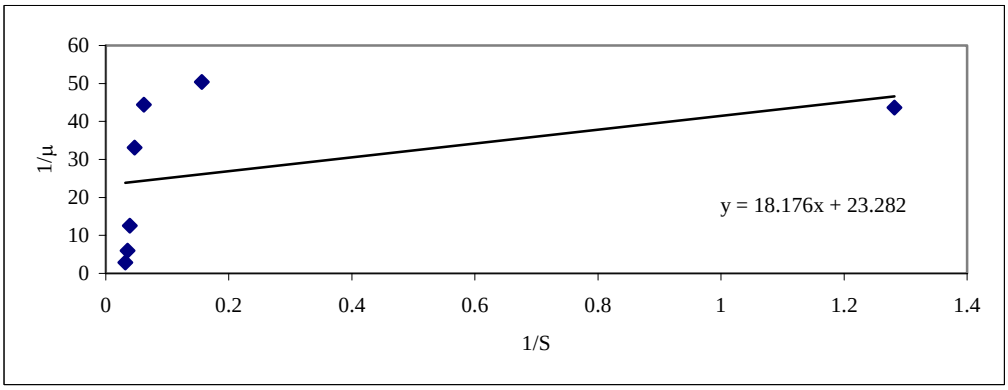


Figure H.4.2: Relationship between cell growth and substrate concentration

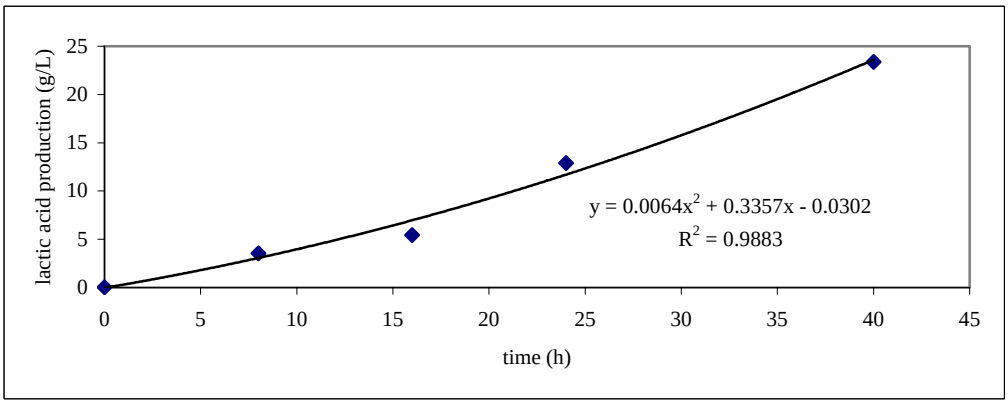


Figure H.4.3: Lactic acid production versus fermentation time

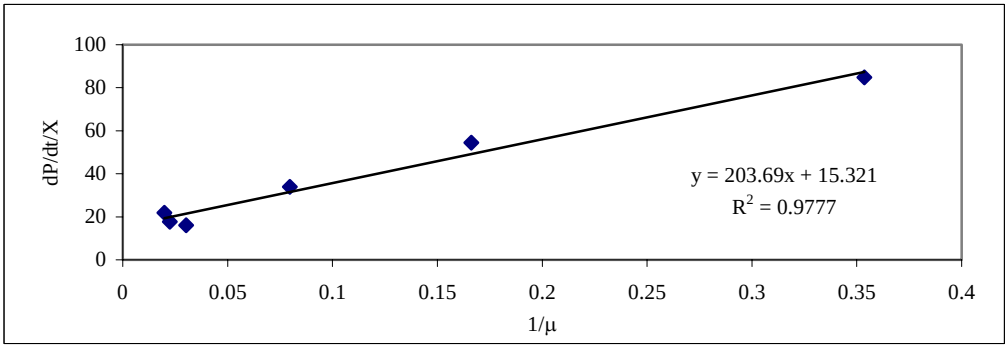


Figure H.4.4: Relationship growth rate with lactic acid production

APPENDIX H.5

KINETIC PARAMETERS (pH 8.5)

Table H.5.1: Cell concentration (X), substrate concentration (S) during the course of fermentation using liquid pineapple waste

Time (h)	X (g/L)	S (g/L)	$\frac{dX}{dt}$	μ h^{-1}	$\frac{1}{S}$	$\frac{1}{\mu}$
0	0.00396	31.3	0.0007	0.176768	0.031949	5.65714286
8	0.00396	29.03	0.000652	0.164646	0.034447	6.07361963
16	0.00804	28.3	0.000604	0.075124	0.035336	13.3112583
24	0.02004	26.4	0.000556	0.027745	0.037879	36.0431655
40	0.02796	24.9	0.00046	0.016452	0.040161	60.7826087
56	0.03204	16.1	0.000364	0.011361	0.062112	88.021978
72	0.02004	0.87	0.000268	0.013373	1.149425	74.7761194

Table H.5.2: Cell concentration (X), lactic acid production (P) during the course of fermentation using liquid pineapple waste

Time (h)	P (g/L)	$\frac{dP}{dt}$	X (g/L)	μ h^{-1}	$\frac{dP/dt}{X}$
0	0.02	0.0313	0.00396	0.176768	7.90404
8	1.31	0.1913	0.00396	0.164646	48.30808
16	1.75	0.3513	0.00804	0.075124	43.69403
24	7.54	0.5113	0.02004	0.027745	25.51397
40	17.12	0.8313	0.02796	0.016452	29.73176
56	20.31	1.1513	0.03204	0.011361	35.93321
72	16.68	1.4713	0.00396	0.013373	371.5404

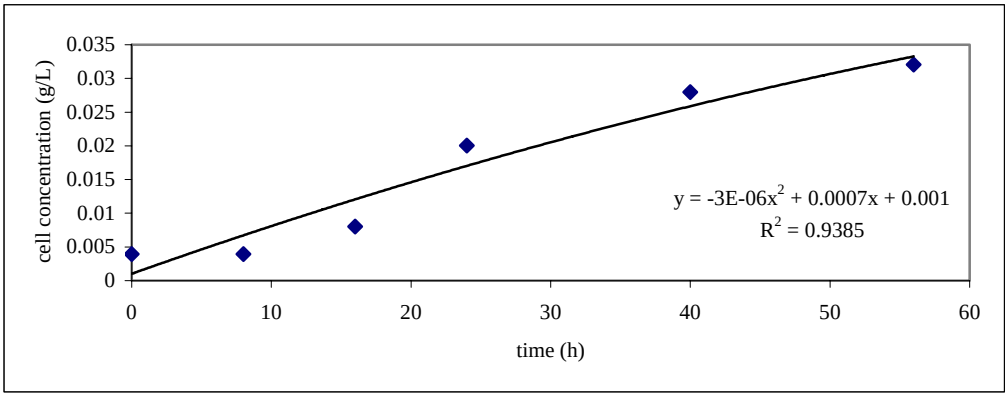


Figure H.5.1: Cell concentration versus fermentation time

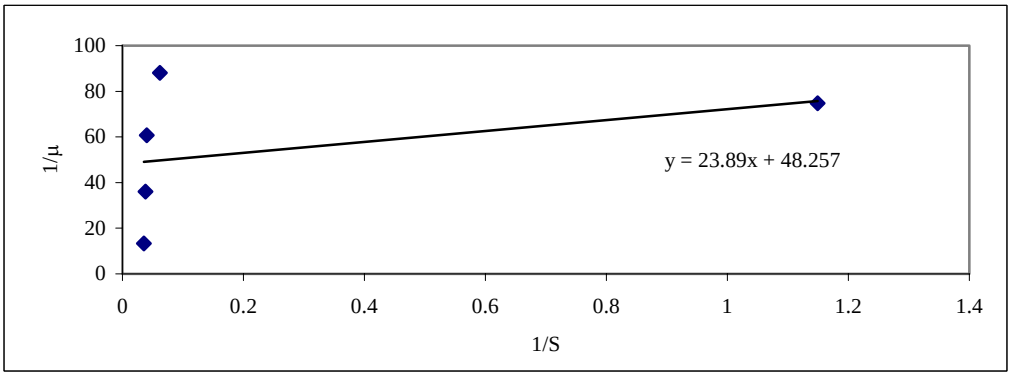


Figure H.5.2: Relationship between cell growth and substrate concentration

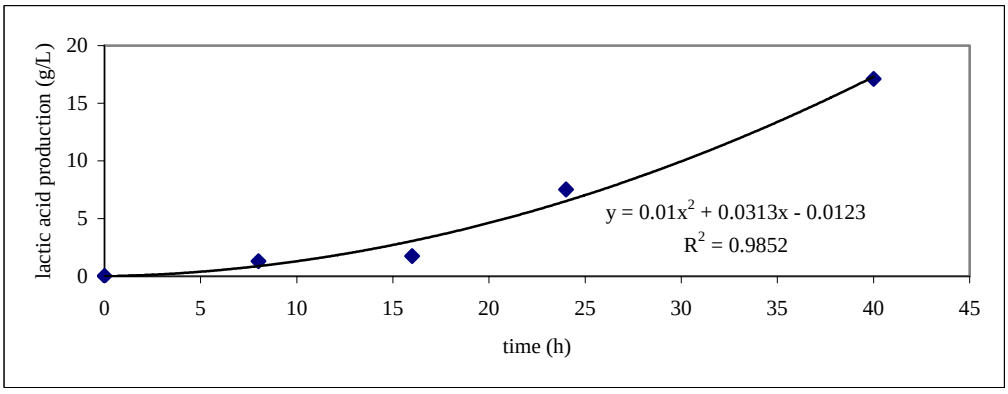


Figure H.5.3: Lactic acid production versus fermentation time

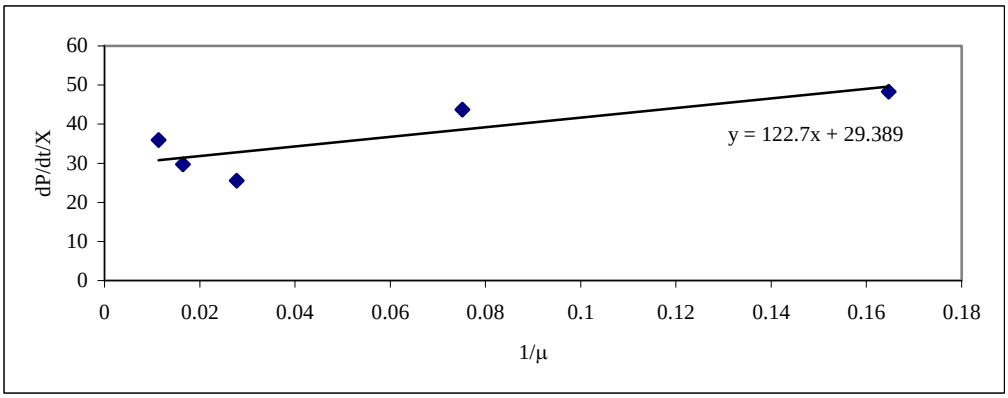


Figure H.5.4: Relationship growth rate with lactic acid production