STUDIES ON CALCIUM CARBONATE LOADED LIQUID CORE CAPSULES AS A pH CONTROLLER IN LACTIC ACID FERMENTATION

NURUL AININA BINTI ZULKIFLI

UNIVERSITI MALAYSIA PERLIS

2014

Studies on Calcium Carbonate Loaded Liquid Core Capsules as a pH Controller in Lactic Acid Fermentation

by

NURUL AININA BINTI ZULKIFLI

(1331110963)

A thesis submitted in fulfillment of the requirements for the degree of Master of Science in (Bioprocess Engineering)

School of Bioprocess Engineering

UNIVERSITI MALAYSIA PERLIS

2014

ACKNOWLEDGEMENT

I would like to thank Allah the Almighty for blessing and giving me the strength to accomplish this thesis. In particular, I wish to express my sincere appreciation to my project's supervisor, Mr. Lee Boon Beng of the Universiti Malaysia Perlis for his encouragement and guidance. The most sincere appreciation and gratitude to Malaysian Toray Science Foundation (9002-00015) for financial support, School of Bioprocess Engineering and Universiti Malaysia Perlis (UniMAP) for providing the research facilities, and the Malaysian Ministry of Education (Mybrain-15) for scholarship support.

The gratitude is also conveyed to my co-supervisor Late of Assoc. Prof. Dr Mohamed Zulkali Mohamed Daud, teaching engineers and technicians for their assistance and cooperation. Special thanks are expressed to my parents and family members for their prayers of study period, support, and motivation during the last two years.

My thankfulness also goes to my friends for their assistance, motivations and suggestions throughout my studies. Last but not least, my sincere appreciation is also extended to all my colleagues and others who have provided assistance in various forms and in many occasions.

TABLE OF CONTENTS

		PAGE
THESIS DECLARATION		i
ACKNOWLEDGEMENT		ii
TABLE OF CONTENTS		iii
LIST OF TABLES		viii
LIST OF FIGURES		ix
LIST OF ABBREVIATIONS		xii
LIST OF SYMBOLS		xiii
ABSTRAK		xiv
ABSTRACT		xv
CHAPTER 1: INTRODUCTION		
1.1	Background of Study	1
1.2	Problem Statement	3
1.3	Objectives of Research	4
1.4	Scope and Significance of Research	5
1.5	Organization of thesis	5
CHAPTER 2: LITERATURE REVIEW		
2.1	Encapsulation Technology	7
	2.1.1 Importance of encapsulation technology	8
	2.1.2 Capsule formation methods	9

2.1.3 Capsule formation materials 12

2.1.3 (a) Alginate 13

2.1.3 (b) Viscosifiers 15

2.2 Lactic acid 18

2.2.1 Physical and chemical properties 18

2.2.2 Application of lactic acid 20

2.2.3 Production of lactic acid 21

2.3 Lactic acid bacteria 22

2.3.1 Introduction to LAB 22

2.3.2 LAB fermentation 23

2.3.3 *Lactobacillus casei* 24

2.3.4 Application of encapsulation technology in LAB fermentation 26

2.4 The pH control strategies used in LAB fermentation 27

2.4.1 pH controller 27

2.4.2 Separation technology 28

2.4.3 Fermentation mode pH controller 30

CHAPTER 3: METHODOLOGY

3.1 Introduction 31

3.2 Chemicals 33

3.3 Encapsulation materials and methods 33

3.3.1 Preparation of encapsulation materials 33

3.3.2 Preparation of MRS broth 34

3.3.3 Preparation of MRS agar 34

3.3.4 Preparation of maximum recovery diluents (MRD) solution 36

3.4 Experimental methods 36

3.4.1 Encapsulation set-up 36

3.4.2 Characterization of liquid core capsule properties 38

3.4.2 (a) Liquid core capsule diameter 38

3.4.2 (b) Capsule membrane thickness 39

3.4.2 (c) Sphericity factor (SF) of capsules 40

3.4.3 Stock culture preparation 41

3.4.4 Stock culture activation 42

3.4.5 Analytical method 43

3.4.5 (a) pH 43

3.4.5 (b) Lactic acid (LA) and glucose concentration 43

3.4.5 (c) Development of standard curve for measurement of glucose and lactic acid concentration 44

3.4.5 (d) Viable cell concentration 46

3.4.5 (e) Viscosity measurement 47

3.4.6 Preliminary studies of pH effect using liquid core capsule 48

3.4.6 (a) Effect of calcium carbonate ($CaCO_3$) loading in controlling pH 49

3.4.6 (b) Effect of capsule volume to solution volume (%C/S) ratio for pH control 49

3.4.6 (c) Effect of liquid core capsules diameter for pH control 50

3.4.6 (d) Effect of liquid core capsules membrane thickness for pH control 50

3.4.6 (e) Reusability of the liquid core capsules for pH control 50

3.4.6 (f) Performance of liquid core capsule in solutions of different viscosity 51

3.5 Fermentation 51

3.5.1 LAB fermentation in shake flask 51

3.6 Design of LAB fermentation experiments 54

CHAPTER 4: RESULT AND DISCUSSION

4.1 Introduction 55

4.2 Characterization of liquid core capsule properties 55

4.2.1 Diameter of liquid core capsule 56

4.2.2 Membrane thickness of liquid core capsule 57

4.2.3 Sphericity factor of liquid core capsule 59

4.3 Preliminary studies based on pH change in LA solutions 61

4.3.1 Effect of calcium carbonate loading in liquid core capsule 61

4.3.2 Effect of capsule volume to solution volume (%(C/S)) ratio 62

4.3.3 Effect of diameter of liquid core capsule 63

4.3.4 Effect of membrane thickness of liquid core capsule 65

4.3.5 Reusability of the liquid core capsule 66

4.3.6 Effect of solution viscosity 67

4.3.6 (a) Viscosity of different LA-acidified solutions 68

4.3.6 (b) pH of LA-acidified solutions 70

4.4 Use of the liquid core capsule to regulate pH in Lactic acid bacteria (LAB) fermentation 76

4.4.1 Effect of diameter of liquid core capsule on fermentation 76

4.4.2 Effect of membrane thickness of liquid core capsule on fermentation 80

4.4.3 Effect of medium viscosity on fermentation 83

4.4.4 Scale-up study 86

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion 89

5.2 Recommendations for future project 91

5.3 Commercialization potential 92

REFERENCES 95

LIST OF PUBLICATIONS 104

LIST OF AWARDS 105

LIST OF TABLES

NO.		PAGE
2.1	Characteristics of lactic acid	19
2.2	Comparison among of LAB used in the lactic acid production	25
3.1	Specification of sodium-alginate	33
3.2	Specification of CMC	33
3.3	The ingredients of MRS agar and broth of LAB fermentation	35
3.4	LAB fermentation parameters	54
4.1	Viscosity of MRS broth with added different concentrations of starch.	70
4.2	Application of liquid core capsule in different concentration of starch solution	73
4.3	Application of liquid core capsule in different concentration of CMC solution	74
5.1	Price of pH regulator capsules	94

LIST OF FIGURES

NO.		PAGE
2.1	Dropping extrusion dripping method	11
2.2	Pre-gel dissolving method	11
2.3	Concentric nozzle method	12
2.4	Chemical structures of (a) M-block and (b) G-block in alginate	13
2.5	Principal chemical structure of amylose	16
2.6	Principal chemical structure of amylopectin	16
2.7	Principal chemical structure of cellulose	17
2.8	Principal chemical structures of CMC	18
2.9	The isomer forms of lactic acid	20
3.1	Flow chart of the research methodology.	32
3.2	Autoclave equipment used for sterilization of fermentation materials.	35
3.3	10mL of syringe image.	37
3.4	Variation of hypodermic needle diameter image.	37
3.5	Encapsulation set-up by extrusion method.	38
3.6	Image analysis of the liquid core capsules.	39
3.7	Four measurements of capsule membrane thickness.	40
3.8	Image of liquid-core capsules with SF (a) less than 0.07 and (b) more than 0.07 before being analyzed by Sigma ScanPro5 software.	41
3.9	Preparation of stock culture of *L.casei* strain *Shirota.* This stock culture was stored in freezer at temperature 4^{o}C.	42
3.10	(a) Preparation of dilution series was conducted to identify the bacteria cultured; (b) image of bacteria cultured was spread in MRS agar after incubated at 48 hours in incubator.	42
3.11	pH measurement image.	43

3.12	Image of reflectometer and analytical test strip for glucose and LA concentration.	44
3.13	Standard curve of 52 g/L MRS broth (glucose concentration)	45
3.14	Standard curve of 20 g/L lactic acid 90 % (liquid)	45
3.15	Standard curve of MRS broth concentration in dilution series.	46
3.16	Standard curve of LA-acidified MRS broth concentration in dilution series.	46
3.17	Total viable cell count by spread-plate method	47
3.18	Image of viscometer and spindle for viscosity analysis.	48
3.19	Submerged fermentation of LAB in Erlenmeyer shake flasks	54
3.20	Image of orbital shaker	54
4.1	Diameter of the liquid core capsules produced by different dripping nozzles.	56
4.2	Diameter of the liquid core capsules produced from dripping nozzle with diameter of 0.8 mm at varied curing times	57
4.3	Membrane thickness of the liquid core capsules produced by different dripping nozzles.	58
4.4	Membrane thickness of the liquid core capsules produced from dripping nozzle of 0.8 mm in diameter at varied curing time.	59
4.5	Sphericity factor (SF) of the liquid core capsules produced by different dripping nozzles	60
4.6	Sphericity factor (SF) of the liquid core capsules produced at varied curing time.	61
4.7	The pH of 20 g/L lactic acid (LA) solution against time where LA solution was containing capsule with diameter of (a) 3.18 mm, (b) 4.50 mm, (c) 4.54 mm, (d) 4.57 mm, and (e) 8.35 mm.	62
4.8	The pH of 20 g/L lactic acid solution versus time (hours) when liquid core capsules of different membrane thickness were dropped into the LA solution. The capsules were produced from (a) 0.8 mm and (b) 1.2 mm of dripping nozzle.	63
4.9	The pH of 20 g/L lactic acid solution versus time when liquid core capsules of different $CaCO_3$ loadings were tested..	64
4.10	pH of 20 g/L lactic acid solution versus time when the solution was tested with different C/S ratio (were indicated in the legend in the	66

parentheses)

4.11 The pH of the lactic acid bacteria fermentation broth which contained liquid core capsules after two times shifting to fresh fermentation medium in three consecutive days. 67

4.12 The viscosity of starch solutions of different concentrations. 68

4.13 Viscosity of CMC solutions of different concentrations. 69

4.14 pH of the starch solutions against time. The starch solutions were prepared with 20 g/L lactic acid. 71

4.15 pH of CMC solutions acidified by 20 g/L lactic acid against time. 72

4.16 pH of MRS broth at different time. The MRS broth was prepared with 20 g/L LA. 75

4.17 Image of liquid core capsules in MRS broth after 24 hours. 76

4.18 Effects of capsule size (i.e. 3.18, 4.50 and 4.54mm) on growth kinetics of LAB in pH against time. 77

4.19 Effects of capsule size (i.e. 3.18, 4.50 and 4.54mm) on growth kinetics of LAB in (a-e) kinetic study of microbial growth. 79

4.20 Effects of liquid core capsule membrane thickness (i.e. 0.4, 0.71 and 0.81 mm) on growth kinetics of LAB in pH against time. 80

4.21 Effects of liquid core capsule membrane thickness (i.e. 0.4, 0.71 and 0.81 mm) on growth kinetics of LAB in (a-e) kinetic study of microbial growth. 82

4.22 Effect of medium viscosity on growth kinetics of LAB in pH against time. 83

4.23 Effect of medium viscosity on growth kinetics of LAB in (a-e) kinetic study of microbial growth. 85

4.24 Effects of large scale fermentation on growth kinetics of LAB in pH against time. 86

4.25 Effects of large scale fermentation on growth kinetics of LAB in (a-c) kinetic study of microbial growth 88

LIST OFABBREVIATIONS

AHA	Alpha Hydroxyl Acid
cfu	Colony Forming Unit
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
LA	Lactic Acid
LAB	Lactic Acid Bacteria
L.casei	*Lactobacillus casei*
MRD	Maximum Recovery Diluents
MRS	de Man Rogosa Sharpe
PLA	PolyLactic Acid
rpm	Rotation per Minutes
SF	Sphericity Factor
sp.	Species
w/v	Weight per Volume
C/S	Capsule volume to Solution volume
CSTR	Continuous Stirred Tank Reactor
Ba^{2}	Barium ion
Ba-alginate	Barium alginate
Ca^{2+}	Calcium ion
$C_6H_{10}CaO_6$	Ca-lactate
Ca-alginate	Calcium Alginate
$CaCO_3$	Calcium Carbonate
$CaCl_2$	Calcium Chloride
CO_2	Carbon Dioxide
CMC	Carboxyl Methyl Cellulose
$C_6H_{12}O_6$	Glucose
$CH_3CH(OH)COO$-	Lactate Ion
$C_3H_6O_3$	Lactic Acid (2-hydroxypropionic acid)
O_2	Oxygen
NaCl	Sodium Chloride
H_2O	Water
HCl	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
NH_3	Nitrite Acid
M	Molar
KCl	Potassium Chloride
X	Viable cell concentration

LIST OF SYMBOLS

α	Alpha
β	Beta
cm	Centimeter
°C	Degree Celsius
K_a	Dissociation Constant
g	Gram
g/L	Gram per liter
ΔH_c	Heat of Combustion
d_{max}	Maximum Diameter
mg	Milligram
mL	Milliliter
mm	Millimeter
mmHg	Millimeter of Mercury
d_{min}	Minimum Diameter
min	Minutes
C_p	Specific Heat
SF	Sphericity Factor
d_{max}	Maximum diameter
d_{min}	Minimum diameter
mg/L	Miligram per liter
rev/min	Revolution per minutes
cP	Centipoises
mPa.s	MilliPascal-seconds

Kajian Tentang Penggunaan Kapsul Berteraskan Cecair Kalsium Karbonat Untuk Mengawal pH Dalam Penapaian Bakteria Asid Laktik

ABSTRAK

Kajian ini menyelidik tentang kapsul teras cecair yang mengandungi kalsium karbonat dan carboxyl methyl cellulose (CMC) yang dikelilingi oleh membran alginat. Aplikasi penggunaan kapsul teras cecair meliputi banyak industri. Produk terkumpul di dalam proses penapaian akan menyebabkan populasi bakteria semakin berkurang dan menurunkan pH. Oleh itu, penggunaan kapsul teras cecair diperkenalkan untuk mengawal pH dalam proses penapaian. Objektif penyelidikan adalah menganalisa kriteria kapsul teras cecair (diameter, ketebalan membran). Selain itu, kualiti kapsul untuk mengawal pH dalam penapaian diuji melalui kadar kepekatan kalsium karbonat, nisbah isipadu kapsul teras cecair dalam media, kekerapan penggunaan kapsul teras cecair dan kelikatan media. Objektif terakhir ialah menganalisa kebolehan penggunaan kapsul teras cecair di dalam proses penapaian bakteria asid laktik. Penyelidikan ini menunjukkan kapsul teras cecair yang bersaiz kecil dan nipis (diameter 3.18 mm; ketebalan membran 0.49 mm) telah mencapai pH yang tertinggi, 6.92 berbanding dengan yang bersaiz besar dan tebal (diameter 8.35 mm; ketebalan membran 1.99 mm) mencapai pH 6.72 selepas 24 jam. Selain itu, kepekatan kalsium karbonat, 40 %(w/v) dan nisbah isipadu kapsul teras cecair dalam isipadu media, 1:3 dipilih untuk kajian penapaian bakteria asid laktik. Kemudian, penyelidikan penggunaan kapsul yang sama berulang kali, menunjukkan ia boleh digunakan sebanyak lebih daripada 2 kali dan mencapai pH target (> pH 4). Kesan kapsul teras cecair terhadap kelikatan media (≤1090cP.s) mampu mengawal pH. Tambahan pula, penggunaan kapsul teras cecair ini juga dapat membantu mengawal pH berbanding menggunakan serbuk kalsium karbonat secara langsung di dalam proses penapaian bakteria asid laktik. Begitu juga, mampu meningkatkan dan mengawal kepekatan daya hidup sel dengan baik. Hal sedemikian, dengan menjalankan proses penapaian berskala besar juga didapati ia memberi keputusan yang baik dan seakan sama. Justeru, penggunaan kapsul teras cecair boleh mengawal pH di dalam media-media yang diselidik menunjukkan ia berjaya mencapai aras pH yang dikehendaki.. Begitu juga, pengawalan pH mampu meningkatkan kepekatan daya hidup sel dan penghasilan laktik asid pada akhir penapaian bakteria asid laktik.

Studies On Calcium Carbonate Loaded Liquid Core Capsule As A pH Controller In Lactic Acid Fermentation

ABSTRACT

Liquid-core capsule composed of calcium carbonate ($CaCO_3$) and carboxyl methyl cellulose (CMC) as inner liquid-core surrounded by a cross-linked alginate membrane was prepared in this study. Liquid-core capsules have been widely used in various industrial applications. The performance of fermentation medium is affected by the accumulation of product(s) in the medium which will reduce the pH that may inhibit the growth of the fermenting microbes. The use of liquid-core capsules to control pH of fermentation medium is a new approach. The objectives of this research were to analyse the effect of liquid-core capsule properties (i.e. diameter, membrane thickness) on the efficiency of pH control, and process variables (i.e. calcium carbonate loading, capsule to solution volume ratio, capsule reusability, and medium viscosity) on the efficiency of the capsules to control pH of fermentation medium. Lastly, to evaluate the effect of liquid core capsules properties on the fermentation kinetics of lactic acid bacteria (LAB). The results showed the smallest and thinnest capsules (diameter 3.18mm and membrane thickness 0.49mm) could achieve pH 6.92 which is higher than that (only reached pH 6.72) of the largest and thickest capsules (diameter 8.35mm and membrane thickness 1.99mm) after 24 hours. Therefore, 40% (w/v) of $CaCO_3$ loading and 1:3 ratio to LA solution volume was selected for LAB fermentation study. Then, the capsule reusability test showed the capsule was can used up to two runs with the pH above 4. The effect of capsule in the medium viscosity (up to 1090cP.s) was capable to control the pH at the desired pH range (> pH 4). In LAB fermentation, pH of fermentation was well controlled by the capsule and the results were comparable to those of free $CaCO_3$. Then, the viable cell concentration was well maintained by the capsule. Similar results were obtained when the large scale fermentation was conducted. In short, the pH regulator capsules were capable to control the pH of the tested solutions at the desired level. In order to improve the viable cell concentration and lactic acid production at the end of the LAB fermentation.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Lactic acid bacteria (LAB) are gram-positive, non-sporing, and usually non-motile facultative anaerobes, thus making the strict exclusion of air unnecessary (Altiok, 2004). LAB are classified into 3 groups based on their metabolism of sugars; Group 1 species are obligate homofermenters of hexose to lactate but do not ferment pentoses. Group 2 species are facultative which heterofermenters of hexose to lactate using the glycolysis pathway and pentoses to lactate and acetate using the phosphoketolase pathway. Under glucose limitation, group 2 species heterofermenters of hexose to lactate, acetate, ethanol and formate. Group 3 species are obligate heterofermenters of sugars (Zelal, 2009; Altiok, 2004).

LAB species such as *Leuconostoc, Streptococcus, Lactobacillus, Enterococcus, Aerococcus,* and *Pediococcus* (Chelule, Mokoena, & Gqaleni, 2010) are dominant microorganisms and considered as major contributors to the fermented food industry (Chelule *et al.,* 2010). The benefits of LAB in food fermentation practice include increasing food palatability by enhancing its aroma and flavor, improving food quality by increasing the amount of proteins and vitamins, enhancing the nutritional value and aid digestion of cereals which are normally nutritionally poor, confer preservative and detoxifying effects on food, enhance immunity and strengthen the body against pathogenic bacterial infections, and prolonged of fermented food shelf life (Chelule *et al.,* 2010; Wee *et al.,* 2006). Thus, LAB fermentation is not only a major economic importance, but also a good human health. Example of LAB fermented foods in Asia include kimchi, tapai, tairu, cultured milk, yogurt, and pickled fruits and vegetables.

The LAB growth and productivity of a biological system is strongly influenced by the pH of the working medium (Huang *et al.,* 2003). The optimal pH range for LAB varies between pH 5 and 7 (Hetényi, Németh, & Sevella, 2011). In order to control the pH of the system, several approaches have been introduced in the past. The pH of the biological systems can be controlled using a pH controller, using a separation process to remove the organic acid in the medium, manipulating the fermentation mode, and adding a pH buffer to regulate the medium pH.

Among the methods, the use of pH controller is the simplest approach but it is relatively expensive and requires advanced instrumentation. The separation process requires an expensive membrane and the separation is commonly carried out outside the fermenter, which increases the risk of contamination (Lee *et al.,* 2007). Furthermore, the continuous and fed batch fermentation modes are reported to be effective to control pH of the medium. However, the risk of contamination is higher when continuous mode is used. The use of fed batch mode is not economically feasible as it requires large volume of fermentation medium to adjust the medium pH (Lee *et al.,* 2007).

Generally, lactic acid is produced when bacteria break down glucose during the LAB fermentation. The high acid content yield during the process would lead to low pH level in the fermentation (i.e. pH 4) and this condition inhibits cell growth, enzymatic hydrolysis, and microbial activity in fermentation (Huang *et al.,* 2003). In the food industry, high acid content could alter the taste and quality of fermented foods. Therefore, controlling the pH of LAB fermentation process to within desired levels is important to increase the quality of food and to maintain the population of beneficial micro-flora.

Encapsulation is defined as formation of capsules or beads by biopolymers where the active compound is surrounded by a continuous film or biopolymers coating (Lee *et al.,* 2005; Chan *et al.,* 2009). The core is protected within a continuous semi-permeable membrane (Lee *et al.,* 2005).

1.2 Problem Statement

In the fermentation of lactic acid, the role of pH control is crucial (Amrane and Prigent, 1994). The acidification of the fermentation broth caused by an increase in lactic acid production which in turn decrease growth of lactic acid bacteria. Current methods to control pH include the use of electrokinetic bioreactor, conventional electrodialysis, and using calcium carbonate, ammonium hydroxide or sodium hydroxide as a buffer.

Although the use of electrokinetic bioreactor equipped with pH controller was successfully implemented to control the pH, separate and concentrate the product from the fermentation broths (Li *et al.*, 2004), it is also expensive (Akerberg and Zacchi, 2000). The use of calcium carbonate, ammonium hydroxide or sodium hydroxide as a buffer method increases the complexity and cost of downstream processing because after fermentation, the product must be separated from the medium and the conversion of the salt to an organic acid is complicated because it involves precipitation and acidification using a mineral acid (sulfuric acid). Therefore, a simple, low cost and environmental friendly downstream processing for organic acid production is desirable (Li *et al.*, 2004).

In this study, liquid core pH buffers were encapsulated within a membrane of sodium alginate gel and are designed to replace current pH control methods. The pH buffer will be used to control the pH in the medium of the fermentation modes. Among the various techniques for immobilizing bioactive materials, calcium alginate gel encapsulation are commonly used, because the gelling process can be easily performed under mild conditions and alginate is an inert and non-toxic product (Koyama and Seki, 2003). This encapsulation technology is a new approach in controlling the pH of the biological system. It is inexpensive as they are formed using only different needle diameter syringes. The performance of the capsules to control pH in the LAB fermentation process is evaluated by considering capsule properties such as diameter, membrane thickness, and shape.

1.3 Objectives of Research

The objectives of the project are as following:

i. To analyze the effect of liquid core capsules properties (i.e. diameter and membrane thickness) on the efficiency of pH control.

ii. To quantify the effect of liquid core capsules properties on calcium carbonate ($CaCO_3$) loading, capsule to solution volume ratio, and capsule reusability on the efficiency of the capsules to control pH.

iii. To determine the efficiency of the capsules to control pH of solutions of different viscosity.

iv. To evaluate the effect of liquid core capsules properties on the fermentation kinetics of lactic acid bacteria.

1.4 Scope and Significance of Research

This research focus on the growth kinetics of LAB under controlled pH cultivation environment by studying key parameters of liquid core capsules with different properties. The parameters used in the LAB fermentation are liquid core capsule diameter, capsule membrane thickness, calcium carbonate loading, capsule volume to solution volume (C/S) ratio, reusability of liquid core capsule, and concentration of starch in broth.

1.5 Organization of Thesis

Chapter 1 gives an overall view and ideas of this project. It reveals the study background, problem statement, research objectives, and scope and significance of research. Chapter 2 reviews the encapsulation technology, importance of encapsulation, capsule formation methods, and capsule formation materials to produce liquid core capsules. A general discussion on lactic acid, lactic acid bacteria (LAB), and the selection of LAB strain are also presented. The problems faced by current pH controller technology and the strategies to control pH in LAB fermentation were also discussed. Chapter 3 covers the method and design of a simple extrusion dripping technique to encapsulate calcium carbonate ($CaCO_3$) within a semi-permeable polymeric alginate membrane. Several key factors including the effect of liquid core capsule diameter, capsule membrane thickness, calcium carbonate loading, capsule volume to solution volume ratio, capsule reusability test, and solution viscosity effect in controlling the pH of LAB fermentation are noted as well as the detailed in LAB fermentation and parameters design. In chapter 4, the results of the physical properties of the liquid core capsules and the preliminary studies are presented and discussed. The influence of liquid core capsule properties on the capability of the liquid core capsules to regulate the pH of lactic acid solution are observed and reported. The results on the studies on the influence of liquid core capsule properties on the pH change of different viscous solutions are presented and discussed. The results of the lactic acid bacteria fermentation carried out using the pH regulator liquid core capsules are reported. Chapter 5 concludes the research outcome of the project and some of the prospect of the work in industry in future. Recommendations are also brought forward and highlighted for further studies.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews the encapsulation technology, and its importance, as well as liquid core capsule formation methods and materials. This chapter also discusses lactic acid in general including its background and chemical properties, application and production of lactic acid. A discussion on lactic acid bacteria (LAB), the selection of LAB strain depending on the isomer produced, and LAB growth kinetics are also presented in this chapter. Then, this chapter also reviews the problems faced by current pH control technologies and pH control strategies in LAB fermentation.

2.1 Encapsulation Technology

Encapsulation play important roles in various industries such as food, pharmacy, chemistry and even agriculture. Encapsulation is a process to protect a gaseous, liquid or solid active agent by surrounding it with a semi-permeable membrane (Blandino, Macias, & Cantero, 1999). In this process the conventional method of calcium alginate beads formation (entrapment) is reversed (Blandino *et al.,* 1999). A mixed of active ingredients is dropped into a continuously stirred solution of sodium alginate. The alginate liquid-core capsules are formed due to the cross-linking of the alginate and calcium ion (Keitaro and Minoru, 2004).

Encapsulation technology is used in cell immobilization as an alternative to entrapment process because it has no leakage and give higher cell loading (Park and Chang, 2000). It is also used in the biopharmaceutical industry to immobilize cell